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Intraspecific and geographical variation in rodenticide exposure among common kestrels in Tenerife (Canary Islands)

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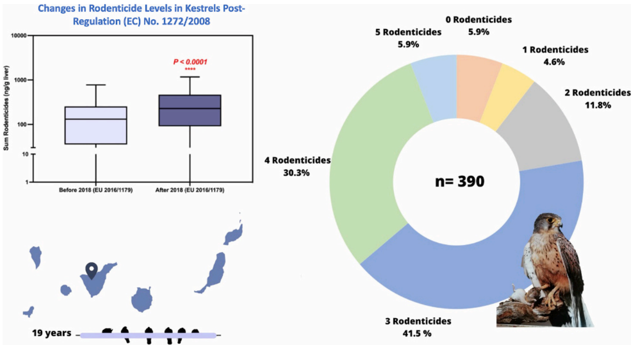
HIGHLIGHTS

- Monitoring of SGARs in a series of 390 kestrels from 11 years from Tenerife
- 93.1 % of kestrels had detectable residues of SGARs.
- 46.9 % of animals had >200 ng/g, with a maximum of 1107.07 ng/g.
- Brodifacoum is increasing its presence and concentration over the years despite current legislation.
- Age, high human density and high livestock development, were determining factors of exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

This study assesses the impact of second-generation anticoagulant rodenticides (SGARs) on the common kestrel (*Falco tinnunculus canariensis*) in Tenerife, Canary Islands. The analysis of 390 liver samples over 19 years using HPLC-MS/MS showed that 93.1 % of kestrels were exposed to SGARs in this island. A notable shift in SGAR profiles was observed, with bromadiolone and flocoumafen decreasing, while brodifacoum levels increased sharply from 2018 onwards. Comparatively, Tenerife kestrels had a higher detection frequency of SGARs (93.1 %) than those in the rest of the islands of the archipelago (68.2 %), with median concentrations nearly double ($\sum$AR = 180.9 vs 102.4 ng/g liver, $P < 0.0001$). Furthermore, on average, kestrels from Tenerife were found to have a higher number of different rodenticide compounds per individual. A Generalized Linear Model (GLM) analysis revealed that several factors contribute to the likelihood of SGAR exposure: being an adult kestrel, the enactment of legal restrictions on SGAR bait concentrations in 2018, higher livestock density, and greater human population density. These findings suggest that both bioaccumulation over the birds' lifespans and environmental

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factors related to human and agricultural activity are influencing the levels of SGARs detected. Alarmingly, 44.7 % of kestrels had SGAR levels above the toxicity threshold established for other raptor species (200 ng/g liver), signaling a high poisoning risk. This is despite EU regulations to protect wildlife, with our findings indicating an increase in both exposure rates and SGAR concentrations since these laws were enacted. The data highlight a critical environmental threat to endemic species on islands like Tenerife. The common kestrel, not considered globally endangered, is nonetheless facing regional threats from SGAR contamination. These results emphasize the urgent need for effective regulations to address the persistent and growing impact of SGARs on island biodiversity.

1. Introduction

The use of artificial or synthetic chemical substances with biocidal properties to control pest populations of some organisms became increasingly widespread after World War II (Carson, 1962). Raptors (i.e., *Accipitriformes, Cathartiformes, Falconiformes, Strigiformes*) are among wildlife organisms adversely affected by anthropogenic contaminants (e.g., biocides). As a result of biomagnification in food webs, the bioaccumulative transfer of contaminants can result in elevated levels of contaminants in these apex predators. Therefore, raptors can be used as powerful sentinels in environmental monitoring programs due to their position at the top of food webs, their long lifespan, low reproductive rate, and extensive home-ranges (Newton, 1998). The value of raptors as efficient biodiversity indicators (Buechley et al., 2019; Sergio et al., 2005) led to the creation of EURAPMON (Research and Monitoring for and with Raptors in Europe), a Research Networking Programme of the European Science Foundation. One of the main objectives of EURAPMON is to link raptor conservation monitoring studies with those using raptors as biological indicators of environmental change (Derlink et al., 2018). Similarly, European environmental monitoring programs (e.g., Finland, Norway, Sweden, UK) use raptors as sentinels to monitor the detrimental effects of environmental pollutants on wildlife and human health (Badry et al., 2019, 2020; Gómez-Ramírez et al., 2014; Helander et al., 2008; Ramello et al., 2022). The use of raptors for the monitoring of environmental pollutants has also been reported in some published works and studies in other European countries, including Germany, Spain, and the Netherlands (Gómez-Ramírez et al., 2014).

The common kestrel (*Falco tinnunculus,* hereafter, kestrel) is considered an effective model for studying behavioral (such as reduced activity, reduced hunting capability, altered intraspecific and interspecific interactions) as well as physiological effects (such as poor health, starvation, impaired motor coordination, low fertilization rate) caused by biocides (Constantini and del Olmo, 2020; Dumonceaux and Harrison, 1994; Martínez-Padilla et al., 2021). Thus, this is one of the most frequently studied raptors in European environmental monitoring programs, even since the early 1960s (Cooke et al., 1982). Kestrels, as well as other raptor species, have been shown to be sensitive to biocides (Costantini and Dell'Omo, 2020). Kestrels, as one of the most common and geographically widespread raptors in the Canary Islands, serve as a key indicator species due to their diurnal habits and presence across diverse habitats, from sea level to 2400 m, excluding only the highest peaks and densest forests (Carrillo, 2007; Carrillo and González-Dávila, 2005; Kangas et al., 2018). Their adaptability to both urban and rural environments, along with their varied diet, positions them as an excellent subject for studying the impact of biocides within the archipelago. The kestrel's role as a bioindicator is underscored by its high admission rates to the Wildlife Recovery Centre in Tenerife, representing a significant proportion of raptor admissions over two decades. Juvenile kestrels, which experience high mortality rates during their dispersal from natal territories, are particularly vulnerable to a range of anthropogenic threats, including poisoning and habitat destruction (Carrillo, 1991; Rodríguez et al., 2010; A. Village, 1990b).

SGARs are a major concern for wildlife conservation, particularly for predatory birds such as raptors (Newton, 1998). A pivotal change occurred in May 2018 when new regulations mandated the reclassification of anticoagulant substances exceeding 30 μg/g as reprotoxic, leading to stricter controls on their use and availability (Frankova et al., 2019). This legal adjustment has resulted in most rodenticide baits being produced with concentrations at or below 30 μg/g, aiming to reduce the risk of secondary poisoning in non-target species.

The accumulation of anticoagulant rodenticides (SGARs) in kestrels has been well-documented, highlighting significant health risks to these raptors in the Canary Islands (Luzardo et al., 2014; Rial-Berriel et al., 2021a). These findings are particularly relevant in light of the Poisoning Control and Prevention Strategy implemented in the Canary Islands since 2014 (BOC, 2014), which has enhanced the detection of environmental pollutants affecting wildlife. Despite the strategy's effectiveness, there is still a lack of comprehensive data on the impact of these substances on the kestrel population of Tenerife, an island with a rich ecological diversity and the highest human population density in the archipelago (Fernández-Palacios and Andersson, 2000).

The objectives of this long-term study were: i) to assess the incidence and concentration of SGARs in the common kestrel population of Tenerife, providing a detailed analysis of the prevalence and patterns of these rodenticides over an 19-year study period; ii) to investigate the relationship between SGAR exposure and various demographic factors, including age and sex, as well as the physiological health as indicated by body condition scores in the kestrel population; iii) to explore the spatial distribution of SGAR concentrations within the kestrel population, examining potential variances across different regions of Tenerife and the implications of land use and anthropogenic impact; and iv) to evaluate the temporal trends of SGAR exposure in kestrels in relation to regulatory changes, particularly the impact of amended bait dosages on the occurrence and levels of these compounds over time.

2. Material and methods

2.1. Study area, sampling, and ethical statement

The volcanic island Tenerife (27° 55′ and 28° 40′ N, 16–17° W) is in the Atlantic Ocean, 292 km from Morocco. Its most diverse habitats and vegetation units make it the largest (2034 km^2) and the highest (3715 m a.s.l.) of the Canary Islands (del Arco Aguilar et al., 2010). Urban areas occupy up to 24.2 % of its territory, with a population of 928,604 inhabitants in 2020. The island is the most densely populated in the Canary Islands (ISTAC, 2022). This island has 43 protected areas covering 48.6 % of its surface, as well as private properties with gardens or orchards and cultivation areas (vineyards, potatoes, tomatoes, banana plantations, orchards, greenhouses) particularly on the windward slopes (Beltrán, 2001; Carralero, 2001). Therefore, the common distribution of private properties and cultivation areas facilitate the arbitrary application of rodenticides by owners rather than specialized personnel.

Our study examined the liver of kestrels as the primary organ for accumulation of rodenticides (Thomas et al., 2011a, 2011b). Liver samples were obtained from necropsies of 390 kestrels. Most birds were admitted to the Wildlife Recovery Centre (WRC) of “La Tahonilla” (Tenerife, Canary Islands) between 2003 and 2009 ($n = 130$) and between 2017 and 2021 ($n = 260$). Additionally, birds were also collected from airport wildlife control units that had been involved in various types of aircrafts collisions. Our database does not contain georeferenced information regarding the exact location of all birds found, but in all cases, we collected a description of the location where the bird was found. The date and immediate cause of death were noted at the time of collection. Birds were either dead (20.8 %) or dead after hospitalization (68.5 %) or were euthanised (10.5 %) when their clinical signs and injuries were irreversible when brought to the WRC. No birds were sacrificed for the purpose of this study. Dead animals were kept frozen at −18 °C until necropsy. Livers were also frozen at −18 °C until the preparation of the extraction and chemical analysis.

An extensive and systematic post-mortem analysis was performed to detect potential effects of SGAR, such as weakness, anaemia, subcutaneous haemorrhage, lethargy, anorexia, and dyspnoea (Dumonceaux and Harrison, 1994; Murray, 2018). These signs were observed in 18.5 % of kestrels of our study. Several other causes of death in kestrels, were also found in this study, including trauma (21.0 %), blindness, drowning, starvation, shooting, lung disease and glue trapping. Despite performing necropsies on all individuals, the cause of death could not be determined with certainty in some individuals. We used the size and thickness of the pectoral muscle as an indicator of the body condition (i.e., the amount of protein reserves) of the examined kestrel (Dumonceaux and Harrison, 1994). By examining the gonads of the carcasses, we were able to identify the sex of the birds (180 females, 206 males, 4 indeterminate). We determined the age of the corpses according to the chromatic characteristics of the plumage using the code of age classification of birds (EURING codification; (EURING, 2020)) and the descriptions by Forsman (1999). In accordance with EURING coding scheme, we identified five ages, namely age 1: chick ($n = 7$), unable to fly freely (age < 35 days for kestrels); age 3: juvenile ($n = 252$), first-year bird, able to fly, born in the breeding season of this calendar year; age 5: adult ($n = 43$), bird in its second year, born last calendar year and now in its second calendar year; age 6: adult ($n = 53$), full-grown bird, born before last calendar year but year of birth unknown; age 8: adult ($n = 35$), bird after the third year, born more than three calendar years ago (including current year) and year of birth unknown.

2.2. Analysis of anticoagulant rodenticides in liver

LC-MS grade formic acid (FA), acetonitrile (ACN) and methanol, were purchased from Honeywell, (Morristown, NJ, USA), while ultrapure water was produced in our laboratory (Gradient A10 Milli-Q, Millipore, Molsheim, France). Standards for 9 ARs (5 SGARs: Brodifacoum, Bromadiolone, Difenacoum, Difethialone, Flocoumafen; and 4 FGARs: Chlorophacinone, Coumatetralyl, Diphacinone, Coumachlor) and an internal procedural standard (P-IS, Warfarin) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure compounds (98 %–99.5 % purity), and stock solutions were prepared at 1 mg/ml in ACN and stored at −20 °C until use. A matrix-matched calibration curve containing all rodenticides studied was prepared from the stock solutions.

2.2.1. Sample preparation

Post-mortem analysis of all kestrel livers was performed, and 1 g of liver was used for extraction of the analytes. After adding P-IS to the sample (or blank matrix for calibration points and quality controls) the mixture was then diluted with ultrapure water (4 ml) for extraction using the modified micro-QuEChERs method, as previously described (Rial-Berriel et al., 2020a, 2020b). Validation of the method indicated the presence of a strong matrix effect. Therefore, calibration curves were prepared with chicken liver for human consumption that tested negative for the analytes of interest. In the same way as the samples, each curve point was extracted using 2 ml of acidified ACN (0.5 % FA). Similarly, QC samples were prepared at 1 ng/g, and analyzed every 30 samples.

2.2.2. UHPLC-QqQ quantitative analysis

An Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled with an Agilent 6460 triple quadrupole mass spectrometer was used to separate and detect the analytes. The method was previously fully validated for liver tissue according to the Standard Practice Guidelines for Method Validation in Forensic Toxicology (SWGFT, 2013) and the SANTE analytical guide (CE, 2019). Recovery rates ranged between 80 and 120 % for all analytes with good linearity ($R^2 > 0.99$) and LOQs ranged from 0.4 to 1.6 ng/ml. A detailed description of the chromatographic and acquisition conditions as well as basic details of the procedure can be found in previous publications (Acosta-Dacal et al., 2021; Rial-Berriel et al., 2020b).

2.3. Data acquisition for the study of exposure determinants

Despite the absence of GPS coordinates for each kestrel, we had access to detailed descriptions of the collection sites, including the specific locality and municipality. Leveraging this data, along with statistics from the Canary Islands Institute of Statistics (ISTAC, 2022), and additional information from the Canary Islands Government, we generated a suite of locality-related variables. We conducted a descriptive analysis for each variable, determining median values which were then employed as thresholds to dichotomize the dataset into two categories for statistical comparison: values below and those at or above the median.

The variables analyzed encompassed a range of ecological and socioeconomic factors, including the area of the municipality (cut-off = 88.8 km^2), population size (36,727 inhabitants), population density (577 inhabitants per km^2), adjusted population density considering buildable space (873 inhabitants per km^2), per capita income (€22,663/year), income per square kilometer (€13,807,774), hectares of protected natural areas within the municipality (2465 Ha), percentage of municipal land designated as protected (33.9 %), hectares under cultivation (621.7 Ha), per capita cultivated area (201.9 m^2), percentage of land cultivated (11.4 %), and specific agricultural metrics such as banana plantation density (8333 m^2/km^2), vineyard (123 m^2/km^2), family orchards (5546 m^2/km^2), vegetable and tuber cultivation (17,162 m^2/km^2), fruit cultivation (9355 m^2/km^2), cereal (2022 m^2/km^2), and greenhouse crops (3172 m^2/km^2), along with total livestock numbers (359 animals) and livestock density (4 heads/km^2).

Furthermore, the year 2018 was a significant temporal marker, delineating the pre- and post-enactment phases of the regulatory change that mandated the reduction of SGAR concentrations in baits from 50 to 30 mg/kg. This dichotomization allowed for an assessment of the regulation's impact on SGAR levels detected in the kestrels.

2.4. Statistical analysis

All statistical analyses were conducted using R software (R Core Team, 2021). The initial step involved a thorough assessment of the distribution of variables. The Kolmogorov-Smirnov test revealed that the concentrations of Second-Generation Anticoagulant Rodenticides (SGARs) and distances did not follow a normal distribution, even after log transformation of the data. Therefore, these variables were represented using the median and interquartile range (p25th - p75th), in addition to the mean ± SD for descriptive purposes.

For comparative analyses between common kestrels from Tenerife and those from previous studies in other Canary Islands, nonparametric tests were employed due to the non-normal distribution of the data. Specifically, the Mann-Whitney U test was used for pairwise comparisons. To control for Type I error inflation due to multiple comparisons, a Bonferroni correction was applied to the p-values.

A Generalized Linear Model (GLM) with a binomial error distribution and logit link function was fitted to explore predictors of kestrel exposure to SGARs. We included 376 common kestrels in the analysis, as 13 had missing data. This method was chosen to analyze the presence or absence of ARs as the binary outcome of having a concentration above or below the threshold set at 200 ng/g in liver tissue, as suggested by various authors for potentially lethal effects in raptor species (Newton et al., 1999; Rattner et al., 2020; Thomas et al., 2011a, 2011b). All potential explanatory variables were dichotomized based on median values (coded as 0/1). The explanatory variables ultimately included in the model were age class (adult/juvenile), legal modification (before and after 2018, the date of its enactment), cattle density, and population density. Additionally, the sum of SGAR concentrations in liver was explored as a continuous dependent variable (continuous outcome), but the explanatory variables for this outcome were the same, hence we decided not to include these analyses in the Results and Discussion section. The forward selection procedure was utilized for model construction, with the Akaike Information Criterion (AIC) guiding the selection process. Prior to the inclusion of explanatory variables in the GLM, potential correlations were assessed using Spearman's correlation test, and highly correlated variables were excluded from the model to prevent multicollinearity. The level of statistical significance was set at $p \leq 0.05$ for all tests.

3. Results and discussion

3.1. Characteristics of the sampled population

This study involved sampling 390 common kestrels during 2 sampling phases (2004–2009 and 2017–2021), an 11-year period over 19-years. Fig. 1 shows the characteristics of the sampled population, including the causes of admission. The proportion of males ($n = 180$; 46.1 %) and females ($n = 206$; 52.8 %) was very similar. However, about two-thirds of the sampled group of kestrels were juveniles (EURING 3; $n = 252$, 64.0 %). The mortality rate of birds, including birds of prey, has been reported to be high within the first year of life due to inexperience in hunting, competition with other adults for occupied territories, and interspecific inexperience (identifying humans and enemy predators as well as factors associated with human activity is crucial to their survival). It has been established that for different species of raptors, death rate can range between 30 and 40 % during the first year of life (Newton et al., 2016), although it varies greatly between the species (Village, 1990a). In kestrels survival to adulthood has been reported to be even lower, with mortality rates ranging from 60 to 70 % (Hiraldo et al., 1996; Newton, 1979). The representation of kestrels sampled from southern ($n = 121$, 31 %) and northern ($n = 269$, 69 %) parts of the island showed a noticeable disparity, as did the proportion between individuals from urban ($n = 94$, 24 %) and rural areas ($n = 296$, 76 %). This imbalance is likely attributable to anthropogenic factors. Particularly, the island's northern slopes, especially the coastal strip, house the densest human populations and the highest levels of agricultural, livestock, and industrial activities. Therefore, kestrels in these areas encounter a more diverse range of threats, potentially increasing mortality rates. Additionally, areas with a higher density of human population correlate with an increased chance of discovering deceased, injured, or malnourished birds (Carrillo, 1991, 2007; Carrillo and González-Dávila, 2005).

3.2. Descriptive analysis of the levels of anticoagulant rodenticides

The descriptive results and comparisons between the incidences and concentrations of the rodenticides in the livers of the common kestrels in Tenerife and the rest of the Canary Islands, over the respective study periods, are presented in Table 1. This table demonstrates the frequency and mean concentrations of each SGAR (brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen) found in the two groups. In addition to the five SGARs identified, our analytical method also incorporated testing for four First Generation Anticoagulant Rodenticides (FGARs). However, none of these were detected in our study.

The detection pattern we found fully coincides with that described previously in the Canary Islands for birds of prey, including kestrels, as well as for other birds, reptiles and mammals found in the Canary Islands (Rial-Berriel et al., 2021a).

There is no doubt that the widespread use of SGARs for rodent control leads to the exposure of non-target species, with raptors being among the animals exposed at the highest rate (Nakayama et al., 2019; Sánchez-Barbudo et al., 2012; Van den Brink et al., 2018). However, the very high frequency and concentrations of SGARs found in kestrels on the island of Tenerife were striking. Only 27 kestrels were negative for anticoagulant rodenticides (17 juveniles, 8 adults, 2 chicks; 19 females, 8 males) (Fig. 1), giving a positivity percentage of 93.1 % ($n = 363$). Furthermore, 68 individuals (17.4 %) showed clear signs of internal and external bleeding. However, it is important to note that not all adverse

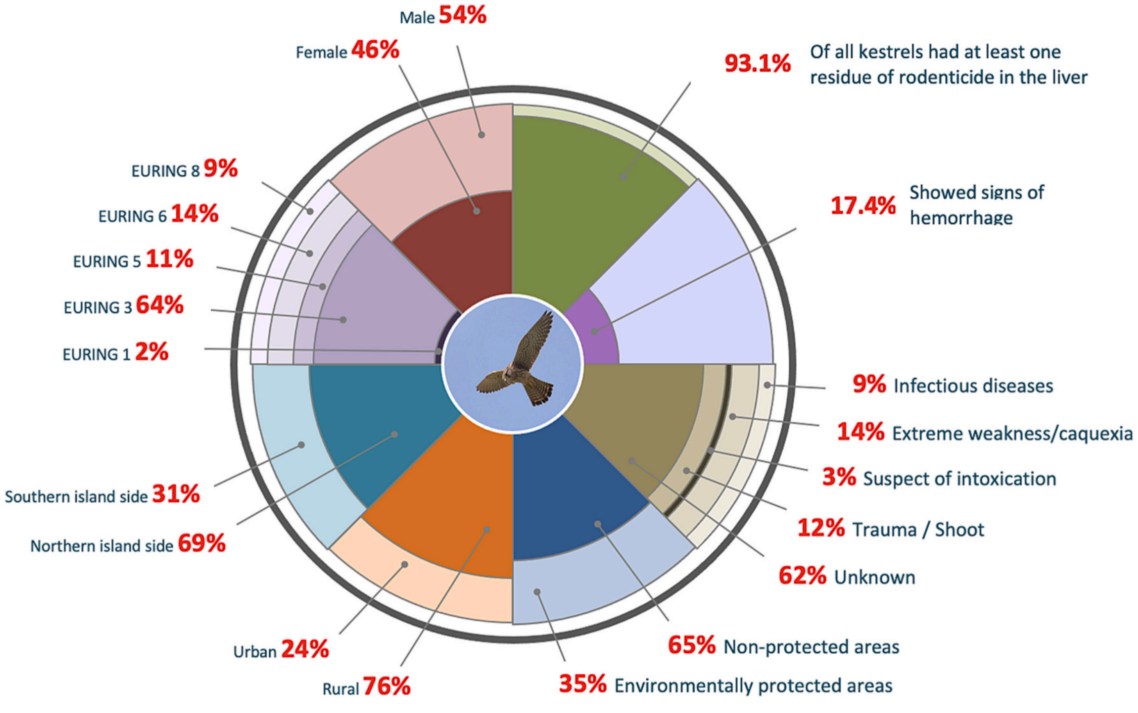

Fig. 1. Characteristics of the sample population of 390 common kestrels (*Falco tinnunculus*) of Tenerife (Canary Islands).

Table 1

Incidence and concentrations of the rodenticides in liver of the common kestrels in the Canary Islands.

	Tenerife (*n* = 390) 2003–2009 and 2017–2021				Rest of the Canary Islands (*n* = 86) 2009–2021					
	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	*P1*	*P2*
Brodifacoum	89.7 **	189.3 ± 229.8	97.6 *	18.1–289.5	58.7	133.0 ± 263.4	34.8	12.7–88.9	0.0046	0.0119
Bromadiolone	85.6 *	67.9 ± 88.8	33.1	12.5–89.9	61.3	232.5 ± 745.2	30.6	5.1–67.1	0.0326	–
Difenacoum	52.8 **	15.8 ± 26.3	5.5 *	3.1–16.7	29.0	12.2 ± 16.1	4.1	1.4–25.0	0.0087	0.0432
Difethialone	19.2 *	65.8 ± 149.8	7.1 **	3.6–38.3	8.0	3.4 ± 2.9	2.5	1.0–6.7	0.0404	0.0045
Flocoumafen	30.5 **	17.4 ± 36.2	5.1	2.9–13.7	4.8	5.6 ± 7.3	5.6	0.5–10.8	0.0018	–

effects of SGARs are related to blood coagulation disorders; rather, recent studies have shown that SGAR exposure can influence disease susceptibility, immune function, and several other effects independently of coagulopathy (Rattner et al., 2018; Van den Brink et al., 2018).

Previous studies reported high concentrations of SGARs in birds of prey in the Canary Islands (Rial-Berriel et al., 2021a). To highlight the significant exposure observed, we compared the current SGAR detection frequency and concentrations in Tenerife's kestrels with our previously published data for the species in the other Canary Islands. Instead of combining the datasets, these earlier results served as a benchmark for comparison. In Tenerife, we found that 93.1 % of kestrels (n = 363) had detectable levels of SGARs, marking a notable increase from the 68.2 % detection rate reported for the species on the other islands (Rial-Berriel et al., 2021c). The median value for the sum of ARs in Tenerife was practically double the value previously reported for the entire Canary archipelago ($\sum$AR = 180.9 vs 102.4 ng/g liver for Tenerife and the rest of the Canary Islands respectively; $P < 0.0001$) (Fig. 2). Additionally, in Tenerife, kestrels not only showed significantly higher values than in the rest of the archipelago, but the average number of rodenticides per animal was also significantly higher (Fig. 2, inset).

Furthermore, it's noteworthy that the median SGARs value for this group of 390 individuals is remarkably close to the toxicity/lethality cut-off values established for certain raptor species (200 ng/g liver). While these cut-offs are not universal and are based on probabilistic data derived from species different than kestrels, they are used as references due to the lack of other reference values (Thomas et al., 2011a, 2011b).

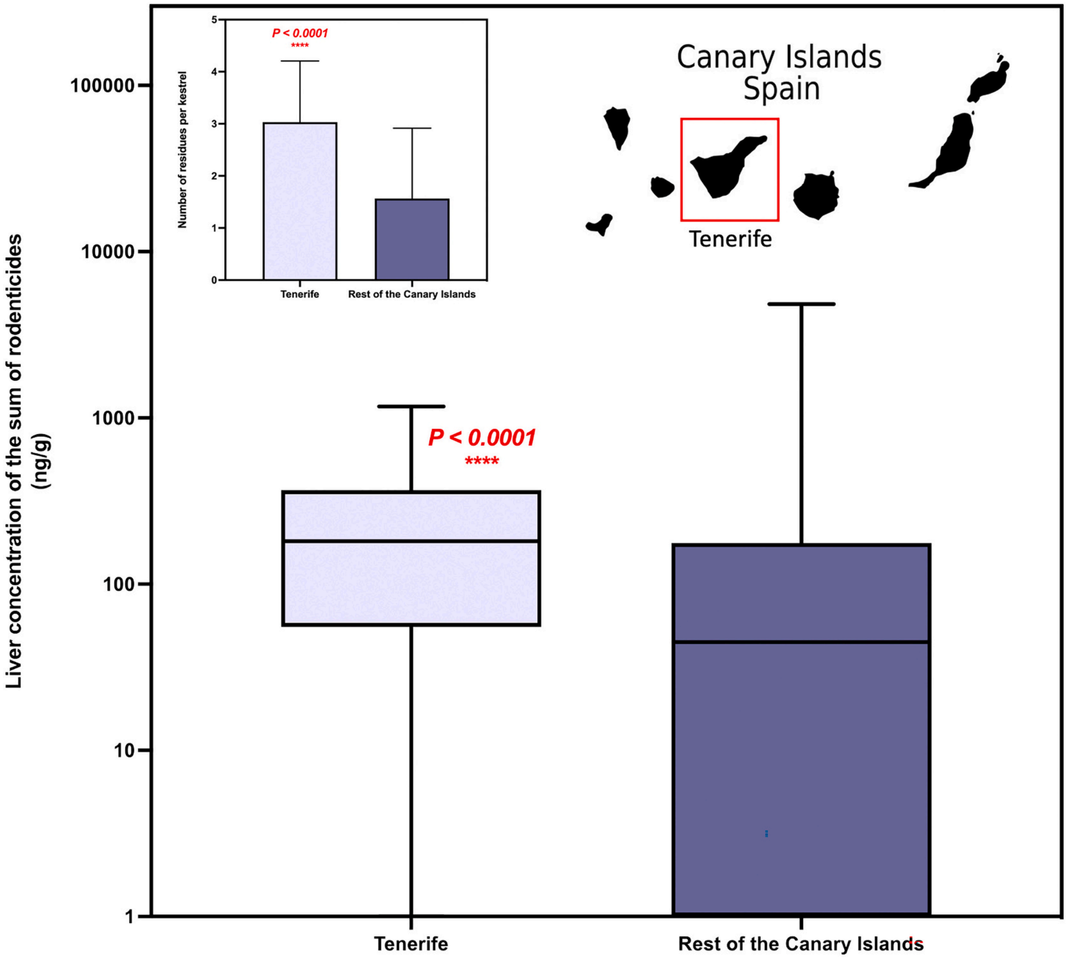

Fig. 2. Box-and-whisker plot showing the comparison of anticoagulant rodenticides in kestrel livers on Tenerife with those in the rest of the Canary Islands. The lines represent the medians, the boxes indicate the 25th to 75th percentiles, and the minimal and maximal values are shown at the ends of the bars. (Inset graphs) *Left.* Average number of ARs found per animal, comparing both territories; *Right.* Location of Tenerife in the Canary Islands archipelago.

Remarkably, a total of 185 individuals (46.9 %) exceeded the highest cut-off value (200 ng/g liver). Brodifacoum stands out due to its high concentrations and frequent detection. Out of a total of 363 kestrels showing rodenticides in the liver, 350 (96.4 %) contained brodifacoum, either alone or in combination with other rodenticides. These concentrations and frequency are significantly higher than those previously reported for the rest of the Canary Islands (Rial-Berriel et al., 2021a, 2021b; Ruiz-Suárez et al., 2014). Brodifacoum, being the most toxic rodenticide for birds and mammals, is of particular concern, and recently its use has been restricted to indoor and immediate outdoor areas of buildings, with its application in open spaces prohibited (EC, 2022). The mean value for brodifacoum in our study was 189.3 ng/g liver (Table 1), which is a very high value, akin to what would be achieved by consuming 1–3 μg of brodifacoum per gram of feed along a 24 h-period, as found in experimental studies with American kestrels (Rattner et al., 2020). Furthermore, liver concentrations above 200 ng/g liver were found in 110 kestrels, which, in the literature, correlate with a variety of clinical manifestations in kestrels, ranging from visible bruising in non-feathered areas to frank bleeding in the oral cavity (Rattner et al., 2020). Detection frequencies and concentrations of difenacoum and difethialone were also significantly higher in this group of Tenerife kestrels (Table 1) compared to the rest of the Canary Islands, and published previously (Rial-Berriel et al., 2021a, 2021b). According to the US Environmental Protection Agency, brodifacoum and difethialone pose the greatest overall potential risk to birds and non-target mammals (Erickson and Urban, 2004). Even though bromadiolone and flocoumafen levels were similar in kestrels from other Canary Islands in kestrels, the frequency of detection on Tenerife was significantly higher, with bromadiolone detected in 334 individuals and flocoumafen detected in 119.

It is also important to note that the average number of rodenticides per animal were also high, and they were significantly higher than those previously reported for kestrels in the Canary Islands (median 2.98 vs. 1.42 rodenticides per animal; $P < 0.0001$) (Fig. 2, inset). A total of 85.6 % ($n = 334$) of the positive cases had >1 rodenticide, and most animals had 3 (41.5 %, $n = 162$)) or 4 (30.3 %, $n = 122$)) different rodenticides simultaneously (Fig. 3, left). Combinations of >1 rodenticide are commonly found in raptors, but usually at a much lower percentage (40–60 %), as reported in recent literature, including the Canary Islands (Coeurdassier et al., 2019; Hong et al., 2019; Huang et al., 2016a; Murray, 2011; Rial-Berriel et al., 2021a; Thornton et al., 2022). Brodifacoum in combination with bromadiolone and difenacoum was the most encountered combination followed by the same combination with flocoumafen (Fig. 3, right). Given its predominance in this group of kestrels, brodifacoum can be found in 99.4 % of combinations containing two or more rodenticides.

3.3. *Temporal trends of the exposure of kestrels to SGARs*

Our longitudinal study spans two distinct periods, 2004–2008 and 2017–2021, totaling 11 years within a 19-year framework. This design facilitated an analysis of the temporal dynamics of SGAR exposure among common kestrels in Tenerife.

Previous research by Rial-Berriel et al. (2021a) indicated an uptick in certain SGARs, notably brodifacoum, within Canary Islands wildlife. Our findings mirror this trend in Tenerife kestrels, as depicted in Fig. 4. Notably, since 2018, the annual average SGAR burden per bird has frequently exceeded the conservative benchmark of 200 ng/g liver tissue. Thus, Post-2018, we observed a surge in both the concentrations of SGARs and the proportion of samples surpassing this threshold (Fig. 5). This period coincides with amendments to Regulation (EC) No. 1272/2008, mandating reprotoxic labelling for anticoagulant baits exceeding 30 μg/g. Despite the regulation's intent to reduce bait concentrations, our data indicate an increase in brodifacoum levels post-regulation, suggesting compensatory usage patterns by end-users (Table 2) (Frankova et al., 2019; Rial-Berriel et al., 2021a).

Moreover, the regulatory changes coincide with intensified efforts to manage feral cat populations on the islands, potentially influencing rodenticide application rates due to altered rodent-predator dynamics (Mahlaba et al., 2017). The implications of such ecological shifts are evident in the heightened rodenticide residues we detected.

The pattern of increased SGAR exposure is not unique to Tenerife. Parallel increases in brodifacoum in raptors have been documented globally, with regional studies reporting similar trends. For example, recent findings from south-eastern France indicate an increase when compared to previous periods (Moriceau et al., 2022). In Western Canada, the mean concentrations of bromadiolone have markedly increase, likely due to regulatory changes that permit only bromadiolone for

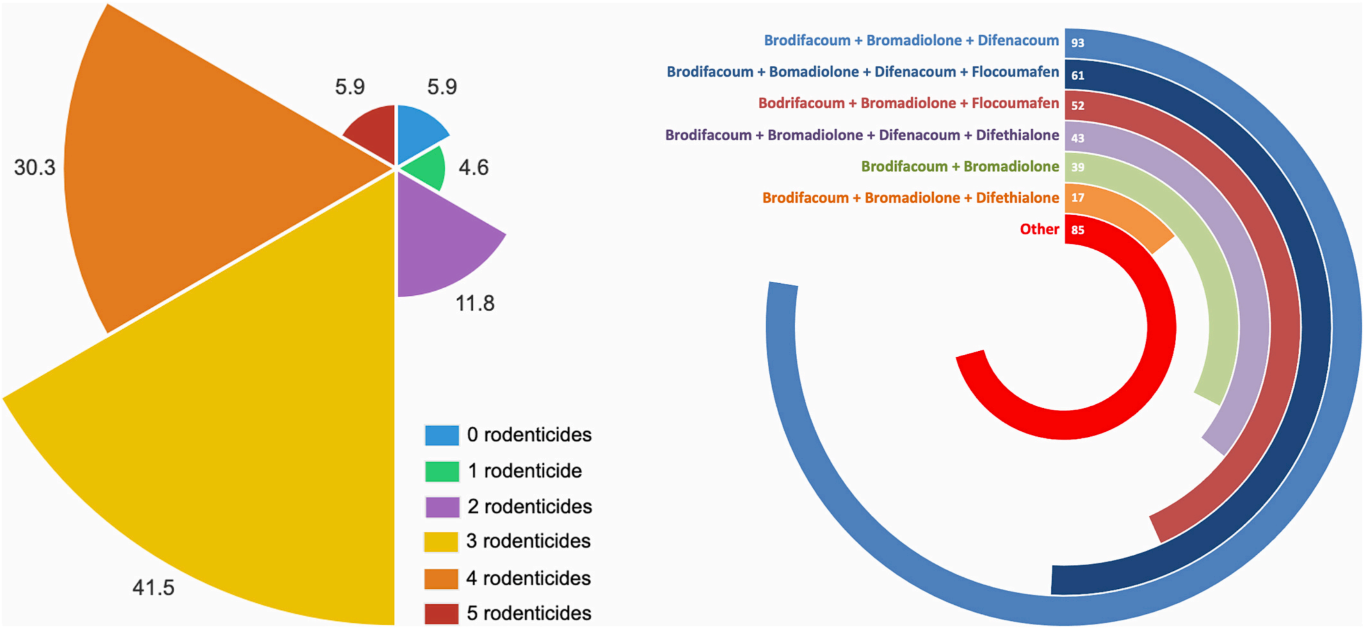

Fig. 3. Left. Number of anticoagulant rodenticides per animal, expressed as a percentage. Right. Most frequent combinations of rodenticides found in kestrels with more than one AR. Figures indicate the number of individuals with that combination.

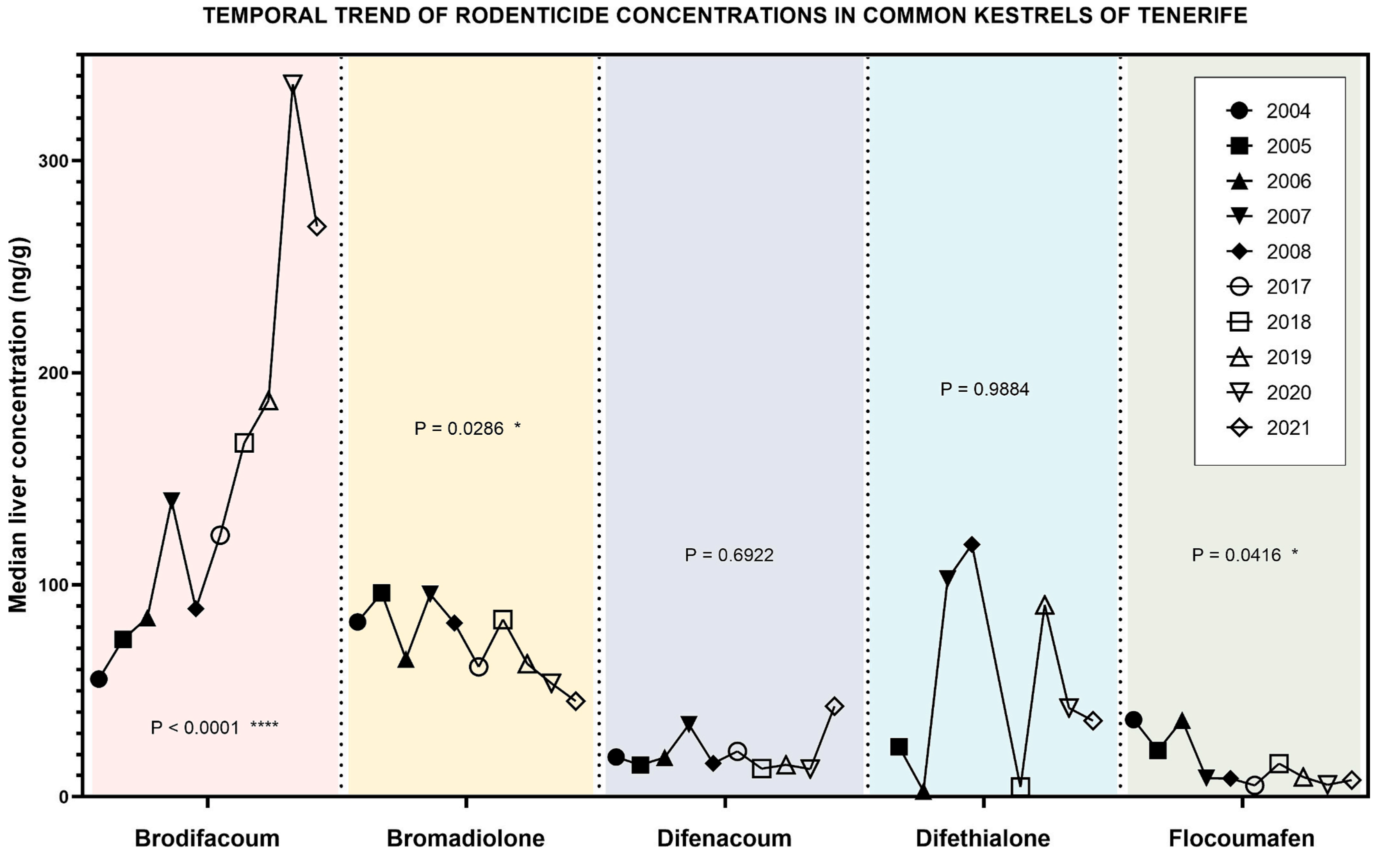

Fig. 4. Temporal trend of rodenticide concentrations in common kestrels from Tenerife.

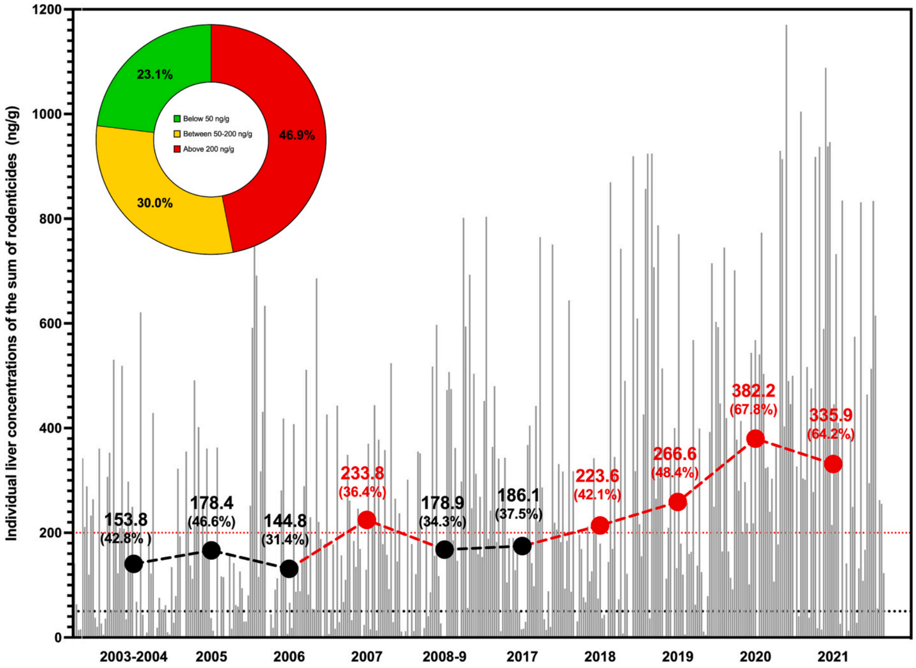

Fig. 5. Individual values of the sum of anticoagulant rodenticides of all kestrels sampled. The dots indicate the median value of all the animals sampled in the specified year or period, as well as the percentage of individuals with values above 200 ng/g liver (in brackets). The dashed red line indicated the threshold value of 200 ng/g liver. When the median value for all birds exceeds the threshold value, the dots and numbers appear in red. Inset graph. Chart indicating the percentage of individuals with liver rodenticide sum values below 50 ng/g liver; from 50 to 200 ng/g liver; and above 200 ng/g liver.

Table 2
Analyses of anticoagulant rodenticides concentrations detected in the liver of common kestrels prior and following the application of the new UE regulation regarding the commercialization of rodenticide baits.

	Before March 2018 (EU 2016/1179)				After March 2018 (EU 2016/1179)				
	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	*P*
Brodifacoum	85.8	98.5 ± 142.7	23.7	8.2–123.9	91.5	246.2 ± 255.3	147.2 ****	42.7–378.2	< 0.0001
Bromadiolone	84.5	77.4 ± 102.7	39.8 *	15.0–730.0	78.8	61.7 ± 78.4	25.2	11.5–376.3	0.0482
Difenacoum	32.2	19.6 ± 33.7	5.1	3.0–16.1	66.8	14.7 ± 23.5	6.3	3.1–16.8	0.9423
Difethialone	9.0	84.1 ± 198.7	4.7	2.6–28.1	23.9	61.7 ± 137.9	7.2	3.9–39.6	0.2608
Flocoumafen	49.7	21 ± 42.8	6.5 *	3.4–17.2	16.1	9.9 ± 16.9	4.3	2.6–9.9	0.0307

outdoor applications (Elliott et al., 2022). In contrast, brodifacoum levels decreased over the same period, likely in line with risk mitigation measures. A similar pattern has been observed on Reunion Island (Coeurdassier et al., 2019). These trends extend beyond Tenerife and underscore a broader issue. This is of special concern, especially since various studies have suggested that SGARs may contribute to a significant population decline in kestrels across Europe (Harris et al., 2020; PECBMS, 2021; Roos et al., 2021a, 2021b). Our data, coupled with recent population assessments (Martínez-Padilla et al., 2021; Carrillo-Hidalgo, unpubl. data), suggest that the kestrel population may also be experiencing a decline in Tenerife.

3.4. Integrated analysis of kestrel exposure to anticoagulant rodenticides

Our research has elucidated a complex interplay of factors influencing the presence of anticoagulant rodenticides (ARs) in the common kestrel population on Tenerife. The statistical models we refined reveal that both biological traits and human-induced changes significantly determine AR levels in these raptors. The logistic regression models (Tables 3 and 4) reveal that age and recent legal modifications are prominent determinants of AR presence in kestrels.

Adult kestrels are more likely to exhibit detectable levels of ARs compared to juveniles, with a 93 % increased likelihood [OR: 1.93, $P = 0.004$]. This finding aligns with previous observations of age-related variations in SGAR levels among raptors and suggests that adult kestrels, due to their foraging behavior and longer exposure times, accumulate higher concentrations of these compounds. For example, Roos et al. (2021b) found that juvenile kestrels possess higher levels of difenacoum than adults, whereas Huang et al. (2016b) reported higher levels in adult owls (*Tyto alba*). Despite these variable findings, the prevalence of SGARs remains high, with 88–93.8 % detected for brodifacoum and bromadiolone, and 19.7–51.4 % for other rodenticides. Given this widespread presence, concerns have been raised about the use of these rodenticides, even when their usage is restricted to interior or perimeter areas of buildings, or when bait concentrations are limited.

Table 3
Best adjusted models explaining the presence (threshold set at 200 ng/g) of anticoagulant rodenticides in the common kestrels from Tenerife.

		Estimates	SE	OR (95%CI)	*p*	AIC
M1	Intercept	−1.19	0.23	0.30 (0.19–0.48)	<0.001	498.60
	Legal modifications Yes-No	0.77	0.22	2.15 (1.39–3.33)	<0.001	
	Age Adult-juvenile	0.66	0.23	1.93 (1.23–3.03)	0.004	
	Cattle density 1–0	0.46	0.22	1.59 (1.03–2.45)	0.036	
	Population density 1–0	0.36	0.22	1.44 (0.94–2.20)	0.093	
M2	Intercept	−1.04	0.21	0.35 (0.23–0.54)	<0.001	499.42
	Legal modifications Yes-No	0.78	0.22	2.17 (1.40–3.36)	<0.001	
	Age Adult-juvenile	0.68	0.23	1.97 (1.26–3.09)	0.003	
	Cattle density 1–0	0.49	0.22	1.63 (1.06–2.50)	0.027	

Note: Model outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), odds ratio (OR) and correspondent 95 % confidence interval (95 % CI), and p-values from a Binomial Logistic Regression model. The Akaike's Information Criterion for the model is also reported. Response variable: threshold set at 200 ng/g. Number of kestrels in the analysis = 376.

Table 4
Summary information of the variables considered for inclusion in the models categorized based on the threshold set at 200 ng/g.

Variables	<200 ng/g N (%)	>200 ng/g N (%)
Age		
Juvenile	145(38.6)	102(27.1)
Adult	54(14.4)	75(19.9)
Law implementation		
Yes (after 2018)	103(27.4)	127(33.8)
No (before 2018)	96(25.5)	50(13.3)
Cattle density		
0 (< median)	126(33.5)	92(24.5)
1 (>median)	73(19.4)	85(22.6)
Population density		
0 (< median)	113(30.1)	81(21.5)
1 (> median)	86(22.9)	96(25.5)

As previously mentioned, the implementation of legal modifications has also had a significant impact, though not in the desired manner. Kestrels found post-regulation exhibit more than twice the likelihood of AR presence [OR: 2.15, $P < 0.001$]. This increase in detection frequency and concentration of brodifacoum post-2018 is a critical finding, considering the regulatory intent to mitigate the risks associated with SGARs by mandating reprotoxic labeling for higher concentration baits.

In our study, we expanded the scope of investigation to consider the impact of socio-demographic factors and land use patterns on the presence of SGARs in the common kestrel population. Our findings reveal that among the variables considered, cattle density emerged as a significant factor. Specifically, kestrels found in areas with higher cattle density are 59 % more likely to have detectable levels of SGARs [Odds Ratio (OR): 1.59, $P = 0.036$]. This relationship indicates that practices associated with livestock farming, such as the use of rodenticides to safeguard feed or control rodent populations, could inadvertently increase the levels of SGARs found in kestrels. Furthermore, our analysis has shown a direct link between the density of agricultural cultivation and livestock presence on the island. However, it is noteworthy that the density of crops alone was not a significant predictor for the presence of

SGARs when considering the established threshold of 200 ng/g. This finding implies that while agricultural activities are related to the presence of SGARs in the environment, it is specifically the practices related to livestock management that are more influential in determining the exposure of kestrels to these rodenticides.

Contrary to expectations, human density did not show a statistically significant correlation in our models, despite being a factor in model performance. This nuance highlights the complexity of the relationship between human density and wildlife exposure to contaminants and suggests that local factors may modulate this relationship in Tenerife. The results of several studies indicate that urban environment and human density are clear determinants of wildlife exposure to SGARs (Alabau et al., 2020; Burke et al., 2021; Lettoof et al., 2020; Lohr, 2018; López-Perea et al., 2019). However, it is crucial to note that the effects of these variables may be shaped by specific local factors.

When considering multiple variables simultaneously, age remains the sole significant predictor of high AR levels in kestrels. This finding underscores the importance of considering life history traits when assessing the risk of contaminant exposure in wildlife. While agricultural and livestock factors appeared influential in univariate analyses, their significance diminished when age was accounted for. This suggests that the accumulation of ARs over time is a critical factor, and age-related bioaccumulation may be a more significant determinant of AR levels than previously understood.

However, our study's interpretive power is subject to certain limitations. Notably, we cannot definitively ascertain whether the rodenticides found in juvenile kestrels (EURING code 3) were accumulated in the areas where these individuals were located at the time of discovery. Given that most juveniles of this species disperse around the island of Tenerife after a variable period of 20–40 days in their parents' territory, the exact localities, habitats, and feeding sites during their dispersal are not known. This uncertainty means that SGAR concentrations in these juvenile kestrels could have been accumulated throughout the dispersal period, rather than being indicative of the contamination levels in the specific areas where they were found. Moreover, the adults (EURING codes 5, 6, and 8) found before the dispersal dates of juveniles may provide insights into geographical differences in SGAR exposure across Tenerife. Our study did not find significant differences between areas, suggesting that AR contamination is widespread across the island. This pervasive presence of ARs, despite recent regulatory changes aimed at protecting wildlife, indicates that these measures are not effectively curtailing the environmental impact of these substances.

Our integrated analysis has delineated the multifaceted nature of AR exposure in kestrels, highlighting the interplay between regulatory changes, biological traits, and anthropogenic land use. These insights are crucial for informing future conservation strategies and regulatory decisions aimed at mitigating the impact of these environmental contaminants on wildlife. Nonetheless, the absence of spatial clustering in exposed individuals and the widespread contamination revealed by our study call into question the efficacy of current regulations and underscore the need for a re-evaluation of wildlife protection policies in the face of persistent environmental contaminants.

4. Conclusions

Our study has revealed a concerning prevalence of anticoagulant rodenticides (ARs) in Tenerife's kestrel population, with most individuals showing levels near or above lethal thresholds, especially concerning given the high toxicity of compounds like brodifacoum and bromadiolone. An upward trend in AR detection has been observed, particularly following regulatory changes intended to reduce bait concentrations, suggesting these efforts have not been fully successful.

The research underscores livestock farming as a significant factor in AR exposure for kestrels, more so than general agricultural activities. Adult kestrels are particularly at risk, likely due to bioaccumulation effects over time. Interestingly, human population density does not correlate strongly with AR exposure, indicating that other local factors may be influencing the risk.

The current regulatory framework appears inadequate in protecting kestrels and potentially other wildlife from AR exposure. This calls for more robust and effectively enforced regulations, with a need for conservation strategies and policies that reflect the complex nature of AR exposure, integrating biological and socio-economic considerations. Further studies are essential to deepen our understanding of the interplay between land use, rodent control practices, and wildlife exposure to ARs.

CRediT authorship contribution statement

Guarantor of integrity of the entire study: OPL, JCH
Study concepts and design: OPL, JCH
Sample providing: JCH
Literature research: OPL, BMC, JCH, CRB, AAD
Laboratory work: CRB, AAD, BMC, MZ, OPL
Data analysis: OPL, BMC, CRB, JCH
Statistical analysis: OPL, BMC, LAHH, MZ
Manuscript preparation (original draft): OPL, JCH, BMC, AAD, CRB
Manuscript editing: OPL, BMC, JCH, CRB, AAD, BMC, LAHH, MZ
Project administration: OPL
Funding acquisition: OPL, MZ, LAHH

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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