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Comparing anticoagulant rodenticide exposure in barn owl (*Tyto alba*) and common kestrel (*Falco tinnunculus*): A biomonitoring study in an agricultural region of southeastern Spain☆

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A B S T R A C T

Second-generation anticoagulant rodenticides (SGARs) are commonly used for rodent control, affecting various non-target wildlife species. Here, blood samples from common kestrels (*Falco tinnunculus*, $n = 70$ chicks) and barn owls (*Tyto alba*, $n = 54$ chicks and 12 adults) from Southeastern Spain were analysed using HPLC-TQ. SGAR prevalence was 68.6% in kestrel chicks, 50% in barn owl chicks and 100% in adult barn owls, with multiple SGARs in both species. Prothrombin time analysis in barn owls revealed a positive correlation with blood ΣSGARs, suggesting a potential adverse effect on coagulation. Analysis of variables potentially influencing SGAR prevalence indicated that, for kestrels, it was only related to the extent of artificial surface, showing no differences across study sites. In owlets, the highest prevalence occurred in the most urbanized study site, with human population density being a key factor. This study highlights species-specific differences in SGAR exposure, likely influenced by ecological traits. Barn owls probably encounter contaminated prey near anthropized areas, with widespread SGAR use and higher presence of target rodents. Conversely, kestrels, hunting a variety of prey often near human settlements, face consistently elevated exposure from multiple sources. Understanding these variations is crucial for effective conservation and minimizing SGAR impact on non-target wildlife.

1. Introduction

Anticoagulant rodenticides (ARs) are highly toxic, persistent and bioaccumulative pesticides used to control rodent populations. These products are primarily employed in agricultural, industrial, and domestic settings to mitigate damage to crops, food contamination, and infrastructure impairment (Jacob and Buckle, 2018). However, the use of ARs entails significant risks, including the potential for accidental exposure to humans and non-target animals, as well as the negative impact on the ecosystems (Lefebvre et al., 2017; Nakayama et al., 2019).

ARs alter blood coagulation by impairing the vitamin K cycle and the activation of coagulation factors, which can cause internal bleeding that can lead to death (Horak et al., 2018; Watt et al., 2005). Additionally, ARs can affect the normal behaviour of poisoned rodents, making them

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weaker and slower in their movements, and consequently, more vulnerable to predation (Littin et al., 2000). Currently, the most frequently used ARs correspond to second-generation compounds (SGARs) such as bromadiolone, brodifacoum, difenacoum, flocoumafen, and difethialone (Jacob and Buckle, 2018). Their anticoagulant action is more potent and specific than first-generation ARs (FGARs), but this also leads to a longer presence of these substances in the consumer's body and a more lasting impact on the environment (Erickson and Urban, 2004; Vandenbroucke et al., 2008). Therefore, the risk of poisoning in non-target animals, including predators and scavengers, may be higher.

In Europe, eight AR compounds are currently registered: coumatetralyl, warfarin, chlorophacinone, brodifacoum, bromadiolone, difenacoum, flocoumafen, and difethialone (European Union, 2012). In Spain, these compounds are currently in use, with the exception of warfarin (Ministerio de Sanidad, n.d.). The Biocidal Products Directive (BPD) (Regulation (EC) n. 528/2012, European Union, 2012) identifies ARs as candidates for substitution due to their high toxicity and persistence. However, most ARs are still authorized and widely used due to the lack of more efficient and eco-friendly alternatives.

In this context, non-target wildlife, especially top predators inhabiting agroecosystems, would be readily exposed, both through the consumption of contaminated target rodents and due to the spread of ARs throughout the food chain. AR contamination has been frequently studied in raptors (Christensen et al., 2012; Elliott et al., 2022; Sánchez-Barbudo et al., 2012; Weir et al., 2018), which are particularly sensitive to these substances compared to other avian species (Nakayama et al., 2020; Rattner et al., 2011). Probably, birds of prey are repeatedly exposed from early life stages (Spadetto et al., 2024), yet sublethal effects of chronic AR exposure remain poorly studied. Overall, AR exposure remains a conservation concern potentially harming raptor populations worldwide (Gomez et al., 2022), which points out the importance of assessing the extent of AR contamination and its possible effects. In addition, birds of prey occupy the highest trophic levels, making them notably sensitive to ecosystem disturbances. Consequently, they are considered excellent biomonitoring species for assessing the presence and impact of environmental contaminants, such as ARs (Badry et al., 2020; Movalli et al., 2017).

The common kestrel (*Falco tinnunculus*) and the barn owl (*Tyto alba*) are two medium-size predatory species that play a key role in controlling rodent populations across Eurasian agroecosystems, contributing to the ecological balance in agricultural and rural landscapes (Montoya et al., 2021). In agricultural areas, rodent-predatory species have experienced significant declines over the past years, primarily due to agricultural intensification (Grande et al., 2018). Increased farming mechanization resulted in habitat fragmentation and land use changes, with subsequent loss of suitable nesting and foraging sites. In addition, the excessive use of pesticides that accumulate throughout the food chain, such as ARs, have further impacted raptor populations (Buck et al., 2020; Roos et al., 2021; Ruiz-Suárez et al., 2014).

This study is intended to assess environmental exposure to ARs in diurnal (*F. tinnunculus*) and nocturnal (*T. alba*) raptors from an agricultural Mediterranean region. More specifically, we aimed: (1) to assess interspecific, interannual, and age-related differences in the prevalence and levels of ARs; (2) to explore potential associations between ARs and landscape-scale environmental variables (land use, human population density and livestock farming); and (3) to investigate the relationship between the prothrombin time (PT, a blood coagulation parameter) and blood AR concentrations as a biomarker of effect.

2. Materials and methods

2.1. Study sites

This study was conducted in the Region of Murcia (SE Spain), characterized by a typically Mediterranean semiarid climate. The mean annual precipitation is about 250–300 mm per year (Spanish National Agency of Meteorology - AEMET, 2024). The most relevant economic activities include the cultivation of fruits, vegetables, and other agricultural products (CARM Región de Murcia, 2022). The average population density is 135.4 inhabitants/km^2 (National Institute of Statistics (INE), 2021), with significant variations within the region. Despite the great development of intensive farming (Rupérez-Moreno et al., 2017), extensive agricultural landscapes still remain across the region. Hence, this scenario allows for assessing AR exposure along a gradient of agricultural intensification. Five distinct study sites were selected for sampling, each characterized by different geographic and land use features (Fig. 1): a) "Intensive irrigation farming", in the southern part of the Campo de Cartagena plain. Today, this landscape is mostly dominated by a wide variety of irrigated crops, such as orchards and vegetables (Rupérez-Moreno et al., 2017); b) "Rainfed tree farming", corresponding to the northern sector of the Campo de Cartagena plain, known as Campo de Murcia, this study site is located beyond the Segura River valley. This site is represented by traditional cultivation of rain-fed cereals and almond trees, although irrigated citrus orchards have expanded through this area during the last decade (Esteve-Selma et al., 2015); c) "Urban-agricultural landscape", which occupies a large valley irrigated by the Segura River and currently devoted to citrus orchards and subsistence farming of vegetables, still using small farming plots and traditional methods. This site surrounds the city of Murcia and is characterized by a high human population density compared to other agricultural areas (Ros Sempere et al., 2010), having also undergone a significant process of urbanization in recent years; d) "Salt steppe", referred as Saladares del Guadalentín and placed between the Sierra Espuña and Carrascoy mountains, it comprises a landscape of saltmarsh patches along the Guadalentín River, surrounded by an agricultural landscape and human structures. Recognized as a Site of Community Importance (SCI) and a Special Protection Area (SPA), this area is subjected to special safeguards to preserve its ecological significance and biodiversity (Pardo et al., 2003); e) "Rainfed cereal farming", covers the northern part of the municipality of Lorca, in the southwest of the Region. It is characterized by hilly terrain, with elevations and valleys creating a rolling landscape. Agriculture in this zone tends to be more extensive and traditional, emphasizing the cultivation of cereals and other rainfed crops. It is also characterized by a relatively low population density, partly due to its rugged topography and its relative distance from major urban centres (Molina Molina et al., 2006).

2.2. Target species and sample collection

The common kestrel is a small diurnal bird of prey belonging to the Falconidae family. It inhabits a wide range of open and semi-open habitats, including agricultural landscapes, meadows, pastures, urban environments, and open forests (Garratt et al., 2011). As a generalist predator, its diet primarily consists of small mammals and insects, but it can also include small birds, reptiles, and amphibians (Montoya et al., 2021; Navarro-López and Fargallo, 2015). The barn owl is a medium-sized owl of the Tytonidae family. It is a specialist of open farmlands and its presence is closely influenced by the availability of foraging and nesting sites, the latter often corresponding to abandoned buildings or other human structures (Séchaud et al., 2021). It is predominantly a rodent-eating species, although it may occasionally capture other prey, such as small birds and insects (Jiménez-Nájar et al., 2021; Moysi et al., 2018). Although widely distributed throughout the Palearctic region, populations of both species are declining at a continental (BirdLife International, 2020a, 2020b) and national scale. Indeed, the common kestrel is currently classified as endangered in Spain (Martínez-Padilla et al., 2021), whereas the barn owl is listed as nearly threatened (SEO/BirdLife, 2021).

During the breeding seasons of 2021 and 2022, 12 adult barn owls, 54 barn owl chicks and 70 common kestrel chicks were sampled. Nestlings belonged to 23 kestrel breeding territories and 19 barn owl territories. Due to territorial occupation, two kestrel and 3 barn owl nests

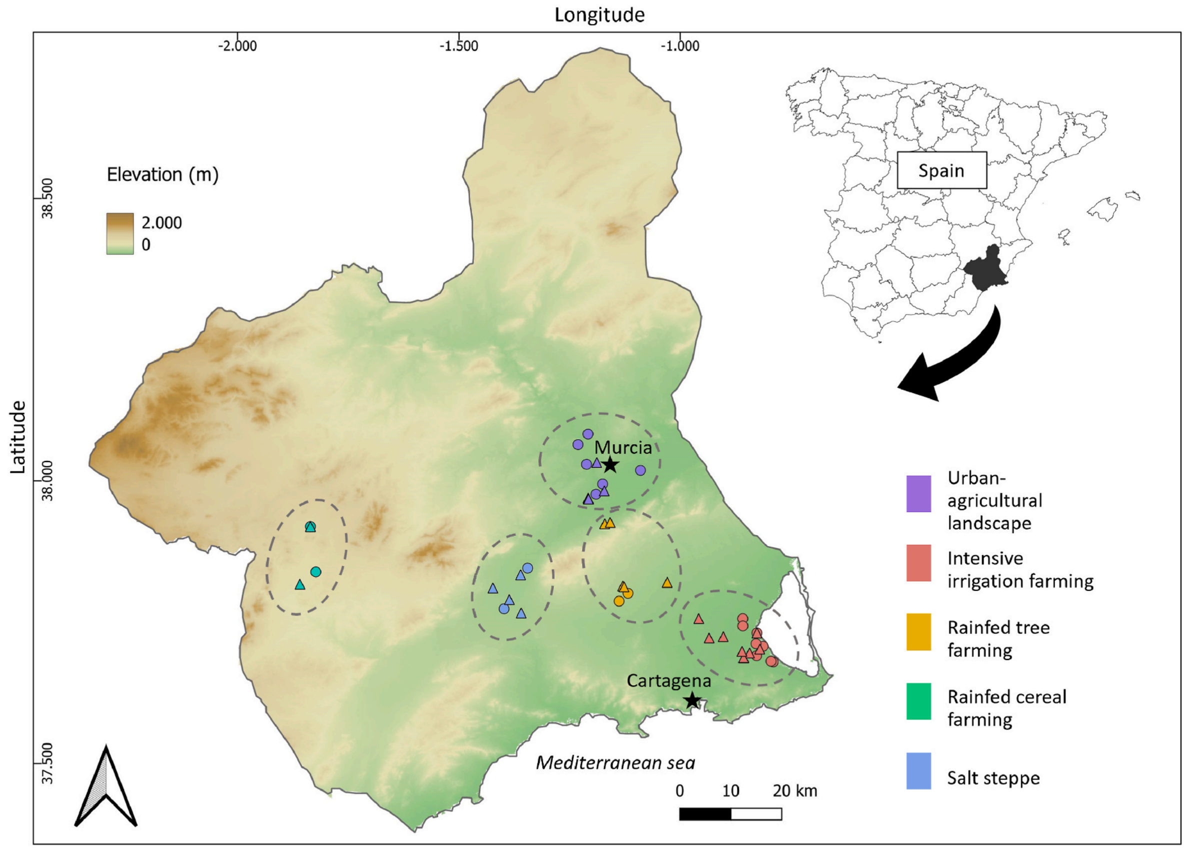

Fig. 1. Distribution of barn owl (dots) and common kestrel (triangles) nests sampled in the Region of Murcia (SE Spain) to assess anticoagulant rodenticide (AR) exposure. The colour assigned to the nests represents each of the five study sites selected to assess geographic variations in AR exposure. The main cities of the region are also indicated (star symbol). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were sampled in both study years, while the remaining 37 nests were sampled only once, in either 2021 or 2022 (Table S1). Prior to blood sampling, breeding territories were monitored through regular visits to estimate laying, hatching, and fledging dates. Nests were accessed when chicks were already feathered but before the fledgling stage (20–35 days post-hatching for kestrels and 40–70 for barn owls). Adult barn owls were captured inside the nest along with the owlets. These surveys were framed within a breeding raptor monitoring program conducted in the Region of Murcia. From 16 barn owl breeding territories, we additionally collected and analysed pellets (Moreno, 1986; Román, 2019) to assess the contribution of a SGAR-target prey item (genus *Rattus*) to diet and its potential relationship with SGAR exposure in our study area. Diet composition was assessed for each territory in terms of prey abundance and biomass. The latter was calculated as the number of individuals multiplied by the mean body mass of each prey species, according to the literature (Faurby et al., 2018; Storchová and Hořák, 2018).

Blood sampling (approved by the Ethical Committee for Animal Experimentation of the University of Murcia; code 657/2020) was carried out following the protocol described by Espín et al. (2021) and guidelines of EU Directive 2010/63/EU for animal experiments. According to these, up to 1.5 mL and 2 mL of blood were collected from the brachial vein of kestrels and barn owls, respectively, using a sterile syringe with a 25G needle. The blood was then introduced into a heparinized tube. In the case of barn owls, a blood aliquot (450 μL) was transferred to a tube containing 50 μL of 0.109 M sodium citrate buffer. After sampling, chicks were immediately returned to their nests to minimize stress. The samples were kept refrigerated until arrival at the laboratory, no later than the end of the day. The sodium citrated samples were then centrifuged for 15 min at 2500 g to obtain citrated plasma. Finally, blood and plasma samples were frozen at −80 °C for later analysis.

2.3. *Rodenticide analysis*

Reference standards of bromadiolone, brodifacoum, chlorophacinone, coumatetralyl, coumachlor, difenacoum, diphacinone, flocoumafen and coumafuryl were purchased from Sigma-Aldrich (USA), whereas difethialone was acquired from Dr. Ehrenstorfer GmbH (Germany). A standard mixture containing all ARs was prepared combining a portion of the stock solution for each compound with an appropriate volume of HPLC-grade methanol to generate a calibration curve as described in Spadetto et al. (2024).

Blood samples underwent analysis using the method described by Spadetto et al. (2024): 1000 μL of acetonitrile and 25 μL of coumachlor (internal standard) were added to 250 μL of blood, and the tube was vortexed for 1 min. After that, extraction salts were introduced, specifically 0.25 g of NaCl and 1 g of Na_2SO_4 for each sample. The tube was manually shaken for 1 min and then centrifuged at 2500 g for 5 min. The resulting supernatant was collected and transferred to a tube containing purification products (12.5 mg of PSA, 37.5 mg of C18, and 225 mg of Na_2SO_4). Extraction salts and purification products were purchased from Sigma-Aldrich (USA). The tube was vortexed for an additional minute and centrifuged at 2500 g for 5 min. Finally, the supernatant was drawn using a syringe and transferred into a chromatography vial after filtering it through a 0.45 μm nylon syringe filter.

The extracts were analysed for the aforementioned ARs using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump; Agilent Series 1260, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C18 analytical column of 150 × 2.1 mm and 2.6-μm particle size (Phenomenex Kinetex R 2.6 μm EVO Polar C18 100 A) and an Ultivo G6303 triple quadrupole mass spectrometer from Agilent, equipped with an electrospray ionization (ESI) interface, as described by Spadetto et al. (2024). The limits of quantification (LOQ) ranged between 0.01 and 2.5 ng mL^{-1}. The recovery values of the analytical technique were above 70%, with relative standard deviation < 15%.

2.4. Coagulation assays

Prothrombin time (PT) was chosen as a parameter to assess coagulation efficiency, as it increases rapidly after exposure to ARs and has already been used to evaluate AR effect on blood coagulation in birds of prey (Hindmarch et al., 2019; Hopf-Dennis et al., 2022; Rattner et al., 2011; Spadetto et al., 2024).

Coagulation assays were carried out using a coagulometer (Clot 2B, RAL SA, Barcelona, Spain) as described in detail in Spadetto et al. (2024). Due to the limited sample volume available for analysis, the tests were conducted once. Initially, the fibrinogen test ensured sample quality and prevented errors in PT interpretation (Rattner et al., 2010b). Briefly, a kit from Spinreact S.A.U (Spain) was employed, which is based on the Clauss method (Clauss, 1957). Citrated plasma samples were diluted 1:10 with imidazole buffer. Subsequently, 200 μL of the dilution were transferred to a tube containing a mixer, along with 20 μL of kaolin. The tube was then incubated for 3 min at 37 °C before adding 100 μL of bovine thrombin and measuring the time required for clot formation.

The PT assay is based on the addition of thromboplastin and calcium ions to the sample, which starts the blood coagulation process by simulating the natural response to blood vessel injury. Thromboplastin is a substance that contains tissue factor, a protein primarily released by connective tissue cells and essential for triggering the blood coagulation process (extrinsic pathway). The thromboplastin reagent was prepared in the laboratory using the Quick method modified by Griminger et al. (1970). To conduct the PT test, 50 mg of chicken thromboplastin were reconstituted in 2.5 mL of $CaCl_2$ in a Falcon tube. The mixture was agitated with a mixer for 15 min and centrifuged at 1800 rpm for 20 min. The supernatant was diluted 1:1 with $CaCl_2$. Then, 200 μL of the prepared reagent were transferred to a tube with a mixer and incubated for 3 min at 37 °C. After this, 100 μL of citrated plasma were added to start the reaction, and the coagulometer recorded the time required for clot formation.

2.5. Statistical analysis

To assess land use configuration for each sampled nest, we generated a 1-km radius buffer using Quantum GIS software version 3.16.16 (QGIS Development Team, 2022). This buffer size was selected based on previous research on common kestrel and barn owl home ranges (Arlettaz et al., 2010; Boileau et al., 2006; Taylor, 1994; Village, 1982). We extracted data on land use cover from the CORINE Land Cover 2018 (European Environment Agency (EEA), 2018), which was complemented with the SIOSE land use map (Instituto Geográfico Nacional (IGN), 2016) for a finer scale agricultural soil classification. By using this dataset, land uses were categorized into four main classes: natural vegetation, artificial areas, agricultural land and water bodies. Within the agricultural land category, two further distinctions were established: total non-irrigated agricultural land (cereal crops, rain-fed tree plantations, vineyards, and olive groves) and total irrigated agricultural land (irrigated arable land, citrus and non-citrus orchards). Furthermore, a category named "mixed crops" was established (vegetable gardens and small cultivated plots), which is widespread in certain areas of the Region of Murcia (e.g., our urban-agricultural site). We then calculated the percentage of land area within each 1-km buffer for each land use class and additional subgroup (Table S2). Moreover, we extracted data on human population density (2021 last census) of the census tract whereby each nest is located (National Institute of Statistics (INE), 2021), which was used as a variable in this analysis. Lastly, we retrieved data on the livestock load in 2021 for the Region of Murcia (Ministerio de Agricultura, Pesca y Alimentación) and calculated the animal density (cattle, pigs, sheep and goats, equids, poultry, and total), as well as the total number of farms within each buffer.

FGARs were detected at low concentrations and in a small number of individuals (see Table S3), so we exclusively focused on SGARs. We computed median and range values for the five SGARs analysed in blood samples. In order to explore the influence of environmental variables on SGAR blood levels, we calculated the total concentration of SGARs (ΣSGARs) as the sum of the concentrations of the identified compounds for each individual. To analyse the relationship between the variations in SGAR prevalence and in blood ΣSGAR concentration with the selected environmental factors (see Table 1), we employed the information-theoretic approach introduced by Burnham and Anderson (2002). Linear Mixed Models (LMM) were implemented using the "lme" function from the "nlme" package (Pinheiro et al., 2023), with environmental variables treated as fixed effects and territory as a random factor. We compared all models to a null model and conducted these comparisons employing the corrected Akaike information criterion (AICc). To determine the strength of evidence, we calculated delta AICc, while AICc weights were calculated to depict the relative likelihood of each model (Burnham and Anderson, 2002).

To compare ΣSGAR concentration among species and sampling years, we also employed the “lme” function. For comparing SGAR prevalence among species and years, we utilized the “glmer” function from the “lme4” package (Bates et al., 2015), with a logit link and binomial error distribution. The same functions were applied to compare ΣSGARs and prevalence among age classes exclusively for the barn owl (chicks and adults). In all these cases, the breeding territory was also considered as a random factor.

For the 16 barn owl territories with available diet information, regression analyses were performed to examine the relationships between ΣSGAR concentrations and prevalence and the percentage of *Rattus* prey in the diet, expressed both as a proportion of the total prey and total biomass.

We applied the Spearman’s correlation test to assess the relationships between ΣSGARs and PT and Mann-Whitney *U* test to compare PT between individuals with detected and undetected levels of SGARs. All statistical analyses were performed using R software version 4.3.1 and significance levels were established at $p < 0.05$.

Table 1
Models applied to explore how specific environmental factors might influence blood concentration of SGARs (ΣGARs) and SGAR prevalence. Land use and livestock variables were calculated within 1 km buffer zones.

Model notation	Model description	Variable type	Assessment
m_study site	Corresponding to the five study sites	Qualitative	Geographical differences
m_art_areas	Artificial areas (%)	Quantitative	Land-use effect
m_agr_land	Agricultural land (%)	Quantitative	Land-use effect
m_non_irr_crops	Total non-irrigated crops (%)	Quantitative	Land-use effect
m_irr_crops	Total irrigated crops (%)	Quantitative	Land-use effect
m_mosaic_crops	Mosaic crops (%)	Quantitative	Land-use effect
m_swine_density	Pigs per km^2	Quantitative	Animal farm effect
m_sheep-goat_density	Sheep and goats per km^2	Quantitative	Animal farm effect
m_cattle_density	Cattle per km^2	Quantitative	Animal farm effect
m_livestock_density	Total livestock per km^2	Quantitative	Animal farm effect
m_tot_farms	Total no. of animal farms	Quantitative	Animal farm effect
m_human_density	Inhabitants per km^2	Quantitative	Human density effect
Null_model	Null model	–	Model comparison

3. Results and discussion

3.1. Concentrations and detection frequency of SGARs

SGAR detection rates and concentrations are detailed in Table 2. In the barn owl, no interannual differences were detected in terms of total concentration (ΣSGARs) ($p = 0.151$) and prevalence ($p = 0.156$), so data from the two monitoring years were combined and analysed together. SGARs were detected in 50% of the nestlings, with 33.3% testing positive for one compound, 11.1% for two, and 5.6% for three. Overall, 16% of the nestlings had multiple SGARs in their blood. Adult barn owls were treated separately from nestlings due to significantly higher ΣSGARs (median = 1.21 vs 0.18 ng mL^{-1}, respectively; $p < 0.001$) and prevalence (100% vs 50%, $p < 0.001$). Moreover, 66.7% of adult barn owls tested positive for multiple SGARs, with 41.7% for two compounds, 16.7% for three, and 8.3% for four. Thirty-three percent of adult barn owls tested positive for a single SGAR compound. Regarding the common kestrel, data from the two monitoring years were also combined as no significant differences were found in ΣSGARs ($p = 0.122$), and, although prevalence was significantly higher in 2022 compared to 2021, we believe this result is due to the limited number of nests sampled in 2021 ($n = 6$) compared to 2022 ($n = 20$). At least one SGAR was detected in 68.6% of kestrel nestlings ($n = 48$), being 32.9% positive for multiple SGARs (22.9% for two compounds and 10% for three).

Most studies on AR exposure in birds of prey are based on opportunistic collection and analysis of liver samples from animals found dead in the field (López-Perea and Mateo, 2018). This approach is useful to estimate the presence of ARs in certain areas and try to diagnose the cause of death. However, the results may be biased as, for example, carcasses are more easily found near urbanized areas, where wildlife is exposed to increased risks (e.g. roadkill, collision or ARs exposure) and higher human population density also increases the chances of carcass finding. Collecting samples from animals found dead or arriving at rehabilitation centres is therefore a valid and non-invasive tool for estimating AR exposure, but it should be noted that a sample from dead or symptomatic animals may not accurately represent the entire at-risk population (Quinn, 2019). Here, active breeding monitoring allowed us to target data collection, which was intended to facilitate the identification of contamination sources and correlation with human activities. Furthermore, blood sample collection enables the assessment of recent exposure to ARs, as these compounds have a relatively short blood half-life (27–34 h in plasma in chicken; reviewed by Horak et al., 2018).

AR levels observed in blood samples are generally lower compared to liver samples (Murray, 2020), primarily due to the brief persistence of ARs in the bloodstream. This can limit the ability to detect these compounds in blood, although analytical techniques are becoming increasingly efficient and sensitive. Furthermore, there is no established toxicity threshold for blood, posing challenges in the interpretation of blood concentration results. For this reason, we believe that these active monitoring studies based on blood sample collection are useful to estimate AR prevalence and, consequently, the recent presence of these compounds in the environment and studied wildlife.

Studies in Spain based on the use of blood samples in nestlings of both avian predators and scavengers have also demonstrated high AR exposure, particularly in red kites (*Milvus milvus*), Egyptian vultures (*Neophron percnopterus*), and bearded vultures (*Gypaetus barbatus*), with SGAR detection rates exceeding 40% (Oliva-Vidal et al., 2022). The red kite is a medium-sized opportunistic bird of prey, capable of exploiting available food resources, and chicks of this species have also been found to be subject to AR contamination in France and Germany, showing AR prevalences of 30% and 22.6%, respectively (Badry et al., 2021; Powolny et al., 2020). In a previous study conducted in the Region of Murcia, we highlighted an almost absolute AR prevalence (98.6%) in long-eared owl (*Asio otus*) nestlings (Spadetto et al., 2024). To our knowledge, AR exposure in the common kestrel and barn owl has been poorly studied using blood samples. We found high SGAR prevalences for both species, confirming the widespread presence of AR compounds in the study area. By contrast, American kestrel chicks (*Falco sparverius*) showed lower prevalence rates (1.7%), possibly due to different diets or because blood samples from siblings were pooled, diluting the compounds which are generally detected at low concentrations (Buechley et al., 2022). Higher prevalences were observed in central Spain for these species. For example, Martínez-Padilla et al. (2017) found a 16.9% prevalence of bromadiolone in common kestrel nestlings, following a control campaign against a vole outbreak promoted by regional authorities. Similarly, SGAR prevalence in barn owls and kestrels of unknown age from the same study area was 19.4% and 11.6%, respectively (Rial-Berriel et al., 2020).

Here, no significant differences were found between barn owl and kestrel nestlings in ΣSGARs ($p = 0.157$) or prevalence ($p = 0.338$), although both were lower in barn owls than in kestrels (ΣSGAR median = 0.18 vs. 0.49 ng mL^{-1} and prevalence = 50% vs. 68.6%, respectively). The highest blood concentration in kestrels was found for flocoumafen (11.26 ng mL^{-1}), followed by brodifacoum (5.56 ng mL^{-1}), while in the barn owl, it corresponded to brodifacoum in an adult (3.84 ng mL^{-1}) and bromadiolone in an owlet (3.72 ng mL^{-1}). These AR concentrations are consistent with findings from other studies based on blood sample collection (Martínez-Padilla et al., 2017; Rial-Berriel et al., 2020). It is remarkable that ΣSGAR levels in the barn owl are significantly higher in adults compared to nestlings, and that the prevalence in adult individuals is absolute (100%). This outcome has also been observed in other studies (Buechley et al., 2022; Oliva-Vidal et al., 2022), and it is likely due to the fact these compounds accumulate throughout their lifetimes, as evidenced in the liver (Roos et al., 2021; Slankard et al., 2019).

Although at varying extents, the five SGARs analysed were detected in both species. In barn owl nestlings, bromadiolone prevailed (35%), followed by brodifacoum (19%) and flocoumafen (11%). In kestrel nestlings, flocoumafen was the most detected compound (46%), followed by brodifacoum (30%) and difenacoum (26%), with bromadiolone detected in only 10% of individuals. In long-eared owl nestlings from the same study area, the most detected compounds were flocoumafen, brodifacoum, and difenacoum (as reported here for kestrel chicks), followed by a notable presence of bromadiolone (38% of cases) (Spadetto et al., 2024). Recently, the same SGAR compounds have been detected with very high prevalences in foxes found in the neighbouring

Table 2
Concentrations of SGARs (ng mL^{-1}) in blood samples from barn owls and common kestrels collected across the Region of Murcia (SE Spain). The values shown for each SGAR compound refer to the prevalence, number of positive samples (n+) and concentrations as median and range [min-max] in individuals with detected levels of SGARs.

Species	Age	Bromadiolone	Difenacoum	Flocoumafen	Brodifacoum	Difethialone	ΣSGARS
BARN OWL	Nestlings ($n = 54$)	35.2% (n+ = 19)	5.6% (n+ = 3)	11.1% (n+ = 6)	18.5% (n+ = 10)	1.9% (n+ = 1)	50.0% (n+ = 27)
		0.11 [0.05–3.72]	0.54 [0.09–0.62]	0.03 [0.03–0.08]	0.24 [0.06–0.5]	0.72	0.18 [0.03–3.75]
	Adults ($n = 12$)	50.0% (n+ = 6)	8.33% (n+ = 1)	25.0% (n+ = 3)	100% (n+ = 12)	16.7% (n+ = 2)	100% (n+ = 12)
		0.09 [0.06–0.30]	0.1	0.1 [0.08–0.11]	1.11 [0.09–3.84]	0.47 [0.26–0.78]	1.21 [0.09–4.63]
COMMON KESTREL	Nestlings ($n = 70$)	10.0% (n+ = 7)	25.7% (n+ = 18)	45.7% (n+ = 32)	30.0% (n+ = 21)	n.d.	68.6% (n+ = 48)
		0.36 [0.10–1.30]	1.15 [0.13–0.66]	0,29 [0.07–11.26]	0.22 [0.11–5.56]	–	0.49 [0.07–11.52]

"*n*" = number of samples; "n.d." = not detected.

province of Alicante (Southeastern Spain) (Carrera et al., 2024). Hence, these second-generation compounds appear to be the predominant ARs employed in the selected study area. On the other hand, difethialone seems to be less frequently used, being detected only in 3 barn owls (one owlet and two adults) and absent in the sampled kestrels and long-eared owls in the study area (Spadetto et al., 2024). It should be noted that the FGARs diphacinone and coumafuryl have been also detected in a few individuals (Table S3), although these compounds are not permitted in Europe (EC Regulation 528/2012) (European Union, 2012). Our findings confirm the extensive SGAR use in the Region of Murcia and the exposure of various non-target predator species.

As no significant differences in SGAR prevalence were found between the two studied species, they both stand as suitable sentinels for biomonitoring ARs in Mediterranean agroecosystems. Indeed, both species have very broad distribution ranges, as the barn owl is found worldwide (except in Arctic regions), and the common kestrel is present throughout the Palearctic region, which facilitates coordinated monitoring programs. Moreover, these predatory birds readily adapt to using nest boxes as breeding sites (Fay et al., 2019; Paz Luna et al., 2020), making their chicks easy to capture and handle for sample collection. The barn owl is a scarcer and more challenging species to monitor in our study area, and territorial occupancy strongly depends on annual weather factors and food availability. Despite its widespread decline, finding nests in other regions is still relatively easy and there are several initiatives involving the placement of nest boxes (Bourbour et al., 2022; Fay et al., 2019). On the other hand, the common kestrel's trophic plasticity allows it to survive and breed even in years when rodents are scarce, resulting in a relatively stable population that is easy to monitor and study.

3.2. Anthropogenic environmental factors affecting SGAR prevalence

Several studies in various wildlife species have attempted to correlate the presence of ARs with specific environmental variables potentially influencing the risk of exposure. Correlations have been identified with the presence of intensive farming, both in Germany and Spain (Carrillo-Hidalgo et al., 2024; Geduhn et al., 2015; López-Perea et al., 2019; Rial-Berriel et al., 2021), as well as with agricultural land (Elliott et al., 2022; Sainsbury et al., 2018; Serieys et al., 2015), indicating widespread use in the context of these activities. The use of ARs is also prevalent around buildings and warehouses, particularly in urban areas and human settlements. Therefore, another identified risk factor for non-target predator species is high human population density within the species' home range (Alabau et al., 2020; Badry et al., 2021; López-Perea et al., 2015).

Regarding our results, we observed that the impact of the considered environmental variables on SGAR prevalence in barn owl and kestrel nestlings depends on the species (Table 3). In common kestrels, prevalence remains consistent across study sites (Fig. 4 and Table S4), being the percentage of artificial surfaces within the territory the only explanatory variable (Table 3 and Fig. 2). The high species' adaptability to anthropogenic environments appears to contribute to their increased susceptibility to SGAR exposure. Conversely, in barn owl nestlings, SGAR prevalence was overall lower (50%) and five environmental variables resulted explanatory. Population density emerged as the most significant (Table 3 and Fig. 3), indicating a greater risk for individuals living in proximity to human settlements, especially in densely populated areas. As observed, barn owls prefer non-cultivated open habitats such as fallow lands for hunting (Séchaud et al., 2021), but this foraging habitat preference seems not to preclude the consumption of AR contaminated prey when their home range overlaps with densely populated areas (Castañeda et al., 2021; Hindmarch et al., 2017; Taylor, 1994). Other explanatory variables for prevalence in barn owls appear to be directly linked to human population density. Specifically, these

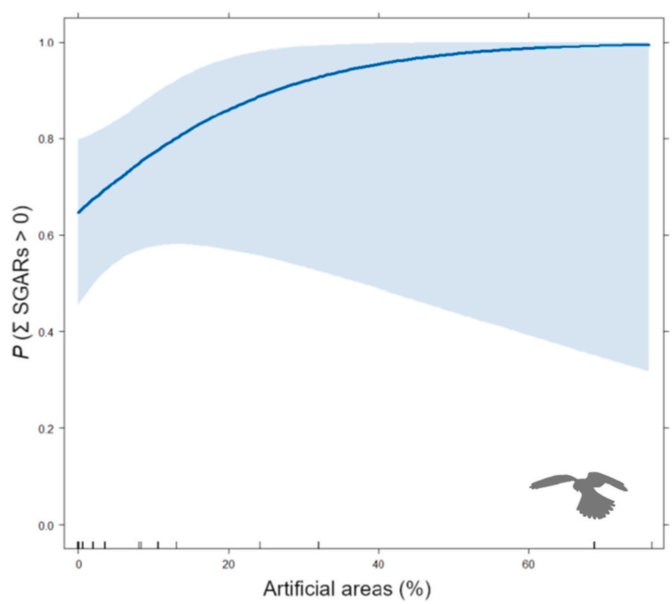

Fig. 2. Predictor effect plot illustrating the effect of artificial surfaces (% calculated in a 1-km buffer around each nest) on SGAR prevalence in common kestrel nestlings in the Region of Murcia (Spain). Shaded area represents 95% confidence interval.

Table 3
Ranking of the models used to explain variations in SGAR prevalence in kestrel and barn owl nestlings, based on Akaike's information criterion (AIC).

Common kestrel					Barn owl				
	k	AICc	ΔAICc	*w*		*k*	AICc	ΔAICc	*w*
Art_areas	3	866.817	0.0000	0.3606	Human_density	3	660.966	0.0000	0.9714
Null_model	2	886.411	19.594	0.1354	Mixed_crops	3	755.249	94.283	0.0087
Mixed_crops	3	902.382	35.564	0.0609	Art_areas	3	765.851	104.884	0.0051
Swine_density	3	904.260	37.443	0.0555	Study_site	5	780.864	119.898	0.0024
Non_irr_crops	3	905.300	38.483	0.0527	Tot_farms	3	782.150	121.184	0.0023
Livestock_density	3	905.613	38.796	0.0518	Null_model	2	782.679	121.712	0.0022
Human_density	3	907.002	40.184	0.0484	Cattle_density	2	782.679	121.712	0.0022
Sheep-goat_density	3	907.483	40.666	0.0472	Non_irr_crops	3	787.649	126.682	0.0017
Agr_land	3	907.664	40.847	0.0468	Agr_land	3	802.186	141.220	0.0008
Irr_crops	3	907.884	41.066	0.0463	Sheep-goat_density	3	802.597	141.631	0.0008
Tot_farms	3	908.013	41.196	0.0460	Livestock_density	3	803.739	142.772	0.0008
Cattle_density	3	908.094	41.276	0.0458	Swine_density	3	803.985	143.018	0.0008
Study_site	6	964.460	97.643	0.0027	Irr_crops	3	805.021	144.054	0.0007

k = number of parameters estimated; AICc = corrected Akaike's Information Criterion; ΔAICc = difference between AICc of each model and the minimum AICc; *w* = Akaike's weight.

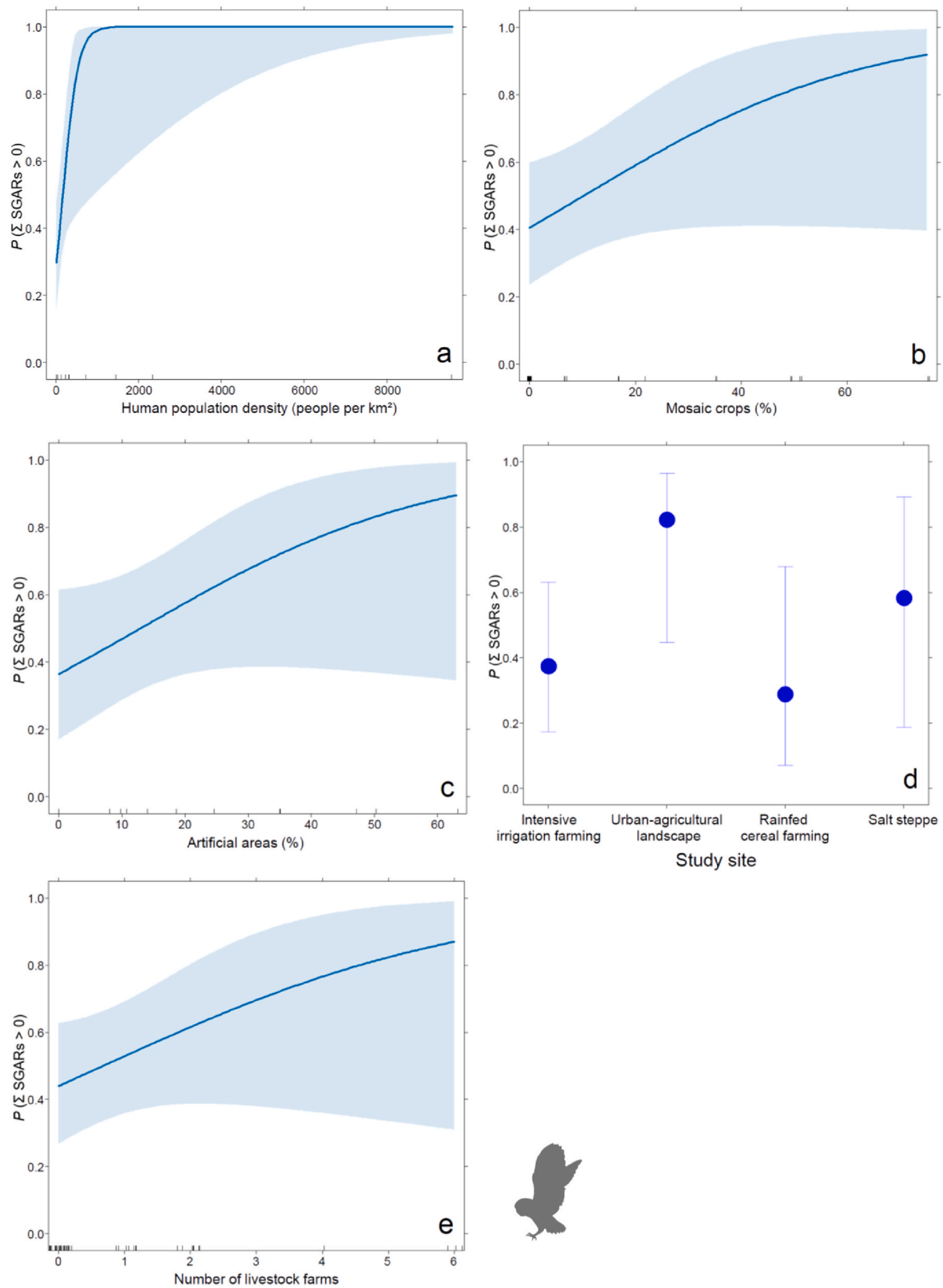

Fig. 3. Predictor effect plots illustrating the effect of human population density (a), complex cultivation pattern (b), artificial areas (c), study site (d) and total number of livestock farms (e) on SGAR prevalence in barn owl nestlings in the Region of Murcia (Spain). Shaded areas (a, b, c, e) and whiskers (d) represent 95% confidence intervals.

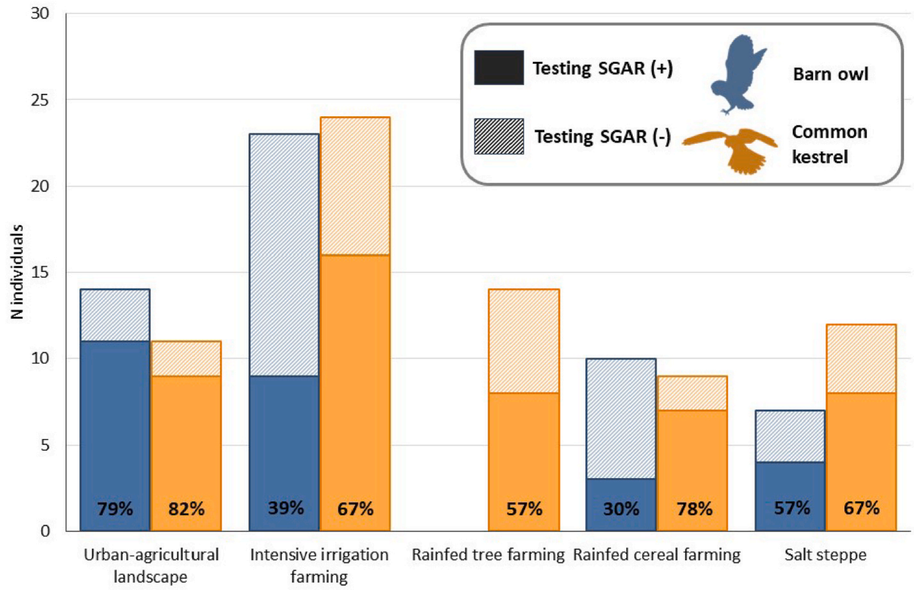

Fig. 4. SGAR prevalence for barn owl ($n = 54$) and common kestrel ($n = 70$) nestlings across different agricultural landscapes in the Region of Murcia (SE Spain). For an easier comparison, percentage values (%) of SGAR positive cases for each species and study site are provided within colour-filled portions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

include the study site, the percentage of artificial areas and mixed crops (Table 3 and Fig. 3). In fact, the "urban-agricultural" study site, where the highest SGAR prevalence was found (78.6%, see Table S4), is placed around the city of Murcia and presents the highest human population density in the entire study area. The landscape in this study site is characterized by diversified irrigated crops, including small citrus plantations, vegetable crops, home gardens and green areas, thus suggesting widespread use of AR products by local people. Furthermore, a relationship between SGAR prevalence and the percentage of artificial surfaces was evident for barn owls, as also observed for kestrels (Fig. 3). This underscores that the primary risk factor for barn owls is proximity to urban densely populated areas. Regarding livestock farms, a correlation with the total number of farms within the buffer was found, albeit slightly exceeding the null model weight (Table 3 and Fig. 3). This suggests that livestock farming could potentially explain SGAR prevalence in barn owls. The establishment of industrial-scale farms is a growing practice in the study area, though further research is warranted to confirm this relationship.

The same variables were studied to determine whether they had effects on ΣSGAR concentrations. However, none of these factors was explanatory for the barn owl, while for the kestrels the only explanatory factor appears to be the study site, with higher concentrations in the "urban-agricultural" area (Table S5 and Fig. S1).

Interestingly, no apparent relationship was found between the percentage of AR positive cases and agricultural land uses. Although surveyed nests were placed within predominantly agricultural landscapes and often characterized by intensive cultivation, this does not necessarily imply a higher exposure risk. Indeed, while in Europe these products are often used for protecting agricultural lands (Hughes et al., 2013; Tosh et al., 2011), they are not authorized in Spain as plant protection products. Despite suggestions of illegal use in this context from other studies (Elliott et al., 2022; Huang et al., 2016), our results indicate a higher likelihood of application of compounds near buildings, urban areas, gardens, and warehouses rather than directly on intensively cultivated land. Nonetheless, it is possible that SGARs are not correctly applied by the general public or non-specialized professional personnel (e.g., inside or around buildings, with proper removal and disposal of poisoned rodents and by using bait boxes), making them easily accessible to non-target species.

3.3. Feeding habits and SGARs

The differences in SGAR prevalence between the two species, though not significant, can be explained by their dissimilar dietary habits and metabolism. Despite inhabiting the same environment, both birds of prey show different foraging strategies that lead them to different levels of contact with human activities and AR baits in their breeding environment. Hence, the overall lower SGAR prevalence in barn owl chicks may have multiple causes. This nocturnal predator showed high AR exposure in other studies due to its rodent-based diet and its preference for agricultural and rural landscapes. However, barn owls inhabiting farmlands with scarce human settlements may be less exposed to ARs as agricultural use is not permitted. In fact, our analyses of barn owl's pellets revealed a predominant reliance on rodents of the *Mus* genus (81.7% of total consumed prey items, $n = 2907$), primarily represented by *Mus spretus*, which is not considered a pest or a target rodent species. Small mammals collectively contributed to 95.0% ($n = 3380$) of the barn owl's diet, thus comprising most of the owl diet, with marginal contributions from birds (3.8%, $n = 134$), invertebrates (0.9%, $n = 33$), lagomorphs (0.1%, $n = 5$) and reptiles (0.1%, $n = 5$) (Table S6). Variations in diet composition are observed across study sites, with substantial higher prevalence of *Rattus* rodents in the "urban-agricultural" study site (16.8% of the total consumed prey items) compared to the other areas (<4%). This interesting finding reflects the higher presence of rats near human settlements, which may result in a higher availability of SGAR contaminated prey for barn owls inhabiting anthropized areas. Indeed, given their status as target rodents, *Rattus* species are highly susceptible to AR contamination. Specifically, in terms of ΣSGARs, results indicate a positive correlation with the percentage of *Rattus* prey in the owls' diet, based on prey count (Table S7 and Fig. S2). Regarding SGAR prevalence, abundance of *Rattus* spp. based on both prey count and biomass was explanatory, highlighting a positive association between the likelihood of detecting SGARs and the significance of these rodents in the barn owl's diet (Table S7 and Fig. S3).

On the other hand, the common kestrel is adapted to living near humans in both agricultural and urban environments, being capable of hunting in open habitats as well as near buildings and urban areas. These foraging preferences increase the risk of feeding on SGAR poisoned prey. Additionally, the kestrel shows a more diversified diet, which includes invertebrates, reptiles, and songbirds. It is essential to note that these types of non-target prey can easily become contaminated with ARs (Elliott et al., 2014; Spurr and Drew, 1999; Walther et al., 2021). In addition, invertebrates can display behavioural changes after ARs consumption, leading to faster emergence and reduced activity, making them easier prey for predators (Parli et al., 2020). These data point to the possibility of secondary and even tertiary AR contamination through non-target species, resulting in the spread of these toxic substances at various levels of the food chain. Therefore, these considerations may contribute to explain the overall higher prevalences observed in the common kestrel in this study.

3.4. *Effects on blood coagulation*

The impairment of blood clotting has often been studied as it represents the direct and most immediate effect of ARs. In fact, coagulation parameters such as PT and Russel's Viper Venom Time (RVVT) rapidly show alterations in case of AR ingestion. PT is the first coagulation parameter affected by AR intoxication, serving as a valuable biomarker to assess the effects of these compounds (reviewed by Rached et al., 2020). In birds of prey, an increase of more than 25% or two standard deviations (SD) from the baseline PT value of the species is considered an indicator of AR contamination, providing useful information about exposure to these compounds. However, the baseline value is species-specific and largely unknown for most avian species, except for the American kestrel and the Eastern-Screech Owl (*Megascops asio*), utilized as model species for diurnal and nocturnal raptors, respectively (Hindmarch et al., 2019; Rattner et al., 2014a, 2015). Nevertheless, it is important to note that measuring PT in birds requires avian thromboplastin, a reagent not commercially available. In fact, the use of commercial kits containing mammalian thromboplastin has been shown to cause a significant increase in PT measurement in avian species (reviewed by Webster, 2009). The production of this thromboplastic extract and measurement techniques may vary between laboratories, complicating result interpretation and the comparison of different studies.

All nestling ($n = 30$) and adult ($n = 9$) barn owls showed a fibrinogen concentration exceeding 50 mg mL^{-1} (range 53.9–215 mg mL^{-1}) and were thus considered suitable for PT analysis. The mean and standard deviation (±SD) of PT was 15.1 ± 1.1 s, almost identical for both nestlings (15.0 ± 1.0 s) and adults (15.4 ± 1.4 s), with a range of 11.8–16.9 s. Additionally, there were no significant differences in PT values between individuals with and without detected levels of SGARs (Mann-Whitney $U = 132.0$; $p = 0.143$). In fact, the mean PT values for non-SGAR-detected and SGAR-detected individuals were very similar, 15.0 ± 0.8 and 15.2 ± 0.3 s, respectively. We cannot assert acute intoxication as all samples coagulated, which is consistent with our expectations according to the ΣSGAR levels in the blood. However, a positive and significant correlation was found between ΣSGARs and PT ($Rho = 0.395$, $p = 0.006$) (see Fig. 5), which is an interesting finding in free-ranging nestlings given that the initial AR dose and time of exposure were unknown. Surprisingly, variations in PT were observed, including samples testing negative for SGARs (Fig. 5). It remains unclear whether these variations fall within a physiological range for barn owls or indicate recent exposure. In fact, while plasma half-life of ARs is relatively short in bird species (16.5 ± 10.0 h for warfarin in Eastern barn owl *Tyto javanica*; Khidkhan et al., 2024), experimental studies suggest that PT returns to baseline levels within seven days after AR exposure in raptors (Rattner et al., 2010a, 2012a). Considering our results, future research should standardize the PT measurement technique in avian species and conduct additional field studies with larger sample sizes. Particularly, studying baseline PT values for frequently exposed wild species like raptors is essential, as chronic exposure and accumulation of ARs may lead to a significant impairment of coagulative function, potentially increasing vulnerability to even minor traumas.

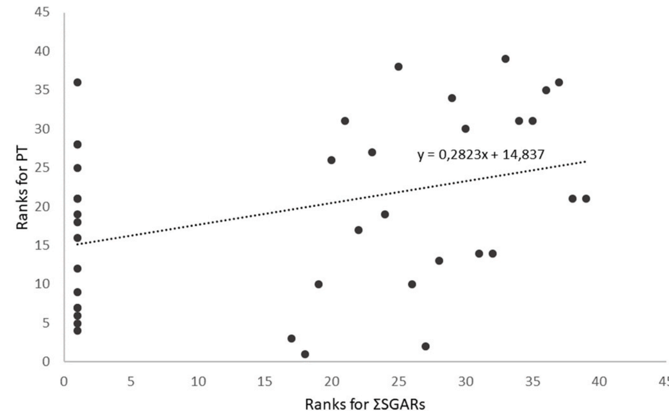

Fig. 5. Correlation between the ranks for prothrombin time (PT) and for ΣSGAR values detected in barn owls ($n = 39$) from the Region of Murcia (SE Spain). Ranks were assigned to both variables to visualize their relationship, highlighting the relative position of observations.

3.5. *Risk assessment*

The use of SGARs in Spain is only allowed as biocide inside and around buildings for the general public and professional personnel, while their application in open spaces is reserved for specialized professional personnel (Ministerio de Sanidad, n.d.). However, AR products with low concentrations (<30 ppm) are distributed for sale to the general public, allowing anyone to use them in private settings (urbanizations, gardens, warehouses) without any surveillance over their application. Moreover, the widespread availability of these products on the market raises concerns about their potential impact on the growing AR resistance and how non-target species may be affected in the medium-to long-term by these products with low AR concentration. Indeed, it is alarming that SGAR prevalence in common kestrels has actually increased after 2018 in the island of Tenerife (Spain), suggesting a lack of effectiveness of the regulations implemented by the European Union (Carrillo-Hidalgo et al., 2024).

The effects of AR exposure have been studied through *in vivo* studies on representative species of both diurnal and nocturnal raptors to establish a toxic concentration level and investigate interspecific differences in toxicity to these substances. Specifically, as aforementioned, the American kestrel has been used as a model for diurnal birds of prey (Rattner et al., 2015, 2014b; 2012b, 2011) and the Eastern screech-owl for nocturnal ones (Rattner et al., 2014a, 2012a) demonstrating pronounced differences in sensitivity to ARs among raptors and other bird species. For instance, the LD50 of diphacinone was 20–30 times lower in the American kestrel than in the Northern bobwhite quail (*Colinus virginianus*), revealing higher risks for predatory birds (Rattner et al., 2012a). These results have been confirmed by recent *in vitro* studies using liver microsomes to assess the activity of the VKOR enzyme complex in different avian species in response to AR exposure (Khidkhan et al., 2024; Nakayama et al., 2020). It also appears that differences in sensitivity to ARs are due to variations in the expression of cytochrome P450, which is involved in the hepatic metabolism of ARs (Khidkhan et al., 2024; Watanabe et al., 2015). In addition, exposure to realistic doses of different AR compounds appears to cause additive effects, manifesting in an increased anticoagulant effect in terms of both duration and magnitude (Rattner et al., 2020).

Our findings suggest that predatory birds in our study area experience recurrent exposure to SGARs starting from their early life stages (i.e. nestlings), indicating chronic exposure, frequently involving multiple AR compounds. As evidenced by other studies targeting animals of different age classes opportunistically collected in the field, ARs bioaccumulate over time in the liver and other tissues as a result of this continuous exposure (López-Perea and Mateo, 2018). Although ARs are not the primary cause of death in most cases, they can have harmful effects at various levels, ultimately affecting the animal's fitness (Murray, 2018). Firstly, any injury can be life-threatening to an animal with impaired blood clotting capacity. Often, these animals fall victim to accidents, especially collisions with vehicles, considered a major cause of raptor mortality (Gomez et al., 2023; Panter et al., 2022). It is possible that their chances of surviving such incidents, even when rescued and taken to a rehabilitation centre, are reduced in conjunction with reduced haemostatic function. Additionally, ARs have been classified in Europe as toxic for reproduction (European Union, 2008), based on the teratogenic effect of warfarin (Chetot et al., 2020). Concerning birds, AR transfer to eggs has been demonstrated in the barn owl (Salim et al., 2015), probably having an effect on clutch and brood size and fledging success (Salim et al., 2014). Other sublethal effects are poorly studied and include an association between AR exposure and severe parasitic infestations (Serieys et al., 2015), infectious diseases (Carrera et al., 2024), reduced body condition (Martínez-Padilla et al., 2017) and alteration of the immune system (Serieys et al., 2018). Fraser et al. (2018) showed that ARs affect gene regulation, interfering with leukocyte differentiation, which can damage the immune function of exposed individuals in a way that may remain unnoticed. In the future, it is highly recommended to deepen research on sublethal effects of chronic exposure to low doses, both concerning AR compounds and other environmental contaminants and their interactions. Although the levels found in our study are low and not compatible with acute toxicity, they indicate the ongoing occurrence of these compounds and the widespread exposure of non-target species in the assessed study area. Thus, avian predators would be exposed to continuous AR ingestion, with likely detrimental effects in the medium and long term on individuals, as well as potential effects on population dynamics (Roos et al., 2021; Thomas et al., 2011).

4. Conclusions

Our study revealed that two farmland bird of prey species from a Mediterranean agricultural region were widely exposed to SGARs, apparently at low concentrations but often to multiple compounds, implying repeated exposure episodes that individuals may experience over time. The assessment of exposure risk seems to depend primarily on the ecological characteristics of each species. The common kestrel, a generalist raptor which commonly inhabits and forages in urban areas, exhibited a slightly higher likelihood of exposure, suggesting the possibility of contamination pathways involving non-target species. On the contrary, the barn owl tends to feed in open and natural spaces, and individuals at higher risk were those breeding near urban centres with high population density. Here, the higher presence of target rodents could pose an additional risk factor for this species. Finally, evaluating the coagulation function emerges as an interesting tool to assess the effects of ARs in free-living birds of prey, although further investigations are needed to confirm preliminary data. These results underscore the importance of a comprehensive understanding of interactions between breeding biology, foraging ecology, and exposure to environmental contaminants to develop effective conservation and management strategies.

5. Recommendations for AR mitigation

Accounting for our results, we propose some recommendations to minimize the risk associated with ARs in non-target wildlife species.

- Adopting safer alternatives: to explore and promote ecologically friendly rodent control alternatives (Hohenberger et al., 2022; Quasim et al., 2023), including biological control through natural avian predators (Paz Luna et al., 2020). Support for ongoing research on the development of these alternative rodent control methods is mandatory.
- Education and training: to conduct awareness campaigns to inform the public about the correct application and management of ARs, including advice on using designated bait boxes and proper disposal of dead rodents. To provide training, especially to professionals (i.e. farmers and livestock breeders), on best practices for rodent control and safe handling of ARs. To encourage the involvement of professionals for managing infestations, reducing the risk of unintentional exposure.
- Environmental monitoring: to implement environmental monitoring programs involving the use of sentinel species to detect the presence of ARs and assess their impact on non-target wildlife. These data can be used to plan more effective and targeted mitigation strategies.
- Regulatory limitations: to review and implement legal restrictions on the use of ARs, limiting their availability or prohibiting their use in ecologically sensitive areas.
- Cross-sector collaboration: to promote collaboration across sectors, including agriculture, public health, and wildlife management, to develop integrated rodent management strategies that minimize impact on non-target species.

The adoption of mitigation and prevention measures, along with the involvement of various stakeholders, can contribute to minimizing the risk associated with the use of ARs, protecting non-target wildlife and preserving ecological balance.

CRediT authorship contribution statement

Livia Spadetto: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Antonio Juan García-Fernández:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Antonio Zamora-López:** Writing – review & editing, Methodology, Investigation, Conceptualization. **José Manuel Zamora-Marín:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Mario León-Ortega:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Miguel Tórtola-García:** Methodology, Formal analysis, Data curation. **Fernando Tecles-Vicente:** Writing – review & editing, Methodology, Formal analysis. **José Fenoll-Serrano:** Methodology, Formal analysis, Data curation. **Juana Cava-Artero:** Methodology, Formal analysis, Data curation. **José Francisco Calvo:** Methodology, Formal analysis, Data curation. **Pilar Gómez-Ramírez:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124944.

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