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Pre- and Postnatal Exposures to Residential Pesticides and Survival of Childhood Acute Lymphoblastic Leukemia

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Simple Summary: Pesticides have been linked to an increased risk of developing childhood leukemia, yet their impact on survival remains unclear. This study examines whether reported use of pesticides at home before and after birth influences five-year survival in children with acute lymphoblastic leukemia. Our data showed that exposure to pesticides during pregnancy, particularly rodenticides, was linked to a higher risk of death after accounting for other prognostic factors. These findings highlight the need to examine preventable environmental factors that may affect childhood leukemia outcomes, with the goal of improving survival.

Abstract: Background: Exposure to pesticides has been associated with an increased risk of developing childhood leukemia. However, the impact of pesticides on childhood leukemia survival has not been examined. We investigated the associations between residential pesticide use during key developmental periods and 5-year survival in children treated for acute lymphoblastic leukemia (ALL). **Methods:** Residential use of insecticides, herbicides, rodenticides, and flea control products from preconception up to 12 months prior to diagnosis and sociodemographic characteristics were collected via parental interview among 837 children diagnosed with ALL between 1995 and 2008 in California, USA. Data on clinical features were abstracted from medical records. Vital status was obtained through linkage to the National Death Index (NDI) up to 2020. Cox proportional hazards regression models were used to estimate hazard ratios (HRs), adjusting for sociodemographic factors and clinical risk group. **Results:** A total of 108 children with ALL (~13%) died within 5 years of diagnosis. Exposure to any pesticides pre- and/or postnatally was slightly higher among deceased compared to alive children (95.4% vs. 91.5%; $p = 0.23$), while use of rodenticides was significantly higher in children who died (25.0%) vs. those who survived (15.5%; $p = 0.02$). In fully adjusted models, exposure to rodenticides was associated with an increased risk of mortality (HR 1.70; 95% confidence interval (CI) 1.08–2.64; $p = 0.02$), especially when the child was exposed during pregnancy (HR 1.90; 95% CI 1.15–3.16; $p = 0.01$) and possibly 12 months before diagnosis (HR 1.60; 95% CI 0.98–2.61; $p = 0.06$). Increased hazards of death were also observed with other types of pesticides during pregnancy, but those associations were not statistically significant. **Conclusions:** This study is the first to report reduced survival among children with ALL previously exposed to rodenticides, particularly during pregnancy, underscoring the need to further evaluate

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mechanisms by which environmental exposures during key developmental stages may later impact cancer outcomes.

Keywords: childhood acute lymphoblastic leukemia; survival; residential pesticides; rodenticides; developmental periods; pregnancy

1. Introduction

In industrialized countries, leukemia stands as the most prevalent malignancy in children, representing 25% of all pediatric cancers. Among its various subtypes, acute lymphoblastic leukemia (ALL) is the dominant contributor, encompassing 78% of all childhood leukemia cases [1]. Despite its relative prevalence, leukemia in children remains a rare disease, with an incidence of 39 cases per million, peaking between the ages of 2 and 5, and exhibiting heightened rates among the Latinx population in the United States [1–3]. Childhood leukemia originates from genetic disruptions, often beginning in utero with oncogenic fusion proteins, followed by additional hits postnatally [2].

Pesticides—used to control unwanted plants, insects, and animals—expose children through ingestion, inhalation, and skin contact from home use, agricultural drift, and contaminated food. Chronic low-level pesticide exposure, particularly from residential and occupational sources during pregnancy, is linked to higher childhood leukemia risk [4–10].

Environmental chemicals may also contribute to cancer initiation and progression via DNA damage, oxidative stress, and immune reactions [2,11,12]. A metanalysis showed that children from lower socioeconomic backgrounds experience a survival gap compared to those from higher socioeconomic backgrounds [13]. Racial disparities also persist, with higher mortality rates among Latinx and Black patients, attributed to factors like genetics, language barriers, and treatment responses [14,15].

Prognostic factors such as socioeconomic status (SES), race, and ethnicity are interlinked with environmental exposures [16], but little is known about the potential independent impact of chemicals on cancer survival. Few studies have examined the link between environmental exposures during perinatal development and pediatric leukemia survival. A Spanish study found maternal smoking during pregnancy and postnatally increased mortality 4-fold, adverse events 8-fold, and treatment-related mortality 14-fold [17]. Data from the California Childhood Leukemia Study (CCLS) showed that paternal preconception smoking and passive smoke exposure reduced 5-year overall survival [18]. Poor perceived air quality and fine particulate matter levels were also associated with lower survival in childhood ALL, lymphomas, and other cancers [19–22]. The impact of pesticides has been investigated for cancer survival in adults but not children [23,24].

This study leverages data from the CCLS, a case–control study designed to investigate environmental and genetic risk factors for childhood leukemia [18]. We aim to evaluate whether pre- and postnatal residential pesticide exposures influence survival outcomes in children diagnosed with ALL in California.

2. Materials and Methods

The CCLS is a case–control study that includes incident cases of childhood leukemia from hospitals across California and matched population-based controls; cases enrolled from 1995 to 2008 were evaluated in this study. Patients with childhood leukemia were enrolled around the time of diagnosis at 17 hospitals if they were younger than 14 years old at diagnosis, had an English or Spanish-speaking parent, lived in one of the study counties at diagnosis, and had no previous cancer. Interviews with a parent, primar-

ily mothers, used a structured questionnaire to collect information on residential use of 12 types of pesticides (yes/no) during three critical developmental periods, including three months before conception, pregnancy, and postnatally (until the child turned three or was diagnosed, whatever occurred first), as well as within a year of the interview following the leukemia diagnosis. The process of extracting sociodemographic data, leukemia type, and vital status through medical record abstraction, clinician validation, and probabilistic linkage to electronic death certificate data was described in a previous study [18]. Of the 837 consenting ALL cases with completed interviews, 108 were linked to death records, with 5 deaths due to external causes.

The primary exposure of interest was pesticide use. Based on their intended pest targets, the 12 pesticide types were grouped into four broad categories: insecticides for controlling various household and lawn insects (5 types), herbicides for targeting various unwanted plants (2 types), flea control for managing fleas on pets and in living areas (4 types), and rodenticides for controlling rodents (1 type). The total number of pesticide types were also categorized into three exposure levels—low (0–2 types), medium (3–4 types), and high (5–12 types)—based on the tertile distribution.

The outcome evaluated was 5-year survival from all causes except external causes. The nonparametric Kaplan–Meier estimator was used to estimate the survival function and survival curves by pesticide exposure group. A directed acyclic graph (DAG) (Figure S1) identified covariates and their relationships with exposure and outcome. Backward elimination further refined the model, excluding birth weight and household dependents due to minimal impact on risk estimates (<10%). The goodness-of-fit tests using Bayesian Information Criterion (BIC) values for the models with and without these covariates are provided in the Supplemental Materials (Table S1). Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs), adjusting for birth year, parental highest education attained (dichotomized—high school or lower vs. some college or more), annual household income (six categories), race and ethnicity (five categories), and National Cancer Institute (NCI) risk group for ALL (categorical: "standard", defined as age > 1 year and age < 10 years and WBC < 50,000/μL; "high", defined as age $\geq$ 10 years or age > 1 year and age < 10 years and WBC $\geq$ 50,000/μL; and "infant", defined as age < 1 year) [25]. Adjusted Cox models were run with and without SES variables to assess their potential impact on survival outcomes.

Stratified analyses by breastfeeding duration and race and ethnicity were conducted to explore potential effect modification. Breastfeeding duration was considered due to its known influence on early immune development [26], while race and ethnicity were analyzed to account for potential sociodemographic disparities in health outcomes [15,27]. Heterogeneity in HRs for pesticide groups across critical developmental periods was tested using log-rank tests. Log-likelihood ratio tests were used to assess the goodness-of-fit and effect modification by breastfeeding and race and ethnicity. Analyses were performed in the R environment, version 4.3.1 (16 June 2023) [28]. All tests were two-sided, and p-values < 0.05 were considered statistically significant.

This study was approved by the Institutional Review Boards at the University of California, Berkeley, and the California Department of Public Health.

3. Results

3.1. Population Characteristics

Among 837 children with ALL, 47% were Latinx, 35% were non-Latinx Whites, and the remainder were either Asian/Pacific Islander, Black, or of unknown origin. A total of 131 (16%) children came from households with an income below $15,000, and 129 (15%) children lived in households with more than six dependents. Additionally, 36% of parents

had a high school education or less. As presented in Table 1, children who died were more likely to have high-risk ALL, to be diagnosed before the age of one year, and belong to families with low educational attainment and low annual income. Racial disparities were evident, with non-Latinx Black children exhibiting the highest percentage of deceased, followed by Latinx children. There was a suggestion that children who were not breastfed were more likely to die compared to those who were breastfed. The distributions of sex (assigned at birth), number of dependents in the household, birthweight, and gestational age were similar between children who survived and those who did not. Overall, Latinx households and those with low annual income and low education attainment were less likely to use pesticides (Table S2).

Table 1. Characteristics of 837 children with acute lymphoblastic leukemia both overall and stratified by 5-year survival status at the end of 2020—the California Childhood Leukemia Study.

Characteristics	Overall *n* = 837	Alive *n* = 729	Deceased *n* = 108
	n (%)	*n* (%)	*n* (%)
Sex (assigned at birth)			
Female	366 (43.7)	324 (44.4)	42 (38.9)
Male	471 (56.3)	405 (55.6)	66 (61.1)
Race and Ethnicity			
Latinx	396 (47.3)	340 (46.6)	56 (51.9)
Non-Latinx White	295 (35.2)	272 (37.3)	23 (21.3)
Non-Latinx Asian/Pacific Islander	73 (8.7)	61 (8.4)	12 (11.1)
Non-Latinx Black	24 (2.9)	16 (2.2)	8 (7.4)
Other/Unknown	49 (5.9)		
Birth Years			
1982–1989	64 (7.6)	48 (6.6)	16 (14.8)
1990–1999	509 (60.8)	442 (60.6)	67 (62.1)
2000–2014	264 (31.5)	239 (32.8)	25 (23.1)
Household Annual Income (USD)			
<15,000	131 (15.7)	112 (15.4)	19 (17.6)
15,000–29,999	149 (17.8)	123 (16.9)	26 (24.1)
30,000–44,999	130 (15.5)	112 (15.4)	18 (16.7)
45,000–59,999	122 (14.6)	102 (14.0)	20 (18.5)
60,000–74,999	63 (7.5)	57 (7.8)	6 (5.5)
75,000+	242 (28.9)	223 (30.6)	19 (17.6)
Number of Dependents in the Household			
1–3	184 (22.0)	155 (21.2)	29 (26.9)
4–5	524 (62.6)	459 (63.0)	65 (60.1)
6+	129 (15.4)	115 (15.8)	14 (13.0)
Highest Parental Education Attained			
High School or Lower	303 (36.2)	260 (35.7)	43 (39.8)
Some College or More	533 (63.7)	468 (64.2)	65 (60.2)
Unknown	1 (0.1)		
Age at Diagnosis (years)			
<1	27 (3.2)	13 (1.8)	14 (13.0)
1–2	195 (23.3)	175 (24.0)	20 (18.5)
3–6	391 (46.7)	360 (49.4)	31 (28.7)
7–9	103 (12.3)	86 (11.8)	17 (15.7)
10–14	121 (14.5)	95 (13.0)	26 (24.1)

Table 1. *Cont.*

Characteristics	Overall n = 837	Alive n = 729	Deceased n = 108
NCI Risk Group			
Standard	561 (67.0)	509 (69.8)	52 (48.1)
High	226 (27.0)	186 (25.5)	40 (37.0)
Infant	26 (3.1)	12 (1.7)	14 (13.0)
Unknown	24 (2.9)		
Birthweight (grams)			
<2500	39 (4.7)	34 (4.7)	5 (4.6)
2500–4000	663 (79.2)	580 (79.6)	83 (76.9)
>4000	135 (16.1)	115 (15.8)	20 (18.5)
Gestational Age (weeks)			
<36	45 (5.4)	36 (4.9)	9 (8.3)
36–41	608 (72.6)	535 (73.4)	73 (67.6)
41+	175 (20.9)	149 (20.4)	26 (24.1)
Unknown	9 (1.1)		
Breastfeeding			
No	138 (16.5)	114 (15.6)	24 (22.2)
Yes	663 (79.2)	585 (80.2)	78 (72.2)
Unknown	36 (4.3)		
Breastfeeding Duration (months)			
6 or Less	520 (62.1)	450 (61.7)	70 (64.8)
More than 6	281 (33.6)	249 (34.2)	32 (29.6)
Unknown	36 (4.3)		

Percentages may not amount to 100% due to rounding. Abbreviations: USD—United States Dollar; NCI—National Cancer Institute.

3.2. Bivariate Analyses

About 92% of all children with ALL were exposed to at least one pesticide type pre- and/or postnatally. The use of pesticides tended to be correlated across different periods. Rodenticide exposure, for instance, showed strong correlations between preconception and pregnancy (correlation coefficient r = 0.82) and between pregnancy and postnatal periods (r = 0.78), suggesting consistent use over time (Figure S2). Conversely, correlations between different pesticide categories were generally low, indicating distinct patterns of use.

Bivariate analysis showed no significant difference in survival between children ever exposed to pesticides and those never exposed (p = 0.23, Table 2). However, survival was lower in children exposed to rodenticides, with 25% exposed among the deceased compared to 15.5% among survivors (p = 0.02). Exposure to rodenticides pre- and postnatally was associated with lower survival rates (80–83%) compared to unexposed children (85–90%) or those exposed to other pesticides (Table S3). The Kaplan–Meier curves showed a statistically significant decrease in 5-year survival in the group exposed to rodenticides at any time (p = 0.015; Figure 1) and during pregnancy (p = 0.022; Figure 2).

Table 2. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models without and with adjustments for socioeconomic status.

Exposure	Alive *n* = 729	Deceased *n* = 108		Model 1—Without SES Adjustment *		Model 2—With SES Adjustment **	
	n (%)	*n* (%)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Any Pesticides							
No	62 (8.5)	5 (4.6)		Ref.		Ref.	
Yes	667 (91.5)	103 (95.4)	0.23	2.06 (0.83–5.11)	0.1	2.22 (0.89–5.54)	0.09
Number of Types Used							
0–2 (Low)	327 (44.9)	40 (37.0)		Ref.		Ref.	
3–4 (Medium)	226 (31.0)	42 (38.9)		1.67 (1.07–2.59)	0.02	1.77 (1.14–2.77)	0.01
5–12 (High)	176 (24.1)	26 (24.1)	0.17	1.47 (0.88–2.44)	0.14	1.56 (0.93–2.62)	0.09
Insecticides							
No	110 (15.0)	15 (13.9)		Ref.		Ref.	
Yes	619 (84.9)	93 (86.1)	0.86	1.10 (0.63–1.91)	0.7	1.15 (0.66–2.00)	0.6
Herbicides							
No	362 (49.7)	54 (50.0)		Ref.		Ref.	
Yes	367 (50.3)	54 (50.0)	1	1.16 (0.78–1.72)	0.5	1.31 (0.87–1.98)	0.2
Flea Control							
No	419 (57.5)	64 (59.3)		Ref.		Ref.	
Yes	310 (42.5)	44 (40.7)	0.8	1.16 (0.72–1.57)	0.8	1.04 (0.70–1.54)	0.8
Rodenticides							
No	614 (84.2)	81 (75.0)		Ref.		Ref.	
Yes	113 (15.5)	27 (25.0)	0.02	1.75 (1.13–2.72)	0.01	1.69 (1.08–2.64)	0.02
Unknown (*n* = 2)							

Percentages may not amount to 100% due to rounding. Abbreviations: HR—hazards ratio; CI—confidence interval; Ref—reference; SES—socioeconomic status. * Adjusted for age at diagnosis, race and ethnicity, and NCI risk group status. ** Adjusted for age at diagnosis, race and ethnicity, NCI risk group status, highest parental education attained, and household income.

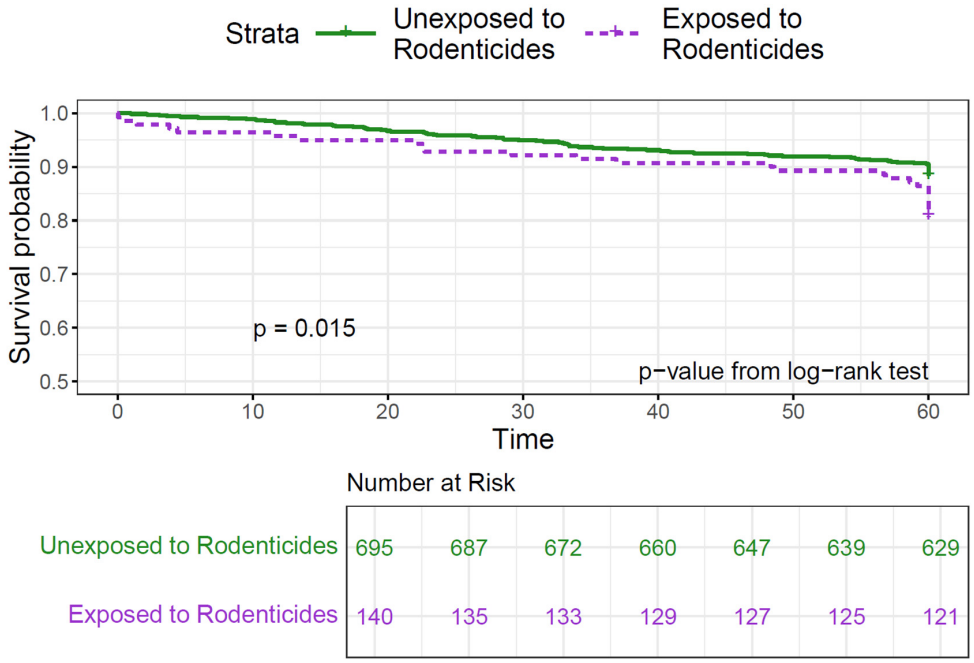

Figure 1. Kaplan–Meier curves for 5-year survival in childhood ALL by rodenticide exposure during any time period.

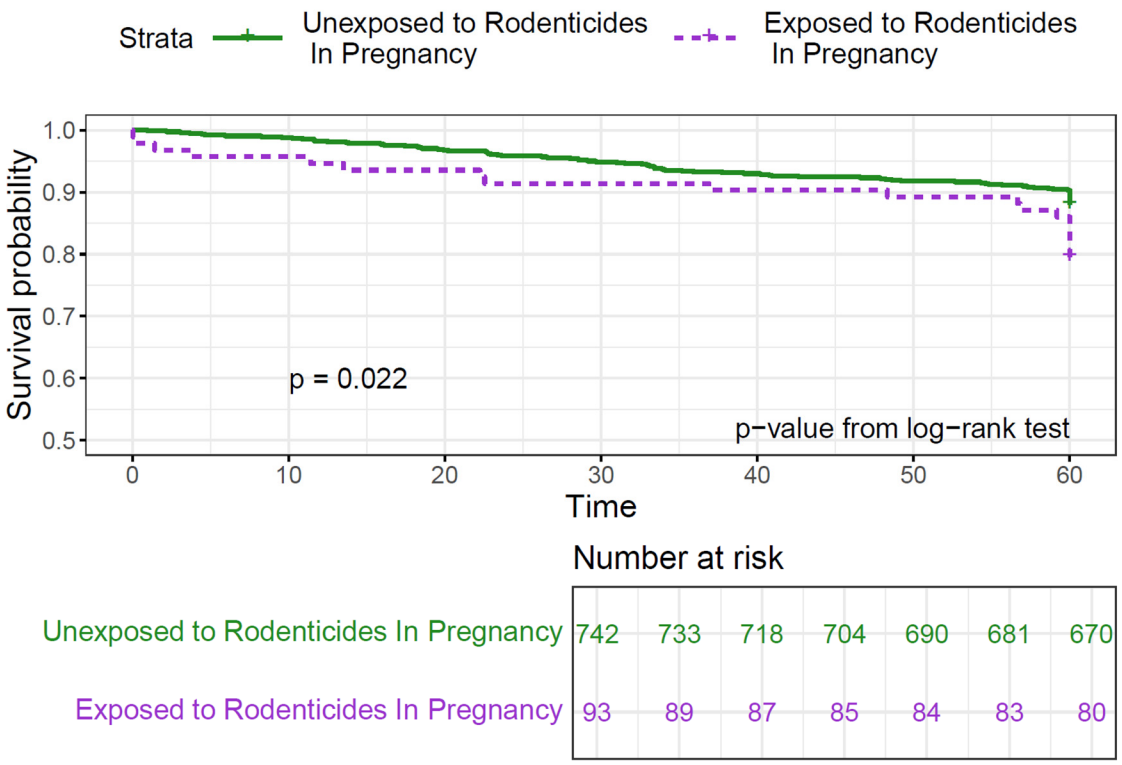

Figure 2. Kaplan–Meier curves for 5-year survival in childhood ALL by rodenticide exposure during pregnancy.

3.3. Multivariate Analyses

Table 2 shows the HRs for 5-year survival of childhood ALL in relation to pesticide exposure at any time, with adjustment for age at diagnosis, race and ethnicity, and NCI risk group (Model 1) and additional adjustment for SES, including parental education and annual household income (Model 2). A two-fold increased risk of mortality was associated with overall exposure to any pesticides in both models but did not reach statistical significance (p = 0.09). Increased risk of mortality was associated with exposure to rodenticides in Model 1 (HR 1.75; 95% CI: 1.13–2.72) and Model 2 (HR 1.69; 95% CI: 1.08–2.64). There was no clear dose–response relationship when examining the number of pesticide types used.

In fully adjusted Cox proportional hazards models examining childhood ALL survival by pesticide exposure during various developmental periods (Table 3), statistically significant increased risks of mortality were seen with exposure to any pesticide during pregnancy (HR 1.6; 95% CI 1.05–2.42), mostly driven by rodenticides (HR 1.91; 95% CI 1.15–3.16) and possibly insecticides and herbicides that conferred 45 to 50% increased risks of mortality, although falling short of statistical significance. Additionally, data suggested that children exposed to rodenticides 12 months prior to the interview had a 60% increased risk of mortality (p = 0.06). No significant differences were observed for other pesticide categories across different developmental periods. Adjusting for highly correlated variables challenged model robustness. Sensitivity analysis adjusting across different time windows yielded consistent trends with the primary findings on rodenticide exposure during pregnancy compared to those unexposed (HR 3.12; 95% CI 1.01–9.65) (Table S4). Similarly, rodenticide exposure during pregnancy yielded an HR of 1.74 (95% CI 1.02–2.99) compared to those who were unexposed, after adjusting for other pesticide use during pregnancy (Table S5).

Table 3. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by periods of exposure.

Exposures	Preconception		Pregnancy		Postnatally		12 Months Before Interview	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Any Pesticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.02 (0.68–1.52)	>0.90	1.60 (1.05–2.42)	0.03	0.84 (0.53–1.35)	0.5	1.35 (0.87–2.09)	0.2
Insecticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.00 (0.67–1.48)	>0.90	1.45 (0.96–2.17)	0.08	0.81 (0.53–1.25)	0.3	1.13 (0.75–1.71)	0.6
Herbicides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.22 (0.79–1.88)	0.4	1.50 (0.98–2.29)	0.06	1.10 (0.73–1.65)	0.7	1.26 (0.84–1.90)	0.3
Flea Control								
No	Ref.		Ref.		Ref.		Ref.	
Yes	0.90 (0.57–1.43)	0.7	1.05 (0.68–1.64)	0.8	0.83 (0.55–1.26)	0.4	1.11 (0.74–1.68)	0.6
Rodenticides								
No	Ref.		Ref.		Ref.		Ref.	
Yes	1.49 (0.85–2.63)	0.2	1.91 (1.15–3.16)	0.01	1.46 (0.91–2.33)	0.1	1.60 (0.98–2.61)	0.06

Abbreviations: HR—hazards ratio; CI—confidence interval; Ref—reference. * Adjusted for age at diagnosis, race and ethnicity, highest parental education attained, household income, and NCI risk group status.

3.4. Effect Modification

Stratified analyses by racial and ethnic group showed that rodenticide exposure was associated with poorer survival among non-Latinx White children (HR 3.35; 95% CI 1.42–7.88), while a weaker association was observed in Latinx children (HR 1.66; 95% CI 0.91–3.01) (Table 4). No significant associations were seen in other racial/ethnic groups. Formal testing for effect modification confirmed that survival varied by race and ethnicity (p for interaction = 0.02).

Table 4. Residential pesticides and 5-year survival in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by race and ethnicity.

Exposure	Non-Latinx White		Latinx		Non-Latinx Black + Asian + Others		Interaction *p*-Value
	HR (95%CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	
Insecticides	1.02 (0.24–4.41) 265/21	>0.90	1.41 (0.69–2.9) 317/47	0.3	0.70 (0.22–2.26) 130/25	0.6	0.8
Herbicides	1.00 (0.41–2.45) 197/15	0.9	0.54 (0.88–2.68) 141/23	0.1	1.40 (0.59–3.28) 83/16	0.4	0.8

Table 4. *Cont.*

Exposure	Non-Latinx White		Latinx		Non-Latinx Black + Asian + Others		Interaction *p*-Value
	HR (95%CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	HR (95% CI) *n* Total/*n* Deaths	*p*-Value	
Flea Control	0.83 (0.36–1.90) 164/12	0.7	1.55 (0.91–2.64) 145/26	0.1	0.55 (0.22–1.39) 45/6	0.2	0.09
Rodenticides	3.35 (1.42–7.88) 46/9	0.005	1.66 (0.91–3.01) 73/16	0.1	0.45 (0.10–1.93) 21/2	0.3	0.02

Abbreviations: HR—hazards ratio; CI—confidence interval. * Adjusted for age at diagnosis, race and ethnicity, highest parental education attained, household income, and NCI risk group status.

Stratified analyses by breastfeeding duration suggested that children exposed to insecticides and who were not breastfed or breastfed for 6 months or less had a higher risk of dying (HR 1.83; 95% CI 0.82–4.08) compared to those unexposed. In contrast, among children breastfed for more than 6 months, insecticide exposure was not associated with increased mortality risk (HR 0.82; 95% CI 0.31–2.12). Formal tests for effect modification, however, did not reach statistical significance (p for interaction = 0.11) (Table S6).

4. Discussion

4.1. Key Findings

Our study, based on comprehensive interview data from Californian families, suggests a significant association between exposure to any residential pesticides during pregnancy and lower survival in children with ALL, after adjusting for clinical and sociodemographic factors. This association was mostly driven by exposure to rodenticides, and to a lesser extent insecticides and herbicides. These findings emphasize the vulnerability of pesticide-exposed patients, highlighting the impact of exposure prior to diagnosis.

To our knowledge, no other childhood cancer study has investigated the relationship between pesticide exposure and survival. Data among adults are also scarce. A 2019 French study examined lymphoma patients with occupational pesticide exposure and found reduced response to immunochemotherapy and lower survival [23]. Another study of Hodgkin's lymphoma patients residing near agricultural fields in California observed no significant association between environmental pesticide exposure and survival [24]. The lack of convergence among adult studies may be due to differences in routes and levels of exposure to agricultural pesticides.

We examined pesticide exposures during key developmental periods and their effects on leukemia survival rates in children. The pregnancy period was particularly impactful, as mortality was associated with exposure to both any pesticide category and rodenticides. Sensitivity analyses adjusting for collinearity indicated that rodenticide exposure during pregnancy significantly increased the hazard of mortality, highlighting pregnancy as a critical period. Other pesticides showed no significant associations with mortality across different time windows.

In general, the results from Models 1 and 2 (Table 2) were similar, showing little impact from social factors. To account for the additional confounding effects of healthcare access and financial burden on treatment outcomes, we ran sensitivity analyses adjusting for hospital site and number of household dependents, which did not substantially alter the observed association between rodenticide exposure and poor survival.

Latinx households and those with lower income and education levels reported using fewer pesticides than other groups (Table S2), which may reflect differences in household practices, access to pesticide products, or awareness of risks associated with pests. Stratified

analyses indicated differences in the association between pesticide exposure and survival, particularly for rodenticide exposure among non-Latinx White children, possibly due to the disproportionately large number of exposed individuals in this group (Table S1). Smaller sample sizes for racial and ethnic groups limit the robustness of these findings, emphasizing the need to further examine socioeconomic disparities and conduct larger-scale studies [13,16,27,29]. In addition, interview data are somewhat limited in characterizing the levels of exposure to pesticides with precision, therefore limiting the interpretation of our results showing no overall dose–response relationship with survival.

Building on evidence linking short breastfeeding duration to childhood leukemia risk [26,30–33], our data suggest higher pesticide-related survival risks in children breastfed for less than six months. Though interaction tests were not significant, this warrants further study on immune modulators' roles in cancer relapse and survival, particularly in mitigating the adverse survival effects associated with rodenticide exposure.

4.2. Biological Effects of Pesticides

Population studies have consistently linked residential pesticide exposure to an elevated risk of developing childhood leukemia, emphasizing both in utero and postnatal exposures [6,34–36]. Like other leukemogenic agents, such as etoposide, benzene metabolites, and lack of bioflavonoids, certain pesticides exert toxicity through oxidative stress and mitochondrial dysfunction. These processes can induce DNA breaks, potentially leading to chromosomal rearrangements (duplications, deletions, and translocations) if not properly repaired [37]. The initial impact often occurs in utero, giving rise to oncogenic fusion proteins. Subsequent insults, determining disease latency, occur post-birth and may involve genetic, epigenetic, or immune factors (e.g., delayed infection-mediated immune deregulation) [2]. Studies suggest that pesticides like organophosphates, carbamates, and pyrethroids—commonly present in insecticides and herbicides—can impair leukocyte function by inducing apoptosis, arresting the cell cycle, and disrupting immune cell functions [38]. Distinct patterns in chromosomal aberrations, cytologic features, and peripheral blood and bone marrow indices (similar to those found in patients with secondary leukemia typically induced by radiation or chemotherapy) have been documented in adult patients with acute myeloblastic leukemia who have been exposed to pesticides (n = 21) vs. those not exposed (n = 40) [39]. The authors suggested that pesticide exposure may worsen leukemia prognosis and survival by triggering harder-to-treat cytogenetic and clinical subtypes. Overall, epidemiological and biological data support the role of certain pesticides in both the development and prognosis of leukemias.

4.3. Rodenticides: Prevalence and Potential Health Risks

Household and agricultural rodenticide use is common, resulting in over 8000 calls to poison centers in 2021 [40]. Most commonly, these calls are related to ingestion, either intentional or unintentional. It is uncertain how often or to what degree incidental small exposures not prompting calls occur. Rodenticides are used in bait stations and have a low risk of volatilization [41,42] that minimizes the likelihood of exposures of acute significance, but undocumented low-dose exposures may occur relatively commonly in the process of opening, moving, or disposing of bait stations. We could not identify any biomonitoring studies examining this kind of low-dose exposure. In addition, there have been many reported cases of illegally imported chemicals used as rodenticides, such as tetramethylenedisulfotetramine and aldicarb, with entirely different mechanisms of action, causing acute illness in the U.S. [43,44]. The degree to which these factors support the biologic plausibility of our findings is uncertain.

Rodenticides include non-anticoagulants like bromethalin, a neurotoxic compound that disrupts oxidative phosphorylation, leading to cytotoxic edema, though human exposure reports suggest it is found in sub-lethal concentrations with no clear dose–response threshold [45,46]. They also contain anticoagulants like brodifacoum, a potent second-generation "super warfarin" that inhibits vitamin K recycling and disrupts blood clotting [47]. It was found in d-Con, the primary rodent-control product used by participants in our study, at a concentration of 0.005% until 2015, when the EPA banned its residential use. However, brodifacoum remains widely used in professional and agricultural settings [48]. Brodifacoum inhibits vitamin K epoxide reductase (VKOR), disrupting the vitamin K cycle, reducing clotting factor synthesis, and prolonging coagulation times [49–51]. Known for its high affinity and prolonged elimination half-life, brodifacoum causes acute poisonings with symptoms resembling fatal leukemia [52]. Our study emphasizes the need to investigate rodenticide exposures' mechanism of effects on leukemia survival, focusing on hematologic and non-hematologic mechanisms tied to vitamin K inhibition [53].

4.4. Limitations and Strengths

Our study leveraged existing data on pesticide exposure during key developmental periods from a case-control study, though reliance on self-reported questionnaires may introduce recall bias influenced by parents' perceptions or societal pressures. However, 73% of ALL cases were diagnosed under the age of 6, likely improving recall accuracy, supported by consistent data across periods, including the reliable 12-month period prior to the interview. High correlation between exposure periods limits our ability to draw definitive conclusions on the relative contributions of prenatal vs. postnatal exposure. While deceased children were not excluded, parents of 50 children who passed away shortly after enrollment did not complete the interview. The survival rate (87%) aligned with national averages (1995–2015) [30,54], and demographic data from the birth registry for these deceased children who did not complete the interview were comparable to the other cases included, supporting representativeness. However, potential differences in neighborhood income between interviewed and non-interviewed families raise concerns about selection bias. Despite adjustments for key sociodemographic factors in our analysis, residual confounding SES factors cannot be ruled out. The causal diagram indicated no need for additional adjustments beyond income, education, and race and ethnicity, and the additional adjustment for hospital sites did not change the results. However, our data did not capture detailed information on access to specialized healthcare, type of medical insurance, or treatment-related factors such as financial resources, drug availability, and access to novel therapies, all of which may influence survival outcomes. Variability in these factors, including timely administration of conventional treatments and management of therapy-related complications, could contribute to disparities in survival [55]. Finally, the limited number of exposed cases, coupled with high correlation of exposure across time periods, constrain the statistical power of our findings and make it challenging to disentangle the independent effect of exposure during a specific time period driving the association.

This study demonstrates key strengths in accounting for potential confounders, largely due to the rich sociodemographic and pesticide exposure data from the CCLS interviews, which provided insights into records of linkage studies, thus enhancing the rigor of our analyses.

5. Conclusions

Our study, featuring detailed data collection and attention to confounders, suggests associations between pesticide exposure—especially for use of rodenticides during pregnancy—and reduced childhood ALL survival. Future studies should aim for more direct exposure assessment methods, larger sample sizes, and a more comprehensive evaluation of leukemia prognosis, including molecular subtypes of ALL and treatment response. By situating our work within the broader context of the impact of environmental exposures on the pediatric cancer continuum from etiology to short- and long-term outcomes, we contribute to the growing body of knowledge on the impact of chemical exposures on childhood leukemia prognosis. This study stands as an initial step towards understanding the effects of pesticide exposure during key developmental stages on the survival outcomes of children with leukemia, urging further research to enhance survival outcomes by addressing preventable environmental factors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers17060978/s1, Table S1: Estimates and goodness-of-fit statistics for model comparison: Cox proportional hazards model for any pesticide exposure among children with acute lymphoblastic leukemia by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S2: Sociodemographic characteristics and pesticide exposure among 837 children with acute lymphoblastic leukemia—the California Childhood Leukemia Study; Table S3: Periods of residential pesticide use by 5-year survival status at the end of 2020 in children with acute lymphoblastic leukemia—the California Childhood Leukemia Study; Table S4. Multivariate analysis of rodenticide exposures adjusted for all time windows among children with acute lymphoblastic leukemia using Cox proportional hazards model by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S5. Multivariate analysis of pesticide exposures in pregnancy period adjusted for other pesticide groups among children acute lymphoblastic leukemia using the Cox proportional hazards model * by 5-year survival status at the end of 2020—the California Childhood Leukemia Study; Table S6. Residential pesticides and 5-year survival at the end of 2020 in childhood acute lymphoblastic leukemia: Cox proportional hazards models * by duration of breastfeeding; Figure S1: Directed acyclic graph (DAG); Figure S2: Correlation matrix between pesticide categories and windows of exposure.

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Institutional Review Board Statement: This study was approved by the Institutional Review Boards at the University of California, Berkeley, and the California Department of Public Health (approval code 2020-167, on 29 August 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the California Childhood Leukemia Study. The Committee for Protection of Human Subjects granted a waiver of consent to also conduct research on outcomes.

Data Availability Statement: The epidemiological and clinical data generated in this study are not publicly available due to the terms of the informed consent signed when subjects were enrolled to the CCLS study but may be available upon reasonable request from the corresponding author. The death data analyzed in this study were obtained from the California Department of Public Health (CDPH) Center for Health Statistics and Informatics (CHSI) and are not publicly available due to terms of CDPH-CHSI.

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Abbreviations

The following abbreviations are used in this manuscript:

ALL	Acute Lymphoblastic Leukemia
BIC	Bayesian Information Criterion
CCLS	California Childhood Leukemia Study
CI	Confidence Interval
DAG	Directed Acyclic Graph
HR	Hazards Ratio
NCI	National Cancer Institute
Ref	Reference
SES	Socioeconomic Status

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Spatial patterns of anticoagulant rodenticides in three species of medium-sized carnivorans in Pennsylvania

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Abstract

Human influences on natural environments are now ubiquitous but manifest in multiple and unique ways depending on local environments and communities. Attempts to control, or mediate, local pests to residences or to agriculture can impart important negative consequences on systems. Secondary exposure to anticoagulant rodenticides (ARs) can cause numerous adverse effects on wild carnivores including death. Few studies have quantified AR prevalence, investigated their pathway of exposure, or associations with specific location types in the northeastern U.S. We hypothesized that ARs would be found in the mesocarnivore community throughout Pennsylvania and have the greatest detection rate in highly urbanized or agricultural landscapes. From 2019 through early 2022, we collected carcasses to obtain liver samples (n = 265) from three species of carnivores: bobcats (*Lynx rufus* (Schreber, 1777)), fishers (*Pekania pennanti* (Erxleben, 1777)), and river otters (*Lontra canadensis* (Schreber, 1777)). We used generalized linear models to test for differences in AR detection rates among species and spatial scales including the six Pennsylvania Game Commission regions and 23 wildlife management units. We detected ARs in all species (44.2% collectively), but detection rates differed among species. Our study is the first to document ARs within North American river otters.

Key words: anticoagulant rodenticides, development, *Lontra canadensis* (Schreber, 1777), *Lynx rufus* (Schreber, 1777), *Pekania pennanti* (Erxleben, 1777), river otter

Introduction

Rodents have been perceived as a threat to human health and livelihood for millennia (Van den Brink et al. 2018). Over the last century, various compounds have been developed to reduce rodent populations, especially those near humans. The most used compounds are anticoagulant rodenticides (ARs), which are further categorized as either first generation (FGAR) or second generation (SGAR) (Rattner et al. 2014), with second-generation compounds developed after resistance to the first generation was identified in some rodents (Jacob and Buckle 2018). In general, second-generation ARs are more potent, longer acting, and more likely to accumulate in tissues than first-generation ARs (Erickson and Urban 2004; Rattner et al. 2014; Elliott et al. 2016), with tissue liver half-lives of up to 350 days (Eason et al. 2002; Fisher et al. 2003; Horak et al. 2018).

Anticoagulant rodenticides inhibit vitamin K epoxide reductase, the enzyme responsible for maintaining adequate vitamin K levels (Watt et al. 2005; Rattner et al. 2014). In 2008, to prevent poisoning of non-target wildlife, children, and domestic animals, the U.S. Environmental Protection Agency (EPA) restricted use of SGARs to agricultural contexts and licensed professionals (e.g., commercial exterminators) (Erickson and Urban 2004; Memmott et al. 2017). Products containing the two most toxic compounds, brodifacoum and difethialone, were still available to the public until 2015 (Murray 2017), and the remaining stockpiles of products containing these two compounds may be available for use for decades to come. Moreover, other second-generation ARs remain readily available (e.g., in hardware or farm supply stores), with widespread exposure to these compounds detected in wildlife around the world. The persistence and toxicity of second-generation ARs render these compounds of particular concern for biomagnification (elevated concentrations) in predators (Horak et al. 2018; López-Perea et al. 2019; Fernandez-de-Simon et al. 2022). Many carnivores readily scavenge carcasses they find on the landscape. Rodents, either recently deceased or suffering rodenticide intoxication, could be encountered, consumed, and provide secondary exposure to carnivores. Notably, Riley and co-workers (Riley et al. 2003) reported acute toxicity from AR exposure as the second leading cause of mortality in coyotes (*Canis latrans* Say, 1823) over a 9-year period in the Santa Monica Mountains National Recreation Area (bordering Los Angeles, California, United States).

The concentrations of ARs that evoke mortality are both poorly understood and apparently highly variable within and among species (Quinn 2019). For example, lethal concentrations from liver samples were found to be as low as 0.17 μg/g for the caracal ((*Caracal caracal* (Schreber, 1776)); Serieys et al. 2019), yet in another wild felid (bobcat (*Lynx rufus* (Schreber, 1777))) individuals with liver concentrations of up to 5.81 μg/g lived for several years (Serieys et al. 2015). A growing area of concern is the sublethal effect of chronic exposure to ARs, which may influence immune function and behavior, potentially affecting an animal's ability to respond to external stimuli (such as predators), thereby putting them at increased risk of mortality beyond the effects of AR intoxication (Serieys et al. 2018*a*, 2018*b*). In laboratory studies, sublethal exposure to ARs produced upwards of 70% mortality when combined with other stressors (Jaques 1959). In a long-term field study of bobcats, secondary AR exposure (at ≥0.05 μg/g wet weight in liver) was associated with severe infestation of notoedric mange (an ectoparasitic disease) (Riley et al. 2007; Serieys et al. 2015). Likewise, a negative association between AR exposure and body condition has been observed in weasels and stoats (Elmeros et al. 2011). Thompson et al. (Thompson et al. 2014) indicated a negative association between AR exposure and fisher survival in California. In studies of humans, dogs, and sheep, the reproductive consequences of AR exposure have included abortions, fetal toxicosis, congenital deformities, and decreased sperm counts (Ginsberg and Hirsh 1989; Robinson et al. 2005; Murray 2017). Although data are limited, Serieys et al. (2015) documented that AR residues transfer from mother to offspring in bobcats. In sum, AR exposure may pose an important challenge for individual fitness and population viability; large-scale analyses evaluating the explicit effects of ARs to populations are difficult to design and execute.

Rodenticide exposure in carnivorous species is widespread where it has been investigated (Riley et al. 2003, 2007; McMillin et al. 2008; Gabriel et al. 2012; Serieys et al. 2015; Rudd et al. 2018; Wiens et al. 2019). The spatial variation in type and extent of exposure, and in the potential for repeated exposure, has been associated with human-dominated landscapes (i.e., commercial, residential, and agricultural areas) because these areas have the highest use of ARs (Cypher et al. 2014). High AR concentrations have been reported in relatively rural and isolated locations when there are activities (e.g., illegal cannabis growing on public lands) where humans deploy large numbers of ARs to control rodents (Gabriel et al. 2012, 2015). Such activities may confound or obfuscate the general patterns associated with AR exposure in wildlife. Nevertheless, on average individual carnivores that live in these areas should have the greatest likelihood of exposure and to any adverse effects of ARs (Hindmarch and Elliott 2018; Serieys et al. 2019). Researchers have shown that some carnivorans like kit foxes (*Vulpes macrotis* Merriam, 1888) may encounter first generation compounds in one habitat type (e.g., undeveloped or agricultural settings) and encounter second-generation compounds in residential or industrial areas (Cypher et al. 2014). Moreover, ARs have been increasingly detected in aquatic or semi-aquatic animals, including some species of freshwater fish, invertebrates, and raptors that forage in aquatic systems (e.g., Bald Eagles (*Haliaeetus leucocephalus* (Linnaeus, 1766))) (Regnery et al. 2020; Niedringhaus et al. 2021). The sources of ARs in these systems are also traceable to urban areas (Regnery et al. 2020).

The northeastern United States supports the highest rural human population densities in the United States, leading to a high degree of human–wildland interface. Eastern forests have relatively high rates of private ownership, which could allow for large and consistent use of ARs with little oversight (L'Roe and Allred 2013). The amount of land cover devoted to agriculture and human populations is large compared to other regions of the United States (Drummond and Loveland 2010; Homer et al. 2020). As a result, carnivorans in the Northeast are likely at high risk of AR exposure. Fishers (*Pekania pennanti* (Erxleben, 1777)) and bobcats are terrestrial predators of conservation and management interest across North America (Powell 1993; Lovallo and Anderson 1996; Powell et al. 2017). Fishers were reintroduced into Pennsylvania (PA) in the late 1990s and have apparently stable or increasing populations across much of the state (Lewis et al. 2012) (Supplementary Fig. S1). Fishers readily scavenge carcasses and are prolific predators of rodents. Bobcats may also scavenge, but are more associated with urban, suburban, and human-modified landscapes than fishers (Lovallo and Anderson 1996; McNeil et al. 2017; Powell et al. 2017). Aquatic and semi-aquatic species have also been identified to have exposure to ARs (Serieys et al. 2019; Niedringhaus et al. 2021); nonetheless, investigations into the exposure of aquatic mammalian species are seldom undertaken. River otters (*Lontra canadensis* (Schreber, 1777)), like fishers, are classified in the family *Mustelidae,* and are semi-aquatic. They, too, will readily scavenge for food in both aquatic and terrestrial environs. Yet river otters primarily prey on fish and aquatic invertebrate species (Liers 1951; Day et al. 2015). Their aquatic habitat and dietary differences provide an important contrast to fishers and bobcats that are indicative of the relative availability of ARs across the landscape. Examining differences in exposure among these three species provides an important test of whether ARs are ubiquitous, confined to specific landcover or habitat types, or a product of the relative life histories and food web of the species.

Here we use fishers, bobcats, and river otters to investigate spatial and inter-specific variation of AR exposure across PA. This set of species represents an important contrast between life histories and major land-use differences that could inform the extent and pathways by which carnivores are being exposed to ARs. These three species are also highly regulated and routinely monitored in PA. Yet, prior to this study, there was no baseline data on AR exposure in these species that could inform or refute the potential roles that ARs may play in their population status or trends. We hypothesized that AR detection would be observable based on differences in habitat use, as indexed by landcover types, and differences in foraging strategy and diets across species. We predicted that fishers would have the highest exposure to ARs followed by bobcats, then river otters. We based this prediction on the observation that fishers occupy terrestrial environments that may be near agricultural or developed areas in Pennsylvania. We expected bobcats to have a reduced exposure because, al-

though they exist in similar habitat as fishers, we hypothesize that they scavenge dead or dying rodents at a reduced rate compared to fishers. Finally, we expected river otters to have the lowest exposure rates because of their use of aquatic food sources, where exposure to ARs would be diminished compared to terrestrial food sources near agricultural or human development. We also evaluated the hypothesis that AR exposure would be highest for all species in areas with the highest total amount of land use dedicated to agriculture or human development (urbanization). Finally, because long-lived animals should have the most opportunity to encounter ARs, we hypothesized that the oldest animals, of all three species, would have the highest rate of exposure.

Materials and methods

Sample collection

We collected carcasses from mesocarnivores from several sources across PA from autumn 2019 through March 2022. Liver samples from 2019 were obtained from carcasses housed in freezers at Pennsylvania Game Commission (PGC) regional offices in PA that had been previously collected as illegal or accidental harvests, nuisance animals, or vehicular collisions. In some instances, these samples had no specific corresponding date or location of collection but were included for analysis to provide a general background estimate of prevalence across the state. Starting in winter of 2020, we worked cooperatively with licensed PA trappers to collect liver samples from recently legally harvested bobcats, fishers, and river otters, and continued to opportunistically collect carcasses and liver samples. The collection of carcasses was coordinated by PGC staff with field support from Wildlife Futures Program personnel. Our design and intent were to gather equal numbers of carcasses from each species in the six PGC regions and to obtain similar numbers from as many wildlife management units ($n = 23$; wildlife management units) as possible. Because samples could only be collected through cooperation with local trappers and regional staff, we could not fully control the numbers or species that came from each wildlife management unit or region of the state. As a result, spatial variation in population numbers (e.g., fishers; Supplementary Fig. S1) and trapping effort led to unequal spatial representation in our data. Both PGC regions and wildlife management units are combinations of ecological, geopolitical, and within-agency administrative units that function to manage wildlife (e.g., allocate license numbers or regulations). Ideally, our study and analyses would focus on the smallest spatial scales that were biologically relevant to the species of research, but wildlife management units are the smallest extent that trappers were required to report their harvested animals. From each carcass, we removed the liver and placed a roughly 100 g portion in a sterile, plastic bag (Whirl-pak™, The Aristotle Corporation, Stamford, CT) that was frozen within 2 h. For each specimen, we recorded the species, sex (if determinable), relative age (juvenile, adult), location of collection to the county or wildlife management units level, and manner of death (e.g., roadkill, legal trapping, etc.). Where possible, we extracted one canine tooth that we submitted to Matson's Laboratory (135 Wooden Shoe Lane, Manhattan, MT 59741, USA) for age estimation (Arthur et al. 1992). For each carcass, we created a unique identifier based on the species, date, and location of collection to match AR data to environmental data.

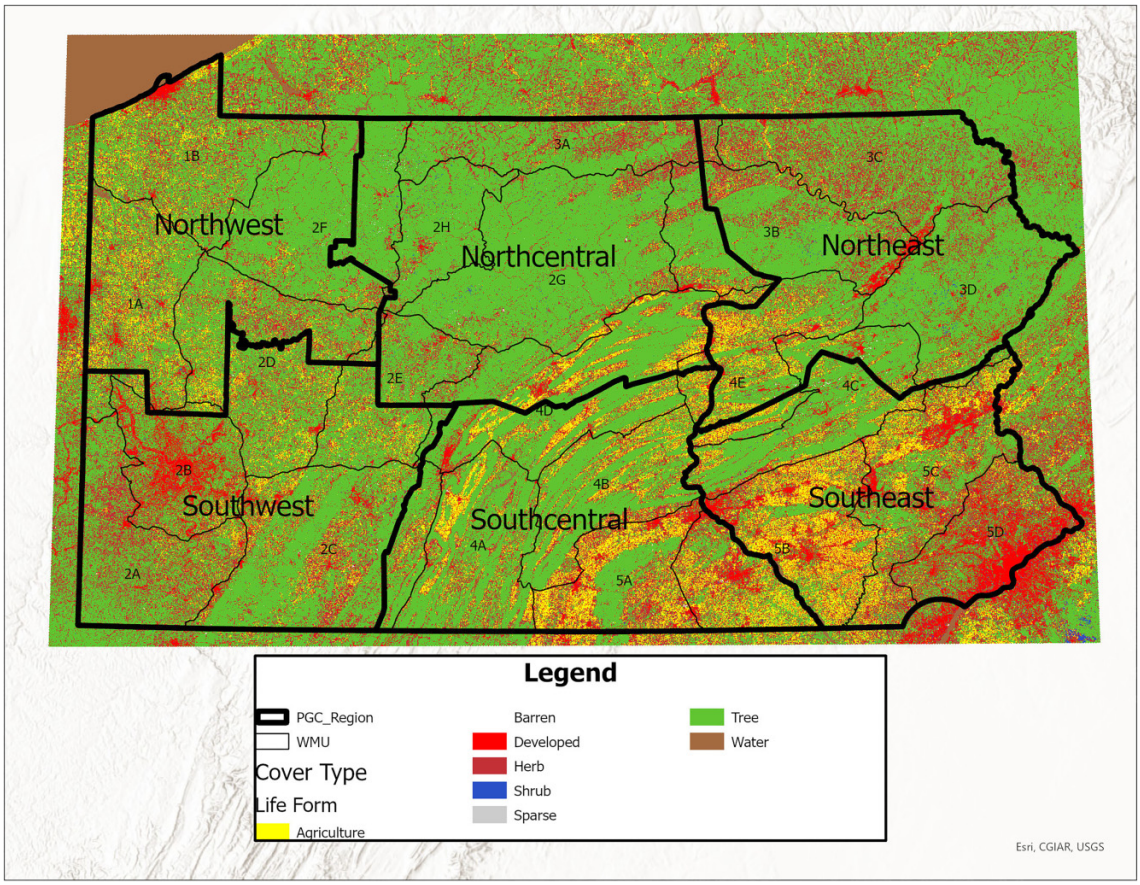
No live animals were handled by the researchers for this work.

Lab analysis

Liver samples were screened for first- and second-generation ARs: brodifacoum, bromadiolone, chlorophacinone, coumachlor, coumafuryl, dicoumarol, difenacoum, difethialone, diphacinone, pindone, and warfarin at the Pennsylvania Animal Diagnostic Laboratory System (PADLS) Toxicology Laboratory at New Bolton Center (Kennett Square, PA). A published QuEChERS extraction (short for quick, easy, cheap, effective, rugged, and safe) and high-performance liquid chromatography (HPLC) method (Vudathala et al. 2010) was used for the analysis of the livers. We established the method detection limit by using 1 g of liver which was spiked at 0, 0.01, 0.05, and 0.500 μg/g on a wet mass basis. One gram of liver was then homogenized with 4 mL of acetonitrile and centrifuged to form a soft pellet. The organic layer was transferred to a clean test tube containing 0.03 g PSA (primary–secondary amine), 0.10 g florisil adsorbent, 0.175 g $MgSO_4$, and 0.05 g basic alumina. An additional 0.050 g of C_{18} sorbent was used to obtain a clean sample suitable for analysis. The tubes were vortexed for 15 s, allowed to rest for 5 min, and then centrifuged at approximately 438 g for 5 min. The supernatant was then transferred to a clean test tube and reduced to dryness under a stream of nitrogen using a warm (60 ± 5 °C) water bath. The residue was dissolved in 0.50 mL of ion pair diluent and then vortexed. The solution was microfuged through a 0.22-micron SpinX filter and the clear filtrate was transferred into an autosampler vial fitted with a high recovery vial for analysis.

Anticoagulant rodenticide detection and quantification was performed using a Shimadzu (Kyoto, Japan) HPLC system, consisting of a CBM-20 A controller, SIL-20AC autosampler, AC-20AT pump, SPD-M20A diode array detector, RF-10AXL fluorescence detector, and a Betasil reverse phase C18 column 150 × 4.6 mm, 5 μm particle size (Thermo Electron Corporation). We conducted all HPLC analyses using 0.03 M tetrabutylammonium hydroxide (TBA) buffer (adjusted to pH 7 with o-phosphoric acid/methanol, 25:75, v/v) as solvent A and methanol as solvent B at a flow rate of 0.6 mL/min in a gradient run. We set the gradient at 30% B for 10 min followed by gradient to 80% B in 4.5 min, and increasing to 90% B in the next 0.5 min. We washed the column with 90% B for 5 min followed by equilibration to initial conditions for 4 min. The fluorescence detector was set at 280 nm excitation and 410 nm emission. We monitored the diode array detector from 240 to 340 nm, with quantification done at 325 nm. We confirmed our results in the abovementioned conditions and with solvents at a slightly different gradient. The gradient consisted of 30% B for 7 min, a gradient to 75% B in the next 3 min, increasing to 80% B in the next 11 min, then holding

Fig. 1. Pennsylvania Game Commission regions (thick black lines) and wildlife management units (thin black lines) with color background depicting eight broad land cover types across Pennsylvania. The spatial data used for the regional and unit lines were accessed through the Pennsylvania Spatial Data Access (PASDA 2022; https://www.pasda.psu.edu/) and cover types were downloaded through LandFire (2022) (https://landfire.gov/). All spatial layers were projected using Lambert Conformal Conic coordinate system and the North American Datum (NAD) 1983.



it for 5 min followed by equilibration to starting conditions for the next 4 min (Vudathala et al. 2010).

We used fluorescence detection to monitor for brodifacoum, bromadiolone, coumafuryl, difenacoum, and warfarin; UV spectra monitored for the presence of chlorphacinone, difethialone, and diphacinone. Each compound had an established limit of quantification (LOQ), which varied by compound as follows: brodifacoum (0.010 μg/g), bromadiolone (0.025 μg/g), chlorophacinone (0.050 μg/g), coumachlor (0.100 μg/g), coumafuryl (0.100 μg/g), dicoumarol (0.100 μg/g), difenacoum (0.010 μg/g), difethialone (0.050 μg/g), diphacinone (0.050 μg/g), pindone (0.100 μg/g), and warfarin (0.100 μg/g). Positive results below the LOQ were recorded as trace amounts. At or above the LOQ, numerical concentrations were recorded in parts per million (ppm), which is equivalent to micrograms per gram (μg/g), on a wet weight basis. For our analyses, we report any amount that was detectable, even those below LOQ, as a positive detection.

Statistical analysis

For most analyses, we used individual exposure rather than population-level exposure to assess patterns of AR distribution and detection. We included in our analysis all detected ARs (trace or quantifiable amounts) to estimate exposure. Where appropriate, we report concentration amounts in μg/g on a wet weight basis for samples that were above trace level. Using Statistical Application Software (SAS; Cary NC), we fit generalized linear models with a binomial distribution to determine if there were differences in detection proportion among the three species. Further, we examined if there were differences in AR detection among PGC regions (Fig. 1) and then we examined both an additive and interactive model using both species and region. We obtained data on landcover for the year 2020 from the Landfire website (landfire.gov) and used the "ENV_LF" categorization of these data (Fig. 1). This classification system places all land within PA as being forest, herbaceous, water, barren, developed, shrub, agriculture, or sparse. For our analyses, we considered the proportion of land in each PGC region and wildlife management units that were classified as developed and agricultural. We also added the values of the developed and agricultural land together to provide a metric that included those two land classes that we hypothesized were associated with rodenticide availability and ultimately detection in wild carnivore populations. For both PGC region and wildlife management units, we obtained shapefiles delineating borders from Penn-

Table 1. Number of samples with detectable anticoagulant rodenticide concentrations by compound types and the percentage of samples detected above the minimum detectable amount and (% of quantifiable compounds >0.1 μg/g on a wet weight basis) for those with quantifiable levels, their mean concentration (Mean Conc), and the minimum and maximum quantifiable concentrations (range) observed for all detections for three species of carnivores (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) in Pennsylvania from 2019 to 2022.

Compound	Detections	Percent > trace (percent > 0.1 μg/g)	Mean conc (μg/g)	Range
Warfarin	2	1.6% (0.0%)	–	0–0
Coumafuryl	0	0.0% (0.0%)	–	0–0
Diphacinone	59	47.9% (52.0%)	0.2	0.05–0.78
Pindone	0	0.00% (0.0%)	–	0–0
Brodifacoum	27	21.9% (16%)	0.05	0.01–0.25
Difenacoum	2	1.6% (0.0%)	–	0–0
Bromadiolone	8	6.5% (15.3%)	0.07	0.03–0.28
Chlorophacinone	8	6.5% (0.0%)	–	0–0
Difethialone	7	5.7% (1.0%)	0.16	0.16
Dicoumarol	10	8.1% (75.0%)	0.54	0.1–1.78
Coumachlor	0	0.0% (0.0%)	–	0–0

Note: Mean concentrations and standard deviations are not reported for compounds only detected at trace levels, below the method's limits of quantification.

sylvania Spatial Data Access (https://www.pasda.psu.edu/). For both wildlife management units and PGC region, we used the "Zonal Histogram" tool in ArcGIS Pro version 9.1 (ESRI Co, Redlands CA, USA) to quantify the total proportion of landcover types within each respective geographic unit. Finally, for each region, we calculated the proportion of AR detections across all three carnivore species and then for each species individually. For wildlife management units, we calculated the proportion of detections for all three species combined but could not do so for each species individually because we had insufficient data across all units.

Using generalized linear models, we tested for a positive relationship between the proportion of a land unit that was developed or agricultural, and finally a combination of developed and agricultural lands per region or wildlife management units and the proportion of samples where we detected ARs in those respective spatial units. At the regional scale, we evaluated AR detection across all three species in addition to each species individually. We recognized that the combined metric would be correlated to either, or both, developed and agricultural lands, but our intention was to evaluate whether the proportion of either developed or agricultural lands better explained patterns of AR detections for any species more robustly than a single cumulative metric. For these analyses, we used a gamma distribution and a log-link transformation. Finally, we evaluated whether the age of the animals was related to AR detections by using generalized linear models to test for a relationship between AR detection and age of the animals.

Results

We collected and generated AR results from 265 livers from three carnivore species in Pennsylvania. Most (65%) were collected from carcasses legally harvested by trappers. Mistakenly killed or illegally killed animals accounted for 16.6% of all carcasses, followed by road-killed animals (12.8%) and the remainder (5.6%) were killed or collected without documentation. We collected 105 (39.6%) river otters, 97 (36.6%) fishers, and 63 (23.7%) bobcats. We detected ARs at both trace and quantifiable levels in all three species examined. Across species, the total trace detection rate was 44.2%, whereas 17.4% had quantifiable levels of ARs. Of the 11 compounds for which we tested, only three were not detected in any liver sample (coumafuryl, pindone, and coumachlor) (Table 1). Diphacinone and brodifacoum were the most detected AR compounds across all species at both trace and quantifiable levels (Table 1). Bromadiolone and dicoumarol were detected at modest levels compared to other compounds. Thirteen individuals were found to have >2 compounds at quantifiable levels and 33 had a single quantifiable compound. One animal had trace levels of four different compounds, five had three, and 11 had two; all other trace detections were positive for one compound (Table 1).

Dicoumarol was found in the highest quantifiable concentrations (0.53 μg/g ± 0.82 SD) (Table 2). Generally, there was high variation among the concentrations found across individuals and species for both the numbers and types of compounds found. Species differences existed within the data at regional and wildlife management units levels. Statewide, species differences were detected ($\chi^2_{[2]} = 61.86$, $P < 0.0001$) and fishers had the highest exposure across the state with 70% of all fishers testing positive for at least one compound (Table 2). Bobcats had the second highest AR detection, followed by river otters (Table 2). Nearly half of all bobcats tested showed at least trace AR levels, whereas 17% of otters were exposed to ARs (Table 2). Evaluating regional differences in exposure to ARs across all species indicated high variation across regions (Table 3). We also had high variation in the numbers of samples we received from each region, with the Southeast providing 8 and the Northeast providing 79 samples, respectively. These differences may be attributed to dif-

Table 2. Total number of samples (Total), samples with any positive anticoagulant rodenticide detections (Detections), the percentage detection within species (Percent detected), and % of all detections (Percent species) pooled across species ($n = 117$) by species (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) for samples collected in Pennsylvania from 2019 to 2022.

Species	Total	Detections	Percent detected	Percent species
Bobcat	63	31	49.21%	26.50%
Fisher	97	68	70.10%	58.12%
River otter	105	18	17.14%	15.38%

Table 3. Total number of samples (Total) and positive anticoagulant rodenticide detections (Detections), and the percentage of samples with detections for each species (bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*)) and region sampled in Pennsylvania from 2019 to 2022.

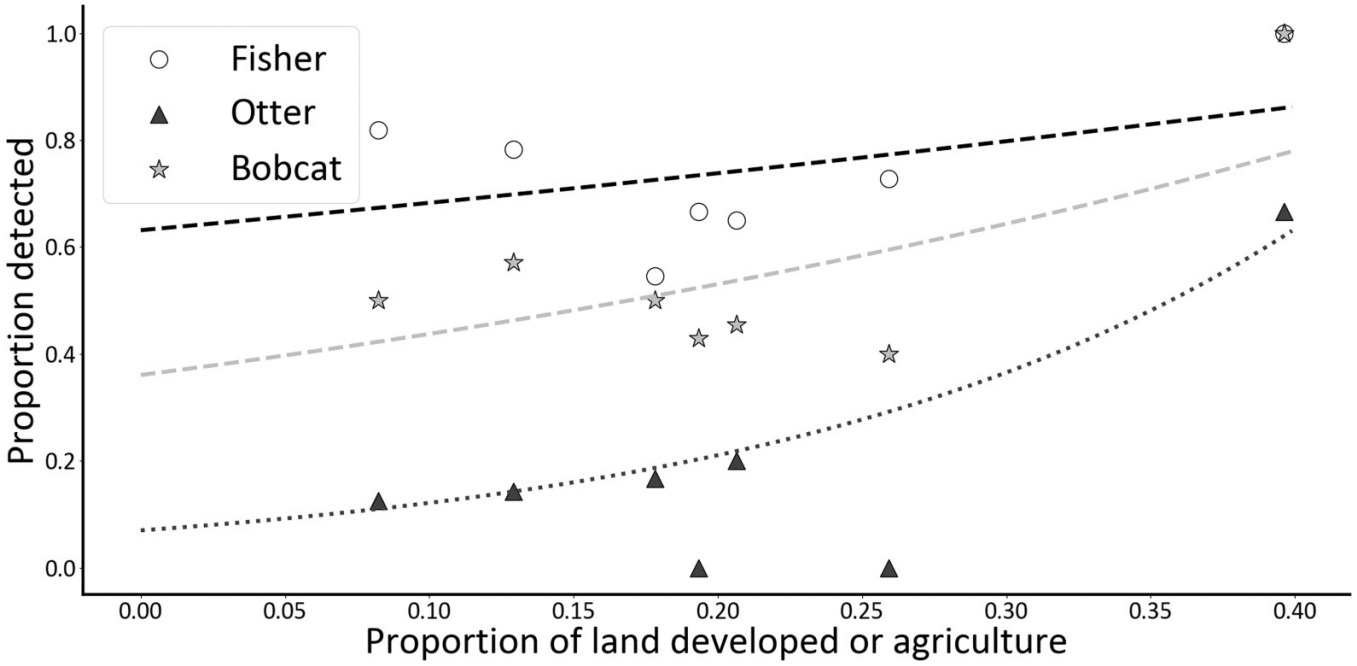
Region	Species	Total	Detections	Percent detected
North Central	Bobcat	16	8	50.0
	Fisher	11	9	81.8
	River otter	8	1	12.5
		35	18	51.4
North East	Bobcat	14	8	57.1
	Fisher	23	18	78.3
	River otter	42	6	14.3
		79	32	40.5
North West	Bobcat	4	2	50.0
	Fisher	22	12	54.5
	River otter	18	3	16.7
		44	17	38.6
South Central	Bobcat	10	4	40.0
	Fisher	11	8	72.7
	River otter	0	0	•
		21	12	57.1
South East	Bobcat	1	1	100.0
	Fisher	4	4	100.0
	River otter	3	2	66.7
		8	7	87.5
South West	Bobcat	7	3	42.9
	Fisher	6	4	66.7
	River otter	4	0	0.0
		17	7	41.2
Unknown	Bobcat	11	5	45.5
	Fisher	20	13	65.0
	River otter	30	6	20.0
		61	24	39.3
Total		265	117	44.2

ferences in species representations or simply due to random chance or collection effort. Nevertheless, regional differences did not provide a significant descriptor of detection ($\chi^2_{[6]} = 10.36$, $P < 0.11$). When we included both species and region in an additive model, we found a similar pattern where region had little explanatory power. Additionally, an interactive model did not converge because the data were too scant. On average, the SE region had the highest exposure of AR detection (mean = 87.5%) across all samples and species.

Regional landcover analyses

The regional analyses revealed that all regions are dominated by forested land cover (62% ± 12%). The SE region has the highest proportion of developed and agricultural land

Fig. 2. Regionally explicit relationships between anticoagulant rodenticide detections for bobcats (*Lynx rufus*), fishers (*Pekania pennanti*), and river otter (*Lontra canadensis*) in Pennsylvania from 2019 to 2022.



(21% and 17%, respectively). The SW region has the second highest proportion of developed lands (12%), whereas the Southcentral region has the second highest proportion of agricultural lands (16.9%) (Fig. 2).

There was a positive relationship between AR detections, using all species, and the proportion of land that was developed or agricultural ($\beta = 2.15 \pm 0.53$, $\chi^2_{[1]} = 6.41$, $P = 0.011$). Additionally, a similar pattern emerged when we considered individual species and used either proportion of land that was developed or agricultural as individual explanatory variables (Table S1). The lone exception was that fishers did not exhibit a positive relationship between AR detection and any land-cover metric we tested (Fig. 3; Supplemental Table S1). For each species, the region with the highest proportion of developed or agricultural land had the highest AR detection rates (Supplemental Fig. S2). The SE region, which has the greatest development and human population densities, is associated with higher proportions of AR exposure regardless of which dependent metrics we examined.

Wildlife managment unit analyses

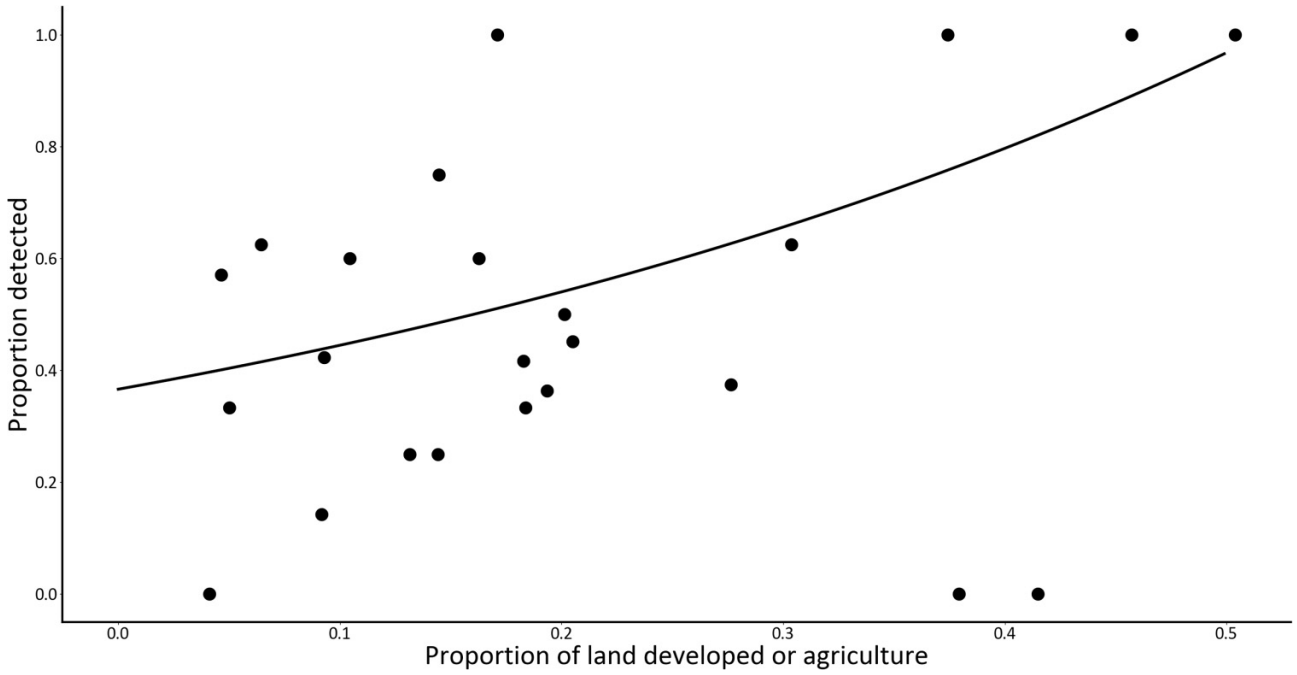
For the wildllife management unit analyses, we confined the analyses to AR detection across all species because we had insufficient data to perform these analyses for each species individually. Using the wildlife management unit-based values, we found that there was a positive relationship between the total detections per wildlife management units and the average concentration of ARs found ($\beta = 7.58 \pm 0.13$, $\chi^2_{[1]} = 6.8$, $P = 0.0089$). Generally, the distribution of land cover we found in the regional analyses was recapitulated in the wildlife management units analyses, but with greater replication at a finer scale. The wildlife management units in the Southeast (around Philadelphia) and Southwest (around Pittsburgh) have the highest proportion of developed and agricultural lands. Northern Wildlife Management Units have relatively undeveloped land and more tree cover. We found strong associations with developed or agricultural lands ($\beta = -2.394 \pm 0.1$, $\chi^2_{[1]} = 8.4$, $P = 0.0038$). The highest rates of detection were found in the Southeast and Southwest Wildlife Management Units (Fig. 3) In contrast, several wildlife management units with relatively low amounts of developed or agricultural lands had relatively high proportions of AR exposure across species, especially fishers.

Age analysis

We were able to estimate ages for 113 (42%) carcasses. Ages for the others could not be estimated either because the head was not present or because we could not extract a tooth. Across all three species, ages ranged from young of the year to 8 years. Of the individuals that had age data, 79% were 2 years or younger (Supplementary Table S2). With respect to species, 17 (73%) of bobcats, 32 (91%) of fishers, and 41 (74%) of otters were 2 years or younger. The oldest bobcats ($n = 2$) were 6 years old; a single fisher was 5 years old, and three otters were estimated to be 8 years old (Supplementary Table S2).

Age, across species, was not an important determinant of the probability that an animal would have any type of AR detection ($\beta = 0.127 \pm 0.009$, $\chi^2_{[1]} = 1.98$, $P = 0.15$). This result appears to be explained by species effects because only otters and bobcats were older than 5 years, and none of those animals tested positive for quantifiable levels of ARs. Only one

Fig. 3. Relationships between anticoagulant rodenticide detections for all detections for all species in Pennsylvania from 2019 to 2022.



fisher and one otter older than 4 years tested positive for ARs. When we tested models with both species and age effects, age was found to be unimportant ($\chi^2_{[1]} = 0.17$, $P = 0.67$). We tested additional models for age considering the individual species, but all failed to show significant results relative to patterns of age and AR exposure.

Discussion

Our study indicates that AR exposure among mesocarnivores is relatively common in Pennsylvania and that they are most detected in areas with high human development. Nevertheless, ARs appear to be present, and to some degree common, in even less human-dominated landscapes. ARs appeared in every species we examined. Our study appears to be the first to take multi-species mesocarnivore approach across a large geographic region. Pennsylvania is among the largest states in the NE United States, which provided us a unique ability to assess ARs within and across regions of different human population densities and land uses. Though our study supports other studies of exposure to ARs within carnivores, it is distinctive in comparing relative rates of exposure among similar sized carnivores with different life histories. Consequently, our study is the first to explicitly test hypotheses about mechanisms of exposure at broad scales. Additionally, our study provides a valuable baseline for AR exposure for three species of mesocarnivores in Pennsylvania. This baseline may be used to assess population trends in mesocarnivores and to build upon our understanding of the health and influence of habitats in which these and other species occur.

We found ARs across all six PGC regions of Pennsylvania and in all three species of carnivores we examined. Fishers were significantly more likely to be exposed to ARs regardless of region compared to bobcat and river otter. Bobcats had relatively higher rates of exposure compared to otters but lower than fishers (Table 2). Detection rates of ARs for fishers in Pennsylvania (70%) were similar, though modestly lower, to those in California (79%) (Gabriel et al. 2012). Yet, detection rates for fishers in Pennsylvania were nearly 30% lower than those detected in Vermont and New Hampshire (97%, $n = 45$) (Buckley et al. 2023). Based on liver samples, Pennsylvania bobcats also had lower rates of AR exposure (49%) compared to bobcats in Southern California (89%) (Serieys et al. 2015). Bobcats in southern California may live in proximity to higher human densities than across Pennsylvania. For example, AR exposure rates were also very high for a study of San Joaquin kit foxes (86%, $n = 30$) near Bakersfield, CA (McMillin et al. 2008). Had we acquired more samples from animals near urban areas, our results may have been similar. Critically, the studies in California, were based on long-term intensive studies of individual populations where animals were tracked and individual mortalities were investigated. Our study focused on animals we could sample coincidentally due to death from other means across the state. Such differences in study approach and intensity may contribute to differences in detection rates that we cannot account for nor quantify. The differences among studies, therefore, should not be taken as a conclusion about relative levels of ARs within those systems.

Importantly, we demonstrate that river otters, along with terrestrial mesocarnivores, were exposed to ARs in the eastern US, although the pathways that lead to exposure may be different or less prevalent than in fishers or bobcats. We have shown a single otter exposure above the method detection

limit for the compound brodifacoum (at 0.01 μg/g), which has not been previously documented in the primary literature. Other otter species and semi-aquatic mammal and bird species are known to be exposed in other systems and our study appears to confirm the presence of ARs in aquatic systems in Pennsylvania and likely throughout the Northeastern United States (Ruiz-Suárez et al. 2016; Serieys et al. 2019; Niedringhaus et al. 2021). In keeping with our general understanding of how carnivores are exposed to ARs, the highest rates of exposure in PA occurred in areas with relatively high rates of human development and agriculture, areas where rodenticides should have the highest use and highest likelihood for contact with wildlife species. We found that at the broad regional scales there were not differences in exposure rates for any species or for all species combined. We did see such differences at the level of the WMU and it indicates that even though exposure rates are high near cities and agriculture, there were still high rates of exposure in some portion of virtually every region.

We hypothesized fishers would have the highest rates of exposure compared to the other carnivores we tested because they are the species most likely to scavenge and prey upon AR-exposed rodents (Gabriel et al. 2012; Gabriel et al. 2015). Bobcats are less likely to scavenge in terrestrial systems but could conceivably capture sick or AR-weakened rodents. Nevertheless, because fishers would scavenge and eat these affected rodents, we expect them to have higher rates of exposure compared to bobcats. Otters forage and live in aquatic systems where less is understood about AR detection and exposure in mammals. Even so, having relatively low human development does not indicate rodenticides are absent from aquatic or terrestrial ecosystems. In our study, regions or wildlife management Units with high forest or non-developed areas still showed carnivore exposure to ARs, and this is likely due to the relative abundance of housing and agriculture throughout PA. More detailed information about carnivore home ranges, use of areas near housing, and sources of ARs is required to fully understand the mechanisms by which they are encountering, presumably, AR-exposed rodents or being exposed directly. Some ARs are flavored (e.g., bacon or cheese) such that they could be attractive to carnivores and consumed directly if they are not used appropriately. The relatively low rates, and concentrations, of exposure in otters could represent a modest AR signal in the aquatic systems (e.g., invertebrates and fish) or could represent exposure from terrestrial systems that ultimately find their way into the aquatic ecosystem. Like fishers, otters are mustelids that will scavenge carcasses, and AR-exposed rodents that wash downstream from terrestrial systems could be consumed. Large cities may also liberally use rodenticide within sewer systems, which could directly introduce ARs into the aquatic system (Regnery et al. 2020). All mechanisms are possible, or even likely, and the presence of ARs in otters cannot exclude any of these.

During our study, we collected more otter carcasses than we predicted and relatively fewer bobcats and fishers. We may have received relatively high numbers of otter samples because otters are inadvertently killed in beaver sets ($n = 29$ for this study) during the PA trapping season, and because all legal otter harvests ($n = 70$) must be reported to local game wardens to obtain a Convention on International Trade in Endangered Species (CITES) permit. Both circumstances provide additional contact with PGC wardens and increase the probability of obtaining samples. Though we did not expect that otters would have high rates of detection, the relatively large numbers of samples from otters do provide an important examination of their rate of exposure to ARs that has not previously been documented. The PGC also must issue a CITES tag for all legally harvested bobcats; however, the method for obtaining tags for bobcats is different from otters and does not place trappers in contact with game wardens under typical legal harvests where trappers simply self-report their harvest. Additionally, the species is less likely to be mistakenly killed in traps set for other species ($n = 2$ for this study). Permitted fisher and bobcat trappers are limited to one of each species per person per season and some trappers were reluctant to relinquish them for study (especially when we requested the head). For all three species, legal trapping occurs only in select portions of the state, and not the same portions for all species, because stable or increasing populations do not occur in all parts of the state. Consequently, there is some bias associated with where legally harvested samples come from with respect to regions and wildlife management units. Fewer animals live in regions without harvest, so there are also fewer opportunities to collect specimens from those regions even through other types of mortalities (e.g., vehicular collisions). At finer spatial scales (wildlife management units or county), more samples are needed to fully address the prevalence of ARs within the carnivore community and within individual species (see Table 3). Undoubtedly, spatial results based on only a few specimens may paint an incomplete picture of actual exposure rates. Future studies should attempt to address issues relative to sample sizes.

We found no strong association between exposure rates and age in the animals we tested. These results are also somewhat contingent on sample size, as we had relatively few animals that were over 2 years old (Supplementary Table S2). The lack of older animals in our sample may indicate that there are few animals in the older age cohorts or that older animals are inherently more wary and so less likely to be trapped. Alternatively, or in addition, the lack of old animals in our samples may be related to another issue. ARs at sublethal doses may negatively affect behavior, impair the decision-making of animals, or make them more risk-prone. Such behavioral effects from ARs could therefore make them more likely to be trapped, hit by cars, or to seek food closer to urban areas where they will be considered a nuisance (López-Perea et al. 2019). Young animals may also share many of these same characteristics, making them more prone to being trapped or killed, accidentally killed, or less effective predators causing them to rely on scavenging or killing less evasive prey (e.g., AR-exposed rodents). Thus, our estimates may be biased toward animals that have been exposed to ARs. Overcoming this limitation would require a non-biased sampling approach. This would involve some form of randomly killing mesocarnivores in the wild, which is not a method that is likely to be used for recovering these relatively elusive and highly managed carnivores

(Keller 2021). Smaller, though more intensive, studies on any of these species could provide more information on exposure and related risks to individuals based on AR exposure. In general, we think that to the degree our sample is biased toward animals that are exposed, the magnitude of the bias is similar across regions. Consequently, the patterns of exposure across PA, and other geographic regions, and the associations to development and agriculture (human population density) are likely robust. More generally, our results fit within our general understanding of where ARs should have the highest contact with wildlife and for which species we predicted a priori should be most exposed.

The exact routes of AR exposure, concentrations in the liver associated with fatalities, and sub-lethal effects on wildlife are still largely unknown. Some animals with high body burdens of ARs may appear relatively healthy, whereas others with relatively low concentrations suffered internal bleeding and ultimately death (Gabriel et al. 2015; Sainsbury et al. 2018). Trace levels of ARs could represent relatively long-ago exposures or relatively small recent ingestions. Additionally, presence of multiple ARs could be the result of one or multiple exposures. Clearly, minor differences exist among data types, but all show similar patterns of exposure across species, regions of PA, and underlying ecological drivers (e.g., association with human development).

Evidence of rodenticide exposure in mesocarnivores is an expected finding in areas of high development such as much of PA (Gabriel et al. 2015; Lohr 2018; López-Perea et al. 2019; Cooke et al. 2022). How sublethal exposures affect individuals and populations is still unknown and needs further study (Quinn 2019). Here we have provided a baseline estimate of exposure, which may be used as a comparison for future studies. In particular, if populations of these carnivores change, then examination of the rates and levels of exposure over time could indirectly implicate or exclude ARs as a mechanism leading to those changes. We assume that ARs have been prevalent in the northeastern United States for at least 50 years. During that time, fishers and otters have been reintroduced to PA and both have shown relatively rapid expansion and population increases (Keller 2021). In particular, fishers seem to continue to thrive and expand into more urban landscapes, which would not be expected if ARs were strongly limiting populations. We cannot estimate how rodenticides may have reduced or minimized this apparent rate of growth and expansion, but ARs appear not to have prevented growth of the populations for any of the species we studied. Coincidentally, regions within PA having relatively low populations of fishers, bobcats, and otters (Keller 2021) also have relatively high rates of AR exposure. Nevertheless, those regions also have less productive habitats for all three species and ARs are confounding the absence of habitat that also occurs within those areas. Cross-regional examination of both AR exposure rates and changes in populations may further elucidate the possibility that these compounds have affected carnivore populations in PA and the eastern US. Future research concerning all three species could focus on diversifying sampling efforts outside of only harvested individuals and ensuring samples are evenly distributed throughout all regions and wildlife management units of the state. Sampling size could be increased to eliminate potential bias due to age, spatial scale, and cause of mortality. These steps may result in a more accurate estimation of detection levels throughout the state, their sources relative to carnivores, and their ultimate effect on populations. Research identifying point source, although challenging, will be beneficial to the resource. Research specific to river otters could focus on environmental sampling, including water, soil, and prey items such as fish, invertebrates, reptiles, and amphibians. Future monitoring for exposure to ARs in all three species could suggest changes in the abundance of rodenticides in these three species but may also serve as sentinels for other species of carnivores, raptors, or ecosystems in general.

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Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Competing interests

We have no competing interests to declare.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2023-0131.

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From: Mourad Gabriel <mgabriel@iercecology.org>
Sent: Thursday, November 6, 2025 9:30 AM
To: lowensvi@icloud.com; Jonathan Evans <JEvans@biologicaldiversity.org>
Subject: Re: Letter to DPR

Hello Lisa and Jonathan, I needed to run this by the other PIs on this project and see if all of the ducks were in a row. What could be added is below.. Is this helpful?

"Current data from investigations into the exposure to ARs in barred owls in Northern California demonstrate continued exposure to anticoagulant rodenticides throughout that landscape. Specifically, Second Generation AR (SGAR) are still being detected in barred owls, varying in age (1-10+years) throughout the Northern California landscape. Specifically, SGAR makes up the majority of exposures, 36% of over 700 owls collected and tested from 2018-2024."

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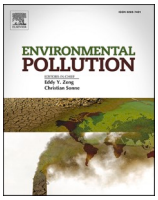

Collateral damage: Anticoagulant rodenticides pose threats to California condors☆

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ABSTRACT

Anticoagulant rodenticides (ARs) are widespread environmental contaminants that pose risks to scavenging birds because they routinely occur within their prey and can cause secondary poisoning. However, little is known about AR exposure in one of the rarest avian scavengers in the world, the California condor (*Gymnogyps californianus*). We assessed AR exposure in California condors and surrogate turkey vultures (*Cathartes aura*) to gauge potential hazard to a proposed future condor flock by determining how application rate and environmental factors influence exposure. Additionally, we examined whether ARs might be correlated with prolonged blood clotting time and potential mortality in condors. Only second-generation ARs (SGARs) were detected, and exposure was detected in all condor flocks. Liver AR residues were detected in 42% of the condors (27 of 65) and 93% of the turkey vultures (66 of 71). Although concentrations were generally low (<10 ng/g ww), 48% of the California condors and 64% of the turkey vultures exposed to ARs exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww), and 10% and 13% exceeded the 20% probability of exhibiting signs toxicosis (>80 ng/g ww). There was evidence of prolonged blood clotting time in 16% of the free-flying condors. For condors, there was a relationship between the interaction of AR exposure index (legal use across regions where condors existed) and precipitation, and the probability of detecting ARs in liver. Exposure to ARs may complicate recovery efforts of condor populations within their current range and in the soon to be established northern California experimental population. Continued monitoring of AR exposure using plasma blood clotting assays and residue analysis would allow for an improved understanding of their hazard to condors, particularly if paired with recent movement data that could elucidate exposure sources on the landscape occupied by this endangered species.

1. Introduction

Anticoagulant rodenticides (ARs; first- and second-generation rodenticides; hereafter FGAR and SGAR) have been used for decades to manage rodent pest populations, but can pose physiological risks to raptors and scavenging birds through secondary poisoning (Rattner et al., 2014a; Elliott et al., 2016; Hong et al., 2019) because they accumulate in their prey (e.g., *Rattus*; Elliott et al., 2014; Poessel et al., 2015; Geduhn et al., 2016). Secondary poisoning of birds can produce a range of deleterious physiological effects (e.g., anemia, hemorrhage, pallor of mucus membranes, depressed mentation and weakness), including direct mortality (Kelly et al., 2014a; Rattner et al., 2014a; Elliott et al., 2016; Hong et al., 2019; Rattner and Harvey, 2021). The widespread legal application of ARs, coupled with potential off-label illegal use, are the principal sources to wildlife (Gabriel et al., 2012; Elliott et al., 2014; Rattner et al., 2014b; Series et al., 2015).

Raptor exposure to ARs is globally widespread. A recent review (Elliott et al., 2016) found that average AR incidence (percentage of bird

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livers with at least one detectable SGAR) was 63% across 14 published studies (n = 2565 raptors) in nine countries. Within North America, SGARs are among the most frequent and abundantly used AR group (Murray, 2017; Quinn et al., 2019; Niedringhaus et al., 2020) and are routinely detected in wildlife because of their longer half-lives (Erickson and Urban, 2004; Herring et al., 2017). Birds that consume SGAR-poisoned animals have a higher likelihood of secondary poisoning and bioaccumulation compared to those exposed to first-generation ARs because of differences in AR toxicity and bioaccumulative potential (Murray, 2011; Thomas et al., 2011; Kelly et al., 2014b). Additional studies have shown that the degree of scavenging in raptors and other avian scavengers is positively correlated with AR exposure and mortality (Hughes et al., 2013; Hong et al., 2019).

The occurrence of ARs within raptor and avian scavenger food chains increases the likelihood of non-target AR exposure and potential adverse effects in those birds. The California condor (*Gymnogyps californianus*), a federally endangered scavenger, is one such species that is routinely exposed to contaminants through their food base (e.g., Pb; see Church et al., 2006; Finkelstein et al., 2012; Kelly et al., 2014a). California condors may consume ARs because they forage on agricultural pests and mammalian predators that have been exposed to ARs by primary or secondary routes, similar to other large facultative and obligate scavengers (e.g., golden eagle *Aquila chrysaetos*, turkey vulture *Cathartes aura*; Langford et al., 2013; Kelly et al., 2014b). With a global population of approximately 334 individuals, any additional physiological stressors could adversely affect their population. Therefore, identifying and understanding external factors that influence condor mortality or impairment are important for effective management of the current population, evaluating potential new release sites, and developing effective public outreach to protect the population.

We evaluated AR exposure in California condors across their current range in the central/southern California and Arizona flocks, as well as in a surrogate avian scavenger (turkey vultures *Carthartes aura*) in both existing condor range and at the future condor release site designated by the Northern California Condor Restoration Program. We determined AR exposure by sampling liver (long-term exposure: months) from deceased condors, blood (recent exposure: days – weeks) from free-ranging live condors, and liver and blood samples from surrogate turkey vultures. We then examined how legal AR application and environmental factors (e.g., precipitation) influenced variability in condor AR exposure and liver tissue concentrations, and whether ARs may be related to condor mortalities. We also assessed potential condor physiological responses to AR exposure, through common biomarker responses, prothrombin time (PT) and Russell's viper venom time (RVVT), to detect coagulopathy. These biomarkers can be used to demonstrate both the physiological response to ARs and as an indication of AR exposure (Rattner et al., 2014b; Rattner et al., 2015; Hindmarch et al., 2019).

2. Methods and study area

2.1. Quantify plasma and liver AR exposure in live and deceased California Condors throughout their current foraging range

California condors are captured on a semi-annual (typically 2–3 times) basis at Pinnacles National Park (PINN; Fig. S1) and undergo regular blood sampling to check health indices and measure exposure to environmental contaminants, particularly lead (Pb). During 2017–2018, a subset of blood samples was collected for AR analysis (n = 44) and blood clotting time biomarkers by venipuncture of the tarsal vein. Samples for AR analysis were stored in ethylenediaminetetraacetic acid (EDTA) blood tubes, and samples for clotting time biomarker testing were collected in citrated blood tubes; tubes were stored on ice and centrifuged at the end of the day (15 min at 2500 g) and plasma fractions were pipetted into individual cryovials (see Rattner et al., 2015) for details). The EDTA and the citrated plasma samples were frozen at −20 °C and shipped on dry ice within one day to the laboratory where they were stored at −80 °C. Condor liver samples (n = 65) from 2006 to 2018 that had been collected as part of the necropsy of deceased condors were requested from research laboratories that were holding samples. Deceased condors were from the PINN/Ventana Wilderness Society (n = 24), southern California (n = 33), and Arizona flocks (n = 8). All liver samples were kept at −80 °C until AR analysis. Additional citrated blood plasma samples (n = 10) were collected under the above protocols from captive condors at the Oregon Zoo to serve as reference coagulation samples by Oregon Zoo staff in 2018. All condor field protocols were covered by endangered species permit TE157291-1 and approved National Park Service Institutional Animal Care and Use Permit PWR-PINN-Condor-2016.A3, and turkey vultures sampling occurred under state (California: 010619, SC-4741; Oregon: 052-17, 094-19) and Federal permits (MB28361A-0, 09379, 21417) and an approved Institutional Animal Care and Use Permits (Protocol 08/09.W.89.A, 2017-001).

2.2. Quantify plasma and liver AR exposure in Turkey vultures within the potential foraging range of northern California Condors

To gain insight into potential condor AR exposure within current condor range as well as potential risk to the future northern California flock (Fig. S1), we collected turkey vultures (n = 71) as surrogates within the current California condor range near Pinnacles National Park (n = 16) and in northern California (n = 20) and southern Oregon (n = 35) using a 12-gauge shotgun and steel shot in 2018 and 2019. This approach allowed us to compare the concentrations and types of ARs to which condors and surrogate scavengers were exposed within the same spatial/temporal period. We collected whole blood (n = 48) from each sacrificed vulture using 20-25-gauge heparinized needles by cardiac puncture. Blood was centrifuged immediately after sampling (15 min at 2500 g), and plasma fractions were pipetted into individual cryovials. All vulture carcasses and plasma were frozen on dry ice in the field and held in a −20 °C freezer until being transferred to either a −20 °C or −80 °C laboratory freezer (carcasses and plasma respectively). We utilized additional live turkey vulture plasma samples (n = 23) collected between 2009 − 2013 (stored at −80 °C) by the Yurok Tribe Wildlife Department (see West et al., 2017 for collection details, Fig. S1).

2.3. Tissue sampling and rodenticide analysis

For both condors and turkey vultures, we examined the frequency of AR exposure based on detecting at least one AR in the liver and or blood plasma (Rattner et al., 2014a; Gabriel et al., 2018). Liver tissue from each condor or vulture was excised and homogenized in liquid nitrogen using a cryomill (SPEX SamplePrep, Metuchen, New Jersey), and an approximately 5 g aliquot was used for quantitative AR analysis. With long term frozen storage, moisture often sublimates from biological tissues, resulting in variable % moisture content. This can influence contaminant concentrations when analysis and reporting are done on a wet weight (ww) basis. We addressed this issue by freeze-drying a subsample of each liver sample to determine moisture content. Final liver AR concentrations were adjusted from ww to dry weight (dw) using the individual percent moisture content. To facilitate comparisons of liver AR concentrations data published in ww, we approximated liver fresh ww concentrations by back calculating ww concentrations using a fresh liver moisture content derived from turkey vultures (mean = 69.9 ± 0.5%, n = 71). Liver tissue aliquots and plasma of condors and vultures were analyzed by the Texas A&M Veterinary Medical Diagnostics Laboratory (College Station, Texas) for quantification of eight ARs, including 4 first-generation ARs (chlorophacinone, coumatetralyl, diphacinone, warfarin), and 4 second-generation ARs (brodifacoum, bromadiolone, difenacoum, difethialone). Rodenticides were quantified by high-performance liquid chromatography-tandem mass spectrometry using an Agilent 1200 series C and 6400 triple quad system (Series et al.,

2015). Plasma samples (~1 ml) were analyzed for ARs on a ww basis. Quality control blanks all reported zero ARs detected, analytical spike recovery averaged 107.7 ± 6.0% (n = 8), and the limit of quantification (LOQ) across all ARs averaged 5.1 ± 0.1 ng/g ww for liver and 2.1 ± 0.4 ng/g ww for plasma and limit of detection (LOD) was between 0.5 and 1.0 ng/g ww for liver and 0.2–0.4 ng/g ww in plasma. If an AR was detected in a sample, but below the LOQ and ≥ the limit of detection (LOD; the lowest concentration in a sample that could be detected but not necessarily quantified as an exact concentration), we used the reported concentration rather than a ½ LOD which is commonly used but has no statistical underpinning (Zoffoli et al., 2013). Across all birds, only SGARs were detected, and as such, the molecular weights were generally similar and summing ARs is less problematic than if we had detected both FGARs and SGARs, which have vastly different molecular weights, toxic potencies and tissue half-lives (Horak et al., 2018; Rattner and Harvey, 2021). In all subsequent analyses, we summed SGARs (ΣSGAR) to simplify interpretation.

Physiological Biomarkers in California Condors and Surrogate Obligate Avian Scavengers.

Prothrombin time and RVVT of citrated California condor plasma samples (n = 47) were used to evaluate evidence of physiological response to potential AR exposure. Thrombin clotting time (TCT) was used as an indicator of fibrinogen concentration in plasma samples. Fibrinogen formation is insensitive to deficiency of vitamin K-dependent clotting factors caused by ARs, but its deficiency resulting from improper blood sample collection can prolong clotting time and confound AR toxicity studies (Rattner et al., 2010). Thus, it is important to verify that fibrinogen concentration is adequate to promote clot formation (~75 mg/dL). Reagents, conduct, and performance of these assays in various species of raptors has been previously described (e.g., (Rattner et al., 2011; Rattner et al., 2015; Hindmarch et al., 2019). The mechanical clot endpoint in these assays was determined using a Start4 fibrometer (Diagnostica Stago Inc., Parsippany, NJ). Nearly all samples were assayed in duplicate (a few [see summaries below] had inadequate volume and were assayed as a single determination), and values were averaged.

For the TCT assay, condor samples were run over a two-day period. Of the 47 samples assayed, 46 produced clots, and of those analyzed in duplicate (n = 44), the average coefficient of variation (CV) ± standard deviation (SD) was 4.1 ± 6.0%. Nine aliquots derived from a pool of citrated chicken plasma were assayed at various intervals during the two-day period to verify assay performance and yielded an average CV ± SD of 2.0 ± 1.8%. For the RVVT assay, there was adequate sample volume for analysis of 46 of the 47 samples and they were analyzed in a single day. Of the 42 condor samples assayed in duplicate, the average CV ± SD was 0.7 ± 0.6%. Seven aliquots derived from a pool of citrated chicken plasma were assayed for RVVT at various intervals and yielded a CV ± SD of 0.7 ± 0.9%. For the PT assay, there was adequate sample volume for analysis of 46 of the 47 samples and they were analyzed in single day. Of the 42 condor samples assayed in duplicate, the CV ± SD was 2.4 ± 3.0%. Seven aliquots derived from a pool of citrated chicken plasma were assayed for PT at various intervals and yielded a CV ± SD of 2.6 ± 1.4%.

2.4. Landscape analysis

To understand the potential influence of landscape variables on condor AR exposure and concentrations, we used ArcMap 10.4.1 (ESRI, Redlands, California, USA) to quantify precipitation and SGAR use (application rates) associated with the region in which a condor either was found deceased or was using prior to mortality. We restricted the time frame for the spatial analysis of each condor to the one-year period immediately prior to death. We selected this time frame because it represents a plausible approximation of when AR exposure would have occurred based on their half-lives in liver (Erickson and Urban, 2004; Herring et al., 2017). We did not assess the influence of landscape factors on AR exposure and concentrations for condors in Arizona because there are no available AR application data of adequate spatial resolution. Some deceased condors (70%) were not equipped with Global System for Mobile Communications (GSM)/Global Positioning System (GPS) transmitters, precluding us from utilizing individual level movement data for each bird. Rather, we used all available location data from other condors within the flock from which the deceased condor was associated during the one-year period prior to its death. We utilized data from Movebank (www.movebank.com) to understand spatial movement patterns for each condor in the central and southern California flocks. We determined the proportion of location detections per county relative to the total number of location detections for all GSM/GPS transmitted condors across the one-year period. We verified the viability of estimates of the percent of time spent in each county through correlation between estimates from all condor movement data and instances where we had actual data from individual condors ($F_{1,6.80} = 104.38$, $P < 0.0001$, $R^2 = 0.37$).

We developed an index of potential condor AR exposure based on both county level AR application rates and the proportion of time condor flocks spent in each county (hereafter AR exposure index). This was accomplished employing legal county-specific data on pesticide use (California Department of Pesticide Regulation, 2020) to estimate total AR application rates within each county for the year prior to each condors' death. We selected only those ARs that condors were exposed to as determined by our liver exposure data (i.e., brodifacoum, bromadiolone, and difethialone; see Results). For each county, total active ingredient (kg) of all ARs applied were summed across the year prior to mortality and adjusted for the area of the county (i.e., weighted average).

Rodent control is one of the primary drivers of AR use (Rattner et al., 2014a; Elliott et al., 2016). As a result, AR use fluctuates with rodent population cycles (Luque-Larena et al., 2013; Lopez-Perea and Mateo, 2018). Additionally, precipitation is a major factor influencing rodent populations (Brown and Heske, 1990; Meserve et al., 2003; Gillespie et al., 2008), so we used county-specific precipitation measurements for the rainy season (Oct–April) prior to each condors' death as a proxy for potential changes in county level rodent populations, which may have influenced AR application (both legal and illegal). Total county level precipitation was calculated from the PRISM Climate Group (2020). Total precipitation (mm) for each county were summed across each rainy season and adjusted to account for the proportion of time each condor flock spent in that county.

3. Statistical analysis

3.1. AR exposure

We evaluated factors influencing condor and vulture AR exposure using species-specific logistic regression models. Each bird's classification as "exposed" (at least one quantifiable AR detected) or "unexposed" (failure to detect ARs in liver) was used as the response variable. We then tested the probability of various factors influencing AR exposure using a logistic regression model with sex, region, and year of death as independent variables. For condors, we also included the AR exposure index and rainy season precipitation prior to the condor's death, along with the interaction between AR exposure index and precipitation to determine if precipitation influenced the relationship between AR exposure in condors and AR exposure index. We did not include an AR exposure index or precipitation in vulture models because no location data existed to define the spatial area covered by vultures. Regions for condor analysis were aligned with condor flocks and are based on GSM/GPS telemetry movement data (Southern California flock: Kern, Los Angeles, Santa Barbara, Tulare, and Ventura counties; Central California flock: Monterey, San Benito, and San Luis Obispo counties). Turkey vulture regions were associated with collection sites (Pinnacles National Park, northern California, and southern Oregon). We combined the

Pinnacles and Ventana flocks for analysis because of their propensity to move and feed together on the landscape (Bakker et al., 2017). Year was not included in vulture model because we did not sample vultures in each region every year.

To evaluate the factors influencing condor and vulture AR concentrations in birds with quantifiable AR concentrations, we used species-specific linear mixed-effects models. Liver AR concentration (ΣSGAR ng/g dw) was the response variable, with AR exposure index, precipitation, sex, region, and year of death as independent variables, and an AR exposure index × precipitation interaction. We included sampling site as a random effect in the turkey vulture models to avoid confounding effects associated with sampling multiple birds from the same location, whereas condor samples were considered independent because of the spatial and temporal differences in deaths.

3.2. Influence of AR exposure on condor cause of death

To assess if ARs influenced the probability of condors dying from a specific cause, we used cause-of-death determinations determined by necropsy by the California Condor Recovery Program and the U.S. Fish and Wildlife Service National Forensics Laboratory (see Viner et al., 2020 for details). Most condor mortalities were the result of either Pb poisoning (67%) or a range of other causes that were not numerous enough to be analyzed individually (4%–7% per cause of death). Therefore, we combined data for other causes of death (e.g., drowning, electrocution, entanglement, trauma) in a single category, thereby categorizing cause-of-death as either (1) "Pb toxicosis", or (2) "other causes". We then tested whether ARs influenced the probably of death via Pb toxicosis or other causes using a logistic regression model with liver ΣSGAR concentration (ng/g dw) and sex as independent variables. We included a liver ΣSGAR × sex interaction to determine if sex influenced the relationship between ΣSGAR concentrations and cause-of-death. Across all models we natural log-transformed all ΣSGAR, AR exposure index, and precipitation data to improve normality of the residuals and homogenize the variance structure.

3.3. AR biomarker response

To evaluate physiological responses to AR concentrations, we used general linear-mixed effects models to contrast fibrinogen concentrations, PT and RVVT in free-flying condors versus captive condors. We included condor ID as a random effect to avoid confounding effects associated with sampling the same condor on multiple occasions. For free-flying condors, we considered AR exposure to be indicated by physiological response values outside the upper extremes for control condors, following the general guidelines that prolongation of prothrombin times by two standard deviations above the arithmetic mean is suggestive of AR exposure (Shlosberg and Booth, 2006; Hindmarch et al., 2019).

4. Results

In this study, only second-generation AR compounds were detected. There were no AR detections in the 44 California condor plasma samples, but SGARs were present in 10% (5/48) of turkey vulture plasma samples (Table 1). Of the five turkey vultures with detectable SGARs in plasma, only one had a concentration above the limit of quantification (3.40 ng/g ww), whereas the other four had trace levels (<1.40 ng/g ww; Table 1). All five of these vulture plasma samples contained brodifacoum, and one also contained bromadiolone, and another contained difethialone. In contrast to blood plasma, SGARs were detected in liver of 42% (27/65) of the condors and 93% (66/71) of the turkey vultures (Table 1). Liver ΣSGAR concentrations ranged from 4.0 to 466.7 ng/g dw (estimated ww range 1.2–135.5 ng/g) in condors and 3.4–932.8 ng/g dw (estimated ww range 0.9–287.8 ng/g) in vultures (Table 1). The geometric mean (± standard error) liver ΣSGAR concentrations were 57.4 ± 16.1 ng/g dw (estimated ww 17.3 ± 4.8 ng/g) in condors and 81.5 ± 13.1 ng/g dw (estimated ww 24.5 ± 3.9 ng/g) in vultures (Table 1). Of the condor livers with detectable concentrations of SGARS, 56% had brodifacoum, 30% had bromadiolone, and 4% had difethialone. Four detectable SGARs were measured in turkey vulture livers – 97% had brodifacoum, 45% had bromadiolone, 17% had difethialone, and 3% had difenacoum. Of the California condors and turkey vultures that had detectable concentrations of ARs, 48% and 64%, respectively, exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww (Thomas et al., 2011);), and 10% and 13%, respectfully, exceeded the 20% probability of exhibiting signs of toxicosis (>80 ng/g ww; Thomas et al., 2011).

4.1. Variables influencing AR exposure

There was a significant interaction between the AR exposure index and precipitation ($\chi^2_1 = 4.91$, P = 0.03; Fig. 1), indicating that the relationship between the likelihood of condor exposure to SGARS and AR exposure index was influenced by precipitation. However, the probability of detecting SGARs in condor livers was neither related to sex ($\chi^2_1 = 0.18$, P = 0.67), flock ($\chi^2_2 = 3.21$, P = 0.07), nor death year ($\chi^2_1 = 1.53$, P = 0.22). To facilitate interpretation of the interaction on the probability of condors having detectable concentrations of SGARs in their livers, we plotted the conditional slope coefficients for the effect of AR exposure index on the probability of condors having detectable concentrations of ARs across the range of the precipitation. This illustrates that the magnitude and direction of the relationship between AR exposure index and the probability of condors having detectable concentrations of SGARs changes depending upon the amount of precipitation. With very low precipitation (<150 mm) the effect of AR exposure index on the probability of detecting SGARs in condor livers is marginally negative, and as rainfall increase that relationship is neutral, but with elevated rainfall (>460 mm), that relationship becomes positive (Fig. 1). Turkey vulture AR concentrations did not differ among regions ($\chi^2_2 = 0.35$, P = 0.84) nor by sex ($\chi^2_1 = 0.56$, P = 0.45).

4.2. Variables influencing AR concentrations

California condor liver ΣSGAR concentrations were positively correlated with year of death ($F_{1,12} = 4.77$, P = 0.05 (Fig. 2), but were not correlated with the AR exposure index ($F_{1,12} = 0.85$, P = 0.38), precipitation ($F_{1,12} = 1.04$, P = 0.33), region ($F_{1,12} = 0.04$, P = 0.85), sex ($F_{1,12} = 3.83$, P = 0.07), nor the AR exposure index × precipitation interaction ($F_{1,11} = 0.00$, P = 0.97). Turkey vulture liver ΣSGAR concentrations were not influenced by region ($F_{2,1.79} = 6.46$, P = 0.15) or sex ($F_{1,59.06} = 0.52$, P = 0.47).

4.3. Influence of AR exposure on condor cause of death

Liver ΣSGAR concentrations were positively associated with the likelihood of Pb toxicosis being the proximate cause of condor death ($\chi^2_1 = 3.92$, P = 0.05) and probability of condors succumbing to Pb toxicosis with increasing ΣSGAR concentrations was higher in female than male condors ($\chi^2_1 = 4.58$, P = 0.03). However, the 95% confidence intervals for both the ΣSGAR and sex odds ratios overlapped 1 slightly (odds = 2.49 [0.88–7.02] and 11.96 [0.94–151.77], respectively) indicating uncertainty around the effects likely due our limited sample size. Geometric mean ΣSGARs were on average 2.3-fold higher in condors that died of lead poisoning than condors that died of other causes (78.3 versus 34.5 ng/g dw, respectively).

4.4. AR biomarker response

Fibrinogen concentrations were 29% higher ($F_{1,35.77} = 5.21$, P = 0.03) in captive condors than in free-flying condors ($F_{1,35.19} = 4.82$, P = 0.03; Fig. 3A), but there were no differences in prothrombin time or

Table 1
Second-generation anticoagulant rodenticide (SGAR) residues in plasma (ng/g ww) and liver (ng/g ww unless specified) of California condors and turkey vultures. Condor samples were collected as part of ongoing health monitoring or from deceased condors (liver) throughout the current free-flying condor range with the United States (Arizona = AZ, Pinnacles NP/Ventana Wildlife Society = PINN/VWS, southern California = SCAL). Turkey vultures were sampled as a surrogate for condors within the current range of condors near Pinnacles National Park (PINN) or in northern California (NCA) and southern Oregon (SOR). Values below the limit of quantification are reported as "trace" and ND refers to not detected.

Species	Region	Tissue	*n*	Overall SGAR detection frequency %	Brodifacoum		Bromadiolone		Difethialone		Difenacoum		Geomean ΣSGARs (ng/g dw)	Geomean ΣSGARs (ng/g ww)
					% Positive[a]	Geomean (range)	% Positive	Geomean (range)	% Positive	Geomean (range)	% Positive	Geomean (range)		
California condor	All	Plasma	44	0	ND	–	ND	–	ND	–	ND	–	–	–
		Liver	65	42	56	16.3 (4.2–117.8)	30	11.2 (1.2–79.0)	4	89.1	ND	–	57.4	17.3
	AZ	Liver	8	25	100	7.7 (4.2–14.0)	ND	–	ND	–	ND	–	25.5	7.7
	PINN/ VWS	Liver	24	50	33	12.1 (8.0–27.0)	50	12.8 (3.3–79.0)	ND	–	ND	–	53.0	16.0
	SCAL	Liver	33	39	69	22.1 (5.6–117.8)	15	7.5 (1.2–47.4)	8	89.1	ND	–	70.8	21.3
Turkey vulture	All	Plasma	48	10	80	trace	20	1.9 (trace-1.9	20	1.4 (trace-1.4)	ND	–		3.4
	NCA	Plasma	23	0	0	–	ND	–	ND	–	ND	–	–	–
	PINN	Plasma	14	56	44	trace	11	trace	11	trace	ND	–	–	1.7
	SOR	Plasma	11	0	0	–	ND	–	ND	–	ND	–	–	–
	All	Liver	71	93	97	19.2 (trace-252.6)	45	9.1 (1.3–119.9)	17	6.3 (1.5–60.1)	3	3.0 (trace-12.7)	81.5	24.5
	NCA	Liver	20	95	95	6.6 (trace-43.7)	50	8.2 (2.3–54.5)	ND	–	ND	–	37.7	11.4
	PINN	Liver	16	94	93	42.1 (4.4–252.6)	53	63.4 (4.7–119.9)	20	10.3 (2.0–60.1)	7	12.7	152.9	46.1
	SOR	Liver	35	91	100	24.8 (1.8–167.4)	38	6.1 (1.3–33.9)	25	5.3 (1.5–12.1)	3	trace	95.4	28.8

[a] % Positive is the proportion of samples that tested positive for a specific AR divided by the total number of samples positive for ARs.

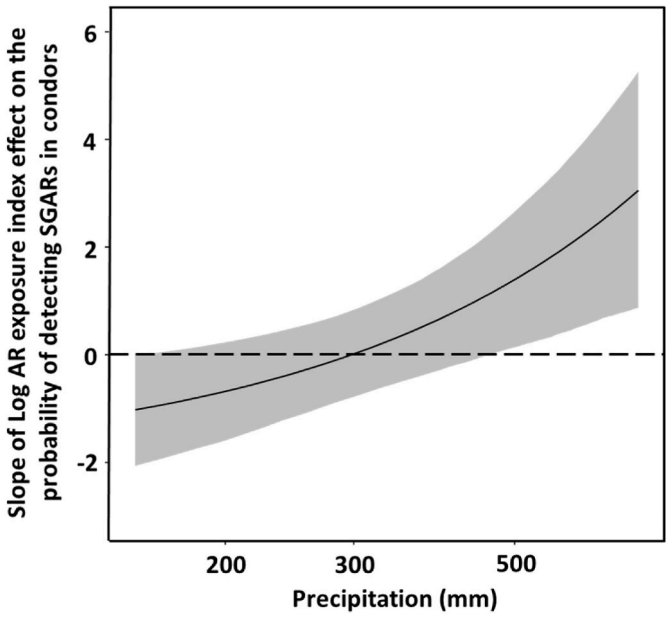

Fig. 1. Conditional effects of precipitation (mm) on the estimated coefficient of AR exposure index on the probability of California condors (*Gymnogyps californianus*) having detectable concentrations of SGARs in their livers after accounting for year of death, location, and sex. Shaded areas indicate the 95% confidence intervals around the estimated coefficient. The horizontal dashed line indicates a zero-coefficient value.

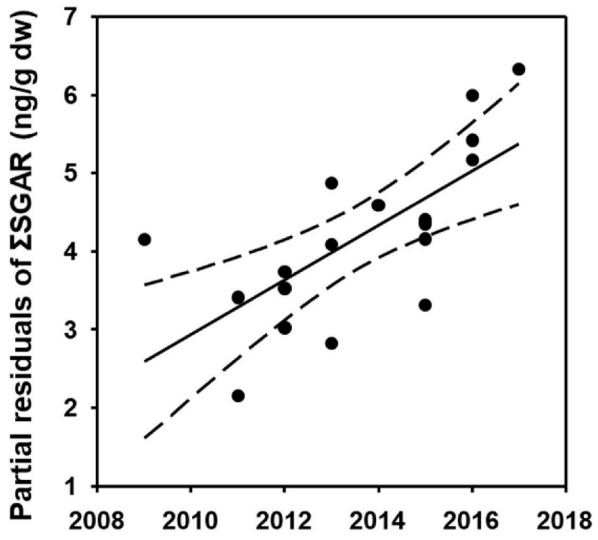

Fig. 2. Partial residual plot of California condor (*Gymnogyps californianus*) ΣSGARs (ng/g dw) and year of California condor death, accounting for the AR exposure index, precipitation, region, and sex. Dashed lines indicate the 95% confidence interval.

Russell's viper venom time between captive and free-flying condors ($F_{1,40.67} = 1.88$, $P = 0.17$) and ($F_{1,40} = 0.09$, $P = 0.76$), respectively (Fig. 3B and C). However, 16% and 9% of the free-flying condors had prothrombin time and Russell's viper venom times, respectively, that exceeded the captive bird mean values by two standard deviations (Fig. 3B), suggestive of anticoagulant exposure and effect in those birds (Hindmarch et al., 2019; Shlosberg and Booth, 2006).

5. Discussion

This is among the first and most widespread assessments of

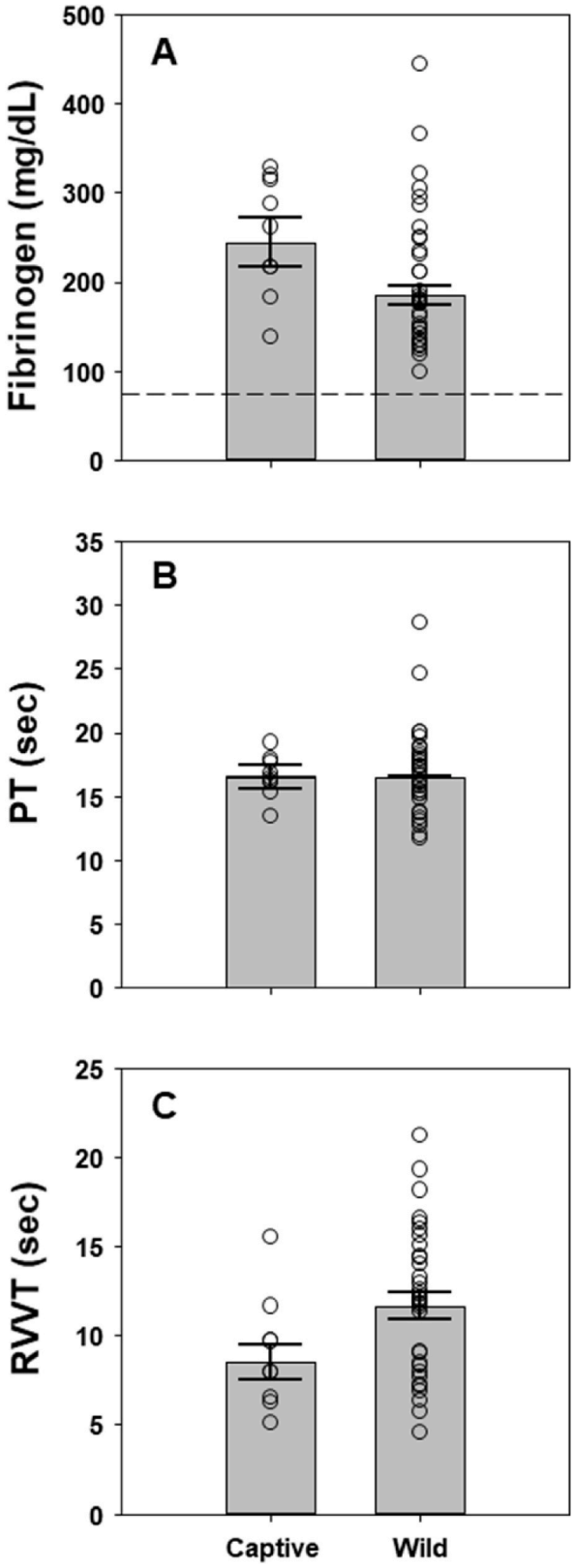

Fig. 3. Fibrinogen concentrations (mg/dL; A), and clotting assay results for prothrombin time (PT; B), and Russell's viper venom (RVVT; C) for captive and free-flying California condors (*Gymnogyps californianus*). Results are model-derived least-squares means ± standard error. Open circles represent individual data points. Dashed line in fibrinogen figure represents the concentration that readily promotes clot formation (~75 mg/dL).

anticoagulant rodenticide exposure in California condors, detailing the incidence rate of exposure as well as some potential factors that influence its probability and residue concentrations in liver. We also found potential evidence of adverse physiological effects associated with AR exposure in free-flying condors. We found wide-ranging exposure in California condors, with residues varying 117-fold, and 25%–50% of birds from all current condor flocks within the United States (central and southern California, Arizona) being exposed. Forty-two percent of the condors and 93% of surrogate vultures contained quantifiable levels of SGARs in their livers. Although concentrations were generally low (<10 ng/g ww), 48% of the exposed California condors and 64% of the exposed turkey vultures exceeded the 5% probability of exhibiting signs of toxicosis (>20 ng/g ww; derived from research in other more abundant species of raptors (Thomas et al., 2011). Furthermore, 10% and 13% for condors and vultures, respectively, exceeded the 20% probability of exhibiting signs of toxicosis (>80 ng/g ww). As such, there was some evidence of delayed blood clotting in 16% of the free-flying condors. Notably, we also found that the probability of AR exposure in condors increased with greater SGAR application rates, mediated by precipitation, and liver ΣSGAR concentrations increased across the period of the study. Lastly, initial evidence suggests increased liver ΣSGAR concentrations were also associated with a higher probability of Pb toxicosis as the primary source of condor mortality, indicating that exposure to these two contaminants may be correlated or that there may be an unknown additive or synergistic effect on physiology between the two contaminants.

Different tissues are indicative of a range of AR exposure time frames in birds, with plasma generally reflecting more recent exposure (weeks) and liver residues reflect longer term exposure (up to a year; (Horak et al., 2018; Murray, 2020). We found no evidence of recent AR exposure in condors based on blood plasma concentrations. However, liver samples from deceased condors indicated considerable AR accumulation, either from chronic low-level exposure or from infrequent acute exposures. These findings highlight some of the challenges of monitoring AR exposure in wild birds. Importantly, other indices of exposure may help evaluate the prevalence of AR impairment. For example, we detected that 16% and 9% of the free-flying condors had prothrombin times and Russell's viper venom times respectively outside the upper extreme value of captive condors, possibly indicating recent AR exposure and illustrating the utility of using plasma samples for examining potential physiological effects of ARs. Similarly, we only detected ARs in 10% of the turkey vulture plasma samples, but 93% of their liver samples were positive for ARs. Future efforts to monitor ARs in condors may require a hybrid approach where clotting assays could be used to assess potential recent exposure (Shlosberg and Booth, 2006; Hindmarch et al., 2019) with liver tissue from deceased condors providing a more robust understanding of specific long term AR exposure.

Benchmark probabilities serve as a valuable tool in assessing risk associated with AR exposure when species specific data do not exist (Rattner et al., 2014a; Herring et al., 2017). Although, none of the vultures died from AR toxicosis and only one condor death in this study was directly attributed to AR exposure, California condors and turkey vultures exceeded the 20% probability of exhibiting signs of toxicosis in 10% and 13%, respectively, of liver samples with quantifiable ΣSGAR residues. In the case of the single condor mortality, the ΣAR concentration was 19 ng/g ww, and the ARs detected included the most potent brodifacoum (Erickson and Urban, 2004; Herring et al., 2017). Notably, 48% of the condors that had detectable concentrations of liver ARs exceeded the quantity observed in the condor that succumbed to AR toxicosis. This illustrates well-known inter-individual differences in sensitivity, with many factors affecting onset of toxicosis (Rattner and Harvey, 2021). Although the concentrations associated with the 20% probability of exhibiting signs of toxicosis (80 ng/g ww (Thomas et al., 2011); may be considerably below liver concentrations observed in laboratory studies of birds (e.g., 550–2100 ng/g ww; Newton et al., 1990; Gray et al., 1994; Rattner et al., 2020), they may reflect concentrations associated with the more stressful and challenging experiences of wild birds (Rattner et al., 2020) or differences in toxicity associated with chronic low-level exposure (Rattner and Harvey, 2021). Importantly, the Thomas et al. benchmark probabilities of exhibiting signs of toxicosis need to be used judiciously (Thomas et al., 2011). These benchmarks simply indicate that there is a specific probability that signs of exhibiting toxicosis "may" begin at that concentration, and that we should expect species to vary greatly in their sensitivity to SGARs (Thomas et al., 2011).

Second generation ARs are the most common form of ARs detected in raptors and avian scavengers (Murray, 2011; Thomas et al., 2011; Kelly et al., 2014b; Elliott et al., 2016) because they are more frequently used than FGARs (Murray, 2011: Rattner et al., 2014a; Elliott et al., 2016) and have longer half-lives (Erickson and Urban, 2004; Herring et al., 2017). As such, we only detected SGARs in California condors and turkey vultures, suggesting SGARs may be used ubiquitously throughout the study area. Correspondingly, we found an interactive relationship between precipitation and the AR exposure index (brodifacoum, bromadiolone, difenacoum) within 12-months prior to the condor's death, and the probability that condor livers would contain at least one detectable SGAR. This relationship indicates that the probability of detecting SGARs in condor livers is influenced by SGAR availability, but that relationship is mediated by precipitation. Although the exact mechanisms behind this relationship are not entirely understood, it seems plausible that varying levels of precipitation may regulate small mammal populations (Brown and Heske, 1990; Meserve et al., 2003; Gillespie et al., 2008), potentially influencing the movement of SGARs through condor food webs. Although the specific sources and locations of AR exposure in condors and vultures are unclear, the use of SGARs in the urban/rural interface has been demonstrated as an important exposure source for other taxa (e.g., non-migratory mammalian species (Nogeire et al., 2015; Poessel et al., 2015; Series et al., 2015; Lohr, 2018). Illegal use of ARs in the growing of marijuana is also a possible source (Gabriel et al., 2012; Franklin et al., 2018; Gabriel et al., 2018), in addition to the off label use of ARs by the public purchased through farm supply stores and online sales where sales are not tracked (Quinn et al., 2019). The relationship between legal applications of SGARs and exposure suggest illegal sources of ARs may play a smaller, more localized role in condor exposure to ARs.

Migration and vast foraging ranges of birds like condors can further complicate determinations of the geographic origin of AR exposure. This is particularly the case when measuring exposure using liver because AR concentrations can spike immediately after acute and substantial exposure (Horak et al., 2018) or accumulate over months of chronic low-level exposure (Rattner et al., 2014a; Herring et al., 2017). As a result, AR concentrations in liver may reflect cumulative exposure from areas substantially removed from the sampling location (Kirk and Mossman, 1998). Subsequently, turkey vulture AR exposure could occur locally, on their wintering grounds, or during migration. The monthly home ranges of California condors can span 25–90 km^2 (Rivers et al., 2014) and on average they travel 70 km per day (Hall et al., 2021). Despite this, the probability that deceased condor livers had detectable concentrations of ARs was related to the ΣSGAR cumulative annual application rate and precipitation within the specific regions utilized during the year prior to their death. This suggests that future condor releases and establishment of new managed flocks, such as in northern California, could benefit from assessing the agricultural application rates. Data from surrogate turkey vultures sampled in northern California and southern Oregon suggest that there is a high likelihood that condors will be exposed to ARs; however, legal application of ARs is considerably lower in northern California than compared to the areas of where condors currently exist (California Department of Pesticide Regulation, 2020).

Use of ARs in the state of California are under some of the strictest environmental regulations in the United States (London et al., 2008; Quinn et al., 2019). Yet even with additional regulations applied by the

California Department of Pesticide Regulation in July of 2014 to minimize non-target exposure in wildlife (Quinn et al., 2019), ΣSGAR concentrations in condor liver increased concurrent with increasing legal ΣSGAR application. In fact, geometric mean ΣSGARs increased by 2.5-fold in condor liver since the 2014 pesticide restrictions. Importantly, during that timeframe, SGAR application rates increased 1.2-fold (California Department of Pesticide Regulation, 2020). A new law (Assembly Bill 1788) was enacted in September of 2020 to further reduce use of SGARs in California, and future monitoring will be helpful in determining the effectiveness of this mitigation effort. However, as with the ban of Pb-based ammunition in California for hunting and shooting of pest species in 2019 (Assembly Bill 711), the success of this new law depends on effective enforcement and potential human component of disregarding regulations/laws.

Lead toxicosis is one of the leading causes of death in California condors and is the primary limiting factor for their population recovery (Finkelstein et al., 2012). However, the probability of a condor dying of Pb toxicosis increased with higher ΣSGAR concentrations in liver, although there was some uncertainty around the magnitude of the effect. The mechanisms responsible for this are unclear, but contaminant mixtures can influence an individual's susceptibility to deleterious effects of certain compounds (Heys et al., 2016; Rattner and Harvey, 2021). For instance, binary mixtures of heavy metals have been found to increase mortality rates in sentinel species (e.g., *Daphnia magna*; Le et al., 2013; Vandenbrouck et al., 2009) and have interactive effects on hormone profiles in birds (e.g., common raven *Corvus corax*; Herring et al., 2018). Thus, AR exposure may reduce the overall health of birds such that additional stressors such as Pb poisoning result in their death. Alternatively, AR exposure may merely be correlated with Pb exposure in the wild because consumed mammals such as shot coyotes (*Canis latrans*) and ground squirrels (Spermophilus spp.) potentially contain both AR residues and Pb fragments. Both coyotes and ground squirrels occur in condor diets (Collins et al., 1999; Finkelstein et al., 2020) and are routinely poisoned with ARs (Poessel et al., 2015; Vyas et al., 2017) and shot with Pb-based ammunition (Stauber et al., 2010; Herring et al., 2016). A third explanation for this relationship could be that with increased Pb exposure, condors become anemic due to inhibition of enzymes involved in hematopoiesis (Finkelstein et al., 2012), coincident AR exposure with coagulopathic consequences could result in increased blood loss, exacerbating effects of Pb toxicosis. It is important to note that the causes and outcomes of this interaction between AR exposure and Pb toxicosis are still speculative, but indicate the importance of more definitive studies to elucidate these mechanisms.

6. Conclusions

Management and recovery of California condors is dependent on understanding risks to individuals, flocks, and the overall population. Anticoagulant rodenticide exposure appears to be common in all current condor flocks within the United States and there is potential for exposure in the future free-flying flock in northern California. While the understanding of AR exposure in condors is largely limited to liver tissue residues from deceased condors, continued monitoring of exposure using blood clotting assays is one means of detecting AR exposure in plasma (Hindmarch et al., 2019; Rattner and Harvey, 2021) at a much reduced cost relative to high-performance liquid chromatography-tandem mass spectrometry although the time frame for AR exposure would be very recent (<1 week; Rattner et al., 2014b; Rattner et al., 2020). Plasma samples collected antemortem or postmortem may help confirm suspected cases of AR toxicosis and mortality (Murray, 2020) and improve our understanding of recent AR exposure moving forward with condor recovery efforts. Further elucidating where on the landscape condors are being exposed to ARs would also help in developing plans for mitigate exposure.

Author statement

Garth Herring: Conceptualization, Data curation, Funding acquisition, Methodology, Formal analysis, Project administration, Writing-Original draft preparation. **Collin Eagles-Smith:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing-Original draft preparation. **Rachel Wolstenholme:** Data curation, Funding acquisition, Methodology, Writing- Review and Editing. **Alacia Welch:** Data curation, Funding acquisition, Methodology, Writing- Review and Editing. **Chris West:** Data curation, Methodology, Writing-Review and Editing. **Barnett A. Rattner:** Conceptualization, Data curation, Funding acquisition, Methodology, Writing- Original draft preparation.

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

Link to data is included in the manuscript after the acknowledgements.

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Data availability—Data can be accessed through Sciencebase.gov at https://doi.org/10.5066/P9LWEXGW and https://doi.org/10.5066/P9NHPLHX.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119925.

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BIRDS

Turkey vultures in California are testing positive for rat poison

Despite statewide restrictions on the deadly poisons, new research shows they're still infiltrating the food web.

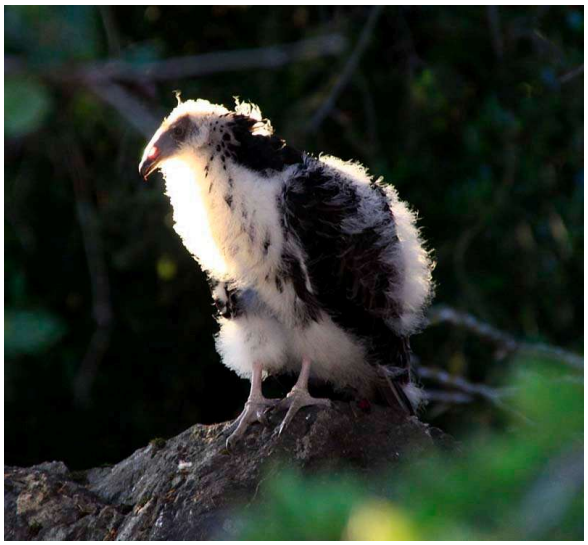
Chi En Kim March 5, 2025

A turkey vulture in Los Angeles, California.
Chon Kit Leong/Alamy

In humankind's ongoing war against rats, rodents are far from the only casualties.

Over the last few years, a **common** class of chemicals known as anticoagulant rodenticides has come under fire for its heavy toll on wildlife. Despite statewide restrictions on these pesticides in California, **a recent study found that** as many as 13% of turkey vultures in the Los Angeles area tested positive for the chemicals. Given the birds' unique ecological perch as nature's carnivorous cleanup crew, the results reveal just how thoroughly anticoagulant rodenticides pervade the ecosystem. They are a reminder of how human actions can have vast environmental consequences, often compounded by climate change — and, in this case, for fundamentally limited returns.

Anticoagulant rodenticides work by causing their victims to bleed to death, often internally. Afflicted animals show signs of anemia and often bleed from their nostrils, mouth and anus before they die. Animal cruelty aside, these substances are problematic because they can persist in carcasses and the environment for **up to a year**. This means that a poisoned rat can in turn poison its predator, and that predator's predator as well, long after the first fatal nibble. The upshot is vast collateral damage: raptors, foxes, **coyotes**, **bobcats** and **mountain lions** — all of which help keep rodent populations in check — have been sickened or killed by these toxins. Occasionally, pets fall victim, too.



A healthy fledgling turkey vulture.
Courtesy of Todd Backman

The same fledgling turkey vulture after it was found sickened by rat poison and collapsed in the patio of a El Cerrito, California, home. ***Courtesy of Patricia Jones***

"I consider them to be like our modern-day DDT, due to the fact that they have infiltrated the entire food web," said Lisa Owens Viani, the director of Raptors are the Solution, a nonprofit that champions wild predators rather than rodenticides as a pest-control solution.

Thanks to the advocacy efforts of groups like **Owens Viani's**, in 2020, California signed into law **a ban** on the most harmful anticoagulant rodenticides by the general public and pest control companies. **In 2023** and **again in 2024**, the state passed additional legislation that added older versions of these rodenticides to that ban.

"I consider them to be like our modern-day DDT, due to the fact that they have infiltrated the entire food web."

Immediately after the first ban was passed, **raptor deaths by poison dropped nearly 15%**, according to data from the California Department of Fish and Wildlife, though the numbers have fluctuated in subsequent years. But the new study, in which tested turkey vultures for the chemicals after the initial bill was enacted, showed that anticoagulant rodenticides still pervade the environment.

According to study author Miguel D. Saggese, an avian and wildlife researcher at Western University of Health Sciences in Pomona, California, the results "provide further evidence that there is still a problem out there for non-target species."

SCAVENGERS LIKE TURKEY VULTURES, with their diverse carrion diet, are good sentinels of rodenticides' footprint across an entire ecosystem. Still, the results might be an underestimate. The new study examined blood samples from live-captured vultures, so the results provide only a snapshot of the birds' most recent encounters with the chemicals. Liver necropsies, which are more telling of chronic exposure, tend to register higher contamination rates — one 2022 study found that **93%** of turkey vultures in Northern California and southern Oregon had anticoagulant rodenticides in their bodies — though necropsy results can skew toward animals that have already perished from the poisons.

Turkey vultures are not a threatened species, but their exposure sounds an alarm for their more vulnerable neighbors. **Spotted owls**, bald eagles and the iconic California condor are already at risk of extinction, and anticoagulant rodenticides are likely a contributing factor. In the past, monitoring efforts have detected the toxins among these birds of prey. The prevalence among turkey vultures indicates that the chemicals need to be eliminated from the environment to ensure the health of wildlife in the West, whether or not the animals are endangered.

A bleeding great horned owl from the Morro Coast at Audubon's Sweet Springs Preserve the day before its death from pesticides.
Courtesy of David Lamkin

A poisoned red-tail hawk that was bleeding right until its death.
Courtesy of WildCare

California is the only state with legislation restricting anticoagulant rodenticides. But even the Golden State's bills have gaping concessions: The agriculture industry and food producers are exempt from the bans, as are public health agencies. And some people still set out illegal rat bait boxes anyway, regardless of what the law says.

Still, there's another compelling reason to renounce anticoagulant rodenticides: They're not all that effective at reining in rats. Experts say that a more durable solution is to not give rodents a reason to come by in the first place — by sealing off food sources and fortifying trash bins. Not only do the relatively slow-acting poisons falter against the prolific reproduction of rodents, they also kill off the rats' natural predators, which are humanity's most valuable allies against rodent infestations. Ultimately, the chemicals we employ to control rat populations end up helping rat populations slide out of control. "None of it makes any sense," Owens Viani said. "I just feel like it's kind of a scam that's been perpetrated to the public."

"I just feel like it's kind of a scam that's been perpetrated to the public."

And climate change is making things worse for pesticides-strained raptors. "Climate change is the very MOTHERSHIP of ecological stressors," wrote Allen Fish, a raptor biologist and former director of the Golden Gate Raptor Observatory, in an email. Already-weakened species may lack the wherewithal to deal with dwindling food sources and shrinking habitats. Meanwhile, warming temperatures allow rats to remain active during mild winters, eating and mating instead of laying low underground, and society's typical response — doling out even more rodenticides — only increases secondary poisoning events.

Anticoagulant rodenticides may well prove the last straw for some species' survival. "It's an ongoing environmental catastrophe that's happening right before our eyes," Owens Viani said.

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RESEARCH ARTICLE

High rates of anticoagulant rodenticide exposure in California Barred Owls are associated with the wildland–urban interface

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ABSTRACT

Pesticide use is pervasive and the exposure of non-target wildlife has been well documented over the past half-century. Among pesticides, anticoagulant rodenticides (AR) have emerged as a particularly important threat in forests of the western United States, with exposure and mortality reported for several species of conservation concern. To further quantify this threat, we collected specimens of Barred Owls (*Strix varia*) and Barred Owl x Spotted Owl hybrids from the Klamath and Cascade Mountains and Sierra Nevada in California, USA to use as indicator species for environmental contamination with AR and to infer exposure of closely related and ecologically similar Northern and California Spotted Owls (*S. occidentalis caurina,* and *S. o. occidentalis,* respectively). We tested 115 Barred Owl and 12 Barred Owl x Spotted Owl hybrid livers for 8 AR compounds and found high rates of exposure (62%) across our study area, and greater than previous studies in the Pacific Northwest. In addition, we sampled 7 ovaries from 7 females and 100% tested positive for AR. Female Barred Owls were more likely than males to be exposed (78% and 50%, respectively). Unlike previous studies, we found no clear link between illegal cannabis cultivation and AR exposure. However, Barred Owls sampled in proximity to the wildland–urban interface (WUI) were more likely to be exposed to AR. Though the exact source (e.g., cannabis cultivation or application around human dwellings) and location are unknown, the association of AR exposure with the WUI was supported from GPS data from Barred Owls, Northern and California Spotted Owls, and hybrids using the WUI for foraging. The high rate of AR exposure in Barred Owls and hybrids provides mounting evidence of an additional stressor that ARs may pose to Spotted Owls—including the first evidence for California Spotted Owls—and fauna native to western forest ecosystems.

Keywords: Barred Owl, brodifacoum, environmental contamination, pesticides, Spotted Owl, *Strix varia*, *Strix occidentalis*, wildland–urban interface

LAY SUMMARY

- Anticoagulant rodenticides have emerged as an important threat in forests of the western United States, and it is vital to understand how and where wildlife is exposed.
- As indicator species for Spotted Owl exposure, we screened 115 Barred Owls and 12 Barred Owl x Spotted Owl hybrids, collected from northern California, USA for 8 anticoagulant rodenticides.
- 62% of owl specimens (72 Barred and 7 hybrid) were exposed to anticoagulant rodenticides, in particular to the acutely toxic, second-generation class.
- Females and owls sampled close to the wildland–urban interface were more likely to be exposed to anticoagulant rodenticides.
- GPS-tagged Barred and Spotted Owls commonly foraged in the wildland–urban interface, suggesting Spotted Owls are also likely at risk of exposure.
- The high rate of AR exposure in Barred Owls and hybrids provides mounting evidence of an additional threat to Spotted Owls.

Las altas tasas de exposición a rodenticidas anticoagulantes en *Strix occidentalis occidentalis* se asocian con la interfaz urbano-silvestre

RESUMEN

El uso de plaguicidas es generalizado y la exposición no deseada de la vida silvestre ha sido bien documentada durante el último medio siglo. Entre los pesticidas, los raticidas anticoagulantes (RA) han surgido como una amenaza particularmente importante en los bosques del oeste de los Estados Unidos, con exposición y mortalidad reportadas para varias especies de interés para la conservación. Para una cuantificación más extensa de esta amenaza, recolectamos especímenes de *Strix varia* y de híbridos de *S. varia* x *S. occidentalis* de las montañas Klamath y Cascade y de la Sierra Nevada en California, EEUU, para usarlas como especies indicadoras de contaminación ambiental con RA y para inferir la exposición de *S. o. caurina* y de *S. o. occidentalis*, dos especies estrechamente relacionados y ecológicamente similares. Evaluamos los hígados de 115 individuos de *Strix varia* y de 12 híbridos de *S. varia* x *S. occidentalis* para 8 componentes de los RA y encontramos altas tasas de exposición (62%) a lo largo del área de estudio, y mayores tasas que la de los estudios previos del noroeste del Pacífico. Además, tomamos muestras de 7 ovarios de 7 hembras y el 100% dio positivo para RA. Las hembras de *S. varia* tuvieron más probabilidad de estar expuestas que los machos (78% y 50%, respectivamente). A diferencia de estudios anteriores, no encontramos un vínculo claro entre el cultivo ilegal de cannabis y la exposición a RA. Sin embargo, los individuos de *S. varia* muestreados en las proximidades de la interfaz urbano-silvestre (IUS) tuvieron más probabilidades de estar expuestos a RA. Aunque se desconoce la fuente (e.g., el cultivo de cannabis o la aplicación alrededor de las viviendas humanas) y la ubicación exacta, la asociación entre la exposición a RA con la IUS se basó en datos de GPS de *S. varia*, *S. o. caurina*, *S. o. occidentalis* e híbridos que utilizan la IUS para buscar alimento. La alta tasa de exposición a RA en *S. varia* y en los híbridos proporciona evidencia creciente de que los RA pueden representar un factor de estrés adicional para *S. occidentalis*—incluyendo la primera evidencia para *S. o. occidentalis*—y la fauna nativa de los ecosistemas forestales del oeste.

Palabras clave: brodifacoum, cannabis, contaminación ambiental, interfaz urbano-silvestre, pesticidas, Strix occidentalis, Strix varia

INTRODUCTION

Pesticide use is pervasive with an estimated 2.5 billion kilograms applied globally each year (Alavanja 2010). The exposure of non-target wildlife to pesticides has been well documented over the past half-century (Grier 1982, Peakall and Kiff 1988), with anticoagulant rodenticide (AR) identified as a particularly widespread and important conservation issue (Stone et al. 1999, Erickson and Urban 2004). Though exposure to AR may result in direct mortality, lesser-understood sub-lethal exposure can also have subtle detrimental effects on non-target wildlife (Riley et al. 2007, Thomas et al. 2011, Serieys et al. 2018). Most accounts of wildlife exposure to AR compounds have occurred in urban or agricultural settings, where the use of rodenticides is frequently permitted for the benefit of human health and mitigation of agricultural damage (Erickson and Urban 2004). However, exposure to AR in remote forest settings is increasingly being reported in the western United States, where multiple species of conservation concern have documented cases of exposure and mortality (Gabriel et al. 2012, 2018, Thompson et al. 2014, Franklin et al. 2018, Wiens et al. 2019). Non-target avian and mammalian predators are particularly vulnerable to secondary AR exposure through the consumption of prey that has ingested rodenticide baits (Stone et al. 1999, Erickson and Urban 2004). Poisoned rodents may be easier prey, because internal hemorrhaging greatly reduces joint mobility, causes lethargy, and reduces escape responses (Brakes and Smith 2005). Mitigating the threat of ARs to non-target wildlife in these forested settings requires understanding which species are exposed, as well as where and how exposure occurs.

Within the past decade, exposure of non-target wildlife to AR has been documented via an unexpected route: illegal cannabis cultivation in remote forests in the western U.S. (hereafter "western forests"; Gabriel et al. 2012, Wengert et al. 2018). Growers use ARs, in addition to other pesticides, to prevent rodent damage to cannabis plants, grow-site infrastructure, and food caches (Gabriel et al. 2012, Thompson et al. 2017). Hundreds of illegal cannabis cultivation sites have been found and eradicated in the foothills and mid-elevation slopes of the southern Sierra Nevada and the Klamath/Cascade Mountains, and an average of 4.5 kg (enough to kill ~22,000 rats from an LD_{50} of 0.27 mg kg^{-1}; Erickson and Urban 2004) of AR are found per site (Wengert et al. 2018). These sites are often located far from other human developments and roads in remote parts of the forests where detection is unlikely (Thompson et al. 2017). However, another source of AR exposure in non-target forest wildlife is from more expected applications around human structures and dwellings located in or near forested settings in what is known as the wildland–urban interface (WUI; Radeloff et al. 2005), defined as where houses meet or are intermixed with undeveloped wildland vegetation. In addition to habitat conversion, exposure of non-target wildlife to ARs is an emerging conservation challenge for wildlife living in close proximity to the WUI (Riley et al. 2007, Serieys et al. 2018).

Whether the exposure is occurring via cannabis cultivation or human communities, exposure to AR in western forests appears to threaten multiple species of conservation concern. For example, high rates of AR exposure have been reported in dead or dying Pacific Fishers (*Pekania pennanti*) in coastal California and the southern Sierra Nevada (85%, n = 101; Gabriel et al. 2012, 2015, Thompson et al. 2014) and in Northern Spotted Owls (*Strix occidentalis caurina*) found dead in coastal California (70%, n = 10; Gabriel et al. 2018). Given the lethal and potential sub-lethal effects of AR, exposure to these pesticides may exacerbate, or even be among the causes of, long-term population declines of both Northern Spotted Owls (Dugger et al. 2016) and California Spotted Owls (*S. o. occidentalis*; Tempel et al. 2013, 2014, Conner et al. 2016) when combined with other key stressors including megafires (Jones et al. 2016), historic habitat loss (Dugger et al. 2016), and competition with invasive species (Long and Wolfe 2019, Wood et al. 2020a). However, given the status of species of conservation concern for both Spotted Owl subspecies, testing Spotted Owls for AR exposure with large sample sizes of liver or blood sampling is difficult and not practical (e.g., obtaining permits).

To characterize Spotted Owls' risk of AR exposure, we used Barred Owls (*S. varia*) as indicator species (Caro and O'doherty 1999) for the presence of AR within the southern Klamath and Cascade Mountains and the Sierra Nevada in northern California. Barred Owls are a closely related and ecologically similar relative of Spotted Owls (Gutiérrez et al. 2007, Wiens et al. 2014) and were first documented within the range of the Northern Spotted Owl in the 1960s (Livezey 2009) and the core range of the California Spotted Owl in the early 2000s (Dark et al. 1998). Barred Owls compete with congeneric Spotted Owls where they occur sympatrically, and there is strong evidence they are one of the causes of declines in Spotted Owl populations (Wiens et al. 2014, Long and Wolfe 2019). Previous work has reported high rates of AR exposure in Barred Owls in Oregon and Washington (48%, n = 40; Wiens et al. 2019), and in coastal California (40%, n = 84; Gabriel et al. 2018). Barred Owls are likely a reasonable, if not conservative, indicator species for AR exposure in Spotted Owls due to a complete overlap in diet and habitat with Barred Owls being less focused on rodent prey than Spotted Owls (Wiens et al. 2014).

In this study, we leveraged biological samples collected as part of an experimental Barred Owl removal study in both the Klamath/Cascades and the Sierra Nevada, which offered a rare opportunity to collect a large sample size at a regional scale. This large sample size allowed us to assess AR exposure across a gradient of conditions likely to influence AR prevalence in the environment, including human density and cannabis cultivation. Furthermore, this is the first study to assess AR exposure in California Spotted Owls through the use of Barred Owls as an indicator species. Because the most useful viable method of testing AR exposure requires the recovery of intact liver tissue from a freshly dead carcass, the collected Barred Owls are a unique opportunity to understand the extent to which both Northern and California Spotted Owls are potentially exposed to ARs within the two sub-regions of our study area. We also GPS-tagged Barred Owls and both Northern and California Spotted Owls to assess the extent to which foraging activities occurred in areas characterized by elevated AR exposure in lethally removed Barred Owls. Finally, we tested the potential of in-utero transfer of AR in *Strix* owls by screening ovaries of AR-positive Barred Owls.

We hypothesized that exposure to AR in forest predators, such as Barred Owls and Barred Owl x Spotted Owl hybrids (hereafter "hybrids"), is influenced by biological factors, such as age and sex, and environmental factors, such as proximity to human communities and the intensity of cannabis cultivation. To test these hypotheses, we quantified the exposure of Barred Owls and hybrids to a suite of AR compounds and evaluated the degree to which exposure was associated with a suite of biological and environmental factors. We predicted higher exposure rates in hybrids, assuming hybrids would have similar foraging behavior to Spotted Owls, which have a dietary niche more focused on rodents than that of Barred Owls (Wiens et al. 2014). We predicted that younger and female Barred Owls would have higher rates of AR exposure as a result of larger dispersal movements (Greenwood 1980). We also predicted that owls exposed to ARs would be in worse physical condition than owls not exposed to ARs, given the potentially deleterious effects of sub-lethal exposure to AR. Among environmental factors, we predicted that Barred Owls collected in areas more likely to be used for cannabis cultivation or closer in distance to either known cultivation sites or the WUI, would have greater exposure to AR. Thus, in addition to characterizing the prevalence of AR in *Strix* owls in two new regions, we aimed to elucidate how behavior and human land use patterns influence AR exposure.

METHODS

Study Area

We collected Barred Owls and hybrids from the southern Klamath and Cascade Mountains and from the Sierra Nevada in northern California (Figure 1) on National Forest lands, national park lands, and private commercial timberlands primarily owned by Sierra Pacific Industries. There was considerable variation in climate, elevation, topography, and vegetation, though both sub-regions were

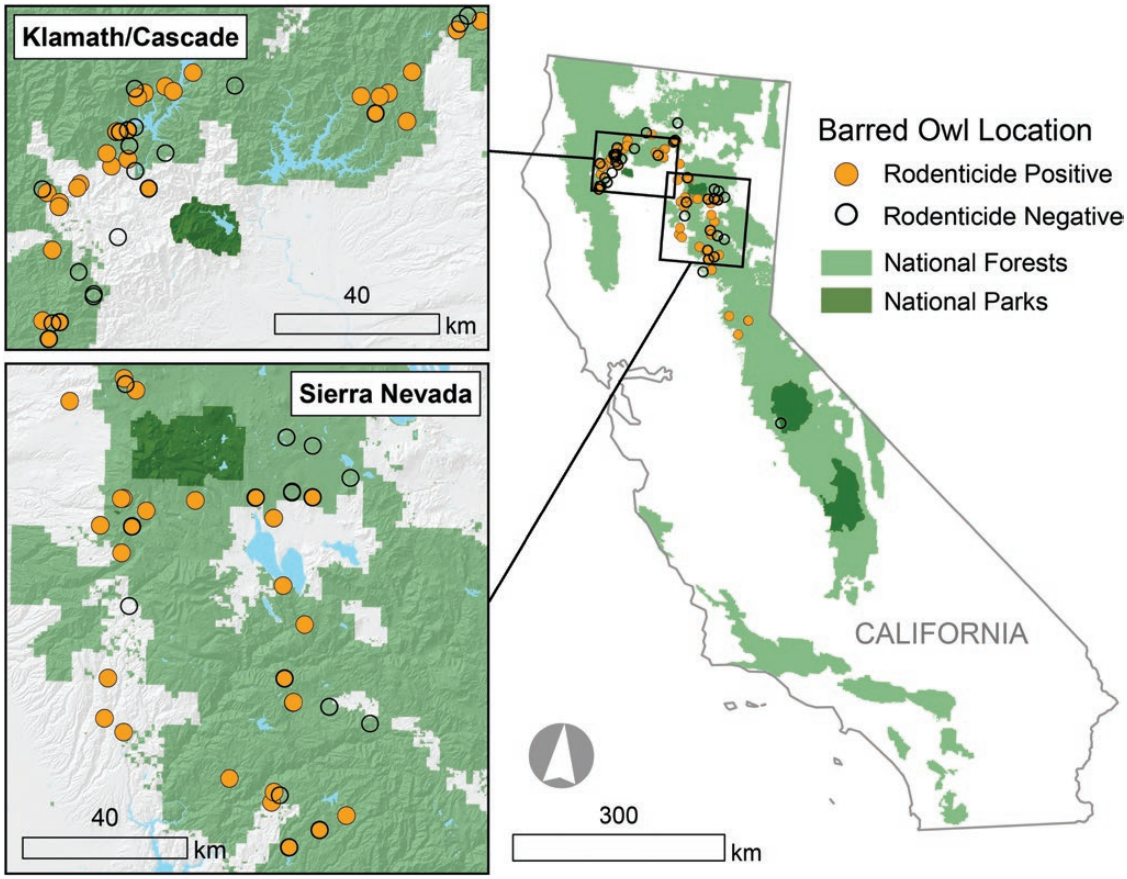

FIGURE 1. Locations of Barred Owls and Barred Owl x Spotted Owl hybrids collected from 2018 and 2019 and screened for anticoagulant rodenticides. Insets at the left show both the Klamath/Cascade and Sierra Nevada sub-regions in California, USA.

predominantly composed of mixed coniferous forest, dominated by ponderosa pine (*Pinus ponderosa*), sugar pine (*P. lambertiana*), incense cedar (*Calocedrus decurrens*), Douglas fir (*Pseudotsuga menziesii*), and white fir (*Abies concolor*). Neither the U.S. Forest Service (S.C. Sawyer, personal communication) nor Sierra Pacific Industries (B.P. Dotters, personal communication) use ARs on lands they manage within our study area. However, there are houses in WUIs adjacent to lands where owls were collected, and it is not known whether ARs are used in these areas.

Tissue Collection and AR Screening

We lured territorial Barred Owls and hybrids by broadcasting digitally recorded Barred Owl vocalizations and collected them with a 12-gauge shotgun following methods described by Diller et al. (2014). We collected Barred Owls and hybrids under federal and state Scientific Collecting Permits (United States Fish and Wildlife Service permits MB24592D-0, MB53229B-0 and California Department of Fish and Wildlife permits SC-002114, SC-11963). We froze owls immediately after collecting them and stored the specimens in a −20°C freezer until we delivered them to the Museum of Vertebrate Zoology (University of California, Berkeley), where we extracted livers and ovaries. We were careful to avoid contamination between the two organs by separating them immediately after they were removed from the abdominal cavity and placing them in separate containers. We thawed all specimens for a similar amount of time to extract tissues, and we left no specimen thawed for over 24 hours. We shipped tissue samples to the California Animal Health and Food Safety Laboratory System (CAHFS; University of California, Davis) where they were screened for 8 commonly used ARs: warfarin, diphacinone, chlorophacinone, coumachlor, brodifacoum, bromadiolone, difethialone, and difenacoum. The first 4 belong within less-acutely toxic first-generation ARs (FGAR); the latter 4 are more acutely toxic second-generation ARs (SGAR) that were created in the 1970s due to rodents developing resistance to first-generation ARs (FGARs; Buckle et al. 1994). High-performance liquid chromatography-tandem mass spectrometry was used to screen tissue samples for AR exposure (whether or not any ARs were detected) and to quantify the concentration of ARs detected (Marek and Koskinen 2007). We classified AR exposure in livers and ovaries using the limit of detection (LOD), which allowed us to detect the presence of

AR in any sample with a concentration above 0.005 μg g^{-1} wet weight (ww). We quantified AR concentrations in liver and ovary samples using the limit of quantification (LOQ), which was 0.050 μg g^{-1} ww for brodifacoum and 0.020 μg g^{-1} ww for all other ARs in owl livers, and 0.200 μg g^{-1} ww for all ARs in owl ovaries (Riley et al. 2007). Any sample above these LOQs could have concentrations quantified. These concentrations all fall below the 0.1 μg g^{-1} ww threshold for mortality rate of 10% of individuals previously reported in Barred Owls (Thomas et al. 2011). When samples had concentrations greater than the LOD and below the LOQ, we designated those individuals as having "trace" exposure.

Calculating Biological Variables

We identified owls in the field as Barred Owls or hybrids based on both plumage and territorial vocalizations. Individuals with vertical barring on the breast feathers and horizontal barring around the nape that produced distinct 2-phrase, 8-note calls (Odom and Mennill 2010) were identified as pure Barred Owls. Individuals with bars and spots on their breast feathers and that produced territorial calls that were not distinctly Spotted Owl or Barred Owl calls were identified as hybrids (Hamer et al. 1994). We classified age as either adult (≥3 yr), sub-adult (1–2 yr), or juvenile (0 yr), based on adults having wider terminal bands than sub-adults on all flight feathers, and juveniles lacking most or all body contour feathers (Mazur and James 2020, J. D. Wiens, personal communication). We determined sex by examining gonads in the lab, and we assessed body condition by characterizing the amount of subcutaneous fat content into four categorical values, with no fat being our baseline ("0"), slight fat ("1"), moderate fat ("2"), and heavy fat ("3"). Because fat reserves in owls change throughout the year (Massemin et al. 1997, DeLong 2006), we obtained a corrected fat index by calculating the residuals of a linear regression of fat against the month of the year (Supplemental Material Figure S1).

Calculating Environmental Variables

We assigned owls that were collected north of the Pit River to the Klamath/Cascade sub-region, and owls sampled south of this river to the Sierra Nevada sub-region (Figure 1). We used this designation to differentiate Barred Owls collected within the range of the Northern Spotted Owl (Klamath/Cascade) or of the California Spotted Owl (Sierra Nevada; Barrowclough et al. 2005). We calculated remaining environmental variables within 2,000 ha circular buffers around collection locations that approximated Barred Owl home range size in the region that we measured using GPS-tagged individuals in a previous study (see Wood et al. 2020a). We used a combination of law enforcement databases (IERC 2019) to calculate the number of known cannabis cultivation sites detected from 2004 to 2019 within the circular buffers. We also related AR exposure to a measure of the probability of illegal cannabis cultivation within the buffers, estimated from a maximum entropy (MaxEnt) model (G. M. Wengert personal communication) parameterized with variables indicative of the suitability of growing cannabis on California's public and private lands. The important variables in this predictive model included elevation, slope, precipitation, canopy cover, stand age, and distances to disturbance, freshwater, roads, and private lands, and used a resolution of 90 m for individual cells. From the MaxEnt model, we obtained an averaged index of cannabis cultivation suitability (ranging from 0 to 1) for each buffer to assess whether owls were more likely to be exposed in areas with more suitable conditions for cannabis cultivation.

Additionally, we calculated the distance of each Barred Owl removal location to the WUI based on 2010 census data (Radeloff et al. 2005, http://silvis.forest.wisc.edu/data/wui-change/), where owls that occurred within the WUI were assigned a distance of 0 km. Both intermix (where housing and vegetation intermingle) and interface (where housing occurs in the vicinity of contiguous wildland vegetation) components of the WUI spatial dataset were used. Four thresholds are defined in the WUI data provided by Radeloff et al. (2005) based on the level of housing density: high, moderate, low, and very low. We chose to use the low density WUI threshold requiring at least 6.17 housing units km^{-2} because of concordance we observed with this threshold and buildings visible in a building footprint spatial layer developed from Microsoft (https://www.microsoft.com/en-us/maps/building-footprints). Finally, we calculated landownership as the proportion of the circular buffers that were composed of National Forest lands. Descriptive statistics of the environmental variables is listed in Supplemental Material Table S1.

Characterizing Barred and Spotted Owl Foraging Activities

To characterize the distribution of Barred Owl foraging locations relative to environmental factors related to AR exposure (in this case WUIs, see below), we GPS-tagged 7 Barred Owls and 3 hybrids between May and August of 2017 and 2018 in the northern Sierra Nevada. We used visual and vocal lures to attract Barred Owls and hybrids and captured them with dho-gaza nets, and applied Argos-enabled GPS backpack tags (Lotek Wireless, Newmarket, Ontario, Canada). We programmed tags to record 4–6 nighttime locations per week between April and August, and then to record 1 location per week between September and March.

We also used locations from 24 GPS-tagged Northern Spotted Owls and 106 California Spotted Owls to characterize their use of areas associated with elevated AR

exposure in Barred Owls—and thus the potential for Northern and California Spotted Owl exposure rates to mirror Barred Owl rates. Northern Spotted Owl locations were collected in the Klamath Mountains between March and August of 2017, and California Spotted Owl locations were collected in the Sierra Nevada between May and August of 2015 through 2020 as part of previous studies (Jones et al. 2016, Atuo et al. 2018, Kramer et al. 2020). We used vocal lures to locate Spotted Owls and captured them either by hand-grab, pan-trap, or snare-poles, and applied GPS backpack tags (Lotek Pinpoint VHF 120, Newmarket, Ontario, Canada). Spotted Owl tags were programmed to record 5 hourly nocturnal locations per night between March and August. From these data, we calculated the mean proportion of locations that occurred within the WUI for both Northern and California Spotted Owls, as well as the proportion of individuals of each subspecies with at least one location in the WUI. We assumed the majority of these locations were primarily foraging locations as owls are nocturnal predators, but we acknowledge that other behaviors such as territory defense, resting, and returns to roosts and nests may be included in these locations.

Additionally, we calculated the proportion of all known Northern Spotted Owl activity centers and all California Spotted Owl activity centers in the Sierra Nevada whose home ranges at least partially overlapped with the WUI to assess the risk of Spotted Owl exposure to ARs via the possibility of foraging in the WUI. We used 2.1 km radius home ranges for Northern Spotted Owls and 1.6 km radius home ranges for California Spotted Owls (Wiens et al. 2014, Blakey et al. 2019). Activity centers were defined as nest locations or geometric centers of daytime roost locations and were obtained from the California Department of Fish and Wildlife (https://www.wildlife.ca.gov/Data/CNDDB/Spotted-Owl-Info). We also used both Northern and California Spotted Owl designated ranges (USFWS 2017) to calculate the proportion of WUI within each Spotted Owl subspecies' range (only including the Sierra Nevada for California Spotted Owls).

Statistical Analysis

We used a set of generalized linear models (McCullagh and Nelder 1989) within an information-theoretic framework (Burnham and Anderson 2002) to test for associations between AR exposure and biological and environmental factors. Because most exposures were at the trace level, we modeled exposure as a binomial response (exposed = 1 and not exposed = 0). Biological factors consisted of species (pure Barred Owl versus hybrid), age, sex, and the index of body condition. Juvenile and un-aged owls were omitted from the generalized linear model because of small sample sizes. Environmental factors consisted of sub-region, proximity to the WUI, number of known cannabis cultivation sites within home ranges, the average index of predictive cultivation for each Barred Owl home range from the MaxEnt model, and landownership.

We used a multi-stage secondary candidate strategy to select top-ranked models (Morin et al. 2020). First, we ran all combinations of biological models and all combinations of environmental models separately. We then identified supported models as those within 5 AIC_c (second-order Akaike Information Criterion corrected for small sample sizes) of the most supported model for each set of models. Second, we combined and evaluated support for variables in the top models from both the biological and environmental sets. In both model-selecting stages, models with uninformative variables (e.g., confidence intervals of variables overlap with zero) were not considered (Leroux 2019). We used the package *MuMIn* in R Studio 1.3.1073 (R Core Development Team 2017) for these analyses.

We also conducted a general Getis Ord-General G high/low cluster analysis (Getis and Ord 1992) to assess the degree to which AR exposure was more clustered than expected at random, less clustered than expected at random, or randomly distributed. We ran separate analyses for owls collected in the Klamath/Cascade sub-region and those collected in the northern Sierra Nevada (where the majority of Sierra Nevada removals were conducted), and only used locations for where owls were exposed, realizing that mates could be non-exposed. To reduce potential biases associated with sampling multiple owls from the same territory, owls collected within 2.52 km (the radius of a 2,000 ha Barred Owl home range in the region; Wood et al. 2020a) of other owls were combined to single points based on the geometric centers of the points. We also conducted a Moran's I spatial autocorrelation analysis with the same condensed points to assess the degree of concordance between different clustering procedures. All spatial analyses were conducted using ArcMap 10.6.1 (ESRI Inc., Redlands, California, USA).

RESULTS

Barred Owl Collections and Liver Analysis

We screened 127 livers (115 Barred Owls and 12 hybrids) for ARs (Figure 1), of which 62% (79 of 127, 72 Barred Owls, and 7 hybrids) tested positive for at least one AR. Brodifacoum and bromadiolone were the only two ARs detected, with 97% (77 of 79) of exposed individuals having exposure to brodifacoum, 15% (12 of 79) to bromadiolone, and 13% (10 of 79) to both. Eighty-seven percent of the AR exposures were at the "trace" level (below quantification limits), with 13% (seven females, and two males) having quantifiable concentrations of AR. Seven of those samples had quantifiable concentrations of brodifacoum

TABLE 1. Generalized linear modeling results from our final stage of model selection used to examine variability in Barred Owls and Barred Owl x Spotted Owl hybrids exposure to anticoagulant rodenticides in northern California in 2018 and 2019. Model covariates include sex and proximity to the wildland–urban interface (WUI). *k* is the number of parameters, and w_i is Akaike's weight. Results for initial modeling steps are provided in Supplemental Material Tables S2 and S3

Model	*k*	ΔAIC_c [a]	w_i
Sex + WUI	3	0.00	0.869
Sex	2	3.95	0.121
WUI	2	9.28	0.008
Intercept only	1	11.94	0.002

[a]Akaike's information criterion corrected for sample size (AIC_c) of top model was 139.5.

(median = 0.084 μg g^{-1} ww, SD = 0.033, min = 0.050, max = 0.150) and three had quantifiable concentrations of bromadiolone (median = 0.150 μg g^{-1} ww, SD = 0.102, min = 0.120, max = 0.310). A total of 7 ovaries were tested for AR contamination and 100% were positive at trace levels (6 contained brodifacoum, 3 contained bromadiolone, and 2 contained both), and all ovaries were from females whose livers also tested positive for the same ARs.

Factors Associated with AR Exposure

After excluding 4 juveniles (because of small sample sizes), 5 un-aged owls, and 2 owls lacking fat scores, 116 individuals (107 Barred Owls and 9 hybrids) were used to conduct the generalized linear model to predict AR exposure. No pairwise combination of variables were highly correlated (all Pearson's *r*'s < 0.6), although distance to WUI and cannabis cultivation suitability were moderately and negatively correlated ($r = -0.42$, $P < 0.01$) – suggesting that cannabis cultivation was more likely to occur near the WUI. The highest ranked model in the biological-only modeling step contained only sex; all other biological variables occurred in models within 5 AIC_c but they were considered uninformative as the 95% confidence intervals overlapped zero and not considered further (Supplemental Material Table S2). The highest ranked model in the environmental-only modeling step contained only distance to WUI; all other environmental variables occurred in models within 5 AIC_c of the top model but they were considered uninformative as the 95% confidence intervals (95% CI) overlapped zero and not considered further (Supplemental Material Table S3). In our second (i.e. combined) modeling step, the top model contained sex and distance to WUI (w_i = 0.869; Table 1). Based on this model, females (78%) were more likely to be exposed to ARs than males (50%; β = –1.448, 95% CI: –2.391 to –0.590; Figure 2). In addition, the probability of AR exposure declined with distance from the WUI (β = –0.146, 95% CI: –0.271 to –0.029) – in other words, Barred Owls sampled near the WUI were more likely to be exposed (Figure 2). Based on this modeling process, there was little support for an association between AR exposure and known grow sites, the predictive index for the suitability of cannabis cultivation, age, species (purebred versus hybrid), body condition, or landownership.

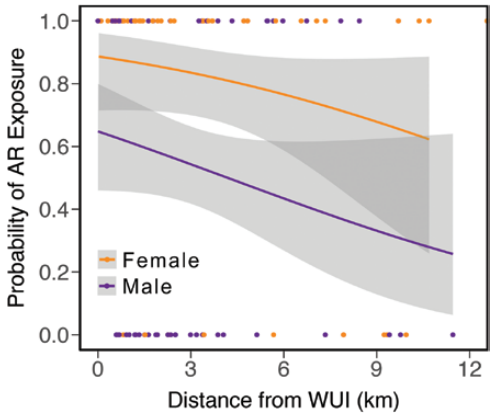

FIGURE 2. Predicted probability of Barred Owls and Barred Owl x Spotted Owl hybrids being exposed to anticoagulant rodenticides (AR) in northern California in 2018 and 2019 plotted against the distance from the wildland–urban interface (WUI; Radeloff et al. 2005). The predicted probability of AR exposure is shown as the solid lines, whereas the 95% confidence intervals are shaded in gray. Colored dots at the top and bottom of the figure represent the raw data of individual owls that were exposed to AR (top) and not exposed to AR (bottom).

We detected little evidence for clustering among locations where Barred Owls were exposed to AR in either the Klamath/Cascade Mountains or the Sierra Nevada. This was the case based on both the Getis Ord-General G high/low cluster analysis (Klamath/Cascade P = 0.27, Sierra Nevada P = 0.83), and the Moran's I analysis (Klamath/Cascade P = 0.39, Sierra Nevada P = 0.58), which indicates that AR was randomly distributed across space in both sub-regions (without considering other environmental variables).

Distribution of GPS-Tagged Owl Locations Relative to the WUI

We tracked the 7 GPS-tagged Barred Owls and 3 hybrids for an average of 229 days (range: 52–392), obtaining an average of 40 foraging locations (range: 15–72) per individual. An average of 2% of Barred Owl and hybrid GPS locations (range: 0–18) occurred within the WUI, and 50% of tagged individuals had at least 1 foraging location within the WUI (Figure 3). We tracked the 24 GPS-tagged Northern Spotted Owls for an average of 65 days (range: 29–79) and obtained an average of 228 foraging locations per individual (range: 94–276). Among Northern Spotted Owls, an average of 2% of GPS locations occurred within the WUI (range: 0–43) and 33% had at least one foraging location within the WUI (Figure 3). We tracked the 106 GPS-tagged California Spotted Owls for an average of 58 days (range: 4–161) and obtained an average of 132 foraging

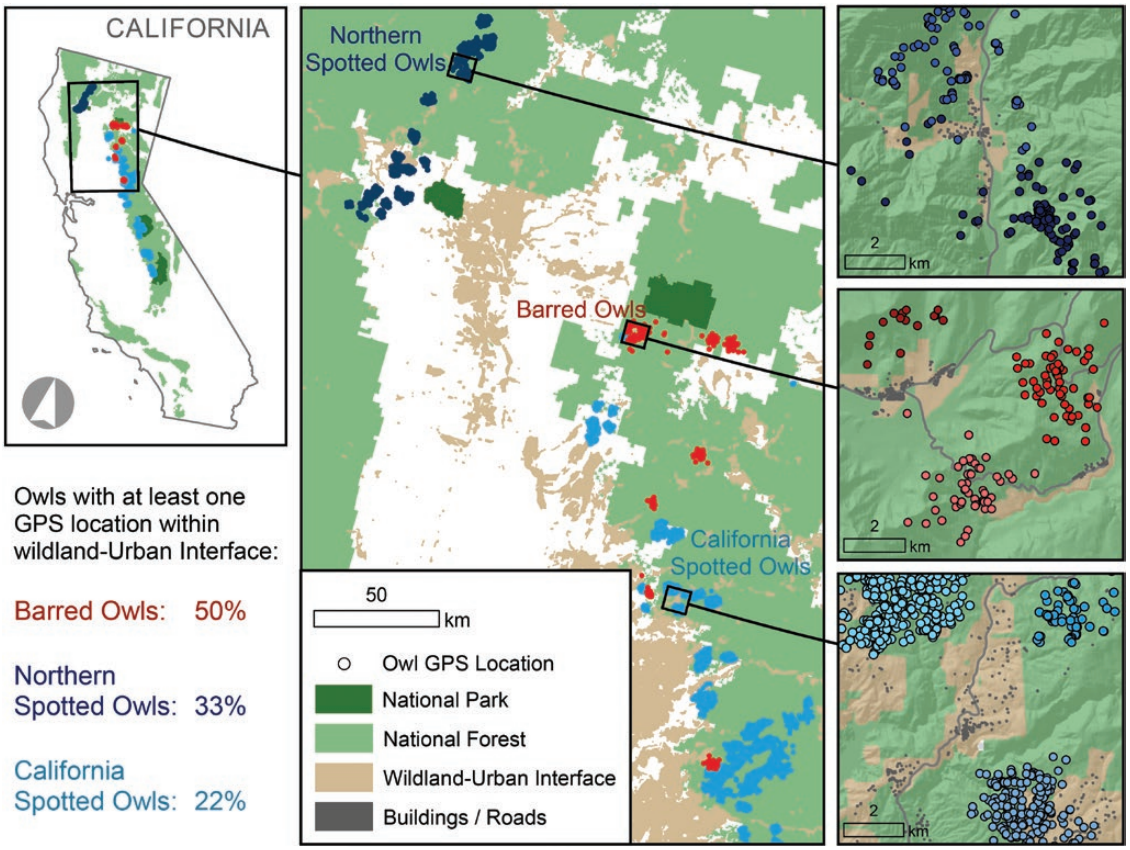

FIGURE 3. Locations and summary statistics of GPS-tagged Barred Owls (n = 10), Northern Spotted Owls (n = 24), and California Spotted Owls (n = 106) in relation to the wildland–urban interface (WUI) in the Klamath/Cascade Mountains and Sierra Nevada in northern California, USA. Dark blue dots on the California map represent GPS-tagged Northern Spotted Owls, red dots represent Barred Owls and hybrids, and light blue dots represent California Spotted Owls. Different color shades in the inset maps represent the GPS locations of individual owls.

locations per individual (range: 9–348). Among California Spotted Owls, an average of 2% of GPS locations occurred within the WUI (range: 0–219) and 22% of tagged individuals had at least one foraging location within the WUI (Figure 3). Based on all known Northern and California Spotted Owl activity centers in the Sierra Nevada, 35% (range: 0.001–1363 ha) and 28% (range: 0.003–751 ha) of individual home ranges overlapped at least partially with the WUI, respectively. However, only 4.3% and 11.9% of Northern and California Spotted Owl ranges overlapped with the WUI, respectively.

DISCUSSION

A high proportion of Barred Owls and hybrids were exposed to AR in both the Klamath/Cascade Mountains and the Sierra Nevada, with exposure being widespread and no evidence for spatial clustering among AR-positive individuals (Figure 1). Females were more likely to be exposed than males and tended to have higher quantifiable concentrations of AR. This is of conservation concern, because we documented, for the first time, AR-positive ovaries and a potential for in-utero transfer of AR in *Strix* owls. AR exposure was not clearly linked to illegal cannabis cultivation, but Barred Owls sampled in proximity to the WUI were more likely to be exposed to ARs. The exposure of such a high proportion of Barred Owls, an apex forest predator, signifies that AR is a pervasive toxicant in western forest ecosystems and contributes to mounting evidence of potential AR exposure in Northern Spotted Owls—and the first potential evidence in California Spotted Owls. Although our sample of hybrids was small, we found similar rates of AR exposure between pure Barred Owls and hybrids, suggesting that Barred Owls may serve as reasonable indicator species for AR environmental contamination and to infer exposure in Spotted Owls. Further support of Barred Owls as reasonable indicator species for Spotted Owl exposure to ARs is provided by the similar use of WUIs by GPS-tagged Barred Owls and Northern and California Spotted Owls. Thus, our study supports previous work showing widespread AR exposure in predators inhabiting remote western forests (Gabriel et al. 2012, 2018, Thompson et al.

2014, Franklin et al. 2018, Wiens et al. 2019), but also suggests that exposure is higher within and around WUIs.

Barred Owl Exposure to AR

Barred Owls collected in our study area were exposed to brodifacoum and bromadiolone. This has important conservation implications because both of these compounds are SGARs and due to the threat they pose to non-target wildlife, their use in California was prohibited in 2014 without a licensed professional, as was their application more than 15 m from human structures (California Code of Regulations Title 3, Section 6471). Indeed, it is unlikely that the high percentage of Barred Owl exposure to AR in our study area comes entirely from legal applications of SGARs, because from 2015 to 2018 only 8.26 kg of brodifacoum were reported to have been sold in the entire state of California (California Department of Pesticide Regulation, https://www.cdpr.ca.gov/docs/mill/nopdsold.htm about the same mass as found at just 2 average illegal cannabis cultivation sites in California (Wengert et al. 2018). Thus, it appears that even with stricter regulations, the legal or more-likely illegal applications of dangerous SGARs and exposure of non-target wildlife remain a challenge for conservation, as does identifying the main sources of illegal applications. Additionally, the proportion of Barred Owls and hybrids exposed to SGARs in our study area (62%) was greater than proportions reported in coastal California (40%, n = 84; Gabriel et al. 2018) and Oregon and Washington (48%, n = 40; Wiens et al. 2019), suggesting that the use of SGARs could be more intense in our study area.

Similar to what has been documented in Oregon and Washington (Wiens et al. 2019), most of our AR-positive specimens had trace liver concentrations below the quantifiable level. As of yet, the sub-lethal effects of ARs and the causes and consequences of trace concentrations in Barred and Spotted Owls have not been studied, although the majority of trace concentrations could be explained by at least 3 non-exclusive possibilities. First, owls with high AR concentrations may have acutely died due to these toxicants and therefore were not available for sampling. If so, our samples may be biased toward the low end of an exposure, with the 9 owls with high concentrations of AR suggesting that concentrations greater than trace levels can occur in Barred Owls. Second, owls may have consumed prey that varied in their concentrations of AR and over different periods of time, which resulted in the majority of, but not all, exposures being at the trace level. However, due to the unknown kinetics of toxicant uptake or sequestration, or degradation mechanisms of AR in *Strix* owls, this possibility will need to be explored further. Third, given that all Barred Owl ovaries tested positive for AR, trace levels could be the result of in-utero transfer of ARs rather than or in addition to the consumption of contaminated prey—a phenomenon that has been reported in Barn Owls (*Tyto alba*; Salim et al. 2015). However, we recognize AR presence in the ovaries still does not necessarily confirm the maternal transfer and that this possibility will need to be explored further by comparing plasmatic vs. ovarian tissue exposure to AR and/or testing eggs directly.

Although the majority of our specimens had trace levels of AR, 9 owls (7 female, 2 male) had concentrations of up to 0.150 μg g^{-1} ww for brodifacoum, and 0.310 μg g^{-1} ww for bromadiolone. These concentrations are both higher than the 0.1 μg g^{-1} ww threshold reported in Barn and Barred Owls, when clinical signs of AR toxicosis begin to show and reflected a mortality rate of 10% of individuals (Thomas et al. 2011). Though not documented in Barred Owls, sub-lethal exposure to SGARs can reduce clutch size and fledgling success in Barn Owls (Salim et al. 2014). In addition, sub-lethal internal hemorrhaging has been documented in Golden Eagles (*Aquila chrysaetos*) and Northern Spotted Owls with liver concentrations of brodifacoum as low as 0.030 and 0.050 μg g^{-1} ww, respectively (Stone et al. 1999, Franklin et al. 2018), and Pacific Fishers have died with signs of AR toxicosis with liver concentrations as low as 0.040 μg g^{-1} ww (Gabriel et al. 2012). More research into the effects of sub-lethal exposure on specific species of concern may be merited, especially because Barred Owl populations are expanding (Wood et al. 2020a) despite high rates of AR exposure. Indeed, no atypical behaviors were observed while collecting Barred Owls who had confirmed trace levels of AR in their tissues. However, the effects of widespread sub-lethal exposure could be more severe in Spotted Owls due to the stress of competitive interactions with more dominant Barred Owls (Wiens et al. 2014), as stress can exacerbate deleterious effects of AR, such as internal hemorrhaging (Cox and Smith 1992).

Biological and Environmental Factors influencing AR Exposure

In contrast to previous studies (Gabriel et al. 2018, Wiens et al. 2019), we found that females were more likely to be exposed to AR than males. Though information is limited for Barred Owls, this may be explained by female Spotted Owls, and female birds in general, having greater dispersal distances on average than those of males (Greenwood 1980, Jenkins et al. 2019). Thus, female Barred Owls, and likely female Spotted Owls, may encounter more sources of ARs that translate to higher rates of exposure and potentially higher concentrations of AR, which also suggests that individuals could have brought AR exposure from natal areas located far from where they were collected. This trend could additionally be explained by Barred Owl females' dependence on males delivering food to them while they are on the nest for a substantial amount of time

every year (Mazur and James 2020). The fact that females had higher rates of exposure is cause for concern because if ovaries testing positive for ARs does indeed signify maternal transfer, it is possible that this transfer is widespread among owls in this study area. However, further research on the possibility of maternal transfer of AR is necessary through the direct testing of eggs.

Higher rates of AR exposure in Barred Owls and hybrids sampled near the WUI indicated that those owls whose home ranges were closer to human development were more likely to be exposed to AR. Indeed, 50% of Barred Owls and hybrids (and 33% of Northern Spotted Owls and 22% of California Spotted Owls) had at least one point in the WUI. Moreover, Barred Owls and hybrids with higher concentrations of AR were collected on average 2 km closer to the WUI than owls with trace AR concentrations, and 3 km closer than owls that were not exposed to AR. However, the mechanism of exposure in the WUI, and whether it is due to cannabis cultivation within the WUI or applications around homes or both, remains unknown. Furthermore, we do not necessarily know where AR-positive owls collected outside of the WUI were exposed. For instance, the half-life for brodifacoum can be as long as 350 days in rats, but predators (including owls) tend to have longer degradation times (up to three times in duration), as demonstrated with the 2–3-day half-life of diphacinone in rats and the 11.7-day half-life of diphacinone in Eastern Screech Owls (*Megascops asio*; Herring et al. 2017). Therefore, it is possible that sampled owls could have been exposed any time over the last 3–4 yr, especially given the apparently recent immigration of some sampled individuals to our study area resulting from vacancies created by removals (D.F. Hofstadter and B.P. Dotters, unpublished data). Nevertheless, we might expect that such discordance between exposure and collection sites resulting from dispersal movements might erode a true association between the WUI and AR exposure, rather than create a false association of WUI and exposure.

Contrary to predictions, AR exposure was unrelated to either of our 2 metrics of illegal cannabis cultivation—an observation that could also have several non-mutually exclusive explanations. First, after California enacted the partial ban on SGARs in 2014, this class of AR was no longer as commonly reported at illegal cannabis cultivation sites, though other toxicants (like FGARs and neurotoxins) were often reported instead (Thompson et al. 2017). Second, illegal cannabis cultivation is by nature clandestine and many grow sites go undetected every year (M. W. Gabriel and G. M. Wengert, personal communication), which could have obscured an actual association to AR exposure. Finally, AR poisoned owls may die near grow sites due to exposure to AR as well as more acutely lethal compounds like neurotoxins, and thus never get sampled. Despite these uncertainties, exposure rates were high in owls sampled several kilometers from the WUI, and particularly so for females—a pattern we consider most likely attributable to either the past or recent use of ARs for illegal cannabis cultivation given low housing densities in these areas (Figure 2).

Threats to Spotted Owls and Western Forest Ecosystems

Our study area adds two new regions to the list of western forests where a high rate of Barred Owls have been exposed to ARs in both remote forested settings and in proximity to the WUI. The 62% of Barred Owls exposed to AR demonstrates that ARs have contaminated the food webs of northern California forests and suggest that AR could pose a threat to wildlife, including Spotted Owls. Although our sample size of hybrids was small, the fact that we did not have any evidence for a difference in exposure rates between pure Barred Owls and hybrids suggests that similar rates of AR exposure could also result in Spotted Owls—a possibility further supported from our GPS foraging locations. In fact, previous work reported 40% (n = 84) of collected Barred Owls and 70% (n = 10) of Northern Spotted Owls that were found dead in coastal California had also been exposed to AR, with Spotted Owls all exposed at trace levels (Gabriel et al. 2018). Spotted Owls prey more selectively on rodents than Barred Owls (Wiens et al. 2014) such that, in regards to diet, Spotted Owls may be more at risk for exposure. However, we found that the proportion of Spotted Owls that frequent the WUI was lower than Barred Owls and also that only a small portion of the WUI overlaps with the U.S. Fish and Wildlife Service designated ranges for both subspecies. Therefore, Spotted Owl behavior and habitat selection may buffer them more from exposure than Barred Owls, which often select suburban habitat containing mature trees (Clement et al. 2019).

In addition to other threats facing Spotted Owl populations, including megafires (Jones et al. 2016), a deficit of large trees (Jones et al. 2018), habitat homogenization (Hobart et al. 2019), and competition with Barred Owls (Wiens et al. 2014, Long and Wolfe 2019), the effects of AR exposure, in comparison, could easily go undetected. Moreover, there is a likely possibility of synergistic effects with sub-lethal effects of AR and other threats faced by Spotted Owls. For example, large disturbances to habitat are correlated to increased cortisol levels in Pacific Fishers (Kordosky 2019) and California Spotted Owl energy expenditure is increased with the presence of Barred Owls in the northern Sierra Nevada (Wood et al. 2020b). Therefore, there is a possibility of environmental stressors accentuating synergistic effects of AR in owls and other forest wildlife.

Our results provide additional evidence that AR exposure could be a more significant threat to forest species of conservation concern than previously thought, and also that it is positively associated with the WUI. This threat is augmented by the long half-life and sub-lethal effects that these toxicants can have (Herring et al. 2017) Exposure in apex predators, like Barred Owls, likely indicates that contamination by AR is pervasive in forest food chains. Indeed, the ubiquity of AR contamination has been documented in many cases, ranging from earthworms and snails being exposed through the soil (Booth and Fisher 2003), to birds eating exposed insects (Masuda et al. 2014), to exposed rodents eaten by various predators, and even to streams, where fish exposed to AR have been reported (Kotthoff et al. 2019). Furthermore, there is the biological significance of low concentrations of AR in various wildlife taxa (Stone et al. 1999, Gabriel et al. 2012, Franklin et al. 2018), suggesting the high rates of trace exposure in Barred Owls and hybrids indicate a significant threat to wildlife, including Spotted Owls.

We believe that future studies should focus on the WUI to elucidate more details on the mechanism of AR exposure, and whether tighter regulations of SGAR applications within the WUI could help to lower this exposure. In fact, as of September 2020, California regulation has recently changed to become stricter regarding the use and application of SGARs (California Assembly Bill No. 1788, Chapter 250). This provides an opportunity to further examine whether further AR exposure is a consequence of legal or illegal applications. Finally, more work is also needed to better understand potential sub-lethal effects and the in-utero transfer of ARs in *Strix* owls, as well as addressing the consequences of high rates of AR exposure in apex predators for forest food webs.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *Ornithological Applications* online.

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Ethics statement: Barred Owl, Spotted Owl, and hybrid handling and tagging procedures were consistent with approved IACUC protocol A005367-R01. Barred Owl and hybrid collection procedures were consistent with approved IACUC protocol A006106-A01.

Conflict of interest statement: The authors declare no conflicts of interest.

Author contributions: MZP, CMW, and MWG conceived the study; MZP and CMW secured funding; DFH, NFK, BPD, KGK, KNR, and CMW contributed to specimen collection; DFH, NFK, and EDF extracted tissue samples; DFH, NFK, CMW, SAW, WJB, BPD, and KNR captured owls and attached GPS tags; MWG and GMW contributed to the cannabis measurements; DFH wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Data availability: Our data is deposited in Dryad. Analyses reported in this article can be reproduced using the data provided by Hofstadter et al. (2021).

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A negative association between bromadiolone exposure and nestling body condition in common kestrels: management implications for vole outbreaks

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Abstract

Background: Vole outbreaks have been extensively described, along with their impacts on humans, particularly in agricultural areas. The use of rodenticides is a common legal practice to minimise crop damage induced by high vole density for biocidal use. However, rodenticides can have negative direct and indirect impacts on non-target species that feed on voles. We studied whether the use of a second-generation anticoagulant rodenticide (SGAR), bromadiolone, can be detected in the blood of fledglings of wild common kestrels Falco tinnunculus in two areas of central Spain, exploring its possible indirect effects.

Results: We found that 16.9% of fledglings had a detectable concentration of bromadiolone in their blood, with an average concentration of 0.248 ± 0.023 ng mL^{-1} . Fledglings with bromadiolone in their blood, regardless of the concentration, had 6.7% lower body mass than those without detectable bromadiolone.

Conclusion: The use of bromadiolone was detectable in the blood of alive non-target species. Detected bromadiolone in blood may reduce the body condition of nestlings, potentially reducing their fitness. The source of bromadiolone found in nestlings needs to be determined in future studies to derive accurate management advice. However, we urge the discontinuation of official SGAR distribution to farmers and their use in agrarian lands to minimise damage of voles on crops, particularly where common kestrels breed, and encourage the use of alternative effective practices. © 2016 Society of Chemical Industry.

Keywords: poisoning; raptors; rodenticide; voles; wild populations.

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October 24, 2025

RODENT FERTILITY CONTROL AS AN ALTERNATIVE TO POISON

1. GOOD BITES™ RODENT FERTILITY CONTROL

Good Bites™ are palatable rodent-attractive pellets made with nuts, seeds and nutritional grains. The active ingredient is a commercially available supplement, *Tripteryguim wilfordii* Hook F (TwHF).

The Thunder God Vine grows in mountainous regions of China. Extracts made from its leaves and roots have been used for centuries in Traditional Chinese Medicine (TCM) primarily to reduce inflammation (Gao et al 2021).

TwHF active ingredients have rapid onset but because TwHF active ingredients have short half life's, 15 minutes, they do not accumulate in animal tissues and do not pose a risk of non-target exposure (Liu et al 2015). If a predator consumes a mouse or rat even as it leaves a bait station its reproduction will not be effected.

Mice and rats are attracted to the palatable pellets and return to repeatedly feed. Impact on the population is observed in 1-3 months. Due to mice and rat small body mass and their rapid metabolism of TwHF active ingredients there is minimum risk posed to children and adults who would have to eat pounds of pellets daily to begin to achieve an effective dose, practically impossible given the small amounts of pellets available in single feeding stations.

Monitoring of Good Bites™ consumption is essential to its efficient deployment to reach the most mice or rats. Good Bites™ are very appealing to other wildlife such as squirrels, chipmunks, birds and raccoons. We therefore developed a Good Box pellet feeding station to allow only mice or rats to consume Good Bites™.

An additional effect of Thunder God Vine root powder is to induce reversible infertility. In other words, it acts as a ***contraceptive***. This characteristic prevents development of resistance as reported with poisons.

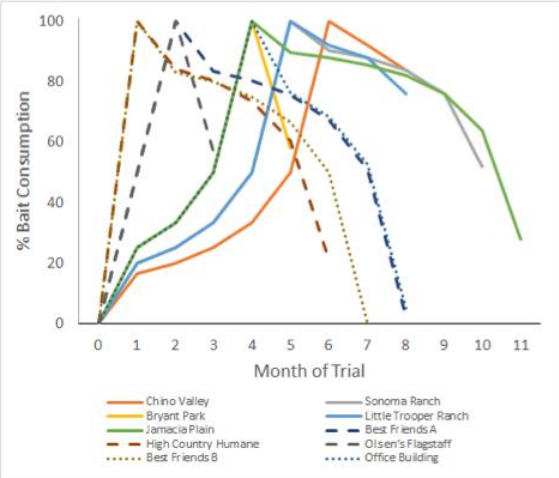
Active ingredients

Good Bites™ (GB) pellets are cereal based and entirely plant derived. They are made of peanut butter, wheat flour, cornmeal, quick oats and table sugar and are highly palatable and attractive to mice and rats. The active ingredient in GB pellets is from Tripterygium wilfordii capsules purchased from Amazon. These capsules contain Tripterygium wilfordii root powder used for hundreds of years in traditional Chinese medicine to provide relief from rheumatoid arthritis inflammation and pain. The active ingredient is triptolide. Triptolide, at ten billionth of a gram or 0.00000001 gram, causes infertility in mice and rats. Triptolide is a contraceptive as the infertility it causes reverses if mice and rats are no longer eating GB pellets. Triptolide is rapidly inactivated by the liver in mice and rats with a half-life of less than 15 minutes. Thus, rodent predators such as raptors are not affected if they catch and eat a mouse or rat that has eaten GB pellets. Triptolide acts on both the rodent ovary and testis, stopping ovulation and sperm development, respectively.

2. PROJECT RESULTS

At present we have conducted over 20 individual studies with 4,321 pounds of pellets deployed and 8,913 data points collected over 2 years from the following settings: retail stores, grain and mill facilities, zoos, sanctuaries, animal shelters, urban neighborhoods reserves and conservation areas. Using scientific published protocols, rodent populations were measured via camera captures, trapping plates, and live captures and correlated to pellet consumption. We developed a web-based application to measure consumption in all locations to determine time to rodent feeding acclimation, population reduction percentages, and sustained reduced populations. There were no adverse effects reported in birds, raccoons, dogs, cats, squirrels, or other small animals. Rodent population reductions because of Good Bites feeding ranged from a low of 10% and high of 99% over a range of 3 to 17 months and sustained for up to 20 months to date.

Consistent among all projects is a pattern (graphed below), wherein consumption climbs to a peak (acclimation) then declines with the reduced rodent population due to reduction in fertility (birth control). Variation in time to decline is dependent upon rodent migration opportunity: Open (parks, ranches, etc.), Semi-Open (buildings, homes), and Closed (interior enclosed buildings)



Pilot Project	Environment	Time to Peak	Reduction	Time to reduction
Chino Valley	Granary - open	6 months	16%	2 months*
Best Friends	Animals – semi-closed	2 months	98%	6 months
Best Friends	Animals - closed	1 month	100%	6 months
Sonoma Ranch	Agriculture - open	5 months	48%	5 months*
Bryant Park	City Park - open	4 months	42 %	1 month *
High Country Humane	Animals – semi-closed	1 month	79%	5 months*
Marco Island	City Park - open	Still climbing	N/A	N/A**
Little Trooper Ranch	Animals - open	5 months	24%	3 months*
Olsen's Flagstaff	Retail – semi-closed	2 months	43%	1 month*
Office Building	Office - closed	4 months	95%	4 months*
Jamacia Plain	Residential - open	4 months	72%	9 months

Summary of all active projects to date:

Category	Project	% Rodent Reduction	Months	Migration
Retail	Flagstaff, AZ	95%	9	Semi-Open
Grain & Mill	Chino Valley, AZ	98%	17	Semi-Open
Urban	Fairfield, CT	53%	7	Open
	Berkeley, CA	10%	5	Open
	Marco Island, FL	48%	12	Open
	Lincoln Park, Chicago, IL	63%	3	Open
	Harlem, NYC, NY	Data collected by NYC staff data 6/26		Open
	Boston, MA	71%	16	Open
	Manhattan, NYC, NY	42%	6	Open
Zoos, Ranches & Sanctuaries	Petaluma, CA	49%	24	Open
	Salt Lake City, UT	39%	3	Semi-Open

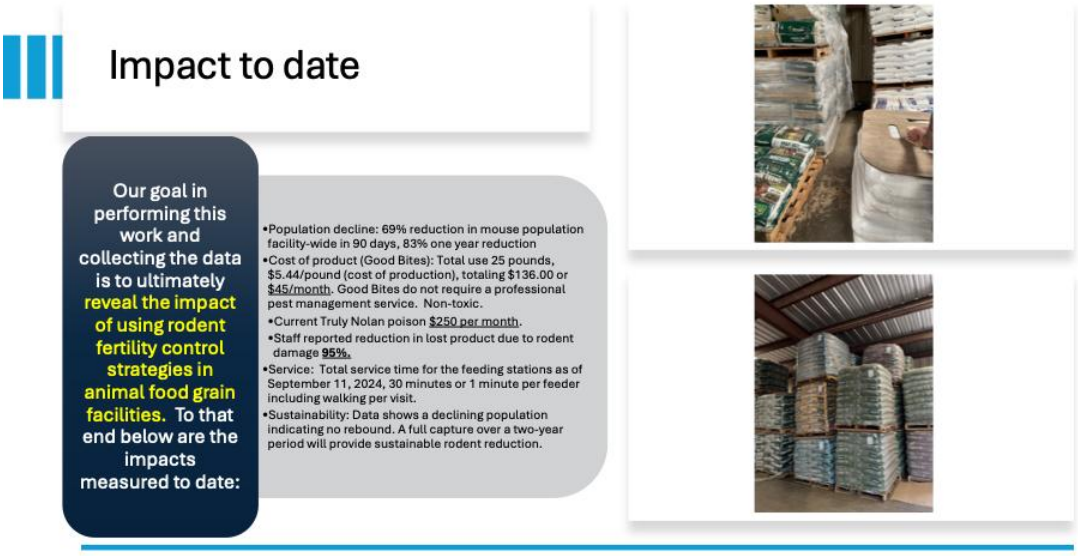
	Kanab, UT	68%	7	Semi-Open
Animal Rescues	Flagstaff, AZ	92%	15	Semi-Open
	Kanab, UT	99%	5	Closed

Website data sets are being loaded in the following formats for all projects: Completion 11/1/25

GRAIN PROCESSING – Olsen's Grain & Mill

- The problem we were asked to address
  - Rodent damage to warehoused feed and grain
  - Rodent damage to milling equipment
  - Reduce Pest Management costs
- Proposed solution
  - Identify problem areas, begin feeding Good Bites, move rodents away from warehousing and assist in mill exclusion and movement
- Execution of the plan
  - Map rodent movements, test feeding stations for best practices
  - Measure feed and grain losses
- Results
  - **95% reduction** in finished product losses

- **Data Collection Detail**
  - Field cameras in use
- Filtered to exclude initial pellet deployment and locate consumption by location
- Feeding stations, EVO Express with Discovery Box inserts, EVO inserts, Good Box
- Initial deployment May 2024
  - Total 1 year population reduction 83%
- Service transferred to Olsen's staff June 1, 2025



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Other publications on the active ingredient in Good Bites:

TwHF, 900 publications available on PubMed database.

BIOLOGY LETTERS

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Research

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THE ROYAL SOCIETY PUBLISHING

Pathogen biology

Urban rat exposure to anticoagulant rodenticides and zoonotic infection risk

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Anticoagulant rodenticides (ARs) deployed to control rodent pest populations can increase the risk of pathogen infection for some wildlife. However, it is unknown whether ARs also increase infection risk for target rodents, which are common hosts for zoonotic (animal-to-human transmitted) pathogens. In this study, we tested whether rats exposed to ARs were more likely to be infected with zoonotic pathogens, specifically *Leptospira* spp. or *Escherichia coli*, after controlling for known predictors of infection (i.e. sex, age, body condition). We collected biological samples from 99 rats trapped in Chicago alleys and tested these for *Leptospira* infection, *E. coli* shedding and AR exposure. We found that rats that had been exposed to ARs and survived until the time of trapping, as well as older rats, were significantly more likely to be infected with *Leptospira* spp. than other rats. We found no significant association between *E. coli* shedding and any predictors. Our results show that human actions to manage rats can affect rat disease ecology and public health risks in unintended ways, and more broadly, contribute to a growing awareness of bidirectional relationships between humans and natural systems in cities.

1. Introduction

Anticoagulant rodenticides (ARs) are one of the most common types of substance used to control rodent pest populations; however, little is known about potential unintended, *sublethal* AR effects on rodents. In other species, AR exposure has been associated with numerous sublethal effects (in addition to acute toxicity). For example, sublethal AR exposure can increase infection risk in urban predators (e.g. bobcats, *Lynx rufus*; mountain lions, *Puma concolor*; coyotes, *Canis latrans*; [1–3]) and has been linked to higher parasite and pathogen burdens in birds (e.g. great bustards, *Otis tarda*; [4]). Wildlife exposed to ARs may be more susceptible to infection because ARs have been shown to disrupt immune function [5]. Like the species above, rodents might also experience greater infection owing to AR exposure; in turn, this is relevant to human health as rodents are common hosts for zoonotic pathogens [6–8], especially in human-dominated areas [9]. ARs do not kill immediately; first-generation ARs require multiple feedings to provide a lethal dose, and second-generation ARs—more potent compounds that can kill after a single dose—typically lead to death in 5–10 days [10]. If infection risk is heightened during the period between AR exposure and death, widespread AR use might increase population transmission of pathogens among rodents. Additionally, this could pose a risk of zoonotic pathogen transmission.

Understanding any unintended effects of rodent control on rodent disease dynamics is important because commensal rats carry dozens of zoonotic pathogens [11,12], come in close proximity to people [13], and have a near-global distribution [14]. Brown rats (*Rattus norvegicus*) and black rats (*R. rattus*) can

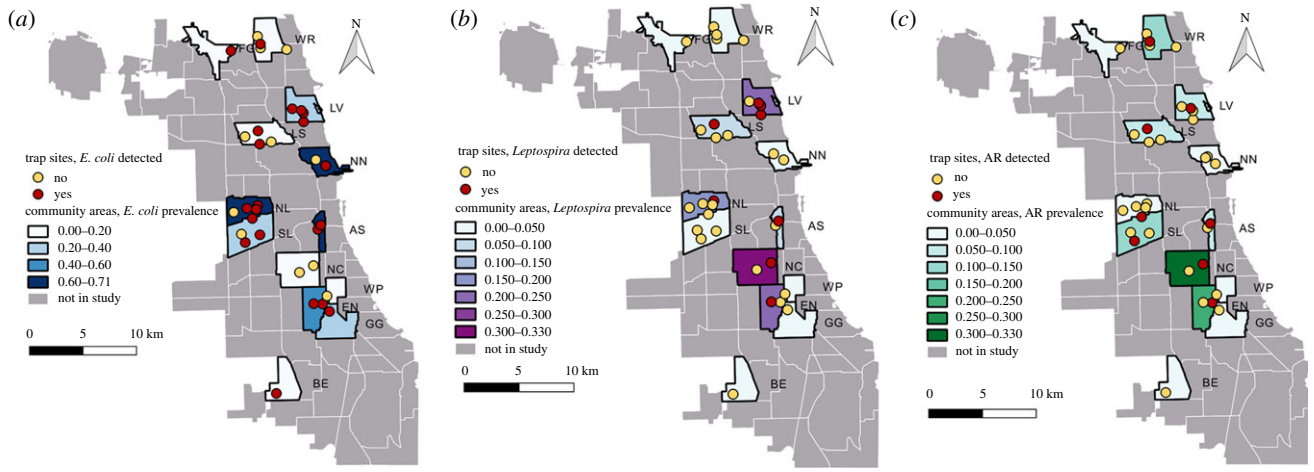

Figure 1. Maps of study community areas (polygons) and trap sites (circles) in Chicago. Colours show the prevalence (shading) or the presence (darker circles) of rats with (*a*) *E. coli* shedding, (*b*) *Leptospira* spp. infection, and (*c*) anticoagulant rodenticide (AR) exposure. Abbreviations correspond to table 1.

carry several environmentally transmitted pathogens that cause human disease (e.g. *Leptospira interrogans*, pathogenic *Escherichia coli*; [15]). Leptospirosis in particular poses a large public health burden, causing an estimated 434 000–1 750 000 cases and 23 800–95 900 deaths in humans annually [16]. Among major cities in the USA, *Leptospira* seroprevalence in rats ranges from 44.1 to 65.3% [17]. Environmental features and management practices can modulate *Leptospira* prevalence. For example, in Chicago, IL, rats trapped in high-income areas with more standing water complaints were more likely to be infected with *Leptospira* spp. [18], while in Vancouver, Canada, rodent control via rat trapping was associated with higher *Leptospira* prevalence [19]. Importantly, low-income urban residents can be disproportionately exposed to rat-associated zoonoses [20] and lower-income countries are often reliant on ARs for rodent control [21]. It is thus crucial to understand how other widespread management practices such as use of ARs could also influence infection dynamics in rats.

In this study, we tested if rats exposed to ARs were more likely to be infected with zoonotic pathogens, specifically *Leptospira* spp. or *E. coli*, after controlling for known physiological predictors of infection. We focused on these pathogens because they are zoonotic, transmitted through the environment, and present in our study population [18]. Based on previous work in urban carnivores, we predicted the probability of *Leptospira* spp. infection and *E. coli* shedding would be higher for rats with detectable concentrations of common ARs in liver tissue relative to other rats. We also predicted the probability of *Leptospira* spp. infection and *E. coli* shedding would be higher for rats that were female, older, and in poorer body condition because these biological factors are known predictors of infection [18,22–24]. Our results will help design best practices for rodent management to protect public health and advance our understanding of how pest management affects urban wildlife ecology.

2. Methods

As part of a previous study [18], 254 rats were trapped in 13 community areas in Chicago, a city with numerous rat complaints (figure 1). Trapped rats were measured, examined for injuries, weighed, and sexed. Rats were considered to be brown rats based on ear and tail morphology, but this assumption was not verified with genetic analyses. A subset of 202 rats were necropsied and screened for environmentally-transmitted bacterial pathogens [18]. Rat kidney tissue was tested for *Leptospira* spp. using polymerase chain reaction (PCR) and rat colon contents (i.e. faeces) were tested for *E. coli* using aerobic culture [18] at Wyoming State Veterinary Laboratory. From these rats, we selected 99 (table 1) to be screened for seven commonly used ARs (first-generation: chlorophacinone, coumachlor, diphacinone, warfarin; second-generation: brodifacoum, bromadiolone, difethialone). Rats were chosen for screening such that sample sizes would be roughly balanced by capture location, sex, age and infection status. Liver screening was performed by the Animal Disease Diagnostic Laboratory at Purdue University (West Lafayette, IN) using high performance liquid chromatography. Method detection limits (lowest concentration that can be confidently identified) for each AR in liver tissue were as follows: chlorophacinone and diphacinone: 0.25 ppm; coumachlor and warfarin: 0.5 ppm; brodifacoum, bromadiolone and difethialone: 1.00 ppm. Animal use was deemed exempt from Lincoln Park Zoological Society IACUC approval because rat samples were procured through pest management professionals (protocol number 2019–005).

We used generalized linear mixed models (GLMMs; binomial distribution, logit link) to test whether infection status varied by rodenticide exposure status (binary; we considered a rat exposed to poison if at least one AR was detected in the liver) as well as other biological predictors previously found to influence rat infection status. We constructed two GLMMs, one with a response variable of *Leptospira* infection status (positive or negative) and the other with a response variable of *E. coli* shedding status (positive or negative). Explanatory variables for each model included AR exposure status, sex, age class and body condition. We estimated rat age in days based on their mass using growth curve equations, following the methods of [25], and binned rats as younger (30–65 days) or older (greater than 65 days; electronic supplementary material, dataset). We quantified body condition using the scaled mass index [26] using tip-to-tip length (i.e. tip of nose to tip of tail) because it was most highly correlated with mass (see the electronic supplementary material for more detail). While injuries have also been found to be associated with infection [15], we did not include this as a variable because we observed only a few, mild wounds in the study population. Given the low sample size, only main effects of the explanatory variables were considered. We also included capture location (i.e. community area) as a random effect to account for non-independence among samples from the same neighbourhood. Analyses were performed using the glmmTMB package [27] in the R statistical environment v. 4.0.3 [28].

Table 1. Sex, age class and anticoagulant rodenticide poisoning status of rats, separated by trapping location (community area).

	sex		age class		poisoning status	
community area	F	M	younger (30–65 days)	older (>65 days)	AR detected	AR not detected
Armour Square (AS)	5	10	14	1	1	14
Beverly (BE)	1	0	1	0	0	1
Edge Water (ED)	1	1	1	1	1	1
Englewood (EN)	0	4	3	1	1	3
Forest Glen (FG)	1	0	1	0	0	1
Greater Grand Crossing (GG)	2	2	2	2	0	4
Lake View (LV)	14	6	16	4	2	18
Logan Square (LS)	11	5	14	2	1	15
Near North Side (NN)	5	2	7	0	0	7
New City (NC)	2	1	1	2	1	2
North Lawndale (NL)	4	1	4	1	0	5
South Lawndale (SL)	11	2	8	5	2	11
Washington Park (WP)	0	1	1	0	0	1
West Ridge (WR)	7	0	6	1	1	6

3. Results and discussion

We analysed infection status as a function of AR exposure, sex and age class for 99 rats that were trapped in 14 community areas (table 1). Ten liver samples were positive for AR residues (6 females, 4 males; 2 older, 8 younger). Specifically, seven were positive for second-generation ARs (brodifacoum: $n = 3$, bromadiolone: $n = 3$, difethialone: $n = 1$) and three were positive for first-generation ARs (diphacinone: $n = 3$). *Leptospira* prevalence was higher for AR-exposed rats (30%, 3/10) than for unexposed rats (7.9%, 7/89), and *E. coli* prevalence was higher for AR-exposed rats (50%, 5/10) than for unexposed rats (42%, 37/89; figure 2).

GLMMs indicated that AR exposure status was a significant predictor of *Leptospira* infection status (odds ratio = 7.02, 95% CI = 1.10–45.0, $p = 0.04$), as was age class (figure 2 and electronic supplementary material, table S1). Older rats (greater than 65 days) were significantly more likely to be infected with *Leptospira* spp. than younger rats (30–65 days; odds ratio = 5.88, 95% CI = 1.20–28.9, $p = 0.03$). Neither sex nor SMI was a significant predictor in the model. The marginal R^2 (i.e. proportion of variance explained by fixed effects) for the *Leptospira* infection model was 0.21, while the conditional R^2 (i.e. proportion of variance explained by both fixed and random effects) was 0.33. No explanatory variables were significant predictors of *E. coli* shedding status. The marginal R^2 for this model was 0.01, while the conditional R^2 was 0.12.

We found that rats exposed to ARs that survived until the time of trapping were significantly more likely to be infected with *Leptospira* spp. than other rats. Though it is known that ARs can promote infection risk in non-target wildlife, our results demonstrate increased zoonotic infection risk in target rodents. This result is significant for public health and urban ecology because commensal rodents are abundant reservoirs of zoonotic pathogens in cities. More generally, this relationship between rodenticide exposure and infection risk demonstrates an unintended effect of wildlife management on a target species that can feed back to human health.

AR-exposed rats may be more susceptible to infection in the period between exposure and death because of immunomodulatory effects of ARs. Rats exposed to warfarin for 30 days exhibit increased lymphocytes, basophils and monocytes [29,30], suggesting immune dysfunction. In carnivores, AR exposure has been associated with immune dysfunction consistent with cytokine-mediated inflammatory processes, including the suppression of neutrophils [31]. These phenotypic changes might interfere with rodents' ability to mount an effective defence when exposed to infectious leptospires in the environment. Although we quantified rat exposure to rat poison as a binary status, the detection limit in our study exceeded concentrations deemed indicative of acute AR poisoning in other species (200 ng g^{-1} or 0.2 ppm; [4]), suggesting they were high enough to interfere with physiological processes. If rats are more likely to become infected with *Leptospira* spp. after consuming ARs, infection would have to occur before the poison kills the rat (approx. 1 week). Experimental work has demonstrated successful *Leptospira* infection 7 days post-infection [32,33], yet further work is needed to examine *Leptospira* spp. infection dynamics at a shorter timescale and determine how long rats can survive following AR exposure.

Alternatively, infected rats might be more likely to consume poisoned bait. For instance, infected rats could be more attracted to bait stations if they have less energy to actively forage for other food. However, rats are considered asymptomatic, chronic carriers of *Leptospira* ([17]; though see [34]), suggesting it is unlikely that infected rats are more likely to consume AR bait. Future work could also investigate behavioural and physiological changes in poisoned and infected rats to clarify causal mechanisms.

Interestingly, the only other study, to our knowledge, to examine AR poisoning and infection risk in target rodents found that common voles (*Microtus arvalis*) infected with

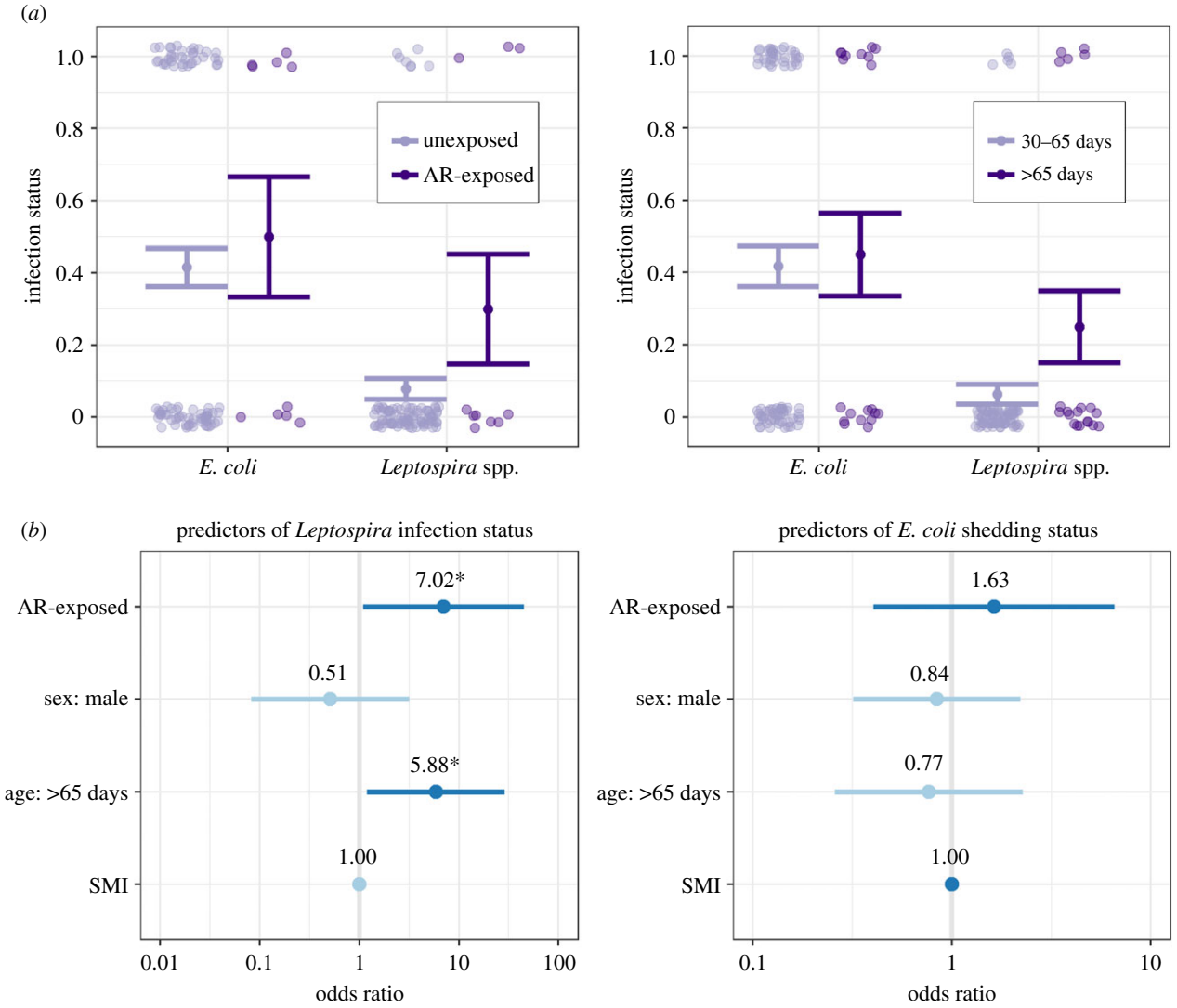

Figure 2. (*a*) Pale-shaded points display binary infection status and solid points and lines represent means and standard errors of infection prevalence. (*b*) Points and lines represent odds ratios and 95% confidence intervals for predictors of infection status from GLMMs. Darker blue lines indicate odds ratios greater than 1, while lighter blue lines indicate odds ratios less than 1. 95% confidence intervals that cross the vertical line at 1 indicate that a predictor is not significant. Asterisks indicate $p < 0.05$.

Francisella tularensis had lower concentrations of the AR chlorophacinone relative to uninfected voles [35]. These results likely differ from ours because all poisoned voles were found dead rather than trapped and *F. tularensis* infection is fatal in voles. However, these differences highlight the need to understand interactions among ARs, pathogens, and hosts with different ecologies. Future epidemiological surveys and experimental work could help identify which types of pathogenic infections are affected by AR exposure.

We also found that older rats were significantly more likely to be infected with *Leptospira* spp. than younger rats. This aligns with previous research and is likely attributable to a greater chance of exposure and infection over time [22]. We might not have found significant associations with other biological factors because of small sample size, which could also explain the relatively large confidence intervals around the odds ratios (figure 2). Contrary to our predictions, we found no association between AR exposure and *E. coli* infection. We may not have detected an increased risk of *E. coli* infection in poisoned rats because our methods could only detect active shedding of *E. coli* in faeces, rather than true infection. Although this is informative for public health, rats could have been infected with *E. coli* but not actively shedding, which might have confounded our results. In addition, while we accounted for non-independence among rats within the same community area using a random effect (under the assumption that community areas are statistically independent from one another, supported by the small home ranges of rats (less than 200 m) [36]), our results may have been confounded by spatial autocorrelation.

Our results add to a growing literature showing environmental hazards of managing rats using ARs, and highlight potential unintended and unpredicted effects of AR exposure on the ecology of rat-associated pathogens of public health importance. Apart from disease ecology, urban rats have exhibited genetic resistance to ARs for decades. Resistant rats carry genetic mutations in the *Vkorc1* gene that interfere with anticoagulant effects on blood clotting [37], rendering the rats less susceptible to anticoagulants. Rats have exhibited genetic resistance even as new generations of ARs are developed [38,39], demonstrating how lethal management can have evolutionary consequences for zoonotic hosts [40]. AR resistance may have important consequences for leptospiral shedding if ARs act as modulators of immune and inflammatory responses and resistant rats are less likely to die

following AR exposure. Instead of relying on ARs, integrated pest management might offer a more sustainable approach by improving urban sanitation and rodent exclusion [41]. Such an approach would align with One Health principles and prevent mortality of urban carnivores, which provide ecosystem services such as rodent population control. More broadly, our results contribute to a growing awareness of bidirectional relationships between humans and natural systems in cities: in our case, that human actions to manage rats can affect rat disease ecology and public health risks in unintended ways.

Ethics. Animal use was deemed exempt from Lincoln Park Zoological Society IACUC approval because rat samples were procured through pest management professionals (protocol no. 2019-005).

Data accessibility. The dataset used in our analysis is available on Zenodo at https://zenodo.org/badge/latestdoi/387547164 [42].

Authors' contributions. M.H.M. led the conceptualization of the study and the collection of biological samples. C.A.S. contributed to project design and led the statistical analysis. M.H.M. and C.A.S. wrote and edited the manuscript, approved the final version of the manuscript agree to be held accountable for the content therein.

Competing interests. The authors declare that they have no competing interests.

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GROWTH PERFORMANCE OF NESTLING BARN OWLS, *Tyto Alba javanica* IN RAT BAITING AREA IN MALAYSIA

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ABSTRACT

The growth of nestling barn owls, *Tyto Alba javanica* in immature oil palm in Malaysia was investigated under rat baiting with three different rodenticides. Four treatment plots were established with three plots baited each with warfarin, brodifacoum and a protozoan based biorodenticide, *Sarcocystis singaporensis* plus a fourth non-baited control plot. Three rat baiting campaign were carried out during the study, the first rat baiting campaign was conducted in October 2008, the second was in March 2009 (except for biorodenticide baiting was conducted a month earlier), and the last third baiting campaign in October 2009. The baiting campaigns coincided with the breeding season of barn owl. Nestlings body measurements namely: body mass, culmen length, tarsus length, wing length and tail length were taken after the third baiting campaign, from September 2009 to January 2010. Measurements were recorded every three days from hatching up until 49 days old, i.e., several days before fledging. Nestlings in control plot showed superior for all parameter taken compared to rodenticides treated plots. Body mass of nestlings in control plot were heavier by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The culmen and tarsus length of nestling barn owls reached the adult size during the growth period; while culmen length in control plot was longer by 3.07%, 5.28%, and 1.41% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The tarsus length of nestlings in control plot was also longer by 2.40%, 3.08% and 3.36% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. In contrast with culmen and tarsus length, wing and tail length still grew until day 49 i.e., several days before fledging. The wing and tail length in control plot was shorter by 15.77% and 13.73% compared to adult size. Teratogenic sign was shown by one nestling in brodifacoum treated plot, where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Wing and tail length in brodifacoum treated plot was shorter by 15.26% and 18.24%, respectively compared to control plot.

Keywords: *Tyto Alba javanica,* growth performance, nestling, warfarin, brodifacoum, *Sarcocystis singaporensi,* teratogenic sign.

INTRODUCTION

The barn owl, *Tyto Alba* is widely distributed around the world, occurring in all continents in a wide range of habitats, except Antarctica and the smaller Pacific Islands (Smith and Cole, 1989). There are 36 subspecies including *T. Alba javanica*, found in Peninsular Malaysia and also in Sumatra and Java Islands of Indonesia (Taylor, 1994). Due to its wide distribution, the barn owl has been extensively studied (Bunn *et al*., 1982; Newton *et al.*, 1991; Eason *et al.*, 2002). In Malaysia, *T. Alba javanica* is commonly propagated to control rats in plantation (Lenton, 1984; Smal, 1989; Hafidzi *et al*., 1999). Since 1970's, its distribution had rapidly expanded and the status changed from rare in the late 1960s to common (Duckett, 1984; Duckett and Karrupiah, 1990). The increase of *T. Alba javanica* population in the Peninsular Malaysia was associated with the phenomenal increase in oil palm acreage. This brought about rat outbreaks which translate into readily available food source. Previously, when rat damage reaches threshold levels planters usually resort to warfarin, the first generation anticoagulant rodenticide to deal with the infestation (Duckett, 1984). However prolonged exposure to warfarin, triggers resistance to the latter, prompting more planters to switch to brodifacoum, a second generation anticoagulant rodenticide introduced in the early 1980s. The use of brodifacoum has caused marked decline in rat populations in oil palm (Duckett, 1984; Wood and Fee, 2003). The downside of bodifacoum is it was proven toxic to *T. Alba* from field observations and laboratory studies. The potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to barn owl as non-target animal by direct consumption or secondary poisoning from build-up of rodenticide residues (Newton *et al*., 1990; Shore *et al*., 1999; Dowding *et al*., 2010). Based on that fact, several workers try to find safer rodenticide to replace brodifacoum with an equally effective but have less impact on non target organism. One such alternative is the biorodenticide based on *Sarcocystis singaporensis*, a protozoan pathogen that has been proven effective against rat in the rice field but does not causes harm to humans other animals, such as fish, other mammals and birds apart form rats of the genus *Bandicota* and *Rattus* (Jakel *et al*., 1996; Jakel *et al*., 2006). Although there have been many studies on the effects of chemical rodenticides on adult barn owl, very little information exist about the effects of chemical rodenticides on growth and development of nestlings. Therefore the objective of this study is to evaluate the effects of regular rat baiting on the growth performance of the nestlings barn owl, *T. Alba javanica* in an immature oil palm area in Malaysia.

MATERIALS AND METHODS

Location and period of study

The study was conducted in immature oil palm at FELCRA oil palm plantation scheme in Seberang Perak ($4^0$02´N, $100^0$53´E), Perak, Malaysia from September 2008 to January 2010. The study sites constitute part of the replanting area started in mid 2007.

Rat baiting and treatment

Twenty two artificial nest boxes, made of wood, were set up in April to June 2008 in the immature palm by Felcra management. Four treatment plots were established. The area for each plot is no less than 100 ha. Three plots were baited with warfarin, brodifacoum and the biorodenticide *Sarcocystis singaporensis*, respectively. The fourth was left untreated and served as the rodenticide-free control plot. The average nest box density was 1 box for 25 ± 3.83 ha. The first baiting campaign for all three rodenticides was carried out on 20-25th October 2008. The second baiting campaign for warfarin and brodifacoum on 10-12th March 2009, while second baiting campaign for biorodenticide was carried out on 25-27th January 2009. Third baiting campaign was carried out on 28 September to 3rd October 2009 for all three rodenticides. The baits were placed at the base of the palm tree. In the first campaign, a single round of baiting was carried out while two baiting rounds were conducted in the second and the third baiting campaign.

Data collection

48 nestlings were selected for this study: 14 from control plot, 12 from warfarin treated plot, 9 from brodifacoum treated plot and 13 from biorodenticide treated plot. They were weighed at three day interval for up to 49 days i.e., several days before fledging. The day of hatching was designated as day zero (Janiga, 1992) for monitoring growth rate. Hatched siblings were individually marked using different colored ribbons tied to the leg for age determination during later stages of growth. All observations were carried out in the nests from 5 to 7 p.m during the breeding season, i.e., from September 2009 to January 2009.

Nestling growth metrics

For nestling growth metrics, five measurements were taken, namely: body mass, culmen length, tarsus length, wing length and tail length. Body mass was measured using Apex A-5001, a portable digital weighing scale (accurate to 1 g); culmen length was measured using Mitutoyo Caliper, from the tip of the upper mandible to the base of the culmen, to the nearest 1 mm. Tarsus length was measured from the top of the tarsus (just below the tibio-tarsal joint) to the joint at the base of the middle toe, to the nearest 1 mm. Tail length was measured from the fold of skin between the central tail-feathers and the tip of the longest tail-feathers, to the nearest 1 cm. Wing length was measured from the bend of the folded wing to the tip of the longest primaries (Weick, 1980; Janiga, 1992).

Statistical analysis

Data of similar-aged nestlings from all the nest boxes were pooled to calculate the mean for different growth and to analyze the pattern of growth changes in the measured variables using Kruskal-Wallis test. Means are presented in ± SE. For hypothesis testing $P < 0.05$ was considered significant.

For the growth in nestlings, logistic growth curve was used (Starck and Ricklefs, 1998), by the given equation:

$$W = A/(1 + exp(- K(t – ti)))$$

Where W = the growth variable, A = asymptote, K = the growth rate constant, t = age of nestling, and ti = the inflection point of the growth curve. The logistic growth equations were fitted to the data using the nonlinear regression procedure of the SAS package version 9.1.

RESULTS

The body mass

Of the 48 nestling barn owls measured, only 32 were successfully measured up to day 49 while the rest died. Of the 32 nestlings, 12 were from the untreated control plot, eight from the warfarin treated plot, two from the brodifacoum treated plot and ten from the biorodenticide treated plot. Since only two nestlings in the brodifacoum treated plot survived during the measurements, the growth comparisons were made based on the age of these nestling with other nestlings in the other treatment plots.

From 226 measurements for nestlings from hatching up to fledging in the untreated control control plot (n = 12), 153 measurements in the warfarin treated plot (n = 8), 67 measurements in the brodifacoum treated plot (n = 2) and 174 measurements in the biorodenticide treated plot (n = 10), nestlings grew from 18.00 ± 0.37 g (n =6), 18.20 ± 0.58 (n = 5), 18.25 ± 0.49 (n = 4), 18.20 ± 0.37 (n = 5) at hatching, to a peak mass of 631.60 ± 12.96 g (day 46, n = 5), 597.30 ± 5.24 g (day 45, n = 3), 565.00 ± 16.05 g (day 43, n = 2) and 604.67 ± 16.00 g (day 45, n = 4) in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-1). Nestlings in control plot have a heavier body mass compared to the average adult body mass by 4.16% (545.90 ± 9.04, n = 10), and lighter by 3.71%, 7.86%, 2.55% for nestlings from warfarin, brodifacoum and biorodenticide treated plots respectively compared to adult body mass. Nestlings in control plot were heavier in body mass by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. Kruskal-Wallis test showed that there was no significant difference for body mass of nestlings in all treatments irrespective of days from day 1 to day 49 for the nestlings.

Table-1. Body mass (mean ± SE) of nestling barn owls in rodenticide treated areas.

Age (days)	**Body mass** (g)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	18.00 ± 0.37 ns	18.20 ± 0.58	8.25 ± 0.48	18.20 ± 0.37
7	73.67 ± 4.58 ns	84.00 ± 2.94	9.00 ± 3.52	81.00 ± 1.15
14	223.67 ± 11.99 ns	216.00 ± 13.50	218.33 ± 7.62	215.00 ± 11.53
22	379.80 ± 10.26 ns	359.33 ± 16.72	355.50 ± 21.56	364.75 ± 15.46
28	449.40 ± 10.16 ns	436.00 ± 9.30	428.50 ± 20.56	434.75 ± 15.17
34	546.80 ± 13.46 ns	536.67 ± 8.38	525.50 ± 24.57	546.00 ± 14.71
43	615.00 ± 7.75 ns	588.00 ± 16.04	565.00 ± 16.03	590.50 ± 10.60
49	568.60 ± 15.58 ns	525.67 ± 18.24	503.00 ± 13.03	532.00 ± 15.35

Generally, absolute rates of growth varied throughout the nestling period (Figure-1) and the most rapid rates occurred between days 10 - 35. Although the growth constant did not vary and ranging from 0.146 to 0.150 g per day, but the asymptote calculated using SAS Version 9.1 indicates the highest asymptote was found in nestlings from the control plot, followed by the biorodenticide, warfarin, and brodifacoum treated plots, respectively. The highest increase in body mass differ from one treatment to another, whereby the control plot was recorded on day 19.20 ± 0.26, in warfarin day 18.87 ± 0.31, in brodifacoum on day 17.99 ± 0.40, in biorodenticide on day 18.75 ± 0 (Table-2/ Figure-1).

Table-2. Body mass (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas.

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	613.5 ± 5.98	0.150 ± 0.004	19.20 ± 0.26
B	585.8 ± 6.62	0.146 ± 0.005	18.87 ± 0.31
C	554.9 ± 8.72	0.145 ± 0.006	17.99 ± 0.40
D	586.1 ± 6.60	0.147 ± 0.005	18.75 ± 0.30

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

The culmen length

The culmen length of nestling barn owls grew from 7.50 ± 0.10 mm (n = 6), 7.60 ± 0.10 mm (n = 5), 7.50 ± 0.13 mm (n = 4), 7.60 ± 0.10 mm (n = 5) in control warfarin, brodifacoum, and biorodenticide treated plots respectively at hatching to 22.70 ± 0.20 mm (n = 65, 22.00 ± 0.17 mm (n = 3), 21.50 ± 1.00 mm (n = 2) and 22.38 ± 0.37 mm (n = 4) for corresponding treatment plots at day 49. The culmen grew full length to reach the adult size (22.80 ± 0.17, n = 10) during the growth period when measurement were taken at day 49. Culmen length in the control plot was longer by 3.18%, 5.58%, and 1.43% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. Kruskal-Wallis test showed there was no significant difference for culmen length of nestlings in all treatments irrrespective of days, from day 1 to day 49 for the nestlings.

Patterns in growth of culmen were similar to that of body mass that exhibits a sigmoidal curve, where the culment grew slowly in the first 10 days and then rapidly to day 35, slowing down again to day 49. The asymptote of culmen length tend to be similar for nestlings in all treatments ranging from 22.926 ± 0.23 mm, 22.160 ± 0.20 mm, 22.040 ± 0.37 mm and 22.878 ± 0.23 mm for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from 0.110 ± 0.004 mm, 0.105 ± 0.004 mm, 0.102 ± 0.005 mm and 0.112 ± 0.005 mm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in culmen length was also quite similar from one treatment to another, ranging from day 10.768 ± 0.31, day 10.171 ± 0.28, day 10.125 ± 0.46, and day 10.500 ± 0.39 in nestlings from control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-4/ Figure-2).

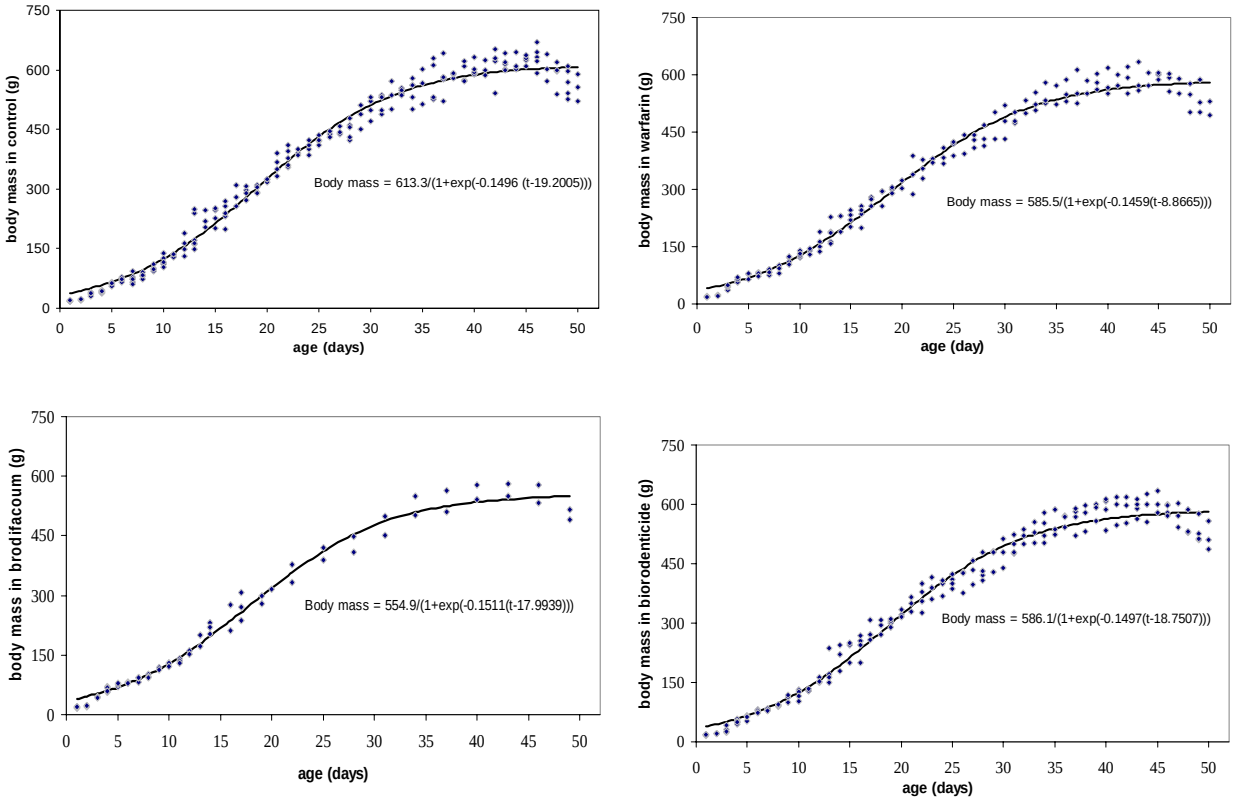

Figure-1. Logistic growth curve of the changes in body weight of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

Table 3. Culmen length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Culmen length** (mm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	7.50 ± 0.10 ns	7.60 ± 0.10	7.50 ± 0.13	7.60 ± 0.10
7	8.92 ± 0.15 ns	9.00 ± 0.16	9.00 ± 0.15	9.00 ± 0.18
14	13.00 ± 0.29 ns	12.83 ± 0.17	12.67 ± 0.17	13.17 ± 0.17
22	18.30 ± 0.20 ns	18.00 ± 0.29	18.00 ± 0.50	18.38 ± 0.23
28	20.60 ± 0.30 ns	19.33 ± 0.33	19.50 ± 0.50	20.75 ± 0.14
34	21.00 ± 0.45 ns	20.33 ± 0.17	20.25 ± 0.25	21.25 ± 0.25
43	21.90 ± 0.10 ns	21.33 ± 0.17	20.75 ± 0.75	22.00 ± 0.20
49	22.70 ± 0.20 ns	22.00 ± 0.17	21.50 ± 1.00	22.38 ± 0.37

Table-4. Culmen length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	22.926 ± 0.23	0.110 ± 0.004	10.768 ± 0.31
B	22.160 ± 0.20	0.105 ± 0.004	10.171 ± 0.28
C	22.040 ± 0.37	0.102 ± 0.005	10.125 ± 0.46
D	22.878 ± 0.23	0.112 ± 0.005	10.500 ± 0.39

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

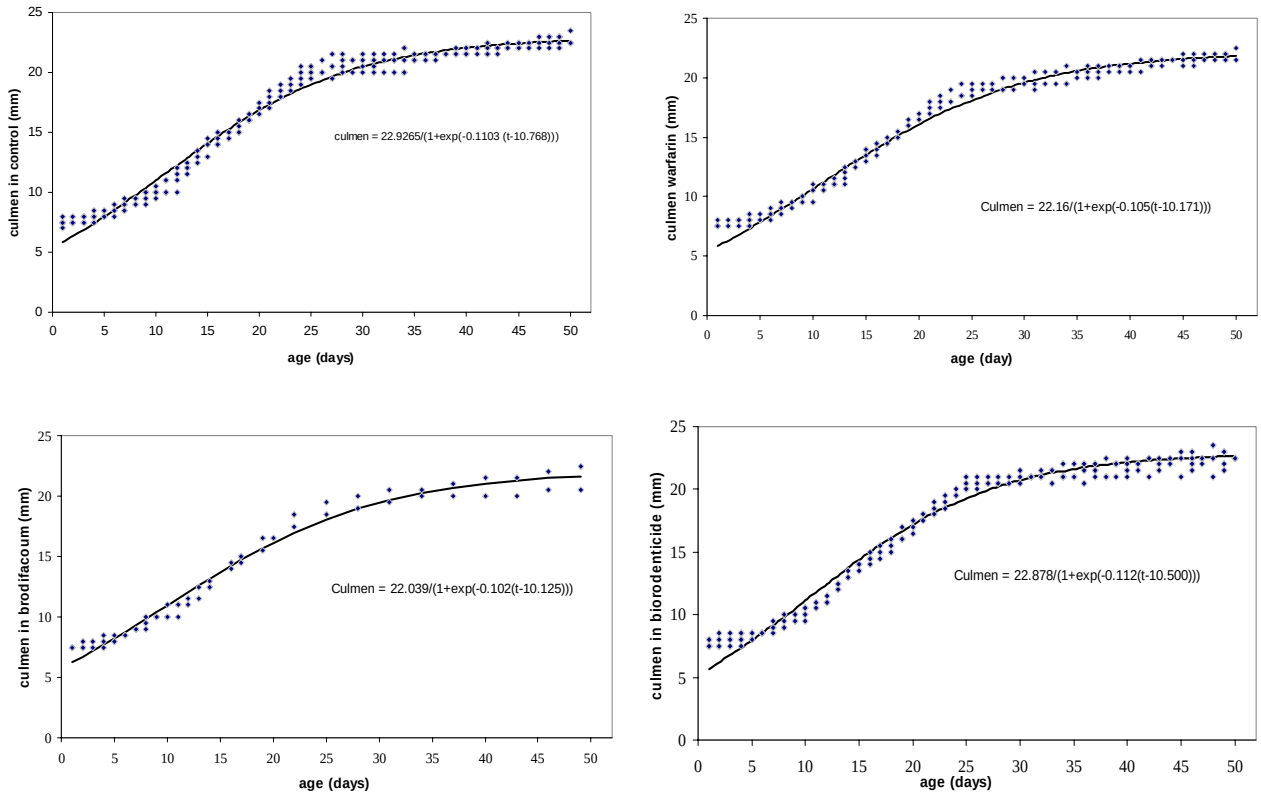

Figure-2. Logistic growth curve of the changes in culmen length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

The tarsus length

The tarsus length of nestlings grew from 16.33 ± 0.33 mm (n = 6), 16.70 ± 0.30 mm (n = 5), 16.00 ± 0.41 mm (n = 4), 16.20 ± 0.37 mm (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to 86.40 ± 0.81 mm (n = 5), 84.33 ± 1.20 mm (n = 3), 83.75 ± 1.00 mm (n = 2) and 83.50 ± 1.32 mm (n = 4) for the corresponding treatment plots in day 49 (Table-5). Same like culmen, tarsus length of chicks grew and reached adult size (86.45 ± 0.20, n= 10) during the growth period. Tarsus length in the control plot was longer by 2.45%, 3.16% and 3.47% compared to warfarin, brodifacoum and biorodenticide treated plots, respectively. The tarsus length in control plot was longer than rodenticides treated plot. However, there was no significant different irrespective of days when tested with Kruskal-Wallis analysis of variance.

Table-5. Tarsus length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Tarsus length** (mm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	16.33 ± 0.33 ns	16.70 ± 0.30	16.00 ± 0.41	16.20 ± 0.37
7	23.00 ± 0.37 ns	22.60 ± 0.24	22.33 ± 0.67	22.50 ± 0.31
14	46.00 ± 2.08 ns	46.33 ± 0.88	45.33 ± 1.45	44.33 ± 1.45
22	67.00 ± 1.58 ns	67.67 ± 0.33	69.00 ± 1.00	69.00 ± 0.91
28	78.40 ± 1.21 ns	78.00 ± 0.58	78.00 ± 3.01	77.50 ± 1.19
34	83.00 ± 1.22 ns	81.67 ± 1.20	80.00 ± 2.51	80.25 ± 0.85
43	85.40 ± 1.21 ns	83.00 ± 1.00	83.00 ± 2.00	82.50 ± 1.04
49	86.40 ± 0.81 ns	84.33 ± 1.20	83.75 ± 2.76	83.50 ± 1.32

The tarsus length grew slowly in the first seven days and then rapidly to day 30, slowing down again to day 50. The tarsus length reached asymptote around 30 to 35 days after nestling hatched, ranging from 86.93 ± 0.43

mm, 84.44 ± 0.45 mm, 84.39 ± 1.03 mm and 83.66 ± 0.52 mm for control warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from 0.14 ± 0.0043 mm, 0.15 ± 0.003 mm, 0.15 ± 0.006 mm and 0.15 ± 0.004 mm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in tarsus length differs from day 13.34 ± 0.15, day 12.86 ± 0.16, day 13.03 ± 0.30, and day 12.90 ± 0.18 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-6/ Figure-3).

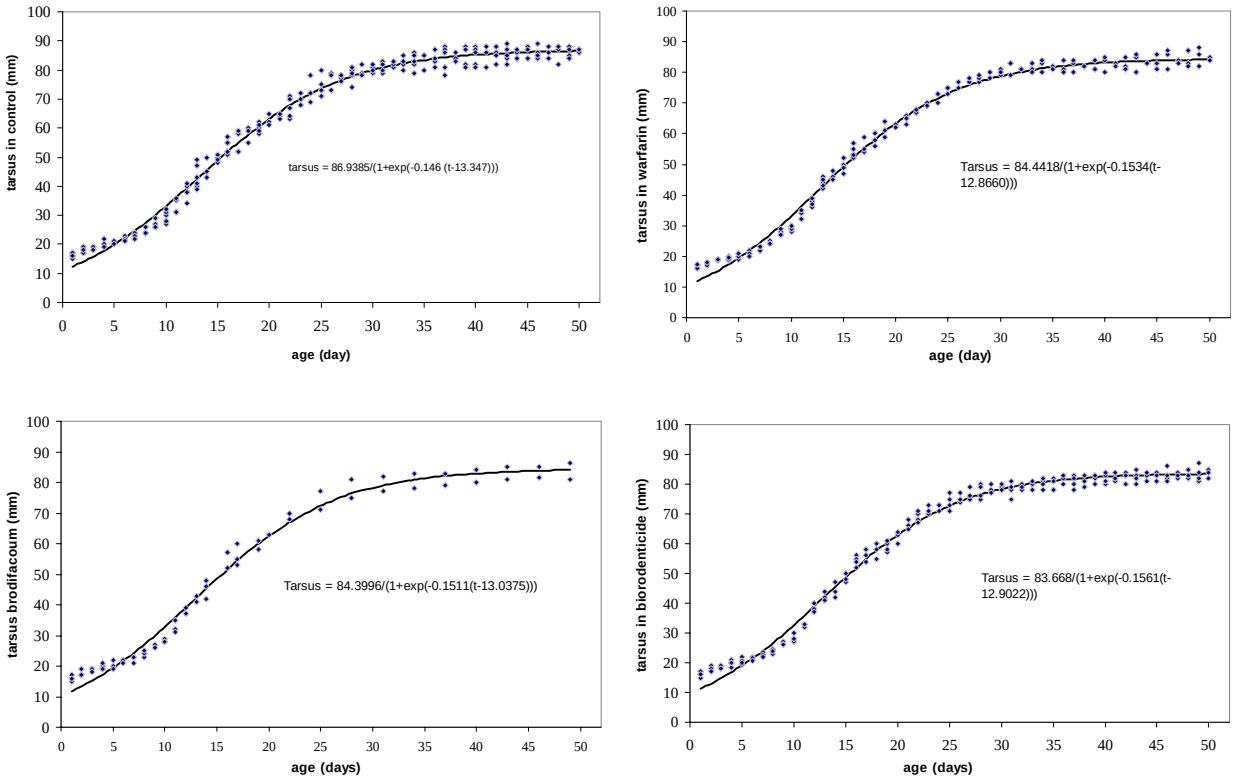

Figure-3. Logistic growth curve of the changes in tarsus length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Table-6. Tarsus length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	86.93 ± 0.43	0.14 ± 0.003	13.34 ± 0.15
B	84.44 ± 0.45	0.15 ± 0.003	12.86 ± 0.16
C	84.39 ± 1.03	0.15 ± 0.006	13.03 ± 0.30
D	83.66 ± 0.52	0.15 ± 0.004	12.90 ± 0.18

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

The wing length

The wing length of nestlings grew from 1.48 ± 0.03 cm (n = 6), 1.50 ± 0.04 cm (n = 5), 1.50 ± 0.04 cm (n = 4), 1.44 ± 0.05 cm (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to 26.02 ± 0.21 cm (n = 5), 25.93 ± 0.20 cm (n = 3), 22.05 ± 0.36 cm (n = 2) and 26.07 ± 0.23 cm (n = 4) for the corresponding treatment plots at day 49 (Table-7). Unlike tarsus and culmen that reached the adult size during the growth period, wing length still grew up to day 49, several days before fledging, and wing length was shorter by 15.77%, 16.06%, 28.62%, and 15.60% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size (30.89 ± 0.14, n=10). Wing length in brodifacoum treated plot was shorter by 15.26% compared to control plot.

Table-7. Wing length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	Wing Length (cm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	1.48 ± 0.03 ns	1.50 ± 0.05	1.50 ± 0.04	1.44 ± 0.05
7	3.30 ± 0.04 ns	3.24 ± 0.05	3.27 ± 0.03	3.20 ± 0.02
14	6.80 ± 0.12 ns	6.90 ± 0.03	6.90 ± 0.21	6.90 ± 0.17
22	14.96 ± 0.14 ns	14.87 ± 0.17	14.60 ± 1.20	14.80 ± 0.26
28	20.94 ± 0.26 ns	21.07 ± 0.18	19.30 ± 2.91	20.83 ± 0.17
34	24.08 ± 0.19 ns	23.77 ± 0.09	20.75 ± 3.97	23.97 ± 0.17
43	25.56 ± 0.19 ns	25.07 ± 0.17	21.55 ± 4.16	25.53 ± 0.15
49	26.02 ± 0.21 ns	25.93 ± 0.20	22.05 ± 4.36	26.07 ± 0.23

The asymptote reached by the wing length were 26.28 ± 0.11 cm, 25.86 ± 0.13 cm, 22.15 ± 0.23 cm and 26.24 ± 0.10 cm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. For the growth constant, ranging from 0.167 ± 0.003 cm, 0.169 ± 0.003 cm, 0.174 ± 0.005 cm and 0.165 ± 0.002 cm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in wing length was differ from one treatment to another, ranging from day 20.18 ± 0.11, day 19.99 ± 0.13, day 18.41 ± 0.24, and day 20.12 ± 0.10 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively.

Teratogenic signs showed by a nestling in brodifacoum treated plot, where up to 49 days old it had malformed primary feathers rendering it flightless (Figures 4 and 5). No nestlings in control, warfarin and biorodenticide treated plots shown teratogenic sign as showed by nestling in brodifacoum treated plot.

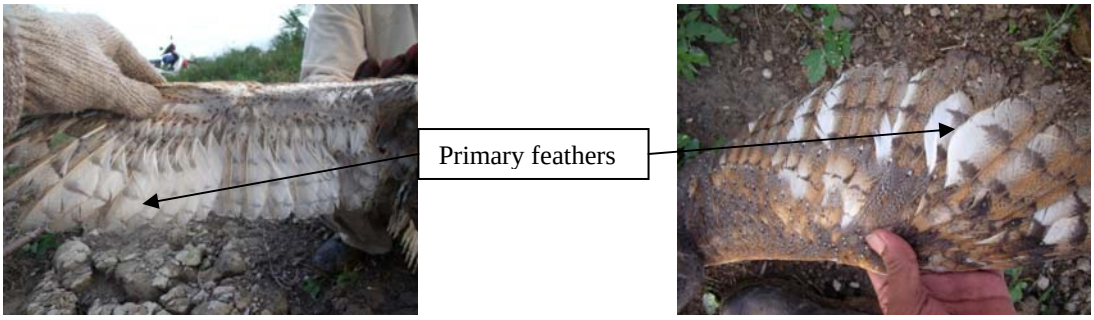

Figure-4. Normal nestling.

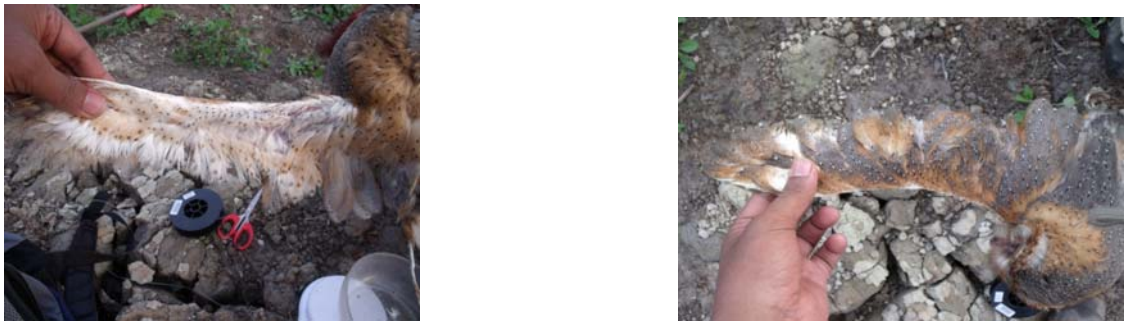

Figure-5. Teratogenic sign showed by nestling in brodifacoum treated plot that has no primary feathers.

The tail length

The tail length of nestlings grew from 0.00 ± 0.00 cm in all treatment at hatching to 11.62 ± 0.17 cm (n = 5), 11.47 ± 0.15 cm (n = 3), 9.50 ± 1.00 cm (n = 2) and 11.85 ± 0.21 cm (n = 4) for control, warfarin, brodifacoum and biorodenticide treated plots at day 49 (Table-9). Same like wing length, the tail length still grew up to day 49. Tail length was shorter 13.73%, 14.85%, 29.47%, 13.88% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size (13.47 ± 0.14, n = 10). The tail length of nestlings in brodifacoum treated plot also shorter by 18.24% compared to nestling in control plot.

Table-8. Wing length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	26.28 ± 0.11	0.167 ± 0.003	20.18 ± 0.11
B	25.86 ± 0.13	0.165 ± 0.003	19.99 ± 0.13
C	22.15 ± 0.23	0.174 ± 0.005	18.41 ± 0.24
D	26.24 ± 0.10	0.165 ± 0.002	20.12 ± 0.10

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

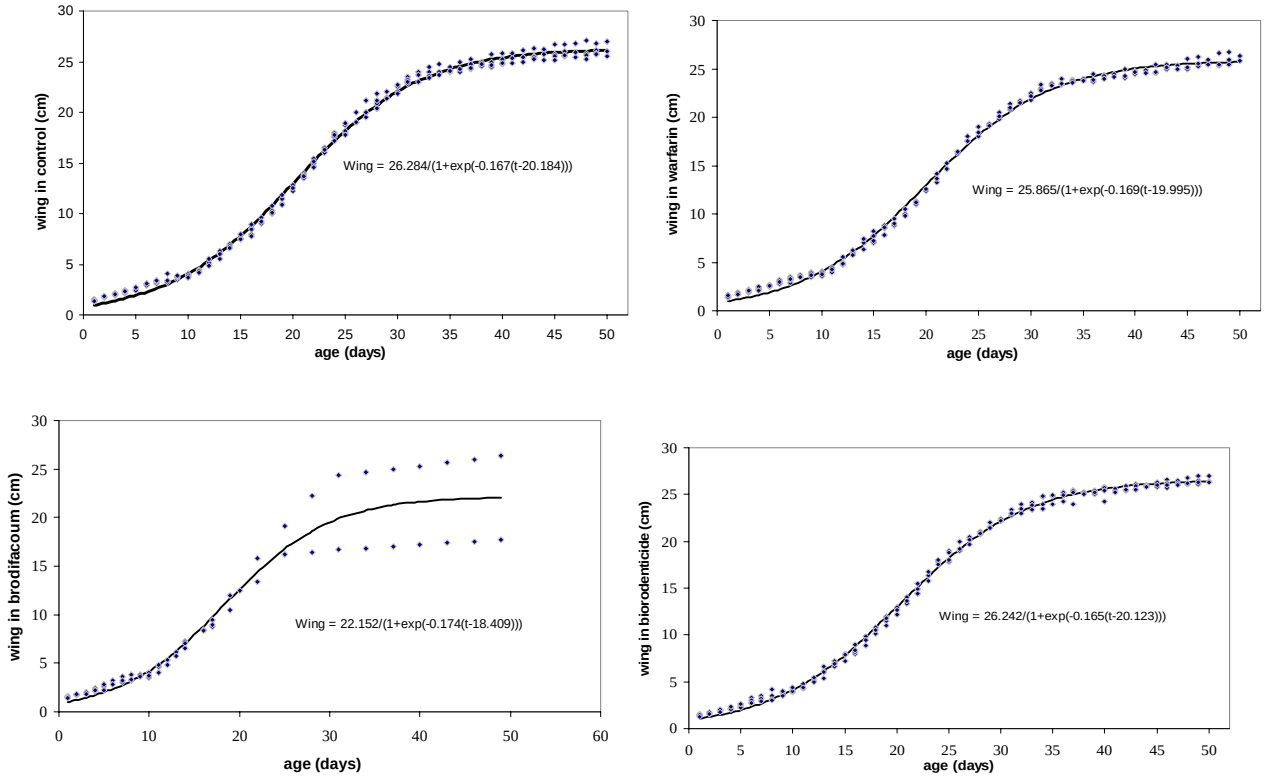

Figure-6. Logistic growth curve of the changes in wing length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Table-9. Tail length (mean ± SE) of nestling barn-owls in rodenticide treated areas.

Age (days)	**Tail length** (cm)			
	Control	**Warfarin**	**Brodifacoum**	**Biorodenticide**
1	0.00 ± 0.00 ns	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	0.35 ± 0.02 ns	0.46 ± 0.05	0.37 ± 0.05	0.43 ± 0.02
14	1.23 ± 0.09 ns	1.23 ± 0.03	1.27 ± 0.03	1.30 ± 0.06
22	4.78 ± 0.07 ns	4.83 ± 0.08	4.80 ± 0.10	4.80 ± 0.16
28	7.44 ± 0.10 ns	7.03 ± 0.14	7.50 ± 0.10	7.25 ± 0.09
34	9.68 ± 0.12 ns	9.37 ± 0.18	8.85 ± 0.65	9.43 ± 0.20
43	10.94 ± 0.12 ns	10.87 ± 0.15	9.20 ± 0.80	10.87 ± 0.13
49	11.62 ± 0.17 ns	11.47 ± 0.15	9.50 ± 1.00	11.60 ± 0.17

The nestling's tail length reached asymptote between 40 - 45 days with the constant growth rate ranging from 0.19 ± 0.004 g per day, 0.17 ± 0.004 g per day, 0.24 ± 0.005 g per day and 0.17 ± 0.004 g per day for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in tail length differs from one treatment to another, ranging from day 24.15 ± 0.16, day 25.03 ± 0.19, day 21.82 ± 0.12, and day 24.61 ± 0.16 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-10/ Figure-5).

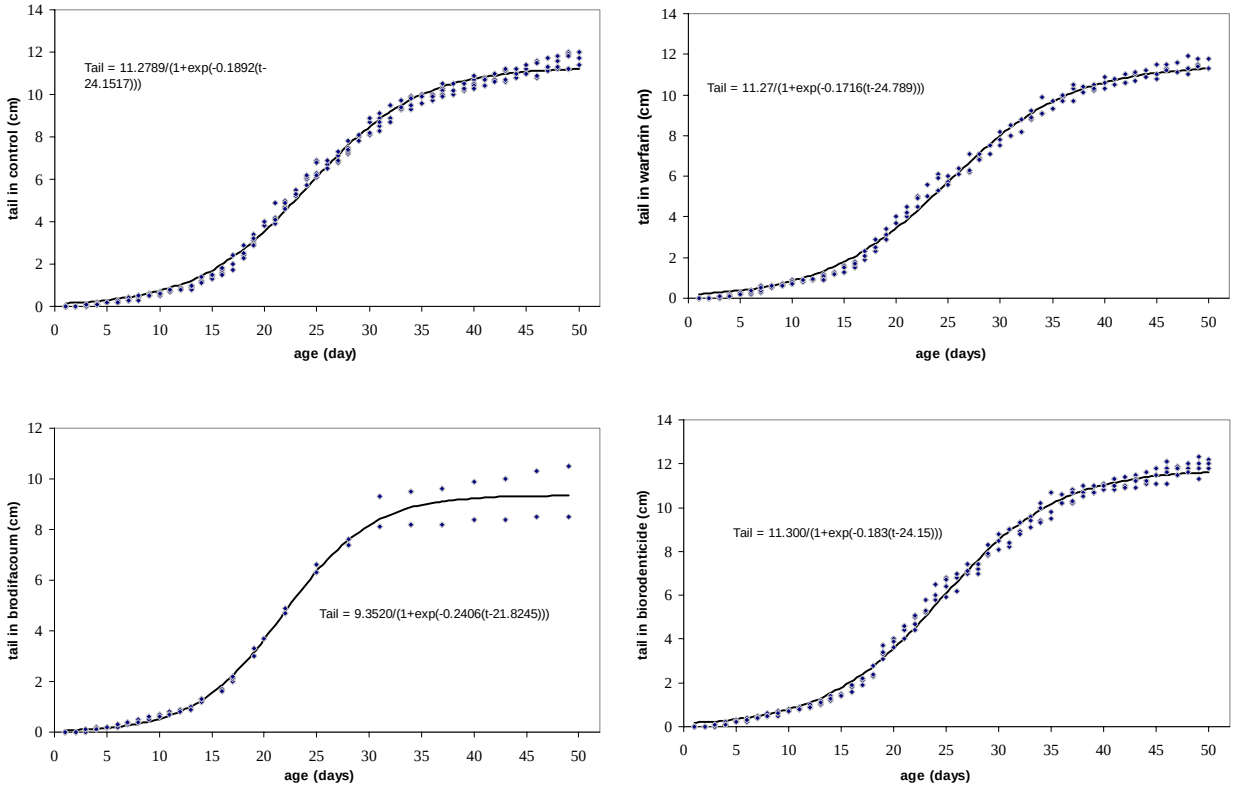

Figure-7. Logistic growth curve of the changes in tail length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

Table-10. Tail length (mean ± SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Treatment	***A*** (g)	***K*** (day^{-1})	***ti*** (days)
A	11.28 ± 0.08	0.19 ± 0.004	24.15 ± 0.16
B	11.46 ± 0.10	0.17 ± 0.004	25.03 ± 0.19
C	9.35 ± 0.09	0.17 ± 0.004	24.61 ± 0.16
D	11.74 ± 0.05	0.24 ± 0.005	21.82 ± 0.12

Remarks: *A* is the asymptote, *K* is the growth constant, and *ti* is the inflection point

DISCUSSIONS

Growth in bird nestlings has been frequently described using weight versus age curves. Growth in birds generally follows a sigmoidal curve, where a small initial increase is followed by a period of relatively rapid growth before leveling off. Information of most interest is the form of the growth curve, its final magnitude or asymptote, and the rate at which it traversed (O'Connor, 1984). It has long been known that proportional differences must exist among growing anatomical parts as a result of genetic, physiological or environmental variation (Starck and Ricklefs, 1998). The constant growth rate in body mass of nestling of barn owls, *T. alba javanica* in untreated control plot was similar as previously reported by Lenton (1984) i.e., K = 0.150-0.162 , and with African subspecies *T. alba affinis* (K = 0.151) (Wilson *et al*, 1987), but higher than Indian barn owl, *T. alba stertens* (K = 0.132) (Nagarajan *et al*., 2002). In rodenticides treated plot, body mass of nestlings is lower than in control plot. This was probably due the stable

number of rat prey in control plot where the adult barn owl can deliver enough food to females and nestlings regularly, in contrast to rodenticide treated plots where rat population experience a crash due to baiting campaign. A study done by Wood (1984) and Liau (1990) found that in unbaited area, the rat population varied between 200 and 600 per ha, with slow fluctuations. However, rat population drop to less than 150 per ha after baiting, and only established six month after control (Wood and Fee, 2003).

The body mass of nestlings in rodenticide treated plots especially brodifacoum reached a lower asymptote than control plot. Although some nestlings survived to fledging age, others were found dead in the nest box. This is because in rodenticide treated plots the rat populations were not as abundant as in the control plot and encourage the males to travel farther and take a longer time to bring rat prey to the nestling. When food is limited the older nestling will out compete the younger siblings for food depriving the latter of food bringing down the average body mass. A study by Durant and Handrich (1998) showed that nestlings have the same body mass when food given is reduced by 17% when compared to nestlings fed enough and show the same pattern for linear growth and fledging. However, when food is reduced to more than 30% than usual, the nestlings showed lower fat accumulation when compared to normal fed nestlings (Lacombe *et al*., 1994).

The culmen and tarsus length in control plot grew rapidly in the first 3 weeks and reached the adult size in the growth period, quite similar to that reported by Wilson *et al* (1987) and Nagarajan *et al.* (2002) where they reported higher growth rates for these bodily parts for young nestlings in Central Mali and India and can reach adult size before fledging. The rapid growth of culmen was also reported in the spotted owl (*Athene brama brama*) (Kumar, 1983). Faster growth of these body parts may be a direct reflection of the use of these organs during the nesting period and immediately after fledging (Holcomb and Twiest, 1968). Rapid leg growth rates as evidenced by tarsus and talon growth rate are another feature of growth in nestling barn owls. Rapid growth of the legs considered by Nagarajan *et al.* (2002) as a selective advantage in competition within broods and also important in post fledging foraging activities, such as collection and handling of food items. In contrast to culmen and tarsus, wing and tail length was shorter than adult size until fledging. Wilson *et al.* (1987) and Nagarajan *et al.* (2002) reported that tail and wing will continue to grow after fledging. The tail and wing would still increase in length after the bird had left the nest and the full ability of young barn owls to catch their own prey would not be achieved until some time had elapsed after leaving the nest (Wilson *et al.*, 1987).

Besides receiving less food than the nestlings in the control plot, nestlings in rodenticide treated plots also face the risk of secondary poisoning by rodenticide residues. If the parents bring home rats that had consumed baits, the young would be exposed to the ingested rodenticide especially brodifacoum, risking them to secondary poisoning. Brodifacoum acts by inhibiting the normal synthesis of vitamin K in the liver (Hadler and Shadbolt, 1975), resulting in an increase in blood clotting time to the point where haemorrhaging occurs (Eason *et al*., 2002). A study showed that the potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to non-target animal either by direct consumption or from build-up of rodenticide residues from indirect consumption of baits (Shore *et al*., 1999; Dowding *et al*., 2010). Mendenhall and Pank (1980) reported that five of six *T. Alba* fed with rats poisoned with brodifacoum died. If the larger adults succumb from rodenticide poisoning the risk to the nestlings would be definitely greater.

Sarcocystis singaporensis is highly host-specific and only lives in the boid snake (*Python reticulatus*) and rodents of the genera *Rattus* and *Bandicota*. The infection of rats is by the sporozoites which eventually invades the muscles to from characteristics cyst in the striated muscles. After inoculation of a lethal quantity of sporocysts, the number of merozoites, the infective stage of the pathogen increase enormously around day 11 post infection especially in the lungs. This induces a fatal pneumonia (Jakel *et al*, 1996).

Warfarin, the first generation anticoagulant, is less toxic than brodifacoum. It is not persistent, and readily metabolized and excreted, and is not retained in the liver beyond 2-4 weeks, while brodifacoum is retained in the liver for 6-12 months (Eason *et al*., 2002). Several studies have shown that birds were almost completely resistant to the effects of warfarin (Papworth 1958). The same indication was also shown by the tawny owl (*Strix aluco*) when given mice that have consumed warfarin on alternate days for three months with no death or apparent behavioral changes (Townsend *et al.*, 1981). Lenton (1984) estimated barn owl nestlings need to consume at least ten medium sizes rats (80g) before a lethal level is reached.

In these study nestlings in rodenticide treated plots, especially brodifacoum showed shorter and lighter measurements in all five anatomical features: body mass, culmen, tarsus, wing and tail length. Previous workers showed that some birds have shorter anatomical parts and lighter in body mass if they are exposed to pesticides or if lived in polluted area. The screech owls (*Otus asio)* administered with fluoride at 40 ppm resulted in a significantly smaller egg and shorter tarsus length (Hoffman *et al*., 1985). The nestlings of the great (*Parus major*) that lives at large non-ferrous smelter and exposed to large amounts of heavy metals have a body mass significantly reduced at the most polluted site although tarsus length, wing length and haematocrit values did not differ significantly among study sites (Janssens *et al*., 2003). The nestling zebra finch (*Taeniopygia guttata*) that were orally dosed with monosodium methanearsonate (MSMA) for 20 days from hatching to fledging showed high mortality if given 24 mg/g, while surviving nestlings showed accumulation of arsenic in blood and specific

tissues, and decreased tarsus length and wing length upon fledging (Albert *et al.*, 2008).

Teratogenic effect was also evidenced in one of the nestling in brodifacoum treated area where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Several pesticides studies also found teratogenic effect on growth and development of birds. The chicken embryos were exposed *benzo [a] pyrene (BP)* via the yolk sac route resulted in retarded growth, as reflected by lower embryonic body weight besides reduced bill length. Abnormal survivors also showed remarkably twisted legs with shortening of the bones, abdominal oedema, haematomas, blisters and a short neck (Anwer and Mehrotra, 1988). Fry (1995) also reported organochlorine, organophosphate, petroleum hydrocarbons, heavy metals, and polychlorinated biphenyls (PCBs) disrupt physiological effects at several levels on birds, including direct effects on breeding adults as well as developmental effects on embryos. The effects on embryos include mortality or reduced hatchability, failure of chicks to thrive (wasting syndrome), and teratological effects producing skeletal abnormalities and impaired differentiation of the reproductive and nervous systems through mechanisms of hormonal mimicking of estrogens. The eggs of Mallard (*Anas platyrhynchos*) that were treated by *Phenyl phosphonothioic acid-o-ethyl-O-[4-nitrophenyl] ester (EPN)* resulted in impaired embryonic growth and was highly teratogenic: 37-42% of the surviving embryos were abnormal with cervical and axial scoliosis as well as severe edema. Brain weights were significantly lower in EPN-treated groups at different stages of development including hatchlings. Hatchlings from *EPN* treated eggs were weaker and slower to right themselves compared to untreated hatchlings (Hoffman and Sileo, 1984).

CONCLUSIONS

Nestlings in rodenticide free area showed consistently heavier body mass and longer in culmen, tarsus, wing and tail length compared to rodenticide treated plots. This was associated with nestlings in rodenticides free getting sufficient food during the growth stage. The food shortage in rodenticide treated plots affect the growth of nestling and exposed to a greater risk of death especially for nestlings less than 20 days old if food shortage continues. Nestlings in brodifacoum treated area did not only face the risk of food shortages but also the risk of secondary poisoning as a result of consuming bait ingested rats. Even one nestling has teratogenic signs where it has no primary feathers in its wings rendering it flightless and the size of the tail is shorter than nestling in rodenticide free area. However, nestlings in warfarin and biorodenticide treated plots have comparable anatomical parts except body mass if compared to rodenticide free area, an indication that there was no apparent evidence of secondary poisoning effect of warfarin and biorodenticide on nestling of barn owls.

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Comparison of the breeding performance of the barn owl Tyto alba javanica under chemical and bio-based rodenticide baiting in immature oil palms in Malaysia

Citation

Mohd Naim, and Mohd Noor, Hafidzi and Kasim, Azhar and Abu, Jalila (2011) *Comparison of the breeding performance of the barn owl Tyto alba javanica under chemical and bio-based rodenticide baiting in immature oil palms in Malaysia.* Dynamic Biochemistry, Process Biotechnology and Molecular Biology, 5 (spec. issue 2). pp. 5-11. ISSN 17490-0626

Abstract

The breeding performance of barn owl, Tyto alba javanica, in areas treated with rodenticides in immature oil palms in Malaysia was investigated. Four plots were established, each at least 100 ha in size and treated with warfarin, brodifacoum, a biorodenticide (Sarcocystis singaporensis) and a non-baited control plot. Three rat baiting campaigns, which coincided with the barn owl breeding season, were carried out in October 2008, February and March 2009, and in October 2009. The nest boxes were distributed at a mean density of one unit per 25 ± 3.83 ha. The clutch size, hatching and fledging rates of barn owls in each plot was monitored monthly from September 2008 to January 2010. There was no significant difference in mean clutch size for all four treatments. The lowest percentage of hatching success was recorded in the brodifacoum-treated plot in all three breeding seasons. Fledging success was highest in the control plot, followed by the S. singaporensis-, warfarin- and brodifacoum-treated plots. The mean clutch size and mean hatching success was not significantly correlated with mean rat damage (clutch size, $r = 0.754$, $p > 0.05$; mean hatching success, $r = 0.832$; $p > 0.05$). The mean fledging success was significantly correlated with mean rat damage ($r = 0.969$; $p < 0.05$). Brodifacoum achieved the lowest level of rat damage but not significantly lower than warfarin and S. singaporensis. This indicates that S. singaporensis is a better rodenticide than warfarin and brodifacoum in controlling rats and yet achieved the highest reproductive rates in the baited areas as reflected by the rate of fledging success.

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Comparison of the breeding performance of the barn owl Tyto alba javanica under chemical and bio.pdf

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CALIFORNIA

RESTAURANT INDUSTRY IMPACT

INDUSTRY AT A GLANCE IN CALIFORNIA

86,779
restaurant locations

$220.1B
restaurant & foodservice sales

Largest
private employer in the state

Every dollar spent in CA restaurants contributes
$1.89
to the state economy

1,853,800
restaurant & foodservice jobs

$33.1B
total taxes paid by restaurants in the state

WE ARE RESTAURANTS IN AMERICA

2nd largest
private sector
employer

Nation's **most diverse industry**

$1.4T
restaurant & foodservice sales

9 in 10
restaurants are
small businesses

Source: National Restaurant Association, based on data from the Bureau of Labor Statistics and U.S. Census Bureau; 2024 data

JOBS & ENTREPRENEURIAL OPPORTUNITIES IN EVERY COMMUNITY

CALIFORNIA RESTAURANTS

CALIFORNIA RESTAURANT INDUSTRY IMPACT

U.S. SENATORS	EATING & DRINKING PLACES Establishments	Employees*
Alex Padilla (D) Adam Schiff (D)	**86,779**	**1,490,100**

	U.S. REPRESENTATIVES	EATING & DRINKING PLACES Estab.	Employees*		U.S. REPRESENTATIVES	EATING & DRINKING PLACES Estab.	Employees*
1	Doug LaMalfa (R)	1,445	23,079	27	George Whitesides (D)	1,187	21,523
2	Jared Huffman (D)	2,014	28,741	28	Judy Chu (D)	2,036	31,368
3	Kevin Kiley (R)	2,080	39,300	29	Luz M. Rivas (D)	1,102	13,921
4	Mike Thompson (D)	1,707	33,448	30	Laura Friedman (D)	2,567	49,878
5	Tom McClintock (R)	1,682	27,543	31	Gilbert Ray Cisneros, Jr. (D)	1,586	22,948
6	Ami Bera (D)	1,270	21,288	32	Brad Sherman (D)	1,935	33,405
7	Doris O. Matsui (D)	1,589	26,366	33	Pete Aguilar (D)	1,134	19,929
8	John Garamendi (D)	1,155	15,186	34	Jimmy Gomez (D)	2,000	28,690
9	Josh Harder (D)	1,195	19,556	35	Norma J. Torres (D)	1,572	26,623
10	Mark DeSaulnier (D)	1,468	23,111	36	Ted Lieu (D)	2,564	55,012
11	Nancy Pelosi (D)	3,712	57,791	37	Sydney Kamlager-Dove (D)	1,428	22,932
12	Lateefah Simon (D)	2,058	25,557	38	Linda T. Sánchez (D)	1,628	21,832
13	Adam Gray (R)	1,021	15,152	39	Mark Takano (D)	1,130	20,153
14	Eric Swalwell (D)	1,424	20,253	40	Young Kim (R)	1,579	26,566
15	Kevin Mullin (D)	1,832	27,761	41	Ken Calvert (R)	1,559	36,041
16	Sam T. Liccardo (D)	1,936	31,135	42	Robert Garcia (D)	1,703	32,416
17	Ro Khanna (D)	2,187	32,737	43	Maxine Waters (D)	1,264	22,036
18	Zoe Lofgren (D)	1,427	20,410	44	Nanette Diaz Barragán (D)	1,302	19,520
19	Jimmy Panetta (D)	1,880	32,956	45	Derek Tran (D)	2,159	33,437
20	Vince Fong (R)	1,400	25,798	46	J. Luis Correa (D)	1,829	37,318
21	Jim Costa (D)	1,133	16,831	47	Dave Min (D)	2,339	51,464
22	David G. Valadao (R)	1,000	16,114	48	Darrell Issa (R)	1,346	31,913
23	Jay Obernolte (R)	1,213	19,017	49	Mike Levin (D)	1,742	35,209
24	Salud O. Carbajal (D)	2,208	40,354	50	Scott H. Peters (D)	2,546	61,580
25	Raul Ruiz (D)	1,092	20,202	51	Sara Jacobs (D)	1,885	30,646
26	Julia Brownley (D)	1,385	26,490	52	Juan Vargas (D)	1,146	17,570
					TOTAL	**86,779**	**1,490,100**

*California's 1,490,100 eating and drinking place jobs represent the majority of the state's total restaurant and foodservice workforce of 1,853,800 jobs, with the remainder being non-restaurant foodservice positions.

NATIONAL RESTAURANT ASSOCIATION | CALIFORNIA RESTAURANT ASSOCIATION

For more information: Restaurant.org | CalRest.org

Source: National Restaurant Association, based on data from the Bureau of Labor Statistics and U.S. Census Bureau; 2024 data

RAPTORS ARE THE SOLUTION

Menu

Poison Free 2023 (Queen Anne Pilot)

PARKER ECO PEST CONTROL

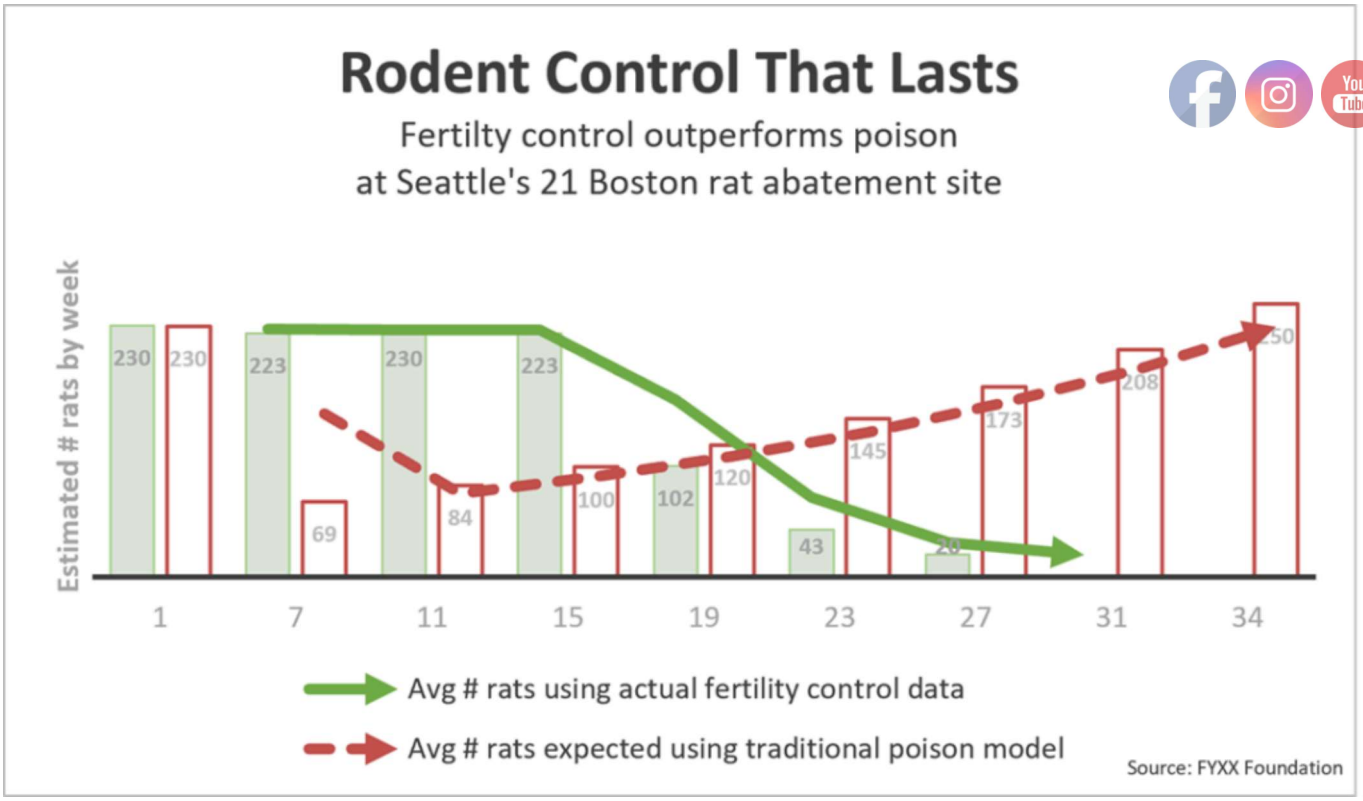
RAPTORS ARE THE SOLUTION SEA-RATS

POISON FREE BY 2023 OWL WISE LEADER

An Upper Queen Anne, Seattle, mixed-use business district demonstration pilot study managed by Raptors Are The Solution to illustrate cost savings and effective rat population management using a non-toxic, rat birth control solution called ContraPest®, as a replacement for a certain class of rat poisons that are harming and killing non-target wildlife and pets. We were able to reduce the rat population by 91 percent on this site in just a few months.

Read the latest news coverage about this project.

Queen Anne is working to become a showcase neighborhood for other communities to follow in their own poison-free rat birth control campaigns. The pilot study team aims to have its documented, successful protocols available for others to use, in the Community and Pest Control Playbook later this year.



A note about our inaugural Queen Anne demonstration pilot study participant TWENTY-ONE BOSTON

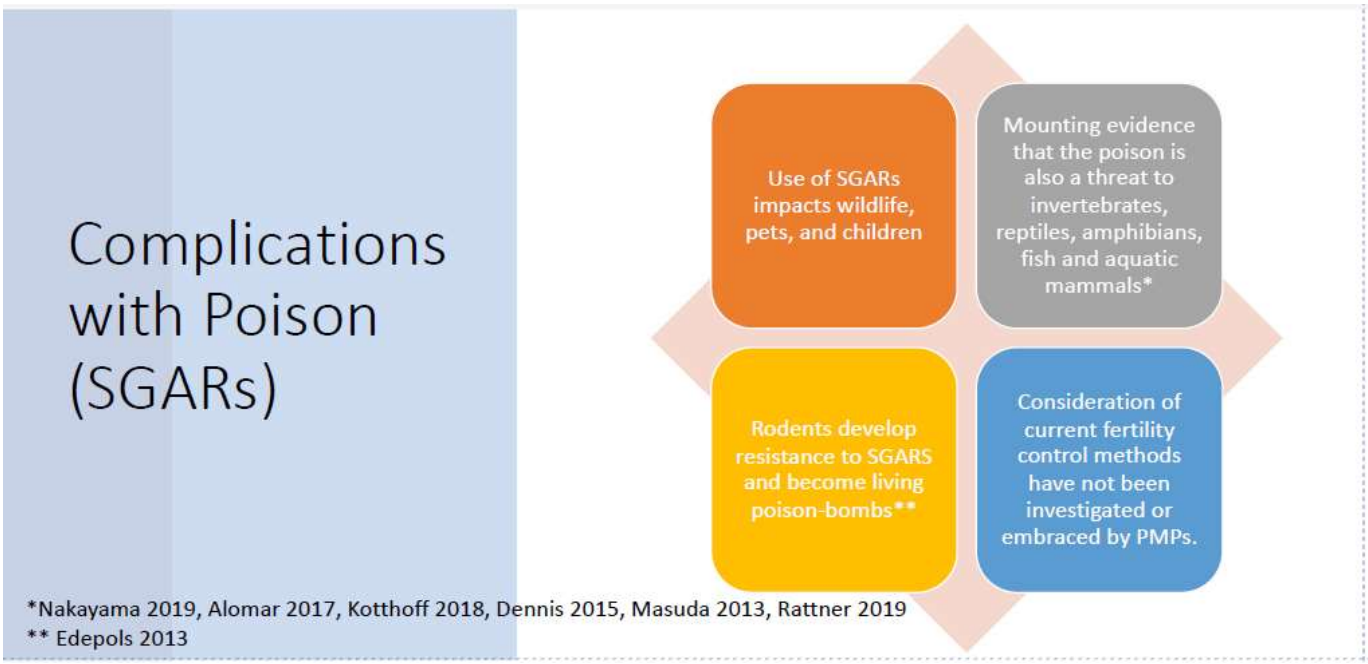
Demolition of Queen Anne's Safeway grocery store happened to make room for 325 apartments situated on top of a new 50,000 square foot grocery store and underground parking**.** Per the Seattle department of construction and inspections, the TWENTY-ONE BOSTON project was required to set out rat poison bait stations prior to ground disturbance caused by demolition. Queen Anne Pilot Study's first participant was developer and landowner Maria Barrientos, marking Seattle's first developer to use a non-toxic fertility control rat abatement strategy for demolition. We are proud to be reaching this trailblazing milestone together as we create a safer urban habitat for wildlife and pets.

At the 7th month mark of managing the future build site's resident Norway Rat population with rat birth control, the pilot study achieved, and held steady, a 91% reduction of the original rat population. The construction superintendent went so far as to remark that he's never demolished a building where he didn't see a single rat. Typically, legacy food-centric buildings are infested with rats by the time demolition happens. The project team received no complaints of migrating rats from the demolition site and nearby businesses benefited greatly from the rat birth control deployed at the Safeway site, taking wintertime rat activity to zero.

See the chart above, which illustrates the pilot study's rat birth control success compared to traditional blood thinning rat poison performance, which has a rebound effect on rat popula after the 4th generation. This happens because rats learn that their colony mates die after e

the bait and stop taking it, thereby returning to original rat population numbers because exponential reproductive growth of rats can no longer be managed with bait that is avoided.

The Problem With Blood-thinning Rat Poisons



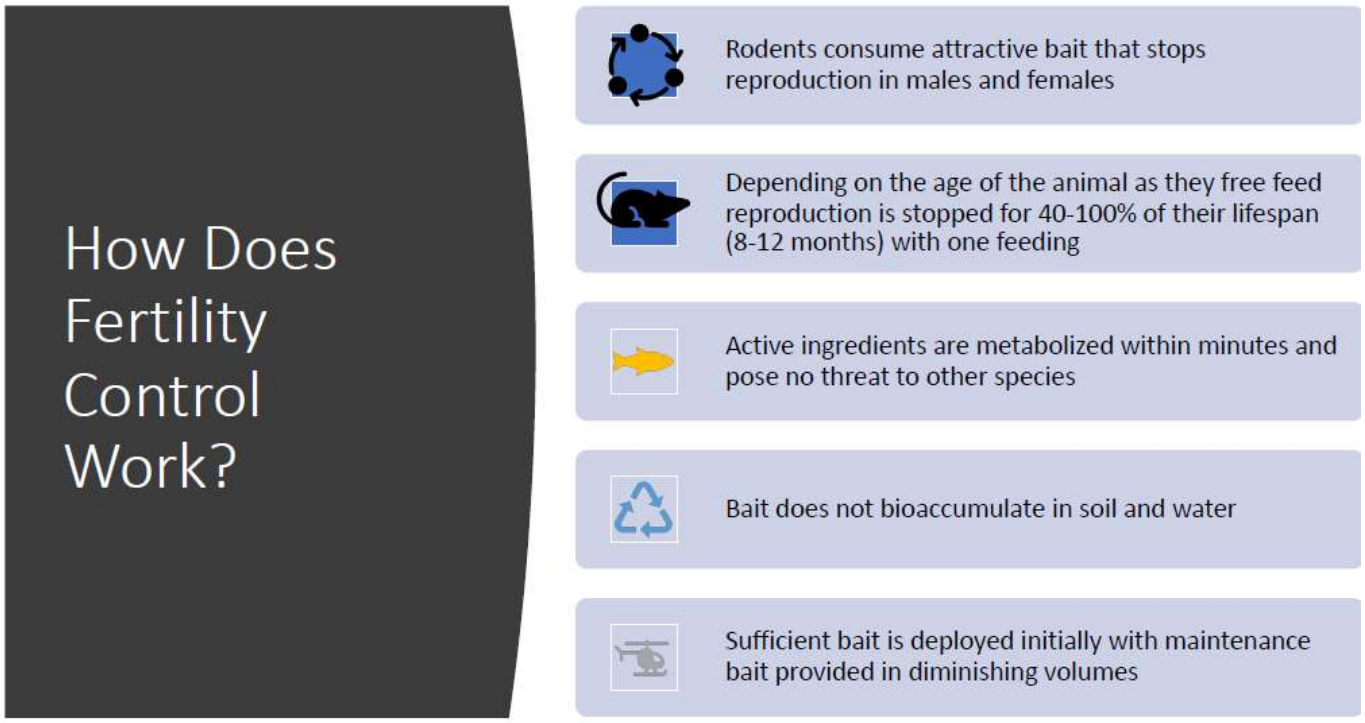
Source: FYXX Foundation

What can we do to stop anticoagulants from infiltrating the food web and killing non-target species?

Replace commercially used blood-thinning rat poisons that are killing non-target wildlife and pets with an effective, non-toxic rat birth control rodent management solution, while improving sanitation and exclusion.

During a year-long pilot study, a mixed-use business district in Washington D.C. decreased its rodent population 99% by utilizing the rat birth control solution, called ContraPest®, which we are using for the Queen Anne pilot study.

Extensive scientific studies have shown that ContraPest® does not bioaccumulate inside the body of non-target species (it is not a hormone) and therefore does not impact predators that eat rats that have ingested this product.



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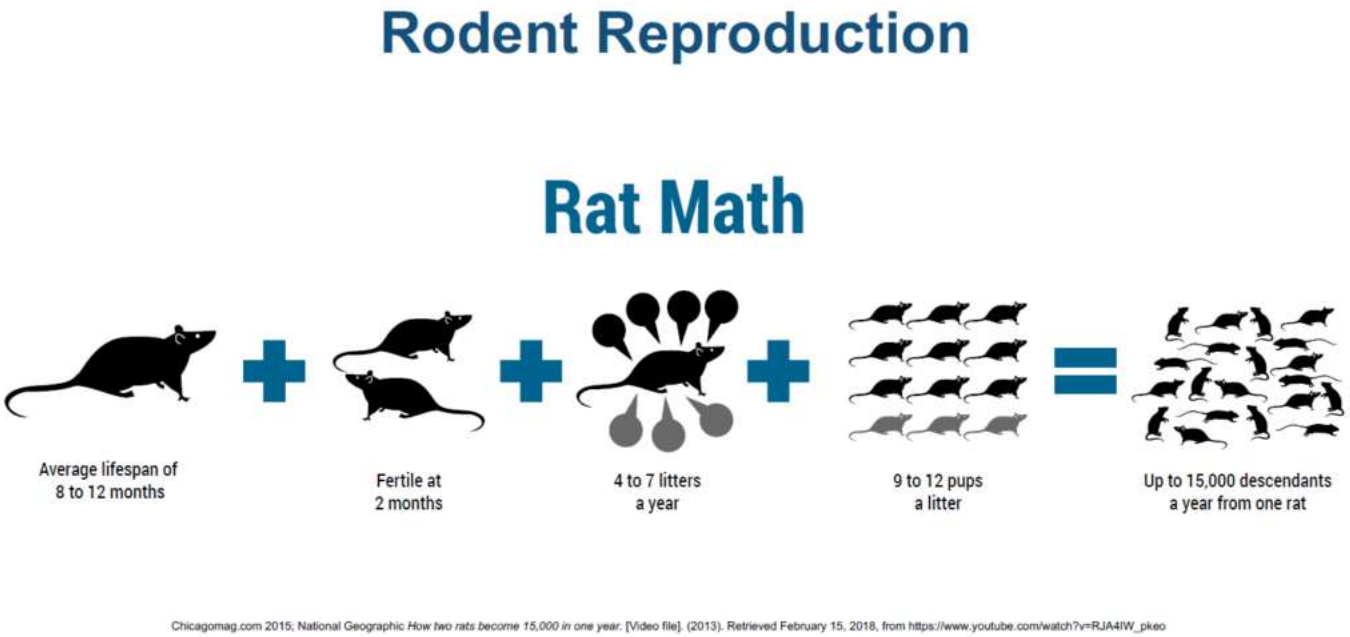

Chicagomag.com 2015; National Geographic *How two rats become 15,000 in one year.* [Video file]. (2013). Retrieved February 15, 2018, from https://www.youtube.com/watch?v=RJA4IW_pkeo

How Deployment and Monitoring of a Business District Pilot Study Could Work

An eco-conscious pest management partner and activist team should:

- Ensure that ALL poison bait stations are properly disposed*;
- Deploy rat birth control bait stations throughout the alleyways and non-toxic rat deterrents inside businesses, if needed;

- Clean and monitor the bait stations on a monthly basis for consumption, and refill as needed. The amount of necessary fertility control product will decrease over time as the rat population decreases, thereby decreasing pest control costs for business owners.
- Collect bait consumption data for analysis that shows population decline.
- Address business concerns around maintenance of the bait stations or the very unlikely case of increased rat activity.
- Our current goal is to remove blood-thinning poisons from our Upper Queen Anne business district (encompasses Queen Anne Ave N., Boston to Galer) which covers 735,000 square feet of commercial space with rat birth control for an estimated monthly cost is $2,600 a month. If every business in the area participates it breaks down to just $18 per business per month.

Get Involved

Are you an Upper Queen Anne business that would like to participate in the pilot study, or someone who would like to remove rat poisons from their Seattle neighborhood? Call or e-mail Tanea Stephens at 206-579-4545 or searatschapter@gmail.com to get started.

Residential Rat Birth Control

People we talk with want to rid their homes of blood-thinning rat poisons. Because there is so much demand, beginning summer 2022, "In Harmony" will offer a nationwide residential DIY rat birth control subscription service. To be placed on a list for more information once it becomes available, sign up here. For those who want a hands-off approach to managing rats, Parker Eco Pest Control now offers Seattle metro residential full service rat birth control management. To get started with residential full service rat birth control, sign up here.

***Note**: when you decide to ditch rat poison for sanitation, exclusion, and non-toxic rodent control products, NEVER throw your old poison bait boxes in the garbage where they end up in the landfill, potentially poisoning rats, eagles, vultures, and other wildlife. If your pest control company does not pick up your old bait boxes, and you are in Seattle, send an e-mail to searatschapter@gmail.com to request a free bait box pick-up and drop-off to the King County Toxic Waste Disposal facility.

The Genesis Story

Tanea Stephens, a RATS Volunteer and Queen Anne resident became concerned about rodenticides in her neighborhood when a rare Snowy Owl visitor set up residence near the business district in October 2020. She took inventory of the toxic rat poisons and found 70 AR bait boxes along the ½ mile stretch of Upper Queen Anne's business district alleyways. After becoming the Washington state chapter representative for RATS and brainstorming with pilot study partner FYXX Foundation, the idea for Poison Free By 2023 was born. If the study garn the anticipated results, Queen Anne will become the showcase neighborhood for our ***Poison Free***

by 2023 citywide campaign and provide a case study that shows rats can be effectively managed without lethal poisons that harm non-target wildlife, pets and children.

Update: One year after the Queen Anne departure, Urban Raptor Conservancy reports that it tested 29 pellets that the Snowy owl cast onto rooftops and beneath roost trees and rodent bones were abundant in the pellets; a few were tested for toxicology and found evidence of anticoagulant rodenticides.

You can learn more about: our Snowy Owl visitor in this Queen Anne & Magnolia News Article; the pilot study in another Queen Anne & Magnolia News feature and Stranger Article; and a Queen Anne & Magnolia News feature on the developer deployment.

Top 3 False Claims by Pest Control Companies

FALSE: The poisons we use are safe. There is no proof that our poisons kill non-target wildlife.

FACT: There is no such thing as a safe rat poison. Current bait box poisons are infiltrating the entire food web and not only killing rats. There have been hundreds of scientific studies showing accumulation of rat poison in species of all kinds. Children under age six and domestic pets are known to have been poisoned.

FALSE: If we can't use anticoagulant rodenticides (ARs), there will be a public health crisis.

FACT: Improving sanitation and installing exclusion helps remove rat attractants, and there are non-toxic alternatives that can be used to manage rodent populations. We are only asking people and companies to stop using four of the most deadly AR poisons.

FALSE: Rats consume poison inside the box and therefore it cannot poison children or pets.

FACT: The loose, often bright blue bait is consistently found outside bait boxes. Rats carry rat poisons outside boxes where children and pets are attracted to them. Poisoned rats eat the bait both inside and outside the box. They leave the boxes, sickened and sluggish, becoming easy prey for raptors and other wildlife.

RAPTORS ARE THE SOLUTION

Menu

Got Rats?

Check out our "Got Rats" poster series for tips on controlling rats without poison.

Our **Got Rats?** posters, created with the help of experts including a pest control company, highlight numerous ways to keep rodents away without using poison and other harmful methods. Click links to share, download, or print!

Got Rats?

Got Restaurant Rats?

Got Rats in Your Crawl Space?

Got Rats in Your Barn?

Got Rats in Your Car?

Got Rodents on your Family Farm?

Raptors are the solution but... they can't be the entire solution. There are a variety of effective alternatives to poison. We recommend taking an "integrated pest management" approach to rodents that emphasizes exclusion and sanitation.

Rodent fertility control is a promising solution. Learn more about it.

Preferred pest control companies that do not use poison

For large scale (warehouse/industrial) rat issues

Preferred pest control products

Start by finding their source of food, shelter, and water and exclude them from it, or hire a company to help you. One of the primary attractants and sources of food for rodents is trash and waste. More tips.

Follow our best practices guide to keep rodents out of dumpsters and waste in!

Tips for restaurants

Tips if you have rats in your crawl space

Other tips on discouraging rats:

- Remove invasive ivy—it's a luxury hotel for rats. They thrive in it! Replace with native plants that offer habitat for other wildlife.
- Pick up bird seed waste in your yard. Instead of bulk seed, use *seed blocks or cakes* that leave less seed on the ground.
- Do not leave pet food out.
- Make sure garbage bags are tied tightly and secured.
- Backyard chicken coops attract rats. Consider installing rat-proof flooring in your coop.
- Consider installing a barn owl box—but ONLY if everyone in your neighborhood commits to not using poison. See the Hungry Owl Project for more information and tips.
- Orkin now offers a shield for your home that protects against rat entry.

For information about gopher problems, check out Gophers Limited.

A few warnings:

Any time you see a "bait box" with an exit hole—beware. If the box contains poison, it should be labeled as such. If it contains a trap instead, it must be labeled—otherwise, the box likely includes

poison.

Rats and mice can “check in,” eat poison, and then check out, easily becoming food for a hawk, an owl, other wildlife, or your pet cat or dog. Bait boxes are **not** safe for wildlife unless you get them from a company that uses snap traps inside them. (Most do not.) They should **never** be used out in the open where songbirds can get caught in them.

Most large pest control companies still use poison in their bait boxes. If you hire a pest control company, insist that they not use poison (or glue traps), or switch to a company using ethical methods. Demanding poison-free solutions helps reduce the market for poison. If there is a big enough market for exclusion and humane solutions, the poison will eventually stop. Remember that the poison cycle equals profits for pest control.

Finally, please do *not* use glue or sticky traps. They are **cruel and inhumane** and also catch songbirds, small owls, and other small animals who often have to be euthanized as a result (and only if they’re lucky). An animal caught on a glue trap, whether a rodent or a non-target animal, often suffers enormously and for a long period. It will eventually die of starvation, suffocation, pain, stress, and/or horrible injury from trying to escape. Several countries have banned glue traps, for good reason, but the United States still allows their use. Read more here (Humane Society of the United States) and here (Royal Society for the Prevention of Cruelty to Animals UK).

RAT POISONS NOT ONLY KILL WILDLIFE: THEY CAN ALSO WEAKEN AND SICKEN THEM.
Known "sublethal" impacts include:

- Hemorrhaging beneath the skin and extensive bruising. Internal hemorrhaging in bones, body wall, heart, and elsewhere in the body. Possible heart failure.[1]
- Hemorrhaging of the heart, liver, kidney, lung, intestines, and muscles.[2]
- Anticoagulants associated with inflammatory response and immune suppression in bobcats.[3]
- Anticoagulants associated with multiple system effects in bobcats.[4]
- Multiple AR exposure events associated with notoedric mange.[5]
- Barn owl clutch size, brood size, fledging success, and nest box occupancy lower in fields treated with anticoagulants.[6]
- Increased vulnerability to other causes of death such as vehicular collisions and predation.[7]
- Coyotes exposed to multiple FGARs and with high FGAR residues tended to be in poorer body condition.[8]
- Chronic anemia, making animals more susceptible to diseases, including mange, and other stressors. [9]
- Reproductive impacts. Female sheep exposed to anticoagulants had more aborted or stillborn lambs (up to 50%); male sheep had lower sperm motility.[10]
- Decreased food intake[11] and decreased body weight.[12]
- Neonatal transfer to young kits. Decreased resilience to environmental stressors.[13] Fetuses more susceptible to brodifacoum toxicity than adults.[14]

- Increased parasite and pathogen burdens.[15]
- Shorter wings, tails, bones, bills, and birth defects.[16]
- Rodents poisoned by anticoagulants are more likely to be eaten by predators.[17]
- Raptors may preferentially prey upon sickened rodents: The energetically beneficial behavior of favoring substandard prey may increase raptor encounters with rodenticide exposed animals if prey vulnerability has resulted from poisoning.[18]
- Exposure to brodifacoum may have prolonged effects that increase toxicity of subsequent AR exposure.[19]
- Bromadiolone and chlorophacinone residues from secondary poisoning can be transferred to the eggs of *T. alba.* [20]
- Increased stress and reduced body condition.[21]

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JOURNAL ARTICLE

Brodifacoum Toxicity in American Kestrels (*Falco sparverius*) with Evidence of Increased Hazard on Subsequent Anticoagulant Rodenticide Exposure

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Abstract

A seminal question in ecotoxicology is the extent to which contaminant exposure evokes prolonged effects on physiological function and fitness. A series of studies were undertaken with American kestrels ingesting environmentally realistic concentrations of the second-generation anticoagulant rodenticide (SGAR) brodifacoum. Kestrels fed brodifacoum at 0.3, 1.0, or 3.0 µg/g diet wet weight for 7 d exhibited dose-dependent hemorrhage, histopathological lesions, and coagulopathy (prolonged prothrombin and Russell's viper venom times). Following termination of a 7-d exposure to 0.5 µg brodifacoum/g diet, prolonged blood clotting time returned to baseline values within 1 wk, but brodifacoum residues in liver and kidney persisted during the 28-d recovery period (terminal half-life estimates >50 d). To examine the hazard of sequential anticoagulant rodenticide (AR) exposure, kestrels were exposed to either the first-generation AR chlorophacinone (1.5 µg/g diet) or the SGAR brodifacoum (0.5 µg/g diet) for 7 d and, following a recovery period, challenged with a low dose of chlorophacinone (0.75 µg/g diet) for 7 d. In brodifacoum-exposed kestrels, the challenge exposure clearly prolonged prothrombin time compared to naive controls and kestrels previously exposed to chlorophacinone. These data provide evidence that the SGAR

brodifacoum may have prolonged effects that increase the toxicity of subsequent AR exposure. Because free-ranging predatory and scavenging wildlife are often repeatedly exposed to ARs, such protracted toxicological effects need to be considered in hazard and risk assessments. *Environ Toxicol Chem* 2020;39:468–481. © 2020 SETAC

Keywords: Wildlife toxicology, Toxicokinetics, Rodenticide, Biomarkers, Species extrapolation

Issue Section: Hazard/Risk Assessment

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