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Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat

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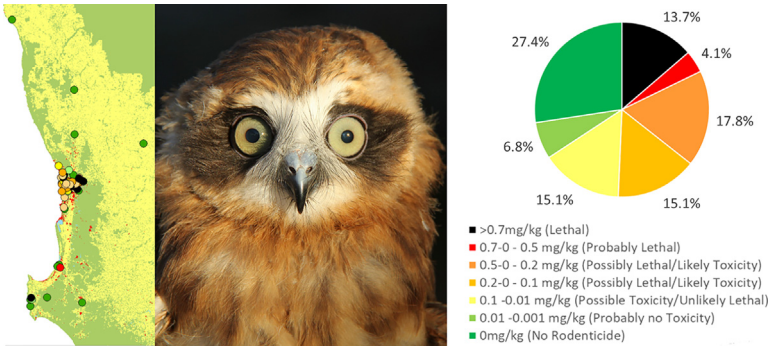
HIGHLIGHTS

- Anticoagulant rodenticide (AR) exposure rates are poorly studied in Australian wildlife.
- ARs were detected in 72.6% of Southern Boobook owls found dead or moribund in Western Australia.
- Total AR exposure correlated with proximity to developed habitat.
- ARs used only by licensed pesticide applicators were detected in owls.
- Raptors with larger home ranges and more mammal-based diets may be at greater risk of AR exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are commonly used worldwide to control commensal rodents. Second generation anticoagulant rodenticides (SGARs) are highly persistent and have the potential to cause secondary poisoning in wildlife. To date no comprehensive assessment has been conducted on AR residues in Australian wildlife. My aim was to measure AR exposure in a common widespread owl species, the Southern Boobook (*Ninox boobook*) using boobooks found dead or moribund in order to assess the spatial distribution of this potential threat. A high percentage of boobooks were exposed (72.6%) and many showed potentially dangerous levels of AR residue (>0.1 mg/kg) in liver tissue (50.7%). Multiple rodenticides were detected in the livers of 38.4% of boobooks tested. Total liver concentration of ARs correlated positively with the proportions of developed areas around points where dead boobooks were recovered and negatively with proportions of agricultural and native land covers. Total AR concentration in livers correlated more closely with land use type at the spatial scale of a boobook's home range than at smaller or larger spatial scales. Two rodenticides not used by the public (difethialone and flocoumafen) were detected in boobooks indicating that professional use of ARs contributed to secondary exposure. Multiple ARs were also detected in recent fledglings, indicating probable exposure prior to fledging. Taken together, these results suggest that AR exposure poses a serious threat to native predators in Australia, particularly in species using urban and peri-urban areas and species with large home ranges.

1. Introduction

Anticoagulant rodenticides (ARs) are commonly used in residential, commercial, and agricultural settings for the control of rodent pests (Rattner et al., 2014b). They block the recycling of vitamin K in the

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liver, which subsequently disrupts normal blood clotting in vertebrates (Park et al., 1984). ARs are often divided into first generation anticoagulant rodenticides (FGARs) and second generation anticoagulant rodenticides (SGARs) based on their chemical structure and when they were first synthesized. Unlike FGARS, SGARs are often lethal with a single feed and are substantially more persistent in liver tissue (Erickson and Urban, 2004).

AR exposure and subsequent mortality have been detected in non-target wildlife in all parts of the world where exposure has been tested (Laakso et al., 2010). Predatory bird species are particularly vulnerable to AR poisoning due to a greater susceptibility to most ARs than other bird species (Herring et al., 2017) and a prey base which frequently contains rodents targeted by the use of ARs. In some raptor species, mortality from AR exposure may have population-level impacts (Thomas et al., 2011). Unlike in Europe and North America, where the non-target impacts of ARs have been extensively studied, relatively little research has been conducted on AR exposure in Australian wildlife (Lohr and Davis, 2018; Olsen et al., 2013). This knowledge gap exists despite several lines of evidence suggesting that patterns of regulation and usage in combination with differences in faunal assemblages may increase the incidence and severity of non-target AR poisoning in Australia relative to better-studied areas of the world (Lohr and Davis, 2018).

Within Australia, patterns in the spatial distribution of AR exposure have not been studied in any wildlife species. A number of studies have addressed the spatial ecology of anticoagulant rodenticide exposure in non-target wildlife but have been primarily limited to North American mammals. Of these, some have focused on impacts within specific habitat types (Cypher et al., 2014; Gabriel et al., 2012). Studies examining patterns of AR exposure between urban and rural habitats have found correlations between the use of urban habitat and exposure rates in San Joaquin kit foxes (Mcmillin et al., 2008) and bobcats (Riley et al., 2007). A model developed to predict exposure patterns in San Joaquin kit foxes found that exposure was most likely in areas of low density housing on the urban/rural interface (Nogeire et al., 2015). Similar dynamics have been suggested but not tested in predatory bird species. Studies in North America and Europe have noted that predatory bird species which use more developed habitats tend to have greater rates of AR exposure than those which predominantly use more natural landscapes (Albert et al., 2010; Christensen et al., 2012). Additionally, a study in Spain noted a positive correlation between human population density and AR exposure in a sample of 11 species of predatory birds and mammals (López-Perea et al., 2015). The greater use of rodenticides and higher prevalence of targeted commensal rodents in human-dominated landscapes relative to natural areas is likely to drive these observed and suggested differences in non-target exposure. However, because AR usage patterns differ between urban and agricultural environments (Lohr and Davis, 2018) a need exists to evaluate the possibility of differences in non-target exposure patterns between different types of anthropogenic landscapes.

To address this knowledge gap, I sought to compare anticoagulant rodenticide (AR) exposure across intact native bushland and two different types of anthropogenic landscapes. Additionally, I undertook the first large-scale targeted testing of wildlife for AR exposure in the continent of Australia (Lohr and Davis, 2018). Testing was conducted on Southern Boobooks (*Ninox boobook*), which provide an excellent model to quantify the spatial distribution of threatening processes associated with fragmentation due to their presence across multiple habitat types and high abundance relative to other predatory bird species. To the best of my knowledge, no studies have directly addressed the relative impacts of different types of human land use on AR exposure in non-target wildlife. Understanding how different types of human land use impact the likelihood of AR exposure in non-target wildlife will be critical in evaluating risks to wildlife on a continental scale and will enable more effective targeting of measures to mitigate secondary toxicity.

2. Methods

Southern Boobooks are medium-sized hawk owls found across the majority of mainland Australia and adjacent parts of Indonesia and New Guinea (Olsen, 2011). They are assigned a conservation status of "Least Concern" by the IUCN ("*Ninox boobook*", 2018). Some taxonomies consider Southern Boobooks to be synonymous with the closely-related New Zealand Morepork (*Ninox novaseelandiae*) found in Tasmania and New Zealand but recent genetic and bioacoustic evidence suggests otherwise (Gwee et al., 2017). Boobooks are dietary generalists, consuming a wide variety of vertebrate and invertebrate prey (Higgins, 1999; Trost et al., 2008). These dietary habits make them an ideal model species for broad assessment of contamination of food webs by persistent pollutants like ARs. Their presence in most habitat types across Australia, with the exception of treeless deserts (Higgins, 1999), facilitates examination of differences in exposure across multiple habitat types and allows for future replication of this study at sites across the continent.

2.1. Specimen collection

Dead boobooks found in Western Australia were solicited from a network of volunteers, wildlife care centres, and government departments and were opportunistically collected when encountered. Boobooks euthanized by veterinarians and wildlife rehabilitators due to severe disease or injury were included. Dates and locations where each boobook was initially collected were recorded from the collector when possible. If liver tissue was identifiable and had a mass >3 g, it was removed and stored frozen at 20 °C until analysed for AR residues. A total of 73 usable boobook livers were stored for testing. While an effort was made to obtain boobooks from a diversity of geographical areas and habitat types throughout Western Australia, most samples originated in the more densely settled urban and peri-urban areas in the south-west of Western Australia in and around the city of Perth.

2.2. Rodenticide analysis

Liver samples were analysed by the National Measurement Institute (Melbourne, Australia) for residues of three FGARs (warfarin, coumatetralyl, and pindone) and five SGARs (difenacoum, bromadiolone, brodifacoum, difethialone, and flocoumafen) registered for use in Australia by the Australian Pesticides and Veterinary Medicines Authority. For each sample, 10 ml of reverse osmosis water and one gram of liver tissue were added to a 50 ml analytical tube and shaken for 15 min on a horizontal shaker. A 10 ml volume of 5% formic acid in acetonitrile solution was then added and the tube was shaken for an additional 30 min. QuEChERS extraction salt was added and the tube was shaken for an additional two minutes. The tube was then centrifuged for 10 min at 5100 rpm. After pipetting 3 ml of the supernatant into a 15 ml analytical tube, 5 ml of hexane was added and the tube was shaken for two minutes 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 transferred to a 2 ml QuEChERS dispersive tube, shaken for one minute, and centrifuged at 13,000 rpm for three minutes. The QuEChERS supernatant was then filtered using 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 prior to LC-MS/MS 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 to quantify concentrations of each rodenticide. Recovery rates for each AR, were calculated using chicken liver samples spiked with analytical standards (Table 1).