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# Widespread exposure of powerful owls to second-generation anticoagulant rodenticides in Australia spans an urban to agricultural and forest landscape

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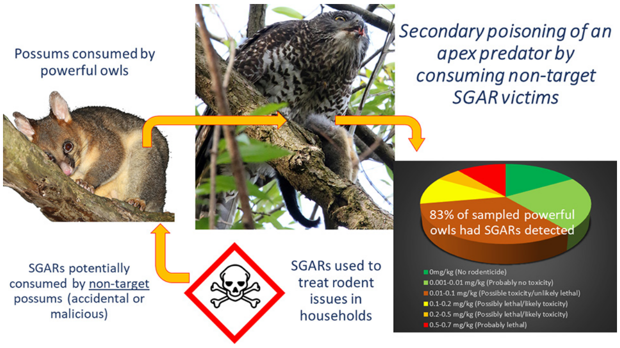
## HIGHLIGHTS

- Powerful owls feed almost exclusively on arboreal marsupials not rodents.
- SGARs were detected in 83.3% of powerful owls.
- Brodifacoum was detected in all owls where a rodenticide was detected.
- Non-target poisoning of possums may lead to secondary poisoning of powerful owls.
- Non-target poisoning may be leading to high prevalence of SGARs in predators.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The powerful owl (*Ninox strenua*) is a threatened apex predator that consumes mainly arboreal marsupial prey. Low density populations reside in urban landscapes where their viability is tenuous. The catalyst for this research was the reported death of eight powerful owls around Melbourne, Australia, in less than one year (2020/2021). Eighteen deceased owls were toxicologically screened. We assessed toxic metals (Mercury Hg, Lead Pb, Cadmium Cd and Arsenic As) and anticoagulant rodenticides (ARs) in liver ($n = 18$ owls) and an extensive range of agricultural chemicals in muscle ($n = 14$). Almost all agricultural chemicals were below detection limits except for p,p-DDE, which was detected in 71% of birds at relatively low levels. Toxic metals detected in some individuals were generally at low levels. However, ARs were detected in 83.3% of powerful owls. The most common second-generation anticoagulant rodenticide (SGAR) detected was brodifacoum, which was present in every bird in which a rodenticide was detected. Brodifacoum was often present at toxic levels and in some instances at potentially lethal levels. Presence of brodifacoum was detected across the complete urban-forest/agriculture gradient, suggesting widespread exposure. Powerful owls do not scavenge but prey upon arboreal marsupials, and generally not rodents, suggesting that brodifacoum is entering the powerful owl food web via accidental or deliberate poisoning of non-target species (possums). We highlight a critical need to investigate SGARs in food webs globally, and not just in species directly targeted for poisoning or their predators.

## 1. Introduction

Apex predators play a vital role in the structure and function of ecosystems through applying top-down pressure on lower order consumers and smaller predators (Ripple et al., 2014; Sergio et al., 2014). As long-

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lived species, with low fecundity and an almost exclusively carnivorous diet, apex predator populations are however at substantial risk from anthropogenic processes (Thinley et al., 2021). With land clearing for agriculture and urbanization occurring at an unprecedented rate globally (McKinney, 2006), the risks to apex predator populations continue to increase. This is particularly the case when apex predators use large home-ranges that encompass a mosaic of land-use types including natural, urbanized, and agricultural landscapes to access their required prey. Human-modified environments contain substantially elevated risks for apex predators such as electrocution, persecution, collision, poisoning and infectious diseases (Chace and Walsh, 2006).

As the human population increases, the nexus between humans and apex predators intensify. The consequent pressures placed on biodiversity through these human/wildlife interactions has led, in many instances, to human-wildlife conflict resulting in either death, disease or serious injury to apex predators. One such example of this is the use of pesticides, which are used to control species that impact human livelihoods or health (Mateo-Tomas et al., 2012; Pay et al., 2021). Although the widespread use of poisons has been heavily regulated in many countries, thousands of non-target animals are unintentionally exposed every year across the globe (Berny, 2007; Christensen et al., 2012; López-Perea et al., 2015; Olea et al., 2009), and in some instances intentional poisoning occurs (Hong et al., 2021). Various anthropogenic processes also increase the exposure of wildlife to heavy metals (Ali and Khan, 2019). The use of pesticides (including rodenticides) and exposure to heavy metals, can have significant and long-term detrimental effects on wildlife populations through bioaccumulation and biomagnification as these contaminants are often persistent in the environment (Grúz et al., 2018). A growing number of studies show almost universal occurrence of toxicants among wild predators inhabiting human modified habitats (Blanco et al., 2004; Gabriel et al., 2012; Helander et al., 2008; Hofstadter et al., 2021; Thomas et al., 2011; Wiens et al., 2019).

The accumulation of heavy metals, urban and agricultural toxicants (hereafter referred to as contaminants) in the environment has been well documented, with many avian predators suffering from their effects (Christensen et al., 2012; Hofstadter et al., 2021; Lohr, 2018; Pay et al., 2021; Shore et al., 2003; Thomas et al., 2011). The transfer through and accumulation of contaminants in food webs is a prominent environmental issue (Sun et al., 2020). Terrestrially feeding raptors are susceptible to the accumulation of metals and synthetic poisons such as ARs (Hofstadter et al., 2021; Wiens et al., 2019) and DDT, as evidenced by its role in the near global decline of peregrine falcons (*Falco peregrinus*) and other raptors (Carson, 1962; Fry, 1995; Grier, 1982; Olsen et al., 1992; Tubbs, 2016). Top order avian predators are both the sentinels of, and vulnerable to, the accumulation of contaminants derived through their diet (Herring et al., 2017; Riley et al., 2007; Thomas et al., 2011). They are therefore a critical group to assess when investigating the prevalence of contaminants in food webs across differing land-uses (Pay et al., 2021). Both sublethal and lethal effects have been documented, which have implications at the individual and population level, influence demographic processes (e.g., reproductive success) and represent welfare and conservation concerns (Hofstadter et al., 2021; Salim et al., 2014; Salim et al., 2015).

A large dietary component of many birds of prey are species often considered pests by humans (Hindmarch and Elliott, 2018), and pests are often controlled using poisons (Pay et al., 2021). Globally, this includes many rodent species that have been deliberately poisoned for decades due to their impacts in agricultural and urban settings. Anticoagulant rodenticides are used commonly around the world to control rodents and second-generation anticoagulant rodenticides (SGARs) are highly persistent, with the potential to cause secondary poisoning in wildlife (López-Perea and Mateo, 2018). The first-generation anticoagulant rodenticides (FGARs) (e.g., warfarin) require multiple feeds to cause death in rodents and are less persistent. The more potent SGARs (e.g., brodifacoum, bromadiolone, difenacoum) require only a single consumption to cause death in rodent species, with the bait and residues remaining in the carcass after death (Rattner et al., 2014). Given many avian predators occupy large home-ranges that often include human dominated land-use types, there is a considerable risk of secondary poisoning of avian predators due to vertebrate pest control in these landscapes (Lohr and Davis, 2018).

Here, we use the powerful owl (*Ninox strenua*) as a case-study to examine the prevalence of contaminants in an apex predator. Powerful owls are one of very few native apex predators still residing in urbanized landscapes in Australia and are of conservation significance. They are highly territorial, non-migratory, and occur at low densities in urban, agricultural, and forested environments. These owls have large home-ranges, with many individual home-ranges encompassing human-modified environments, particularly urban and urban fringe areas that offer sufficient canopy cover and prey (Cooke et al., 2002a; Kavanagh, 2004; Pavey, 1995). Powerful owls are long-lived, top order predators that primarily prey on arboreal native possums and gliders (Cooke et al., 2006). In recent years these owls have been documented more frequently in human-modified environments (e.g., Bradsworth et al., 2017; Carter et al., 2019; Isaac et al., 2014) accompanied by an increase in reported mortalities. We collected the remains of 10 powerful owls between 2004 and 2019. In the year from early 2020 to early 2021 we collected eight dead powerful owls around greater Melbourne. This unprecedented number of dead owls was the catalyst for this research. Specifically, we investigate whether these owls were exposed to environmental contaminants, and whether this could contribute to the increase in detected mortalities.

Human-induced disturbances certainly play a role in increased species mortality and in many cases these risks are well documented (Loss et al., 2015). The presence of toxicants in powerful owl populations, however, is virtually unknown and given its use of human-modified environments, warrants critical evaluation. Specifically, our aims were to:

1. Determine the prevalence of toxicants among powerful owls across an urban-forest/agriculture gradient;
2. Explore whether the degree of urbanization along the urban-forest/agricultural gradient influences the concentrations of toxicants found in owls; and,
3. Consider conservation implications for future management of avian predators in human-modified landscapes.

We predicted heavy metals and agricultural chemicals would be detected in the owls as they are living in increasingly urbanized landscapes. We expected minimal ARs to be present as powerful owls rarely consume rodents. We also predicted that powerful owls from increasingly urban areas or agricultural areas would be exposed to higher levels of toxicants than those from more forested areas of the urban-forest/agriculture gradient.

## 2. Materials and methods

### 2.1. Study site and sample collection

Seventeen tissue samples were removed opportunistically from dead owls collected primarily across Victoria, Australia, with one sample from New South Wales (total, n = 18). Of these, eight were found dead in 2020/2021; 10 had been collected 2004–2019.

All 18 samples were from areas with some urbanization and many also included agricultural and forested areas. To classify the land-use associated with the sampling localities we created a buffer in GIS with a radius of 1.5 km around each collection point, the distance which encompasses most of the area they use while capturing prey (Bradsworth et al., 2017; Carter et al., 2019). We used the 'Vicmap Vegetation 1:25,000' and 'Road Locality' spatial layers (https://www.data.vic.gov.au/), for tree cover and road density analysis. The 'Vicmap Vegetation 1:25,000' layer classes every point of the landscape as either having dense, medium, sparse or no tree cover. As these variables are highly correlated, we only use dense and no tree cover in subsequent analysis. We calculated the linear distance of roads within the 1.5 km buffer and converted this to road length (km) per km$^2$. Higher road densities indicate increasingly urban parts of the landscape (Fig. 1). Of the dead powerful owls analysed in this study, 16 had locational data which was deemed accurate enough to investigate the role of land-use associations with exposure to toxicants.

### 2.2. Tissue samples

Dead powerful owls were collected and frozen in a −20 °C freezer at Deakin University prior to delivery to Melbourne Veterinary School (The University of Melbourne) for pathological examination and sample collection to determine cause of death (if possible) and investigate the presence of any contaminants. Most owls (15/18) underwent a post-mortem examination to assess body condition (observation of pectoral muscle mass and subcutaneous, abdominal and coronary band fat reserves), evidence of trauma (skin damage, fractured bones, haemorrhage), ecto-and internal parasites, and examination of body systems. Most samples arrived frozen, limiting histopathological analysis and potentially compromising the ability to detect and diagnose bruising and haemorrhage caused by rodenticide intoxication (Stroud, 2012). The presence of the bursa of Fabricius and thymic lobes were used to identify immature/juvenile birds. Muscle and liver samples were collected from each owl and placed into clean plastic jars, weighed, and stored at −20 °C for toxicology analysis.

### 2.3. Toxicological screening

Muscle and liver samples were analysed at the Australian Government's National Association of Testing Authorities (NATA) accredited National Measurement Institute (NMI) for a variety of contaminants. Screening was conducted on liver tissue for residues of eight rodenticides (warfarin, coumatetralyl, bromadiolone, brodifacoum, flocoumafen, difenacoum, difethialone and pindone) (n = 18 owls). Rodenticides were quantified in liver samples by first homogenising it with Milli Q water. The sample was then shaken and again extracted with 5% formic acid in acetonitrile. The supernatant was then neutralized by using QuEChERS kits. QuEChERS rapid method was modified to include additional clean-up stages to endure the extract was clean and suitable for Tandem Quadrupole Detector (TQD) Liquid Chromatograph-Mass Spectrometer (LC-MS/MS). Isotopically labelled standards were used to correct for analyte recovery and matrix dependent signal suppression for rodenticides (see Appendix Table A.1 for Limits of Reporting (LOR's)).

The liver tissue of 18 owls was also tested for heavy metals, including arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg). The samples were homogenised, and then digested with concentrated nitric and hydrochloric acids by heating on a digestion block with a temperature controller. Elements were then determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and/or Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

Muscle tissue samples from 14 of these powerful owls were analysed for the presence of multiple agricultural contaminants including fungicides, herbicides, insecticides and pesticides, organophosphates, organochlorines, carbamates, acaricides, phenols and synthetic pyrethroids (see Appendix Table A.1 for full list and LOR's for each). Muscle was used in these analyses due to limited liver sample mass, with the rationale that muscle analysis would detect persistent organic pollutants or contaminants that were at systemically high enough concentrations to have potentially contributed to bird death following an acute exposure event.

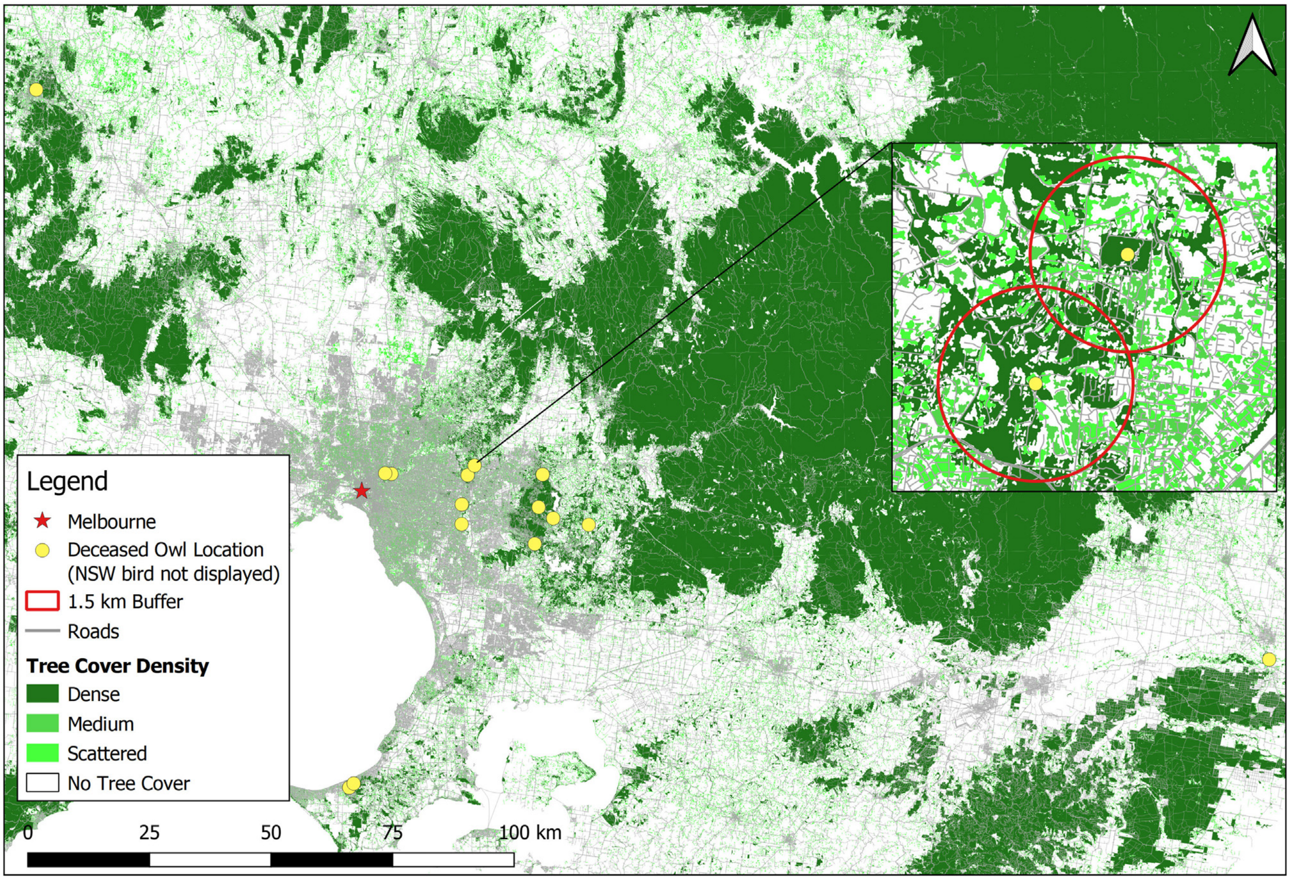

**Fig. 1.** Location of 16 dead powerful owls collected in Victoria (2004–2021). The CBD of Melbourne (star) is surrounded by considerable amounts of grey shading indicating extensive road networks and high degrees of urbanization. The inset shows two powerful owl collection locations and the 1.5 km buffer around each used to define the landscape properties for that location. Note: two dead birds were recovered from the same location and as such are represented by one spot on the map.

A portion of the muscle sample was extracted using a modified QuEChERS extraction technique. Approximately 1 g of homogenised sample was then spiked with internal standard then extracted with 10 ml of acetonitrile (with 2% formic acid). A portion of the solvent was then diluted and cleaned-up by SpinFiltr dSPE Cleanup. A portion of the extract was vortexed in the SpinFiltr cleanup tube containing magnesium sulphate, primary-secondary amine (PSA), C18 and ChloroFiltr sorbents then centrifuged and filtered. Extracts were then analysed by Liquid Chromatograph with Mass Spectrometer detection (LC-MSMS) and Gas Chromatograph with Mass Spectrometer detection (GC-MSMS).

All results from rodenticide, toxic metal and agricultural screening were provided by the NMI as mg/kg wet weight.

### *2.4. Statistical analysis*

To define our sites on a gradient of land-uses associated with urbanization, forest cover and agriculture we utilised a hierarchical cluster analysis and SimProf procedure in PRIMER version 7. We used dense tree cover, no tree cover and road density data in the 1.5 km buffer around each owl location for this analysis. These variables are sensitive to differences in the amount of urbanization, agriculture and forest, and therefore can describe the landscape effectively. The variables were all normalized before generating a resemblance matrix based on Euclidean distances.

Non-metric multidimensional scaling plots were produced to visualise the sites on a gradient of urbanization as well as show clusters identified during the SimProf procedure. Correlation analysis was then conducted to look for trends in key toxicants against axes in the nMDS as well as against dense tree cover, no tree cover and road density. This was conducted to establish if trends in toxicant levels were associated with position along the urban to forest/agriculture gradient.

## 3. Results

Findings from analysis of owl samples are summarized in Appendix Table B.1, highlighting the year and location of collection, age, weight, sex, history of carcass, and gross pathology.

### *3.1. Anticoagulant rodenticides*

Anticoagulant rodenticides were detected in 15 of 18 owls (83.3%) examined in this study. Five of the ARs analysed were not detected in any samples (warfarin, coumatetralyl, difenacoum, difethialone and flucoumafen) (see Appendix Table B.1 for case details). The most frequently detected rodenticide was the SGAR brodifacoum, which was detected in all 15 owls that had detectable rodenticide concentrations. Three owls (year and location: 2019 Fairfield; 2020 Monbulk; 2021 McCrae) had detectable levels of pindone (A FGAR used mainly for the management of rabbits in Australia) and two owls (2011 Longford; 2020 Monbulk) had the SGAR bromadialone detected (Table 1).

All owls which registered a rodenticide detection (n = 15) had detectable levels of the SGAR brodifacoum. Ten had a single AR detected in their livers, four (2011 Longford; 2019 Fairfield; 2021 McCrae (2 birds)) had two types of AR, and one owl (2020 Monbulk) had three different rodenticides detected in its liver.

To investigate the potential toxic or lethal effects of anticoagulant rodenticides, we added the concentrations of all detected SGARs (brodifacoum and bromadialone) in each individual to produce a value for total SGAR concentration. Despite concerns about interpretation of summed AR values (e.g. Rattner and Harvey, 2021) this has been a relatively common approach in the recent, relevant literature (e.g. Lohr, 2018; López-Perea and Mateo, 2018; Pay et al., 2021) and thus provides the ability to compare across studies. The total liver concentrations of SGARs in the 15 samples where SGARs were detected averaged 0.136 mg/kg (range 0.007 to 0.60) with a standard error of 0.052 mg/kg. There is considerable debate as to what levels of SGARs are either toxic or lethal in predatory birds, but here we use Lohr's (2018) categories to describe potential toxicity or lethality of SGARs in owls. Two of the owls (11.1%) in this study had liver concentrations of SGARs over 0.50 mg/kg (2020 Vermont; 2020 Donvale), which Lohr (2018) suggests is the suspected lethal threshold for owls (Fig. 2). If considered from a point where Lohr (2018) proposes that toxic effects may start to manifest and potentially lead to lethal outcomes (liver concentrations over 0.1 mg/kg), 27.8% of birds were potentially affected. In total, 11 of the 18 powerful owls (61.2%) examined had concentrations of SGARs over the level of 0.01 mg/kg, at which Lohr (2018) suggests that toxic effects may be expected to start occurring (Fig. 2). Considering many factors affect concentrations of SGARs in livers including recovery from more substantial SGAR exposure, it is difficult to interpret health impacts. Given many birds were obtained frozen and with substantial trauma it was not possible to assign haemorrhage to particular SGAR concentrations.

### *3.2. Agricultural chemical assessment*

A broad screen for 187 agricultural chemicals detected few contaminants in muscle with the exception of persistent organochlorine pesticides (OCPs), which were detected in 10 of the 14 owls. Of the 25 OCPs analysed, the prevalence of detection was low, with p,p′-DDE (a metabolite of DDT), dieldrin and heptachlor epoxide detected. p,p′-DDE was the most prevalent OCP detected and was found in 10 of 14 (71.4%) owls at an average concentration of 0.654 mg/kg. One owl had a muscle p,p′-DDE concentration of 5.4 mg/kg (2020 Vermont) and for the remaining birds levels were less than the LOR of 0.02 mg/kg (see Appendix Table B.1 for case details).

Dieldrin was detected in 2 of 14 owls (14.3%), at concentrations of 0.054 (2020 Macclesfield) and 0.024 mg/kg (2021 Glen Waverley). Heptachlor epoxide was detected in one owl at 0.039 mg/kg (2020 Vermont). These three powerful owls also had detectable p,p′-DDE.

### *3.3. Heavy metals*

Cadmium and Hg were the most prevalent, Pb was detected in few owls, and As was not detected in any samples. Cadmium was detected in the liver of 17 of 18 (94.4%) owls at an average concentration of 0.048 mg/kg (SE = 0.009 mg/kg) with a maximum of 0.14 mg/kg (2020 Vermont). Mercury was detected in the liver of 14 of 18 owls (72.2%) with an average concentration of 0.087 mg/kg (SE = 0.016 mg/kg) and a maximum of 0.21 mg/kg (2020 Macclesfield). Lead was detected in 3 of 18 owls (16.7%) (2004 Mt. Evelyn; 2020 Donvale; 2020 Vermont) at an average concentration of 0.053 mg/kg (maximum of 0.1 mg/kg ± 0.024 mg/kg SE).

### *3.4. Toxicant distribution along a gradient of urbanization*

To assess how SGARs, p,p′-DDE, Cd and Hg may be associated with the gradient of urbanization to agriculture and forest, we used the dense tree

**Table 1**

Detection of rodenticides in liver samples from 17 dead powerful owls in Victoria and 1 dead owl from New South Wales between 2004 and 2021. Wet weight concentrations are presented (minimum and maximum concentration) with a positive detection above the LOR of 0.005 LOR for the AR. ND = not detected.

| | Warfarin | Coumatetralyl | Brodifacoum | Bromadialone | Difenacoum | Difethialone | Flocoumafen | Pindone |
|---|---|---|---|---|---|---|---|---|
| Percent exposed (number of owls) | 0 | 0 | 83.3 (15) | 11.1 (2) | 0 | 0 | 0 | 22.2 (4) |
| Maximum concentration (mg/kg) | ND | ND | 0.600 | 0.043 | ND | ND | ND | 0.007 |
| Minimum detected concentration (mg/kg) | ND | ND | 0.007 | 0.022 | ND | ND | ND | 0.005 |

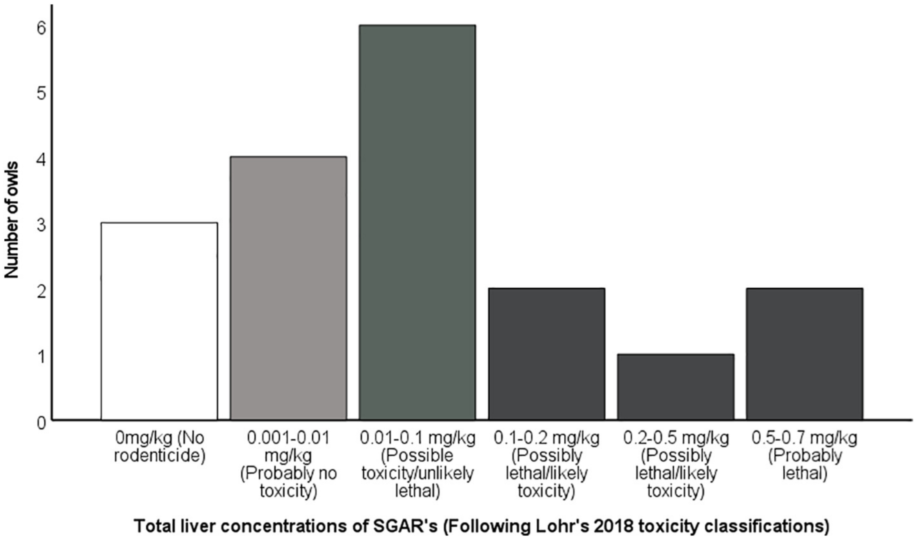

**Fig. 2.** Total liver concentrations of SGARs scored using Lohr's (2018) toxicity classifications for 18 powerful owls examined, 2004–2021. White bars represent no detected rodenticides, light grey bars potential toxic effects only, dark grey bars potential lethal classes.

cover, no tree cover and road density data to categorise our sites. After 999 permutations of the SimProf procedure we identified four distinct clusters of sites. The sites were then plotted using nMDS to show the dispersion of sites along gradients of land-use (Fig. 3). The x-axis of the nMDS represents a gradient in tree cover from areas with large amounts of no tree cover (agriculture and extremely high-density urban areas) through to areas of high dense tree cover (forests) (Fig. 3). The y-axis in the nMDS represents a gradient in road density and as such represents the degree of urbanization ranging from low density urbanization to high density urbanization (Fig. 3). The clusters effectively describe the position of dead owls in the landscape with one cluster representing highly urban birds in areas with high road densities and moderate amounts of dense tree cover, a second cluster represents semi-urban zones with areas of agriculture and forest associated with them, a third cluster represents urban fringe areas with moderate road densities but high dense tree cover, and a final cluster represents urban fringe areas characterised by low road densities and high dense tree cover (Fig. 3).

Total SGAR concentrations (mg/kg) were not associated with either the x-axis of the nMDS ($r_P = -0.157$, $n = 16$, $P = 0.562$) or the y-axis of the nMDS ($r_P = -0.290$, $n = 16$, $P = 0.277$). SGAR concentrations were also not associated with the density of roads ($r_P = -0.350$, $n = 16$, $P = 0.184$), the proportion of the 1.5 km buffer around each owl's location consisting of dense tree cover ($r_P = -0.041$, $n = 16$, $P = 0.879$) and no tree cover ($r_P = 0.084$, $n = 16$, $P = 0.756$). This suggests that position in the urban gradient and indicators of urbanization such as tree cover and road density does not influence the level of exposure of powerful owls to SGARs. Total liver concentrations of SGARs >0.01 mg/kg (the threshold suggested by Lohr, 2018 as potentially having toxic effects) occurred in all habitat groupings revealed in the SimProf analysis except for the high urban group (Fig. 4). There were however only two birds from high urban landscapes. Similarly, there was no association with p,p′-DDE levels in muscle samples and the x-axis of the nMDS ($r_P = -0.169$, $n = 12$, $P = 0.600$) and the y-axis of the nMDS ($r_P = -0.143$, $n = 12$, $P = 0.658$). Levels of p,p′-DDE were not related to road density ($r_P = -0.242$, $n = 12$, $P = 0.449$), the amount of dense tree cover ($r_P = -0.170$, $n = 12$, $P = 0.598$) or the amount of no tree cover ($r_P = 0.073$, $n = 12$, $P = 0.882$). This suggests that exposure to p,p′-DDE is not associated with position in the urban gradient or elements associated with increasing urbanization (i.e. road density), forest (i.e. dense tree cover) and agriculture (i.e. no tree cover).

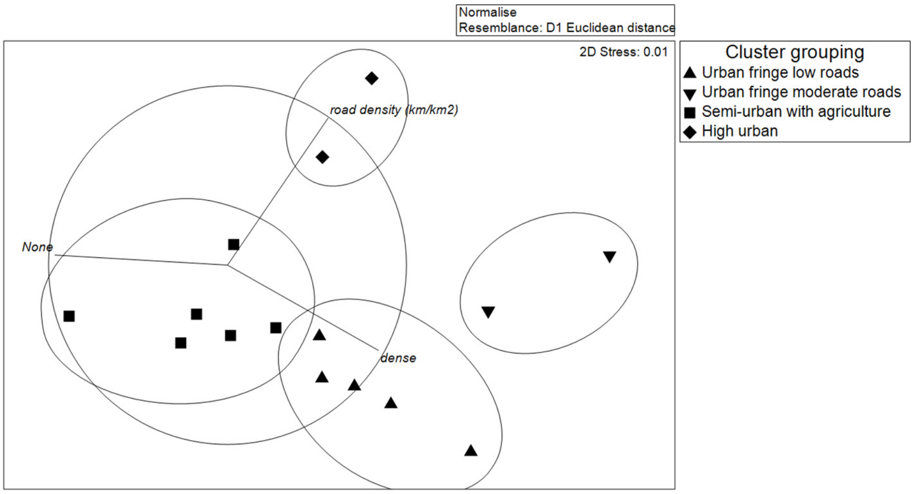

**Fig. 3.** Non-metric Multidimensional Scaling (nMDS) plot of sites where Victorian powerful owls were retrieved between 2004 and 2021 (only owls with accurate spatial locations are included; n = 16). Cluster groupings are derived from SimProf analysis. Vectors for all the environmental variables are overlaid to indicate their effect in the nMDS. Dense refers to the proportion of dense tree cover in the 1.5 km buffer, and none refers to the proportion of no tree cover in the 1.5 km buffer.

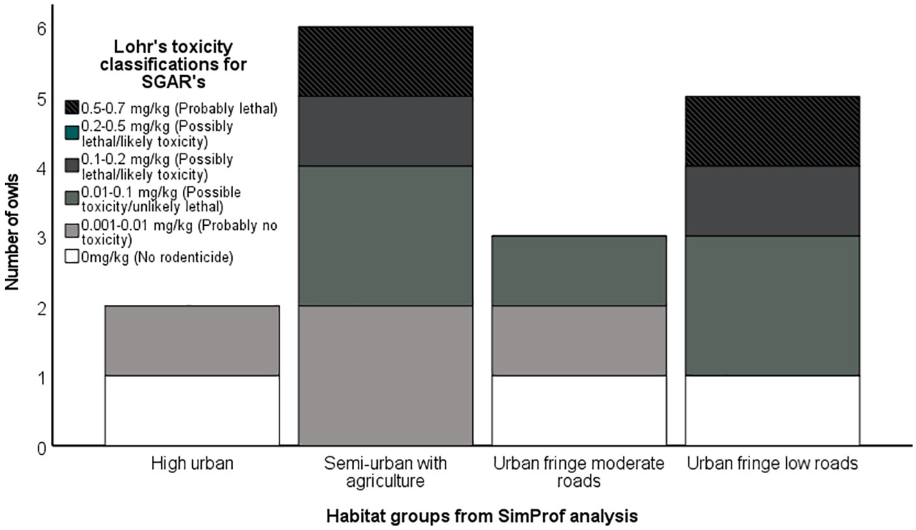

**Fig. 4.** Prevalence of total liver rodenticide levels across the different urban landscape habitat groupings in 16 powerful owls examined in Victoria, 2004–2021. Lohr's (2018) toxicity classes in owls are used to categorise each level.

There was no association with Cd concentrations in liver tissue and the x-axis of the nMDS ($r_P = -0.157$, $n = 16$, $P = 0.561$) and the y-axis of the nMDS ($r_P = 0.007$, $n = 16$, $P = 0.980$). Concentrations of Cd were also not related to road density ($r_P = -0.086$, $n = 16$, $P = 0.752$), the amount of dense tree cover ($r_P = -0.158$, $n = 16$, $P = 0.560$) or the amount of no tree cover ($r_P = 0.160$, $n = 16$, $P = 0.555$). There was a negative association between Hg concentrations in liver tissue and the x-axis of the nMDS ($r_P = -0.605$, $n = 16$, $P = 0.013$) but no association with the y-axis of the nMDS ($r_P = -0.187$, $n = 16$, $P = 0.487$). There was a weak negative but non-significant trend between Hg concentrations and road density ($r_P = -0.485$, $n = 16$, $P = 0.057$). There was no association between dense tree cover and Hg concentrations ($r_P = -0.418$, $n = 16$, $P = 0.107$). Concentrations of Hg were positively associated with the amount of area with no tree cover ($r_P = 0.611$, $n = 16$, $P = 0.012$). This suggests Cd levels are not associated with landscape characteristics of the urban-forest/agricultural gradient. Hg concentrations however are influenced by some of these factors. Hg concentrations tended to be associated with the semi-urban areas with increasing levels of agriculture, which were characterised by low road densities and increasing levels of no tree cover.

## 4. Discussion

Like many top-order predators, powerful owls are long-lived, have relatively low fecundity and populations occur at low densities. Any additional threatening processes, such as poisoning with toxicants, could have significant ramifications for the viability of populations in urbanizing landscapes. Even if not lethal or acutely toxic, toxicants could have potentially sub-clinical health impacts on fitness, reproduction, and immune function (Rattner et al., 2014). The prevalence of toxicants and in particular SGARs in the food web of powerful owls therefore requires urgent attention and like other parts of the world, increased regulation of the sale of SGARs in Australia should be considered (Eisemann et al., 2018; Lohr and Davis, 2018).

The detected prevalence and extent of ARs and persistent OCPs is of concern. Apex predators are critical to the health of ecosystems but are also disproportionately subjected to contaminants that move through food webs (Hofstadter et al., 2021; Lohr, 2018; Pay et al., 2021; Thomas et al., 2017). When apex predators utilize landscapes that incorporate intense human activities such as urbanization and agriculture, their risk of exposure to contaminants can be elevated, and as such this potentially constitutes a threat for species of conservation concern.

### *4.1. Could non-target poisoning of possums lead to SGARs in powerful owls?*

The high prevalence of SGARs detected in dead powerful owls is of significant conservation concern. The detection of rodenticides in raptors that prey specifically on species that are targeted for poisoning (e.g., rats, mice and rabbits) is unsurprising and has been reported in many predators globally (Lohr, 2018; López-Perea and Mateo, 2018). Powerful owls, however, do not generally prey on rabbits or rodents making these results unusual and alarming. Instead, powerful owls consume whichever medium or large arboreal mammal is the most available at a given locality (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) supplemented by a range of other small to medium-sized, mainly arboreal vertebrates (Debus and Chafer, 1994). They largely hunt in the canopy and mid-story layer and have rarely been documented coming to ground to take prey. The arboreal common ringtail possum (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*) are the predominant diet of powerful owls (Cooke et al., 1997; Cooke et al., 2002b; Hollands, 2004; Kavanagh, 2002; Kavanagh, 2004; McNabb, 1996; Soderquist et al., 2002; Tilley, 1982; Wallis et al., 1998), with greater gliders (*Petauroides volans*) and sugar gliders (*Petaurus breviceps*) (both arboreal species) also taken in areas where these species occur (Bilney et al., 2011; Cooke et al., 2006; Kavanagh, 2002; Kavanagh, 2004; Olsen et al., 2011). Birds and fruit bats (*Pteropus* spp.) have also been detected in their diet occasionally, especially in landscapes where arboreal mammalian prey species are at low densities (Pavey et al., 1994; Soderquist, 1999). Rodents, however, have only been documented in the diet of powerful owls in two highly urban parklands in the CBD of Melbourne and, in these locations, they were not the dominant dietary items with possums still the main diet (Fitzsimons and Rose, 2010; Menkhorst and Loyn, 2005).

Given that powerful owls prey primarily on common ringtail possums and common brushtail possums across their entire range, the high prevalence of SGARs detected in this study suggests that these owls may be contaminated through consuming possums that have been exposed to rodenticides. The SGAR most frequently detected in the powerful owls was brodifacoum, the active ingredient in most retail rat poisons available throughout Australia. Although rodenticides are primarily used to poison rodents, non-target possums may also consume this bait. While primarily herbivorous, urban common brushtail possums are considered likely to consume a range of foodstuffs (e.g. vegetables, food scraps, bird eggs; see Adams et al., 2013), whereas common ringtail possums are strict herbivores

(Pahl, 1984). The common brushtail possums' predisposition to a more varied diet might make them more likely to consume rodenticide baits.

Given that brushtail possums utilize roof cavities of houses for shelter, causing considerable issues for human occupants (Eymann et al., 2006), it is extremely likely they will encounter rodenticides placed in roof cavities by homeowners or pest controllers. Rodenticides have been detected in both common ringtail and common brushtail possum species (Grillo et al., 2016; WHA, 2021), but the transfer of these rodenticides to powerful owls through consumption of possums, has not been suggested previously and raises serious concerns. As evidence of this exposure pathway, the database maintained by Wildlife Health Australia (WHA, 2021), which collates data from veterinary surveillance partners nationally, contains >10 cases of confirmed and suspected SGAR consumption and toxicity in common brushtail and common ringtail possums, across Australia (WHA, 2021). Evidence is lacking, however, about how frequently possums are exposed to ARs and this represents a critical avenue for further research and monitoring in Australia.

Proximity to urban development can facilitate owl exposure to toxicants (Lohr, 2018). We examined powerful owls from different land use types but the prevalence of brodifacoum did not vary with position along the urban to agricultural/forest gradient, in which most birds sampled had detectable levels of SGARs, regardless of the degree of urbanization. This indicates that powerful owls inhabiting all areas, not just those residing in highly urban or agricultural areas, are exposed to rodenticides. Given that SGARs (mostly brodifacoum) can be purchased for domestic, commercial, industrial and agricultural use in and around buildings from many hardware stores or supermarkets, the secondary effects on food webs cannot be ignored.

### 4.2. Other toxicants in powerful owl food webs

Pindone was also detected in some powerful owls. Pindone is a first-generation AR used primarily for the control of rabbits in Australia. Given powerful owls rarely hunt from the ground or prey on rabbits, we hypothesise that pindone may also be consumed by common brushtail possums. Pindone in common brushtail possums does not necessarily cause haemorrhaging or death in the possums (Eason and Jolly, 1993) but it is still present in their bodies and so may be ingested by their predators (sensu Mendenhall and Pank, 1980).

Despite the use of DDT having been banned in Australia since 1987 (Falkenberg et al., 1994), OCPs were detected at relatively low concentrations in owl muscle. The most prevalent OCP detected was p,p′-DDE and was detected in the skeletal muscles of over 70% of our samples, at a maximum concentration of 5.4 mg/kg. Levels would be expected to be higher in fatty tissues such as liver or brain. OCPs, and specifically p,p′-DDE, are related to a number of negative reproductive effects in birds, such as reduced eggshell thickness or malformed embryos, which may result in increased nestling mortality, consequent reductions in reproductive success with potential effects on populations (Aver et al., 2020; Fry, 1995; Lundholm, 1997; Tubbs, 2016). While Tanabe et al. (1998) suggest that bird liver p,p′-DDE concentrations need to be greater than 20 mg/kg to pose a threat to individual reproduction, egg shell thinning has occurred in some species at egg concentrations over 3 mg/kg (Aver et al., 2020; Tanabe et al., 1998). Given the use of most OCPs are banned in Australia, and given the low levels we report, it is likely these levels will naturally decline over time.

Toxic metal concentrations (As, Cd, Pb and Hg; the metals most often associated with poisoning of humans and animals; Grúz et al., 2019) in powerful owl liver samples were also at relatively low concentrations. Accumulation properties of these metals suggest they may be good bioindicators of environmental contamination (Grúz et al., 2019), indicating generally low environmental exposure in this sample of owls. Cadmium concentrations were well below the level of 3 mg/kg in liver tissue whereby it is considered that environmental levels are elevated (Nighat et al., 2013; Scheuhammer, 1987), but it is unknown whether Cd concentrations in the kidneys of these owls was below the 10 mg/kg potentially associated with toxic effects (Burger, 2008). While the tissue concentration of mercury considered toxic varies greatly across species, a concentration of 2.4 mg/kg has been suggested to cause breeding impairments in birds (Jackson et al., 2011; Scheuhammer et al., 2007). Mercury concentrations in the owls we studied were well below these levels. Mercury concentrations did however indicate a signal towards being higher in areas with no tree cover and low road cover, which is indicative of increased agriculture. Mercury fluxes from agricultural lands are higher than for many other land-uses (Denkenberger et al., 2012), which has presumably contributed to the slightly elevated levels in owls from these areas. Lead was detected in three individuals in this study, and all were lower than the 2 mg/kg in liver tissue considered as a threshold for toxicity in birds (Pain et al., 1995). Arsenic was not measured above the detection limit. Importantly, cumulative heavy metal loads can increase long-term physiological stress in birds, indicated by altered haematological parameters, thus the impact of individual metals should not be the sole consideration (Bauerová et al., 2017). The toxic metal levels in powerful owl liver presented in this study would not suggest any immediate concern but provide a reference point for future research to investigate if these change with increased urbanization and industrial intensification.

### 4.3. Management implications

This research provides evidence of exposure of powerful owls to multiple environmental contaminants including ARs, OCPs and heavy metals. It is difficult to determine the health impacts of their combined toxic effects on fitness, reproduction, immune function and disease resistance. Efforts to reduce exposure of owls to these toxicants however, can help mitigate these risks to owl populations. Reducing exposure to OCPs and heavy metals may not immediately be required as levels are relatively low, yet efforts to reduce SGAR exposure are warranted. We have demonstrated that the SGAR brodifacoum is prevalent in powerful owls and in some instances at potentially lethal levels. Furthermore, the presence of brodifacoum is not limited to particular land-use types such as agricultural or urban areas but was detected across most of the owls examined here, indicating that brodifacoum use is widespread across the landscape. We propose that SGARs may be entering the powerful owl food web through either accidental or deliberate poisoning of possums that inhabit buildings and are regarded as pests by human occupants. While there is evidence of possums being poisoned by SGARs, it is unknown whether SGARs are affecting possums at the population level. It is unlikely that SGAR exposure in powerful owls is facilitated through consumption of rodents, however, other Australian owls consume rodents and have had considerable exposure to SGARs. Therefore, efforts to limit the transfer of SGARs into food webs are critically needed for predatory species. There is a critical need in Australia and many other parts of the world to investigate bioaccumulation and transfer of SGARs in food webs, and not just in species that are the targets for poisoning or their predators. As our research suggests, there is considerable potential for toxicants to move through food webs due to accidental or deliberate poisoning of non-target species.

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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. **Yun Jin:** Investigation. **Clare Death:** Conceptualization, Writing – Review and Editing. **Michael A. Weston:** Writing – Review and Editing. **Nicholas Carter:** Visualization, 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.

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