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Silent killers? The widespread exposure of predatory nocturnal birds to anticoagulant rodenticides

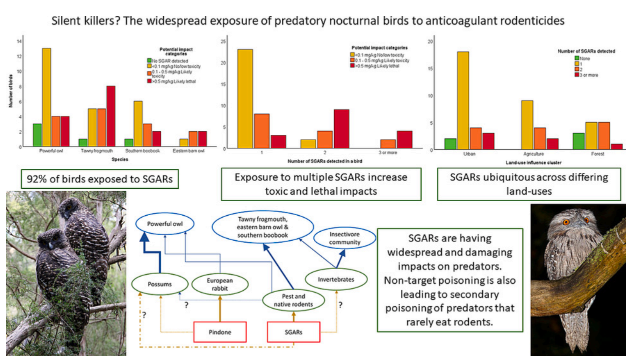
Raylene Cooke [a,*], Pam Whiteley [b], Clare Death [c], Michael A. Weston [a], Nicholas Carter [a], Kieran Scammell [a], Kaori Yokochi [a], Hao Nguyen [d], John G. White [a]

[a] *Deakin University, Geelong, School of Life and Environmental Sciences, Faculty of Science, Engineering and the Built Environment, 221 Burwood Highway, Burwood 3125, Vic., Australia*
[b] *Wildlife Health Victoria: Surveillance, Melbourne Veterinary School, The University of Melbourne, 250 Princes Highway, Werribee 3030, Vic., Australia*
[c] *Melbourne Veterinary School, The University of Melbourne, 250 Princes Highway, Werribee, Vic., Australia*
[d] *National Measurement Institute, 1/153 Bertie Street, Port Melbourne 3207, Vic., Australia*

HIGHLIGHTS

- Anticoagulant rodenticides were detected in 92 % of nocturnal avian predators.
- Multiple rodenticide exposures in an individual increased the likelihood of mortality.
- Predators with largely non-rodent diets were also heavily exposed to rodenticides.
- Predators from different landscape types were exposed to rodenticides.
- Regulation of rodenticides is critical for the conservation of native predators.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) influence predator populations and threaten the stability of ecosystems. Understanding the prevalence and impact of rodenticides in predators is crucial to inform conservation planning and policy. We collected dead birds of four nocturnal predatory species across differing landscapes: forests, agricultural, urban. Liver samples were analysed for eight ARs: three First Generation ARs (FGARs) and five SGARs (Second Generation ARs). We investigated interspecific differences in liver concentrations and whether landscape composition influenced this. FGARs were rarely detected, except pindone at low concentrations in powerful owls *Ninox strenua*. SGARs, however, were detected in every species and 92 % of birds analysed. Concentrations of SGARs were at levels where potential toxicological or lethal impacts would have occurred in 33 % of powerful owls, 68 % of tawny frogmouths *Podargus strigoides*, 42 % of southern boobooks *N. bookbook* and 80 % of barn owls *Tyto javanica*. When multiple SGARs were detected, the likelihood of potentially lethal concentrations of rodenticides increased. There was no association between landscape composition and SGAR exposure, or the presence of multiple SGARs, suggesting rodenticide poisoning is ubiquitous across all landscapes sampled. This widespread human-driven contamination in wildlife is a major threat to wildlife health. Given the

* Corresponding author.
E-mail address: raylene.cooke@deakin.edu.au (R. Cooke).

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high prevalence and concentrations of SGARs in these birds across all landscape types, we support the formal consideration of SGARs as a threatening process. Furthermore, given species that do not primarily eat rodents (tawny frogmouths, powerful owls) have comparable liver rodenticide concentrations to rodent predators (southern boobook, eastern barn owl), it appears there is broader contamination of the food-web than anticipated. We provide evidence that SGARs have the potential to pose a threat to the survival of avian predator populations. Given the functional importance of predators in ecosystems, combined with the animal welfare impacts of these chemicals, we propose governments should regulate the use of SGARs.

1. Introduction

Humans have had a long and challenging relationship with pest rodents throughout history (Singleton et al., 2001; Stenseth et al., 2003). A small number of commensal rodents, mainly in the genera *Rattus* and *Mus* have caused extensive crop damage globally and continue to threaten food security and the health and wellbeing of human populations (Macdonald et al., 1999; Mason and Litten, 2003; McDonald and Harris, 2000; Randall, 1999; Stenseth et al., 2003). The commensal habits of rodents has also led to conflict in urban settings, requiring a considerable focus on control (Himsworth et al., 2013; Parsons et al., 2017). As urbanisation and anthropogenic land-uses have expanded globally, so too has the conflict with commensal rodents. Consequently, there has been a long history of attempting to manage rodent pests to limit their damage and impacts (Whitmer, 2022).

Refinement and development of management approaches saw the emergence of warfarin in the late 1940s, a first-generation anticoagulant rodenticide (FGAR) (Hadler and Buckle, 1992). Anticoagulant rodenticides (ARs) work by blocking the Vitamin K cycling pathway, inhibiting the blood clotting processes, which ultimately results in internal haemorrhaging when a high enough dose is reached (Meehan, 1984; Radostits et al., 1999; Thijssen, 1995). FGARs require multiple feeds over several days by the rodent to cause death (Rattner et al., 2014); and in some cases, this has led to bait avoidance issues (Takeda et al., 2016). While revolutionary for the control of rodents, increasing resistance to warfarin (Buckle et al., 1994; Fisher et al., 2019) forced the development of other FGARs such as chlorophacinone and diphacinone and more potent single feed anticoagulant rodenticides in the form of second-generation anticoagulant rodenticides (SGARs) (Clark, 1978). SGARs such as brodifacoum and bromadiolone have had strong uptake for the management of rodent populations globally because of their extremely high potency (Erickson and Urban, 2004; Jacob and Buckle, 2018). Although a single feed is generally enough to provide a lethal dose of these poisons, the animals will usually not die for at least 4–5 days, a period during which more poison can be consumed and exposure time to predation extended (Eason et al., 2002; Mason and Litten, 2003). Along with their high potency, SGARs also have a relatively long latent period in the body, and concerns have been raised about the risks of SGARs bioaccumulating and magnifying in the tissues of predators (Eason et al., 2002; Newton et al., 1990; Oliva-Vidal et al., 2022; Rattner et al., 2014). Additionally, there may be subclinical impacts associated with lower concentrations of both FGARs and SGARs on fitness, reproduction, and immune function (Rattner et al., 2014; Vyas et al., 2022).

There is a growing body of literature documenting increases in secondary poisoning across multiple species, particularly predators, and non-target poisoning associated with rodenticides. Most of this literature pertains to Europe and North America (Nakayama et al., 2019) with relatively limited investigation into the ecological impacts of rodenticides in the southern hemisphere, with the notable exception of New Zealand (e.g., Eason et al., 2001, 2002; Littin et al., 2002) and several recent studies in Australia (e.g., Lohr, 2018).

Given the high dependency on rodenticides, and limited regulation associated with their use in many southern hemisphere countries such as Australia (Pay et al., 2021), there is an urgent need to investigate how prevalent rodenticides are in ecosystems. Several recent studies in Australia report high concentrations of rodenticide in predatory bird species. Lohr (2018) documented concerning levels of exposure to SGARs in southern boobook owls (*Ninox boobook*) in Western Australia and demonstrated a positive relationship with the degree of urbanisation, suggesting that poisons were potentially entering the food chain from domestic settings. Pay et al. (2021) found high SGAR concentrations in Tasmanian wedge-tailed eagles (*Aquila audax fleayi)* and demonstrated a link with proximity to agriculture and increasing human population density. Further to this, Pay et al. (2021) suggested that there may be a broader contamination of Australian food chains as wedge-tailed eagles do not primarily prey upon rodents, indicating non-rodent species may be consuming rodenticides and acting as vectors of ARs to predators. Cooke et al. (2022) found high exposure of powerful owls (*Ninox strenua*) to SGARs, particularly brodifacoum, and demonstrated this poisoning was widespread across differing human landscapes. Like the findings from Tasmanian wedge-tailed eagles, Cooke et al. (2022) highlighted that the exposure pathway for powerful owls is unlikely to be via rodents and more probably via possum species, ultimately suggesting accidental or deliberate poisoning of endemic possums, particularly in urban settings. These studies offer strong evidence that secondary poisoning in predators is more prevalent than previously considered in Australian ecosystems and has highlighted a substantial knowledge gap in our understanding of the prevalence, route of transfer and impact of rodenticides in Australia. Exposure of endemic Australian rodents to ARs is also not well understood.

This lack of understanding has meant that regulations on the use of rodenticides including the highly potent and bioaccumulating SGARs have remained minimal in Australia compared to many northern hemisphere countries. Although rodenticide use in Australian agricultural settings is largely restricted to around buildings and storage facilities, in urban settings rodenticides are available to the public to control urban rodent problems with relatively few restrictions. These products can be purchased at major retail stores including supermarkets and used in and around homes and buildings, creating a pathway for urban wildlife to potentially be exposed to either accidental or deliberate non-target poisoning, or secondary poisoning from rodenticides. There is limited consumer awareness of these risks, including the need for monitoring and removal of sick and dead poisoned rodents as part of integrated pest management. Australia is also one of the most urbanised nations in the world, and the urban footprint is continuing to expand (United Nations, 2023) suggesting that rodenticide exposure and prevalence in ecosystems could increase if exposure is primarily coming from urban settlements.

The key target rodent species for control in Australia are three invasive introduced species: house mouse (*Mus musculus*), black rat (*Rattus rattus*), and in some areas, brown rats (*R. norvegicus*). Brown rats have a more restricted distribution than black rats and house mice, mainly being found in close proximity to city centres (Adams et al., 2023), near waterbodies and on some offshore islands (Seebeck and Menkhorst, 2000). Black rats, however, are extremely common in urban settings and around buildings in agricultural settings, with recent research also highlighting their strong utilisation of bushland habitats (Adams et al., 2023). In Australia, black rats have also been identified as major pests in some agricultural settings where they require extensive control e.g., macadamia crops (White et al., 1997; White et al., 1998). House mice in Australia are highly eruptive resulting in plague years where they impact agricultural productivity (Singleton et al., 2005) and cause concern among the urban-dwelling public. All three species are largely nocturnal in their behaviour, and as such poisoned rodents may pose an elevated risk to nocturnal predators. In Australian ecosystems, many top-order mammalian predators have declined appreciably, particularly in close proximity to urban and agricultural settings. This has led to a situation where the community of native nocturnal predators capable of consuming rodents in urban and agricultural settings is largely comprised of birds, as such, the community of nocturnal avian predators may have increased susceptibility to secondary poisoning involving rodenticides. In addition to the likely greater exposure rates, predatory bird species are also thought to be more sensitive to ARs than other birds (Herring et al., 2017; Rattner and Harvey, 2021; Rattner et al., 2012).

In this study we investigate the prevalence of rodenticides in four Australian nocturnal bird species: powerful owl, southern boobook, eastern barn owl (*Tyto javanica*) and tawny frogmouth (*Podargus strigoides*). Eastern barn owls and southern boobooks are both raptor species that primarily prey upon small mammals such as rats and mice (Heywood and Pavey, 2002; McDowell and Medlin, 2009; McNabb, 2002; Morton, 1975; Trost et al., 2008). Powerful owls are a much larger raptor and feed primarily on larger arboreal marsupials such as possums and gliders (Cooke et al., 2006; Kavanagh, 1988; Pavey, 1992). Tawny frogmouths, on the other hand, are not a raptor species and feed primarily on insects, frogs and spiders but will take the occasional mouse (Rose and Eldridge, 1997). All four species can reside in urban, agricultural, and forested environments. The three owl species are rarer and at much lower densities than the tawny frogmouth, which is considered common in urban landscapes with moderate tree densities (Weaving et al., 2011; Weaving et al., 2014; Weaving et al., 2016).

Recent research (Cooke et al., 2022) found the presence of rodenticides in dead powerful owls. Given that powerful owls rarely eat rats or mice, it raised serious questions as to the extent of exposure of nocturnal birds to rodenticides, particularly the SGARs commonly used to manage commensal rats and mice. Our aims therefore were to:

1. Determine the prevalence and concentration of rodenticides in four nocturnal bird species;
2. Establish whether differing land-use composition (urban, agricultural and forest) results in different levels of exposure of nocturnal birds to rodenticides; and
3. Based on findings, suggest management options to reduce exposure of nocturnal birds to rodenticide poisoning.

We predicted that eastern barn owls and southern boobooks will have higher prevalence and concentrations of rodenticides than powerful owls and tawny frogmouths due to their respective typical diets. Eastern barn owl and southern boobook diets comprise largely of small mammals, thus increasing their potential for exposure. Powerful owl and tawny frogmouth diets, on the other hand, generally don't include many small mammals, and therefore their potential for exposure should be much lower. We also predicted that prevalence and exposure to rodenticides will be more concentrated in birds from areas with greater urban and agricultural land-use compared to those from more forested landscapes.

2. Materials and methods

2.1. Study site and sample collection

Sixty liver tissue samples were collected from carcasses of nocturnal birds found opportunistically: eastern barn owl $n = 5$; southern boobook $n = 12$; tawny frogmouth $n = 19$ and powerful owl $n = 24$ (18 of the powerful owl samples were reported previously in Cooke et al., 2022). Samples were collected across the Australian state of Victoria except for two eastern barn owl samples from South Australia and one powerful owl sample from New South Wales. Forty-one of the samples were collected between 2020 and 2022 with the other 19 samples collected between 2003 and 2019. Samples were collected opportunistically by the authors, members of the public, animal shelters, Deakin University students and veterinary surgeries. A wide campaign of emails, media and social media was used to alert the public to the study and to call for specimens. Data recorded for each specimen included the species, date when it was found and where possible, a location. While all species are present in areas where humans reside and visit, they are rare species (apart from tawny frogmouths) and finding dead individuals is relatively uncommon, thus limiting the capacity for large sample sizes.

2.2. Tissue samples

All carcasses were collected and frozen at −20 °C at Deakin University prior to delivery to Melbourne Veterinary School at The University of Melbourne for pathological examination and sample collection. Most samples were not fresh and often arrived frozen, limiting any histopathological analysis, and compromising the ability to detect and diagnose bruising and haemorrhage caused by rodenticide intoxication (Stroud, 2012). Liver samples were collected from each bird and placed into clean plastic jars, weighed, and stored at −20 °C for rodenticide analysis.

2.3. Toxicological screening

All liver samples were analysed at the Australian Government's accredited laboratory the National Measurement Institute (NMI) with accredited methods for determination of rodenticides in liver samples. Prior to delivery to NMI, liver samples from all birds were macerated to a smooth paste and stored at −20 °C. All equipment was thoroughly cleaned between samples to limit any potential of cross contamination (See Supplementary 1 for instrument parameters, reagents and standards used).

Each of the 60 liver samples were then screened for residues of eight rodenticides that are available for use in Australia, three FGARs (warfarin, coumatetralyl and pindone) and five SGARs (bromadiolone, brodifacoum, flocoumafen, difenacoum and difethialone). Two grams of liver sample from each bird was weighed in a 50 ml analytical tube. The sample was homogenised with 5 ml of Milli Q water followed by vigorous shaking on a horizontal shaker for 5 min. The sample was further extracted with 10 ml of 5 % formic acid in acetonitrile. The tube was shaken for an additional 30 min. Agilent EN-QuEChERS extraction salts were added to the tube, and the tube was shaken for 2 min before being centrifuged at 5100 rpm for 10 min at 2 °C. 3 ml of the supernatant was pipetted into a 15 ml analytical tube, 5 ml of hexane was added, and the tube was shaken for 2 min then centrifuged for 10 min at 5100 rpm. The hexane layer was removed using a vacuum pipette and discarded. A 1 ml aliquot of the supernatant was carefully transferred to a 2 ml QuEChERS dispersive tube. The sample was vortexed for 10 s then shaken vigorously on the horizontal shaker for 2 min before being centrifuged at 13000 rpm (micro centrifuge) for 3 min. The QuEChERS supernatant was filtered through a 0.45 μm filter. After filtration, 3 μl of coumachlor was added as an internal standard to 497 μl of the filtered extract and vortexed before being transferred to a LCMS-MS vial for analysis.

A Waters TQS Tandem Quadrupole Detector Liquid Chromatograph-Mass Spectrometer (LC-MS/MS) and an ACQUITY UPLC CSH C18 100 × 2.1 mm column were used for detection and quantification of concentrations of each rodenticide. For each analytical batch, a matrix blank, solvent blank, seven points (0.0–0.030 mg/kg) matrix matched calibration and four spike levels (0.001, 0.002, 0.005 and 0.010 mg/kg) were performed to ensure all the required QA and QC were met for the reportable results. Duplicate results were performed for every 10th sample. Recovery rates for each AR were calculated using chicken liver samples spiked with analytical standards. Chicken liver was chosen as it is the closest matrix match available commercially to use for the blank and control. The limits of detection (LOD) were 0.0005 mg/kg for warfarin and coumatetralyl, and 0.001 mg/kg for all other rodenticides, with limits of reporting (LOR) of 0.001 mg/kg for warfarin and coumatetralyl, and 0.005 mg/kg for all other rodenticides. Values below the LOR and above the LOD are reported as trace detections indicating presence but at low concentrations.

2.4. Statistical analysis

For each rodenticide type we calculated the proportion of each species exposed i.e., the proportion of samples over the LOD. Where a particular rodenticide was detected in a species, we also calculated basic summary statistics including the mean, median and standard error of the concentration across samples.

We combined the detected concentrations of all SGARs to produce a total SGAR concentration metric. As all SGARs have a similar mode of action, weight, and potency (Rattner and Harvey, 2021), summing their concentrations can provide an indication of exposure to rodenticides, while acknowledging that each compound differs in terms of impacts on birds. FGARs were not included in this combination as their molecular weights are vastly different (Rattner and Harvey, 2021).

While acknowledging that rodenticide concentrations in liver tissue have been questioned for their capacity to diagnose lethal levels of exposure and that different species of predatory birds have different sensitivities to ARs (Thomas et al., 2011), we used the toxicity thresholds in liver tissue described in Lohr (2018), which were developed based on numerous other studies, as a measure of potential impact. These categories are:

1) Lethal (>0.7 mg/kg);
2) Probably lethal (0.5 - <0.7 mg/kg);
3) Possibly lethal and likely toxic (0.2 - <0.5 mg/kg);
4) Possibly lethal and likely toxic (0.1 - <0.2 mg/kg);
5) Possibly toxic but unlikely lethal (0.01 – <0.1 mg/kg);
6) Probably no toxicity (LOD - <0.01 mg/kg); and.
7) No detected rodenticide (below LOD).

In some cases, it was necessary to combine some of these groupings to account for low sample sizes.

When recording the number of SGARs detected in individual liver samples, we used the categories of: No SGARs detected, one SGAR, two SGARs, and three or more SGARs. A Chi square test of independence was used to investigate any association between species and the number of SGARs detected. Another Chi square test of independence was then undertaken to establish whether there was a relationship between the number of SGARs detected and the impact categorisation based on the total SGAR concentration. For the latter Chi square test, individuals with no exposure ($n = 5$) were removed.

All 60 samples were from areas with some degree of urbanisation but many also include agricultural and forested areas. To categorise the landscape type that each bird came from, we established a 1.5 km buffer around each animal's location in QGIS version 3.16.7 (QGIS.org, 2023). We used a buffered distance of 1.5 km to represent the broad area a bird may have used for foraging, while allowing for characterisation of surrounding landscape influences. Acknowledging that each species may range differently, and that individual home ranges do vary, we based this distance on powerful owl movement data in Bradsworth et al. (2017, 2022) and Carter et al. (2019). Our key objective was not to accurately reflect the area an animal was hunting in, but rather to characterise the composition of the landscape around where the sample was found. We used a Victorian government timeseries landcover layer for 2015–2019 (DELWP, 2023) and defined land cover in each buffer as the proportion of urban, modified open, natural open, modified forest, natural forest, and aquatic land cover types. K-means clustering was then used in R version 4.1.0 (R Core Team, 2022) to establish broad land use groupings ('land use clusters' hereafter) for each bird. Of the 60 birds, we were able to obtain reasonably precise locations and spatial data for 56 of them. Two eastern barns owls from South Australia and a powerful owl from New South Wales were excluded as we did not have comparable land cover layers from these states. Further to this, one powerful owl from urban Melbourne was excluded due to lack of a precise location.

To establish whether landscape type influenced the total SGAR concentrations in birds, we combined all species together due to sample size limitations. While this limited the capacity to establish species-specific trends, it allowed for a larger sample size, thus a more robust indication of whether land-use clusters influence SGAR concentrations in liver samples across the studied bird species. We used a single factor ANOVA to assess any impact of the land-use clusters on total SGAR concentration. Chi square tests of independence were used to investigate any association between the number of SGARs detected and land-use clusters. Correlations were then used to examine the association between the total SGAR concentration and proportions of individual land cover types that were used to define the land use clusters. All statistical tests were conducted using SPSS version 29 (IBM Corporation, 2022).

3. Results

3.1. Prevalence and concentration of rodenticides across species

In 60 liver samples analysed (five eastern barn owls, 12 southern boobooks, 19 tawny frogmouths and 24 powerful owls), at least one AR was detected in 55 birds (92 %), and more than one AR was detected in 21 birds (38 %). FGARs were rarely detected in any species, with no detection of warfarin or coumatetralyl in any species (Table 1). This may largely reflect the rapid breakdown and excretion of FGARs rather than a lack of prior exposure. Pindone, a FGAR largely used for the control of European rabbits (*Oryctolagus cuniculus*) in Australia (Fisher et al., 2015), was detected in 42 % of powerful owls at concentrations at which toxicity is unlikely to occur (range 0.001–0.007 mg/kg ww (wet weight)), again potentially highlighting the more rapid breakdown of FGARs rather than low levels of exposure. On the other hand, SGARs were considerably more prevalent than FGARs, likely reflecting their popularity as well as longer latency in the body than FGARs. Difenacoum was detected in 40 % of eastern barn owls, 11 % of tawny frogmouths and 13 % of powerful owls, but was not detected in any southern boobooks (Table 1). Brodifacoum was detected in 55 of 60 birds (92 %) and was the most prevalent SGAR across all species. Bromadiolone was the second most detected SGAR (19 of 60 birds) and was found in 80 % of eastern barn owls, 17 % of southern boobooks, 32 % of tawny frogmouths and 21 % of powerful owls. Difethialone was not detected in any eastern barn owl or southern boobook but was detected in 16 % of tawny frogmouths and 8 % of powerful owls (Table 1). Flocoumafen was detected in 40 % of eastern barn owls and 5 % of tawny frogmouths, however, was not detected in any of the southern boobook or powerful owl liver samples. Overall, these results indicate SGARs are prevalent across all the species investigated, with brodifacoum being the most encountered SGAR.

Pooling SGAR concentrations allowed for a broad assessment of the potential impact of SGARs. Using 0.5 mg/kg ww of SGARs as a threshold where mortality is likely to occur (Lohr, 2018), we found 4/24 (17 %) powerful owls, 8/19 (42 %) tawny frogmouths, 2/12 (17 %) southern boobooks and 2/5 (40 %) eastern barn owls were likely to have had potentially lethal SGAR concentrations (Fig. 1). Moreover, SGAR concentrations 0.1–0.5 mg/kg ww (likely to be suffering toxicological impacts, Lohr, 2018) were found in 4/24 (17 %) powerful owls, 5/19 (26 %) tawny frogmouths, 3/12 (25 %) southern boobooks, and 2/5 (40 %) eastern barn owls (Fig. 1). Overall, these results suggest SGAR concentrations from a substantial fraction of individuals in all studied species were likely to be having either toxicological or lethal impacts with some variation between species (powerful owl 33 %; tawny frogmouth 68 %; southern boobook 42 %; eastern barn owl 80 %).

Table 1
Summary statistics of rodenticide concentrations of each rodenticide in liver samples of four species of Australian predatory nocturnal birds (2003–2022). Values are based on birds with a detected concentration of each rodenticide. Total SGAR concentrations are based on the summed concentrations of SGARs in each individual bird. ND = not detected; ww = wet weight. Values in the first row for each species represent the number of birds with detected levels versus the number of birds sampled.

	First generation anticoagulant rodenticide			Second generation anticoagulant rodenticide					
	Pindone	Coumatetralyl	Warfarin	Difenacoum	Brodifacoum	Bromadiolone	Difethialone	Flocoumafen	Total SGAR
LOD (mg/kg)	0.001	0.0005	0.0005	0.001	0.001	0.001	0.001	0.001	
Eastern barn owl (n = 5)	0/5(0 %)	0/5(0 %)	0/5(0 %)	2/5(40 %)	5/5(100 %)	4/5(80 %)	0/5(0 %)	2/5(40 %)	5/5(100 %)
Maximum (mg/kg ww)	ND	ND	ND	0.012	0.055	5.414	ND	0.005	5.438
Minimum (mg/kg ww)	ND	ND	ND	0.010	0.001	0.329	ND	0.001	0.031
Mean (mg/kg ww)	ND	ND	ND	0.011	0.020	1.643	ND	0.003	1.340
Median (mg/kg ww)	ND	ND	ND	0.011	0.012	0.414	ND	0.003	0.404
SE (mg/kg ww)	ND	ND	ND	0.001	0.010	1.258	ND	0.002	1.027
Southern boobook (n = 12)	0/12(0 %)	0/12(0 %)	0/12(0 %)	0/12(0 %)	11/12(92 %)	2/12(17 %)	0/12(0 %)	0/12(0 %)	11/12(92 %)
Maximum (mg/kg ww)	ND	ND	ND	ND	1.034	15.092	ND	ND	15.229
Minimum (mg/kg ww)	ND	ND	ND	ND	0.002	0.082	ND	ND	0.002
Mean (mg/kg ww)	ND	ND	ND	ND	0.188	7.587	ND	ND	1.568
Median (mg/kg ww)	ND	ND	ND	ND	0.009	7.587	ND	ND	0.009
SE (mg/kg ww)	ND	ND	ND	ND	0.097	7.505	ND	ND	1.370
Tawny frogmouth (n = 19)	0/19(0 %)	0/19(0 %)	0/19(0 %)	2/19(11 %)	18/19(95 %)	6/19(32 %)	3/19(16 %)	1/19(5 %)	18/19(95 %)
Maximum (mg/kg ww)	ND	ND	ND	0.009	1.014	8.114	0.006	0.001	8.172
Minimum (mg/kg ww)	ND	ND	ND	0.001	0.007	0.082	0.002	0.001	0.007
Mean (mg/kg ww)	ND	ND	ND	0.005	0.273	2.787	0.004	0.001	1.204
Median (mg/kg ww)	ND	ND	ND	0.005	0.154	0.206	0.003	0.001	0.356
SE (mg/kg ww)	ND	ND	ND	0.004	0.073	1.659	0.001	N/A	0.593
Powerful owl (n = 24)	10/24(42 %)	0/24(0 %)	0/24(0 %)	3/24(13 %)	21/24(88 %)	5/24(21 %)	2/24(8 %)	0/24(%)	21/24(88 %)
Maximum (mg/kg ww)	0.007	ND	ND	0.025	0.600	0.654	0.008	ND	0.850
Minimum (mg/kg ww)	0.001	ND	ND	0.001	0.001	0.022	0.005	ND	0.001
Mean (mg/kg ww)	0.004	ND	ND	0.010	0.119	0.266	0.006	ND	0.185
Median (mg/kg ww)	0.004	ND	ND	0.004	0.031	0.043	0.006	ND	0.051
SE (mg/kg ww)	0.001	ND	ND	0.007	0.039	0.144	0.002	ND	0.058

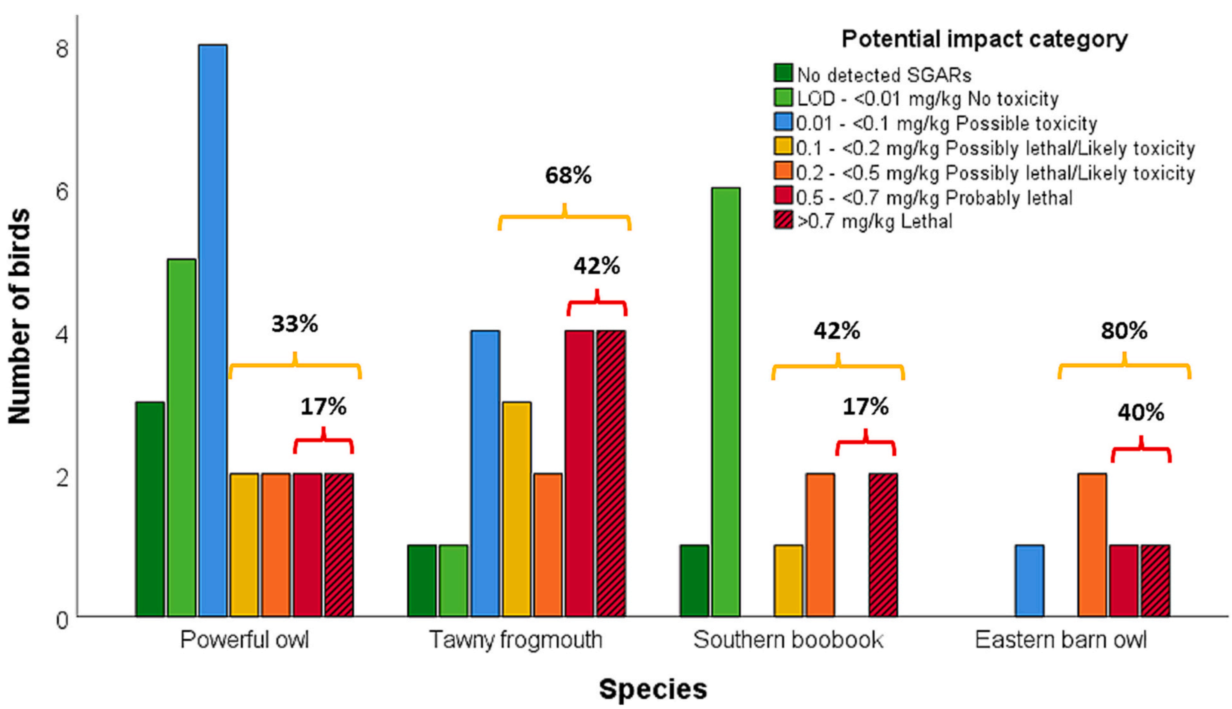

Fig. 1. Total SGAR concentrations for powerful owls, tawny frogmouths, southern boobooks, and eastern barn owls established against Lohr's (2018) potential impact categories. Percentages above the final two categories (red bracket) represent likely lethal outcomes, and across the last four categories (orange bracket) represent likely toxic or lethal outcomes.

The number of SGARs detected in an individual liver sample reflects different exposure events for either the predator or their prey and highlights the use of different toxins in the bird's habitat. Studied bird species showed a consistent trend in the number of SGARs detected ($\chi^2 = 10.147$, DF = 9, $P = 0.339$, $n = 60$). Most species had one rodenticide detected, in each case this was brodifacoum (Fig. 2). Eastern barn owl was the only exception, with detections of two, three or more rodenticides being more common, however, the sample size was too small for this to be reflected as a statistically significant difference.

When the exposure to multiple SGARs and likely impacts of SGARs on birds were examined together, there was a strong relationship between the number of SGARs that the birds were exposed to (excluding

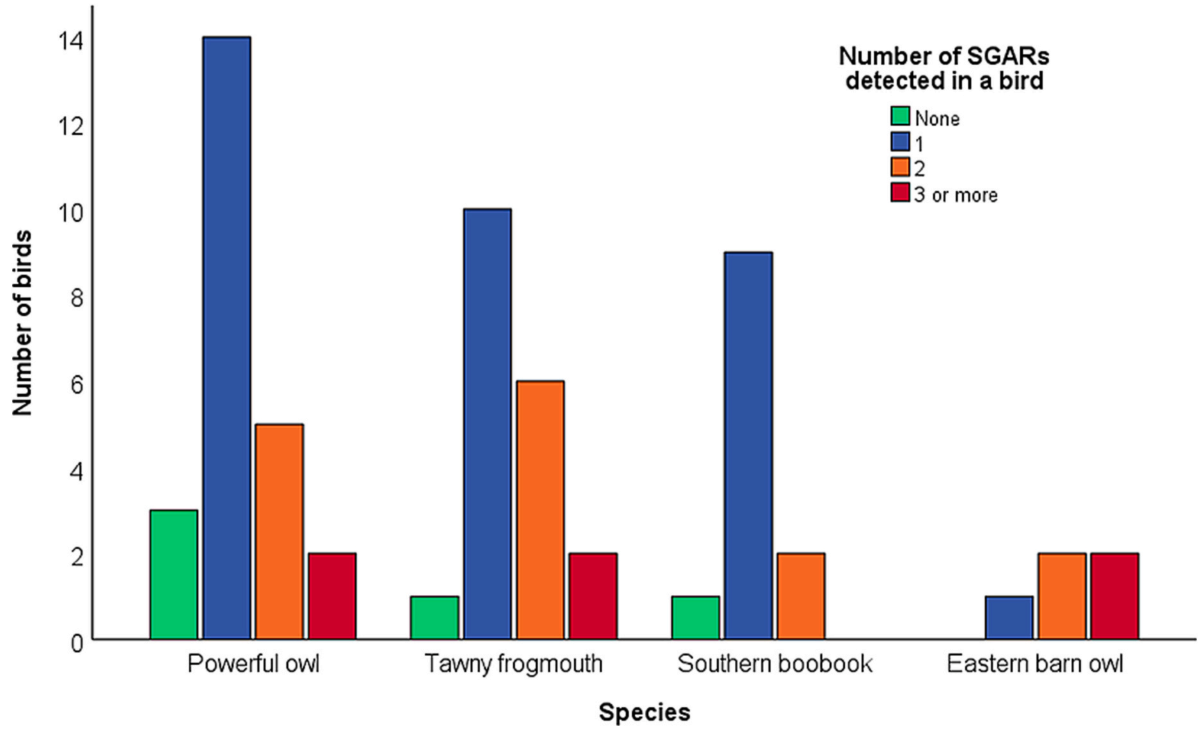

Fig. 2. The number of SGARs detected in individual powerful owls, tawny frogmouths, southern boobooks, and eastern barn owls. Where three or more SGARs were detected in an individual sample we have combined these animals into one grouping.

birds with no exposure) and the potential impact category to which the bird belonged ($\chi^2 = 22.660$, DF = 4, $P < 0.001$). When exposed to a single SGAR, low toxicity impacts (<0.1 mg/kg ww) were more likely to occur and potentially lethal concentrations (>0.5 mg/kg ww) were less likely to occur than expected (adjusted standardized residuals 4.2 and −4.2, respectively). When exposed to two, or three or more SGARs, low toxicity impacts were less likely to occur (adjusted standardized residuals −2.9 and −2.4, respectively), and potentially lethal impacts were more likely to occur than expected (adjusted standardized residuals 3.1 and 2.1, respectively) (Fig. 3). When we excluded birds with

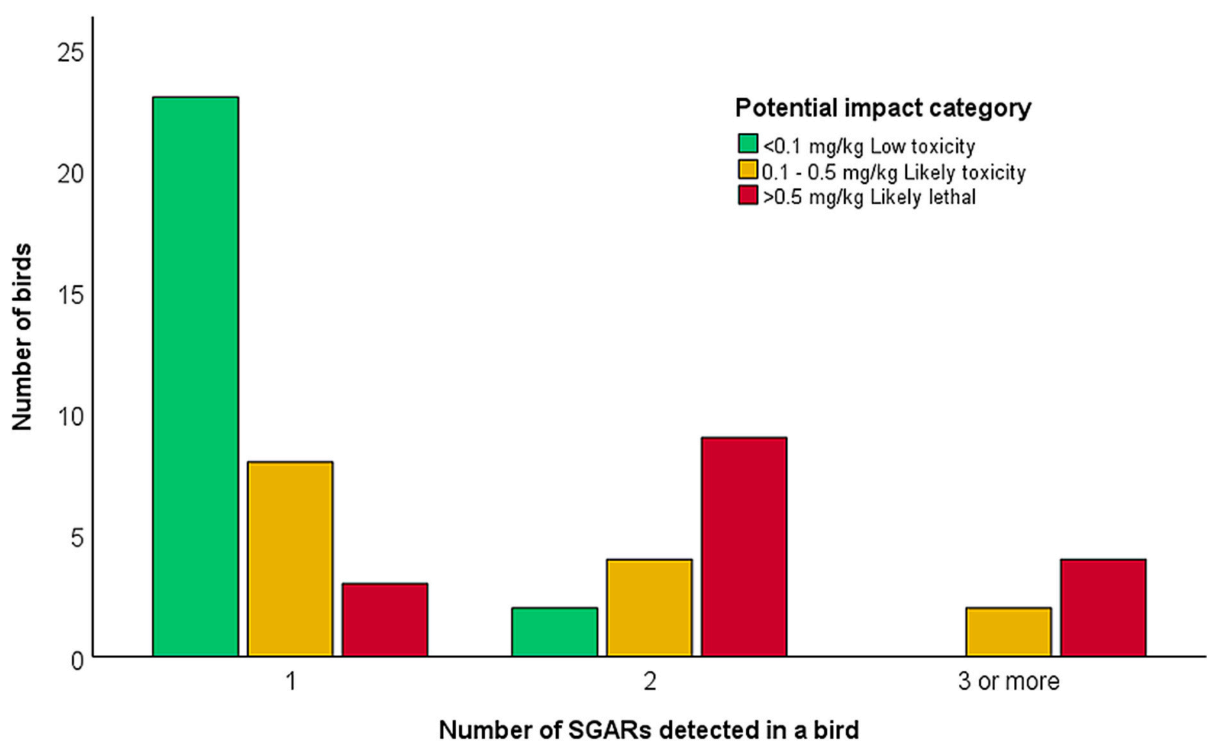

Fig. 3. The relationship between the number of SGARs birds (all species) were exposed to and the potential impact on those birds. Lohr's (2018) potential impact categories have been merged to accommodate low sample sizes. SGAR concentration over 0.5 mg/kg ww are considered likely lethal, between 0.1 and 0.5 mg/kg ww are considered likely toxic, and concentrations below 0.1 mg/kg ww are considered as having no toxicity.

no SGAR exposure, there was a positive correlation between the number of SGARs detected in the sample and the total concentration of SGARs in the sample (all species pooled, $r = 0.409$, $n = 55$, $P = 0.002$, n = 55).

3.2. Prevalence and concentration of rodenticides across land-use types

The K-means cluster analysis indicated that three clusters best defined the landscapes birds were utilizing based on gap statistics and sums of squares. Three of the land cover type variables central to defining the land-use clusters were urban, natural forest and modified open. We defined our land-use clusters and assigned each bird as: urban influenced (means ± standard errors: 80.2 ± 2.9 % urban, 17.7 ± 3.1 % natural forest, 7.2 ± 2.0 % modified open); agricultural influenced (59.4 ± 4.9 % modified open, 21.6 ± 3.7 % natural forest, 7.2 ± 2.0 % urban); or forest influenced (69.8 ± 3.9 % natural forest, 17.7 ± 3.1 % urban, 9.9 ± 1.8 % modified open) (Fig. 4). In total 27 birds were urban influenced, 15 agricultural influenced and 14 forest influenced.

Land-use clusters did not have an influence on the total concentration of SGARs ($F_{(2,53)} = 0.267$, $P = 0.538$) or the number of SGARs detected in birds ($\chi^2 = 7.541$, DF = 6, $P = 0.274$, $N = 56$, Fig. 5) across nocturnal bird species, suggesting that exposure to SGARs is relatively consistent across all land-use clusters. Further to this, when each land cover type was investigated separately, there was no correlation between any of the land cover type proportions and total SGAR concentrations (urban $r = -0.92$, $P = 0.500$, 95 % CI of −0.346–0.175; natural forest $r = 0.029$, $P = 0.830$, 95 % CI of −0.235–0.290; modified open $r = 0.127$, $P = 0.350$, 95 % CI of −0.140–0.378). This adds further support for the suggestion that SGAR exposure is ubiquitous across all land-use types sampled.

4. Discussion

In 1962, Rachel Carson's book “Silent Spring” (Carson, 1962) introduced the world to the impacts of pesticides on humans and non-target species. This catalysed investigations into pesticides such as DDT, which were bioaccumulating in raptors and devastating their populations (Cade et al., 1988; Ratcliffe, 1993). We now face an era where global data is highlighting the devastating impacts of SGARs on numerous non-target species (López-Perea et al., 2015; Lopez-Perea and Mateo, 2018; Olea et al., 2009; Rodríguez-Estival and Mateo, 2019). Our findings indicate potentially major impacts of rodenticides, especially SGARs, on populations of four nocturnal predatory bird species. Detectable concentrations of SGARs were found in 55 of 60 (92 %) birds assessed for liver concentrations of rodenticides. Likely toxicity or potentially lethal concentrations occurred in 33 % of powerful owls, 68 % of tawny frogmouth, 42 % of southern boobook and 80 % of eastern barn owl (based on Lohr's (2018) risk categories for SGARs in liver tissue). Regardless of the concentrations, this remarkably high prevalence highlights considerable movement of these pesticides through the food chain, with the potential for them to act as a threatening process for predator populations (Hofstadter et al., 2021; Pay et al., 2021). Under Australian law, a key threatening process has the potential to cause a threatened species or ecological community to become threatened, or to act on at least two threatened species (DCCEEW, 2023). Broader assessments of the prevalance of SGARs among fauna and ecosystems may reveal that such contamination qualifies as a key threateneing process in some cases. Given the critical ecological role of apex predators, this impact could be catastrophic at the ecosystem level (Ripple et al., 2014; Rodríguez-Estival and Mateo, 2019).

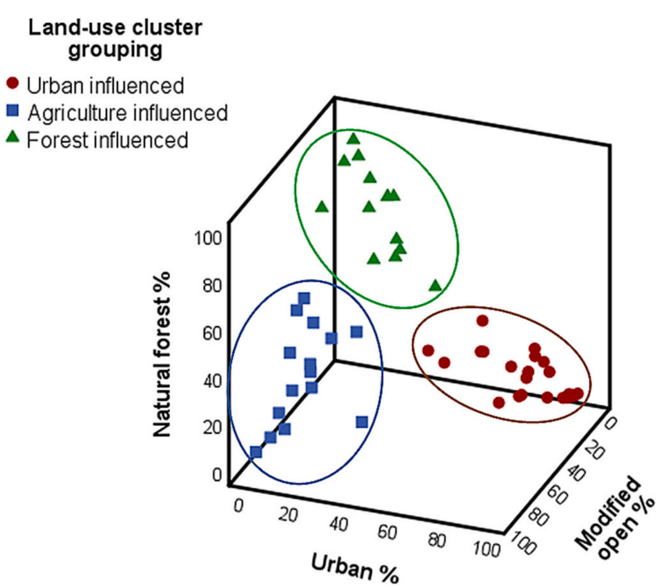

Fig. 4. Land-use influence clusters for birds based on K-means cluster analysis. The clusters are defined by three land cover types (natural forest, urban and modified open).

4.1. First-generation anticoagulant rodenticides

While FGARs, particularly products containing warfarin, are easily available for purchase from hardware stores and supermarkets in Australia, the prevalence of FGARs was low and not detected in the tawny frogmouth, southern boobook, or eastern barn owl. This may reflect a relatively low usage rate, or more likely reflects the short latency period of FGARs in the body (Rattner and Harvey, 2021). Unfortunately, Australia does not have data available on the volume of sales of ARs or their application (Lohr and Davis, 2018) limiting our capacity to link usage with prevalence in wildlife. Interestingly, pindone, a FGAR and the only rodenticide registered in Australia for the management of European rabbits (Eason and Jolly, 1993), was detected in 42 % of powerful owls and was the only FGAR detected in this species. While at low concentrations where toxicity is unlikely, it indicates a potential route via rabbits to powerful owl populations. While powerful owls do not regularly take prey from the ground, there have been limited reports of rabbits in the diet of powerful owls (Tilley, 1982) and the detection of pindone in powerful owls suggests that consumption of rabbits may be more common than previously thought (Fig. 6). Additionally, rabbit management with pindone is likely to be concentrated in areas where rabbits are considered an issue, such as urban fringe areas with large open areas of grassland. There is also the potential that possums may consume pindone coated-carrot baits, transferring pindone to powerful owls that prey on them (Fig. 6). The overall low concentrations of FGARs likely reflect the shorter half-life and reduced bioaccumulation in animals compared to SGARs (Vandenbroucke et al., 2008), and care should be taken when interpreting a lack of detectable FGARs in livers as this does not equal a lack of exposure or impact on an animal.

4.2. Second-generation anticoagulant rodenticides

In contrast, SGARs were detected in very high proportions of liver tissues in all four predatory nocturnal bird species studied (88–100 %). Brodifacoum, by far the most commonly available rodenticide in Australian stores, was detected in 92 % of birds tested. None of the studied species are likely to eat the poison directly, thus their poisoning is considered as secondary poisoning (Fig. 5).

Of particular concern is that the detection of multiple SGARs was common in liver samples we analysed, occurring in 21 of 55 birds where an SAGR was detected (38.2 %). While concerning, the exposure to multiple SGARs is lower in this study when compared to some studies from the USA and Canada with exposure to multiple SGARs occurring in >50 % of birds and as high as 91 % (Elliot et al., 2022; Murray, 2020). As most rodenticide products contain only one active ingredient, detection of multiple SGARs indicate multiple exposure events either for the

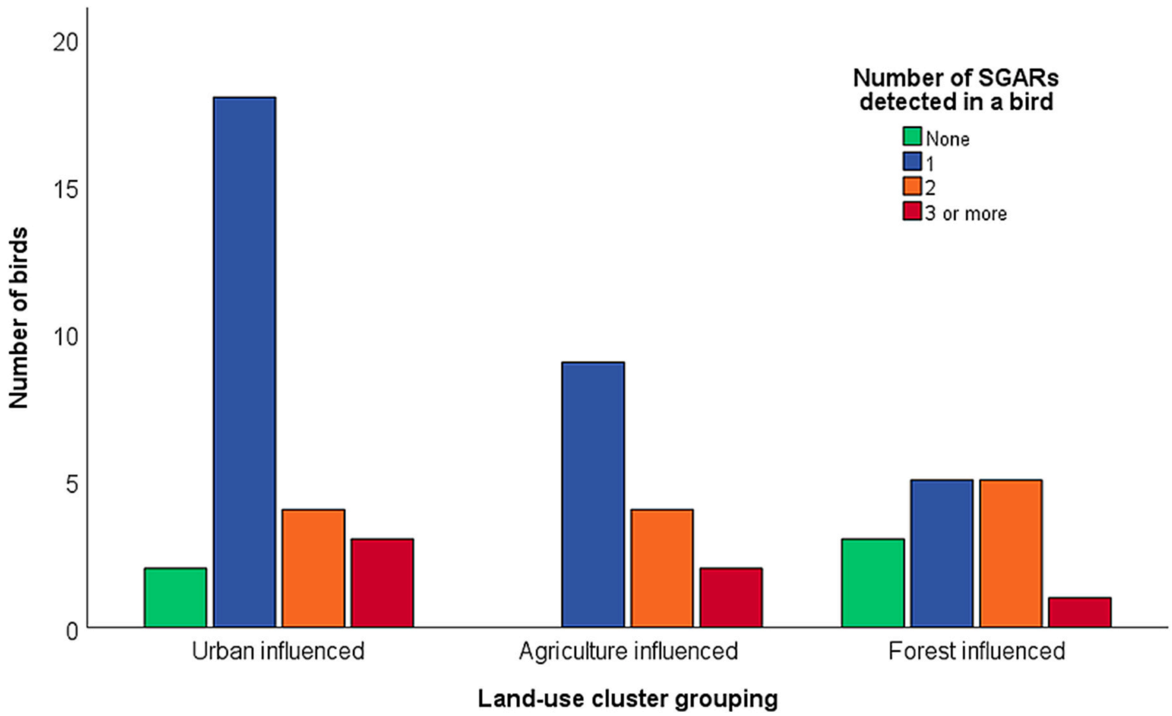

Fig. 5. The number of SGARs detected in individual birds associated with land-use cluster groupings. Data for powerful owl, tawny frogmouth, southern boobook, and eastern barn owl have been merged.

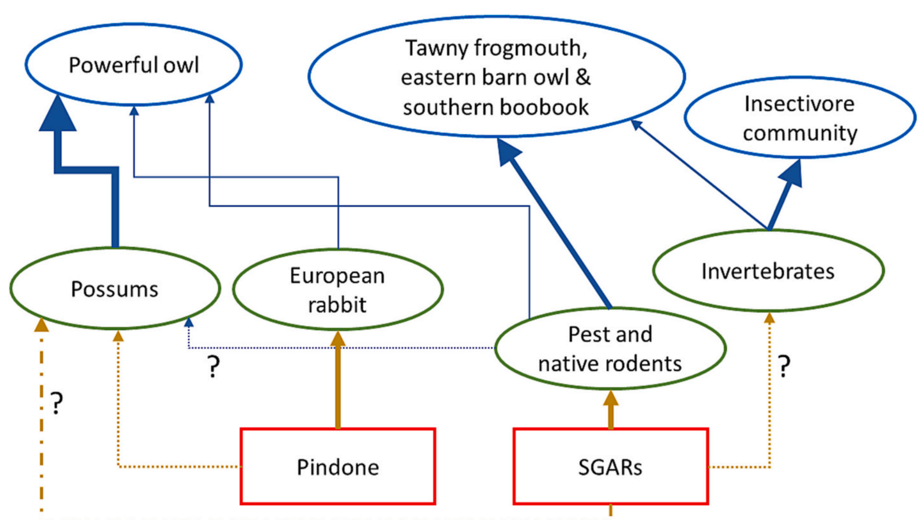

Fig. 6. Potential pathways for pindone and SGARs through the food web. Red boxes indicate the rodenticide, brown lines indicate primary animal exposure routes (solid lines are targets for the poison, dashed lines are likely non-target routes, and dotted lines are speculated). Blue lines indicate potential routes into secondary consumers of rodenticides (wider lines indicate dominant dietary route, dotted lines indicate potential but unknown routes).

predator or their prey, further confirming that secondary poisoning of these four bird species is common. While no data are publicly available on the amount and location of SGAR sales and use in Australia, the majority of baits that are easily available to both domestic households and commercial pest controllers have brodifacoum as their active ingredient (Pay et al., 2021). Given the relatively high rate of brodifacoum use in Australia, it is likely that birds that had more than one SGARs detected in their livers would have had multiple exposures to brodifacoum prior to their exposure to another SGAR.

Contrary to our expectation, all studied species had high proportions of birds with SGAR exposure, even tawny frogmouths and powerful owls, species that are not thought to be major predators of rodents (e.g., Cooke et al., 2006; Rose and Eldridge, 1997). Exposure of tawny frogmouths to SGARs is likely via poisoned mice, which they will consume occasionally even though their diet consists primarily of arthropods (Rose and Eldridge, 1997). A further alternative is that exposure for frogmouths could be via invertebrates that have been exposed to ARs (e.g. Alomar et al., 2018; Hoare and Kelly, 2006). Invertebrate exposure to ARs has never been documented in Australia but should not be discounted as a possible transfer route. If invertebrate communities are exposed to SGARs then it would be probable that the insectivorous animal community could also be exposed to secondary poisoning (Fig. 6). Widespread exposure of powerful owls to SGARs is particularly concerning as it suggests the potential for broader, non-rodent routes of transfer of rodenticides in the food chain. While rodents have been documented in the diet of powerful owls in two studies (Fitzsimons and Rose, 2010; Menkhorst and Loyn, 2005), they were not the dominant dietary items and these two studies were consistent with others, in that the vast majority of powerful owl literature reports medium to large arboreal marsupials as the dominant prey items (Bilney et al., 2011; Chafer, 1992; Cooke et al., 2006; McNabb et al., 2018; Pavey et al., 1994; Seebeck, 1976; Tilley, 1982; Traill, 1993; Van Dyck and Gibbons, 1980). We suspect that possums, particularly common brushtail possums (*Trichosurus vulpecula*), may be exposed to SGARs by directly eating baits in urban settings. In Australia, SGARs are approved for use in and around domestic, commercial, industrial, and agricultural buildings (APVMA, 2023). There are currently no restrictions requiring the use of rodent specific bait stations, as such many rodenticides are sold in packs that can be thrown into roof cavities. The common brushtail possum is common in urban settings, lives in and around households often nesting in roof cavities, and has a broad diet (Adams et al., 2013). They are also known to occasionally eat carrion (Vandersteen et al., 2023) which suggests the potential of another exposure route via eating dead, poisoned rodents (Fig. 5). Although rodenticides have been detected in possums (Grillo et al., 2016; WHA, 2021), there is currently no study that has examined the extent of the exposure of native possum species to SGARs; exploring this potential pathway will lead to a better understanding of how SGARs are reaching this apex predator species.

4.3. The influence of landscape type

Unexpectedly, our findings did not show a clear relationship between the extent of exposure to SGARs (both in types and concentration) and the land-use clusters that a bird came from. This indicates exposure to SGARs is widespread and is occurring across all landscape types at similar rates. It is important to note that none of the sampled birds came from remote areas of pristine forest. This is largely because dead birds are extremely unlikely to be found in areas which people do not often visit. Regardless of the landscape type each bird came from, all birds collected in this study had some degree of urban or agricultural influence in areas they occupied. This supports previous studies that have indicated that domestic and agricultural use of rodenticides may be a major route into raptor populations (Elliot et al., 2022; Lohr, 2018; Lohr and Davis, 2018; López-Perea et al., 2015). Agricultural and urban land-uses substantially increase the proportion of the landscape over which animals are likely to be exposed to rodenticides and potentially adds substantial additional pressures to species surviving in human dominated landscapes (Gabriel et al., 2012; Hofstadter et al., 2021; Thomas et al., 2011; Wiens et al., 2019). While our data do not show major trends against different land-uses, our sample size may have limited our capacity to detect fine scale trends. Further collection of samples over a longer time frame and wider landscapes should occur in Australia to allow for investigation of landscape scale influences.

4.4. Management of SGARs

SGARs, which are designed to effectively kill rodents, are also effective at killing other species via non-target poisoning or indirect, secondary poisoning. Globally, SGARs are increasingly being detected through many food webs, indicating considerable non-target and secondary poisoning (Berny, 2007; Christensen et al., 2012; López-Perea et al., 2015, 2019; Olea et al., 2009; Riley et al., 2014; Rodríguez-Estival and Mateo, 2019; Sainsbury et al., 2018). The high liver concentrations of SGARs detected in this research suggests that in many cases animals are likely to have either died or exhibited toxic effects as a result of secondary poisoning (Vandenbroucke et al., 2008). Given predators are at relatively low densities (Ripple et al., 2014), this additional pressure on populations likely constitutes an additional threatening process that needs to be managed (Rodríguez-Estival and Mateo, 2019). This is particularly the case in human modified landscapes where many species are dealing with the pressures of habitat loss and fragmentation, as well as other anthropogenic impacts such as electrocution, persecution, collision, poisoning and infectious diseases (Battin, 2004; Chace and Walsh, 2006; Di Minin et al., 2016; Tozer et al., 2012). This research has highlighted an alarming issue of rodenticide exposure in wildlife in Australia and provides a further demonstration of the impact of SGARs in human dominated landscapes.

Much of the world is moving to tighten regulations on the availability and use of SGARs (e.g. California Assembly Bill number 1788 (2020)). Throughout the USA, only FGARs are registered as ready-to-use bait station products for the consumer market (USEPA, 2022). In British Columbia, Canada, a licence is required to purchase SGARs, and this is only available to essential services or pest controllers (British Columbia, 2023). In the United Kingdom, the Rodenticide Stewardship Regime was developed and funded by rodenticide user groups and interested agencies, with steering committee representatives from professional pest controllers, rodenticide manufacturers, farmer and gamekeeper groups, government, wildlife groups and universities (CCRU UK, 2023). In Australia and many areas in the southern hemisphere, the public can use anticoagulant rodenticides with few limitations, especially in urban settings. There now exists so much global research highlighting the impacts of rodenticides, particularly SGARs, on species and ecosystems, that their availability for use by the public in many parts of the world seems unjustifiable. Like the use of DDT in previous decades, the use of SGARs has been effective in managing pest species, but at a considerable cost. Management of these poisons will require considerable and concerted actions to limit both non-target poisoning and secondary poisoning. To reduce non-target impacts we suggest regulations associated with the delivery of SGARs (Cooke et al., 2022; Pay et al., 2021). All baits must be delivered in tamper proof, rodent specific bait stations, something that is not currently mandated in Australia and many parts of the world. Secondly, sale of SGARs to the public should also be restricted or banned. Domestic use of rodenticides is increasingly recognised as a key source of non-target and secondary poisoning of wildlife (e.g., Rodríguez-Estival and Mateo, 2019) and is likely further enhanced by homeowners using rodenticides in “off-label” ways. To combat this misuse, the use of SGARs should be restricted to professionally accredited pest managers with very strict use criteria, including the use of rodent specific bait stations. Further to this, restricting the use of rodenticides to indoor areas, with no outdoor applications could help limit interactions of non-target species with rodenticides.

While restricting the use of SGARs is a critical step in reducing their impacts on food webs, it is acknowledged that people still need options to control rodents. We strongly encourage the uptake of integrated rodent management strategies that do not require the use of any ARs as all ARs pose a risk to wildlife. Where the use of a rodenticide is unavoidable, we propose the preferential use of FGARs to manage rodent problems; due to their shorter half-life, they pose a lower risk in the environment than SGARs.

Finally, our research also highlights the need for more routine testing of animals for the presence of rodenticides. Prior to recent research (e.g., Cooke et al., 2022; Lohr, 2018; Pay et al., 2021), almost no public data was available on rodenticide poisoning of wildlife in Australia, and the importance and potential scale of the issue were unknown. There is a need for a national rodenticide stewardship program that includes surveillance in wildlife, similar to that instituted in the United Kingdom (hse.gov.uk), and this could potentially be funded from a levy on product sales as proposed in Elliot et al. (2016). Frameworks for ongoing testing would also allow for the evaluation of the impact of any policy changes around the regulation of SGAR use.

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CRediT authorship contribution statement

Raylene Cooke: Conceptualization, Writing – Original Draft, Writing – Review and Editing, Project administration. **Pam Whiteley:** Conceptualization, Investigation, Writing – Review and Editing. **Clare Death:** Conceptualization, Writing – Review and Editing. **Michael A. Weston:** Writing – Review and Editing. **Nicholas Carter:** Visualization, Writing – Review and Editing. **Kieran Scammell:** Investigation. **Kaori Yokochi:** Writing – Review and Editing. **Hao Nguyen:** Laboratory Analysis, Writing – Review and Editing. **John G. White:** Conceptualization, Writing – Original Draft, Writing – Review and Editing, Formal analysis.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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