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# Pesticides threaten an endemic raptor in an overseas French territory

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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.

## 1. Introduction

On numerous islands worldwide, rodents are considered invasive species that are responsible for various damages to human health, foodstuff and products and/or ecosystems and biodiversity, notably because they threaten endemic species (Buckle and Smith, 2015). On Réunion Island, an overseas French territory in the Indian Ocean, Norway rats (*Rattus norvegicus*), black rats (*Rattus rattus*), and house mice (*Mus musculus*) were introduced and currently occupy a wide range of habitats. They are found in urban settings, where they may spoil foodstuffs, or in rural areas, where they feed on agricultural crops, especially sugarcane plantations, which is the main agricultural product of the Island, leading to losses estimated at 10–15% of the annual harvest (Grollier and Soufflet, 2011). Moreover, rats are vectors of zoonotic pathogens that are transmissible to humans, such as leptospirosis, for which they exhibit a high seroprevalence (Guernier et al., 2016). Finally, rodents are partially responsible for the decline in endemic endangered species, such as the Réunion petrel (*Pseudobulweria aterrima*). The application of pesticides, notably anticoagulant rodenticides (ARs), is currently the main method implemented for controlling rodents and has helped to eradicate some invasive species for island biodiversity conservation (Buckle and Smith, 2015). The development of rodent resistance to the first generation of ARs (FGARs, e.g., chlorophacinone) has led to the use of second generation ARs (SGARs), namely, bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone, which are more toxic and effective than FGARs for killing resistant rodents. However, they are also more persistent in animal tissue and more toxic to birds and mammals (Erickson and Urban, 2004), which has led to the high secondary exposure or poisoning of rodent predators (López-Perea and Mateo, 2018). On Réunion Island, campaigns to spread ARs have been organized since 1977 to limit rodent damage (Grondin and Philippe, 2011). However, the use of these pesticides is related to an increase in suspected cases of poisoning of the Réunion harrier, *Circus maillardi* (Grondin and Philippe, 2011).

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This species, endemic to Réunion, is the last breeding raptor on the Island and is classified as endangered (International Union for the Conservation of Nature, 2018), with a breeding population estimated at 150 pairs (Grondin and Philippe, 2011). Its diet may vary according to the habitat it occupies, but it is commonly composed of 50 to 70% rodents (Grondin and Philippe, 2011). It has been widely reported that AR application is related to the secondary exposure and poisoning of raptors (López-Perea and Mateo, 2018), but to date, there are no data quantifying the exposure of the Réunion harrier to ARs. In this study, we aimed to measure the AR concentrations in the livers of Réunion harrier carcasses that have been collected by the Société d'Etudes Ornithologiques de la Réunion (SEOR) since 1999. To determine whether AR exposure could be a threat for this bird, the measured concentrations were compared to toxicity threshold concentrations ($> 100\,\text{ng}\,\text{g}^{-1}$), for which a significant likelihood of toxicosis has been evidenced for raptors (Newton et al., 1999; Thomas et al., 2011). Then, the AR concentrations were related to factors for possibly explaining the variations in harrier exposure or poisoning, such as age, the sex of the individuals and the landscape composition characterized by habitat variables as well as the local density of breeding individuals or the year of carcass discovery. Identifying the exposure patterns and drivers represents a crucial step in adapting rodent control methods for making them compatible with wildlife conservation.

## 2. Materials and methods

### 2.1. Sample collection

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

### 2.2. Liver analysis

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

### 2.3. Statistical analysis

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

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

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

Then, we checked whether landscape composition influences harrier exposure. Given the low number of birds with accurate discovery locations ($n = 22$), analyses were conducted at the township level ($n = 42$). For each township, we computed the proportion of sugarcane and urbanised areas. Data were extracted from a classification map (CIRAD, 2018) that was rasterised on a 25 m × 25 m pixel raster grid and then aggregated at a 250 m resolution. In addition, we tested whether harrier exposure was linked to the spatial distribution of the breeding Réunion harriers to better target conservation efforts. We assumed that this distribution was relatively stable during the last 20 years. We used a large-scale survey to assess the population size and distribution of territorial pairs (unpublished data). The survey was based on 184 point counts separated by approximately 2000 m and sampled during the breeding season (from May to June) in 2017. The counting was conducted only in acceptable weather conditions, i.e., no or light wind and rain. We applied spatial smoothing (function spatstat::ppp.smooth(), $\sigma = 5000\,\text{m}$) to the raw count data of the territorial pairs of harriers (Baddeley et al., 2016). This allowed the production of a smoothed map as a proxy of the density of the territorial pairs, reflecting their distribution on the island (hereafter harrier density), which was then averaged for each township. We ran generalized mixed model (GAMM) with the ΣARs (Gaussian family with identity link) or the proportion of exposed/poisoned cases (binomial family with identity link) as the response variables in which an individual was the sampling unit. We compared 18 models per response variable (including the null model), with the full model including a smooth term for year, harrier density, the proportion of sugarcane crops and the

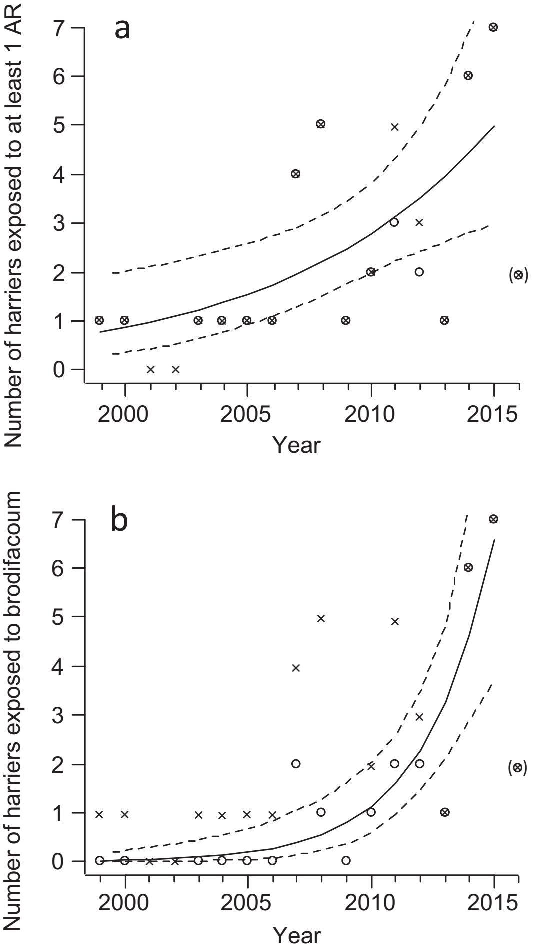

**Fig. 1.** Inter-annual variations in the number of Réunion harriers positive for (a) at least one anticoagulant rodenticide and (b) brodifacoum alone. The total number of carcasses collected per year is represented by x in (a) and (b). The trends were estimated with a GAM (without accounting for any 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.

proportion of urbanised surface areas (or an interaction of those two). Township was included as a random factor. Then, the GAMMs with similar response variables were run for each a.i. separately. Given the relatively limited sample size ($n = 42$), we restricted the maximum number of degrees of freedom of smoothing terms to $k = 5$ to avoid overfitting. The models were ranked according to their AIC scores. All statistics were performed with R.3.3.3 (R Core Team, 2018) and the libraries ade4 (Dray et al., 2018), gamm4 (Wood and Scheipl, 2017), geoR (Ribeiro and Diggle, 2016), lme4 (Bates et al., 2015), mgcv (Wood, 2017), spatstat (Baddeley et al., 2016) and multcomp (Hothorn et al., 2008).

## 3. Results

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

### 3.1. Proportion and number of Réunion harriers exposed to ARs

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

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

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

### 3.2. Hepatic concentrations of ARs in the harriers

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

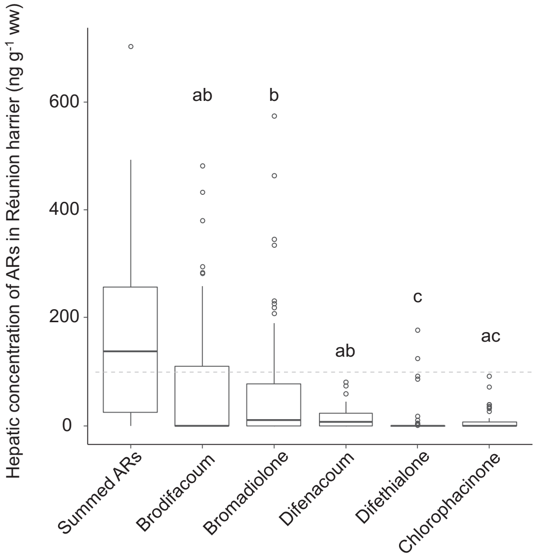

**Fig. 2.** Concentrations of the anticoagulant rodenticides measured in the livers of the Réunion harriers collected between 1999 and 2016 (n = 58). Different letters indicate significant differences in the concentrations between the compounds. The horizontal dashed line at 100 ng g$^{-1}$ represents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

### 3.3. Potential cases of poisoning: trends for the harriers with AR concentrations > 100 ng g$^{-1}$

Thirty-six harriers (62%) had ΣARs > 100 ng g$^{-1}$. For 16, 11 and 2 of the birds, respectively, the brodifacoum, bromadiolone or difethialone concentrations alone were > 100 ng g$^{-1}$. For 2 of the birds, the bromadiolone and brodifacoum concentrations were both > 100 ng g$^{-1}$, whereas for 9 of the harriers, only the ΣARs was > 100 ng g$^{-1}$. Thus, for further analyses, only the ΣARs, brodifacoum and bromadiolone were considered. Inter-annual trends were detected for the number of potential cases of poisoning per year, which increased from 1999 to 2015 (Poisson GAM, edf = 1.5, p = 0.002, Fig. 4). The proportion of potential cases per year tended to increase from 2008 to 2016 (binomial GAM, edf = 2.1, p = 0.054), whereas the decreasing trend from 1999 to 2007 exhibited large variability and thus must be considered with caution. The rising evidenced is particularly alarming for the last 3 years, as 56% of the harriers with ARs > 100 ng g$^{-1}$ were found from 2013 to 2016. In 2013 and 2016, all the harriers collected were potentially poisoned by brodifacoum (Fig. 4). When considering potential poisoning by brodifacoum alone, both the number of cases and their proportion per year increased over time (Poisson GAM, edf = 1, p < 0.001; binomial GAM, edf = 1, p < 0.01, respectively, Fig. 4). For bromadiolone, no temporal trend was detected for the number (Poisson GAM, edf = 1.4, p = 0.74) or the proportion of poisoned birds per year (binomial GAM, edf = 3.74, p = 0.28). Intra-annual variations of the ΣARs also occurred for the number of poisoned harriers and their proportion per month. In both cases, a non-linear trend was found, with a peak occurring in July (Poisson GAM, edf = 2.9, p < 0.01 and binomial GAM, edf = 2.7, p < 0.05, respectively). However, for brodifacoum and bromadiolone, no difference in the number (Poisson GAM, edf = 2.8, p = 0.22 and edf = 1.8, p = 0.33, respectively) or proportion (binomial GAM, edf = 1, p = 0.69 and edf = 1, p = 0.20, respectively) of potential poisoning cases was detected between months. The poisoned harriers were spatially aggregated (Clark-Evans test, R = 0.50, p = 0.001), with 14 cases on the eastern part of the island, 6 in the south, 5 in the west and 3 in the north. Otherwise, no spatial clustering was detected for the harriers poisoned only by brodifacoum or bromadiolone (Clark-Evans test,

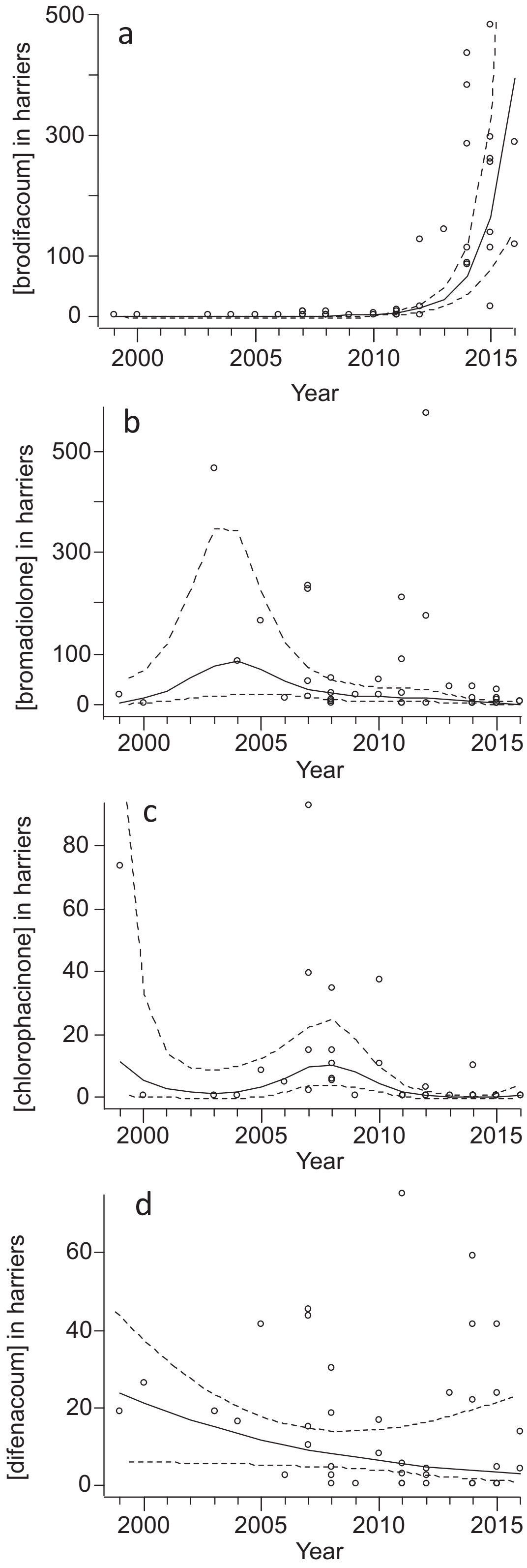

**Fig. 3.** Inter-annual variations in the hepatic concentrations of Réunion harrier (ng g$^{-1}$ ww) for brodifacoum (a), bromadiolone (b), chlorophacinone (c) and difenacoum (d). The trends were estimated with a GAMM (without accounting for any other fixed factor). Dashed lines represent the 95% confidence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

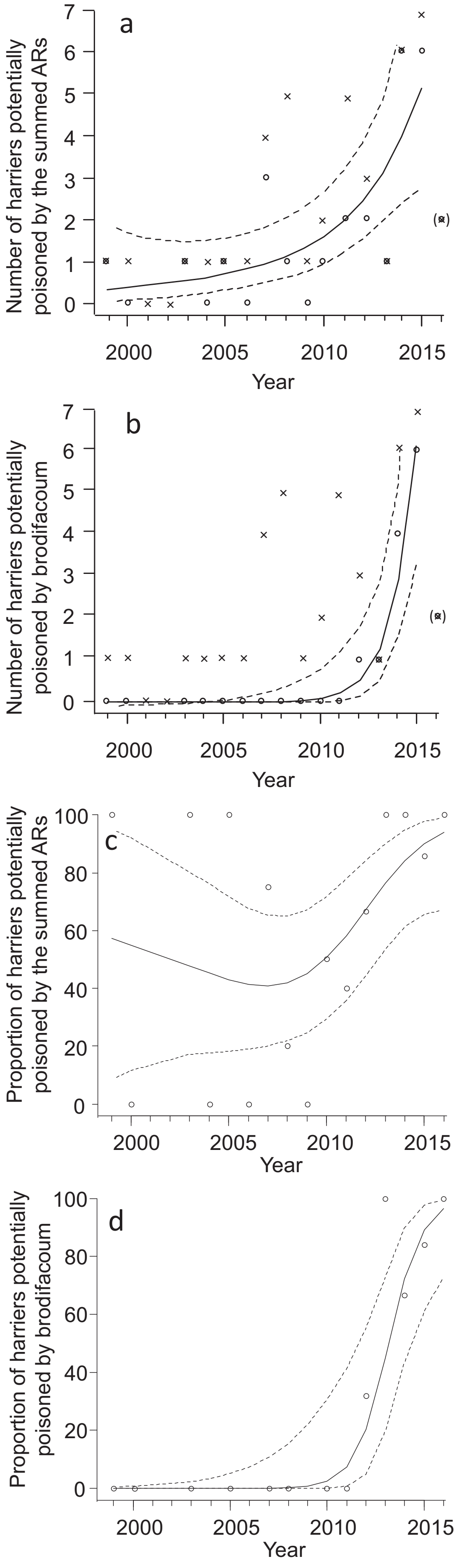

**Fig. 4.** Inter-annual variations in the number of Réunion harriers potentially poisoned by the total amount of ARs (a) or by brodifacoum alone (b) and in the proportion of harriers potentially poisoned by the summed ARs (c) or brodifacoum calculated from the total number of carcasses collected in 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 ng $g^{-1}$. Dashed lines represent the 95% confidence intervals of the estimates; n = 15 for the number and n = 42 for the proportions.

R = 0.88, p = 0.083; R = 0.86, p = 0.10, respectively).

### 3.4. Is there a link between harrier exposure and landscape composition and/or the local density of breeding harriers?

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

## 4. Discussion

### 4.1. Exposure of Réunion harriers to ARs versus continental raptors

Our data are the first to report the rate of exposure to ARs of the Réunion harrier and more generally of wildlife on this island. The proportion of exposed harriers was very high, as 93% of the birds exhibited residues in their livers at concentrations compatible with poisoning for 62% of them. As in most schemes implemented at large scales for the monitoring of wildlife poisoning (e.g., SAGIR network in metropolitan France, WIIS in the UK), the sampling of the present survey was based on the opportunistic discovery of dead or weakened individuals without any assessment of the sampling effort over space and time. The likelihood of carcass discovery might be linked to human population density and activity patterns, an awareness of people regarding the conservation of wildlife and/or a willingness to collect dead or weakened birds. Thus, the trends we documented, notably, those related to the number of exposed or potentially poisoned harriers, may represent a biased picture of the actual exposure of the overall population. However, both the number of birds exposed and the high concentrations measured during the last years of the survey unequivocally showed that ARs are a threat for this species. Although the exposure rate to ARs in the present study is one of the highest reported, it is consistent with the rates determined previously for raptors, i.e., 58% on average with peaks over 90% (López-Perea and Mateo, 2018). As reported for raptors and wildlife, we observed that the SGARs were the most prevalent ARs in the Réunion harrier, which is explained by their longer persistence in tissues compared to FGARs. Furthermore, multiple exposures were frequently noticed, but the proportion measured in our study, 81%, is among the highest assessed to date (Christensen et al., 2012) and questions the factors driving the exposure of the Réunion

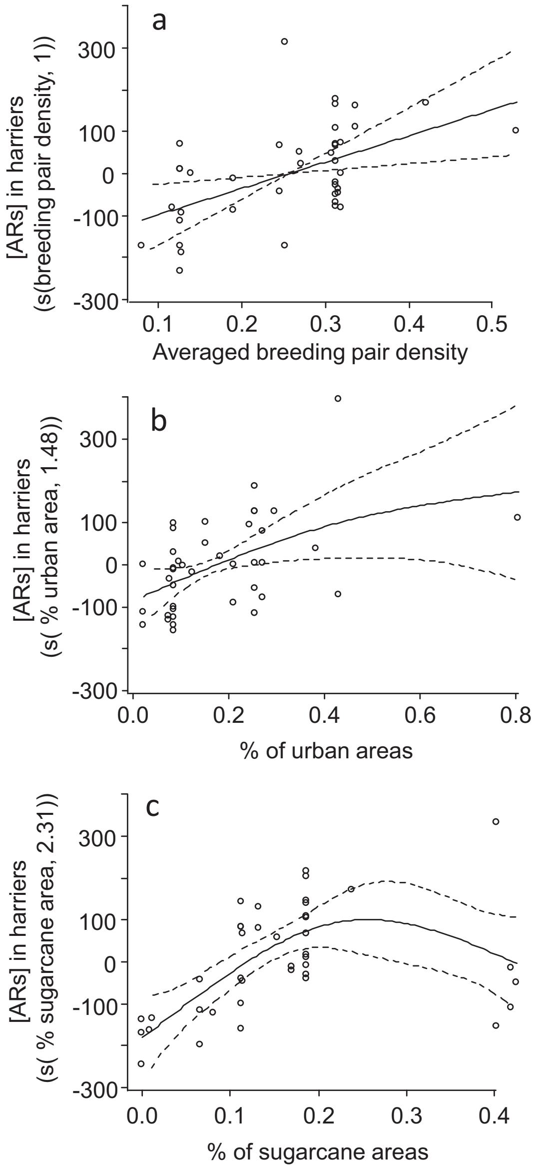

**Fig. 5.** Relationship between the summed concentrations of anticoagulant rodenticides measured in the Réunion harriers and the estimated density of the harrier breeding pairs (a), the proportion of urbanised areas (b) and the proportion of sugarcane areas (c) at the township level. The trends were estimated by a GAMM. The Y axis represents the smooth term, *s*, generated by the GAM and is estimated subject to the constraint that $\Sigma s(x_i) = 0$, 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.

harrier.

### *4.2. AR usage and landscape as drivers of harrier exposure to and potential poisoning by ARs*

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

We found that higher concentrations of $\Sigma$ARs in the harriers were related to larger urban areas in townships, which is consistent with the findings of Hindmarch and Elliot (2018). Our results strongly suggest that Réunion harriers were exposed to ARs used as biocides, but no data are accessible about the products distributed on Réunion Island during the study period. The fact that different a.i. are used in the biocidal products distributed on the market could explain why no influence of urban areas was evidenced for each a.i. Moreover, when precise information on the products applied is available, a sound interpretation may be biased by the fact that some AR bait formulations apparently contain trace levels of other a.i. than those stated on the label (Geduhn et al., 2014). The difethialone occurrence was the lowest among the detected ARs. To our knowledge, this a.i. has never been used on crops; thus, exposure would rather result from biocidal usage in urban areas even if misuses could not be excluded. Like difethialone is, along with brodifacoum, one of the most toxic ARs for birds considering its acute LD50 (0.26 mg/kg body weight for the Northern bobwhite, Erickson and Urban, 2004), its outdoor spread should be restricted, as it is in the UK (Buckle and Prescott, 2018). Difenacoum exhibited the highest occurrence measured in the harriers, while the relatively low concentrations could be explained by its moderate persistence in animal tissue compared to other SGARs and/or by a frequent but limited exposure. Poisoning by difenacoum is unlikely because the concentrations were always $< 100\,\mathrm{ng\,g^{-1}}$, but it may contribute to poisoning when multiple exposures occurred. The absence of available information makes the use of difenacoum questionable as a biocide on Réunion island. However, according to Grollier and Soufflet (2011), it would have been applied on the sugarcane crops, though we found no more details on its spreading features.

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

Moreover, we found a clear positive relationship between the AR concentrations and the harrier breeder density, making the birds inhabiting the most productive resource areas more likely to be exposed. Thus, if areas that host large rodent populations are attractive for the

Réunion harrier because of the high prey availability, they can become ecological traps for rodent predators when ARs are intensively spread, leading to an increase in both exposure and poisoning, as was shown in Spain (López-Perea and Mateo, 2019). Given the uncertainty of the location for most of the individuals, working at the township level might add noise to the detected signals. A more robust design able to characterize the probability of recovering a dead bird regarding links between exposure and the habitat variables would confirm these findings. Similarly, the dosage of the live birds, based on blood sampling, across the island could shed light on the actual exposure and risk for this endangered species.

### *4.3. Diagnosis of harrier poisoning by ARs and consequences for the population*

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

### *4.4. Recommendations for monitoring and mitigating Réunion harrier exposure to ARs*

All these points lead to consider AR poisoning following treatments on sugarcane crops and, likely to a lesser extent, its use as a biocide as a major threat to Réunion harriers. Mitigation actions are urgently required for ensuring its conservation. Thus, with this objective, we recommend the following:

- reinforce the survey of harrier mortality and exposure to ARs based on standardized monitoring. This would lead to an unbiased definition of the spatial and temporal pattern of exposure and thus the definition of areas where priority mitigation actions should be implemented.
- improve knowledge about the spatial distribution of Réunion harriers and their ecology (home range and variability over time, demography and life history traits), which is currently the aim of the ongoing ECOPAP programme (Ecology and Conservation of Réunion Harrier, 2016/2019) led by the SEOR. This would allow for the identification of key foraging areas and the periods where/when the surveying of harrier poisoning has to be reinforced and rodent control measures adapted.
- build an integrated pest management (IPM) plan compatible with Réunion harrier conservation and biodiversity in general in partnership with AR users and regulatory agencies. The general principles of risk mitigation for ARs were recently reviewed by Buckle and Prescott (2018). It seems crucial to collaborate with farmers to mitigate the side effects of ARs by combining mechanical, biological and chemical controls, for example. Such IPM plans should rely on rodent population surveys that include density estimates, research on genetic resistance to ARs and assessments of damage to crops caused by rodents over space and time. Based on our experience, which led to the development of an IPM plan for water vole in metropolitan France (Coeurdassier et al., 2014), FREDON Réunion could be a key partner for developing and transferring methodological innovations to farmers. Risk mitigation measures for biocidal ARs (Buckle and Prescott, 2018) could be implemented in urban areas where harriers exhibit high densities. Raising the awareness of people living in critical areas for harriers and throughout the entire island is also required for preventing high pesticide use in gardens and peri-urban areas. Recent actions have been undertaken by the SEOR to inform people and promote mechanical methods.
- explicitly define which method(s) is (are) permitted to limit the damage caused by rats and mice on sugarcane (and other crops) in the regulation, notably the methods based on rodenticides. In France, the current regulation for the control of rodents for plant protection focuses on metropolitan cases, i.e., only bromadiolone is authorized against voles (Journal Officiel, 2014). Both the agronomic and ecological contexts of overseas territories should be considered by authorities and implemented in regulations when needed. This is essential for proposing solutions adapted to farmers that are environmentally acceptable and clarifying the management of these practices by local authorities. Professional staff trained on sound application practices should register and control the quantities of bait spread by farmers. As less potent ARs are currently available, brodifacoum and difethialone should be avoided on sugarcane crops and the outdoors without forgetting the risk of rodent-resistant strain selection. More generally, the development of alternative methods based on safer pesticides and/or non-chemical solutions is recommended (Witmer, 2018).

## 5. Conclusion

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

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