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Widespread anticoagulant poison exposure in predators in a rapidly growing South African city

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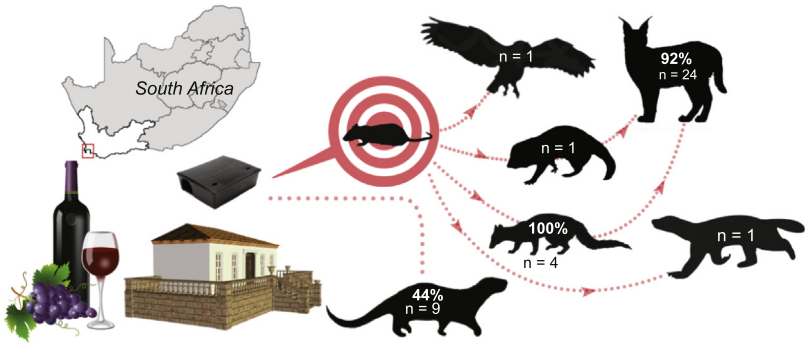
HIGHLIGHTS

- Anticoagulants were detected in six of seven predatory species in Cape Town, South Africa.
- The exposed species fill various aquatic and terrestrial ecological niches.
- Vineyards were the critical link between caracals and urban rat poison exposure.
- Residues concentrations in Cape genets and otters suggest invertebrate vectors.
- The anticoagulants detected were all the most toxic, second-generation compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are used worldwide to control rodent populations. ARs bioaccumulate across trophic levels and threaten non-target wildlife. We investigated the prevalence of AR exposure in seven predator species in the rapidly developing Greater Cape Town region of South Africa – a mosaic of natural, urban, and agricultural areas within a global biodiversity hotspot. We focused sampling on caracals (*Caracal caracal*, $n = 28$) as part of a larger caracal ecology study, but also opportunistically sampled Cape Clawless otters (*Aonyx capensis*, $n = 9$), large-spotted genets (*Genetta tigrina*, $n = 4$), honey badger (*Mellivora capensis*, $n = 1$), water mongoose (*Atilax paludinosus*, $n = 1$), small gray mongoose (*Galerella pulverulenta*, $n = 1$), and Cape Eagle owl (*Bubo capensis*, $n = 1$). We tested livers from all species, and blood from ten caracals, for eight AR compounds to assess prevalence and amount of exposure for each compound. We used generalized linear models to test spatial, demographic, and seasonal risk factors for ten measures of AR exposure in caracals. We detected at least one of the four most toxic AR compounds in six species. Exposure was high for caracals (92%) and all species combined (81%). For caracals, proximity to vineyards was the most important AR exposure risk factor. Vineyards in Cape Town do not use ARs to protect their vines but do host commercial hospitality structures where ARs are used. Vineyards may thus link caracals that forage within vineyards to the rat poisons used in and around their commercial structures. Residue levels were unexpected in large-spotted genets and Cape Clawless otters, suggesting invertebrate

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vectors. ARs may present a cryptic threat to populations already vulnerable to increasing habitat loss, vehicle collisions, poachers and fire. Targeted mitigation should include a mix of environmentally responsible policies that reduce AR use, particularly in areas near wildlife habitat.

1. Introduction

Pesticide exposure is a leading threat to biodiversity globally (McGill et al., 2015). Toxicants used in and around urban and agricultural areas often indiscriminately kill species (Berny, 2007; Elliott et al., 2014; Hindmarch and Elliott, 2018; Riley et al., 2014). Even when used in small amounts, some toxicants are prone to bioaccumulation that can lead to widespread exposure of many nontarget species (Geduhn et al., 2015). While unintended, direct mortality and sublethal pesticide exposure can influence population dynamics (Thomas et al., 2011) and potentially escalate to ecosystem impacts if the toxicant exposure is pervasive across numerous species (Hindmarch and Elliott, 2018).

Globally, anticoagulant rodenticides (ARs) are the principal chemical method used for lethal control of rats and mice (van den Brink et al., 2018). As vitamin K antagonists, ARs slowly deplete vitamin K clotting proteins, causing internal hemorrhage and death up to ten days after lethal ingestion of the poisons (Cox and Smith, 1992). ARs are composed of two classes of compounds; first-generation (warfarin, diphacinone, and chlorophacinone) and second-generation ARs (SGARs; brodifacoum, bromadiolone, difethialone, difenacoum). SGARs have prolonged action and increased potency, and with hepatic half-lives ranging 6–12+ months (Eason et al., 2002), meaning their actual tissue persistence when a sublethal dose is ingested can be greater than six years (Vandenbroucke et al., 2008). Consequently, for predatory species that consume prey species targeted with ARs, both acute and chronic secondary toxicant exposure may occur (Hindmarch and Elliott, 2018; Serieys et al., 2015a).

Over more than two decades of research, a clear consensus is emerging that ARs are a pervasive threat to nontarget wildlife. First, wherever AR exposure testing is conducted, AR exposure is detected (Laakso et al., 2010; Elliott et al., 2014). Second, AR exposure often exceeds 80% in a wide range of mammalian and avian predators (e.g., Christensen et al., 2012; Cypher et al., 2014; Elmeros et al., 2011; Laakso et al., 2010; Lohr, 2018; Serieys et al., 2015a). Third, ARs are implicated in direct and indirect mortality of nontarget wildlife (e.g., Cypher et al., 2014; Fournier-Chambrillon et al., 2004; Gabriel et al., 2012, 2018; McMillin et al., 2008; Poessel et al., 2015; Serieys et al., 2015a, 2018). To date, AR studies are extensive in Europe (e.g., Alomar et al., 2018; Berny and Gaillet, 2008; Christensen et al., 2012; Dowding et al., 2010; Elmeros et al., 2011, 2018; Fournier-Chambrillon et al., 2004; Koivisto et al., 2018; Kotthoff et al., 2018; Lemarchand et al., 2010; Lopez-Perea et al., 2015; McDonald et al., 1998; Ruiz-Suárez et al., 2014, 2016; Sanchez-Barbudo et al., 2012), North America (Albert et al., 2010; Beier et al., 2010; Cypher et al., 2014; Erickson and Urban, 2004; Franklin et al., 2018; Gabriel et al., 2012; Gehrt and Riley, 2010; Hindmarch et al., 2017; Huang et al., 2016; Poessel et al., 2015; Serieys et al., 2015a; Stone et al., 1999), and New Zealand (Eason et al., 2002; Hoare and Hare, 2006; Masuda et al., 2014). In southern Africa, there are no AR studies despite the rich wildlife heritage and the central role that wildlife plays in attracting tourist revenue (Kinzig and McShane, 2015). Rapid population growth and urbanization in sub-Saharan Africa (Güneralp et al., 2017), combined with expanding agricultural activity, increase the risk of unintentional wildlife poisoning as rodenticides are ubiquitous in agricultural and urban landscapes worldwide. Poisoning thus presents an additional threat (Kinzig and McShane, 2015) to wildlife, exacerbating the vulnerability of populations already challenged by fragmentation, isolation, disease, poaching, vehicles, and both intentional (Ogada, 2014) and unintentional contaminant exposure.

We investigated AR exposure in two regions of the Western Cape, South Africa: the Greater Cape Town area (GCT; mixed urban-agricultural-wildland) and the Central Karoo (mixed pastoral-wildland). Sampling effort was focused on the caracal (*Caracal caracal*) in GCT, a common mesocarnivore in the Western Cape that has assumed the role of apex predator throughout much of its range following the extirpation of lion, leopard and hyena (Tambling et al., 2018). To assess whether ARs also pose an ecological risk to other species in GCT, we opportunistically collected liver samples from dead specimens of six other predatory species, from 2013 to 2017. Using these AR data, we tested: i) for the presence and level of exposure to first- or second-generation ARs in nontarget wildlife species living adjacent to urban and agricultural land uses; and ii) whether spatial, temporal, and demographic factors influence caracal risk of exposure to ARs. Our findings can be used to help identify potential contributors of environmental contamination with ARs, allowing for targeted mitigation efforts and improved wildlife health.

2. Methods

2.1. Study areas

We sampled mammalian carnivore species in two study sites in the Western Cape of South Africa that can be broadly categorized as mixed urban and protected natural areas. The urban area, Greater Cape Town (GTC, −33.942989, 18.630957; Fig. 1) is a mosaic of urban, light industrial and agricultural land that surround both small pockets and larger areas of fragmented natural land (e.g., City parks, Table Mountain National Park). The GTC covers an area of approximately 400 km^2 with a mean population density of 1530 people/km^2 (worldpopulationreview.com). Approximately 12% of households are in extremely dense informal settlements (Housing Development Agency, 2013) where population density can exceed 46,000 people/km^2.

The rural study site is located approximately 250 km north-east of GTC in the Central Karoo (CK, −32.666667, 22.250000). The Central Karoo consists mainly of privately-owned small-stock farmland in the semi-desert region of the Western Cape Province, South Africa. Here, population density is exceptionally low with 1.8 people/km^2 and croplands are rare and restricted to small areas immediately adjacent to farm houses. Stock largely comprises free-ranging sheep that feed on native vegetation.

2.2. Trapping, opportunistic mortalities, and sample collection

In GCT, we trapped caracals between 2014 and 2017 using standardized cage-trapping techniques (Serieys et al., 2013). Once captured, we chemically immobilized animals with a mixture of ketamine HCl (7 mg/kg) and medetomidine HCl (0.08 mg/kg). We recorded age class, sex, weight, and morphological measurements (i.e., chest circumference, body length, tail length, ear length, head circumference, etc.). Individuals were classified as juveniles (<2 years) or adults (≥2 years) based on body size, weight, tooth wear and eruption, and reproductive status (Schroeder et al., 2005). Individuals were fitted with Tellus 1C collars (Followit™, Lindesberg, Sweden) that collected GPS locations at three-hour intervals and were equipped with a drop-off component that activated within six months collar fitting. We collected blood samples via cephalic or saphenous venipuncture which we then centrifuged

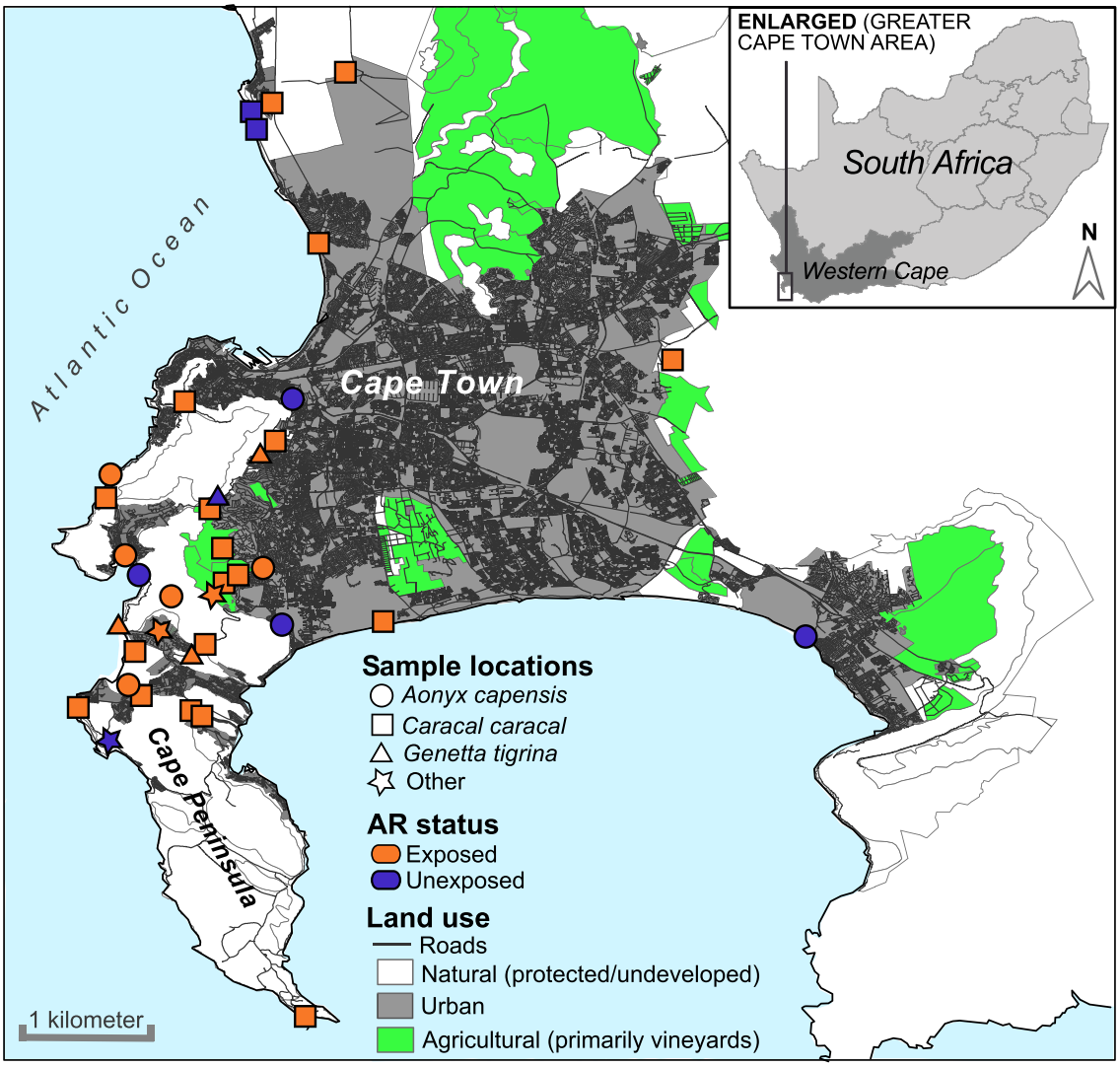

Fig. 1. A map of the primary study area, the Greater Cape Town area in South Africa, where seven predatory species were sampled. Orange symbols represent anticoagulant-exposed individuals. Blue symbols represent unexposed individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

within 24 h to separate serum. Sera were stored at −80 °C until analyzed for ARs. Animal capture, handling, and sampling protocols followed ethical guidelines approved by the American Society of Mammologists and were also approved by the University of Cape Town (2014/V20/LS), Cape Nature (AAA007-0147-0056), and South African National Parks (SERL/AGR/017–2014/V1).

We opportunistically collected wildlife carcasses in GCT, primarily killed as a result of vehicle collisions. Species opportunistically sampled included caracals (*Caracal caracal*, $n = 19$), Cape Clawless otters (*Aonyx capensis*, $n = 8$), large-spotted genets (*Genetta tigrina*, $n = 4$), honey badger (*Mellivora capensis*, $n = 1$), water mongoose (*Atilax paludinosus*, $n = 1$), small gray mongoose (*Galerella pulverulenta*, $n = 1$), and Cape Eagle owl (*Bubo capensis*, $n = 1$). If GPS-collared caracals died while being monitored, we also collected their carcasses ($n = 5$) for necropsy. Carcasses were either necropsied immediately upon retrieval or stored at −20 °C until necropsies could be performed. The cause of mortality, collection date, sex, age class, and GPS location of each carcass was recorded. Canine teeth were extracted from caracal carcasses and used to age specimens via cementum annuli (Matson's Laboratory LLC, Missoula, MT; Crowe, 1972). We removed (a portion of) the liver from each carcass and stored all liver and serum samples at −20 °C. AR compounds are stable and so the length of storage time does not affect compound detection results (Waddell et al., 2013).

In the Central Karoo, we collected liver samples from four caracals killed by hunters or sheep farmers in April 2015 during standard culling operations (Tensen et al., 2018). After culling operations, the carcasses were provided to the project by the Western Cape Nature Conservation Board (Permit no. 0056-AAA007-00161). No animals were killed for the purpose of this study. No ethical clearance was required by the Science Faculty Animal Ethics Committee as the animals were not killed for research purposes.

2.3. Anticoagulant screening

The detection of these compounds in liver, reflects the history of exposure for the individual, and is therefore the preferred tissue for AR studies (Serieys et al., 2015a). The detection of ARs in blood (or serum) is possible but reflects only recent exposure events and is not a reliable indicator of exposure generally (Serieys et al., 2015a, 2018). However, the detection of ARs in blood can provide unique insights into the prevalence of compounds used most pervasively on the landscape, particularly first-generation ARs with short hepatic half-lives (Serieys et al., 2015a). We assessed the presence and concentrations of eight anticoagulant compounds in 2 g of liver tissue or 1 g of serum at the Center for Health and Food Safety at University of California, Davis. Samples were first screened for AR compounds using liquid

chromatography-mass spectrometry (LC–MS/MS). If ARs were detected, then amounts were quantitated using high-performance liquid chromatography (HPLC). The approach is standardized and previously described (Serieys et al., 2015a; Waddell et al., 2013). The compounds tested were a standard panel of commercially available compounds (including within South Africa; Serieys, pers.obs) that included first-generation compounds (warfarin, coumachlor, chlorophacinone, and diphacinone) and second-generation rodenticides (bromadiolone, brodifacoum, difethialone, and difenacoum). Limits of quantitation for these anticoagulants in wet liver tissue were 0.01 μg/g for brodifacoum, 0.05 μg/g for bromadiolone, warfarin, and coumachlor, and 0.25 μg/g for chlorophacinone, diphacinone, and difethialone. In blood, limits of quantitation were 0.001 μg/g for each compound with method detection limits ranging from 0.00028 to 0.00045 μg/g. ARs that were determined to be positive by LC–MS/MS, but were below the limit of quantitation by HPLC, were simply described as above the limit of detection (LOD). However, when conducting analyses (see below), for values that registered as LOD, we substituted a numerical value of 0.001 (μg/g) given that the detection of the compounds themselves is a primary goal of the study and is still informative with respect to model results.

2.4. Spatial risk factors for caracal AR exposure

We investigated the potential relationship between caracal AR exposure and different types of human land use where ARs are expected to occur (Hindmarch and Elliott, 2018; Hindmarch et al., 2017; Lohr, 2018; Poessel et al., 2015; Serieys et al., 2015a). Our limited sample sizes for the other species prohibited evaluating risk factors for exposure using those AR data. To assess spatial risk factors for caracal AR exposure, we measured the Euclidean distance from the sample GPS coordinate to the nearest edge of three common land use types that typically border wildlife habitat, namely, residential areas, vineyards and altered open spaces (e.g. landscaped sports fields and golf courses) in Google Earth Pro (Google LLC, Menlo Park, California, USA). Therefore, for all samples, we had three different distance measurements to test for spatial risk factors for AR exposure.

2.5. Percentage urbanization and agriculture in caracal home ranges

Seven caracals were GPS-collared for a minimum of 30 days during the study period from 2014 to 2017. Five were still GPS-collared when they died. One adult female died within one month from when the GPS-collar dropped off. One individual GPS-collared for five months died approximately 9 months after his GPS-collar dropped off. Despite the time lag, we included his home range information in these analyses because he was an adult male and his movement patterns were largely stable throughout his time collared. Data from these seven caracals were used to explore the relationship between the proportion of urban development and vineyards within home ranges, and the level of AR exposure. We calculated 95% kernel-density estimates (KDE) for estimating home range size (Kie et al., 2010; Walter et al., 2011) in the package 'adehabitatHR' (Calenge, 2006) in R (R Development Core Team, 2014). To assess the percent of urban and agriculture land within each home range, we first created polygons around all urban (e.g. any type of development) and agricultural areas in Google Earth Pro (Google LLC, Menlo Park, California, USA). We then calculated the percent overlap of each home range with urban and agricultural land uses in R (R Development Core Team, 2014).

2.6. Statistical analyses

We measured anticoagulant exposure as (1) whether exposure was detected (total exposure: presence/absence); (2) whether exposure to individual compounds was detected (presence/absence of brodifacoum, bromadiolone, difethialone, difenacoum); (3) the total summed concentration of all compounds detected (total residues); (4) individual residue concentrations of the four most frequently detected compounds (brodifacoum, bromadiolone, difethialone, difenacoum); and (5) the number of individual compounds detected in individuals (0–8). We assessed percent exposure across all individuals collectively, and for species for which we had >3 samples each viz., caracals, Cape clawless otters, and large-spotted genets (Tables 1–2). For caracals, we also present the descriptive statistics for concentrations and compounds detected in males, females, adults, and juveniles for dry (November 1–April 30) and wet (May 1–October 31) seasons.

We used three types of generalized linear models (GLMs) to assess predictors of AR exposure for caracals sampled in the urban site (GCT) where ARs were detected as in Serieys et al., 2015a (Supplemental Table S1). For each model type (see next paragraph), we first performed univariate analyses to identify potential predictors, or risk factors, of exposure. We tested log-transformed distances for the three land use types of interest, sex (male, female), age class (adults ≥ 2; juveniles ≤ 2 years), age (Matson's age in years), and season (wet, dry). For each age dataset (age class, $n = 24$ vs. Matson age in years, $n = 23$), we performed separate analyses to avoid potential confounding effects (Serieys et al., 2015a). All reported models are the result of univariate analyses; none of the anticoagulant measures were best-explained by multivariate models.

For presence/absence of any compound and of the four most frequently detected compounds (bromadiolone, difethialone, and difenacoum), we performed logistic regressions. For total residues and the individual residue concentrations of the four most commonly detected compounds, we performed log-linear regressions. Finally, for the number of compounds detected, we performed Poisson regressions to evaluate risk factors for exposure to multiple compounds (0–8).

We did not correct our alpha for multiple tests because commonly used methods of correction are described as overly conservative with a higher probability of generating Type II errors in comparison with Type I errors (Moran, 2003). All statistical tests were considered significant when $\alpha \leq 0.05$, but some of these may represent false positives. All analyses were performed in R (R Development Core Team, 2014).

3. Results

3.1. Prevalence of exposure and evidence of chronic AR exposure across species

We collected liver samples from 45 individuals (41 from urban GCT and four from the rural CK site) comprising seven species (Table 1). Blood samples from 10 caracals were collected. Cause of death was primarily vehicle collision ($n = 28$; Table 1). Samples from the urban site (GCT) had high exposure prevalence (Table 2) with anticoagulants detected in the liver of all species tested, with the exception of one small gray mongoose sampled within a national park. The four caracals that originated from rural CK (where AR exposure was not anticipated) were not exposed (Table 1). Overall, total AR prevalence across the 41 individuals (seven species) sampled from GCT was 81% ($n = 32$; Tables 1–2).

ARs were present in 92% (95% CI: 77–99%) of GCT caracal livers (exposed, $n = 22$; unexposed, $n = 2$) but in none of the blood samples ($n = 10$). In all but one caracal, the AR detected was a second-generation compound and included: brodifacoum ($n = 22$ exposed), bromadiolone ($n = 19$ exposed), difethialone ($n = 13$ exposed), and difenacoum ($n = 8$ exposed), with the former two also detected at the highest concentrations (Tables 2–3, Fig. 2). In a single caracal case, warfarin was detected, and was the only compound detected in this caracal. We detected multiple compounds within individual caracals, large-spotted genets, and the Cape Eagle owl. In caracals, the majority of samples had ≥3 compounds per individual (range: 0–5; Table 3). Brodifacoum and bromadiolone were the most frequently detected compounds across all species (Table 2), and in higher concentrations (Fig. 2; Table 3).

Table 1
Sample sizes, study areas, percent exposed to any AR compound with 95% confidence intervals and sources of mortality.

Study areas	Species	*n*	% Exposed (95% CI)	Source of mortality
Greater Cape Town (GCT)	Caracal (*Caracal caracal*)	24	92% (77–99)	Roadkill ($n = 18$), unk. disease ($n = 2$), anticoagulant toxicosis ($n = 2$), unk. ($n = 1$)
	Cape clawless otter (*Aonyx capensis*)	9	44% (15–77)	Roadkill ($n = 6$), unk. poison ($n = 1$), unk. ($n = 2$)
	Large-spotted genet (*Genetta tigrina*)	4	75% (22–99)	Roadkill
	Small gray mongoose (*Galerella pulverulenta*)	1	0% (0–95)	Roadkill
	Cape Eagle owl (*Bubo capensis*)	1	100% (5–100)	Roadkill
	Water mongoose (*Atilax paludinosus*)	1	100% (5–100)	Dog attack
	Honey badger (*Mellivora capensis*)	1	100% (5–100)	Roadkill
	All species combined	41	80% (65–91)	
Central Karoo	Caracal	4	0% (0–60)	Routine culling operations

We detected a range of residue concentrations within individuals (all species: range = 0.0–2.13 μg/g; mean = 0.35, SE = 0.09, median = 0.10; Table 3). For eight individuals, concentration was only at the level of detection (LOD) but not quantifiable, indicating low levels of exposure. These included samples from one large-spotted genet (bromadiolone), water mongoose (bromadiolone), honey badger (difenacoum), four cape clawless otters (brodifacoum, bromadiolone, difenacoum), and one caracal (warfarin). The Cape eagle owl was exposed to two compounds: brodifacoum (0.66 μg/g) and LOD amounts of difenacoum. Difenacoum was above LOD for all detections with the exception of one genet (0.083 μg/g). The highest concentrations of AR were detected in two genets (1.82 μg/g and 2.13 μg/g).

3.2. Demographic risk factors for exposure in caracals

We collected liver from six female and 18 male caracals and eight adults and 16 juveniles (Table 4; Supplemental Table S2). Five samples were collected during the dry season, while 19 were collected during the wet season. We also assessed age (in years) for 23 caracals (range: <1–16; median: <1 year; mean = 2 years, SD = 2.7). Generalized linear models revealed no significant relationship between AR exposure response variables and age, age class, or seasonal predictor variables. The only demographic parameter that exerted a measurable effect on AR exposure was sex (Table 5). The mean concentration detected in female caracals was nearly twice (0.67 μg/g, SE = 0.21) that in males (0.36 μg/g, SE = 0.11), while the median concentration in females was three times greater (0.66 μg/g) than the median concentration detected in males (0.21 μg/g; Table 4). These differences were not significant because variance was high within the small sample set. The only exception was difethialone, for which both exposure and residue were significantly greater in female caracals ($p = .008$, Table 4).

3.3. Spatial risk factors for exposure

Seventy-nine percent ($n = 19$) of caracal liver samples were collected from opportunistic carcasses while 21% ($n = 5$) were collected from caracals that died while GPS-collared. In total, however, we had GPS tracking data for a total of seven (29%) caracals. We tested spatial risk factors for exposure using: 1) log-transformed distance from the three land uses, and 2) for the seven caracals for which we had GPS-collar data, we tested for associations between the percent of urban and agricultural areas in their 95% KDE home ranges and the multiple AR exposure measures. Home ranges of collared animals ranged from 5.6 km^2 to 170.0 km^2 (mean: 77.4 km^2, SD = 62.5, median = 62.5 km^2). The variation in home range size reflects our disparate sampling across age class and sex (six males; adult, $n = 3$; juvenile, $n = 5$; adult female, $n = 1$). Due to our small sample size, we were unable to perform analyses that partitioned data by age class or sex.

Despite the limitations in sample sizes for GPS-collared individuals, we found remarkably concordant results between the two spatial datasets and analyses (Table 5). Total residue concentration, exposure to difenacoum, and the concentrations of brodifacoum and difenacoum were negatively correlated with distance from vineyards when we analyzed data from all 24 caracals. Similarly, using our subsample of seven GPS-collared individuals, we found total residue concentrations and the concentrations of brodifacoum and difenacoum were positively correlated with the proportion of home range area that was classified as vineyard (Table 4), but not with the proportion of urban area. The similar results between these two types of spatial analyses reinforce that vineyards play an important role in increasing caracal exposure to ARs.

3.4. ARs and mortality in caracals

A female caracal, approximately 5 months old, died in the trapping cage, and a veterinary pathologist found thoracic bleeding that may have been a consequence of AR exposure. She was exposed to three compounds and her total residues were 0.17 μg/g. Another juvenile caracal, male, was found dead in a stream after having spent 78 consecutive days within vineyard habitat. A necropsy revealed internal bleeding in his abdomen with death attributed to AR toxicosis. He was exposed to three compounds and had the highest caracal residue concentration (1.51 μg/g).

Table 2
Percentage exposure prevalence to the four compounds detected for all species combined, and for the three species for which we had >3 samples. Ninety-five percent confidence intervals are in parentheses.

		Percentage exposure prevalence (95% CI)				
Species	*n*	Any compound	Brodifacoum	Bromadiolone	Difethialone	Difenacoum
All species combined	41	81 (65–91)	69 (52–81)	56 (40–71)	37 (23–53)	27 (15–43)
Caracal	24	92 (77–99)	92 (72–99)	79 (57–92)	54 (33–74)	33 (16–55)
Cape clawless otter	9	44 (15–77)	38 (10–74)	nd	13 (1–53)	nd
Large-spotted genet	4	75 (22–99)	50 (10–74)	0.75 (22–99)	25 (1–78)	25 (1–78)

Table 3
Concentrations (μg/g) of the three most frequently detected compounds in GCT. A fourth compound, difenacoum, was also detected but only at LOD (level of detection but not quantifiable) amounts. Mean, standard error (SE) and median concentrations are shown.

	All species		Caracals only	
	Mean (SE)	Median	Mean (SE)	Median
Total residues	0.35 (0.09)	0.10	0.40 (0.10)	0.21
Brodifacoum	0.20 (0.05)	0.05	0.25 (0.08)	0.10
Bromadiolone	0.09 (0.04)	0.05	0.08 (0.02)	0.05
Difethialone	0.06 (0.02)	LOD*	0.05 (0.02)	0.05

Table 4
Sample sizes, total residue mean (standard error), median, range, and number of compounds detected in 24 caracals in the Greater Cape Town region.

			Concentrations (μg/g)			No. compounds
Variable	Group	*n*	Mean (SE)	Median	Range	Median
Sex	Female	6	0.67 (0.21)	0.66	0.15–1.42	4
	Male	18	0.36 (0.11)	0.21	0.00–1.51	3
Age class	Adult	8	0.49 (0.17)	0.33	0.10–1.49	3
	Juvenile	16	0.41 (0.13)	0.18	0.00–1.51	3
Season	Dry	5	0.34 (0.15)	0.17	0.10–0.94	3
	Wet	19	0.46 (0.12)	0.22	0.00–1.51	3
All	All	24	0.44 (0.10)	0.22	0.00–1.51	3

4. Discussion

4.1. Widespread AR exposure and availability

We detected pervasive ARs in the livers of six predatory species within a rapidly expanding urban area of the Western Cape, South Africa. We did not detect AR exposure in four caracals living in a semi-arid rural region of South Africa (Central Karoo) with extremely low human population densities (1.8 people/km^2). We also did not detect ARs in the ten caracal blood samples. While the detection of ARs in blood can provide unique insights into the pervasiveness of some AR compounds on the landscape (Serieys et al., 2015a), the half-lives of AR compounds in blood are significantly shorter (Vandenbroucke et al., 2008), and so this finding is not surprising. Multiple second-generation ARs were detected within liver samples of individuals, reflecting a history of multiple exposure events and suggesting that AR exposure is chronic. With one exception, we detected only second-generation ARs, possibly because they are used more frequently or because their hepatic half-lives are substantially more prolonged. Our findings of widespread AR exposure echo those for carnivore species in similar North American urban settings where ARs are implicated as a leading cause of mortality for some populations (Cypher et al., 2014; Fraser et al., 2018; Gehrt and Riley, 2010; McMillin et al., 2008; Poessel et al., 2015). Therefore, the threat these compounds pose to predatory species in urban areas of South Africa may also be substantial.

First- and second-generation ARs are readily available to the public at local supermarkets, hardware and agricultural supply stores. Not only is their sale unrestricted, but ARs are often the only option available at local stores for private citizens that attempt rodent control efforts (Serieys, pers. obs.). Mechanical traps are not routinely available at all stores and supermarkets. Anecdotal reports from private citizens and city and park officials suggest there is a general lack of information concerning the correct application of poisons and the wider implications of using the products. Moreover, there is a widespread misconception that black bait boxes prevent environmental contamination, and that specifically, the rats and mice die within the bait boxes immediately after poison ingestion. Furthermore, in high-density (formal and informal) residential areas, rodent infestation is a foremost health concern, and anticoagulants are frequently hand-broadcast (loose pellets) in the environment without protective bait boxes (Nattrass et al., 2018). As the population of South Africa and Cape Town grows, evaluating the ecological consequences of ARs on biodiversity and wildlife will be an important priority given the important conservation status of the region.

4.2. Vineyards are the link between urban ARs and caracals

In southern California, bobcat (*Lynx rufus*) AR exposure was strongly linked with proximity to high-density single-family residential areas that often border park boundaries (Serieys et al., 2015a). Given the widespread availability of ARs in GCT and that often they are the only retail choice for rodent control, we were surprised that AR exposure was not strongly correlated with proximity to residential areas. We also did not detect an association between the proportion of overlap between caracal home ranges and urban areas. These findings are contrary

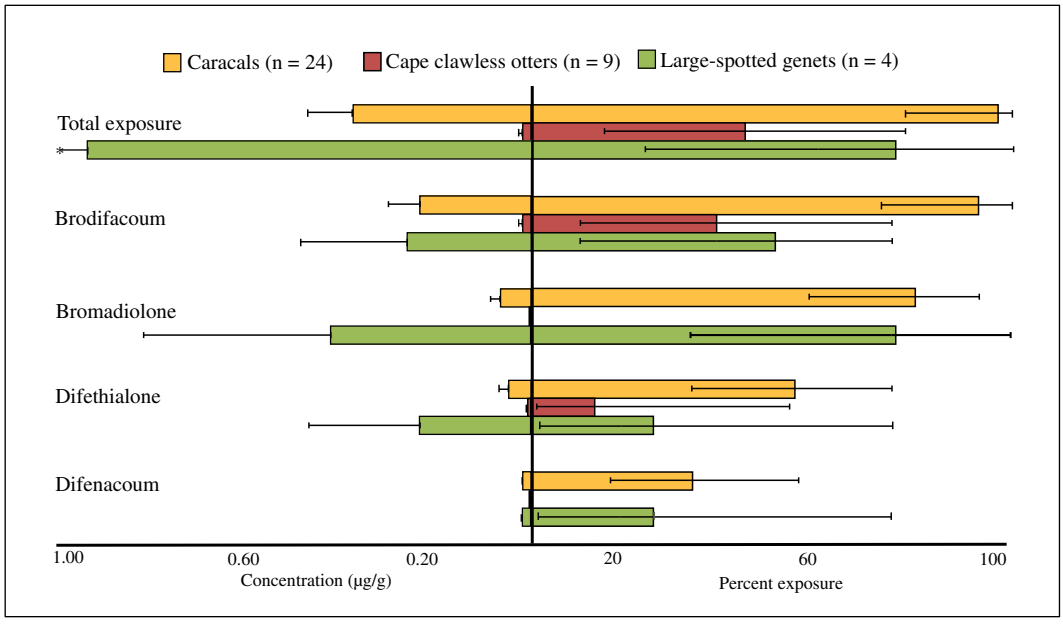

Fig. 2. Percent exposure and 95% confidence intervals (right) and residue concentrations detected and standard error (left) for the three species for which we had >3 samples. We present results for overall (total) exposure, as well as for the four compounds detected across species exposed.

Table 5
Results of GLM analyses conducted with the caracal dataset only to test demographic, seasonal, and spatial risk factors for 10 measures of AR exposure. Only significant results are shown, including the ß coefficient, standard error, t, and *p*-value. A positive ß coefficient indicates a positive association, while a negative ß indicates a negative association.

Predictor	Outcome	ß	se	t	*p*
Sex (reference: male)	Difethialone exposure (yes, no)	0.61	0.21	−2.94	0.008
	Difethialone (μg/g)	−0.11	0.05	−2.29	0.032
Distance from vineyards ($n = 24$ caracals)	Total residues (μg/g)	−0.11	0.03	−3.88	0.001
	Brodifacoum (μg/g)	−0.10	0.02	−5.92	<0.001
	Difenacoum exposure (yes, no)	−0.08	0.03	−2.54	0.019
	Difenacoum (μg/g)	−0.004	0.002	2.54	0.019
Proportion vineyards in home range ($n = 7$ radio-collared caracals)	Total residue (μg/g)	14.46	3.85	3.76	0.020
	Brodifacoum (μg/g)	12.46	2.50	4.98	0.008
	Difenacoum (μg/g)	0.52	0.14	3.76	0.020

to other work in North America, where, for example, mountain lions (*Puma concolor*, Beier et al., 2010) and coyotes (*Canis latrans*; Gehrt and Riley, 2010) had positive correlations between AR residues and the percent urban area in home ranges. In Australia, a similar trend was observed in the Southern Boobook (*Ninox boobook*) – a geographically widespread owl species (Lohr, 2018). Rather, in GCT caracals, proximity to vineyards and the proportion of vineyards in home ranges was the primary land use (that we measured) associated with AR exposure. This finding was particularly unexpected because (in addition to above): 1) vineyards represent a relatively small proportion of the total study area (Fig. 1) and home ranges (mean = 6.0%), and 2) we spent substantial time in every vineyard within the Cape Peninsula (see Fig. 1) during fieldwork and we never observed baits near the vines, even when in fruit.

Rather than being a principal source of AR contamination, vineyards may act as a corridor between wildlife and urban-derived ARs. For example, caracals are extremely adaptable to different types of human disturbance (Tambling et al., 2018), but they do not frequently move through dense urban areas (Serieys, unpublished data). We have, however, regularly observed GPS-collared caracals, including those within this study, to forage in vineyards. All vineyards in GCT have associated hospitality enterprises including restaurants, delis, tasting rooms, bed and breakfasts or private residential accommodations. The vineyards are incentivized to ensure that rodents do not enter tourist and commercial nodes. We have observed black bait boxes adjacent to vineyard-based hospitality structures, as is typically seen elsewhere across GCT in urbanized areas. Vineyards may thus not contaminate the environment with ARs more so than other land uses but rather provide a landscape accessible to caracals where they can be exposed to urban-derived ARs.

While vineyards provide active foraging ground for predatory species such as caracals, residential areas are substantially more difficult to access. Cape Town ranks 15th amongst the 50 most dangerous cities in the world (worldatlas.com, accessed 2018). Residential areas frequently employ common “fortress” security measures that may entail robust concrete walls or electric fencing surrounding homes in entirety. In this environment, we rarely observe GPS-collared caracals to forage in residential areas although species filling similar ecological niches in North America do forage within and near urban areas (Riley et al., 2010; Smith et al., 2016). The effect may be that some residential neighborhoods buffer AR environmental contamination if the landscape is less permeable to wildlife. Future studies in GCT could focus on AR exposure in avian or small arboreal predators (raptors or genets less deterred by fencing) to discern more readily the ecological risks of residential AR use.

4.3. Potential pathways of exposure

With the exception of Cape Clawless otters, the species we tested for AR exposure are known to frequently prey on rodent species (Avenant and Nel, 2002; Ogada and Kibuthu, 2009; Widdows and Downs, 2015) that could be the targets of local pest control campaigns. Further, species such as caracal and the Cape eagle owl are obligate, but opportunist, predators that have diets dominated by small mammal species that are most abundant on a local scale (Avenant and Nel, 2002; Ogada and Kibuthu, 2009; Leighton and Serieys, unpublished data). Caracals have an extremely diverse diet, and locally we have observed them prey on other carnivores such as water mongoose and large-spotted genets (Serieys and Leighton, unpublished data). As is suggested for other obligate predatory species (Riley et al., 2007; Serieys et al., 2015a), we believe that AR exposure would only occur via secondary or tertiary routes in caracal, Cape eagle owls, Cape Clawless otters, and water mongoose.

Large spotted genets are more generalist omnivores that thrive within urban areas (Widdows and Downs, 2015). Therefore, they may consume hand-broadcast baits directly if they forage in and around houses, especially in attics and roofs where they are reported to frequently shelter (Widdows and Downs, 2016). Further, large-spotted genets may also be exposed via secondary routes including rodents and invertebrates. ARs are considered nontoxic to invertebrates (Pain et al., 2000) and after feeding on baits for extended periods, they can retain lethal AR levels (Johnston et al., 2005). Invertebrates are therefore increasingly recognized as potential AR vectors (Alomar et al., 2018; Dowding et al., 2010; Elliott et al., 2014; Johnston et al., 2005; Masuda et al., 2015). In one urban genet population, diet was largely comprised of cockroaches and small mammals that included *Rattus* sp. (Widdows and Downs, 2015), both of which may have frequent contact with AR baits. Further, while our large-spotted genet sample size was small, they also seem able accumulate high levels of AR residues without apparent signs of toxicity, suggesting that genets, similar to some felids (Fraser et al., 2018) may be tolerant to the vitamin-K antagonistic effects of ARs.

We were surprised to detect ARs in 50% of the cape clawless otters that inhabit marine and freshwater systems. We suspect that otters may also be secondarily exposed via invertebrate prey, or alternatively, directly exposed via contaminated water. While previous studies have documented AR exposure in European otters (*Lutra lutra*) in France (Fournier-Chambrillon et al., 2004; Lemarchand et al., 2010), contamination was attributed to the consumption of rodents poisoned during pest control operations in nearby riparian systems (Fournier-Chambrillon et al., 2004). To our knowledge, rodent control operations do not occur in GCT riparian areas. Thus, we suspect that otters are exposed via contaminated waterways as has been documented in other systems (Kotthoff et al., 2018). Otters inhabiting GCT access rivers and wetlands primarily to forage for their principle freshwater prey, the freshwater crab *Potamonautes perlatus*. Many of these water bodies are in close proximity to both formal and informal settlements (Okes, 2017) where pesticides and anticoagulants are regularly hand-broadcast (Nattrass et al., 2018; Okes, 2017). Pesticides and pollutants, including ARs, can collect in freshwater systems through storm water run-off and sewage spills (Kotthoff et al., 2018; Okes, 2017). ARs have also been detected in marine vertebrates and invertebrates after island rodent eradication programs, suggesting that the poison baits can travel via surface runoff into coastal marine systems where they are ingested

by species at varying trophic levels (Masuda et al., 2015). While Cape Clawless otter residue concentrations were low, we detected exposure to multiple compounds revealing how pervasive these poisons are in the urban landscape and that the degree of use may lead to freshwater and marine contamination. For aquatic species such as otters that are exposed to multiple pollutants, even low levels of AR residues may have profound adverse health impacts if they interact with other stressors or contaminants.

4.4. Consequences of exposure

Species vary in their sensitivity to the anticoagulant effects of ARs (Erickson and Urban, 2004; Fraser et al., 2018). Some species, vulnerable to the anti-clotting effects, die directly of exposure (Erickson and Urban, 2004) while others are tolerant to the Vitamin K antagonistic effects of ARs and can withstand sustained sublethal exposure (Fraser et al., 2018; Serieys et al., 2018). Either way, ARs can be an important contributor to mortality in populations. Mounting evidence shows that anticoagulants promote both immune suppression and inflammation in multiple species (Aleksandrov et al., 2015, 2017, 2018; Belij et al., 2012; Fraser et al., 2018; Popov et al., 2013; Serieys et al., 2018). In a free-ranging urban bobcat population in southern California USA, ARs are linked with immune dysregulation and notoedric mange which together were associated with a population decline (Fraser et al., 2018; Riley et al., 2007; Serieys et al., 2015a, 2018) that resulted in a genetic bottleneck (Serieys et al., 2015b). Ongoing work in this region has shown that ARs can therefore have a catastrophic impact on overlapping wildlife populations through direct and indirect mortality (Beier et al., 2010; Gehrt and Riley, 2010; Riley et al., 2010; Serieys et al., 2015a)

Pesticide exposure may also interfere with the reproductive success of wildlife which poses a serious conservation threat if poisons ultimately reduce fitness (Berny, 2007; Hindmarch and Elliott, 2018; Riley et al., 2014). Maternal-fetal transfer of ARs can occur, and in regions with pervasive AR contamination, chronic exposure in predatory species can begin before birth (Serieys et al., 2015a). AR exposure also increases the probability of miscarriage, fetal toxicosis, fetal congenital deformities, and decreased sperm counts in humans (Ginsberg and Hirsh, 1989), dogs (*Canis familiaris*, Munday and Thompson, 2003), and sheep (*Oves aries*, Robinson et al., 2005). We sampled two lactating caracal females and two juveniles (both < 6 months old) that were all exposed to multiple AR compounds. As documented elsewhere (Gabriel et al., 2012; Serieys et al., 2015a), ARs in GCT may impact species from early stages of development and persist for the duration of an animal's life.

4.5. Conservation and management implications

Our geographic area of study is small. Yet, the Greater Cape Town area (GCT) is considered one of the "world's great centers of terrestrial biodiversity" (World Heritage Center, 2004), and our findings likely reflect conditions across many urban and peri-urban agricultural areas of South Africa. Worldwide, anticoagulant rodenticides are increasingly recognized for both directly and indirectly posing a conservation threat to non-target wildlife, including endangered species (e.g., Cypher et al., 2014; Gabriel et al., 2012, 2018). While we infrequently detected AR toxicosis, opportunistic sampling of carcasses biases detection rates, and often underestimates frequency and amounts of exposure as well as the frequency of toxicity-induced mortality (Berny, 2007). The bioaccumulation of the poisons we documented suggests that apex predators to the east and north of GCT, such as leopards (*Panthera pardus*) with IUCN status "vulnerable" (Stein et al., 2016), may also be exposed to ARs through secondary or tertiary poisoning. Our results highlight a novel threat faced by numerous wildlife species living within and adjacent to a large metropole in South Africa. Our results stress the urgent need for more intensive research that investigate prevalence and potential species declines associated with exposure. For example, Cape Eagle and Spotted Eagle (*Bubo africanus*) owls are rarely observed in the most heavily populated regions of Cape Town and their absence has been anecdotally attributed to rodenticide exposure. Further work on ARs will enable conservation managers and officials to better align biodiversity conservation and development priorities in the region. Moreover, targeted research and mitigation may be needed to resurrect and sustain wildlife populations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at: https://doi.org/10.1016/j.scitotenv.2019.02.122.

References

Albert, C.A., Wilson, L.K., Mineau, P., Trudeau, S., Elliott, J.E., 2010. Anticoagulant rodenticides in three owl species from Western Canada, 1988–2003. Arch. Environ. Contam. Toxicol. 58, 451–459. https://doi.org/10.1007/s00244-009-9402-z.

Aleksandrov, A., Mirkov, I., Ninkov, M., Mileusnic, D., Demenesku, J., Subota, V., Kataranovski, D., Kataranovski, M., 2018. Effects of warfarin on biological processes other than haemostasis: a review. Food Chem. Toxicol. 113, 19–32. https://doi.org/10.1016/j.fct.2018.01.019.

Aleksandrov, A., Mirkov, I.L., Ninkov, M., Mileusnic, D., Kataranovski, D.M., 2017. Oral warfarin intake affects skin inflammatory cytokine responses in rats. Environ. Toxicol. Pharmacol. 54, 93–98.

Aleksandrov, P.A., Tusup, M., Mirkov, I., Djokic, J., Ninkov, M., Zolotarevski, L., Kataranovski, D., Kataranovski, M., 2015. Proinflammatory cytokine responses in skin and epidermal cells following epicutaneous administration of anticoagulant rodenticide warfarin in rats. Cutan. Ocul. Toxicol. 34, 149–155. https://doi.org/10.3109/15569527.2014.928307.

Alomar, H., Chabert, A., Coeurdassier, M., Vey, D., Berny, P., 2018. Accumulation of anticoagulant rodenticides (chlorophacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*. Sci. Total Environ. 610, 576–582.

Avenant, N., Nel, J., 2002. Among habitat variation in prey availability and use by caracal *Felis caracal*. Mamm. Biol. 67, 18–33.

Beier, P., Riley, S.P.D., Savjajot, R., 2010. Mountain lions (*Puma concolor*). In: Gehrt, S., Riley, S.P.D., Cypher, B. (Eds.), Urban Carnivores. John Hopkins University Press, Baltimore, MD, pp. 141–155.

Belij, S., Miljković, D., Popov, A., Subota, V.V., Timotijevic, G., Slavic, M., Mirkov, I., Kataranovski, D., Kataranovski, M., 2012. Effects of subacute oral warfarin administration on peripheral blood granulocytes in rats. Food Chem. Toxicol. 50, 1499–1507.

Berny, P., 2007. Pesticides and the intoxication of wild animals. J. Vet. Pharmacol. Ther. 30, 93–100. https://doi.org/10.1111/j.1365-2885.2007.00836.x.

Berny, P., Gaillet, J.R., 2008. Acute poisoning of red kites (*Milvus milvus*) in France: data from the SAGIR network. J. Wildl. Dis. 44, 417–426.

Calenge, C., 2006. The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. Ecol. Model. 197, 516–519.

Christensen, T., Lassen, P., Elmeros, M., 2012. High exposure rates of anticoagulant rodenticides in predatory bird species in intensively managed landscapes in Denmark. Arch. Environ. Contam. Toxicol. 63, 437–444. https://doi.org/10.1007/s00244-012-9771-6.

Cox, P., Smith, R., 1992. Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behavior. Proceedings of the 15th Vertebrate Pest Conference. University of California, Davis, pp. 164–170.

Crowe, D.M., 1972. The presence of annuli in bobcat tooth cementum layers. J. Wildl. Manag. 36, 1330. https://doi.org/10.2307/3799278.

Cypher, B., McMillin, S., Westall, T., Van Horn Job, C., Hosea, R., Finlayson, B.J., 2014. Rodenticide exposure among endangered kit foxes relative to habitat use in an urban landscape. C.A.T.E. 7, 1–21.

Dowding, C., Shore, R., Worgan, A., Baker, Harris, S., 2010. Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). Environ. Pollut. 158, 161–166.

Eason, C.T., Murphy, E.C., Wright, G., Spurr, E.B., 2002. Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. Ecotoxicology 11, 35–48. https://doi.org/10.1023/A:1013793029831.

Elliott, J., Hindmarch, S., Albert, C., Emery, J., Mineau, P., Maisonneuve, F., 2014. Exposure pathways of anticoagulant rodenticides to nontarget wildlife. Environ. Monit. Assess. 186, 895–906. https://doi.org/10.1007/s10661-013-3422-x.

Elmeros, M., Christensen, T., Lassen, P., 2011. Concentrations of anticoagulant rodenticides in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) from Denmark. Sci. Total Environ. 409, 2373–2378.

Elmeros, M., Lassen, P., Bossi, R., Topping, 2018. Exposure of stone marten (*Martes foina*) and polecat (*Mustela putorius*) to anticoagulant rodenticides: effects of regulatory restrictions of rodenticide use. Sci. Total Environ. 612, 1358–1364.

Erickson, W., Urban, D., 2004. Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach. United States Environmental Protection Agency Report http://pi.ace.orst.edu/search/getDocketDocument.s?document=EPA-HQ-OPP-2006-0955-0005, Accessed date: 14 November 2014.

Fournier-Chambrillon, C., Berny, P.J., Coiffier, O.B.P., Dasse, B., Delas, G., Galineau, H., Mazet, A., Pouzenc, P., Rosoux, R., Fournier, P., 2004. Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: implications for conservation of European mink (*Mustela lutreola*). J. Wildl. Dis. 40, 688–695.

Franklin, A.B., Carlson, P.C., Rex, A., Rockweit, J.T., Garza, D., Culhane, E., Volker, S.F., Dusek, R.J., Shearn-Bochsler, V.I., Gabriel, M.W., Horak, K.E., 2018. Grass is not always greener: rodenticide exposure of a threatened species near marijuana growing operations. BMC. Res. Notes 11, 94.

Fraser, D., Mouton, A., Serieys, L., Cole, S., Carver, S., VandeWoude, S., Lappin, M., Riley, S.P., Wayne, R.K., 2018. Genome-wide expression reveals multiple systemic effects associated with detection of anticoagulant poisons in bobcats (*Lynx rufus*). Mol. Ecol. 27, 1170–1187.

Gabriel, M.W., Diller, L.V., Dumbacher, J.P., Wengert, G.M., Higley, J.M., Poppenga, R.H., Mendia, S., 2018. Exposure to rodenticides in Northern Spotted and Barred Owls on remote forest lands in northwestern California: evidence of food web contamination. Avian. Conserv. Ecol. 13. https://doi.org/10.5751/ACE-01134-130102.

Gabriel, M.W., Woods, L.W., Poppenga, R.H., Sweitzer, R.A., Thompson, C., Matthews, S.M., Higly, J.M., Keller, S.M., Purcell, K., Barrett, R.H., Wengert, G.M., Sacks, B.N., Clifford, D.L., 2012. Anticoagulant rodenticides on our public and community lands: spatial distribution of exposure and poisoning of a rare forest carnivore. PLoS One 7, e40163.

Geduhn, A., Jacob, J., Schenke, D., Keller, B., 2015. Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). PLoS One 10, e0139191. https://doi.org/10.1371/journal.pone.0139191.

Gehrt, S.D., Riley, S.P.D., 2010. Coyotes (*Canis latrans*). In: Gehrt, S., Riley, S.P.D., Cypher, B. (Eds.), Urban Carnivores. John Hopkins University Press, Baltimore, MD, pp. 78–95.

Ginsberg, J., Hirsh, J., 1989. Risks to the fetus of anticoagulant therapy during pregnancy. Annu. Rev. Med. 40, 79–86.

Güneralp, B., Lwasa, S., Masundire, H., Parnell, S., Seto, K.C., 2017. Urbanization in Africa: challenges and opportunities for conservation. Environ. Res. Lett. 13, 015002.

Hindmarch, S., Elliott, J., Mccann, Levesque, P., 2017. Habitat use by barn owls across a rural to urban gradient and an assessment of stressors including, habitat loss, rodenticide exposure and road mortality. Landsc. Urban Plan. 164, 1320143.

Hindmarch, S., Elliott, J.E., 2018. Ecological factors driving uptake of anticoagulant rodenticides in predators. In: van den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife, Emerging Topics in Ecotoxicology. Springer, Cham, Switzerland, pp. 229–258 https://doi.org/10.1007/978-3-319-64377-9_6.

Hoare, J.M., Hare, K.M., 2006. The impact of brodifacoum on non-target wildlife: gaps in knowledge. New Zeal. J. Zool. 157–167.

Housing Development Agency, 2013. The Housing Development Agency Report 2012/2013. Department of Human Settlements, Republic of South Africa http://thehda.co.za.

Huang, A.C., Elliott, J.E., Hindmarch, S., Lee, S.L., Masonneuve, F., Bowes, V., Cheng, K.M., Martin, K., 2016. Increased rodenticide exposure rate and risk of toxicosis in barn owls (*Tyto alba*) from southwestern Canada and linkage with demographic but not genetic factors. Ecotoxicology 25, 1061–1071.

Johnston, J., Pitt, W., Sugihara, R.T., Eisemann, J.D., Primus, T.M., Holmes, M.J., Crocker, J., Hart, A., 2005. Probabilistic risk assessment for snails, slugs, and endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. Environ. Toxicol. Chem. 24, 1557–1567.

Kie, J., Matthiopoulos, J., Fieberg, J., Powell, R.A., Cagnacci, F., Mitchell, M.S., Gaillard, J.M., Moorcroft, P.R., 2010. The home-range concept: are traditional estimators still relevant with modern telemetry technology? Philos. Trans. R. Soc. B 365, 2221–2231. https://doi.org/10.1098/rstb.2010.0093.

Kinzig, A.P., McShane, T.O., 2015. Conservation in Africa: exploring the impact of social, economic and political drivers on conservation outcomes. Environ. Res. Lett. 10, 090201.

Koivisto, E., Santangeli, A., Koivisto, P., Korkolainen, T., Vuorisalo, T., Hanski, I., Loivamaa, I., Koivisto, S., 2018. The prevalence and correlates of anticoagulant rodenticide exposure in non-target predators and scavengers in Finland. Sci. Total Environ. 642, 701–707.

Kotthoff, M., Heinz, R., Jurling, H., Severin, K., Hennecke, S., Friesen, A., Koschorreck, J., 2018. First evidence of anticoagulant rodenticides in fish and suspended particulate matter: spatial and temporal distribution in German freshwater aquatic systems. Environ. Sci. Pollut. Res. 1-11. https://doi.org/10.1007/s11356-018-1385-8.

Laakso, S., Suomalainen, K., Koivisto, S., 2010. Literature Review on Residues of Anticoagulant Rodenticides in Non-target Animals. Nordic Council of Ministers TemaNord, Copenhagen.

Lemarchand, C., Rosoux, R., Berny, P., 2010. Organochlorine pesticides, PCBs, heavy metals, and anticoagulant rodenticides in tissues of Eurasian otters (*Lutra lutra*) from upper Loire River catchment (France). Chemosphere 80, 1120–1124.

Lohr, M.T., 2018. Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. Sci. Total Environ. 643, 134–144.

Lopez-Perea, J.J., Camarero, P.R., Molina-Lopez, R.A., Parpal, L., Obon, E., Sola, J., Mateo, R., 2015. Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain. Sci. Total Environ. 511, 259–267.

Masuda, B., Fisher, P., Beaven, 2015. Residue profiles of brodifacoum in coastal marine species following an island rodent eradication. Ecotoxicol. Environ. Saf. 113, 1–8.

Masuda, B., Fisher, P., Jamieson, I.G., 2014. Anticoagulant rodenticide brodifacoum detected in dead nestlings of an insectivorous passerine. New Zeal. J. Ecol. 38, 110–115.

McDonald, R., Harris, S., Turnbull, G., Brown, P., Fletcher, M., 1998. Anticoagulant rodenticides in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) in England. Environ. Pollut. 103, 17–23.

McGill, B.J., Dornelas, M., Gotelli, N.J., Magurran, A.E., 2015. Fifteen forms of biodiversity trend in the Anthropocene. TREE 30, 104–113.

McMillin, S., Hosea, R., Finlayson, Cypher, B.L., Mekebri, A., 2008. Anticoagulant rodenticide exposure in an urban population of San Joaquin kit fox. Proceedings of the 23rd Vertebrate Pest Conference. University of California, Davis, pp. 163–165.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403–405.

Munday, J.S., Thompson, L.J., 2003. Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol. 40, 216–219.

Nattrass, N., Stephens, J., Loubser, J., 2018. The Rat Trap: Contestation Over Rodent Control in Cape Town (Center for Social Science Research Working Paper No. 410). Institute for Communities and Wildlife in Africa, Center for Social Science Research Working Paper. Institute for Communities and Wildlife in Africa.

Ogada, D., Kibuthu, P., 2009. Impacts of agriculture on the diet and productivity of Mackinder's Eagle Owls (*Bubo capensis mackinderi*) in Kenya. Biotropica 41, 485–492. https://doi.org/10.1111/j.1744-7429.2009.00498.x.

Ogada, D.L., 2014. The power of poison: pesticide poisoning of Africa's wildlife. Ann. N. Y. Acad. Sci. 1322, 1–20. https://doi.org/10.1111/nyas.12405.

Okes, N.C., 2017. Conservation ecology of Cape Clawless Otters, *Aonyx capensis*, in an Urban Environment (PhD Dissertation). University of Cape Town.

Pain, D., Brooke, M., Finnie, J., Jackson, 2000. Effects of brodifacoum on the land crab of Ascension Island. J. Wildl. Manag. 64, 380–387.

Poessel, S., Breck, S., Fox, K., Gese, E., 2015. Anticoagulant rodenticide exposure and toxicosis in coyotes (*Canis latrans*) in the Denver metropolitan area. J. Wildl. Dis. 51, 265–268. https://doi.org/10.7589/2014-04-116.

Popov, A., Belij, S., Subota, V., Zolotarevski, L., Mirkov, I., Kataranovski, D., Kataranovski, M., 2013. Oral warfarin affects peripheral blood leukocyte IL-6 and TNFα production in rats. J. Immunotoxicol. 10, 17–24. https://doi.org/10.3109/1547691X.2012.684159.

R Development Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/.

Riley, S.P.D., Boydston, E., Crooks, K., Lyren, L., 2010. Bobcats (*Lynx rufus*). In: Gehrt, S., Riley, S.P.D., Cypher, B. (Eds.), Urban Carnivores. John Hopkins University Press, Baltimore, MD, pp. 121–138.

Riley, S.P.D., Bromley, C., Poppenga, R.H., Uzal, F.A., Whithed, L., Sauvajot, R.M., 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban Southern California. J. Wildl. Manag. 75, 1874–1884. https://doi.org/10.2193/2005-615.

Riley, S.P.D., Serieys, L.E.K., Moriarty, J.G., 2014. Infectious disease and contaminants in urban wildlife: unseen and often overlooked threats. In: McCleery, R.A., Moorman, C.E., Peterson, M.N. (Eds.), Urban Wildlife Conservation: Theory and Practice. Springer, pp. 175–215 https://doi.org/10.1007/978-1-4899-7500-3_10.

Robinson, M.H., Twigg, L.E., Wheeler, S.H., Martin, G.R., 2005. Effect of the anticoagulant, pindone, on the breeding performance and survival of merino sheep, *Ovis aries*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 140, 465–473. https://doi.org/10.1016/j.cbpc.2004.11.011.

Ruiz-Suárez, N., Henríquez-Hernández, L.A., Valerón, P.F., Boada, L.D., Zumbado, M., Camacho, M., Almeida-González, M., Luzardo, O.P., 2014. Assessment of anticoagulant rodenticide exposure in six raptor species from the Canary Islands (Spain). Sci. Total Environ. 485, 371–376. https://doi.org/10.1016/j.scitotenv.2014.03.094.

Ruiz-Suárez, N., Melero, Y., Giela, A., Henríquez-Hernández, L.A., Sharp, E., Boada, L.D., Taylor, M.J., Camacho, M., Lambin, X., Luzardo, O.P., Hartley, G., 2016. Rate of exposure of a sentinel species, invasive American mink (*Neovison vison*) in Scotland, to anticoagulant rodenticides. Sci. Total Environ. 569, 1013–1021. https://doi.org/10.1016/j.scitotenv.2016.06.109.

Sanchez-Barbudo, I.S., Camarero, P.R., Mateo, R., 2012. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Sci. Total Environ. 420, 208–280.

Schroeder, M.A., Robb, L.A., Braun, C., 2005. Criteria for gender and age. In: Silvy, N.J. (Ed.), Techniques for Wildlife Investigations and Management. The Wildlife Society Bethesda, MD, pp. 303–338.

Serieys, L.E.K., Armenta, T., Moriarty, J., Boydston, E., Lyren, L., Poppenga, R., Crooks, K., Wayne, R., Riley, S.P.D., 2015a. Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study. Ecotoxicology 24, 844–862. https://doi.org/10.1007/s10646-015-1429-5.

Serieys, L.E.K., Foley, J., Owens, S., Woods, L., Boydston, E.E., Lyren, L.M., Poppenga, R.H., Clifford, D.L., Stephenson, N., Rudd, J., Riley, S.P.D., 2013. Serum chemistry, hematologic, and post-mortem findings in free-ranging bobcats (*Lynx rufus*) with notoedric mange. J. Parasitol. 100, 989–996. https://doi.org/10.1645/12-175.1.

Serieys, L.E.K., Lea, A., Epeldegui, M., Armenta, T.C., Moriarty, J.S., Carver, S., Foley, J., Wayne, R., Riley, S.P.D., Uittenbogaart, C., 2018. Urbanization and anticoagulant poisons promote immune dysfunction in bobcats. Proc. R. Soc. B 285, 20172533.

Serieys, L.E.K., Lea, A., Pollinger, J.P., Riley, S.P.D., Wayne, R.K., 2015b. Disease and freeways drive genetic change in urban bobcat populations. Evol. Appl. 8, 75–92. https://doi.org/10.1111/eva.12226.

Smith, J.A., Wang, Y., Wilmers, C.C., 2016. Spatial characteristics of residential development shift large carnivore prey composition. J. Wildl. Manag. 80 (6), 1040–1048.
Stein, A., Athreya, V., Balme, G., Henschel, P., Karanth, U., Miquelle, D., 2016. *Panthera pardus*. The IUCN Red List of Threatened Species.
Stone, W.B., Okoniewski, J.C., Stedelin, J.R., 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. J. Wildl. Dis. 35, 187–193.
Tambling, C., Avenant, N., Drouilly, M., Melville, H., Kerley, G., Wilson, S., Balfour, D., 2018. The role of mesopredators in ecosystems: potential effects of managing their populations on ecosystem processes and biodiversity. In: Kerley, G., Wilson, S., Balfour, D. (Eds.), Livestock Predation and Its Management in South Africa: A Scientific Assessment. Centre for African Conservation Ecology, Nelson Mandela University, Port Elizabeth, South Africa, pp. 205–227.
Tensen, L., Drouilly, M., van Vuuren, B., 2018. Genetic structure and diversity within lethally managed populations of two mesopredators in South Africa. J. Mammal. 127, 1–11. https://doi.org/10.1093/jmammal/gyy127.
Thomas, P., Mineau, P., Shore, R., Champoux, L., Martin, P.A., Wilson, L.K., Fitzgerald, G., Elliott, J., 2011. Second generation anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. J. Env. Int. 37, 914–920. https://doi.org/10.1016/j.envint.2011.03.010.
van den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A., 2018. Anticoagulant rodenticides and wildlife: introduction. In: van den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife, Emerging Topics in Ecotoxicology. Springer, Cham, Switzerland, pp. 1–9 https://doi.org/10.1007/978-3-319-64377-9_6.
Vandenbroucke, V., Bosquet-Melou, A., De Backer, P., Croubels, S., 2008. Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. J. Vet. Pharmacol. Ther. 31, 437–445. https://doi.org/10.1111/j.1365-2885.2008.00979.x.
Waddell, L.S., Poppenga, R.H., Drobatz, K.J., 2013. Anticoagulant rodenticide screening in dogs: 123 cases (1996–2003). J. Am. Vet. Med. Assoc. 242, 516–521.
Walter, W., Fischer, J., Baruch-Mordo, S., VerCauteren, K.C., 2011. What is the proper method to delineate home range of an animal using today's advanced GPS telemetry systems: the initial step. In: Krejcar, O. (Ed.), Modern Telemetry. InTechOpen, London, pp. 249–268.
Widdows, C., Downs, C., 2016. Urban roost temperatures of large-spotted-genets: the effect of anthropogenic structures. J. Therm. Biol. 57, 66–71.
Widdows, C., Downs, C.T., 2015. A genet drive-through: are large spotted genets using urban areas for “fast food”? A dietary analysis. Urban Ecosyst. 18, 907–920. https://doi.org/10.1007/s11252-015-0438-8.
World Heritage Center, 2004. Properties Inscribed on the World Heritage List. UNESCO World Heritage Convention, Paris, France http://whc.unesco.org/en/list, Accessed date: 13 March 2018.