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Anticoagulant rodenticides in predatory birds 2012: a Predatory Bird Monitoring Scheme (PBMS) report

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Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability contaminant monitoring and surveillance work on avian predators. By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Anticoagulant rodenticides, and in particular second generation anticoagulant rodenticides (SGARs), can be toxic to all mammals and birds. Predators that feed upon rodents are particularly likely to be exposed to these compounds. The PBMS, together with other studies, has shown that there is widespread exposure to SGARs of a diverse range of predators in Britain and that some mortalities occur as a result. This report describes the PBMS monitoring for SGARs in barn owls (*Tyto alba*) found dead in 2012, summarises long term trends in exposure in this species, and compares the relative prevalence of SGARs in barn owls in England and Scotland. We also report the results of an initial investigation into SGAR contamination in 42 sparrowhawks (*Accipiter nisus*) found dead between 2010 and 2012. Sparrowhawks normally feed on birds and the aim of this investigation was to assess the potential importance of avian foodwebs in exposure of predators to SGARs.

SGARs were detected in 87% of the 63 barn owls that were collected in 2012. The most prevalent compounds were difenacoum, bromadiolone and brodifacoum. The majority of the residues were low (< 0.1 µg/g wet weight). One owl was diagnosed as likely to have been poisoned by SGARs. Most of the sparrowhawk livers that were analysed had detectable liver SGAR concentrations, again mainly difenacoum, bromadiolone and brodifacoum (79%, 55% and 64% of birds, respectively). The proportion of sparrowhawks with detectable residues of one or more SGAR (93%) did not differ significantly from that for barn owls (86%) collected over the same time 2010-12 time period. Co-occurrence of multiple residues in the liver was common in barn owls and sparrowhawks (70% and 74% of birds respectively). Sparrowhawks had significantly lower liver sum SGAR concentrations than barn owls in those birds that had detectable residues.

SGARs have been monitored in barn owls since 1983. Data on long-term trends have been adjusted to account for changes over time in sensitivity of analytical methods. This has meant that very low residues (<0.025 µg/g wet weight), which are now detectable, are not included in the time trend analysis. Overall, the proportion of both adult and juvenile barn owls with detectable liver concentrations of one or more SGAR has increased significantly over the course of monitoring. The proportion of barn owls with detectable SGAR residues over the period 1990-2012 was two-fold higher in England than in Scotland but residue magnitude did not differ between birds from the two areas.

1 Introduction

1.1 Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.



By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. Our monitoring provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory decisions about sustainable use of chemicals (for example, the [EU Directive on the Sustainable Use of Pesticides](#)). In addition, the outcomes from the monitoring work are used to assess whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently, the PBMS has two key objectives:

- (i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides, biocides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value
- (ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the [PBMS website](#).

1.2 PBMS monitoring of anticoagulant rodenticides

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. Predators that feed upon rodents are particularly likely to be exposed to these compounds. The PBMS (see previous reports, also (Newton et al., 1999; Shore et al., 2006; Walker et al., 2008a; Walker et al., 2008b) together with other studies (McDonald et al., 1998; Shore et al., 2003a; Shore et al., 2003b; Dowding et al., 2010) have shown that there is widespread exposure to SGARs in a diverse range of predators in Britain. Defra's Wildlife Incident Monitoring Scheme (WIIS)² and the PBMS have shown that some mortalities result from this exposure.

In response to conservation concerns over the potential impacts of SGARs on predators, the PBMS has monitored trends in exposure to second generation anticoagulant rodenticides (SGARs) in a sentinel species, the barn owl (*Tyto alba*). This has been done since 1983 and the findings from previous years and analyses of long-term trends are given in previous PBMS reports and by Newton et al. (1990, 1999). This report describes the results of PBMS monitoring of barn owls submitted to the PBMS in 2012.

A recent study has demonstrated that, in Scotland, the proportion of sparrowhawks (*Accipiter nisus*) that contained detectable liver SGAR concentrations was similar to that for barn owls (Hughes et al., 2013). This was unexpected because it has been assumed that the main transfer pathway for SGARs is via target and non-target rodents that eat SGAR bait. Secondary exposure in predators would therefore be expected to be highest in species that feed on rodents. Barn owls predominantly eat small mammals (Village, 1990) but sparrowhawks feed largely on birds (Newton, 1986). Hence, the Scottish study suggests that SGAR transfer through avian transfer pathways may also be important. We investigated whether the observations from Scotland were supported from a sample of sparrowhawks taken from across Britain. The results of that investigation are also presented in this report.

In some previous years, the PBMS has measured SGARs in red kites (*Milvus milvus*), a high priority species that has been reintroduced to England, and kestrels (*Falco tinnunculus*) which can accumulate relatively high liver SGAR concentrations (Walker et al., 2012; Walker et al., 2013) and about which there are concern over population declines (<http://www.bto.org/birdtrends2009/wcrkestr.shtml>). However, no red kites or kestrels were analysed for SGARs this year.

Barn owl and sparrowhawk carcasses were submitted to the PBMS by members of the public. The birds died from various causes, but mainly from road traffic collisions, other trauma and from starvation. The provenance of the birds is shown in Figure 1.1. All the barn owls received by the PBMS were autopsied and a subsample of 63 birds (stratified by date found) were analysed; all were birds that had died in 2012. A similarly stratified subsample of 42 sparrowhawks that had died between 2010 and 2012 was analysed. Tissues from all birds were archived in the PBMS tissue and egg archive where they are available for future research purposes. Liver SGAR residues were quantified by Liquid Chromatography Mass Spectrometry and a summary of the analytical methods can be downloaded at http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_Methods.pdf. Anticoagulant rodenticide concentrations are reported as µg/g wet weight (wet wt) throughout this report.

² Annual WIIS reports are available at www.pesticides.gov.uk/environment.asp?id=58

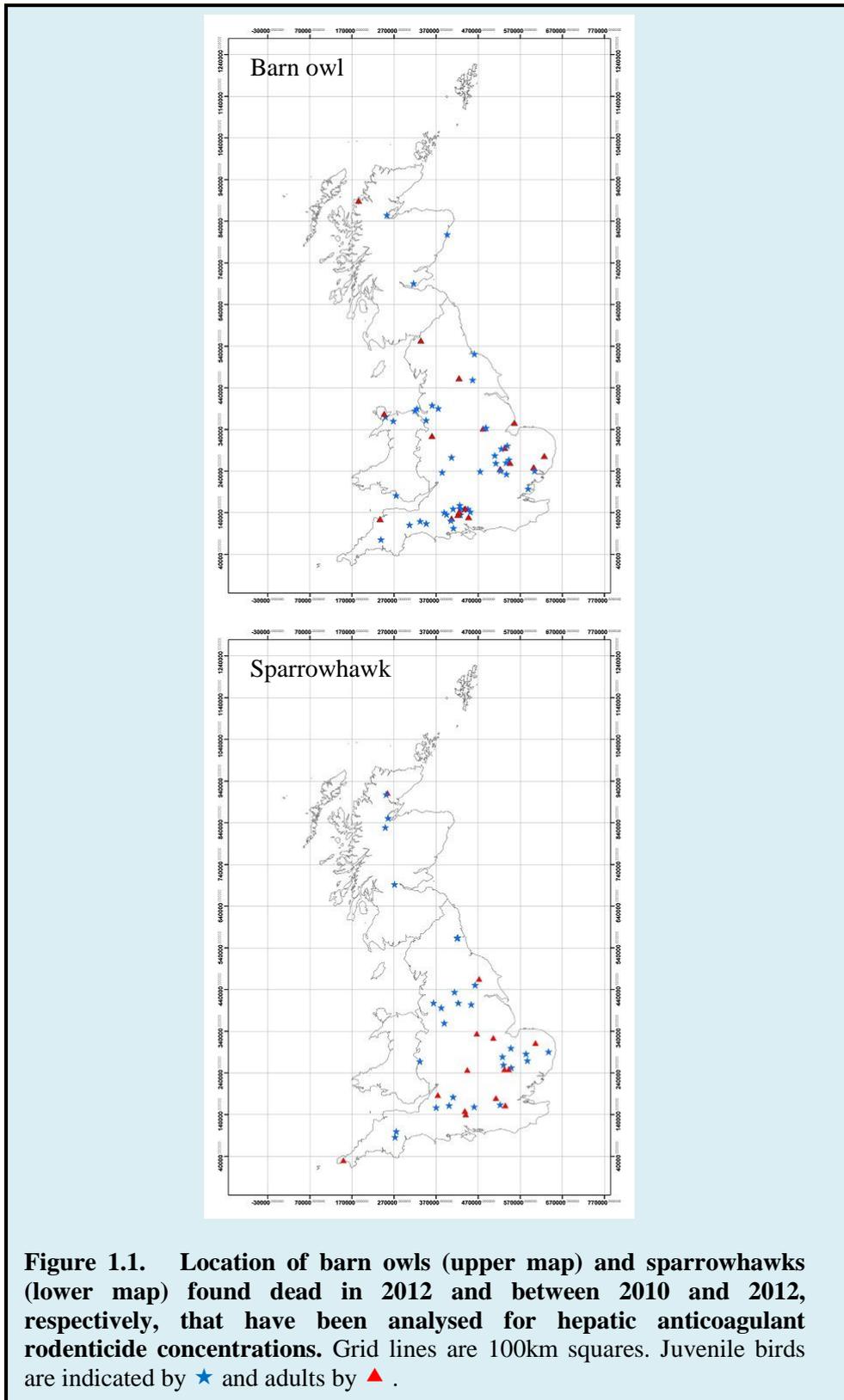


Figure 1.1. Location of barn owls (upper map) and sparrowhawks (lower map) found dead in 2012 and between 2010 and 2012, respectively, that have been analysed for hepatic anticoagulant rodenticide concentrations. Grid lines are 100km squares. Juvenile birds are indicated by ★ and adults by ▲ .

2 Anticoagulant rodenticide concentrations in birds submitted to the PBMS in 2012

Summary statistics for the incidence of detectable concentrations of anticoagulant rodenticides in the barn owls and sparrowhawks that were analysed are given in Table 2.1. Results for individual birds are given in a downloadable addendum to this report (<https://wiki.ceh.ac.uk/display/pbms/Home>).

The data reported here (Section 2) are for concentrations quantified using matrix-matched standards.

Table 2.1. Number (No/) of birds with detectable liver SGAR concentrations and the percentage (%) this comprised of all birds analysed. Total number of barn owls and sparrowhawks analysed was 63 and 42, respectively.

	Limit of Detection ¹	barn owls		sparrowhawks	
		No/	%	No/	%
<i>2nd Generation (SGAR)</i>					
bromadiolone	1.6	43	68	23	55
difenacoum	1.6	45	71	33	79
flocoumafen	1.6	6	9.5	0	0
brodifacoum	1.6	41	65	27	64
difethialone	1.6	1	1.6	1	2
Any SGAR	-	55	87	39	93
Multiple SGARs	-	44	70	31	74

¹ Method LoDs reported in ng/g wet wt.

NB. These figures are calculated based on methods using matrix matched standards.

2.1 Barn Owls collected in 2012

Sixty-three barn owls that had died in 2012 were analysed. Fifty-five (87% of the sample) contained detectable liver concentrations of one or more SGAR (Table 2.1).

The majority of exposure was due to bromadiolone and difenacoum (83% of barn owls analysed). Brodifacoum was also detected in more than half of the owls (65% Table 2.1) but often at low concentrations. Flocoumafen was detected in six of the 63 owls. Overall, more than one SGAR was detected in the liver of 70% of the owls.



The potentially lethal range for SGAR residues in barn owls has variously been described as $> 0.1 \mu\text{g/g}$ wet wt (Newton et al., 1998) and $> 0.2 \mu\text{g/g}$ wet wt ((Newton, et al., 1999) and is so classed on the basis of two sets of observations. The first was that owls diagnosed at post-mortem of having died from rodenticide poisoning (because they had characteristic signs of haemorrhaging from such organs as the heart, lungs, liver, brain and/or subcutaneous areas) almost all had liver residues $>0.1 \mu\text{g/g}$ wet wt. The second was that owls that had been experimentally poisoned had residues of the range $0.2\text{-}1.72 \mu\text{g/g}$ wet wt (Newton, et al., 1999). This range has been used in this report as an indicator of concern that SGARs may have a lethal effect on individuals although more recent analysis (Thomas et al., 2011) suggests that effects on some individuals may be associated with residues $<0.1 \mu\text{g/g}$ wet wt.

Most owls had concentrations below the potentially lethal range. Eight (12.7% of the sample) had liver residues (summed values for all SGARs) greater than $0.1 \mu\text{g/g}$ wet wt and six of these had SGAR concentrations above $0.2 \mu\text{g/g}$ wet wt. The maximum sum SGAR liver concentration was $0.421 \mu\text{g/g}$ wet wt (entirely brodifacoum) in a barn owl that had post-mortem signs of haemorrhage that was not associated with any obvious trauma. Rodenticides were considered to be a probable cause of death in this bird. In the remaining seven owls with liver residues $> 0.1 \mu\text{g/g}$ wet wt., there were no signs of haemorrhaging other than those likely to have been caused by physical trauma.

2.2 Sparrowhawks collected in 2010 to 2012

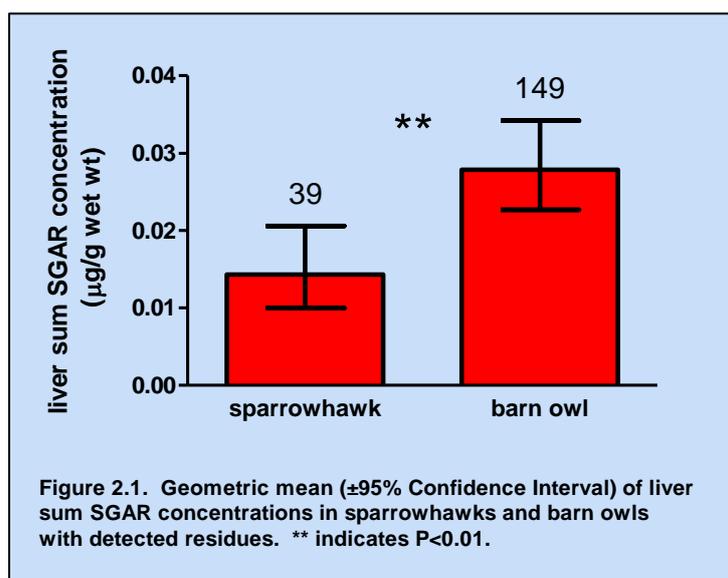
Of the 42 sparrowhawks we analysed, 39 (93%) contained detectable concentrations of anticoagulant rodenticides (Table 2.1). Thirty one birds had been exposed to more than one SGAR. As with barn owls (current report), and in kestrels and red kites from previous years (Walker, et al., 2012; Walker, et al., 2013), the most prevalent SGAR detected in sparrowhawks was difenacoum, although many birds also contained liver residues of bromadiolone and brodifacoum. (Table 2.1). Flocoumafen was not detected in any sparrowhawk livers while difethialone was detected in one bird.



For the period during which sparrowhawks were collected (2010-2012), the PBMS has analysed 173 barn owls for SGARs and 149 birds (86%) had detectable liver residues of one or more SGAR (this report and Walker, et al., 2012, 2013). This proportion was not significantly different (Fisher's Exact test $P>0.05$) from that in sparrowhawks.

Sum liver SGAR concentrations in sparrowhawks with detectable SGAR residues ranged between 0.002 and $0.136 \mu\text{g/g}$ wet wt. Two sparrowhawks had sum SGAR liver residues greater than $0.1 \mu\text{g/g}$ wet wt but, on post-mortem examination, there was no evidence of hemorrhage other than that associated with physical trauma. Therefore the contribution, if any, of rodenticides to the death of these two individuals is equivocal.

Sum liver SGAR concentrations in sparrowhawks with detectable residues were compared with those for barn owls that had been analysed between 2010-12 by the PBMS (Figure 2.1). Data were \log_{10} transformed to meet the assumptions of the two sample student t-test. Sum SGAR liver concentrations in birds with detectable residues were on average two fold higher in barn owls than sparrowhawks ($t_{(186)} = 2.96, P=0.003$).



Our findings are broadly consistent with those of Hughes et al (2013) in that we found that there is widespread low-level contamination of sparrowhawks with SGARs. The prevalence of detectable liver residues did not differ significantly between sparrowhawks and barn owls, as observed by Hughes et al (2013), although the percentage of birds with detectable residues in the present study was almost twice that of birds that came exclusively from Scotland (Hughes et al., 2013).

Previous studies have demonstrated that liver concentrations of contaminants in sparrowhawks can vary with factors, such as body condition ((Wienburg and Shore, 2004). Age and sex may also affect likelihood of exposure to SGARs, particularly in sexually dimorphic species such as sparrowhawks in which males and females may differ in their feeding preferences (Newton, 1986). Furthermore, unlike barn owls, sparrowhawks may occupy habitats in semi-urban as well as rural areas and exposure profiles may differ between areas. We have not investigated the importance of any such factors because of the relatively small sample size of birds available to us. Investigation of key factors that affect liver residues in sparrowhawks is merited using a larger dataset.

3 Long term trends in liver SGAR concentrations in barn owls

3.1 Long term time trends in the prevalence of liver SGAR residues in barn owls

A common limit of quantification (LoQ) was applied to the long-term dataset for SGARs. This was 0.025 µg/g wet wt. and was applied to each of the five compounds as described in Walker et al. (2010). Any detected values below this 0.025 µg/g LoQ were re-assigned as non-detected values for the purposes of time trend analysis and the percentage occurrence of SGARs were then recalculated for each year - these are termed “adjusted % detected” values. The use of adjusted % detected values under-estimates the true occurrence of liver SGAR residues for compounds and years where the limit of quantification was substantially lower, but it eliminates biases in the long-term data due to improvement in the sensitivity of analysis over time. The adjusted % detected values therefore provide a measure of temporal changes but do not necessarily indicate the actual scale of exposure. Adoption of a common limit of detection for different SGARs eliminates detection biases when comparing % detection values for different rodenticides.



All residues reported in this section have been determined using either solvent-matched standards or estimated solvent-matched standard values calculated from matrix matched standards as per Walker *et al.* (2013).

In previous PBMS reports, we have described long-term changes in accumulation of liver SGAR concentrations for data collected on an annual basis. However, ongoing analysis (Shore – *unpub. data*) has indicated that there are significant differences between liver residues in adult and juvenile barn owls (where juveniles were defined as birds hatched in the current or previous calendar year relative to when they died). Inter-year variation in the proportion of adults and juveniles may therefore mask trends in accumulation over time. Here therefore, we report long-term trends in barn owls for adult and juvenile barn owls separately. However, the consequence of this is that sample sizes in any

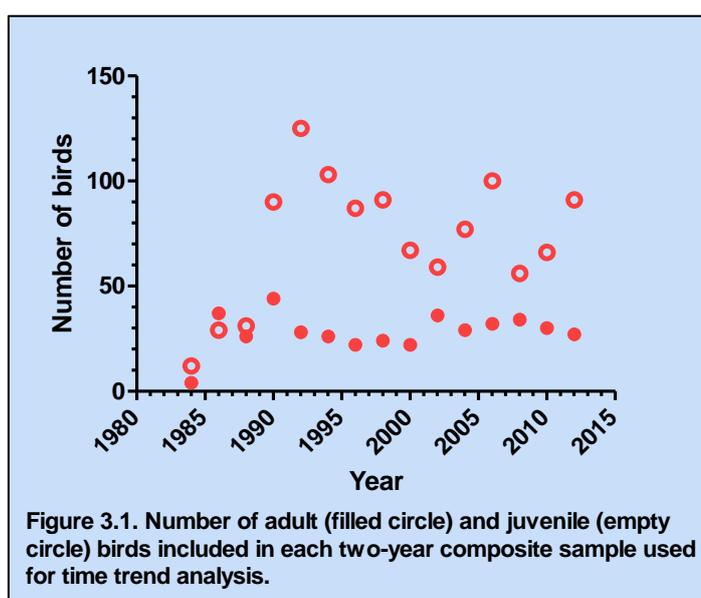


Figure 3.1. Number of adult (filled circle) and juvenile (empty circle) birds included in each two-year composite sample used for time trend analysis.

one year are smaller than previously. We have therefore combined data for pairs of years and report long-term trends on a biennial basis. Sample sizes for pairs of years are illustrated in Figure 3.1.

The adjusted % detected values for one or more SGAR in juvenile and adult barn owl livers has increased from when monitoring began in 1983/1984 in both adults and juveniles (Figure 3.2). The positive correlation between year and % of birds with detected residues was statistically significant in adults and in juveniles (Spearman rank correlation coefficient $r_s > 0.64$, $P < 0.01$ in both age groups). This long-term change primarily reflects an increase over time in the proportion of both adults and juveniles with detectable liver residues of difenacoum and bromadiolone (Figure 3.2; $r_s > 0.56$, $P < 0.05$ in both cases). Brodifacoum has been detected in barn owls during the course of the monitoring period and there is evidence of a significant progressive increase in exposure over time for this compound too, but only in adults ($r_s = 0.561$, $P < 0.05$; Figure 3.2). There were few birds with detected (adjusted) concentrations of flocoumafen (maximum 2% of juveniles and 5% of adults) and no evidence of any significant progressive change over time (data not shown).

The proportion of adult birds that have multiple compounds in their livers has also risen significantly over time ($r_s = 0.52$, $P < 0.05$; Figure 3.3). In terms of potential adverse effects, the 2012 results are consistent with those previously reported (Walker et al. 2013) in that the proportion of adult barn owls with liver concentrations above 0.1 $\mu\text{g/g}$ wet wt. has risen during the course of monitoring ($r_s = 0.662$, $P < 0.05$) but there has been no significant change in the proportion of either adult or juvenile birds with liver residues $> 0.2 \mu\text{g/g}$ wet wt. (Figure 3.3). Overall, the average proportion of adult and juvenile owls analysed that had SGAR residues $> 0.2 \mu\text{g/g}$ wet wt is 6.1% and 3.9% respectively, but the cause of death in many of these birds has not been attributed to anticoagulant rodenticides based on post mortem examination findings.

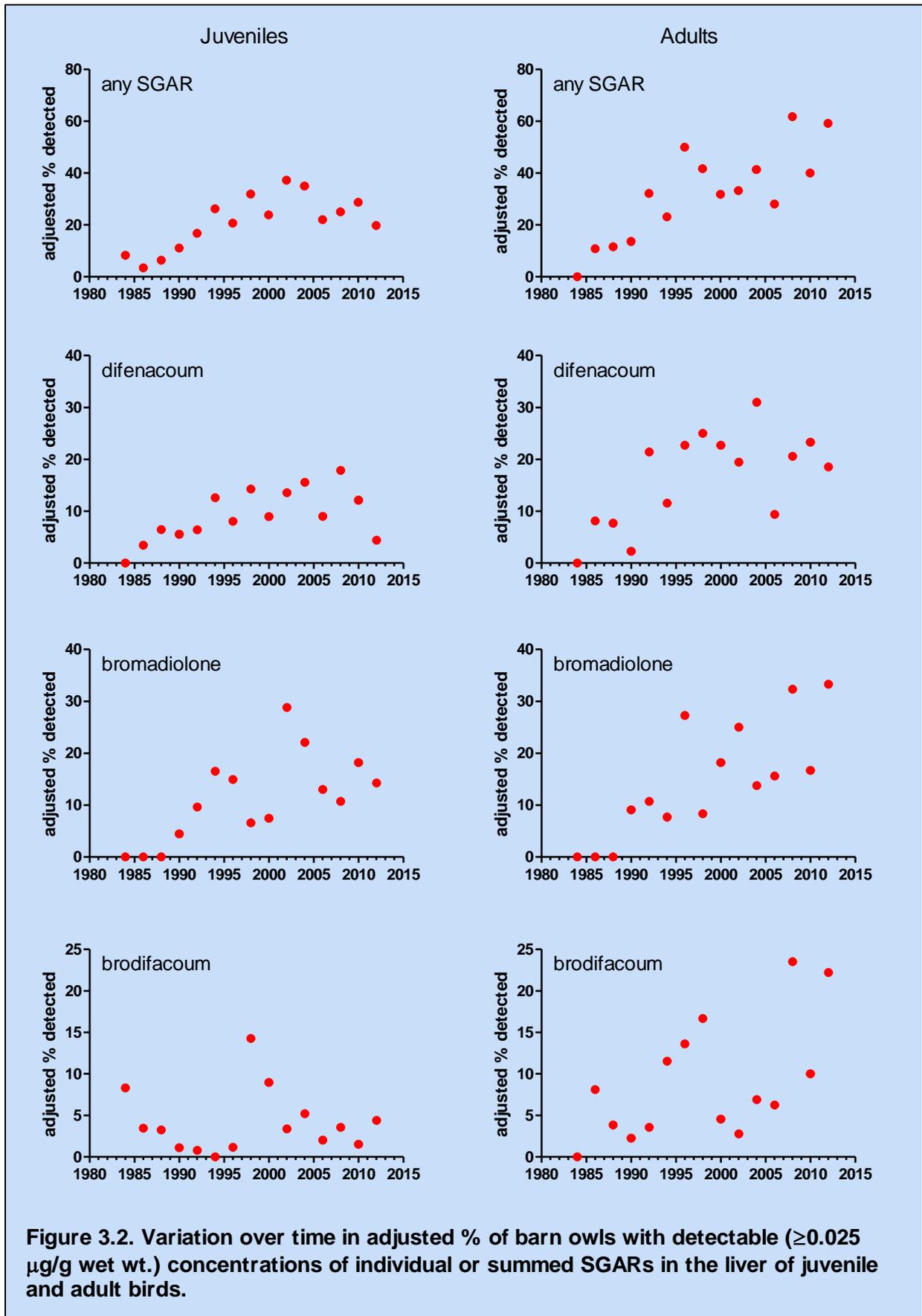
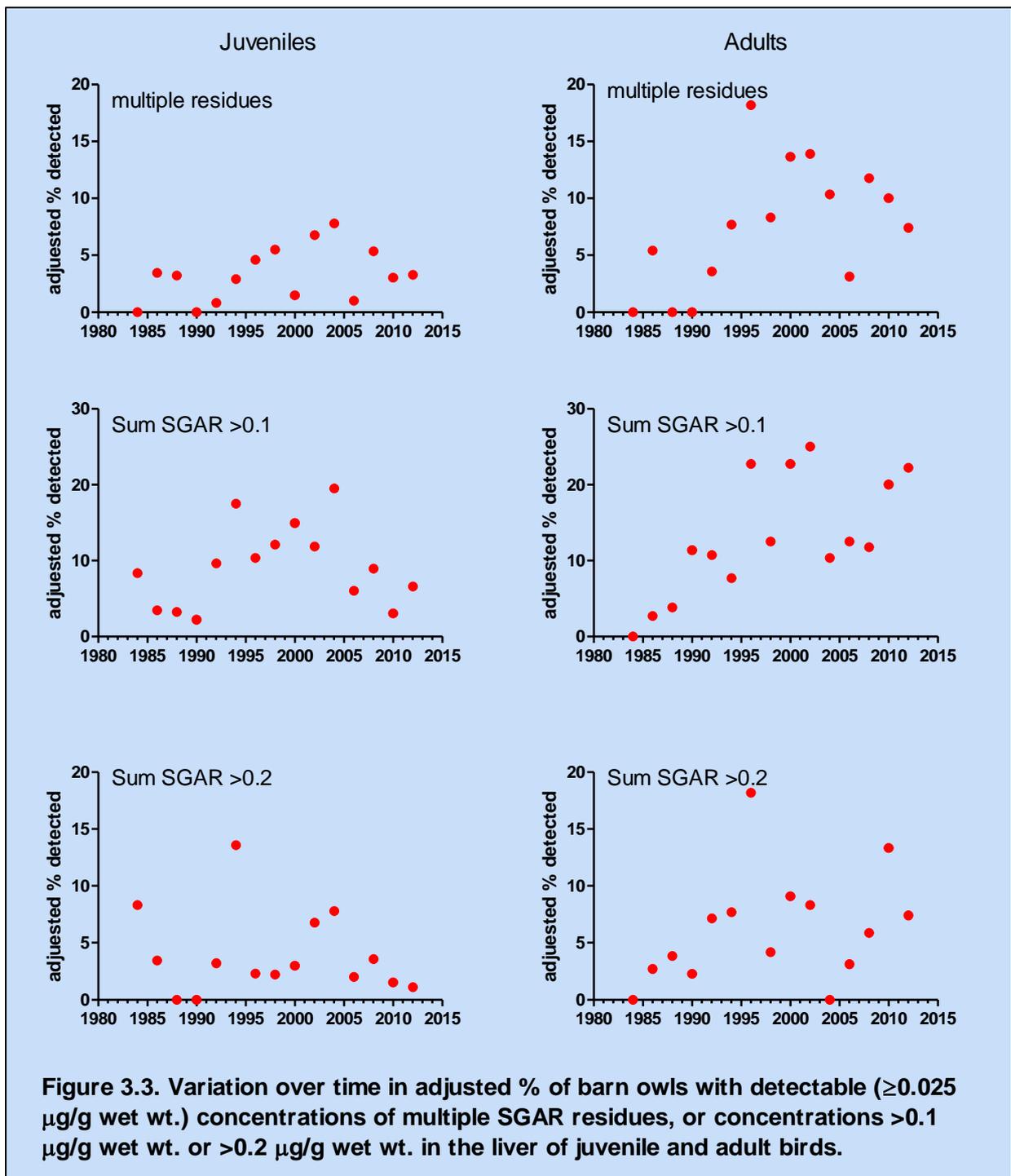


Figure 3.2. Variation over time in adjusted % of barn owls with detectable (≥ 0.025 $\mu\text{g/g}$ wet wt.) concentrations of individual or summed SGARs in the liver of juvenile and adult birds.



3.2 Long term regional analysis of the prevalence of liver SGAR residues in barn owls

The scale of exposure of barns owls in England and Scotland has been compared using the available data pooled for the years 1990-2012 to provide sufficient sample size for analysis. The adjusted % of owls with detected residues of any SGAR was approximately two-fold higher in England than in Scotland and the difference between the countries was significantly different. Similarly difenacoum was more prevalent in owls found in England than those found in Scotland (Table 4.1). Neither bromadiolone nor sum SGAR concentrations were significantly different between Scotland and England; other active ingredients were not compared due to low sample size.

Table 4.1. Number (n) of owls and the number as a percentage of all birds tested (%) from England and Scotland between 1990 and 2012 that had detectable liver SGAR concentrations ≥ 0.025 $\mu\text{g/g}$ wet wt. (common limit of quantification applied to all compounds and samples).

	number (% of whole sample tested) of owls with detected residues				Fisher's Exact test P-value	Mann Whitney U test of residues ³
	England (n=1147) ²		Scotland (n=119) ²			
Bromadiolone	191	(16%)	13	(11%)	0.117	U=942.5; P=0.141
Difenacoum	158	(14%)	6	(5.0%)	0.006	-
Flocoumafen	2	(0.2%)	1	(0.8%)	0.257	-
Brodifacoum	65	(5.7%)	4	(3.4%)	0.396	-
Difethialone	0	(0%)	0	(0%)	- ¹	-
Any SGAR	346	(30%)	20	(17%)	0.002	U=2905; P=0.151
Multiple SGAR	65	(5.7%)	4	(3.4%)	0.396	-

¹ Unable to test due to low sample size

² Sample size for difethialone is 94 and 8 for England and Scotland, respectively.

³ Non-detected values and concentrations less than 0.025 $\mu\text{g/g}$ wet weight were excluded from the analysis.

4 Acknowledgements

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