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Pesticides threaten an endemic raptor in an overseas French territory

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Pesticides threaten an endemic raptor in an overseas French territory

Abstract

The Réunion harrier is an endangered raptor that is endemic to Réunion Island. Anticoagulant rodenticides (ARs) are widely applied on the island to prevent leptospirosis transmission to humans and limit the damage to sugarcane crops caused by rats and house mice. As these pesticides exhibit a high risk of secondary poisoning for rodent predators, we examined whether the Réunion harriers were exposed to and potentially poisoned by ARs. The AR concentrations in the livers of 58 harrier carcasses collected from 1999 to 2016 were measured. Both the temporal and spatial trends were analysed, and the influences of individual and landscape characteristics on the liver concentrations and potential poisoning were determined. AR residues were detected in 93% of the harriers. Difenacoum was the most frequently found (73% of positive cases), while brodifacoum and bromadiolone showed the highest concentrations. Both the numbers of harriers exposed to ARs and of individuals that were potentially poisoned increased over time. This is particularly alarming as the number of harriers potentially poisoned by brodifacoum increased dramatically beginning in 2014. We also showed that the landscape composition of the townships influenced the AR exposure, as the concentrations increased with the proportion of urban areas and showed a peak at 25% of the township under sugarcane cultivation. We conclude that AR poisoning is likely a main threat for Réunion harrier conservation and propose several actions to limit poisoning.

Keywords: anticoagulant rodenticide; *Circus maillardi*; mitigation; poisoning; Réunion harrier

32 Introduction

33 On numerous islands worldwide, rodents are considered invasive species that are responsible
34 for various damages to human health, foodstuff and products and/or ecosystems and
35 biodiversity, notably because they threaten endemic species (Buckle and Smith, 2015). On
36 Réunion Island, an overseas French territory in the Indian Ocean, Norway rats (*Rattus*
37 *norvegicus*), black rats (*Rattus rattus*), and house mice (*Mus musculus*) were introduced and
38 currently occupy a wide range of habitats. They are found in urban settings, where they may
39 spoil foodstuffs, or in rural areas, where they feed on agricultural crops, especially sugarcane
40 plantations, which is the main agricultural product of the Island, leading to losses estimated at
41 10-15% of the annual harvest (Grollier and Soufflet, 2011). Moreover, rats are vectors of
42 zoonotic pathogens that are transmissible to humans, such as leptospirosis, for which they
43 exhibit a high seroprevalence (Guernier et al., 2016). Finally, rodents are partially responsible
44 for the decline in endemic endangered species, such as the Réunion petrel (*Pseudobulweria*
45 *atterima*). The application of pesticides, notably anticoagulant rodenticides (ARs), is currently
46 the main method implemented for controlling rodents and has helped to eradicate some
47 invasive species for island biodiversity conservation (Buckle and Smith, 2015). The
48 development of rodent resistance to the first generation of ARs (FGARs, e.g.,
49 chlorophacinone) has led to the use of second generation ARs (SGARs), namely,
50 bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone, which are more toxic
51 and effective than FGARs for killing resistant rodents. However, they are also more persistent
52 in animal tissue and more toxic to birds and mammals (Erickson and Urban, 2004), which has
53 led to the high secondary exposure or poisoning of rodent predators (López-Perea and Mateo,
54 2018). On Réunion Island, campaigns to spread ARs have been organized since 1977 to limit
55 rodent damage (Grondin and Philippe, 2011). However, the use of these pesticides is related
56 to an increase in suspected cases of poisoning of the Réunion harrier, *Circus maillardi*
57 (Grondin and Philippe, 2011). This species, endemic to Réunion, is the last breeding raptor on
58 the Island and is classified as endangered (International Union for the Conservation of Nature,
59 2018), with a breeding population estimated at 150 pairs (Grondin and Philippe, 2011). Its diet
60 may vary according to the habitat it occupies, but it is commonly composed of 50 to 70%
61 rodents (Grondin and Philippe, 2011). It has been widely reported that AR application is related
62 to the secondary exposure and poisoning of raptors (López-Perea and Mateo, 2018), but to
63 date, there are no data quantifying the exposure of the Réunion harrier to ARs. In this study,
64 we aimed to measure the AR concentrations in the livers of Réunion harrier carcasses that
65 have been collected by the Société d'Etudes Ornithologiques de la Réunion (SEOR) since
66 1999. To determine whether AR exposure could be a threat for this bird, the measured
67 concentrations were compared to toxicity threshold concentrations ($>100 \text{ ng g}^{-1}$), for which a

68 significant likelihood of toxicosis has been evidenced for raptors (Newton et al., 1999; Thomas
69 et al., 2011). Then, the AR concentrations were related to factors for possibly explaining the
70 variations in harrier exposure or poisoning, such as age, the sex of the individuals and the
71 landscape composition characterized by habitat variables as well as the local density of
72 breeding individuals or the year of carcass discovery. Identifying the exposure patterns and
73 drivers represents a crucial step in adapting rodent control methods for making them
74 compatible with wildlife conservation.

75

76 **Materials and methods**

77 *Sample collection*

78 From 1999 until February 2016, 58 Réunion harrier livers were collected. On Réunion Island,
79 the discovery of a dead, injured or weakened harrier was opportunistic and did not rely on any
80 standardized protocol. Following the discovery, the live animals were looked after at the SEOR
81 care centre, but some of them died or were euthanized because of their very poor physical
82 condition. The carcasses were stored at -18°C, and available information on the sex, age,
83 location and date of the finding were recorded. The age was defined as juvenile or adult based
84 on plumage characteristics. The location was not reported for 16 birds, and for 17 other cases,
85 this information was limited to the township where the harrier was found. The date of finding
86 was not available for 16 birds. As necropsies were not performed on the collected birds, the
87 clinical signs used for diagnosing AR poisoning (haemorrhages, lack of blood coagulation)
88 were not registered, and thus, the likelihood of AR poisoning was determined from the AR
89 concentration measured in the liver.

90

91 *Liver analysis*

92 The AR analysis was carried out following LC-MS/MS method (Fourel et al., 2017). Three
93 FGARs, warfarin, coumatetralyl, chlorophacinone, and the 5 SGARs used in European
94 countries, bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone, were
95 analysed. The chromatographic separation was achieved with a semi-porous Poroshell 120
96 StableBond C18 column (2.1*100 mm, 2.7 µm), and the MS/MS detection was carried out by
97 a 6410B triple quadrupole equipped with an electrospray ionization source in negative mode.
98 Two fragment ions were recorded in dynamic multiple reaction monitoring mode, and a
99 calibration curve was built for each analyte. The limits of detection were 1 - 2 ng g⁻¹ wet weight
100 (ww), and the recovery rates were above 70%.

101

102 *Statistical analysis*

103 The response variables were the number of exposed harriers (with at least one AR detected)
104 or the proportion of harriers exposed to the ARs (number of exposed individuals/total number
105 of carcasses discovered) per year or township, the ARs concentrations in the liver, and the
106 number or the proportion (number of individuals suspected to be poisoned/total number of
107 carcasses discovered) of harriers for which AR poisoning was suspected as the main cause
108 of death per year or township. For all the responses, we considered the sum of all the active
109 ingredients (a.i.) detected in one individual (\sum ARs) or each a.i. separately. Exposure was also
110 expressed as a binomial variable depending on whether residues were detected (1) or not
111 detected (0) in a bird. For suspected poisoning, Newton et al. (1999) and Thomas et al. (2011)
112 showed that an AR concentration in the liver $> 100 \text{ ng g}^{-1}$ is compatible with lethal poisoning
113 for raptors. Thus, based on the AR concentrations measured, poisoning was expressed as a
114 binomial variable, i.e., unlikely (0 if $<100 \text{ ng g}^{-1}$) or potential (1 if $\geq 100 \text{ ng g}^{-1}$). To consider a
115 possible cumulative effect of different ARs when they co-occurred, we also considered
116 potential poisoning when the \sum ARs in an individual was $>100 \text{ ng g}^{-1}$.

117 First, the ordination of the different rodenticide concentrations measured in the individuals was
118 explored with a principal component analysis (PCA). Then, we checked whether the AR
119 concentrations were explained by the age or the sex of the individuals with a between-class
120 analysis (BCA) (Dray et al., 2018). The BCA ratio, i.e., the inertia percentage explained by a
121 factor, and the statistical significance of the factors tested were determined with a permutation
122 test (Dray et al., 2018). The influence of these traits on the proportion of harriers with AR
123 residues or on the proportion of poisoning was checked using binomial generalized linear
124 models (GLM).

125 Temporal trends for the hepatic concentration (log-transformed) of each a.i. was investigated
126 with a generalized additive mixed model (GAMM) (Wood, 2017) with individual as a random
127 factor. The temporal trends for the number or proportion of exposed birds per year or month
128 were assessed with GAMM and the appropriate likelihood (Poisson for number of cases and
129 binomial for the proportions). In all cases, a smooth term on year was used as an explanatory
130 variable for each a.i. with the number of degrees of freedom limited to 5. For the number of
131 exposed or poisoned harriers per year, inter-annual trends were checked from 1999-2015
132 because the survey was only conducted in January and February 2016. We tested whether
133 the harriers collected, the harriers exposed or those with \sum ARs $>100 \text{ ng g}^{-1}$ were distributed
134 regularly, randomly or were aggregated over space based on the Clark-Evans test of
135 aggregation (Clark and Evans, 1954).

136 Then, we checked whether landscape composition influences harrier exposure. Given the low
137 number of birds with accurate discovery locations ($n=22$), analyses were conducted at the
138 township level ($n=42$). For each township, we computed the proportion of sugarcane and
139 urbanised areas. Data were extracted from a classification map (CIRAD, 2018) that was
140 rasterised on a 25 m x 25 m pixel raster grid and then aggregated at a 250 m resolution. In
141 addition, we tested whether harrier exposure was linked to the spatial distribution of the
142 breeding Réunion harriers to better target conservation efforts. We assumed that this
143 distribution was relatively stable during the last 20 years. We used a large-scale survey to
144 assess the population size and distribution of territorial pairs (unpublished data). The survey
145 was based on 184 point counts separated by approximately 2000 m and sampled during the
146 breeding season (from May to June) in 2017. The counting was conducted only in acceptable
147 weather conditions, i.e., no or light wind and rain. We applied spatial smoothing (function
148 `spatstat::ppp.smooth()`, $\sigma = 5000$ m) to the raw count data of the territorial pairs of harriers
149 (Baddeley et al., 2016). This allowed the production of a smoothed map as a proxy of the
150 density of the territorial pairs, reflecting their distribution on the island (hereafter harrier
151 density), which was then averaged for each township. We ran generalized mixed model
152 (GAMM) with the \sum ARs (Gaussian family with identity link) or the proportion of
153 exposed/poisoned cases (binomial family with identity link) as the response variables in which
154 an individual was the sampling unit. We compared 18 models per response variable (including
155 the null model), with the full model including a smooth term for year, harrier density, the
156 proportion of sugarcane crops and the proportion of urbanised surface areas (or an interaction
157 of those two). Township was included as a random factor. Then, the GAMMs with similar
158 response variables were run for each a.i. separately. Given the relatively limited sample size
159 ($n=42$), we restricted the maximum number of degrees of freedom of smoothing terms to $k=5$
160 to avoid overfitting. The models were ranked according to their AIC scores. All statistics were
161 performed with R.3.3.3 (R Core Team, 2018) and the libraries `ade4` (Dray et al., 2018), `gamm4`
162 (Wood and Scheipl, 2017), `geoR` (Ribeiro and Diggle, 2016), `lme4` (Bates et al., 2015), `mgcv`
163 (Wood, 2017), `spatstat` (Baddeley et al., 2016) and `multcomp` (Hothorn et al., 2008).

164

165 **Results**

166 Among the 58 Réunion harriers collected, 23 were females and 31 were males, and the sex
167 was not reported for 4 individuals. Adults represented 71% of the birds. Both the sex ratio and
168 age structure remained stable over time (binomial GLM, $p>0.26$). The total number of harrier
169 carcasses collected per year (regardless of whether they were contaminated by ARs and for
170 which the year of discovery was reported) is indicated in Fig. 1.

171

172 *Proportion and number of Réunion harriers exposed to ARs*

173 Ninety-three percent of the harriers (54/58) were exposed to ARs, with difenacoum,
174 bromadiolone, brodifacoum, chlorophacinone and difethialone measured in 73%, 70%, 51%,
175 41% and 18% of the birds, respectively (Fig. A1). Flocoumafen, warfarin and coumatetralyl
176 were never found. Only 12% of the harriers were exposed to one AR, while 30%, 29%, 15%
177 and 7% contained residues of 2, 3, 4 or 5 a.i., respectively. Bromadiolone and difenacoum co-
178 occurred the most frequently and were detected together in 61% of all the individuals. The
179 number of ARs detected was not related to sex or age (Poisson GLM, $p>0.24$), and no temporal
180 trend was found (Poisson GLM, $p>0.15$). The proportion of exposed harriers was not related
181 to sex or age when grouping all of the ARs (binomial GLM, $p>0.81$) or when considering each
182 a.i. separately (binomial GLMs, $0.077<p<0.86$).

183 The number of exposed harriers per year increased from 1999 to 2015 (Poisson GAM, $\text{edf}=1$,
184 $p<0.01$, Fig. 1), while the proportion of exposed birds per year did not show any inter-annual
185 trend (binomial GAM, $\text{edf}=1.81$, $p=0.90$). When taken separately, only difenacoum (negative
186 linear, $\text{edf}=1$, $p<0.05$) and chlorophacinone (non-linear with a peak in 2008, $\text{edf}=3.4$, $p<0.01$)
187 exhibited significant temporal trends for the proportion of exposed individuals per year.
188 Regarding the number of exposed birds per year, those exposed to brodifacoum increased
189 over time (GAM, $\text{edf}=1$, $p<0.0001$, Fig. 1), while the maximum number exposed to
190 chlorophacinone occurred in 2008 (GAM, $\text{edf}=3.8$, $p<0.05$). No intra-annual variation between
191 the months of discovery was found for the number (Poisson GAM, $\text{edf}=2.85$, $p=0.2$) or the
192 proportion (binomial GAM, $\text{edf}=2.1$, $p=0.4$) of exposed individuals per month. When
193 considering each a.i. separately, no trend was found for the number or the proportion of
194 exposed birds per month, with the exception of the harrier number exposed to brodifacoum,
195 peaking in July (Poisson GAM, $\text{edf}=3$, $p<0.05$).

196 The harriers exposed to the ARs were spatially aggregated (Clark-Evans test, $R=0.59$,
197 $p=0.001$). Among the 42 harriers for which the township of discovery was reported (Fig. A1),
198 39 contained ARs, and 40% were found on the eastern part of the island. Only 2 specimens
199 were found in the centre, and they did not contain residues. When checking for each AR
200 separately, spatial aggregation was found for bromadiolone, difenacoum and brodifacoum
201 (Clark-Evans test, $R=0.59$, $p=0.001$; $R=0.50$, $p=0.001$; and $R=0.43$, $p=0.001$, respectively) with
202 patterns similar to that described above.

203

204 *Hepatic concentrations of ARs in the harriers*

205 The highest median concentration was measured for bromadiolone, which was followed by
206 difenacoum and brodifacoum (Fig. 2). The difethialone residues were the lowest for the SGARs
207 (Fig. 2). The PCA revealed a positive association between the difethialone and difenacoum
208 concentrations in the individuals, while these ARs were not related to the three others.
209 Bromadiolone and chlorophacinone were weakly linked and tended to be negatively correlated
210 with brodifacoum. The AR concentrations in the individuals were not explained by age or sex
211 (BCA ratio=0.019 and 0.012; permutation test, $p=0.38$ and 0.69 , respectively). From 1999 to
212 2016, we found significant temporal trends for brodifacoum, bromadiolone, chlorophacinone
213 and difenacoum. The brodifacoum concentrations have increased sharply since 2012 (GAMM,
214 $\text{edf}=2.47$, $p < 0.0001$), and bromadiolone peaked in 2003 (GAMM, $\text{edf}=3.59$, $p < 0.001$) (Fig. 3).
215 A low peak was found for chlorophacinone from 2007-2008 (GAMM, $\text{edf}=3.77$, $p < 0.001$),
216 whereas difenacoum decreased slowly beginning in 1999 (GAMM, $\text{edf}=1$, $p < 0.05$) (Fig. 3).

217

218 *Potential cases of poisoning: trends for the harriers with AR concentrations > 100 ng g⁻¹*

219 Thirty-six harriers (62%) had $\sum \text{ARs} > 100 \text{ ng g}^{-1}$. For 16, 11 and 2 of the birds, respectively, the
220 brodifacoum, bromadiolone or difethialone concentrations alone were $> 100 \text{ ng g}^{-1}$. For 2 of the
221 birds, the bromadiolone and brodifacoum concentrations were both $> 100 \text{ ng g}^{-1}$, whereas for
222 9 of the harriers, only the $\sum \text{ARs}$ was $> 100 \text{ ng g}^{-1}$. Thus, for further analyses, only the $\sum \text{ARs}$,
223 brodifacoum and bromadiolone were considered. Inter-annual trends were detected for the
224 number of potential cases of poisoning per year, which increased from 1999 to 2015 (Poisson
225 GAM, $\text{edf}=1.5$, $p=0.002$, Fig. 4). The proportion of potential cases per year tended to increase
226 from 2008 to 2016 (binomial GAM, $\text{edf}=2.1$, $p=0.054$), whereas the decreasing trend from
227 1999-2007 exhibited large variability and thus must be considered with caution. The rising
228 evidenced is particularly alarming for the last 3 years, as 56% of the harriers with ARs > 100
229 ng g^{-1} were found from 2013-2016. In 2013 and 2016, all the harriers collected were potentially
230 poisoned by brodifacoum (Fig. 4). When considering potential poisoning by brodifacoum alone,
231 both the number of cases and their proportion per year increased over time (Poisson GAM,
232 $\text{edf}=1$, $p < 0.001$; binomial GAM, $\text{edf}=1$, $p < 0.01$, respectively, Fig. 4). For bromadiolone, no
233 temporal trend was detected for the number (Poisson GAM, $\text{edf}=1.4$, $p=0.74$) or the proportion
234 of poisoned birds per year (binomial GAM, $\text{edf}=3.74$, $p=0.28$). Intra-annual variations of the
235 $\sum \text{ARs}$ also occurred for the number of poisoned harriers and their proportion per month. In
236 both cases, a non-linear trend was found, with a peak occurring in July (Poisson GAM, $\text{edf}=2.9$,
237 $p < 0.01$ and binomial GAM, $\text{edf}=2.7$, $p < 0.05$, respectively). However, for brodifacoum and
238 bromadiolone, no difference in the number (Poisson GAM, $\text{edf}=2.8$, $p=0.22$ and $\text{edf}=1.8$,
239 $p=0.33$, respectively) or proportion (binomial GAM, $\text{edf}=1$, $p=0.69$ and $\text{edf}=1$, $p=0.20$,

240 respectively) of potential poisoning cases was detected between months. The poisoned
241 harriers were spatially aggregated (Clark-Evans test, $R=0.50$, $p=0.001$), with 14 cases on the
242 eastern part of the island, 6 in the south, 5 in the west and 3 in the north. Otherwise, no spatial
243 clustering was detected for the harriers poisoned only by brodifacoum or bromadiolone (Clark-
244 Evans test, $R=0.88$, $p=0.083$; $R=0.86$, $p=0.10$, respectively).

245

246 *Is there a link between harrier exposure and landscape composition and/or the local density*
247 *of breeding harriers?*

248 For the Σ ARs in each individual as a response variable, the top model included a smooth term
249 for year, harrier density and the proportions of sugarcane and urban areas in the township of
250 discovery. The Σ ARs increased linearly with the density of breeding pairs (GAMM, $\text{edf}=1$,
251 $p<0.01$, Fig. 5), whereas the landscape influence differed according to habitat type. The harrier
252 exposure increased in a non-linear way with the proportion of urban areas in a township
253 (GAMM, $\text{edf}=1$, $p<0.01$). In the sugarcane areas, the Σ ARs exhibited a peak at 25% of the
254 township area (GAMM, $\text{edf}=2.3$, $p<0.01$) (Fig. 5). The same analyses repeated on the a.i.
255 separately revealed a marginal relationship between the brodifacoum concentrations and both
256 the harrier density and sugarcane areas (GAMM, linear increase, $\text{edf}=1$, $p=0.086$ and non-
257 linear trend with a peak at 30% of sugarcane, $\text{edf}=2.43$, $p=0.088$, respectively). Finally, the
258 proportion of harriers poisoned by brodifacoum increased linearly but marginally with the
259 sugarcane areas in a township (GAMM, $\text{edf}=1$, $p=0.076$).

260

261 **Discussion**

262 *Exposure of Réunion harriers to ARs versus continental raptors*

263 Our data are the first to report the rate of exposure to ARs of the Réunion harrier and more
264 generally of wildlife on this island. The proportion of exposed harriers was very high, as 93%
265 of the birds exhibited residues in their livers at concentrations compatible with poisoning for
266 62% of them. As in most schemes implemented at large scales for the monitoring of wildlife
267 poisoning (e.g., SAGIR network in metropolitan France, WIIS in the UK), the sampling of the
268 present survey was based on the opportunistic discovery of dead or weakened individuals
269 without any assessment of the sampling effort over space and time. The likelihood of carcass
270 discovery might be linked to human population density and activity patterns, an awareness of
271 people regarding the conservation of wildlife and/or a willingness to collect dead or weakened
272 birds. Thus, the trends we documented, notably, those related to the number of exposed or
273 potentially poisoned harriers, may represent a biased picture of the actual exposure of the

274 overall population. However, both the number of birds exposed and the high concentrations
275 measured during the last years of the survey unequivocally showed that ARs are a threat for
276 this species. Although the exposure rate to ARs in the present study is one of the highest
277 reported, it is consistent with the rates determined previously for raptors, i.e., 58% on average
278 with peaks over 90% (López-Perea and Mateo, 2018). As reported for raptors and wildlife, we
279 observed that the SGARs were the most prevalent ARs in the Réunion harrier, which is
280 explained by their longer persistence in tissues compared to FGARs. Furthermore, multiple
281 exposures were frequently noticed, but the proportion measured in our study, 81%, is among
282 the highest assessed to date (Christensen et al., 2012) and questions the factors driving the
283 exposure of the Réunion harrier.

284

285 *AR usage and landscape as drivers of harrier exposure to and potential poisoning by ARs*

286 Two AR usages are distinguished in Europe: Biocides are spread for the control of commensal
287 rodents to protect human health, foodstuffs or equipment, while plant protection products
288 (PPP) are applied to control agricultural pests (Jacob and Buckle, 2018). On Réunion Island,
289 ARs are used indoors and around buildings but are also applied on crops to control rat and
290 mouse populations for both preventing leptospirosis and limiting yield loss (Grollier and
291 Soufflet, 2011). They are spread in lower amounts for the conservation of endangered birds.
292 Our results showed a clear influence of the landscape composition of the townships, notably
293 of both urban and sugarcane areas, on harrier exposure to ARs. How landscape modulates
294 predator exposure to ARs has been reviewed by Hindmarch and Elliot (2018). The authors
295 reported that habitats that are heavily influenced by human activities, such as intensive
296 agriculture or urbanization, are at risk because they are susceptible to hosting high rodent
297 densities and being intensively and permanently treated with ARs. Overall, 75% of the Réunion
298 harrier breeding pairs nest at the interface of sugarcane and semi-natural habitats (Bretagnolle
299 et al., 2000), but they also forage in urbanised habitats, such as fallow or wastelands, and thus
300 may feed on contaminated rodents over a significant portion of their home range.

301 We found that higher concentrations of Σ ARs in the harriers were related to larger urban areas
302 in townships, which is consistent with the findings of Hindmarch and Elliot (2018). Our results
303 strongly suggest that Réunion harriers were exposed to ARs used as biocides, but no data are
304 accessible about the products distributed on Réunion Island during the study period. The fact
305 that different a.i. are used in the biocidal products distributed on the market could explain why
306 no influence of urban areas was evidenced for each a.i. Moreover, when precise information
307 on the products applied is available, a sound interpretation may be biased by the fact that
308 some AR bait formulations apparently contain trace levels of other a.i. than those stated on the

309 label (Geduhn et al., 2014). The difethialone occurrence was the lowest among the detected
310 ARs. To our knowledge, this a.i. has never been used on crops; thus, exposure would rather
311 result from biocidal usage in urban areas even if misuses could not be excluded. Like
312 difethialone is, along with brodifacoum, one of the most toxic ARs for birds considering its
313 acute LD50 (0.26 mg/kg body weight for the Northern bobwhite, Erickson and Urban, 2004),
314 its outdoor spread should be restricted, as it is in the UK (Buckle and Prescott, 2018).
315 Difenacoum exhibited the highest occurrence measured in the harriers, while the relatively low
316 concentrations could be explained by its moderate persistence in animal tissue compared to
317 other SGARs and/or by a frequent but limited exposure. Poisoning by difenacoum is unlikely
318 because the concentrations were always $<100 \text{ ng g}^{-1}$, but it may contribute to poisoning when
319 multiple exposures occurred. The absence of available information makes the use of
320 difenacoum questionable as a biocide on Réunion island. However, according to Grollier and
321 Soufflet (2011), it would have been applied on the sugarcane crops, though we found no more
322 details on its spreading features.

323 The present findings suggest that rodenticide use in crops was responsible for the harrier
324 exposure, as the concentrations of Σ ARs or brodifacoum tended to be higher when the
325 sugarcane crops reached 25% of the township area. Only qualitative data on the main a.i.
326 distributed by the sugarcane farmers from 1999-2011 were available (Grollier and Soufflet,
327 2011) and complemented by FREDON Réunion for 2012-2016 (unpublished data). Until 2013,
328 chlorophacinone or bromadiolone were provided to farmers. Then, brodifacoum was chosen
329 in 2014, and bromadiolone and/or difenacoum were distributed in 2015 and 2016. These data
330 do not fully fit with the inter-annual trend we evidenced for the harrier exposure. Both the
331 chlorophacinone and bromadiolone exposure varied over the years, with a non-linear trend for
332 the bromadiolone concentration and a peak of individuals positive for chlorophacinone in 2008.
333 At the same time, the brodifacoum concentrations in the harriers increased from 2012 onward,
334 i.e., 2 years before its spread on the sugarcane crops. However, 2 cases of potential poisoning
335 by brodifacoum were recorded in total from 1999–2013, while 12 were recorded for 2014-2016
336 when it was used on cane crops. We can assume that the field application of brodifacoum on
337 sugarcane was responsible for the high exposure and potential poisoning of the harriers
338 reported in the last three years.

339 Moreover, we found a clear positive relationship between the AR concentrations and the harrier
340 breeder density, making the birds inhabiting the most productive resource areas more likely to
341 be exposed. Thus, if areas that host large rodent populations are attractive for the Réunion
342 harrier because of the high prey availability, they can become ecological traps for rodent
343 predators when ARs are intensively spread, leading to an increase in both exposure and

344 poisoning, as was shown in Spain (López-Perea and Mateo, 2019). Given the uncertainty of
345 the location for most of the individuals, working at the township level might add noise to the
346 detected signals. A more robust design able to characterize the probability of recovering a
347 dead bird regarding links between exposure and the habitat variables would confirm these
348 findings. Similarly, the dosage of the live birds, based on blood sampling, across the island
349 could shed light on the actual exposure and risk for this endangered species.

350

351 *Diagnosis of harrier poisoning by ARs and consequences for the population*

352 How to interpret pesticide concentrations in wildlife in terms of the poisoning likelihood remains
353 an open question. For ARs, Thomas et al. (2011) showed that toxicosis may occur in raptors
354 below previously suggested concentrations of concern, <100-200 ng g⁻¹. When pooling all
355 individuals regardless of species, Σ ARs in the liver of 80 ng g⁻¹ was associated with a
356 probability of 20% for showing signs of toxicosis, but interspecies differences in sensitivity were
357 pointed out (Thomas et al., 2011). Finally, the threshold for Σ ARs of 100 ng g⁻¹ is commonly
358 used as an acceptable compromise for raptors. In metropolitan France, the protocol adopted
359 by the SAGIR network to diagnose the cause of death for wildlife relies on aetiology, a
360 necropsy conducted by veterinarians and then a chemical analysis of tissue when poisoning
361 is suspected. To date, this protocol has not been strictly applied on Réunion Island and should
362 be implemented systematically when a harrier carcass is found. This would help to better
363 diagnose poisoning and determine the specific threshold concentrations related to poisoning
364 likelihood for the Réunion harrier. Furthermore, the consequences of poisoning on harrier
365 populations cannot be assessed, for instance, using population modelling because
366 demographic parameters such as the survival rate or productivity remain unknown. The
367 breeding population was estimated at ~150 pairs (Grondin and Philippe, 2011). Twelve harriers
368 were potentially poisoned by brodifacoum during the last 3 years of the survey, and the year
369 of discovery was not known for 16 individuals, among which 30% contained brodifacoum. It is
370 generally considered that a low proportion of bird carcasses, <20%, is found in nature (Saucy
371 et al., 2001), which strongly suggests that harrier poisoning by ARs is underestimated.
372 Moreover, among the 36 potential cases of poisoning, 25 were adults, while adult survival is a
373 critical parameter in the dynamic of raptor populations (Saether and Bakke, 2000).

374

375 *Recommendations for monitoring and mitigating Réunion harrier exposure to ARs*

376 All these points lead to consider AR poisoning following treatments on sugarcane crops and,
377 likely to a lesser extent, its use as a biocide as a major threat to Réunion harriers. Mitigation

378 actions are urgently required for ensuring its conservation. Thus, with this objective, we
379 recommend the following:

380 • reinforce the survey of harrier mortality and exposure to ARs based on standardized
381 monitoring. This would lead to an unbiased definition of the spatial and temporal pattern of
382 exposure and thus the definition of areas where priority mitigation actions should be
383 implemented.

384 • improve knowledge about the spatial distribution of Réunion harriers and their ecology (home
385 range and variability over time, demography and life history traits), which is currently the aim
386 of the ongoing ECOPAP programme (Ecology and Conservation of Réunion Harrier,
387 2016/2019) led by the SEOR. This would allow for the identification of key foraging areas and
388 the periods where/when the surveying of harrier poisoning has to be reinforced and rodent
389 control measures adapted.

390 • build an integrated pest management (IPM) plan compatible with Réunion harrier
391 conservation and biodiversity in general in partnership with AR users and regulatory agencies.
392 The general principles of risk mitigation for ARs were recently reviewed by Buckle and Prescott
393 (2018). It seems crucial to collaborate with farmers to mitigate the side effects of ARs by
394 combining mechanical, biological and chemical controls, for example. Such IPM plans should
395 rely on rodent population surveys that include density estimates, research on genetic
396 resistance to ARs and assessments of damage to crops caused by rodents over space and
397 time. Based on our experience, which led to the development of an IPM plan for water vole in
398 metropolitan France (Coeurdassier et al., 2014), FREDON Réunion could be a key partner for
399 developing and transferring methodological innovations to farmers. Risk mitigation measures
400 for biocidal ARs (Buckle and Prescott, 2018) could be implemented in urban areas where
401 harriers exhibit high densities. Raising the awareness of people living in critical areas for
402 harriers and throughout the entire island is also required for preventing high pesticide use in
403 gardens and peri-urban areas. Recent actions have been undertaken by the SEOR to inform
404 people and promote mechanical methods.

405 • explicitly define which method(s) is (are) permitted to limit the damage caused by rats and
406 mice on sugarcane (and other crops) in the regulation, notably the methods based on
407 rodenticides. In France, the current regulation for the control of rodents for plant protection
408 focuses on metropolitan cases, i.e., only bromadiolone is authorized against voles (Journal
409 Officiel, 2014). Both the agronomic and ecological contexts of overseas territories should be
410 considered by authorities and implemented in regulations when needed. This is essential for
411 proposing solutions adapted to farmers that are environmentally acceptable and clarifying the
412 management of these practices by local authorities. Professional staff trained on sound

413 application practices should register and control the quantities of bait spread by farmers. As
414 less potent ARs are currently available, brodifacoum and difethialone should be avoided on
415 sugarcane crops and the outdoors without forgetting the risk of rodent-resistant strain
416 selection. More generally, the development of alternative methods based on safer pesticides
417 and/or non-chemical solutions is recommended (Witmer, 2018).

418

419 **Conclusion**

420 Anticoagulant rodenticides are likely one of the main threats to Réunion harriers. Dead
421 individuals exhibiting high SGAR concentrations have been found in the last 17 years, and the
422 number of potential poisonings, mainly by brodifacoum, increased during this period. The
423 population could be endangered if nothing is done in the short term. Our results strongly
424 suggest that AR applications on sugarcane fields and in peri-urban areas are sources of
425 exposure and poisoning. Thus, the rapid implementation of an IPM plan is critical for harrier
426 conservation. In the very short term, we recommend switching from brodifacoum to less potent
427 a.i. for outdoor applications. As it is also suspected that bromadiolone poisons harriers,
428 difenacoum or chlorophacinone could be acceptable substitutes; however, the target rodents
429 may have developed resistance to these a.i., which generally forces the reuse of brodifacoum
430 or difethialone in the medium term. To our knowledge, the resistance of rodents to less toxic
431 ARs has not yet been studied on Réunion Island. Thus, the current methods of control that rely
432 only on pesticide spread are scarcely compatible with harrier conservation. Based on existing
433 IPM plans implemented for ARs, the management of rodent populations in the sugarcane fields
434 on Réunion Island should consider *(i)* the collective control of rodents by farmers with chemical,
435 biological and mechanical methods; *(ii)* the optimization of AR spreading protocols considering
436 both the efficiency of treatment against the target rodents and conservation issues; and *(iii)* the
437 restriction or even prohibition of ARs in areas where biodiversity issues have been identified.
438 However, health issues need to be accounted for as rats may also carry leptospirosis and are
439 abundant in cities. This has to be kept in mind for developing a global rodent control strategy
440 on the island.

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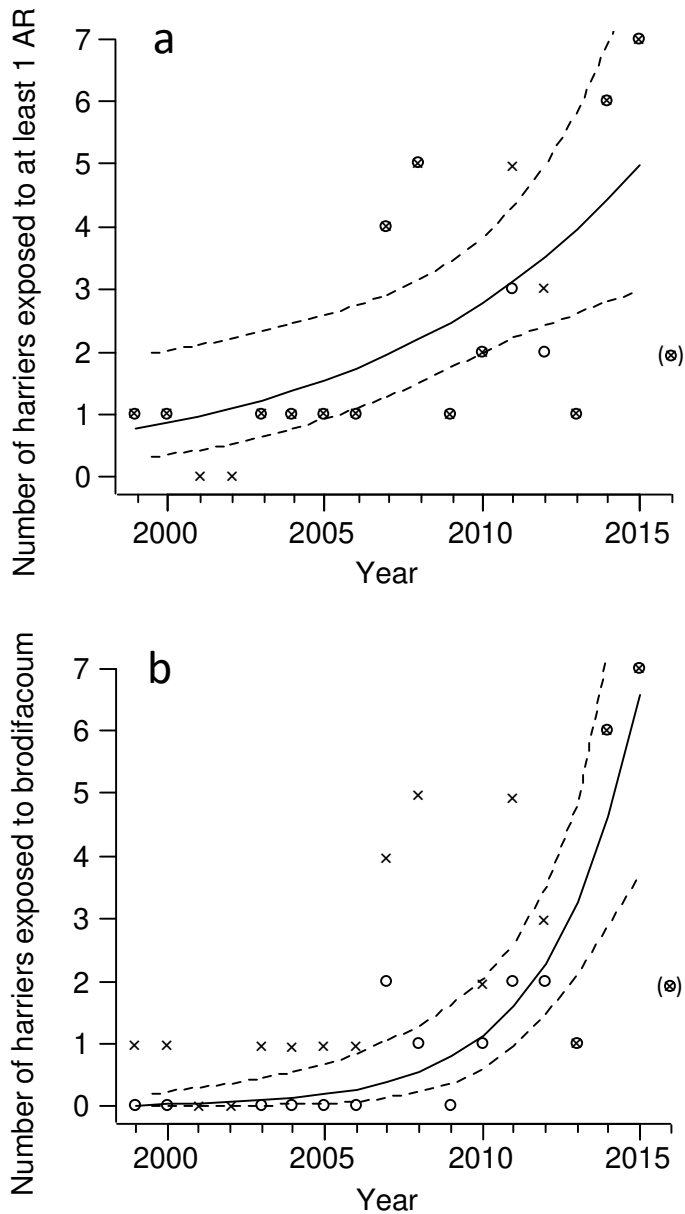


Figure 1. Inter-annual variations in the number of Réunion harriers positive for (a) at least one anticoagulant rodenticide and (b) brodifacoum alone. The total number of carcasses collected per year is represented by x in (a) and (b). The trends were estimated with a GAM (without accounting for any other variable) and without data from 2016 for the numbers of exposed harriers in (a) and (b) because only 2 months were monitored that year (data points in brackets). Dashed lines represent the 95% confidence intervals of the estimates; n=15 for the number and n=42 for the proportions.

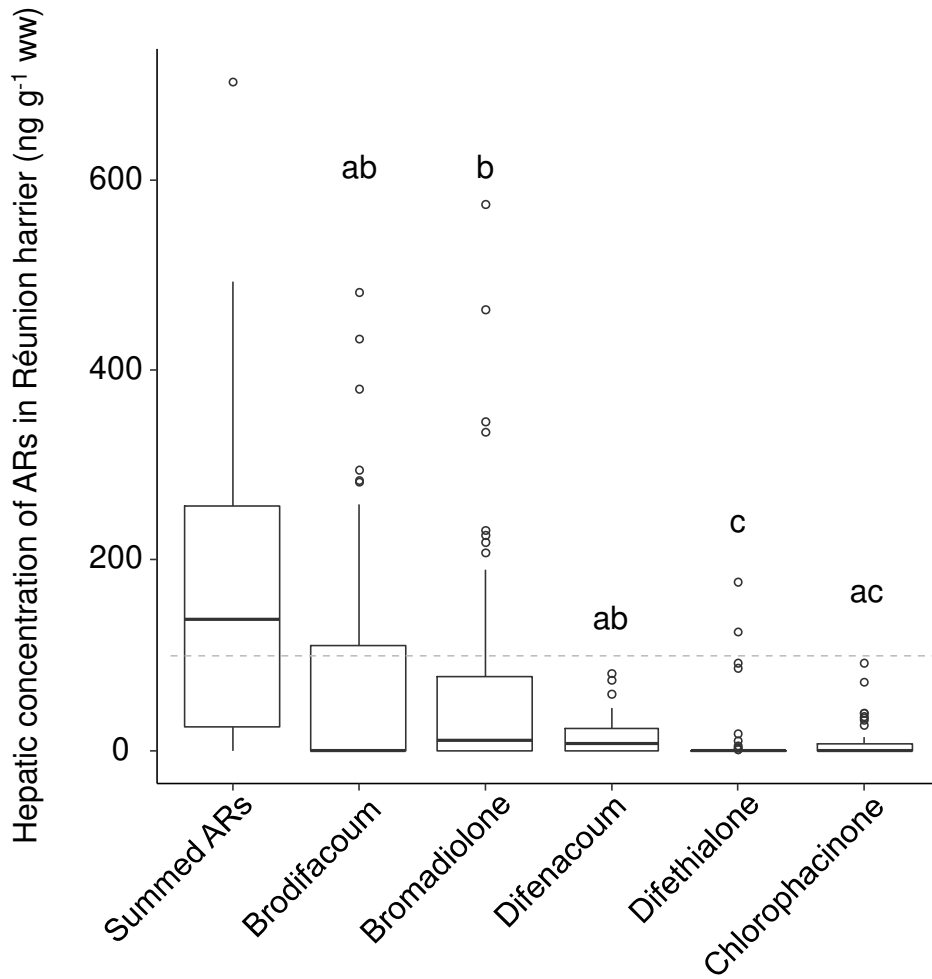


Figure 2. Concentrations of the anticoagulant rodenticides measured in the livers of the Réunion harriers collected between 1999 and 2016 (n = 58). Different letters indicate significant differences in the concentrations between the compounds. The horizontal dashed line at 100 ng g⁻¹ represents the threshold above which potential poisoning is considered according to Newton et al. (1999) and Thomas et al. (2011).

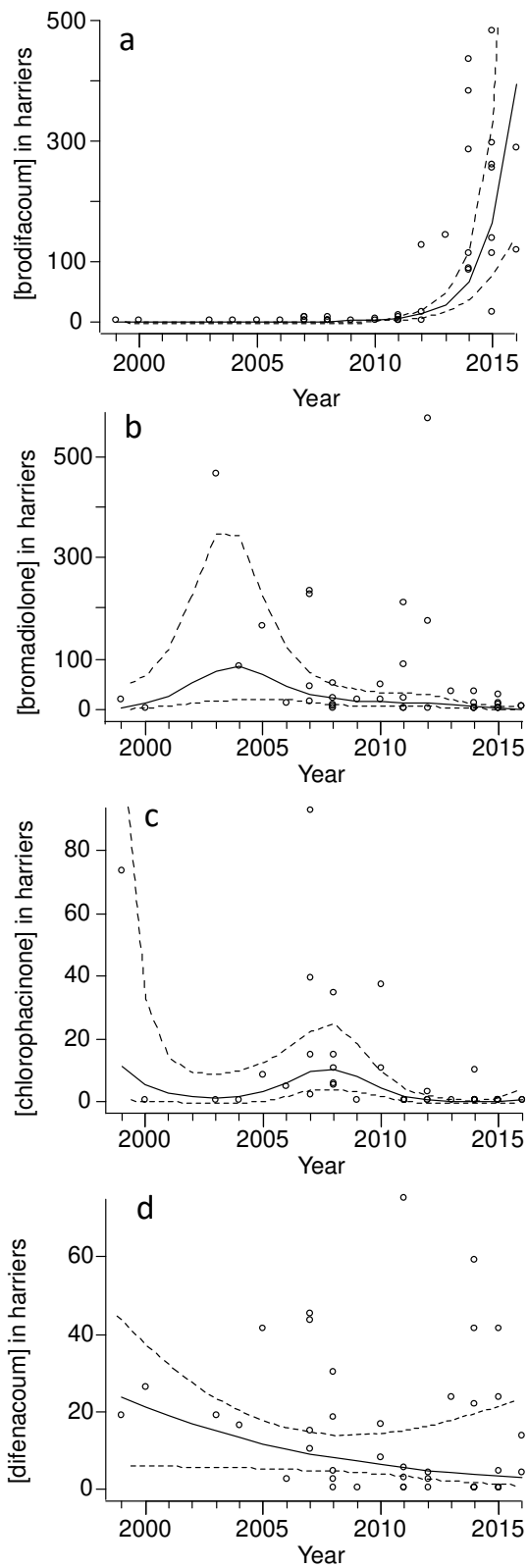


Figure 3. Inter-annual variations in the hepatic concentrations of Réunion harrier (ng g⁻¹ ww) for brodifacoum (a), bromadiolone (b), chlorophacinone (c) and difenacoum (d). The trends were estimated with a GAMM (without accounting for any other fixed factor). Dashed lines represent the 95% confidence intervals of the estimates; n = 42.

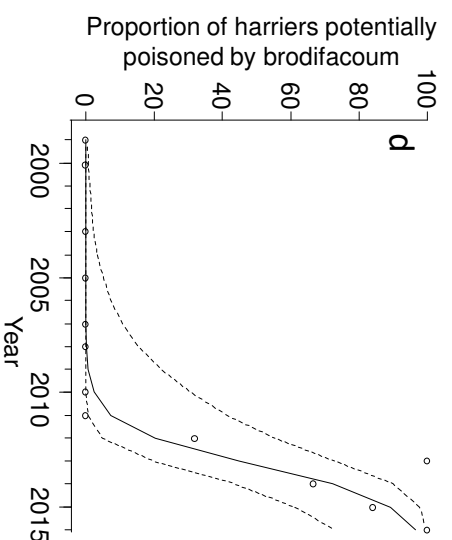
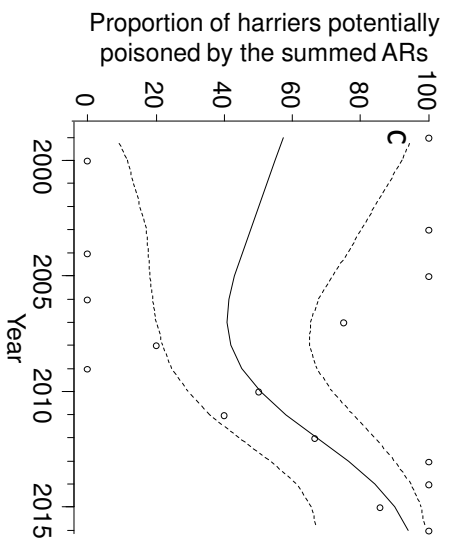
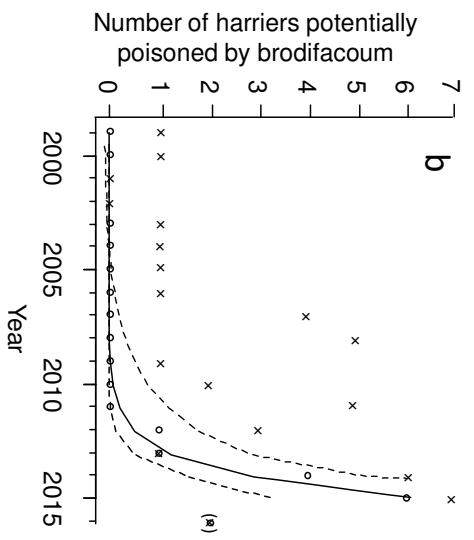
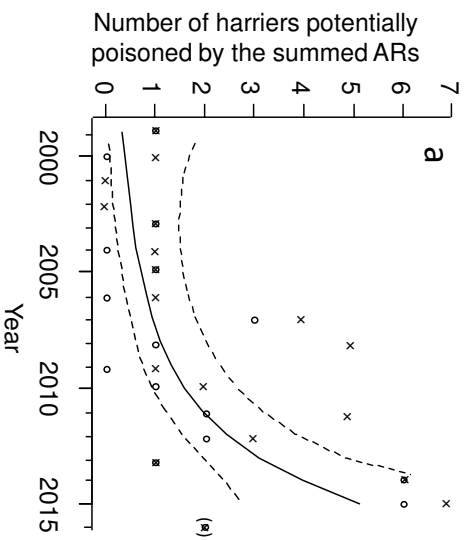


Figure 4. Inter-annual variations in the number of Réunion harriers potentially poisoned by the total amount of ARs (a) or by brodifacoum alone (b) and in the proportion of harriers potentially poisoned by the summed ARs (c) or brodifacoum calculated from the total number of carcasses collected in a year (d). The total number of carcasses collected per year is represented by x in (a) and (b). The trends were estimated with a GAM (without accounting for any other variable) and without the data from 2016 for the numbers of potentially poisoned harriers in (a) and (b) because only 2 months were monitored that year (data points in brackets). Potential poisoning is considered when the liver concentrations of the ARs (summed or each compound separately) $> 100 \text{ ng g}^{-1}$. Dashed lines represent the 95% confidence intervals of the estimates; $n = 15$ for the number and $n = 42$ for the proportions.

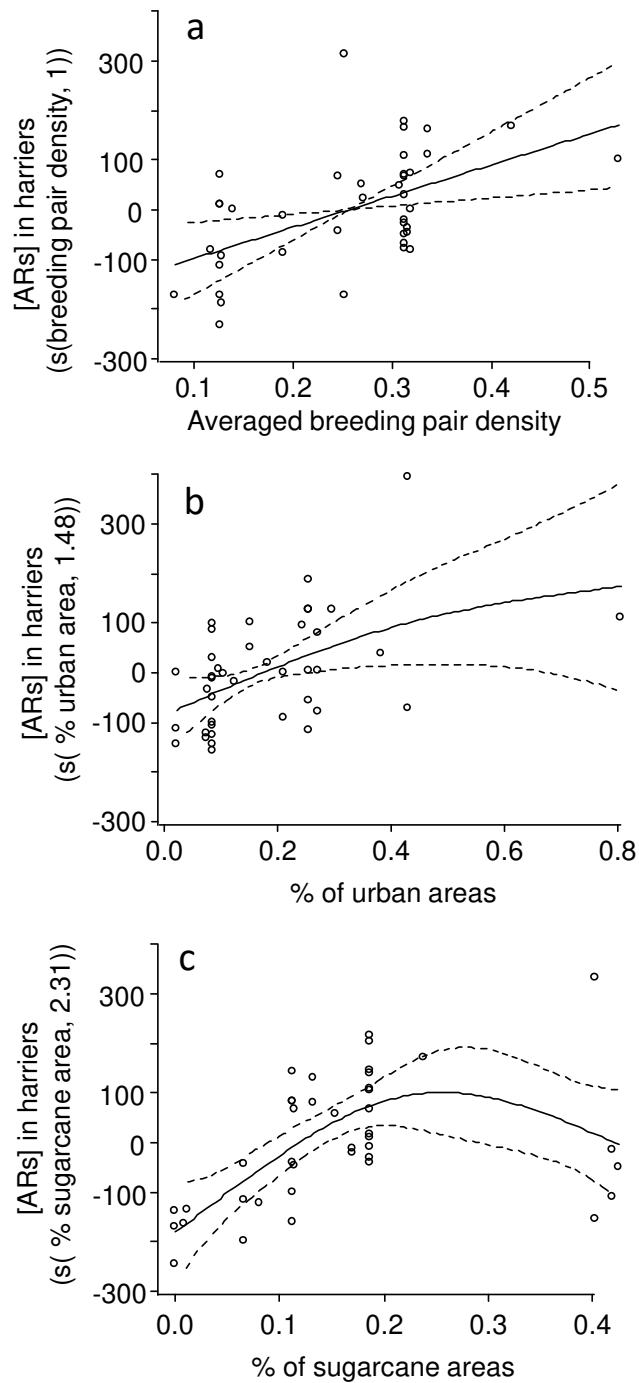


Figure 5. Relationship between the summed concentrations of anticoagulant rodenticides measured in the Réunion harriers and the estimated density of the harrier breeding pairs (a), the proportion of urbanized areas (b) and the proportion of sugarcane areas (c) at the township level. The trends were estimated by a GAMM. The Y axis represents the smooth term, s , generated by the GAM and is estimated subject to the constraint that $\sum s(x_i) = 0$, where x_i are the covariate values. Both the covariate and the estimated degrees of freedom, edf, are indicated in parentheses. The smooth term, s , is centred to ensure model identifiability. Dashed lines represent the 95% confidence intervals of the estimates; $n = 40$.