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Toxic time bombs: Frequent detection of anticoagulant rodenticides in urban reptiles at multiple trophic levels

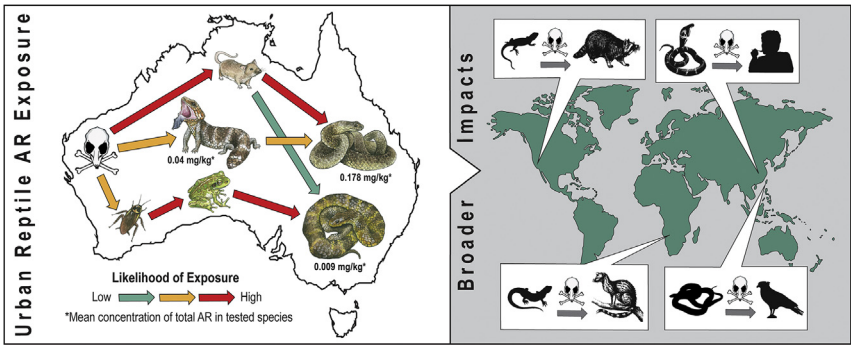
D.C. Lettoof [a,*], M.T. Lohr [b,c], F. Busetti [b], P.W. Bateman [a], R.A. Davis [b]

[a] Behavioural Ecology Lab, School of Molecular and Life Sciences, Curtin University, Brand Drive, Bentley, WA 6102, Australia
[b] School of Science, Edith Cowan University, 100 Joondalup Drive, Joondalup, WA 6027, Australia
[c] Phoenix Environmental Sciences, 1/511 Wanneroo Road, Balcatta, WA 6021, Australia

HIGHLIGHTS

- First detections of ARs in wild reptiles outside of an eradication event
- Frequency of detection varies by trophic tier and dietary preference.
- Exposure in urban reptiles may cause risks to human health where reptiles are regularly eaten.
- Reptiles are potentially good indicators of AR exposure in the food web.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are regularly used around the world to control pest mammals. Second-generation anticoagulant rodenticides (SGARs) are highly persistent in biological tissue and have a high potential for bioaccumulation and biomagnification. Consequently, exposure and poisoning of non-target organisms has been frequently documented, especially in countries with unregulated AR sales and usage. Most of this research has focussed on rodent-predators, usually raptors and predatory mammals, although exposure has also been documented in invertebrates and insectivorous fauna. Few studies have explored non-target exposure in reptiles, despite species sharing similar trophic positions and dietary preferences to other exposed fauna. We tested three abundant urban reptile species in Perth, Western Australia that differ in diet and trophic tiers for multiple AR exposure, the dugite *Pseudonaja affinis* (rodent-predator), the bobtail *Tiliqua rugosa* (omnivore) and the tiger snake *Notechis scutatus occidentalis* (frog-predator). We found frequent exposure in all three species (91% in dugites, 60% in bobtails and 45% in tiger snakes). Mean combined liver concentrations of ARs of exposed individuals were 0.178 mg/kg in dugites, 0.040 mg/kg in bobtails and 0.009 mg/kg in tiger snakes. High exposure frequency and liver concentration was expected for the dugite. Exposure in the other species is more surprising and implies widespread AR contamination of the food web. We discuss the likelihood of global AR exposure of urban reptiles, highlight the potential for reptiles to be important vectors of ARs in the food web and highlight implications for humans consuming wild reptiles.

* Corresponding author.
E-mail address: damian.lettoof@postgrad.curtin.edu.au (D.C. Lettoof).

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1. Introduction

Anticoagulant rodenticides (ARs) are used globally to control pest rodent populations (Shore and Coeurdassier, 2018). ARs are applied in a wide variety of land use settings including commercial and residential areas, agricultural and silvicultural land, and islands with threatened ecological communities (Lohr, 2018; Lopez-Perea et al., 2019; Pitt et al., 2015). Baiting is the standard method for delivering ARs, as the baits can be easily dispersed across a landscape and modified to suit particular target species (Hoare and Hare, 2006b). To avoid bait consumption from non-target species, baits are often deployed inside stations intended to minimise access from other fauna; for example, access holes to the stations may be raised above the ground and sized appropriately for target species (Bettink, 2015). Even so, many non-target species are poisoned or exposed to ARs, either as a result of direct bait consumption or through consumption of target and non-target fauna which have eaten bait (Elliott et al., 2014; Hong et al., 2019; Pitt et al., 2015). Such secondary poisoning events have been documented in avian and mammalian predators, particularly rodent-predators and scavengers (Colvin et al., 1988; Cox and Smith, 1990; Eason and Spurr, 1995; Hindmarch et al., 2019; Hosea, 2000; Lopez-Perea et al., 2019; Sanchez-Barbudo et al., 2012). Only more recently has the true saturation of ARs throughout the food web been identified: when baits are accessible, they are consumed directly by a suite of invertebrates (Alomar et al., 2018; Elliott et al., 2014), birds (Masuda et al., 2014), lizards (Wedding et al., 2010) and small mammals (Brakes and Smith, 2005). Non-target primary consumers of ARs are potentially important vectors for ARs to organisms in higher trophic levels; for example, insectivorous hedgehogs (*Erinaceus europaeus*) in Britain have a similar prevalence of AR exposure to predatory birds (Dowding et al., 2010), and Stewart Island robin (*Petroica australis*) nestlings have died from AR poisoning linked to being fed poisoned invertebrates (Masuda et al., 2014).

ARs work by blocking the recycling of vitamin K in the liver, disrupting the normal blood clotting mechanisms in vertebrates (Park et al., 1984). Commonly used ARs, such as brodifacoum, are classed as second generation anticoagulant rodenticides (SGARs) according to their chemical structure and period of development. SGARs are a significant risk to the vertebrate food web due to their long periods of persistence in liver tissue. In rodent livers, the mean half-life of SGARs can range from 108 days for difethialone to 220 days for flocoumafen (Eason et al., 2002), and up to 307.4 days for brodifacoum (Vandenbroucke et al., 2008). Despite long half-lives and the potential for environmental contamination for some ARs, currently only the United States, Canada and the UK impose substantial restrictions on AR use through permits, best-practice guidelines and limiting the more toxic ARs to indoor use (Bradbury, 2008; Health Canada, 2010; Tosh et al., 2011). In Australia, for example, nine first and second generation ARs can be legally sold to the public, resulting in exposure for humans and wildlife (Lohr and Davis, 2018). It has been estimated that this leads to 1400 human AR exposures per year (Australian Pesticides and Veterinary Medicines Authority, 2017) and high exposure for urban wildlife with 72.6% exposure recorded in Australian Boobooks (*Ninox boobook*) in Perth, Western Australia (Lohr, 2018).

Detection of AR exposure in non-target vertebrates is increasingly documented in the published scientific literature, but previous research on vector and indicator species has focussed on invertebrates, birds and mammals (Lopez-Perea et al., 2019; Serieys et al., 2019; Thomas et al., 2017). Reptiles have largely been ignored despite observations of direct bait consumption (Hoare and Hare, 2006a) including one instance where bait consumption is suspected to have directly caused mortality (Bettink, 2015). In addition to evidence of direct AR exposure, reptiles have potential to be vectors of high AR loads by virtue of their longevity, occupying multiple trophic levels and apparent resistance to anthropogenic contaminants (Hopkins, 2000). Recently, Lohr and Davis (2018) reviewed the literature on interactions between reptiles and ARs and identified the role of reptiles as a vector for ARs as a research priority. Here, we address this recommendation by analysing AR concentrations in the livers of three large-bodied and abundant reptiles in Perth, Western Australia, an urban landscape where secondary exposure to ARs has already been documented in predatory birds (Lohr, 2018). Our study species – the dugite (*Pseudonaja affinis*) a rodent-predator snake, the bobtail (*Tiliqua rugosa*) an omnivorous lizard and the tiger snake (*Notechis scutatus occidentalis*) a wetland snake with a dietary preference for frogs – were chosen as they give insight to AR exposure in reptiles and trophic transfer. Each of our study species differs in dietary preference and trophic tier, and therefore should exhibit different frequencies and concentrations of ARs. We predicted that the dugite would have the highest concentration of ARs, the tiger snake would have limited or no exposure to ARs, and the bobtail would fall between these two.

2. Materials and methods

2.1. Study area and species

The urban footprint of Perth, the capital city of Western Australia, covers over 1050 km^2 with a population of over two million (MacLachlan et al., 2017). The primary land uses of urban Perth are residential and industrial intersected with parks of remnant native vegetation. Currently all ARs available in Australia, with the exception of pindone, are sold directly to the public without requiring a license and there are no records on the volume of sales or frequency of use (Lohr and Davis, 2018). Anecdotal accounts of increased baiting in winter corresponding with increased exposure in Australian Boobooks during winter suggest some degree of seasonality in the use of AR baits (Lohr, 2018) but substantial deployment of ARs has been observed by the authors year-round in and outside of residential and commercial buildings.

We tested AR exposure in three large (200–1100 g) reptile species frequently found within urban Perth and demonstrating differences in diet and trophic tier. Dugites (*Pseudonaja affinis*: Elapidae) are a large snake (>1.7 m) that ontogenetically shift their diet from reptiles to mammals (Cipriani et al., 2017; Wolfe et al., 2018). Bobtails (*Tiliqua rugosa*: Scincidae) are a large (~0.4 m) omnivorous lizard known to eat primarily vegetation as well as a variety of invertebrates and anthropogenic scraps such as pet food and rubbish, in urban areas (Dubas and Bull, 1991; Norval and Gardner, 2019). Both dugites and bobtails occupy the same open woodland and heath habitats, and are frequently found in urban gardens. West Australian tiger snakes (*Notechis scutatus occidentalis:* Elapidae) are a large (~1 m) snake that have a diet comprising of mostly frogs, but occasionally reptiles, mammals and birds (Lettoof et al., 2020a). All three study species spatially overlap in wetland habitats, but tiger snakes are rarely found outside wetlands in Perth. Bobtails can suffer predation from dogs (*Canis lupus sp.), cats* (*Felis catus*), foxes (*Vulpes vulpes*), wedge-tailed eagles (*Aquila audax*) and a range of snake species including dugites (Norval and Gardner, 2019). Evidence of predation on Australian snakes is rare but predators of smaller individuals include carnivorous birds (raptors, kingfishers, corvids), dogs, cats, foxes, monitor lizards (*Varanus sp.*) and other snakes (Shine, 1995). As Australia doesn't have many large-bodied predators, predation on larger snakes is likely to be rare, especially in urban environments with fewer predators present. For this study we only tested adult dugites (>1.2 m) and tiger snakes (>0.7 m).

2.2. Specimen collection

Dugites ($n = 11$) and bobtails ($n = 10$) were collected opportunistically as road kill or non-rotten carcasses donated by wildlife care centres between 2014 and 2018, and tiger snakes ($n = 11$) were wild caught and euthanised between 2018 and 2019 (morphological data presented in Appendix Table 1). Carcasses were stored within 12 h of

collection frozen at −20 °C until the liver was extracted and analysed for AR residues. All specimens were collected within 30 km from the centre of Perth, Western Australia, and within 250 m of residencies or other urban infrastructure (Fig. 1). Specifically, dugites and bobtails were collected from areas surrounded by residential or industrial infrastructure. Tiger snakes were collected from four urban wetlands surrounded by residential infrastructure, with one wetland being partially bordered by an industrial area.

2.3. Samples extraction and purification

Liver samples aliquots of 1 g (wet weight, *w.w.*) were accurately weighed, frozen at −80 °C and then freeze-dried using a SubliMate 2 Bench Top laboratory Freeze dryer (EscoGlobal, Singapore). Freeze dried samples were homogenised in stainless steel vessels using a MM 400 milling system (Retsch GmbH, Germany). Homogenised samples were transferred into centrifuge plastic tubes (15 mL) and two aliquots of 5 mL of acetonitrile were pipetted into the tubes added with a 10 μL (10 ng/μL) solution containing the deuterated surrogates. Analytes were extracted using a sonication bath (15 min sonication for each aliquot). After extraction, samples were centrifuged at 4400 rpm for 5 min using a Heraeus Megafuge 8 centrifuge from ThermoFisher Scientific (Sydney, Australia) and the supernatant was transferred into a new plastic tube added with 2 mL of n-hexane. Samples were then vortexed for 5 min and then centrifuged at 4400 rpm for 5 min and the supernatant discarded. Samples extracts were evaporated near to dryness under a gentle nitrogen stream and then reconstituted in 400 μL of a 50:50 ACN/H_2O solution. The final extracts were transferred in 2 mL Teflon-lined screw cap amber glass vials stored at 0–4 °C until analysis. Bias (average percentage recovery) and precision (percentage relative standard deviation of recoveries, % RSD), determined by processing through the entire analytical procedure spiked samples of organic chicken liver supplied by a local butcher (South Perth, WA). Samples were spiked with a solution containing all AR and surrogate standards to give a final concentration of either 10 ng/g or 75 ng/g of each AR and 100 ng/g of deuterated standards. Unspiked chicken liver samples ($n = 3$) were used as a negative control (i.e., blanks). These samples were extracted and analysed along with the batch of samples. Details regarding chemicals, analytical standards, solutions and calibration standards are summarised in the Supporting information.

2.4. UHPLC MS/MS analysis

Chromatographic separation was achieved with an UltiMate 3000 UHPLC system (Thermo Fisher Scientific Corporation, US) coupled to an Agilent InfinityLab Poroshell 120 SB-C18 column (100 × 2.1 mm, 2.7 μm using acetonitrile and water containing 10 mM ammonium acetate at pH 5.7 at 25 °C and 0.250 mL/min flow rate. Rodenticides were detected using a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific Corporation, US). Analytes ionisation was achieved using an Ion Max NG API source operated in negative mode. The mass spectrometer was operated in multiple reactions monitoring (MRM) mode. UHPLC, Max NG API source and mass spectrometry settings are summarised in the Supporting Information (Tables S1–S3). Data was processed using Xcalibur 4.1.31.9 and Tracefinder 4.1software packages.

2.5. Statistical analysis

We compared the differences in AR concentration between each reptile using a Kruskal-Wallis for each AR that was detected in more than one species, as well as for the total (sum) concentration of ARs for individuals exposed to multiple ARs. For statistical analysis, samples that were recorded below detectable limits were entered as half the detection limit. A Dunn post-hoc test with Benjamini-Hochberg adjusted *p*-values was performed to identify which species differed significantly ($p \leq 0.05$) from each other. We used chi-squared tests to compare differences between species for detection frequency (% of individuals with any AR) and the mean number of ARs detected per exposed individual. All statistical analyses were conducted in R Studio (R Core Team, 2018).

3. Results

ARs were detected in all three species (Table 1). All dugites and seven of ten bobtails were killed by vehicle collisions, the remaining three bobtails were euthanised by wildlife care centres and contained no ARs. 91% of 11 dugites were exposed to ARs and 73% were exposed

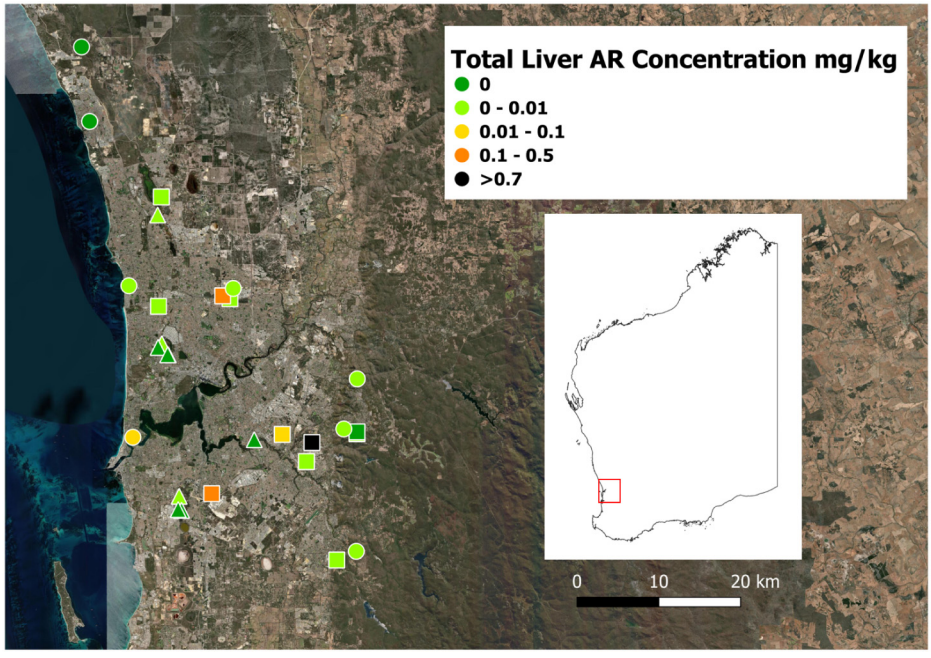

Fig. 1. Locations of individual reptiles screened for ARs in Perth, Western Australia. Squares = dugites (*Pseudonaja affinis*), circles = bobtails (*Tiliqua rugosa*) and triangles = tiger snakes (*Notechis scutatus occidentalis*).

Table 1
Concentration of reptile livers exposed to ARs from greater Perth, Western Australia (mg/kg). n values are the number of each individual exposed to each compound.

Species	Exposed/total tested	Combined concentration of ARs for exposed Mean ± SE (Range)	Brodifacoum		Bromadiolone		Difenacoum		Flocoumafen		Warfarin	
			n	Mean ± SE (Range)	n	Mean ± SE (Range)	n	Mean ± SE (Range)	n	Mean ± SE (Range)	n	Mean ± SE (Range)
Dugite *Pseudonaja affinis*	10/11	0.178 ± 0.074 (0.003–0.704)	7	0.096 ± 0.046 (0.010–0.330)	5	0.202 ± 0.137 (0.002–0.700)	4	0.019 ± 0.012 (0.003–0.053)	0	NA	4	0.007 ± 0.002 (0.004–0.011)
Bobtail Tiliqua rugosa	6/10	0.040 ± 0.031 (0.007–0.182)	6	0.025 ± 0.017 (0.006–0.109)	4	0.020 ± 0.018 (0.001–0.073)	1	0.002	1	0.004	0	NA
Tiger snake *Notechis scutatus*	5/11	0.009 ± 0.002 (0.006–0.014)	5	0.009 ± 0.002 (0.006–0.014)	0	NA	0	NA	0	NA	0	NA

to more than one AR, 60% of 10 bobtails were exposed to ARs and 40% were exposed to more than one AR, and 45% of 11 tiger snakes were exposed to brodifacoum only. The highest combined AR concentration was detected in dugites, which were three times higher compared to bobtails, and 51 times higher compared to tiger snakes (Fig. 2). The most commonly detected AR was the SGAR brodifacoum. The FGAR warfarin was only detected in dugites, which were generally exposed to the most ARs. Flocoumafen was detected only in a single bobtail, and pindone and coumatetralyl were not detected in any samples. Total AR concentrations in livers were significantly higher for dugites than for tiger snakes ($p = 0.008$) and approached significance between bobtails and dugites ($p = 0.069$). Bromadiolone and difenacoum concentrations in livers were significantly higher for dugites than for tiger snakes ($p = 0.048$). There was no significant difference in detection frequencies between species, and the difference in number of ARs in exposed individuals exposed individuals for dugites and tiger snakes approached significance ($p = 0.05$).

Dugite and bobtail carcasses varied in damage and condition which limited our ability to conduct necropsies or identify AR toxicity symptoms such as haemorrhaging, or determine the sex of most individuals (Table A.1). No tiger snakes exhibited any haemorrhaging. Of the best condition carcasses, the livers of two bobtails and one tiger snake were enlarged and pale or mottled, which can be symptoms of AR poisoning; however, none of these individuals contained ARs above detectable limits.

4. Discussion

All three reptile species tested were exposed to ARs, and this is the first study to report ARs in wild reptiles not associated with rodent eradications on oceanic islands. The impacts of AR on reptiles are relatively unknown and what is known has been thoroughly summarised in (Lohr and Davis, 2018). Although we cannot infer any toxicological effects on reptiles from this study, we present insight into AR exposure of multi-trophic reptile species in an urban ecosystem. As predicted, adult dugites (common urban rodent-predators) had the highest frequency of exposure and concentration of ARs, and were the only species exposed to warfarin. In Australia, warfarin is available at nearly all hardware and grocery stores and is not associated with any particular land use. We predict it's detection in dugites is a function of higher exposure from rodent predation. Also, as predicted, adult tiger snakes had the lowest exposure and bobtails fell between the two snakes. The frequency and concentration of ARs in bobtails, and the trace amounts in tiger snakes is concerning; however, not surprising given the public availability of ARs through retail sales (Lohr and Davis, 2018). Bobtails are probably exposed to ARs both directly and indirectly: Perth urban

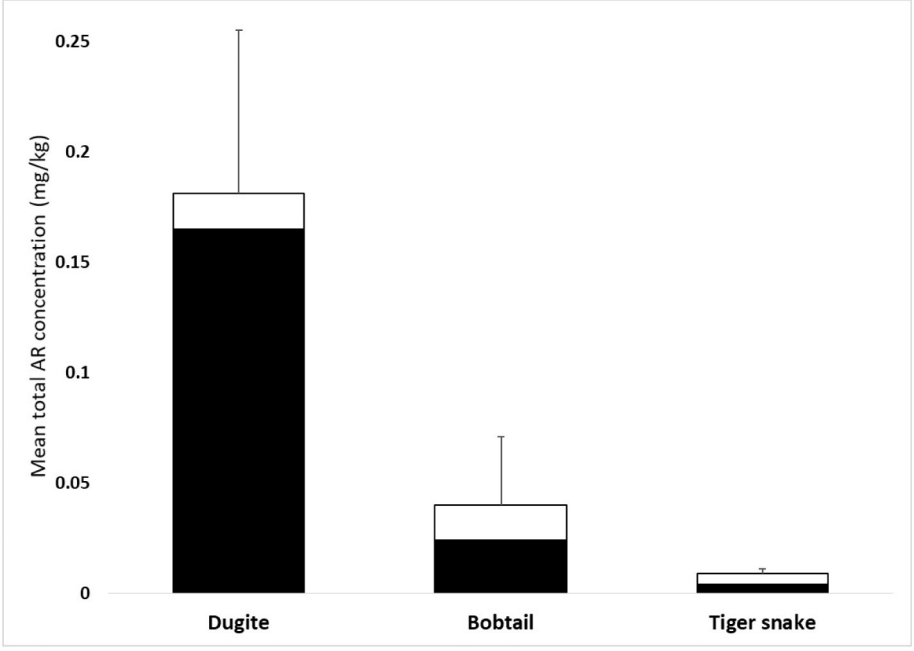

Fig. 2. Mean total AR liver concentration of exposed reptile species tested in Perth, Western Australia. Bar fill represents frequency of exposure (dugites 91%, bobtails 60% and tiger snakes 45%). Error bars = SE.

bobtails are commonly found throughout residential gardens and have been observed inside AR bait boxes (Ashleigh Wolfe, pers. comm.). As bobtails are known to eat anthropogenic food scraps we suspect they, like many other large omnivorous lizards (Bettink, 2015; Merton, 1987), are likely to eat baits found in residential backyards. Bobtails have also been recorded consuming mice (Norval and Gardner, 2019) and thus may be secondarily exposed to ARs from predating poisoned rodents or scavenging carrion, as well as contaminated invertebrates (Alomar et al., 2018; Elliott et al., 2014).

Tiger snakes in Perth predominately eat frogs and rarely eat rodents (Damian Lettoof, unpublished data), and yet we detected a relatively high (45%) prevalence of a single SGAR (brodifacoum) at trace concentrations. The four wetlands from which tiger snakes were collected are surrounded by residential areas, and we suspect several routes of AR exposure for tiger snakes: (1) traces of SGARs may be detectable for years after a single predation of a poisoned rodent as a consequence of persistence in liver tissue (Rueda et al., 2016); (2) urban wetlands are contaminated by storm water run-off (Lettoof et al., 2020b) which may include residentially used ARs (Kotthoff et al., 2019) and result in direct exposure from contaminated water; and (3) the primary prey of tiger snakes, frogs, may be exposed to ARs. Amphibians have never been tested for AR exposure despite being very susceptible to accumulating pesticides via direct contact with contaminated water (Bruhl et al., 2011), or through consumption of exposed invertebrates (Alomar et al., 2018; Elliott et al., 2014). Any combination of these routes of exposure could explain the prevalence of ARs in urban tiger snakes, and probable exposure for each species is illustrated in our graphical abstract.

The liver concentrations we detected in our test species are similar to those found in other wild reptiles. A single whip snake (*Hemorrhois hippocrepis*) was found with a liver concentration of 0.54 mg/kg of flocoumafen, and wild lava lizards (*Microlophus duncanensis*) were found with brodifacoum concentrations ranging between 0.001 and 0.8 mg/kg as well as two individuals at 1.6 and 1.9 mg/kg (Rueda et al., 2016). Although we are unsure if the AR liver concentrations in our study species are lethal or sublethal, the prevalence and mean total AR concentration in dugites is concerning compared to other taxa (see Table 3 in Lohr, 2018 and Tables 9, 10 and 12 in Laakso et al., 2010). The few short-term laboratory and field studies on the acute toxicity or sub-lethal effects of ARs and other pesticides in reptiles have rarely detected physiological impacts (Mauldin et al., 2019; Pauli et al., 2010); however, considering the slow metabolism of reptiles, the testing time periods may have been too short to show acute clinical symptoms. The available literature, nonetheless, suggests that at least some reptile species are more resistant to pesticide toxicity than are other taxa (Pauli et al., 2010). The Western fence lizard (*Sceloporus occidentalis*), for example, required an acute oral dose of the first generation (FG) anticoagulant pindone three to five times the fatal dose for birds and mammals before mortality occurred (Weir et al., 2015).

If reptiles are truly more tolerant of AR toxicity, their potential to be toxic vectors poses a much greater threat to the food web than currently recognised. We frequently detected (45–91%) ARs in all three reptile species, despite the difference of their trophic tiers and diet, and we found a single dugite (9% of tested) with total AR liver concentration of 0.7 mg/kg (a concentration that is considered lethal in raptors (Kaukeinen et al., 2000)). This suggests at least two possibilities: (1) there is pervasive AR contamination throughout the food web (Pitt et al., 2015) in areas of high baiting e.g. urban landscapes, and (2) reptiles accumulating high AR concentrations may present a fatally toxic meal for predators or scavengers. This may not have serious implications for urban wildlife in our study system, as we suspect predation events for urban adult dugites and tiger snakes are rare. On a global scale, however, this has serious implications for regions with higher AR use, biomass and biodiversity of reptiles, and more reptile predators than Perth. For example, North American racoons (*Procyon lotor*) are common scavengers of urban reptile road-kill (Antworth et al., 2005) and are likely to be exposed from eating poisoned reptiles. Urban genets (*Genetta sp.*) in Africa also predate on reptiles (Delibes et al., 1989; Widdows and Downs, 2015), and have been found with AR exposure (Serieys et al., 2019). There is already emerging evidence of this scenario: An island-wide study of ten raptor species in Taiwan found a high prevalence of AR exposure in rodent-eating and scavenging species as well as snake-eating species (Hong et al., 2019), suggesting that snakes may be an important vector of ARs to other trophic levels in this area.

Reptile species richness is highest in tropical and arid regions of the world (Böhm et al., 2013), and the tropical bioregion is also densely occupied by humans. As AR exposure is highest in wildlife living in or in close proximity to urban or agricultural land (Lopez-Perea et al., 2019; Serieys et al., 2018), tropical urban reptile populations are highly likely to be exposed. This highlights AR toxicity as an additional threat to habitat loss and wild harvest (Böhm et al., 2013) for tropical reptile populations and biodiversity. Urban reptiles' high exposure to ARs has further implications for humans. Reptile meat is a common food resource in tropical and subtropical regions of the world (Klemens and Thorbjarnarson, 1995), and is consumed on almost every continent. Although AR use and wildlife exposure has not been well studied in these regions, many reptiles persist in urban environments and would be harvested with close proximity to areas where anticoagulant rodenticides are used. Snakes, as rodent-predators, are of the highest risk of exposure, and thus localities where snakes are regularly eaten by humans, such as Vietnam (Magnino et al., 2009), China (Wang et al., 2014), Malaysia (Cantlay et al., 2017) and Africa (Taylor et al., 2015) are consequently also at high risk of exposure.

Besides tolerance to toxicity, reptiles may be particularly good reservoirs of ARs due to: a) the slow decomposition rate of ARs (Eason and Spurr, 1995), and b) a much lower rate of elimination and depuration of accumulated ARs from reptiles due to slower metabolism compared to other taxa (Campbell et al., 2005; Davenport et al., 1990). There is one excellent example of extreme persistence of ARs in a wild population of lizard: after a heavy baiting program was implemented on Pinzon Island, Galapagos to eradicate rats, a high prevalence of ARs was detected 100–850 days post-baiting in the livers of lava lizards (*Microlophus duncanensis*) (Rueda et al., 2016). Although no population-level poisoning was observed in the lizards, there was an unexpected outcome for the island birds of prey: 22 Galapagos hawks (*Buteo galapagoensis*) and a short-eared owl (*Asio flammeus*) were found dead from brodifacoum poisoning, presumably from predation of toxic lizards, 12–773 days after the baiting event. Lava lizards were found with liver concentrations of brodifacoum between 0.001 and 0.8 mg/kg at 400 days post-baiting, and at 800 days post baiting lizards were still found to have liver concentrations between >0.001 and 0.2 mg/kg. If elimination rates are similar for our test species then it's possible that some of our individuals have been retaining ARs for years. ARs are primarily shed from the body through faeces which has important implications for reptiles as vectors: a) insectivorous reptiles may be recursively exposed to toxic invertebrates which feed on contaminated reptile faeces; and b) reptiles that feed, and subsequently defecate, infrequently, i.e. snakes, should retain ARs substantially longer than mammals and birds.

Reptiles' sensitivity to AR toxicity is fundamentally unknown. Western fence lizards survived oral dosages of brodifacoum up to 1750 mg/kg, a concentration thousands of times higher than the LD50s recorded for most birds and mammals (Laakso et al., 2010). Laboratory experiments also found no observable effect on gopher snakes (*Pituophis catenifer*) that were fed mice that died from a lethal dose of the anticoagulants warfarin and diphacinone (Brock, 1965); while one of 19 iguanas (*Iguana iguana*) orally administered brodifacoum died and showed signs of intoxication with blood in the body cavity, although oddly this individual was from the lowest concentration treatment (Mauldin et al., 2019). There is speculation on why reptiles are relatively more resistant to AR toxicity than are other taxa; suggestions

include a difference in blood coagulation chemistry (Merton, 1987) and naturally slower clotting mechanisms (Dessauer, 1970). In raptors, despite variation between species (Thomas et al., 2011), a liver threshold of 0.1 mg/kg is considered a minimum for toxicity (Rattner et al., 2014). We detected a mean total AR liver concentration above 0.1 mg/kg in exposed dugites (91%), and a single exposed bobtail (17%). As SGARs are more toxic than diphacinone, we logically expect a liver concentration of 0.1 mg/kg to have at minimum a sublethal effect. We also found exposure to multiple rodenticides was relatively common in 80% of exposed dugites and 67% of exposed bobtails (maximum 3 ARs), and suggests accumulation from multiple prey items. Multiple ARs may have a synergistic effect rather than a cumulative effect (Lohr, 2018). For example, laboratory studies have demonstrated rat sensitivity to warfarin vastly increased after chronic exposure to brodifacoum (Mosterd and Thijssen, 1991), and American kestrels (*Falco sparverius*) exposed to the FGAR chlorophacinone experienced prolonged prothrombin times if they were previously exposed to brodifacoum (Rattner et al., 2020).

Sublethal concentrations have been shown to impact both the physiology and behaviour of exposed individuals. General symptoms of intoxicated animals shortly before mortality are anorexia, weakness, lethargy and dyspnea (shortness of breath) (Fitzgerald and Vera, 2006), and thus any reduction in mobility could increase the likelihood of mortality from other causes (Brakes and Smith, 2005). As with other taxa, exposed reptiles may be vulnerable to increased predation (Cox and Smith, 1992), increased mortality from vehicle collisions when on roads (Lohr, 2018; Mendenhall and Pank, 1980; Serieys et al., 2015), and a disruption of their thermoregulation routine (Merton, 1987). Thus, we recognise the potential for livers from dugites and bobtails which were collected as roadkill, or from wildlife rehabilitators, to be biased towards higher AR concentrations, compared to livers of tiger snakes that were collected alive and euthanised. However, if AR exposure does interfere with a reptile's normal behaviour then we also acknowledge that the easily hand-caught tiger snakes could be biased towards higher AR concentrations.

5. Conclusions and future direction

This study offers convincing evidence that urban reptiles of different trophic tiers and diet are exposed to residentially used ARs, and suggests the surrounding food web is more contaminated than previously assumed. We predict a similar AR exposure in reptiles of the same ecological niche in cities where the purchase of ARs are unrestricted and retail available. Based on their probable resistance to toxicity, low elimination rates, and multi-trophic positions, we consider reptiles in proximity to AR sources (i.e. urbanisation) to be good indicators of food web contamination. Our data highlight a novel threat faced by reptile predators and humans consuming wild reptiles captured near human habitation – particularly if liver or fat tissues are consumed.

To further assess the contamination of reptiles in the food web, we suggest investigating AR exposure of small insectivorous species, such as geckos, that have been detected living in bait boxes and are frequently eaten by other wildlife taxa known to be susceptible to AR toxicity. To address the concerns of human exposure we suggest screening for ARs in livers from reptiles sold in meat markets, particularly in countries of Eastern Asia. The frequent AR exposure in a snake that mostly eats frogs suggests frogs may also be contaminated. To the best of our knowledge ARs have never been detected or screened for in an amphibian, thus we recommend testing wild urban amphibian populations in AR exposed areas. Additional research is urgently needed to determine the scope and severity of AR exposure in reptiles in order to mitigate risks to humans and non-target wildlife.

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CRediT authorship contribution statement

D.C. Lettoof: Conceptualization, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition. **M.T. Lohr:** Conceptualization, Visualization, Investigation, Writing - review & editing, Funding acquisition. **F. Busetti:** Methodology, Resources, Writing - original draft. **P.W. Bateman:** Resources, Funding acquisition, Writing - review & editing. **R.A. Davis:** Resources, Funding acquisition, Conceptualization, Writing - review & editing.

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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