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Potential exposure of native wildlife to anticoagulant rodenticides in Gran Canaria (Canary Islands, Spain): Evidence from residue analysis of the invasive California Kingsnake (*Lampropeltis californiae*)

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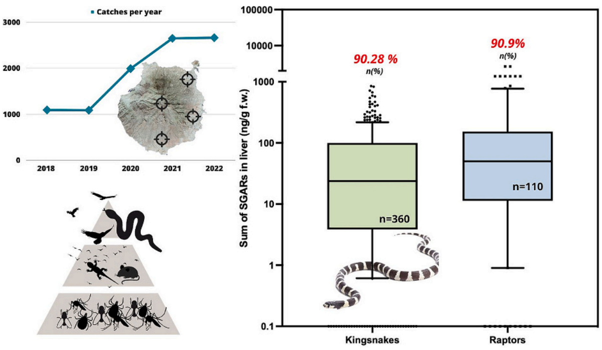
HIGHLIGHTS

- *Lampropeltis californiae* is a good indicator of wildlife exposure to ARs in Gran Canaria.
- High AR frequency of detection and concentration observed in California kingsnakes
- Higher concentrations of ARs are influenced by snakes' body condition and geographic area.
- California kingsnakes seem to tolerate a high degree of ARs exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs), particularly second-generation compounds (SGARs), are extensively used in pest management, impacting non-target wildlife. The California kingsnake (*Lampropeltis californiae*), an invasive species in Gran Canaria, is under a control plan involving capture and euthanasia. This research aimed to detect 10 different ARs in these snakes, explore geographical and biometrical factors influencing AR exposure, and assess their potential as sentinel species for raptors, sharing similar foraging habits. Liver samples from 360

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Non-target animals
Brodifacoum
Bromadiolone

snakes, euthanized between 2021 and 2022, were analysed for ARs using LC-MS/MS. Results showed all detected rodenticides were SGARs, except for one instance of diphacinone. Remarkably, 90 % of the snakes tested positive for ARs, with over half exposed to multiple compounds. Brodifacoum was predominant, found in over 90 % of AR-positive snakes, while bromadiolone and difenacoum were also frequently detected but at lower levels. The study revealed that larger snakes and those in certain geographic areas had higher AR concentrations. Snakes in less central or more peripheral areas showed lower levels of these compounds. This suggests a correlation between the snakes' size and distribution with the concentration of ARs in their bodies. The findings indicate that the types and prevalence of ARs in California kingsnakes on Gran Canaria mirror those in the island's raptors. This similarity suggests that the kingsnake could serve as a potential sentinel species for monitoring ARs in the ecosystem. However, further research is necessary to confirm their effectiveness in this role.

1. Introduction

Anticoagulant rodenticides (ARs) are widely used to control rodent populations, but they also pose a threat to non-target wildlife. ARs can be classified into first-generation anticoagulants (FGARs) and second-generation anticoagulants (SGARs), which differ in toxicity, half-life, and resistance. Both types of ARs cause coagulopathy by inhibiting vitamin K1 and clotting factors. SGARs are more effective and persistent than FGARs, but also more hazardous to the environment (Bermejo-Nogales et al., 2022; Nakayama et al., 2019; Rattner et al., 2014; Ravindran et al., 2022). Many non-target species, such as mammals, birds, and reptiles, are exposed to ARs through various pathways, resulting in adverse effects on their health and survival (Lohr and Davis, 2018; Nakayama et al., 2019; Ravindran et al., 2022; Sánchez-Barbudo et al., 2012; Shore and Coeurdassier, 2018). The European Commission has implemented regulations to limit the use and concentration of ARs in baits, and to promote sustainable alternatives for pest management (EC, 2019, 2017). Wildlife biomonitoring studies are essential to assess the presence and impact of ARs in the environment, and to protect both biodiversity and human health (García-Fernández et al., 2020; Gómez-Ramírez et al., 2014; Grove et al., 2009).

Sentinel species are used in wildlife toxicology to assess the risks of contaminants for specific or similar species (Badry et al., 2021; Chumchal et al., 2022; Grove et al., 2009; Ruiz-Suárez et al., 2016; Sonne et al., 2020). To be an effective sentinel, a species must meet criteria such as exposure potential, geographic distribution, ease of collection, and susceptibility to contaminants (Basu et al., 2007; Golden and Rattner, 2003). Invasive snakes, like the California kingsnake (*Lampropeltis californiae*), the horseshoe whip snake (*Hemorrhois hippocrepis*) or the brown tree snake (*Boiga irregularis*), are generalist predators that have impacted endemic fauna, especially on islands (McElderry et al., 2022; Montes et al., 2021; Wiseman et al., 2019). They are also subject to governmental management (Gallo Barneto et al., 2018; Soto et al., 2022), which facilitates their collection over time. Therefore, they are promising sentinels for studying wildlife contaminant exposure in regions where they are established. Recent research support the suitability of snakes as indicators of environmental and food web contamination (Hoang et al., 2021; Lettoof et al., 2020; Lohr and Davis, 2018).

The California kingsnake (*Lampropeltis californiae*) is a nonvenomous constrictor and a generalist predator native to the southwestern North

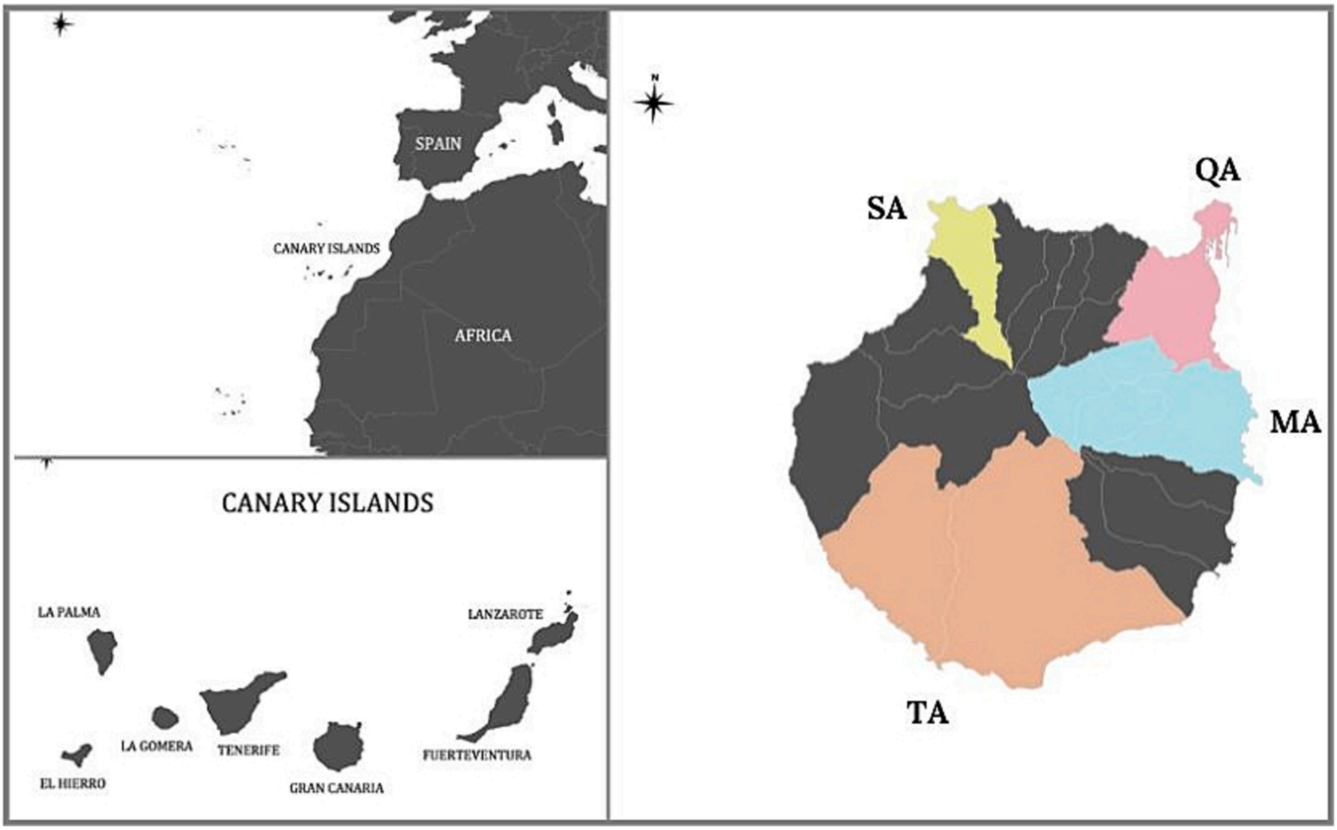

Fig. 1. Geographical location of the Canary Islands archipelago on the left; and Gran Canaria Island with the four distribution areas of the California kingsnake (MA = Main Area, SA = Secondary Area, TA = Tertiary Area, QA = Quaternary Area) on the right.

America and northwestern Mexico (Pyron and Burbrink, 2009; Wiseman et al., 2019). In Gran Canaria (Canary Islands, Spain) it has been classified as invasive, and it preys on various species, especially rodents and three endemic reptiles: the giant lizard (*Gallotia stehlini*), the skink (*Chalcides sexlineatus*) and the Boettger's wall gecko (*Tarentola boettgeri*) (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015; Piquet and López-Darias, 2021). The snake was introduced to the island over two decades ago, probably by accidental or intentional releases of captive individuals. It naturalized in 2007 and has rapidly expanded despite control efforts (Cabrera-Pérez et al., 2012; Gallo and Mateo, 2020; Piquet et al., 2021). It is now established in four main areas of the island the island (Fig. 1) and >2000 snakes have been removed in the past year (BOE, 2022; GESPLAN, 2023a). The island's isolation, climate and lack of predators favour this adaptable and opportunistic snake (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015; Piquet et al., 2021). If not contained, it could colonize other islands in the archipelago (Fisher et al., 2021; Piquet et al., 2021).

These characteristics makes it a suitable sentinel species for assessing the exposure of non-target wildlife species to ARs in the Island of Gran Canaria, particularly for raptor species such as Common buzzards (*Buteo buteo*), European sparrowhawks (*Accipiter nisus*), Barbary falcon (*Falco pelegrinoides*), Common kestrels (*Falco tinnunculus*), Long-eared owls (*Asio otus*), and Barn owls (*Tyto alba*). These birds of prey have exhibited elevated exposure to these contaminants in the archipelago, given their dietary habits centered around rodents, reptiles, and birds, which closely resemble those of snakes (Rial-Berriel et al., 2021a, 2021c; Ruiz-Suárez et al., 2014).

The aims of this study were: a) to characterize the exposure of the California kingsnake to ARs; b) to identify the geographical and biometrical factors influencing snakes' exposure to ARs; c) to investigate the potential suitability of kingsnakes as a sentinel species by comparing their AR exposure to that of raptors.

2. Material and methods

2.1. Study area

This research was conducted on Gran Canaria, belonging to the Canary Islands archipelago, situated off the northwest coast of Africa. These oceanic islands are notable for their geographical isolation and subtropical climatic conditions. Notably, 43 % of the island's central and southwestern landmass is a Biosphere Reserve. This region has an average annual temperature of 22 °C, a variety of ecosystems, and a remarkable prevalence of endemic invertebrate, avian, and reptilian species (Biota, 2023; UNESCO, 2023).

The invasive California kingsnake is predominantly located in four established areas, which are associated with the following municipalities (Fig. 1): main area (MA: municipalities of Telde, Santa Brígida, Valsequillo and San Mateo), secondary area (SA: municipalities of Gáldar and Agaete), tertiary area (TA: municipalities of San Bartolomé de Tirajana and Mogán) and quaternary area (QA: municipality of Las Palmas de Gran Canaria) in addition to other independent dispersal areas (BOE, 2022; Gallo and Mateo, 2020; GESPLAN, 2023a).

2.2. Sampling of snakes and raptors

Snakes were collected randomly by hand or using traps throughout the island, euthanized and consequently frozen at the GESPLAN facility in Gran Canaria between 2021 and 2022 as part of the eradication program *Strategic Plan for the control of California kingsnake in the Canary Island* #STOPCULEBRAREAL (GESPLAN, 2023b). All snakes were alive when they were retrieved from the traps. The frozen snakes were necropsied following the procedures described by the Association for Sustainable Development and Conservation of Biodiversity (ADS) guidelines (GESPLAN, 2023c) at SERTOX facilities, Department of Toxicology, University of Las Palmas. Livers, recognized as the primary organs for rodenticide accumulation (Vudathala et al., 2010), were collected and subsequently stored at −20 °C, until chemical analysis. Snake characteristics including sex (female, male or unknown sex-lack of developed sexual organs), design pattern (banded, striped, or aberrant), coloration pattern (albino, normal: brown/black), weight, fat weight, snout-vent length (SVL in cm), total length (TL in cm) and tail length (in cm) were noted. Additionally, the presence or absence of observed haemorrhagic lesions was also recorded. This involved the identification of external or internal haemorrhagic wounds (superficial or deep), bleeding within body cavities, and generalized haemorrhages.

In parallel, data regarding raptors, including species identification and location, were collected as part of the Poisoning Control and Prevention Strategy in the Canary Islands (BOC, 2014). The study included six raptors endemic to the Canary Islands: *Tyto alba, Asio otus, Falco tinnunculus, Accipiter nisus, Falco pelegrinoides* and *Buteo buteo insularum.* These raptors were either found dead in the wild, or in the recovery centres of Gran Canaria between 2020 and 2022. Necropsies, including the collection of livers and storage, were carried out by the Anatomical Pathology Department, at the University of Las Palmas.

2.3. Analysis of anticoagulant rodenticides in snake and raptor livers'

Certified AR standards (warfarin, diphacinone, chlorophacinone, coumachlor, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen) and procedural-internal standard (P-IS, (±)-Warfarin-d5) of maximum purity (93.1 %–99.8 %) from Dr. Ehrenstorfer (Augsburg, Germany) were used. The solvents used were acetonitrile, methanol (ACN and MeOH, >99.9 % purity) and formic acid (FA, 98 % purity) from Honeywell (Morristown, NJ, USA). While water was produced in the laboratory using a MilliQ A10 water purification system (Millipore, Molsheim, France). QuEChERS Extract Pouch, AOAC Method (6 g de magnesium sulphate and 1.5 g sodium acetate), were purchased in commercial premixes from Agilent Technologies (Palo Alto, CA, USA).

Liver samples were extracted by the methodology previously validated in our research team (Rial-Berriel et al., 2021b, 2020b, 2020a). Liver tissue (1 g) was disaggregated and homogenized with 4 ml of miliQ water at 6500 rpm, 2 × 30 s in a Precellys Evolution homogenizer (Bertin Technologies, Rockville, Maryland., USA). Then, 1 g of the homogenate was manually shaken for 1 min with 2 mL of ACN 1 % FA in a 5 mL Eppendorf tube and submitted to ultrasonication for 20 min (VWR, 50/60 Hz, 120 W). Next, 480 mg of anhydrous magnesium sulphate and 120 mg sodium acetate were added to each sample tube, mix with a vortex for 30 s, and manually shaken for 1 min. Samples were then centrifuged at 3125.16 xg (4200 rpm) and 2 °C for 5 min (5804 R, Eppendorf, Hamburg, Germany). The supernatant was then filtered through a 0.2 μm Chromafil PET-20/15 (Macherey-Nagel, Düren, Germany) into glass amber vials.

Quality Control samples (QCs) were generated utilizing a blank chicken liver matrix to ensure methodological consistency. A comprehensive ten-point calibration curve was constructed, spanning a concentration range of 0.195 to 100 ng/g. This curve was prepared following the same extraction protocol delineated in the preceding section, thereby facilitating more accurate and reliable comparative analyses. Similarly, QCs were prepared at a single concentration of 2 ng/g (with RSD ≤ 20 % and REC = 70–120 % for QCs, LOD/LOQ) (Supplementary Tables 1 and 2). All samples, QCs, calibration points, and blanks were spiked with the P-IS solution prior to extraction and concentrations were presented as wet weights (ww).

To detect and quantify ARs, an Agilent 1290 UHPLC linked to an Agilent 6460 triple quadrupole mass spectrometer was utilized. A heated InfinityLab Poroshell 120 column was used in tandem with an inline filter and UHPLC guard column. The mobile phase employed a gradient of 0.1 % FA and 2 mM ammonium acetate in water (Phase A) and 2 mM ammonium acetate in MeOH (Phase B). Injection volume and flow rate were set at 8 μL and 0.4 mL/min, respectively. The mass spectrometer ran in dynamic multiple reaction monitoring (dMRM) mode across both polarities, with specific cycle, dwell, and run times. Operational parameters for the Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) and the gases used are detailed in the methods. Further validation parameters are available in (Rial-Berriel et al., 2020a, 2020b).

2.4. Statistical analyses

All statistical analyses were conducted in R (R Core Team, 2021). Frequency of detection was determined as the percentage of animals presenting at least one AR in their livers. All the analyses conducted were centered on SGARs. This decision was based on the substantial differences in molecular weights between FGARs and SGARs, as well as the fact that we only detected one FGAR in a single snake (Herring et al., 2022).

To better understand the factors influencing exposure to second-generation anticoagulant rodenticides (SGARs) in California kingsnakes, we utilized a Generalized Linear Model (GLM) with binomial error distribution and logit link function. Given the high prevalence of SGARs in our samples (90 % positive) and aiming to assess kingsnakes as potential sentinel species for wildlife, particularly raptors, we adopted a threshold-based approach. We dichotomized our data at 100 ng/g wet weight (ww), aligning with the 75th percentile of our data (99.1 ng/g ww) and near levels deemed potentially toxic to certain raptors (Newton et al., 1999; Thomas et al., 2011). However, we recognize the limitations of direct comparisons, as our focus is on reptiles, and raptor species vary. Recent literature, including Herring et al. (2022), suggests nuanced interpretations of toxicosis probabilities at different concentration thresholds. Our model included sex, age class, weight, snout-vent length, and distribution areas, analyzing data from 356 snakes, excluding four with missing data.

Furthermore, we examined predictors of variation in the sum of AR concentrations (ΣARs) in the snakes. The response variable, ΣARs, was log-transformed due to its non-normal distribution. The explanatory variables for this model were similar: sex, age class, weight (g), snout-vent length (cm), distribution areas, and necropsy findings (0 = absence, 1 = presence). We also considered including the capture method (manual/trap) as a variable. This decision was influenced by Lettoof et al. (2020), who suggested that AR exposure might alter normal reptilian behavior, potentially leading to a bias in which easily hand-caught snakes exhibit higher AR concentrations.

Given the low limits of quantification of our method (below 1 ng/g), we treated snakes with non-detectable ARs as true negatives. Consequently, we excluded these 35 individuals, in addition to the four with missing data, from the model ($n = 39$).

Explanatory variables were checked for correlations using the Spearman's correlation test before being inserted in both models, and those presenting high correlations (Spearman's rho >0.7) were prevented to appear in the models. Given the high correlation between age class, weight, and SVL, we inserted just SVL in both global models and excluded the other overmentioned explanatory variables.

For model refinement, we performed a multi-model inference using the 'MuMIn' R package (v.1.46.0) (Bartoń, 2022) ranking the best models through the corrected Akaike's Information Criterion (AICc) and ΔAICc <2 (those within two units) (Burnham and Anderson, 2002). Best models were checked for general fit using the 'performance' R package (v.0.10.4) (Lüdecke et al., 2021).

Concentrations of ARs between raptors and snakes were compared using a Mann-Whitney test. Finally, figures were generated using Microsoft® Excel v16.77.1 and GraphPad Prism v9.4.3 (GraphPad Software, CA, USA).

3. Results and discussion

A total of 360 snakes, with the majority captured in 2021 ($n = 338$), was sampled across the different distribution areas (Fig. 2). Of these, 51.1 % ($n = 184$) were females, 41.7 % ($n = 150$) were males, and 7.2 % had unknown sex ($n = 26$). While 52.2 % ($n = 188$) were adults, 32.8 % subadults ($n = 118$), and 15 % ($n = 54$) juveniles, according to the classification used by Wiseman et al. (2019). The most prevalent coloration pattern was normal (79.7 %, $n = 287$), while the most common design pattern was striped (84.7 %, $n = 305$).

3.1. Descriptive analysis of ARs found in California kingsnakes from Gran Canaria

Among the 10 ARs tested, all of them were SGARs (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen). However, diphacinone (8.13 ng/g ww) was an exception as it belongs to FGARs and was detected in just one individual (Fig. 4). This trend appears to be widespread across diverse geographical regions and taxa, with SGARs clearly prevailing in food chains (Lohr and Davis, 2018; López-Perea and Mateo, 2018; Nakayama et al., 2019; Sánchez-Barbudo et al., 2012).

In this study, only 9.7 % ($n = 35$) of the examined snakes exhibited undetectable concentrations of ARs in their liver (Fig. 3). This noteworthy frequency of AR detection in kingsnakes surpasses that observed in the native lizard species across the archipelago (62.7 %), as reported previously (Rial-Berriel et al., 2021a). Additionally, over half of the analysed snakes showed two or more ARs (Fig. 3). Exposure to multiple ARs within the same animal has been extensively described in birds and mammals (Lohr, 2018; López-Perea and Mateo, 2018; Luzardo et al., 2014; Ruiz-Suárez et al., 2016, 2014). Although reports on reptiles are less common, Lettoof et al. (2020) have documented that certain snakes *(Pseudonaja affinis)* and lizards *(Tiliqua rugosa)* were exposed to multiple ARs, with a maximum of three compounds. In our study, the most frequently detected pairwise combination of ARs consisted of brodifacoum-bromadiolone, while the trio with the highest frequency of detection included brodifacoum-bromadiolone-difenacoum. These findings raise concerns, as others have previously suggested the possibility of synergistic effects (Lettoof et al., 2020; Lohr, 2018).

Focusing on brodifacoum, this compound exhibited the greatest concentrations among SGARs (max = 790.6 ng/g ww) and the highest frequency of detection (Table 1, Fig. 4). Either alone or in combination with other rodenticides, brodifacoum was present in 99 % of the positive samples. In contrast, difethialone, another SGAR, was detected least frequently (8.6 %, $n = 31$) (Fig. 4). Brodifacoum was previously detected in 23 carcasses of endemic giant lizards on the Canary Islands (Rial-Berriel et al., 2021a). Notably, a study of three urban reptile species in Australia revealed an AR detection frequency of 91 % of snakes *(Pseudonaja affinis)*, 60 % of lizards *(Tiliqua rugosa)* and 45 % of tiger snakes *(Notechis scutatus),* in which brodifacoum was the most frequently detected compound (Lettoof et al., 2020). This pattern is consistent with the findings of Nakayama et al. (2019), who conducted a global literature review and reported that brodifacoum is the most frequently detected AR in wildlife, followed by bromadiolone and difenacoum. The high detection frequency of brodifacoum could be attributed, in part, to its longer half-life compared to others. This is a troublesome finding, considering that it is one of the most toxic rodenticides in wildlife (Nakayama et al., 2019).

3.2. Study of the factors of the exposure of California kingsnake to anticoagulant rodenticides

This study allowed us to identify the biometric and geographical factors influencing snakes' exposure to SGARs.

Regarding SGARs concentration above 100 ng/g ww, the best GLM model was the one including snout-vent length (SVL) and distribution areas (MA, SA, TA, QA) as fixed factors (Table 2). In detail, for each additional unit increase in snake length, there was a 3.6 % rise in the likelihood of detecting SGARs at these concentrations [OR (95 % CI) = 1.036 (1.021–1.052), $p < 0.001$] (Table 2). The distribution areas QA

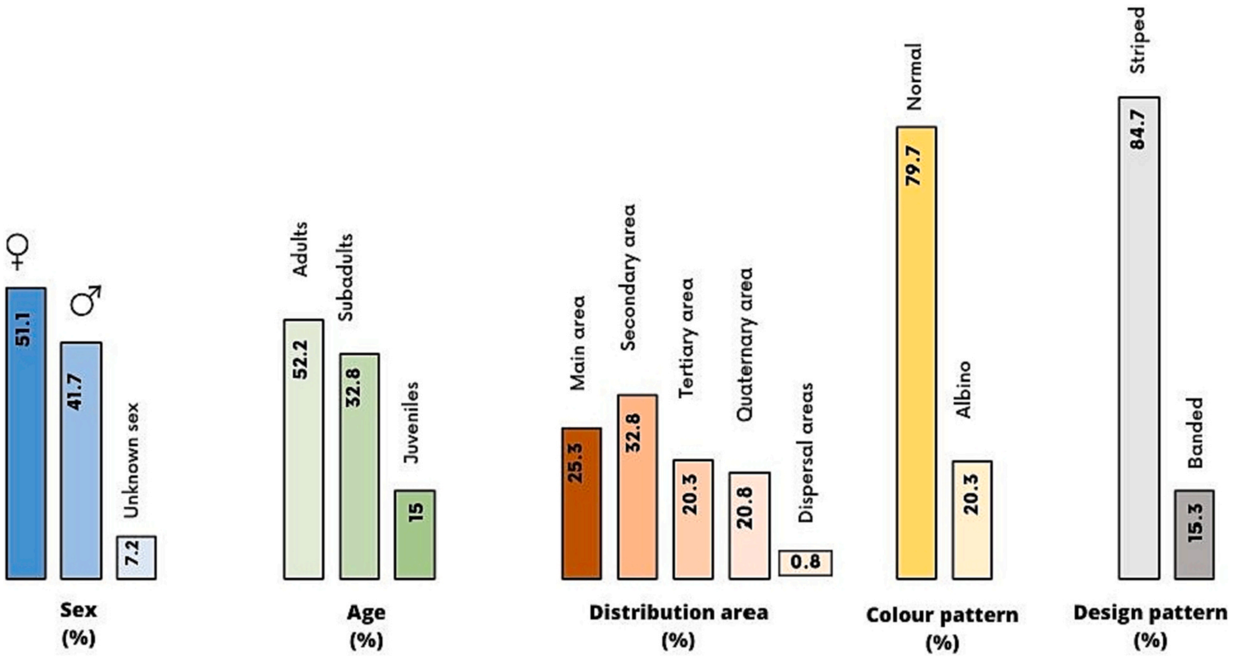

Fig. 2. Characteristics of the series of 360 California kingsnakes (*Lampropeltis californiae*) of Gran Canaria (Canary Islands).

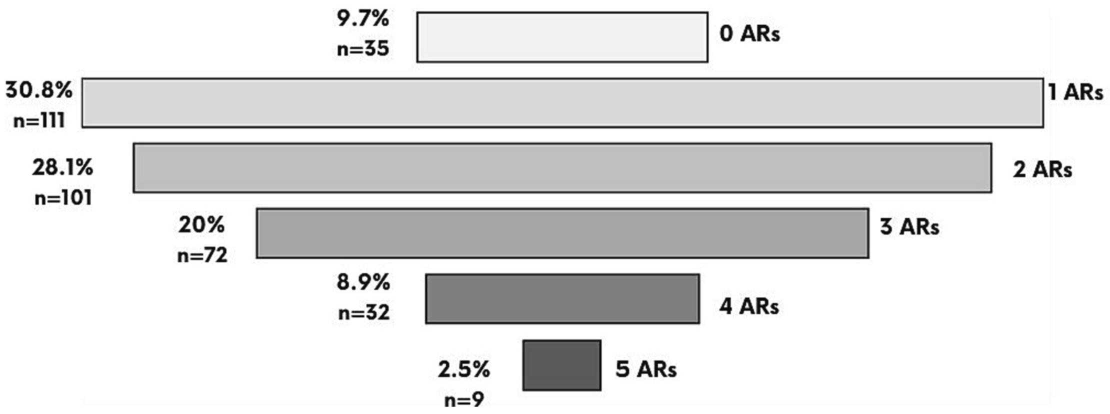

Fig. 3. Funnel plot showing the percentage of snakes exposed to different anticoagulant combinations.

Table 1
Descriptive statistics for the identified SGARs in the snakes of Gran Canaria.

	Brodifacoum	Bromadiolone	Difenacoum	Difetihalone	Flocoumafen
Minimum	0.57	0.34	1.20	1.52	0.34
Maximum	790.6	673.4	168.0	101.4	141.7
Geometric mean	17.77	7.06	5.01	7.21	1.36
SE	5.87	6.72	1.99	4.97	2.40

Note: Descriptive statistics of the SGARs detected in the snakes, including the maximum and minimum values, geometric mean, and standard error of the mean (SE), reported in ng/g ww. in liver. Number of snakes tested positive for SGARs = 325.

and SA exhibited a significant negative effect, compared to MA, indicating that snakes residing in these areas are less likely to exhibit presence of SGARs above the established threshold [(QA = OR (95 % CI): 0.364 (0.169–0.754), p = 0.008); (SA = OR (95 % CI): 0.257 (0.128–0.502), $p < 0.001$); (TA = OR (95 % CI): 0.490 (0.237–0.987), p = 0.049)] (Table 2).

When considering the sum of SGARs (ΣSGARs) as the response variable, the best model was the one that included SVL ($p < 0.001$), distribution area [(QA; $p = 0.001$), (SA; $p < 0.001$), (TA; $p = 0.118$)] and capture method. Specifically for one unit increase in snake length the probability of finding higher concentrations of SGARs was 4 % (Table 3). Snakes inhabiting SA and QA, had 73 % and 55 % lower probability, respectively, of presenting high concentrations of these compounds. (Table 3).

Based on these findings, it is apparent that snake size is an important biometric factor in predicting both variations and higher concentrations of SGARs (Tables 2 & 3), likely to be related to the process of bioaccumulation and biomagnification that occurs in larger animals when they consume greater quantities and larger prey items (Hoang et al., 2021). However, the same study has reported contrasting trends related

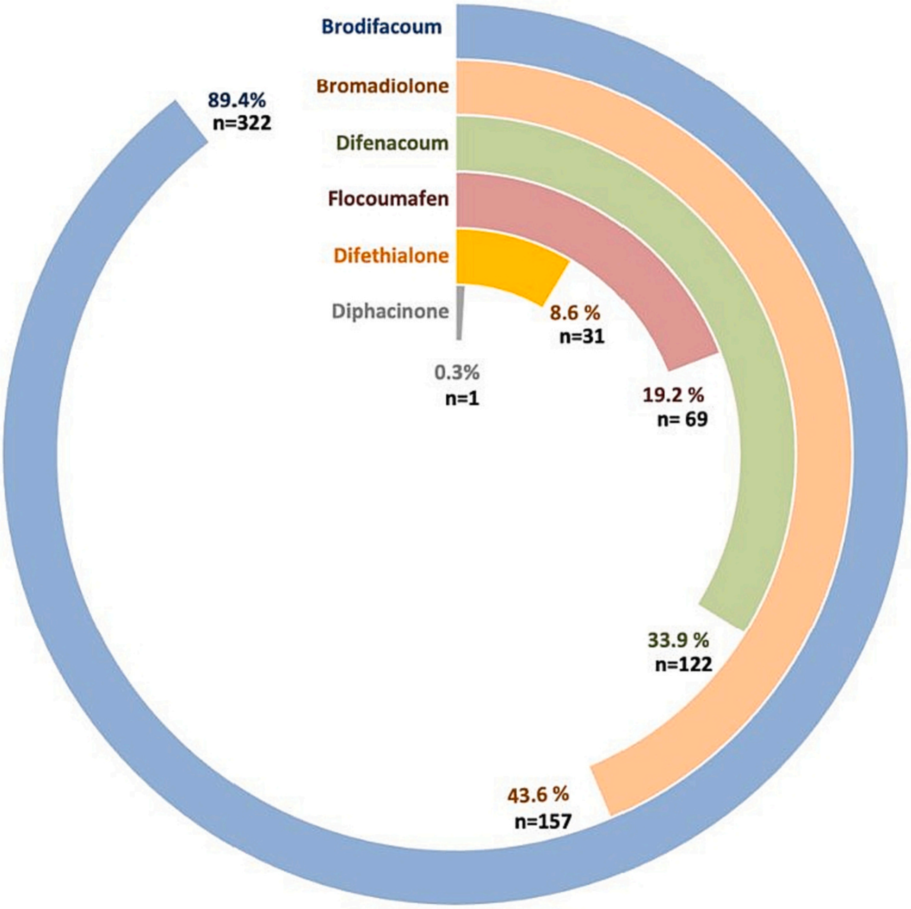

Fig. 4. Doughnut chart showing the frequencies of rodenticide detection found in the total number of snakes sampled ($n = 360$) in Gran Canaria (Canary Islands).

Table 2

Summary of the analysis of exposure to higher concentrations of SGARs (above the threshold set at 99.1 ng/g) in the California kingsnake.

	Est.	SE	OR (95 % CI)	*p*	AICc
Intercept	−3.341	0.660	0.035 (0.009–0.125)	<0.001	
SVL	0.036	0.008	1.036 (1.021–1.052)	<0.001	
Distribution area QA	−1.010	0.380	0.364 (0.169–0.754)	0.008	370.9
Distribution area SA	−1.358	0.348	0.257 (0.128–0.502)	<0.001	
Distribution area TA	−0.713	0.363	0.490 (0.237–0.987)	0.049	

Note: Model outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), odds ratio (OR- exponential of estimates) and correspondent 95 % confidence interval (95 % CI), and *p*-values from a Generalized Linear Model with binomial distribution. The corrected Akaike's Information Criterion for the model is also reported. Response variable: 75th percentile (threshold set at 99.1 ng/g). Number of snakes in the analysis = 356. The comparative table, containing selected models based on the corrected Akaike's Information Criterion (AICc) and ΔAICc <2 (those within two units), can be found in Supplementary Table 4.

Table 3

The best linear model explaining the variation in rodenticide concentrations (ΣSGARs) in the California kingsnake.

	Estimates	SE	Effect size 95%CI	*p*	AICc
Intercept	0.815	0.426	2.26 (0.976–5.228)	0.057	1195.9
SVL	0.038	0.005	1.04 (1.029–1.049)	<0.001	
Distribution area QA	−0.800	0.248	0.45 (0.275–0.733)	0.001	
Distribution area SA	−1.315	0.231	0.27 (0.170–0.423)	<0.001	
Distribution area TA	−0.394	0.251	0.67 (0.412–1.105)	0.118	
Capture method TRAP	−0.326	0.212	0.72 (0.476–1.095)	0.125	

Note: Models' outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), 95 % confidence interval (95 % CI), and p-values from a linear model. The corrected Akaike's Information Criterions for the model is also reported. Number of snakes in the analysis equals to 321. Response variable (ΣSGARs) was log-transformed for analysis. Effect sizes (95 % CI) are proportional changes in the response variable, derived by exponentiating the estimates and 95 % confidence interval.

to size in other organic pollutants, attributing such correlations to growth dilution. Furthermore, given that the categorization into age groups (juvenile, subadult, and adult) relies on this biometric measurement, and since such variables are highly correlated, it is reasonable to anticipate that older individuals will exhibit elevated concentrations of these compounds compared to younger ones.

Regarding the geographical factor, it was found that certain distribution areas had a negative significant association with respect to both response variables, particularly, the second and forth areas of distribution (SA and QA). This is an interesting finding because these areas have a lower density of livestock and crops (ISTAC, 2023) compared to MA. It is well documented that wildlife populations near livestock and agricultural farms have an increased susceptibility to AR exposure (Lohr, 2018; López-Perea et al., 2019; Rial-Berriel et al., 2021a). Therefore, future studies could benefit from more detailed georeferencing the location of snake captures to enable a more accurate assessment.

Finally, the observation of hemorrhagic lesions at necropsy and the capture method were considered factors of interest for the study due to the variability in sensitivity and tolerance of reptiles to these compounds (Lettoof et al., 2020; Lohr and Davis, 2018; Mauldin et al., 2020; Ravindran et al., 2022; Sánchez-Barbudo et al., 2012). However, while they were included in the analysis, there was no discernible effects of these variables on ΣSGAR concentrations (Table 3, Supplementary Table 4).

3.3. *Potential role of snakes as a sentinel of raptors' exposure to ARs*

The frequencies of ARs detection and detection patterns of the primary compounds were similar in raptors form Gran Canaria (2020–2022) and California kingsnakes (2021–2022). Brodifacoum, bromadiolone and difenacoum emerged as the predominant rodenticides in both groups (Table 4). This consistency aligns with previous studies involving archipelago birds of prey (Rial-Berriel et al., 2021a; Ruiz-Suárez et al., 2014). Similarly, raptors inhabiting the island showed rodenticide combinations in a high percentage of individuals (almost 70 %). In both series, combinations of 2–3 rodenticides were the most prevalent. Nonetheless, a substantial disparity in median values between the two groups was evident, as indicated in Table 4. Based on physiological and behavioural effects that can result from exposure to ARs, it becomes conceivable livers of these raptors, whether found dead in the wild or within wildlife recovery centres, may exhibit a tendency towards higher levels of ARs (Lettoof et al., 2020). Furthermore, it is important to clarify that we only analysed liver samples that appeared fresh, excluding any that were in a state of putrefaction or mummification. However, it must be acknowledged that the exact degree of freshness for some samples was uncertain, which could potentially introduce a bias in the observed concentrations. (Herring et al., 2022).

Considering these results, both species exhibit a high probability of exposure to ARs in Gran Canaria. However, these data alone do not provide conclusive evidence regarding their role as sentinels of raptors on the island. Nevertheless, further in-depth research should be conducted to explore their potential sentinel role, as they have certain characteristics that make them a subject of interest for this purpose.

Firstly, these snakes have been extensively documented throughout the island of Gran Canaria, with established populations found in four distinct areas encompassing eight municipalities, in addition to sporadic occurrences in non-established zones. Moreover, their limited home range, in conjunction with their prevalence make them good indicators of local pollution (Gallo and Mateo, 2020; Hoang et al., 2021). Secondly, they occupy a medium-high trophic level in the Canarian ecosystem and are rarely preyed upon, with only occasional attacks recorded (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015). Thirdly, these snakes exhibit the capacity to bioaccumulate pollutants, as evidenced in this study. This is reinforced by prior research suggesting that snakes preying on rats/mice or smaller reptiles may directly bioaccumulate SGARs due to their persistence in liver tissue (Lettoof et al., 2020). Additionally, there are indications of AR biomagnification, akin to observations with other compounds like persistent organic pollutants, where concentrations surpass those found in their prey (Hoang et al., 2021). Lastly, kingsnake exhibit a substantial annual capture rate, with over 2000 specimens captured in the past year (GESPLAN, 2023a). This species has been the subject of research in both its native habitat and within our archipelago region, with ongoing studies continuing in the area.

Table 4
Comparison of ARs levels in kingsnakes and raptors of Gran Canaria.

	Kingsnakes n = 360		Raptors *n* = 110		
	n (%)	Median (ng/g ww)	n (%)	Median (ng/g ww)	*p*-value
Brodifacoum	322 (89.4)	19.2	96 (87.3)	32.3	0.014
Bromadiolone	157 (43.6)	6.8	57 (51.8)	9.5	0.129
Difenacoum	122 (33.9)	3.7	44 (40.0)	3.9	0.551
Difetihalone	31 (8.6)	4.7	21 (19.1)	5.7	0.956
Flocoumafen	69 (19.2)	0.8	19 (17.3)	1.8	0.007
Diphacinone	1 (0.3)	–	1 (0.9)	–	–
ΣARs	90.3	23.8	90.9	49.7	0.003

Note: n (%): Frequency of detection for each compound and ΣARs in both species; Median: Median value in ng/g ww.

In summary, it is important to continue studying the presence of these compounds in Gran Canaria's reptiles, not only considering their role as sentinels but also as potential risks to the local raptor population. These raptors might eventually specialize in snake hunting in the future, which could introduce a new source of high exposure to rodenticides, as evidenced in previous studies involving raptors and high-trophic-level mammals that prey upon snakes or similar reptiles (Hong et al., 2019; Lettoof et al., 2020; Nakayama et al., 2019).

4. Conclusions

The examination of 360 snake livers from Gran Canaria showed a strikingly high prevalence (>90 %) of anticoagulant rodenticides, with brodifacoum being the most frequent and at highest concentrations. Moreover, over a half of the individuals exhibited the presence of at least two or more rodenticides.

Explanatory factors associated to the presence of higher SGAR concentrations were the snake size, with bigger snakes more likely to be exposed to rodenticides compared to smaller ones and the distribution area, with those inhabiting the Main Area showing higher prevalence of elevated concentrations of rodenticides compared to those in the other three areas.

In the context of its potential as an environmental sentinel species, the California kingsnake has exhibited remarkable effectiveness in indicating contamination by these compounds. Moreover, when compared to raptors, with whom they share certain feeding habits, both species showed elevated frequencies of ARs detection and exhibit similar detection patterns. Nonetheless, advanced investigations are imperative to ascertain its role as a sentinel for exposure in other wildlife species, such as birds of prey.

Consequently, it is of paramount importance to persist in the exploration of these compounds in reptilian populations within the Canary Islands and to contemplate their examination in other regions dealing with invasive snake species. Legislative measures and educational initiatives assume pivotal roles in advocating for the responsible application of these biocides, particularly considering worrying wildlife discoveries.

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CRediT authorship contribution statement

1. **Guarantor of integrity of the entire study:** OPL, BMC.
2. **Study concepts and design:** OPL, BMC.
3. **Sample providing:** RGB, M.A. C-P, AMM.
4. **Literature research:** OPL, BMC, RGM, AMM, CRB.
5. **Laboratory work:** CRB, BMC, AAD.
6. **Data analysis:** OPL, BMC.
7. **Statistical analysis:** OPL, BMC, LAH, KSR, MC.
8. **Manuscript preparation (original draft):** OPL, BMC, MZ.
9. **Manuscript editing:** OPL, AAD, BMC, MZ, M.A.C-P, RGB, ASP, KRS, MC.
10. **Project administration:** OPL.
11. **Funding acquisition:** OPL, MZ, LAH.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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