



Forest food web contamination by rodenticides is pervasive but responsive to regulation: Insights from Barred Owl biomonitoring

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ABSTRACT

Anticoagulant rodenticides and their substitutes are widely deployed in urban and agricultural settings, but evidence is mounting that contamination extends into remote forests through diffuse sources. Most wildlife exposure data come from opportunistic carcass collections that systematically overrepresent animals near human activity and in compromised condition. We used experimental removals of Barred Owls (*Strix varia*) from non-urban forests of northwestern California as a minimally biased sentinel sample, analyzing liver tissue from 1003 individuals collected between 2019 and 2025 for 14 rodenticide analytes. In the regional dataset ($n = 837$), anticoagulants were detected in 35.7% of owls (second-generation 35.2%, first-generation 1.2%). Brodifacoum was most prevalent (30.6%), followed by bromadiolone (14.2%). Cholecalciferol, reported separately because it also occurs endogenously and was not screened in previous *Strix* owl surveys, was detected in 18.5% of individuals; 9.9% of owls carried cholecalciferol but no anticoagulant. Bromethalin was not detected, though liver is a suboptimal tissue for this neurotoxicant. Exposure rates, concentrations, and compound richness were higher in females than males and increased across age classes, consistent with bioaccumulation of persistent compounds. In a subadult subsample from Hoopa Valley ($n = 292$), model-predicted anticoagulant exposure fell from ~50% in 2019 to ~20% in 2024, coincident with tightening California restrictions on anticoagulants—a pattern consistent with a measurable regulatory effect on wildlife exposure. Concurrent increases in cholecalciferol detections indicate possible product substitution that warrants monitoring. That restrictions on the most persistent anticoagulants produced a detectable signal within years demonstrates that policy can translate into measurable wildlife benefit.

1. Introduction

Rodenticides are widely deployed to control commensal rodent pests, but their use can unintentionally poison or contaminate non-target wildlife through secondary exposure—the consumption of contaminated prey by predators and scavengers. Most wildlife exposure data originate from urban, suburban, and agricultural settings where pest control is routine (Elliott et al., 2016; Jacob and Buckle, 2018). However, increasing evidence from western North America shows that contamination permeates remote forests as avian predators and other carnivores accumulate

detectable residues far from cities, implicating diffuse sources and food web transfer in ostensibly wild systems (Franklin et al., 2018; Gabriel et al., 2018, 2012; Hofstadter et al., 2021; Wiens et al., 2019). Trespass cannabis cultivation on public lands has been identified as a significant rodenticide source in remote northern California forests, with grow-site density linked to reduced fisher (*Pekania pennanti*) survival and with brodifacoum detected in a Northern Spotted Owl (*Strix occidentalis caurina*) found dead near active growing operations (Franklin et al., 2018; Gabriel et al., 2012; Thompson et al., 2014; Wengert et al., 2021). Rodenticide persistence in tissues and soils means legacy contamination may continue cycling

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through food webs even where active inputs have declined.

Two rodenticide classes dominate current management and wildlife-risk discussions—anticoagulants and non-anticoagulants—encompassing four groups relevant here: first-generation anticoagulants (FGARs), second-generation anticoagulants (SGARs), cholecalciferol, and bromethalin (Mercer et al., 2022). FGARs (e.g., warfarin, chlorophacinone, diphacinone) were introduced in the mid-twentieth century and generally require multiple feedings to reach lethal thresholds while their hepatic half-lives are relatively short, on the order of days to weeks (Eason et al., 2010; Fisher et al., 2003). Resistance to FGARs in target rodent populations drove the development of more potent SGARs (e.g., brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen; Feinstein et al., 2016; McGee et al., 2020; Pelz et al., 2005). SGARs are substantially more lipophilic and persistent, with hepatic half-lives reaching 113 days for brodifacoum in laboratory rats (Fisher et al., 2003) and more than 100 days for flocoumafen in quail liver (Huckle et al., 1989). This prolonged retention elevates the probability of secondary exposure and bioaccumulation in predators and scavengers (Nakayama et al., 2019; Oliva-Vidal et al., 2022; Rattner et al., 2014).

Two non-anticoagulants are increasingly positioned as alternatives. Cholecalciferol (vitamin D₃) is a hypercalcemic toxicant that drives calcium dysregulation culminating in soft-tissue mineralization (Mercer et al., 2022; Peterson and Fluegeman, 2013). Bromethalin is a neurotoxin whose active metabolite (desmethyl-bromethalin) uncouples mitochondrial oxidative phosphorylation in the central nervous system (Coppock, 2013; Mercer et al., 2022). Unlike the other rodenticides discussed here, cholecalciferol also occurs naturally—animals obtain vitamin D₃ through endogenous cutaneous synthesis and via animal-origin foods (Schmid and Walther, 2013). Diagnosis requires concurrent evidence of toxicosis pathology—typically metastatic mineralization and hypercalcemia—and plausible product-use context (Gabriel et al., 2015; Mercer et al., 2022). Non-anticoagulant rodenticides are often characterized as posing lower secondary poisoning risk than SGARs (Eason et al., 2010), but empirical evidence in free-ranging wildlife remains limited (Mercer et al., 2022; Murray and Cox, 2023; Sant et al., 2019), making their occurrence and exposure pathways important to track alongside anticoagulants under shifting regulations.

Policy creates a moving backdrop for exposure risk. In California, access to the most toxic anticoagulants has progressively tightened. In 2014, the Department of Pesticide Regulation (DPR) banned the four SGARs brodifacoum, bromadiolone, difenacoum, and difethialone for consumer use, restricting them to certified pest control operators (Saggeese et al., 2024). The California Ecosystems Protection Act (AB 1788) of 2020, further prohibited use of these four SGARs statewide, with exemptions for agricultural activities, public health emergencies, water infrastructure protection, and offshore island invasive species eradication, among other exemptions. In 2024, the Poison-Free Wildlife Act (AB 2552, effective 2025) extended the statewide prohibition to the FGARs chlorophacinone and warfarin. These constraints were expected to reduce environmental loading of anticoagulants while creating incentives for substitution toward non-anticoagulant products such as bromethalin and cholecalciferol—a pattern consistent with observed increases in reported human bromethalin exposures following earlier federal restrictions on long-acting anticoagulants (Feldman et al., 2019). The net effect on forest food webs remains uncertain, however, as non-anticoagulants also pose risks to non-target wildlife and their secondary-exposure dynamics are comparatively understudied (Murray and Cox, 2023; Rattner et al., 2014).

Regulatory conditions also differ across the California–Oregon border, which forms the northern edge of our study area (Fig. 1). Oregon has enacted no state restrictions beyond the federal baseline, under which SGARs remain available to licensed applicators for professional pest control and agricultural use. By contrast, California prohibits use of the four principal SGARs statewide under AB 1788, with limited exemptions. Legal SGAR use is therefore less constrained on the Oregon side of the border, and because animal dispersal is indifferent to

jurisdictional boundaries, wildlife exposure may be higher near the Oregon border.

Against this regulatory backdrop, we took advantage of an independent conservation effort to assess rodenticide contamination across remote forest landscapes in northwestern California. Barred Owls (*Strix varia*) invaded western North America from their native range in the east and experimental lethal removals were instituted to assess whether reducing competitive pressure could stabilize Northern Spotted Owl populations (Diller et al., 2016, 2014; Franklin et al., 2021; Hobart et al., 2025; Wiens et al., 2021). Ecosystem-level syntheses argue that the consequences of Barred Owl invasion extend beyond Northern Spotted Owls as altered food web structure is expected to generate trophic cascades and broader community reorganization (Arenas-Viveros et al., 2025; Holm et al., 2016; Jirinec et al., 2026; Kryshak et al., 2022). Critical for toxicant surveillance, removals sample individuals healthy enough to defend territories, reducing the bias of opportunistic carcass recovery toward animals near human activity and in compromised condition (Elliott et al., 2024; Wobeser, 2007). Barred Owls' year-round residency within stable home ranges integrates local contamination signals over time, their mammal-dominated diet provides a primary pathway for rodenticide ingestion, and their high densities enable large sample sizes that support robust spatial and temporal inference—together making them effective sentinels of landscape-scale contamination (Gabriel et al., 2018; Hofstadter et al., 2021; Jirinec et al., 2026; Wiens et al., 2019). In northern coastal California, where Barred Owls now overlap and compete with Northern Spotted Owls in some of their last strongholds, quantifying rodenticides in Barred Owls carries direct relevance not only for Northern Spotted Owls, but also for co-occurring mesocarnivores and other raptors sharing the same food webs (Franklin et al., 2018; Gabriel et al., 2018; Jirinec et al., 2026; Wiens et al., 2019). Because Barred Owls are long-lived (≥ 15 years), age class can serve as a window into contamination dynamics as older birds integrate burdens accumulated within established home ranges, while younger birds may reflect recent exposures at potentially distant natal sites (Stapp et al., 2025; Watson et al., 2024)—though legacy persistence of SGARs in tissues means older animals may also archive exposures from well before the sampling period.

We leveraged samples from lethally removed Barred Owls to test four sets of predictions. First, if rodenticide contamination is widespread in remote forest food webs, we should detect multiple compounds across a broad spatial extent, with SGAR dominance reflecting their greater persistence and bioaccumulation potential. Second, if exposure accumulates across the lifespan of sampled Barred Owls through hepatic retention and greater cumulative encounter with contaminated prey, exposure rates, concentrations, and compound richness should increase from younger to older age classes. Third, following prior evidence of sex-biased rodenticide burdens in this species (Hofstadter et al., 2021), we expected females to exhibit higher exposure than males. Fourth, if California's progressive restrictions on anticoagulants (2014–2025) have reduced environmental loading, SGAR exposure in young birds should decline across years, SGAR exposure in resident adults should increase toward the Oregon border where use remains less restricted, and substitution toward non-anticoagulant products should manifest as increasing cholecalciferol or bromethalin detections over time. We tested these predictions using samples from 1003 Barred Owls collected between 2019 and 2025, screening for 14 rodenticide analytes spanning anticoagulants and non-anticoagulants, and examining patterns with independent pesticide-use reporting and the California–Oregon regulatory contrast.

2. Material and methods

2.1. Study area

We sampled Barred Owls in northwestern California (Fig. 1) on federal and state lands, tribal territories, and private ownership in Humboldt, Del Norte, Siskiyou, and Trinity counties. The survey area

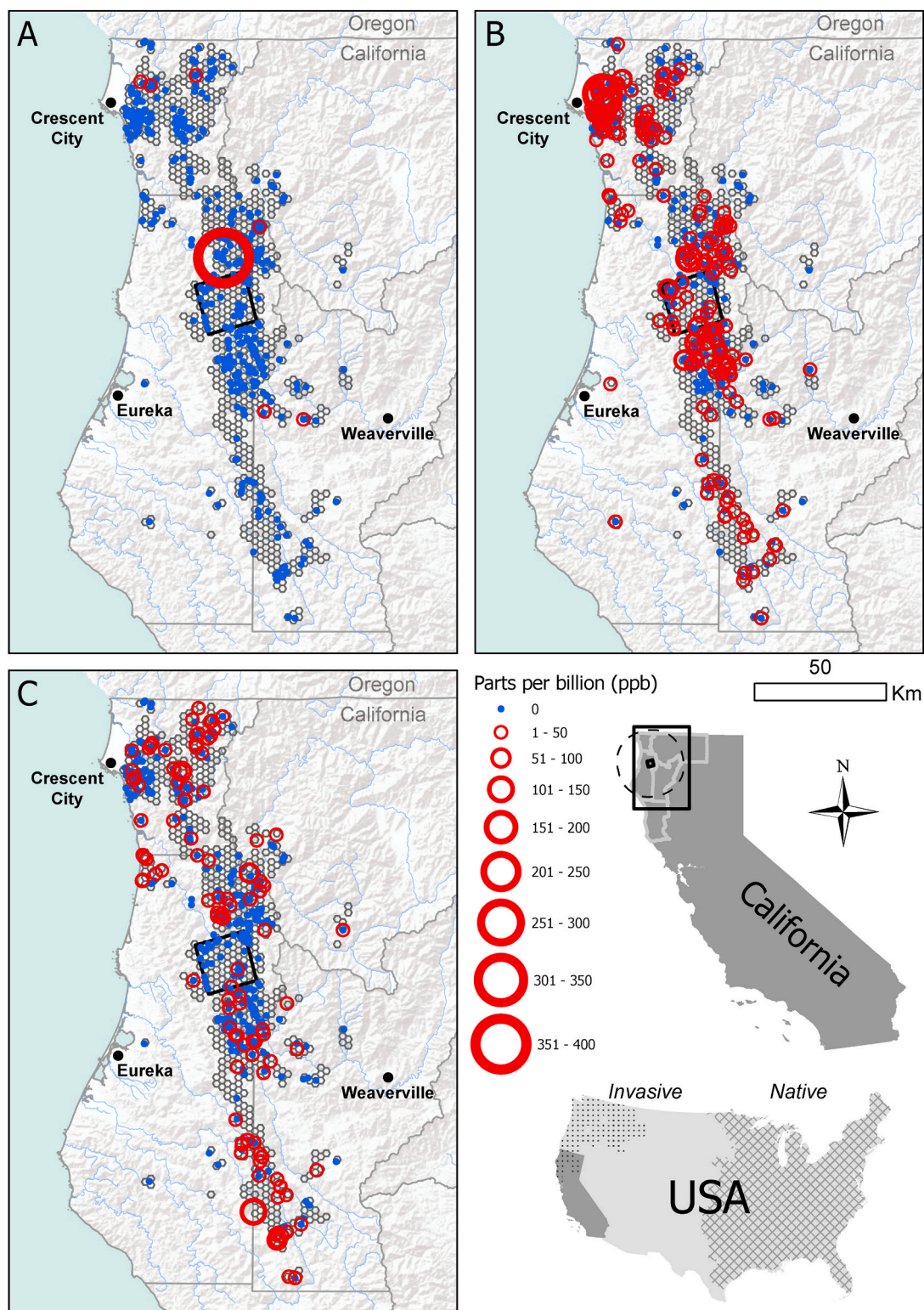


Fig. 1. Study area and spatial distribution of liver rodenticide residues in adult Barred Owls ($n = 531$). Circles are sized by concentration (ppb) with bins shown in the legend; blue filled circles indicate non-detections (0 ppb) and red ringed circles indicate detections (>0 ppb). Panels show (A) first-generation anticoagulants (FGAR), (B) second-generation anticoagulants (SGAR), and (C) cholecalciferol. Gray hexagons depict the extent of Barred Owl removal sampling across northwestern California. The black square outlines the Hoopa Valley Reservation (shown in A–C). The inset map of California highlights the five counties (Del Norte, Humboldt, Trinity, Siskiyou, Mendocino) for which pesticide application totals were summarized from California DPR records. The dotted buffer represents a 100-km zone measured outward from the Hoopa boundary, indicating the plausible natal-origin area for subadults sampled at Hoopa based on observed dispersal distances (Watson et al., 2024). The USA inset shows the species' native (eastern) and invasive (western) ranges. Only adults are mapped because established individuals are more likely to reflect local exposures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was 3990 km², stretching from the coast to ~100 km inland and from the Oregon border to ~230 km south. The region bridges two major ecoregions, the Klamath Mountains and the Coast Range (Omernik and Griffith, 2014), and is defined by rugged topography with steep river canyons and sharp ridgelines with elevations extending from sea level to nearly 2000 m. The climate is Mediterranean with cool, wet winters and hot, dry summers, and a strong maritime-to-continental gradient in precipitation and temperature. This environmental heterogeneity supports exceptional biological diversity as the Klamath-Siskiyou has been recognized among the world's most significant temperate forest regions (Dellasala et al., 1999; Whittaker, 1960). Dominant vegetation includes coastal redwood (*Sequoia sempervirens*) forests near the coast and, inland, mixed-conifer assemblages of douglas-fir (*Pseudotsuga menziesii*), tanoak (*Notholithocarpus densiflorus*), pines (*Pinus* spp.), and incense cedar (*Calocedrus decurrens*), intergrading with oak (*Quercus* spp.) woodlands and montane meadows.

2.2. Barred Owl specimen collection

Barred Owl removals were undertaken as part of ongoing experiments to assess the ability to reduce competitive pressure on federally threatened Northern Spotted Owls and other native wildlife (Hobart et al., 2025) and specimens obtained from these actions were subsequently used to evaluate rodenticide exposure patterns. Research use of specimens did not influence removal decisions or timing. Following established protocols, trained personnel located Barred Owls during standardized nocturnal surveys and, after confirming species identity by audiovisual cues, humanely dispatched individuals using agency-approved methods (Diller et al., 2014). Removal methods were uniform across the study area and throughout the sampling period. Field crews located owls by broadcasting conspecific calls and dispatched them with a 12- or 20-gauge shotgun. Surveys covered all road-accessible portions of the study area, including unimproved Forest Service two-tracks that crews often had to clear of downed trees, and included sites adjacent to small towns, inholdings, as well as very remote areas. Road access rather than proximity to human habitation therefore constrained where owls were collected. A subadult series from Hoopa Valley used for temporal analyses was collected within a single area by a consistent field crew (Fig. 1). All field procedures were conducted under appropriate state (S-193180001-19337-001), federal (MB24592D), and tribal permits and approved institutional animal care protocols (A006106-R03).

Geographic coordinates and morphometric data were recorded at time of removal. Sex was assigned based on vocalizations and morphometrics (e.g., body size, brood patch) and confirmed during necropsy via gonadal inspection. Field crews assigned owls to three age classes based on characteristics of remiges (Pyle, 2022; Pyle et al., 2022): subadult 1 “S1” (all juvenile remiges present, corresponding to formative plumage; ~1–17 months old), subadult 2 “S2” (mixture of juvenile and adult remiges, corresponding to minimum second basic plumage; ~12–36 months), and adult “A” (no juvenile remiges, minimum third basic plumage; >24 months). Specimens were frozen within ~24 h at –20 °C and necropsied days to months later. No screening for infectious disease was used to include or exclude individuals. Responsiveness to playback served as a functional screen, because owls that were moribund or behaviorally impaired were unlikely to be detected and removed. Samples collected from a subset of carcasses for unrelated studies of avian influenza and parasite burden did not inform inclusion here.

We analyzed tissue samples from 1003 Barred Owls across two overlapping datasets. The first comprised 166 subadults collected between 21 March 2019 and 22 June 2022 within the Hoopa Valley Reservation (“Hoopa subadults”; Fig. 1). These specimens were used exclusively for assessing temporal trends in rodenticide exposure. The second (“regional”) dataset included 837 individuals collected between 30 August 2022 and 28 May 2025, encompassing both adults and

subadults from Hoopa (from August 2022 onward) as well as additional collections from the broader study area beginning in August 2023 (Fig. 1). This latter dataset was used to estimate overall exposure rates and conduct all other analyses.

2.3. Tissue sampling and laboratory analysis

We assessed rodenticide exposure from liver tissue. Carcasses were thawed and necropsied under sterile conditions (≤ 24 h from onset of thaw); whole livers were excised, re-frozen at –20 °C, and shipped to the USDA National Wildlife Research Center (NWRC; Fort Collins, Colorado). At NWRC, livers were homogenized and duplicate subsamples of each were extracted by dispersive Solid Phase Extraction (dSPE) and analyzed by Ultra-High-Performance Liquid Chromatography (UHPLC) coupled with a triple-quadrupole mass spectrometer (MS/MS) and electrospray ionization (ESI) source.

NWRC quantified 14 rodenticide analytes: 12 anticoagulants—first-generation (warfarin, chlorophacinone, diphacinone, pindone, coumachlor, coumatetralyl, coumafuryl) and second-generation (brodifacoum, bromadiolone, difethialone, difenacoum, flocoumafen)—and two non-anticoagulants (cholecalciferol and desmethyl-bromethalin, the active metabolite of bromethalin). Extracts were quantified by LC-MS/MS against deuterated internal standards, available for all analytes except pindone, coumafuryl, and coumachlor. Five batches were run on an Agilent 6470A instrument and one on a more sensitive Agilent 6475A, which contributes to the between-batch variation in detection limits described below. Cholecalciferol was quantified from the same extracts under separate LC-MS/MS conditions. Each batch included unfortified control liver (quail or Barred Owl) and control liver fortified at three concentrations, each analyzed in quadruplicate, with an acceptance window of 75–125% of nominal concentration. Detection and quantitation limits were derived from these quality-control samples for each analyte in each batch. Method performance met acceptance criteria in every batch for all second-generation anticoagulants and for cholecalciferol, the compounds accounting for nearly all detections reported here. Pindone, coumafuryl, coumachlor, and desmethyl-bromethalin could be confirmed but not reliably quantified (Table S4). Additional details for laboratory analysis, including MS/MS parameters, calibration, and quality-control performance, are given in Appendix A (Table S1–S4, Fig. S1–S2).

Each sample was analyzed in duplicate. NWRC reported replicate concentrations and flagged values below the detection limit (DL) as not detected (ND). Batch-specific DLs, estimated from quality-control samples, varied across the six analytical batches (Table S1); for brodifacoum, the most frequently detected analyte, batch-specific DLs ranged from 1.4 to 2.6 ppb. Because batch identifiers were not consistently propagated to individual replicates in the combined dataset, we computed a single cross-batch mean DL for each analyte by averaging the six batch-specific values (the “mean” column in Table S1) and used this analyte-specific value for all ND substitutions, in consultation with NWRC. We derived a single concentration per owl per analyte by averaging the two replicates: if both replicates were ND, the concentration was set to 0 ng g^{–1} wet weight (hereafter ppb); if one replicate was ND, that replicate was replaced with $0.5 \times$ (cross-batch mean DL) before averaging with the quantified replicate. Discarding the ND replicate and using only the quantified value would overestimate concentration by treating a mixed-detection pair as a fully quantified sample; substituting $0.5 \times$ DL is the more conservative choice, acknowledging genuine analyte presence while avoiding upward bias. This approach is meant to reduce downward bias when averaging mixed ND/quantified pairs near method limits. As a worked example, the cross-batch mean DL for brodifacoum was 1.75 ppb; an owl with one replicate quantified at 1.5 ppb and the other ND would receive a replicate-averaged value of $[1.5 + (0.5 \times 1.75)]/2 = 1.19$ ppb—a concentration below every batch-specific DL (range 1.4–2.6 ppb) but derived from a genuine detection in one of two subsamples. Replicate-averaged

concentrations reported in Table 1 and used in downstream analyses can therefore fall below the nominal DL; such values reflect real analyte detection in one paired subsample rather than measurement below the method's sensitivity threshold. An analyte was considered detected if the replicate-averaged concentration exceeded 0 ppb after the above ND handling.

2.4. Statistical analysis

We evaluated three response variables: (1) exposure status (binary; > 0 ppb versus 0 ppb), (2) concentration (ppb), and (3) rodenticide richness (count of distinct compounds detected per owl, including cholecalciferol). For sex and age class comparisons, we fit generalized linear models (GLMs) using the *glmmTMB* package (v1.1.14; Brooks et al., 2017); for temporal and latitudinal analyses, we fit generalized additive models (GAMs) using the *mgcv* package (v1.9-4; Wood, 2017), with penalized cyclic or cubic spline smooths as appropriate. All models were fit in R (v4.5.2; R Core Team, 2026). Exposure was modeled with a binomial (logit) error family; concentration with negative-binomial (GLMs) or Tweedie (GAMs) families with a log link; and richness with Poisson or Conway-Maxwell Poisson families with a log link. The specific error family for each model was selected empirically based on dispersion diagnostics and is reported in Appendix A (Table S6–S27). Models with concentration as a response variable were fit to the full sample—including zeros assigned to non-detected individuals—rather than to positive individuals only. Model-estimated marginal means and smooth predictions therefore represent population-average concentrations that incorporate the large fraction of ND and are accordingly lower than the among-positives means reported in Table 1. Under this parameterization, predicted values can fall below the cross-batch mean DL. Juveniles ($n = 7$) and individuals of unknown sex ($n = 2$) were excluded from sex and age comparisons; final sample sizes for all analyses are reported in the Results and Appendix A. In addition to sex-,

age-, and time-based analyses, we evaluated broad latitudinal gradients across the regional dataset.

2.4.1. Sex and age-class effects

We tested sex and age-class differences in exposure, concentration, and richness for anticoagulants and cholecalciferol. Concentrations were integer-rounded prior to modeling to provide the count-valued input required by the negative-binomial family. Estimated marginal means (EMMs) were used for effect visualization and inference, with Tukey-adjusted pairwise contrasts among the three age classes (S1, S2, adult).

2.4.2. Rodenticide application records

To contextualize observed exposure patterns, we summarized annual totals from California Department of Pesticide Regulation application records for the five counties overlapping the regional study area (Del Norte, Humboldt, Trinity, Siskiyou, and Mendocino), filed by registered restricted-use applicators, aggregated to four groups—FGAR, SGAR, cholecalciferol, and bromethalin—and by individual compound. Annual totals (kg) were summed within group and compound by year. These summaries are descriptive and were not linked statistically to owl outcomes. Importantly, DPR records capture only applications by certified commercial applicators and do not include consumer or illicit use, which means that actual environmental loading of compounds that have been available over the counter is almost certainly underrepresented in these data.

2.4.3. Temporal trends in Hoopa Valley subadults

For Hoopa subadults (age classes S1 and S2), we modeled multi-year trends in exposure and concentration with GAMs including year as a smooth term. Fits are reported on the response scale with 95% confidence intervals.

2.4.4. Latitudinal gradients

To assess broad spatial structure within the regional dataset (2022–2025), we modeled latitudinal trends in exposure and concentration as smooth functions of latitude (UTM Zone 10N northing in km) using GAMs, to test the prediction that anticoagulant exposure in residents increases toward the Oregon border. This analysis was restricted to adults because established territory holders are more likely to reflect local exposures than dispersing subadults (Watson et al., 2024). Fits are reported on the response scale with 95% confidence intervals.

2.4.5. Supplementary analyses

We conducted three additional analyses. First, we modeled sex $\times$ age-class interactions for all rodenticides combined (anticoagulants and cholecalciferol), fitting GLMs for both exposure and concentration, with effects summarized via EMMs. Second, to assess within-year patterns, we fit GAMs of concentration as a cyclic smooth function of day-of-year with a random-effect smooth for year. Population-level seasonal effects were obtained by excluding the year term in prediction. Third, we tested whether cholecalciferol detection was associated with anticoagulant detection using a chi-square test of independence on the 2×2 table of exposure status, with the odds ratio and its 95% confidence interval from Fisher's exact method.

2.4.6. Diagnostics and presentation

Residual uniformity, dispersion, and zero-inflation were assessed with *DHARMa* (v0.4.7); GAM fit, including the adequacy of basis dimensions and the distribution of residuals, was evaluated with the *gam.check()* function in *mgcv*. Estimated marginal means, contrasts, and predictions were generated with *emmeans* (v2.0.2) and *ggeffects* (v2.3.2). Full model specifications are provided in Appendix A. Figures display predicted means with 95% confidence intervals.

Table 1

Exposure and concentrations of rodenticide residues detected in livers of Barred Owls ($n = 837$), summarized by rodenticide class and individual active ingredient. Exposure rate is the percentage of individuals with a detectable residue for the listed group, class, or chemical. Mean concentration and concentration range (ppb) are calculated among detections only; dashes indicate no detections.

Group, class, or chemical	Exposure rate (%)	Mean concentration (ppb) ^a	Range concentration (ppb) ^a
Anticoagulants	35.72	14.74	0.97–412.86
First-generation (FGAR)	1.19	42.96	0.83–401.08
warfarin	0.84	59.13	1.49–401.08
chlorophacinone ^b	0.12	2.05	2.05
coumachlor ^b	0.12	0.83	0.83
diphacinone ^b	0.12	12.85	12.85
coumatetralyl	0	—	—
coumafuryl	0	—	—
pindone	0	—	—
Second-generation (SGAR)	35.24	13.48	0.97–346.56
brodifacoum	30.59	10.14	1.11–320.50
bromadiolone	14.22	10.16	0.55–222.50
difethialone	1.91	10.40	1.29–69.05
difenacoum	0.36	1.08	0.97–1.20
flocoumafen ^b	0.12	3.30	3.30
Hypercalcemic rodenticides			
cholecalciferol ^c	18.52	12.16	2.21–132.25
Neurotoxic rodenticides			
bromethalin ^d	0	—	—

^a Concentration in positive individuals only.

^b Single detection.

^c Also an essential nutrient (vitamin D₃).

^d Assayed as the metabolite desmethyl-bromethalin.

3. Results

We analyzed liver tissue from 837 Barred Owls in the regional dataset (2022–2025) to estimate overall exposure and conduct demographic and spatial analyses, and from 292 Hoopa subadults (2019–2024; 166 from the pre-2022 series plus 126 additional subadults from 2022 onward) to assess temporal trends (Table S5).

Of 837 owls in the regional dataset, 35.7% had at least one anticoagulant detected in liver tissue (Table 1), with a mean concentration of 14.7 ppb among positive individuals (range 1.0–412.9 ppb); detections were driven almost entirely by SGARs, with FGARs rare (1.2% of all owls). Brodifacoum was the most frequently detected compound overall followed by bromadiolone; difethialone, difenacoum, and flocoumafen were each detected in fewer than 2% of individuals (Table 1). FGAR detections were similarly sparse: warfarin occurred in 0.8% of owls, and chlorophacinone, coumachlor, and diphacinone were each detected in a single individual (0.1%); coumatetralyl, coumafuryl, and pindone were not detected. Cholecalciferol was detected in 18.5% of all owls (mean 12.2 ppb, range 2.2–132.3), of which 83 (9.9% of all owls) carried no anticoagulant; the proportion of owls carrying at least one rodenticide compound therefore lies between 35.7% and 45.6%, depending on whether cholecalciferol detections are attributed to endogenous or exogenous sources. Cholecalciferol was detected more often in anticoagulant-positive than in anticoagulant-negative owls (24.1% vs. 15.4%; odds ratio 1.74, 95% CI 1.20–2.51; $\chi^2 = 9.54$, $df = 1$, $p = 0.002$). Bromethalin was not detected in any sample (see Discussion for interpretive caveats regarding tissue selection).

3.1. Rodenticide patterns by sex and age class

Females exhibited consistently higher rodenticide exposure and concentrations than males for both anticoagulants and cholecalciferol (Fig. 2; Table S6–S9). Anticoagulant exposure was 43% in females versus 29% in males ($\sim 1.5\times$ higher). Cholecalciferol exposure showed an even larger proportional difference (26% vs. 11%, $\sim 2.3\times$ higher). Mean concentrations followed the same pattern: anticoagulant concentrations averaged 8.0 ppb in females versus 2.9 ppb in males ($\sim 2.8\times$), and cholecalciferol averaged 3.3 vs. 1.3 ppb ($\sim 2.5\times$). All contrasts were supported at $p < 0.001$.

Across age classes, exposure rates and concentrations increased from S1 to S2 to adults for both anticoagulants and cholecalciferol (Fig. 3; Table S10–S13). Anticoagulant exposure rose from 18% (S1) to 29% (S2) to 42% (adults), such that adults were $\sim 2.3\times$ more likely to be exposed than S1 owls. Cholecalciferol exposure showed a similar but lower-prevalence pattern (10%, 12%, 22% for S1, S2, adults; $\sim 2.2\times$ higher in adults). Concentrations also increased with age class: anticoagulant concentrations averaged 2.4, 4.1, and 6.4 ppb ($\sim 2.7\times$ higher in adults than S1), and cholecalciferol averaged 1.1, 1.4, and 2.8 ppb ($\sim 2.5\times$ higher). Pairwise contrasts between S1 and adults were significant for all metrics; S1–S2 and S2–adult contrasts were variable.

Rodenticide richness showed the same demographic gradients (Fig. 4; Table S14–S15). Females carried more distinct compounds than males (0.87 vs. 0.49; $\sim 1.8\times$ higher). Richness increased with age class from 0.33 in S1 to 0.52 in S2 to 0.80 in adults ($\sim 2.4\times$ higher in adults than S1).

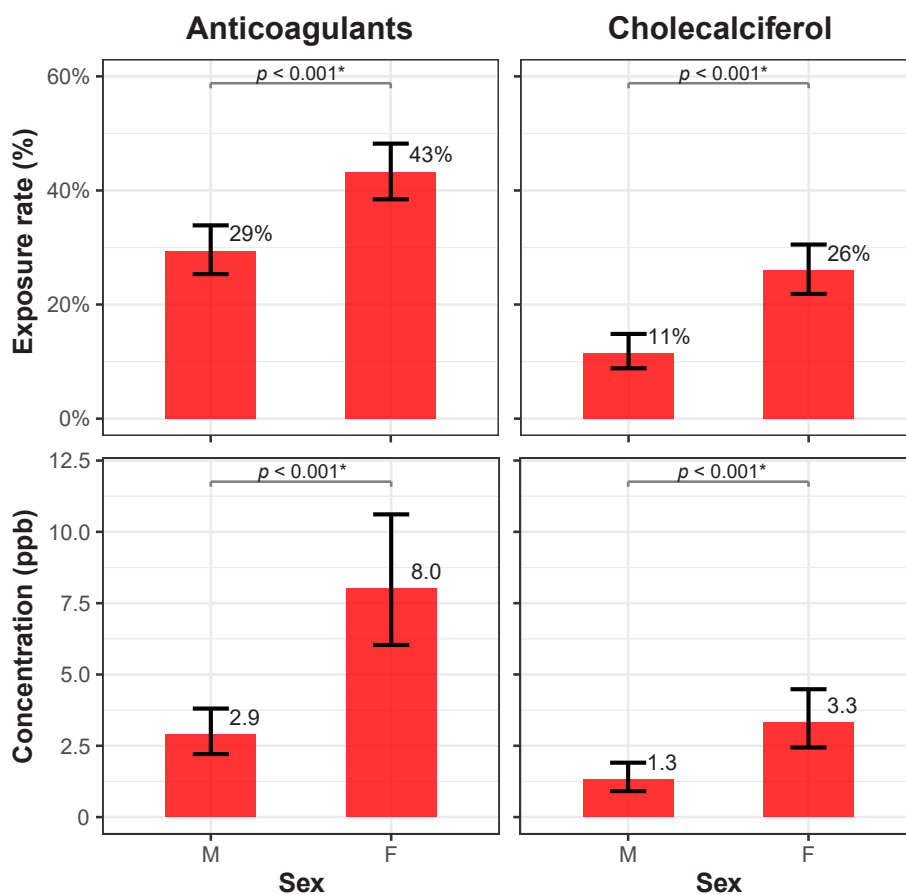


Fig. 2. Sex differences in rodenticide exposure for Barred Owls (*Strix varia*). Top row: exposure rate (percent positive) for (left) anticoagulant rodenticides (ARs) and (right) cholecalciferol, estimated from binomial models. Bottom row: mean liver tissue concentration (ppb) for the same compound classes, estimated from negative-binomial models fit to integer-rounded ppb values that include zeros (non-detects). Bars are model-estimated marginal means with 95% CIs. Brackets annotate p -values from the fitted models.

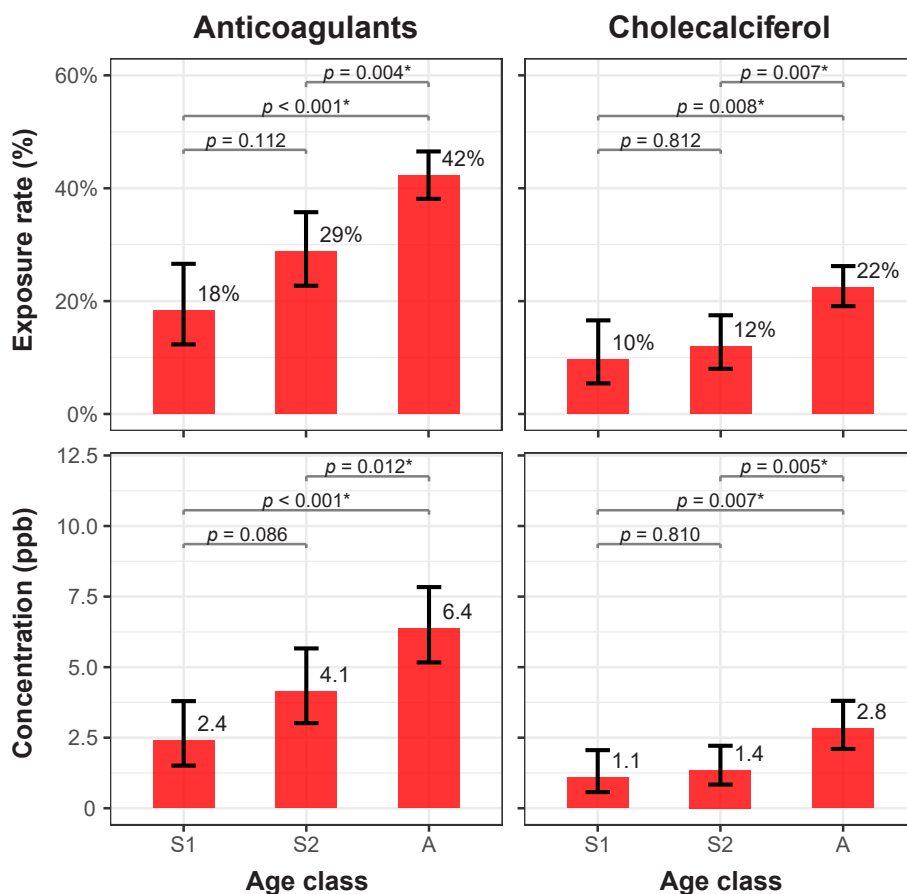


Fig. 3. Age-class differences in rodenticide exposure for Barred Owl (*Strix varia*). Top row: exposure rate (percent positive) for (left) anticoagulant rodenticides (ARs) and (right) cholecalciferol, estimated with binomial models. Bottom row: mean liver tissue concentration (ppb) for the same compound classes, estimated with negative-binomial models fit to integer-rounded ppb values that include zeros (non-detects). Bars are model-estimated marginal means with 95% CIs. Brackets show Tukey-adjusted pairwise contrasts among age classes S1 (young subadult), S2 (older subadult), and A (adult). Brackets annotate p -values from the fitted models.

3.2. Temporal trends in exposure and reported application

DPR restricted-use application records for the five study counties showed a post-2020 shift in reported applications—SGAR totals fell sharply by 2021, while cholecalciferol applications increased modestly and FGAR applications rose sharply from a low base (driven by dipha-chinone), with a small rise in bromethalin (Fig. S3).

Over the same period, Hoopa subadults exhibited concordant

changes (Fig. 5; Table S16–S19)—predicted anticoagulant exposure declined from ~50% in 2019 to ~20% in 2024 (GAM: $edf = 1.44$, $\chi^2 = 21.5$, $p < 0.001$), and concentrations peaked near ~20 ppb around 2020 before falling to ~5 ppb by 2024 ($edf = 2.70$, $F = 7.0$, $p < 0.001$). In contrast, cholecalciferol exposure increased from ~0% to ~6% ($edf = 1.01$, $\chi^2 = 5.14$, $p = 0.017$), with a parallel rise in concentrations ($edf = 1.03$, $F = 1.35$, $p = 0.016$). This reflects the fact that cholecalciferol was undetected before 2023 (0 of 180 birds) and present in 7 of 112 birds in

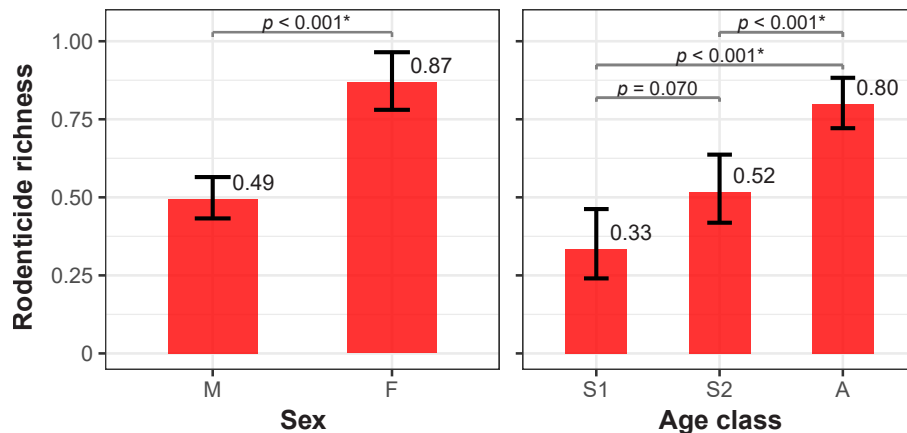


Fig. 4. Rodenticide richness for Barred Owls (*Strix varia*). Richness is the number of distinct rodenticide compounds detected per owl, including cholecalciferol (also a natural nutrient). Left: sex differences. Right: age-class differences (S1 = young subadult, S2 = older subadult, A = adult). Bars show model-estimated marginal means with 95% CIs. Brackets give p -values for pairwise contrasts (sex: M vs F; age class: Tukey-adjusted).

2023–2024 (Table S5).

3.3. Latitudinal gradients

Rodenticide metrics for adults varied nonlinearly with latitude (Fig. S4; Table S20–S23). Anticoagulant exposure showed a shallow U-shaped pattern, declining from southern latitudes toward mid-latitudes around Hoopa Valley and increasing again toward the Oregon border, although support for a latitudinal effect was comparatively weak (GAM: $edf = 1.96$, $\chi^2 = 4.80$, $p = 0.068$). Cholecalciferol exposure exhibited a much stronger latitudinal gradient, with high exposure in the south, a pronounced decline across mid-latitudes, and a rebound toward the north ($edf = 4.72$, $\chi^2 = 40.30$, $p < 0.001$). Anticoagulant concentrations varied strongly and nonlinearly with latitude, including localized peaks around mid-latitudes that did not align with the exposure pattern ($edf = 7.12$, $F = 3.79$, $p < 0.001$). Cholecalciferol concentrations broadly followed the exposure gradient, decreasing from south to mid-latitudes and remaining low with only a modest increase toward the north ($edf = 3.23$, $F = 5.58$, $p < 0.001$).

3.4. Supplementary analyses

Interaction models pooling rodenticide classes indicated that sex differences were modest in S1 but became more pronounced with age: females exceeded males in both exposure and concentration among S2

and adults (Fig. S5; Table S24–S25). Seasonal GAMs indicated higher anticoagulant concentrations in winter with a trough in late summer ($edf = 2.16$, $F = 1.15$, $p = 0.005$; Fig. S6; Table S26). Cholecalciferol concentrations showed little evidence of seasonality after accounting for among-year differences ($edf = 2.70$, $F = 4.43$, $p = 0.616$; Fig. S6; Table S27).

4. Discussion

More than a third (36%) of 837 Barred Owls sampled from predominantly non-urban forests carried at least one anticoagulant rodenticide in their liver tissue, and cholecalciferol, which no previous owl survey had screened for, was detected in a further 10% with no anticoagulant. Exposure was dominated by SGARs. With rare FGAR detections and relatively frequent cholecalciferol occurrence, these patterns reinforce growing evidence that rodenticides permeate forest food webs in western North America well beyond urban and agricultural settings (Franklin et al., 2018; Gabriel et al., 2018, 2012; Hofstadter et al., 2021; Wiens et al., 2019). Depending on the source of cholecalciferol, which the present data cannot resolve (Section 4.3), between 36% and 46% of owls carried at least one rodenticide compound. These prevalences are notable given that our sampling frame excludes moribund and dead individuals, meaning the true population-level burden is almost certainly higher. Anticoagulant prevalence (36%; Table 1) was similar to the 40% reported by Gabriel et al. (2018) in northwestern

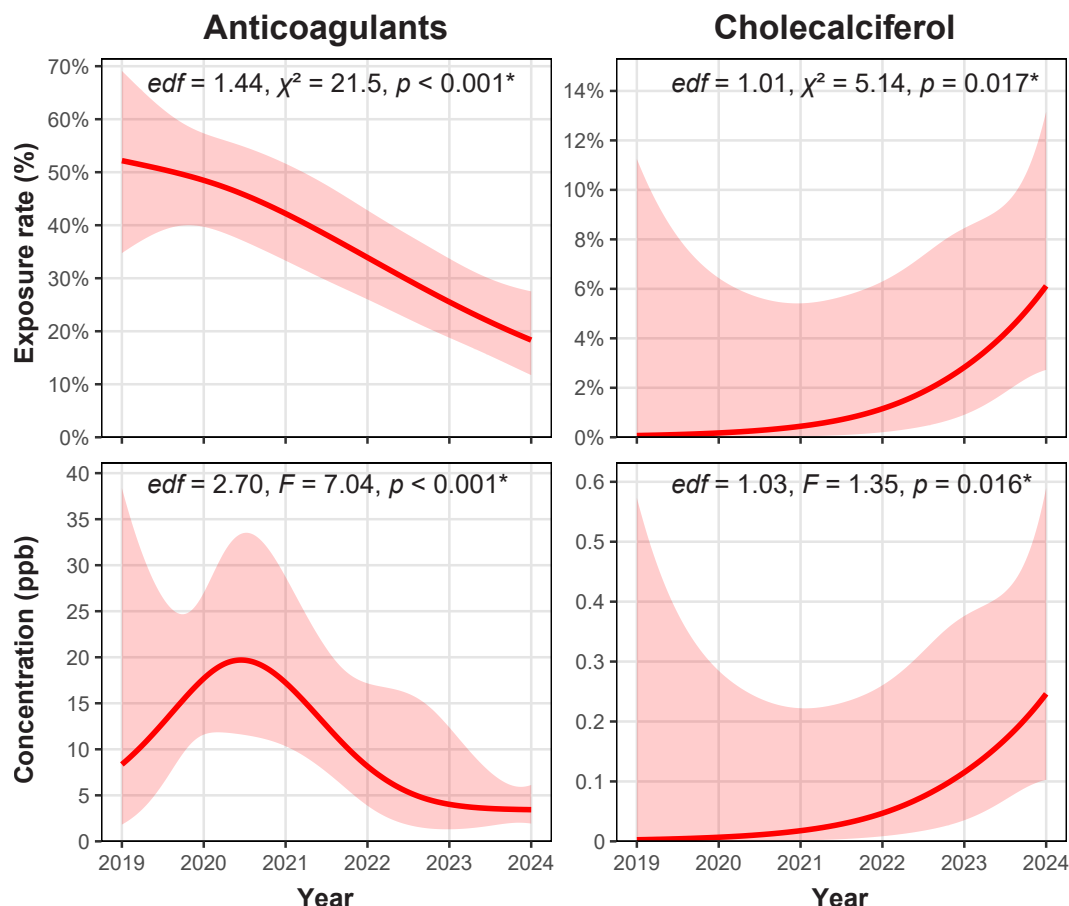


Fig. 5. Temporal trends in rodenticide exposure for subadult Barred Owls (*Strix varia*) removed in the Hoopa Valley study area (S1 and S2 only; $n = 15, 74, 54, 37, 20$, and 92 in 2019–2024). Top row: exposure rate for (left) anticoagulant rodenticides (ARs) and (right) cholecalciferol. Bottom row: liver concentration (ppb) for the same classes, fit to values that include non-detects. All panels are fits from generalized additive models (GAMs); lines are predicted means and bands are pointwise 95% confidence intervals for the expected response. Cholecalciferol was undetected before 2023 (0 of 180 birds) and present in 7 of 112 birds in 2023–2024; the right-hand panels reflect this emergence rather than a gradual rise across the full series. Panel annotations give the estimated degrees of freedom (edf), the smooth test statistic, and the associated p -value.

California and somewhat below the 48% reported by Wiens et al. (2019) across Oregon and Washington, though lower than the 62% reported by Hofstadter et al. (2021) in a northern California sample spanning the wildland-urban interface. That AR prevalence in our later, post-restriction sample remains near the lower end of this previously reported range suggests that anticoagulants continue to move through western forest food webs despite regulatory change (but see Section 4.1).

Mechanistically, SGAR dominance is expected as, relative to FGARs, SGARs exhibit single-feed lethality, high lipophilicity, and prolonged hepatic persistence that elevate secondary exposure and bioaccumulation in predators (Rattner et al., 2014; Van Den Brink et al., 2018). Because exposure risk is tightly coupled to product availability and use, California's progressive restrictions—retail limitations (2014), the SGAR moratorium under AB 1788 (2020), and FGAR restrictions under AB 2552 (2024)—established *a priori* predictions of declining SGARs in the environment and potential substitution toward non-anticoagulants. Our spatiotemporal analyses tested for these regulatory fingerprints across years and along the California–Oregon policy boundary.

4.1. Policy signals

The most striking pattern in the temporal data is a ~60% relative decline in anticoagulant exposure among Hoopa Valley subadults between 2019 and 2024—from approximately 50% to 20% of individuals carrying detectable residues—coincident with California's progressive restrictions on anticoagulants (Fig. 5). Liver concentrations fell concordantly, and county-level pesticide-use reports showed directionally consistent shifts: SGAR application totals decreased while cholecalciferol increased modestly and FGARs rose sharply (Fig. S3). Together, these patterns are consistent with a meaningful regulatory effect on wildlife exposure, with possible product substitution toward cholecalciferol. Because tissue metrics lag shifts in product use, the true environmental response may be faster than these data suggest.

Several mechanisms could link regulation to the observed decline. Removing SGARs from consumer shelves lowers the volume entering residential and agricultural settings and reduces the fraction of contaminated prey moving through forest food webs, and constrained retail supply also limits the material available for diversion to unlicensed cultivation. Trespass cannabis cultivation and the effort spent eradicating it vary from year to year, and fluctuations in rodent prey abundance change how much contaminated prey owls encounter in a given year. These pathways cannot be separated using the present data, but each is consistent with the direction and timing of the change we observed.

We avoided strong causal attribution for several reasons. DPR application records capture only certified commercial use and do not include consumer or household purchases, thus certainly underrepresenting environmental loading for some “legal” compounds. Illicit cannabis cultivation—historically a major source of SGAR contamination in these forests (Gabriel et al., 2012; Wengert et al., 2021)—is entirely unreported in regulatory databases. The scale of illicit use has been substantial as eradication operations in northern California have recovered tens of kilograms of pesticides, including brodifacoum products, from cannabis cultivation sites (Gabriel et al., 2012), quantities that dwarf the reported legal applications across entire counties. Nonetheless, the magnitude and timing of the observed decline—a ~60% reduction in exposure prevalence across years spanning the AB 1788 moratorium—suggest that regulatory action reached beyond certified commercial applicators, possibly by reducing household availability and, indirectly, illicit use. Brodifacoum concentrations in Barred Owls from western Canada likewise declined after federal restrictions in 2013 (Elliott et al., 2022), although responses have been inconsistent across North American jurisdictions (Elliott et al., 2024). The persistence of SGARs in the environment and the continued

operation of unlicensed cultivation sites mean that legacy and ongoing illicit inputs likely contribute to residual exposure (Butsic et al., 2018), though the overall trajectory is encouraging.

Among adult (resident) owls, rodenticide metrics showed class-specific latitudinal structure (Fig. S4). Anticoagulant exposure varied weakly with latitude, whereas cholecalciferol exposure showed a stronger gradient. The predicted increase toward the Oregon border received only weak support, with adult anticoagulant exposure rising modestly toward the north, a pattern consistent with (but not diagnostic of) less restricted SGAR use in Oregon. We treat these patterns as descriptive regional structure and interpret them conservatively, because latitude is a proxy for multiple correlated gradients—jurisdiction, land use, human settlement density, and historical and current cannabis cultivation intensity—that were not partitioned in this analysis.

4.2. Demography and cumulative risk

Across the regional dataset, females exceeded males in exposure, concentrations, and compound richness, and anticoagulant metrics increased across age classes from S1 to S2 to adults (Figs. 2 and 3). These demographic gradients are consistent with the persistence and bioaccumulation potential of SGARs in upper-trophic-level predators (Rattner et al., 2014; Van Den Brink et al., 2018). Our female-biased burdens parallel patterns reported for Barred Owls in California (Hofstadter et al., 2021), but differ from Oregon and Washington studies that found widespread exposure but no detectable sex or age differences (Wiens et al., 2019), suggesting context dependence among landscapes or sampling frames.

Sex differences could plausibly reflect a combination of physiology and behavior. Females bear substantial energetic demands during egg production and incubation, periods during which males provision them and elevated food intake over extended periods could increase cumulative exposure. Accordingly, fat deposition in Tawny Owls (*Strix aluco*) preceded the breeding season and then dropped (Hardy et al., 1981). This pattern matches the seasonality in AR concentration we found here (highest in winter and lowest in late summer; Fig. S6), but whether this reflects reproductive mobilization of lipophilic contaminants (e.g., into developing eggs) warrants investigation. The lack of strong seasonality in cholecalciferol concentrations argues against a purely seasonal reproductive explanation, but targeted sampling of females during egg laying could help resolve this.

Whether these burdens carry population-level consequence depends on how they compare with published toxicity criteria. For Strigidae, liver SGAR concentrations of 14.5 and 107 ppb correspond to 0.20 and 0.50 probabilities of diagnosed coagulopathy (Elliott et al., 2024). Here, 7.6% of owls exceeded the lower value (21.7% of SGAR-positive individuals) and 0.5% exceeded the upper. Both are lower bounds because our sampling frame excludes moribund and dead birds, and because exposure was concentrated in adults and females—the classes contributing most to population growth in long-lived species. The conservation implications of this demographic structure are direct. Northern Spotted Owls, under sustained competitive pressure from Barred Owls, are declining at 2–9% annually across their range, with adult survival identified as the dominant demographic parameter (Franklin et al., 2021; Lesmeister et al., 2026). This vulnerability is further amplified by diet-mediated exposure risk as Spotted Owls consume a higher proportion of rodent prey, particularly flying squirrels (*Glaucomys* spp.) and woodrats (*Neotoma* spp.), whereas Barred Owls have a broader diet that includes a wider range of non-rodent prey (Hamer et al., 2001; Wiens et al., 2014). Consistent with this risk pathway, Gabriel et al. (2018) documented AR exposure in 70% of Northern Spotted Owls sampled, compared with 40% of Barred Owls. Persistent rodenticides therefore represent a potential additive risk—whether acute or chronic—that can compound other stressors in a species already on a trajectory toward extirpation without intervention. Barred Owl removal experiments have

helped stabilize local Northern Spotted Owl occupancy in parts of the range (Diller et al., 2016; Hobart et al., 2025; Wiens et al., 2021). Reducing rodenticide inputs is a parallel and complementary pathway for lowering cumulative risk in the same forest food webs.

The food-web contamination documented here is not limited to owls. The same prey base sustains other forest carnivores whose populations are already vulnerable. For example, 85% of fishers tested across California's two isolated populations were exposed to at least one AR, predominantly SGARs, and direct AR toxicosis was a confirmed cause of mortality (Gabriel et al., 2015, 2012). Because primary consumers encounter bait directly, their body burdens are likely higher than what secondary consumers such as owls accumulate, and rodent mortality from bait consumption can depress prey populations independently of any secondary poisoning pathway. Together, secondary poisoning and prey-base depletion could turn contaminated landscapes into ecological sinks for predator populations—a risk that remains largely unquantified but is plausible given the scale of contamination documented here and in prior work.

4.3. Cholecalciferol: rodenticide or natural baseline?

Interpreting cholecalciferol detections requires care because cholecalciferol is both a rodenticide active ingredient and an essential nutrient. Sources of cholecalciferol in vertebrates are diet and endogenous UV-mediated synthesis. In birds, feather coverage limits direct cutaneous synthesis—the precursor 7-dehydrocholesterol is concentrated in unfeathered skin (Schutkowski et al., 2013)—and 7-dehydrocholesterol of uropygial origin spread onto feathers is photoconverted to cholecalciferol by UV exposure and ingested during preening (Uva et al., 1983). For nocturnal, densely feathered species such as Barred Owls, these UV-dependent pathways are likely limited, and diet is presumably the dominant source. Barred Owls' prey—small mammals and birds—also carry endogenous cholecalciferol. Detection in owl livers, particularly at low concentrations, is therefore not diagnostic of rodenticide exposure.

Our data are consistent with a mix of natural and anthropogenic inputs. First, liver concentrations in positive owls (mean 12.2 ppb, range 2.2–132.3 ppb) overlap closely with values reported for mammalian liver (~20 ppb on average, range 0.5–141 ppb; Schmid and Walther, 2013), making it plausible that owls attain these levels via ordinary dietary and endogenous sources. Second, cholecalciferol was detected across all age classes, including young S1 birds, consistent with a physiological baseline rather than an exposure that accumulates only with time on the landscape.

At the same time, cholecalciferol detections increased from 2019 to 2024 in Hoopa subadults (Fig. 5, Table S5), a pattern not likely attributable to tissue storage time (see Section 4.5), and age-class patterns show rising prevalence and concentrations from S1 to adults (Fig. 3). County-level DPR records indicate modest increases in cholecalciferol applications over the same period. Cholecalciferol was also detected more often in owls that carried anticoagulants than in those that did not, an association not expected from endogenous synthesis alone but consistent with co-exposure to baits deployed in the same landscapes. The simplest explanation is that two sources overlap: a dietary and physiological baseline present in all owls regardless of baiting, and bait-derived inputs—consistent with product substitution—that raise exposure where and when cholecalciferol products are used. Low-ppb detections should not be attributed to rodenticide exposure by default, but cholecalciferol warrants continued surveillance alongside anticoagulants. Future work should collect kidneys and other target tissues for histopathological evaluation of hypercalcemia—the diagnostic hallmark of cholecalciferol toxicosis—to distinguish endogenous baselines from bait-derived inputs.

4.4. Bromethalin: absence of evidence is not evidence of absence

Bromethalin (tested as desmethyl-bromethalin) was not detected in any of 837 liver samples. Liver is not the standard tissue for bromethalin analysis, because desmethyl-bromethalin accumulates preferentially in adipose tissue and, to a lesser extent, the central nervous system (Mercer et al., 2022; Murray and Cox, 2023). Although desmethyl-bromethalin has been detected in liver tissue of clinically affected birds (Sant et al., 2019), concentrations sufficient to produce a liver detection would likely be acutely fatal, meaning lethally exposed individuals are unlikely to respond to playback-based sampling—further biasing estimates toward non-detection. Additionally, bromethalin's short reported plasma half-life and susceptibility to photodegradation reduce detection probability even where exposure has occurred (Sant et al., 2019). Secondary toxicosis in predators feeding on recently exposed prey is considered biologically plausible, and desmethyl-bromethalin was detected in 29.5% of dead raptors in one North American study (Murray and Cox, 2023). Field observations from cannabis cultivation sites in the study region document increasing bromethalin use in recent years (G.M. Wengert, unpublished data), consistent with the modest rise in DPR-reported applications (Fig. S3). Future studies should prioritize adipose tissue to obtain an accurate assessment of bromethalin exposure in forest wildlife, while recognizing that secondary exposure risk for bromethalin may be inherently lower and shorter-lived than for SGARs.

4.5. Study strengths and limitations

The principal strength of this study is its sampling frame. Systematic removals across predominantly non-urban forests decouple specimen collection from human proximity. Barred Owls are effective sentinels of forest contamination (Gabriel et al., 2018; Hofstadter et al., 2021; Wiens et al., 2019), and the scale of our dataset—over one thousand individuals spanning a broad spatiotemporal gradient—provides substantial power for detecting demographic and spatial patterns. Analytical consistency (single laboratory, duplicate subsamples) further supports comparability across the dataset.

However, several limitations qualify our findings. Removals rely on vocalization playback and individuals that are moribund, behaviorally impaired, or already dead from rodenticide toxicosis are less likely to respond, meaning our exposure and concentration estimates are likely conservative for the population at large. Coverage was also restricted to road-accessible terrain, leaving roadless areas unsampled. In contrast, carcass-based datasets are biased toward severely affected animals and can inflate apparent burdens (Wobeser, 2007). Among 208 Barred Owls recovered as carcasses in western Canada, 96% carried an SGAR residue at a mean of 130 ppb (Elliott et al., 2022), roughly triple our exposure rate and an order of magnitude above our concentrations. These opposing biases underscore that exposure metrics are dependent on collection methods and likely not directly comparable across studies. Some early specimens (2019–2022) were stored frozen as whole carcasses for up to several years before necropsy and while vitamin D appears stable in frozen biological tissues for periods of months to several years (Fu et al., 2021; Ko et al., 2026), we cannot entirely exclude the possibility that prolonged storage affected analyte recovery for some compounds. SGAR residues can persist in liver for months to years, so tissue concentrations in adults may archive exposures from well before the current regulatory regime, and temporal trends in tissue data will lag changes in product use (Rattner et al., 2014; Wiens et al., 2019). For cholecalciferol, baseline occurrence and tissue kinetics in *Strix* remain poorly characterized and distinguishing endogenous from exogenous sources at the individual level will require targeted histopathology. For bromethalin, liver is a suboptimal tissue that likely underestimates true exposure. Policy inferences are correlative because DPR data capture only commercial restricted-use applications and do not reflect consumer use or illicit applications. An additional jurisdictional nuance applies to the Hoopa temporal series as the Hoopa Valley Reservation is tribal

sovereign land subject to federal rather than state pesticide regulations, meaning restricted-use SGARs remained legally available to certified applicators there after California's state moratorium took effect. Because subadults sampled at Hoopa likely acquired much of their exposure outside reservation boundaries given observed dispersal distances in this population (Watson et al., 2024), the regulatory environment experienced by individual owls is difficult to characterize precisely, adding complexity to the interpretation of temporal trends at this site. Finally, this study documents exposure and concentration patterns but does not assess sublethal or lethal toxicological effects, which have not been conclusively demonstrated in *Strix* owls in this region.

5. Conclusion

Rodenticides are widespread in forest food webs of western North America, even in landscapes far from urban and agricultural centers. In over 1000 Barred Owls from northwestern California, exposure was dominated by SGARs, structured by sex and age, and accompanied by frequent cholecalciferol detection—patterns consistent with cumulative risk from persistent compounds and shifting product use under tightening regulation. More than one in three apparently healthy, behaviorally responsive owls carried anticoagulant residues, up to 46% carried at least one rodenticide compound, and because sampling excluded impaired individuals, the true population burden is almost certainly higher. The demographic consequences for Northern Spotted Owls, fishers, and other conservation-dependent species sharing these food webs—whether through direct toxicosis, sublethal impairment, or prey-base depletion—remain insufficiently quantified. That a regulatory signal was detectable in owl tissue within four years of California's SGAR moratorium shows sentinel monitoring can keep pace with policy—a feedback loop conservation rarely gets.

CRedit authorship contribution statement

Vitek Jirinec: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mourad W. Gabriel:** Writing – review & editing, Resources, Methodology, Conceptualization. **M. Zachariah Peery:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **J. Mark Higley:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Daniel F. Hofstadter:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Christina P. Varian:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **Angela Rex:** Writing – review & editing, Methodology, Investigation, Data curation. **Alan B. Franklin:** Writing – review & editing, Conceptualization. **Robert H. Poppenga:** Writing – review & editing, Conceptualization. **Leslie W. Woods:** Writing – review & editing, Conceptualization. **Steven F. Volker:** Data curation, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. **Greta M. Wengert:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declarations

Forest food web contamination by rodenticides is pervasive but responsive to regulation: Insights from Barred Owl biomonitoring (ENVPOL-D-26-05998R1)

Ethics declaration

Animal subject

This study was conducted in accordance with the following

guidelines for animal welfare and/or reporting: Stipulations in state (S-193180001-19337-001) and federal (MB24592D) permits. This study was approved by the University of Wisconsin–Madison IACUC (Approval No. A006106-R03).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Claude in order to copyedit the manuscript and debug R code. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. Mark Higley reports a relationship with Hoopa Valley Tribe that includes: funding grants. M. Zachariah Peery reports a relationship with University of Wisconsin–Madison that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2026.129205>.

Data availability

Data will be made available on request.

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Appendix A

Supporting information

for the manuscript

Forest food web contamination by rodenticides is pervasive but responsive to regulation: Insights from Barred Owl biomonitoring

Environmental Pollution

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SUPPLEMENTARY LABORATORY METHODS

Materials

Desmethyl des-2,4,6-tribromo bromethalin (DMDBB) and d₄-brodifacoum were purchased from Toronto Research Chemicals (TRC), Toronto, Ontario, Canada. Coumatetralyl-d₄, d₄-difenacoum, d₄-difethialone, and d₄-flocoumafen were obtained from LGC Standards, Guildford, UK. Pindone, flocoumafen, pyrogallol, 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), vitamin D₃, and d₃-vitamin D₃ were obtained from Sigma-Aldrich, St. Louis, MO. Automated handheld powder dispenser, SDH-1 with 150 mg dispensing disk, XQ Instruments, Singapore. Autosampler vial inserts (0.350-mL) were from Agilent Technologies, Santa Clara, CA. All other materials were described in Franklin et al. (2018).

Liver extraction

Owl livers were extracted using a modified version of the procedure described in Franklin et al. (2018). In addition to the 12 rodenticides described in the 2018 publication, flocoumafen and vitamin D₃ (cholecalciferol) were added to the method – as well as the internal standards (ISTDs) d₄-difenacoum, d₄-brodifacoum, d₄-difethialone, d₄-flocoumafen, and DMDBB. The derivatization procedure for vitamin D₃ was adapted from Yadav et al. (2021) and incorporated into the extraction procedure.

Owl livers were homogenized with a liquid nitrogen cryogenic mill and stored frozen in vacuum sealed bags. Duplicate subsamples of homogenized liver (90–110 mg) were weighed into 1.5-mL microcentrifuge tubes. DI water (0.10 mL) was added and the sample vortex mixed thoroughly to form a uniform suspension. ISTDs were added to all samples (0.020 mL acetonitrile (ACN) containing 37 µg/mL d₅-bromadiolone and DMDBB; 27 µg/mL d₄-diphacinone and d₄-chlorphacinone; 20 µg/mL d₄-coumatetralyl, d₄-difenacoum, d₄-brodifacoum, and d₄-difethialone; 17 µg/mL d₅-warfarin; and 10 µg/mL d₄-flocoumafen and 0.005 mL ACN containing 15 µg/mL d₃-vitamin D₃). QC samples, included with each analysis, were prepared from control liver and fortified at three levels with ACN solutions containing pindone, coumatetralyl, coumafuryl, warfarin, diphacinone, coumachlor, chlorphacinone, difenacoum, brodifacoum, bromadiolone, difethialone, flocoumafen, desmethyl bromethalin, and vitamin D₃. ACN was added to unknown and QC samples to achieve a total ACN volume of 1.20 mL, and then vortex mixed 10–15 s. Excess NaCl (160 mg) was added using an automated powder dispenser, vortex mixed 10–15 s, followed by centrifugation at 12,000 x g for 1 min. A portion of the upper ACN phase was transferred to a 2-mL dSPE tube containing 25 mg C₁₈ sorbent, 25 mg primary-secondary amine (PSA) sorbent, and 150 mg MgSO₄. The mixture was vortex mixed 2–3 s, and then centrifuged at 12,000 x g for 1 min. One portion of the supernatant (0.40 mL) was transferred to a 1.5-mL microcentrifuge tube and evaporated to dryness with N₂ gas in a 40–60°C

water bath (25–30 min.). The analytes were reconstituted with 0.100 mL ACN (mobile phase B) followed by 0.400 mL pH 9.5 20-mM ammonium acetate (mobile phase A), with vortex mixing after each addition. The sample was decanted into an amber glass autosampler vial for analysis by LC-MS/MS Method A. A second portion of the dSPE supernatant (0.15 mL) was transferred to an amber glass screw-top 2-mL autosampler vial with 0.350-mL glass insert. Pyrogallol (200 mg/mL in ACN) and freshly prepared PTAD (100 mg/mL in ACN) were added, capped with a PTFE-lined cap, vortexed briefly, and then reacted at RT for 2–3 hours in a dark location. Analysis was by LC-MS/MS Method B.

LC-MS/MS Method A (13 rodenticides)

The UHPLC-ESI-MS/MS conditions are described in Franklin et al. (2018), with the following modifications: flow rate 0.900 mL/min; gradient program held at 10% B for 0.20 min, increased linearly to 64% over 4.3 min, increased to 100% B over 0.1 min and held for 0.49 min, and finally re-equilibrated at 10% B for 0.34 min. Drying gas (N₂) was 225°C (6 L/min), nebulizer pressure 40 psi, sheath gas 12 L/min at 375°C, and capillary voltage -3500 V. Delta EMV was -100 V for all stored MRMs. Table S2 presents the MS/MS parameters for each analyte. The chromatography is illustrated in Figure S1 for a fortified QC quail liver.

A 7-level calibration curve was constructed from the peak area response ratio (analyte/internal standard) for each analyte ranging from approximately 0.2 to 950 ng/mL. Diphacinone-d₄ was used as the ISTD for coumafuryl, pindone, and coumachlor since deuterium-labelled analogs were not commercially available. Each calibration curve was fit to a second-order quadratic function weighted either 1/X or 1/X² and achieved correlation coefficients (R²) ranging from 0.996 to 0.9999. Detection limits (DL) and quantitation limits (QL) were estimated from 3X and 10X signal-to-noise, respectively, from fortified QC samples from each analysis (Table S1). Table S4 presents the fortification concentrations, accuracies, and precision for each rodenticide.

LC-MS/MS Method B (vitamin D₃)

Each sample (7.5 µL) was chromatographed at 0.800 mL/min on the same column used for Method A heated to 50°C. Mobile phase A was 0.1% formic acid, and mobile phase B was 0.1% formic acid in methanol. The gradient program was held at 85% B for 0.4 min, increased linearly to 100% over 1.10 min, held for 0.40 min, and then re-equilibrated at 85% B. The drying gas was 5 L/min at 300°C, nebulizer pressure 45 psi, sheath gas 11 L/min at 250°C, nozzle voltage 500 V, and capillary voltage 3500 V. Delta EMV was 100 V for all stored MRMs. Table S3 presents the MS/MS parameters for derivatized vitamin D₃ and d₃-vitamin D₃. The chromatography is illustrated in Figure S2 for a fortified QC quail liver.

A 7-level calibration curve was constructed from the peak area response ratio (PTAD-vitamin D₃/PTAD-d₃-vitamin D₃) ranging from approximately 1 to 700 ng/mL. The calibration curve was

fit to a second-order quadratic function weighted $1/X$ and achieved correlation coefficients (R^2) ranging from 0.9998 to 0.9999. Detection limits (DL) and quantitation limits (QL) were estimated from 3X and 10X signal-to-noise, respectively, from fortified QC samples from each analysis (Table S1). Table S4 presents the fortification concentrations, accuracies, and precision for vitamin D₃.

Table S1. Detection Limits (DL) and Quantitation Limits (QL) in rodenticide laboratory analyses. All values are parts per billion (ppb).

Analyte	Limit	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean
pindone	DL	23.000	22.000	26.000	16.000	23.000	31.000	23.500
coumatetralyl	DL	0.600	1.000	0.690	0.600	1.400	0.710	0.833
coumafuryl	DL	0.960	0.300	0.430	0.300	0.690	0.500	0.530
warfarin	DL	2.100	2.000	2.700	1.800	3.800	1.500	2.317
diphacinone	DL	2.700	3.000	6.200	5.700	4.500	4.800	4.483
coumachlor	DL	0.140	0.130	0.200	0.170	0.430	0.290	0.227
chlorophacinone	DL	1.500	1.100	3.600	1.100	3.500	0.790	1.932
difenacoum	DL	1.100	1.100	4.800	0.500	0.910	0.460	1.478
brodifacoum	DL	1.500	1.400	1.400	2.600	1.500	2.100	1.750
bromadiolone	DL	0.380	0.910	2.200	1.500	1.400	0.740	1.188
difethialone	DL	1.700	1.800	2.400	3.000	1.300	3.300	2.250
flocoumafen	DL	0.240	0.260	0.360	0.280	1.000	2.000	0.690
desmethyl bromethalin	DL	3.700	8.400	5.600	3.900	4.400	6.200	5.367
cholecalciferol	DL	5.100	4.500	6.700	1.900	2.300	4.500	4.167
pindone	QL	76.400	74.700	86.500	54.400	77.900	103.000	78.817
coumatetralyl	QL	2.000	3.450	2.290	1.990	4.620	2.350	2.783
coumafuryl	QL	3.210	1.010	1.430	1.010	2.310	1.680	1.775
warfarin	QL	6.900	6.730	9.010	6.110	12.800	4.950	7.750
diphacinone	QL	9.050	10.100	20.500	19.000	15.000	15.900	14.925
coumachlor	QL	0.471	0.445	0.657	0.562	1.450	0.971	0.759
chlorophacinone	QL	5.010	3.690	12.000	3.820	11.700	2.640	6.477
difenacoum	QL	3.800	3.630	15.900	1.670	3.040	1.550	4.932
brodifacoum	QL	5.150	4.710	4.660	8.590	5.080	6.880	5.845
bromadiolone	QL	1.270	3.020	7.290	5.030	4.740	2.460	3.968
difethialone	QL	5.590	5.910	7.970	10.100	4.220	11.100	7.482
flocoumafen	QL	0.803	0.854	1.210	0.950	3.350	6.780	2.325
desmethyl bromethalin	QL	12.400	27.900	18.600	13.100	14.800	20.600	17.900
cholecalciferol	QL	17.100	15.100	22.500	6.450	7.780	14.800	13.955

Table S2. Method A: Agilent G6470A/G6475A MS/MS—13 rodenticides and 10 ISTDs.

Time segment # ^a	Analyte	Precursor ion (m/z)	Product ion (m/z) ^b	Fragmentor (V)	Collision energy (V)	Dwell (ms)	CAV	Retention time (min)
2	coumafuryl	297.1	161.0 240.0	92	17 21	160	2 5	0.5
	d ₅ -warfarin *	312.1	161.0	122	21	20	3	0.9
	warfarin	307.1	250.0 161.0	110	24 20	20	3 5	0.9
3	pindone	229.1	172.0 116.0	105	21 37	60 75	5 2	0.9
	d ₄ -coumatetralyl *	295.1	141.0	126	29	35	2	1.0
	coumatetralyl	291.1	141.0 247.1	126	29 25	35 35	2 5	1.0
4	coumachlor	341.1	284.0 161.0	128	26 22	80 80	2 5	1.4
	d ₄ -diphacinone *	343.1	167.0	119	26	100	5	2.0
5	diphacinone	339.1	167.0 172.0	116	23 19	100 100	5 5	2.0
	d ₄ -chlorophacinone *	377.1	201.0	122	22	100	4	2.4
6	chlorophacinone	373.1	201.0 145.0	116	23 23	100 100	4 5	2.4
	d ₅ -bromadiolone *	530.1	255.0	199	40	50	3	2.8, 2.9 ^c
	bromadiolone	525.1	250.0 181.0	208	41 37	50 50	3 4	2.8, 2.9 ^c
7	d ₄ -difenacoum *	447.2	293.1	197	34	50	3	2.8
	difenacoum	443.2	293.1 135.0	197	34 38	50 50	3 5	2.8
	d ₄ -brodifacoum *	525.1	78.9	212	50	20	5	3.2
	brodifacoum	521.1	135.0 78.9	213	44 52	20 20	4 2	3.2
	d ₄ -flocoumafen *	545.2	386.1	158	29	10	5	3.3
	flocoumafen	541.2	382.1 160.9	192	26 38	10 10	2 5	3.3
8	d ₄ -difethialone *	541.1	78.9	207	50	20	2	3.4
	difethialone	537.0	78.9 151.0	212	50 42	20 20	5 2	3.4
	desmethyl des-2,4,6- tribromo bromethalin *	326.0	279.0	118	29	20	5	3.5
	desmethyl bromethalin	561.7	355.9 401.9	127	54 38	30 30	2 2	3.9

* internal standard (ISTD); ^a Segments 1 and 9 diverted to waste; ^b **Bold** = quantifier product ion;^c Stereoisomers

Table S3. Method B: Agilent G6470A MS/MS—vitamin D₃.

Time segment # ^a	Analyte	Precursor ion (m/z)	Product ion (m/z) ^b	Fragmentor (V)	Collision energy (V)	Dwell (ms)	CAV	Retention time (min)
2	PTAD-d ₃ -vitamin D ₃ *	563.5	301.1	110	17	100	7	1.0
	PTAD-vitamin D ₃	560.5	298.1	110	10	100	7	1.0
			280.0		32	100	7	

* internal standard (ISTD); ^a Segments 1 and 3 diverted to waste; ^b **Bold** = quantifier product ion

Table S4. Fortified QC results—14 rodenticides—accuracy and precision.

Analyte	ISTD	<i>n</i>	Fortification range (ng/g)	Accuracy range (%)	Mean Accuracy (%) [75-125%]	sd (%)	CV (%)
pindone	d ₄ -diphacinone	76	61 - 6600	31.1% - 89.3%	47.4%	10%	22%
coumatetralyl	d ₄ -coumatetralyl	76	23 - 2500	56.8% - 183%	107%	20%	19%
coumafuryl	d ₄ -diphacinone	76	15 - 1600	27.6% - 197%	116%	40%	35%
warfarin	d ₅ -warfarin	76	23 - 2400	85.5% - 115%	98.7%	5.5%	5.6%
diphacinone	d ₄ -diphacinone	76	25 - 2700	78.9% - 121%	98.6%	4.9%	5.0%
coumachlor	d ₄ -diphacinone	76	13 - 1400	68.4% - 339%	174%	61%	35%
chlorophacinone	d ₄ -chlorophacinone	76	25 - 2700	81.5% - 110%	92.8%	5.9%	6.3%
difenacoum	d ₄ -difenacoum	76	20 - 2200	87.2% - 113%	98.6%	4.1%	4.2%
brodifacoum	d ₄ -brodifacoum	76	23 - 2500	87.1% - 118%	101%	5.1%	5.1%
bromadiolone	d ₅ -bromadiolone	76	34 - 3700	80.1% - 114%	100%	4.9%	4.9%
difethialone	d ₄ -difethialone	76	24 - 2500	87.3% - 113%	99.0%	5.1%	5.2%
flocoumafen	d ₄ -flocoumafen	76	16 - 1700	84.3% - 113%	98.6%	4.2%	4.2%
desmethyl bromethalin	desmethyl des- 2,4,6-tribromo bromethalin	76	47 - 5000	83.4% - 325%	146%	43%	30%
vitamin D ₃	d ₃ -vitamin D ₃	76	110 - 12000	92% - 129%	107%	7.7%	7.2%

Control liver: Quail (RodentPro), *n* = 21; Barred Owl, *n* = 55

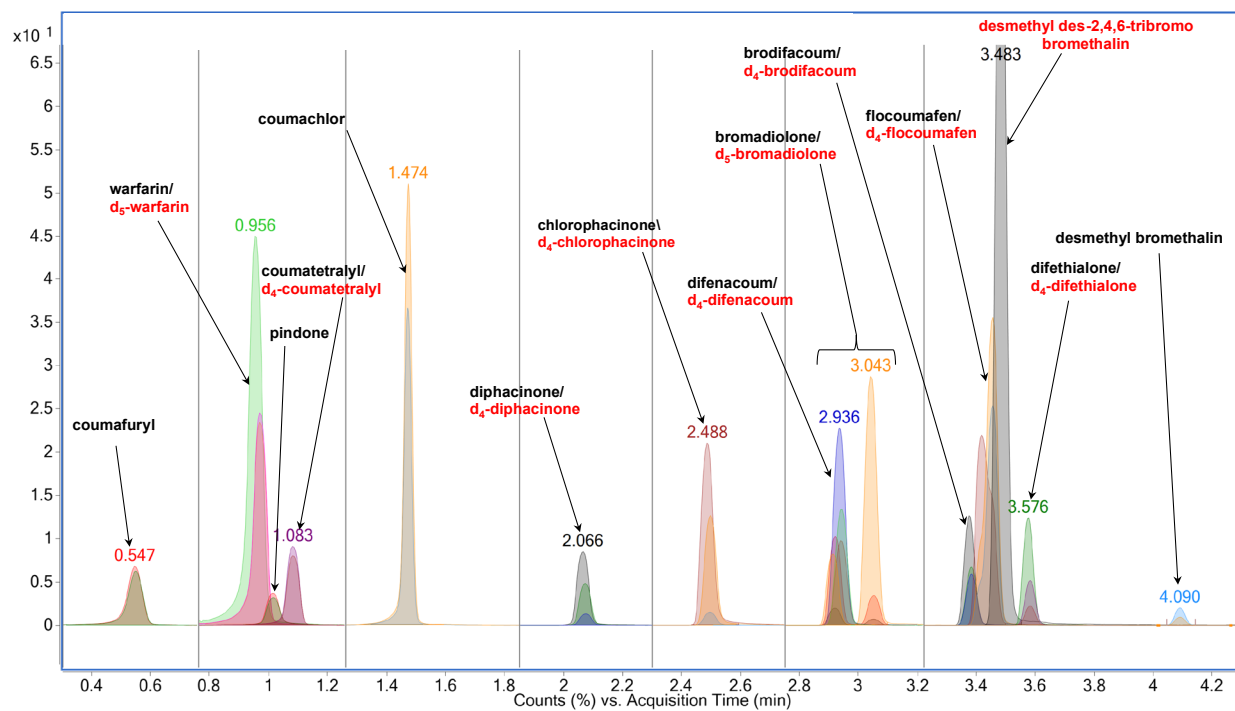


Figure S1. LC-MS/MS Method A: fortified QC quail liver, 13 rodenticides and 10 internal standards.

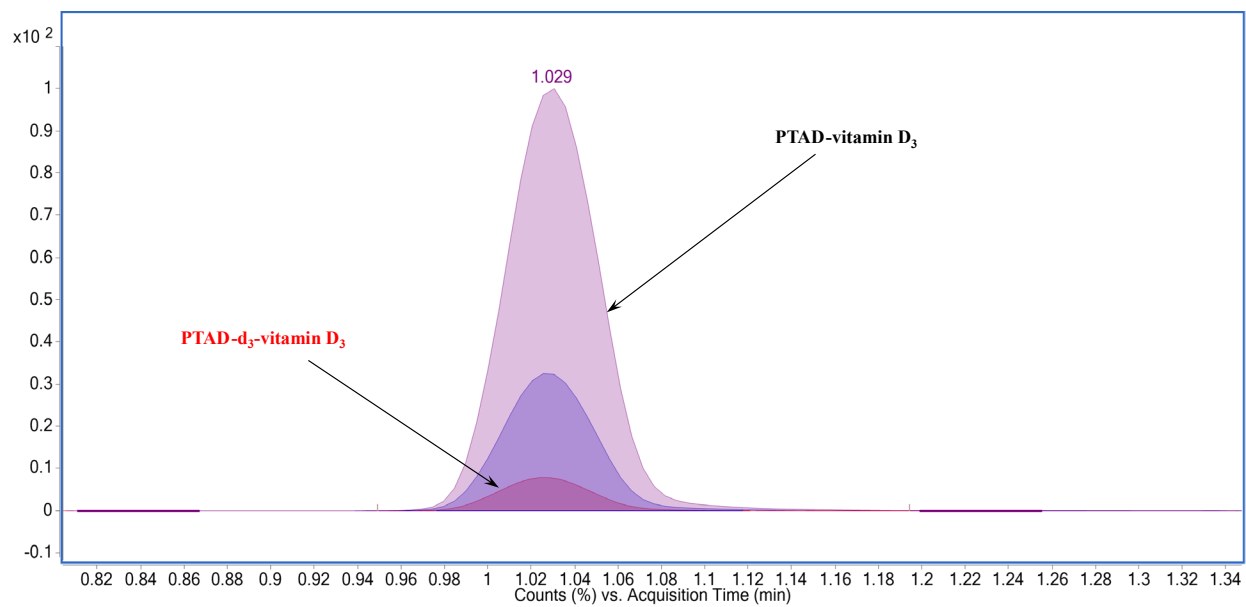


Figure S2. LC-MS/MS Method B: fortified QC quail liver, derivatized vitamin D₃ and d₃-vitamin D₃ internal standard.

SUPPLEMENTARY RESULTS TABLES

Table S5. Sample sizes by year, sex, anticoagulant rodenticide (AR), and cholecalciferol (VD3) exposure for subadult Barred Owls collected on the Hoopa study area.

Year	Total	Males	Females	AR positive	VD3 positive
2019	15	6	9	4	0
2020	74	51	23	41	0
2021	54	38	16	24	0
2022	37	21	16	11	0
2023	20	10	10	5	2
2024	92	57	35	16	5
Sum	292	183	109	101	7

Table S6. Model summary table for AR exposure in Barred Owls by sex (Figure 2). *P*-values less than 0.05 are bolded.

glmmTMB(AR_exposure ~ sex, family = binomial(link = "logit"))				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.42	0.34 – 0.51	-8.31	<0.001
sex [F]	1.83	1.37 – 2.44	4.12	<0.001
Observations	828			
R ² Tjur	0.021			

Table S7. Model summary table for cholecalciferol exposure in Barred Owls by sex (Figure 2). *P*-values less than 0.05 are bolded.

glmmTMB(VD3_exposure ~ sex, family = binomial(link = "logit"))				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.13	0.10 – 0.17	-13.58	<0.001
sex [F]	2.70	1.86 – 3.91	5.24	<0.001
Observations	828			
R ² Tjur	0.035			

Table S8. Model summary table for AR concentration in Barred Owls by sex (Figure 2). *P*-values less than 0.05 are bolded.

glmmTMB(AR_concentration (integer) ~ sex, family = nbinom2(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	2.90	2.21 – 3.80	7.67	<0.001
sex [F]	2.76	1.87 – 4.09	5.08	<0.001
Observations	828			

Table S9. Model summary table for cholecalciferol concentration in Barred Owls by sex (Figure 2). *P*-values less than 0.05 are bolded.

glmmTMB(VD3_concentration (integer) ~ sex, family = nbinom1(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	1.32	0.91 – 1.91	1.46	0.144
sex [F]	2.51	1.79 – 3.52	5.34	<0.001
Observations	828			

Table S10. Model summary table for AR exposure in Barred Owls by age-class (Figure 3). *P*-values less than 0.05 are bolded.

glmmTMB(AR_exposure ~ age-class, family = binomial(link = "logit"))				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.23	0.14 – 0.36	-6.16	<0.001
age-class [S2]	1.79	1.01 – 3.17	2.00	0.045
age-class [A]	3.24	1.96 – 5.37	4.57	<0.001
Observations	828			
R ² Tjur	0.034			

Table S11. Model summary table for cholecalciferol exposure in Barred Owls by age-class (Figure 3). *P*-values less than 0.05 are bolded.

glmmTMB(VD3_exposure ~ age-class, family = binomial(link = "logit"))				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.11	0.06 – 0.20	-7.05	<0.001
age-class [S2]	1.27	0.59 – 2.73	0.62	0.538
age-class [A]	2.71	1.41 – 5.22	2.99	0.003
Observations	828			
R ² Tjur	0.020			

Table S12. Model summary table for AR concentration in Barred Owls by age-class (Figure 3). *P*-values less than 0.05 are bolded.

glmmTMB(AR_concentration (integer) ~ age-class, family = nbinom1(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	2.39	1.51 – 3.80	3.72	<0.001
age-class [S2]	1.73	1.04 – 2.86	2.12	0.034
age-class [A]	2.66	1.70 – 4.15	4.29	<0.001
Observations	828			

Table S13. Model summary table for cholecalciferol concentration in Barred Owls by age-class (Figure 3). *P*-values less than 0.05 are bolded.

glmmTMB(VD3_concentration (integer) ~ age_class, family = nbinom1(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	1.08	0.57 – 2.06	0.25	0.803
age-class [S2]	1.26	0.61 – 2.59	0.62	0.537
age-class [A]	2.61	1.41 – 4.83	3.04	0.002
Observations	828			

Table S14. Model summary table for rodenticide richness in Barred Owls by sex (Figure 4). *P*-values less than 0.05 are bolded.

glmmTMB(rodenticide_count ~ sex, family = poisson(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.49	0.43 – 0.56	-10.33	<0.001
sex [F]	1.76	1.48 – 2.08	6.46	<0.001
Observations	828			
R ² Nagelkerke	0.073			

Table S15. Model summary table for rodenticide richness in Barred Owls by age-class (Figure 4). *P*-values less than 0.05 are bolded.

glmmTMB(rodenticide_count ~ age-class, family = comppois(link = "log"))				
<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.33	0.24 – 0.46	-6.58	<0.001
age-class [S2]	1.55	1.05 – 2.28	2.21	0.027
age-class [A]	2.39	1.70 – 3.37	5.00	<0.001
Observations	828			

Table S16. Model summary table for AR exposure in Barred Owls by year (Figure 5). *P*-values less than 0.05 are bolded.

gam(AR_exposure ~ s(year, bs = "cs", k = 5), family = binomial(link = "logit"), method = "REML", select = TRUE)				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.50	0.39 – 0.64	-5.34	<0.001
Smooth term (year)			21.46	<0.001
Observations	292			
R ²	0.077			

Table S17. Model summary table for cholecalciferol exposure in Barred Owls by year (Figure 5). *P*-values less than 0.05 are bolded.

gam(VD3_exposure ~ s(year, bs = "cs", k = 5), family = binomial(link = "logit"), method = "REML", select = TRUE)				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.01	0.00 – 0.05	-5.83	<0.001
Smooth term (year)			5.14	0.017
Observations	292			
R ²	0.025			

Table S18. Model summary table for AR concentration in Barred Owls by year (Figure 5). *P*-values less than 0.05 are bolded.

gam(AR_concentration ~ s(year, bs = "cs", k = 5), family = tw(link = "log"), method = "REML", select = TRUE)				
<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	8.28	6.29 – 10.91	15.04	<0.001
Smooth term (year)			7.04	<0.001
Observations	292			
R ²	0.048			

Table S19. Model summary table for cholecalciferol concentration in Barred Owls by year (Figure 5). *P*-values less than 0.05 are bolded.

gam(VD3_concentration ~ s(year, bs = "cs", k = 5), family = tw(link = "log"), method = "REML", select = TRUE)				
<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.04	0.01 – 0.17	-4.31	<0.001
Smooth term (year)			1.35	0.016
Observations	292			
R ²	0.019			

Table S20. Model summary table for AR exposure in Barred Owls by latitude (Figure S4). *P*-values less than 0.05 are bolded.

gam(AR_exposure ~ s(UTMN_km, bs = "cs", k = 10), family = binomial(link = "logit"), method = "REML", select = TRUE)				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.73	0.61 – 0.87	-3.56	<0.001
Smooth term (UTMN km)			4.80	0.068
Observations	530			
R ²	0.009			

Table S21. Model summary table for cholecalciferol exposure in Barred Owls by latitude (Figure S4). *P*-values less than 0.05 are bolded.

gam(VD3_exposure ~ s(UTMN_km, bs = “cs”, k = 10), family = binomial(link = “logit”), method = “REML”, select = TRUE)

<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.27	0.21 – 0.33	-11.89	<0.001
Smooth term (UTMN km)			40.30	<0.001
Observations	530			
R ²	0.086			

Table S22. Model summary table for AR concentration in Barred Owls by latitude (Figure S4). *P*-values less than 0.05 are bolded.

gam(AR_concentration ~ s(utmn_km, bs = “cs”, k = 10), family = tw(link = “log”), method = “REML”, select = TRUE)

<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	4.82	4.03 – 5.78	17.10	<0.001
Smooth term (UTMN km)			3.79	<0.001
Observations	530			
R ²	0.008			

Table S23. Model summary table for cholecalciferol concentration in Barred Owls by latitude (Figure S4). *P*-values less than 0.05 are bolded.

gam(VD3_concentration ~ s(UTMN_km, bs = “cs”, k = 10), family = tw(link = “log”), method = “REML”, select = TRUE)

<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	2.45	1.97 – 3.05	8.09	<0.001
Smooth term (UTMN km)			5.58	<0.001
Observations	530			
R ²	0.079			

Table S24. Model summary table for exposure to all rodenticides (including cholecalciferol) in Barred Owls by sex*age-class interaction (Figure S5). *P*-values less than 0.05 are bolded.

glmmTMB(exposure ~ sex * age-class, family = binomial(link = "logit"))				
<i>Parameters</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.37	0.22 – 0.62	-3.79	<0.001
sex [F]	0.78	0.32 – 1.93	-0.53	0.597
age-class [S2]	0.97	0.49 – 1.91	-0.08	0.934
age-class [A]	1.99	1.13 – 3.52	2.38	0.018
sex [F] × age-class [S2]	3.54	1.19 – 10.57	2.27	0.023
sex [F] × age-class [A]	2.95	1.12 – 7.77	2.20	0.028
Observations	828			
R ² Tjur	0.083			

Table S25. Model summary table for concentration of all rodenticides (including cholecalciferol) in Barred Owls by sex*age-class interaction (Figure S5). *P*-values less than 0.05 are bolded.

glmmTMB(concentration ~ sex * age-class, family = nbinom1(link = "log"))				
<i>Parameters</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>P</i>
Intercept	3.55	2.24 – 5.62	5.40	<0.001
age-class [S2]	1.01	0.57 – 1.80	0.04	0.972
age-class [A]	1.78	1.11 – 2.86	2.38	0.017
sex [F]	0.86	0.39 – 1.88	-0.38	0.704
age-class [S2] × sex [F]	2.71	1.08 – 6.80	2.12	0.034
age-class [A] × sex [F]	2.19	0.96 – 4.96	1.87	0.061
Observations	828			

Table S26. Model summary table for AR seasonality in Barred Owls (Figure S6). *P*-values less than 0.05 are bolded.

gam(AR_concentration ~ s(DOY, bs = "cc", k = 10) + s(year, bs = "re"), family = tw(link = "log"), method = "REML", knots = list(0.5, 366.5), select = TRUE)				
<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	5.10	4.34 – 6.00	19.68	<0.001
Smooth term (DOY)			1.15	0.005
Smooth term (year)			0.00	0.604
Observations	837			
R ²	0.001			

Table S27. Model summary table for cholecalciferol seasonality in Barred Owls (Figure S6). *P*-values less than 0.05 are bolded.

gam(VD3_concentration ~ s(DOY, bs = "cc", k = 10) + s(year, bs = "re"), family = tw(link = "log"), method = "REML", knots = list(0.5, 366.5), select = TRUE)				
<i>Parameters</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
Intercept	0.87	0.08 – 8.95	-0.12	0.904
Smooth term (DOY)			4.43	0.616
Smooth term (year)			6.74	<0.001
Observations	837			
R ²	0.020			

SUPPLEMENTARY RESULTS FIGURES

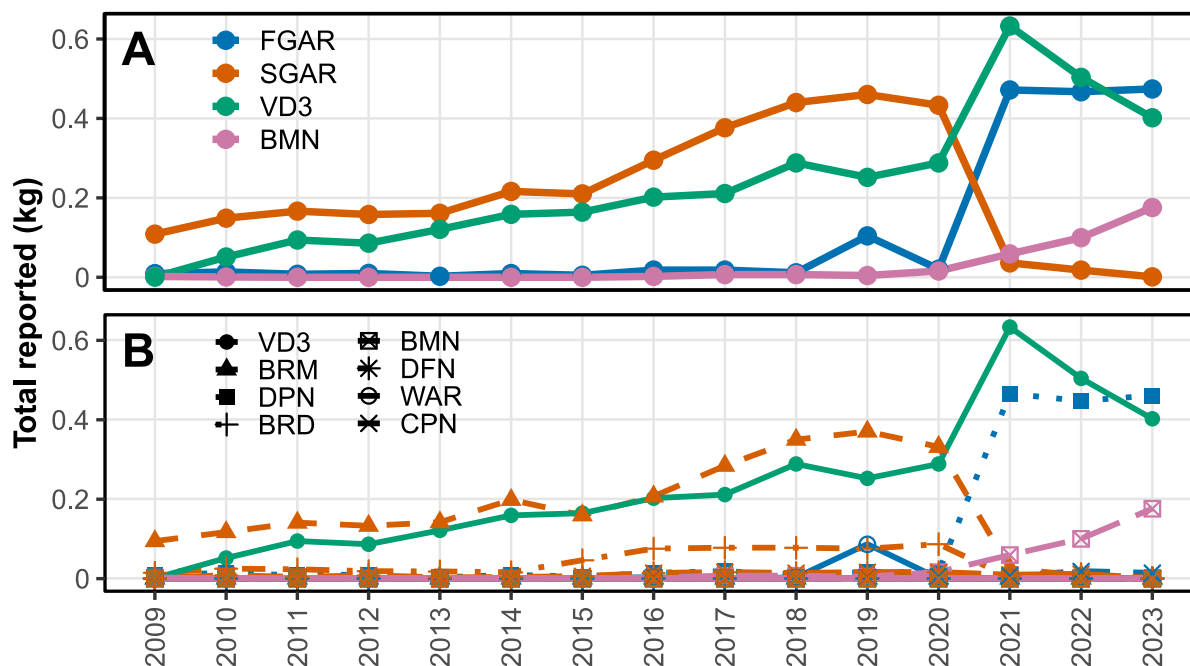


Figure S3. Reported commercial rodenticide applications in the study region from the California Department of Pesticide Regulation (DPR) Pesticide Use Reporting system, 2009–2023. (A) Totals by compound class: FGAR (first-generation anticoagulants), SGAR (second-generation anticoagulants), VD3 (cholecalciferol), and BMN (bromethalin). (B) Totals by individual compound: VD3 = cholecalciferol, BRM = bromadiolone, DPN = diphacinone, BRD = brodifacoum, BMN = bromethalin, DFN = difethialone, WAR = warfarin, CPN = chlorophacinone. Lines show annual sums (kg) across all reported applications in Del Norte, Humboldt, Trinity, Siskiyou, and Mendocino counties (Figure 1). Data are public records obtained from DPR for commercial pesticide applicators only.

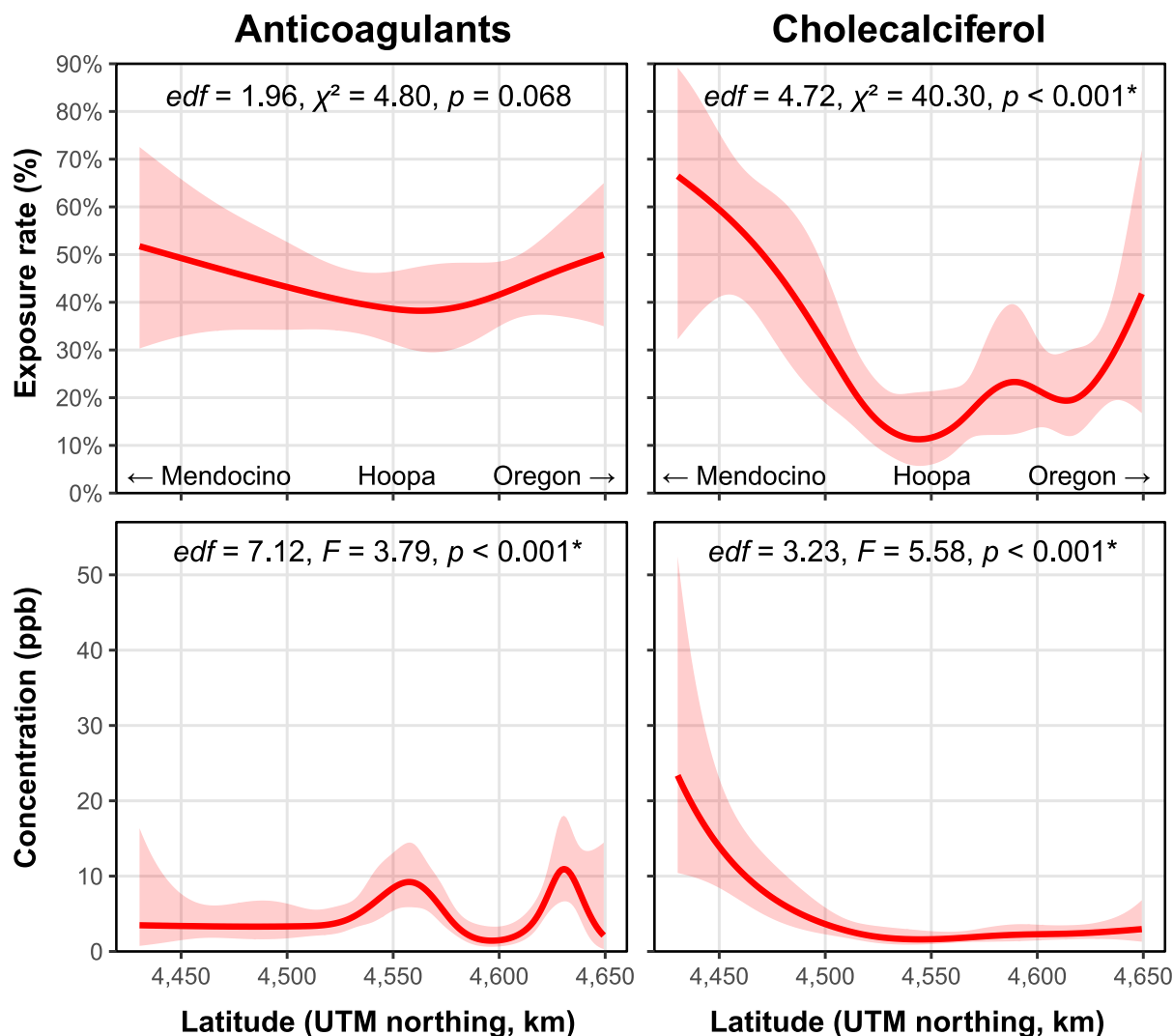


Figure S4. Latitudinal patterns in rodenticide exposure for adult Barred Owls (*Strix varia*). Top row: exposure rate (percent positive) for (left) anticoagulant rodenticides (ARs) and (right) cholecalciferol, modeled as GAMs using UTM northing coordinates as a continuous measure of latitude. Bottom row: tissue concentration (ppb) for the same compound classes, modeled as GAMs fit to ppb values that include zeros (non-detects). Lines show fitted means; shaded bands are 95% CIs. The x-axis spans the study region from Mendocino County border through the Hoopa area (Humboldt County) to the Oregon border (Figure 1). Panel annotations give the estimated degrees of freedom (*edf*), test statistic for the smooth and the associated *p*-value.

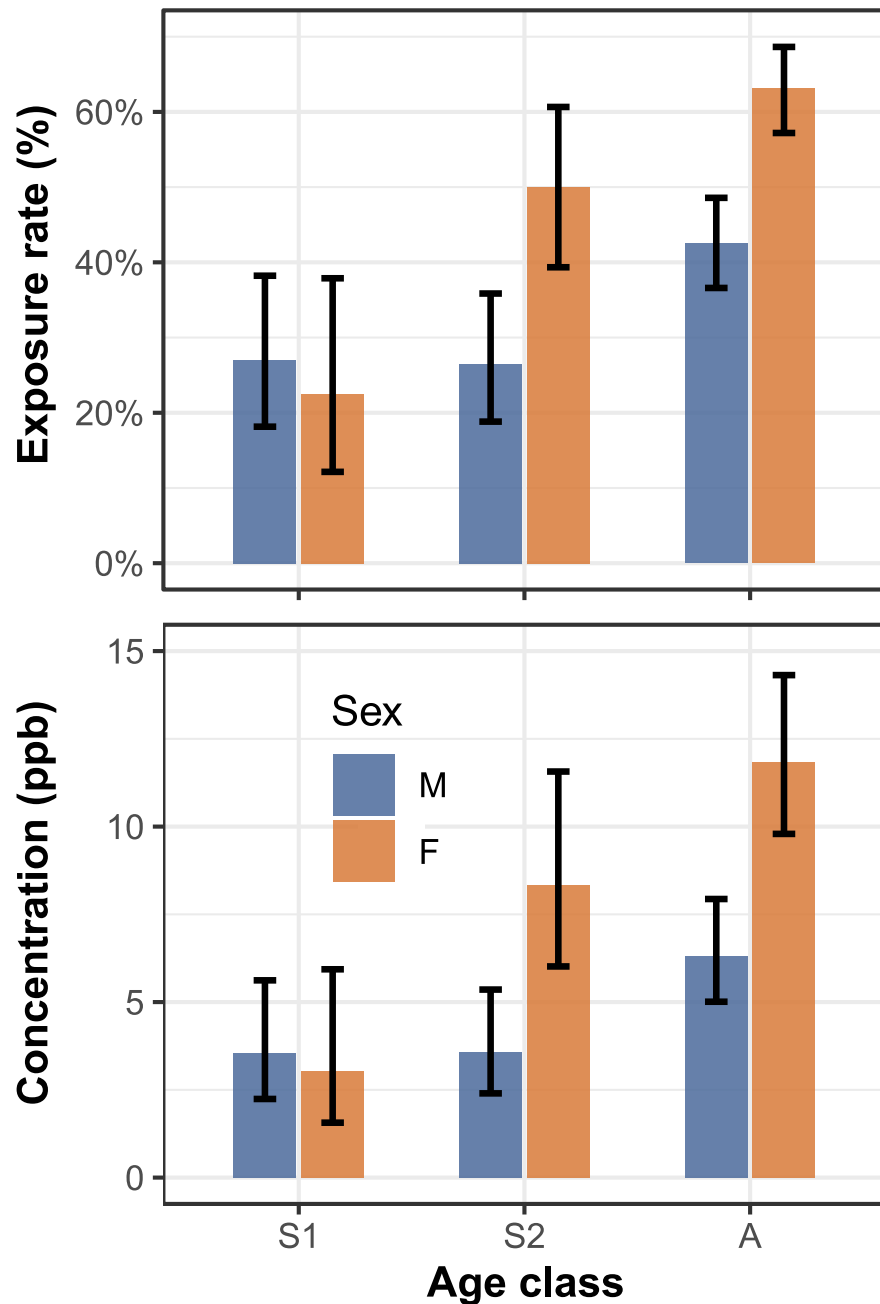


Figure S5. Interaction of sex and age-class for rodenticide exposure in Barred Owls (*Strix varia*). Top: exposure rate (percent positive) to any rodenticide (including cholecalciferol), estimated from a binomial model with sex $\times$ age-class. Bottom: tissue concentration (ppb) of anticoagulants and cholecalciferol combined, estimated from a negative-binomial model fit to integer-rounded ppb values that include zeros (non-detects). Bars are model-estimated marginal means with 95% CIs. Age-classes are S1 = young subadult, S2 = older subadult, and A = adult.

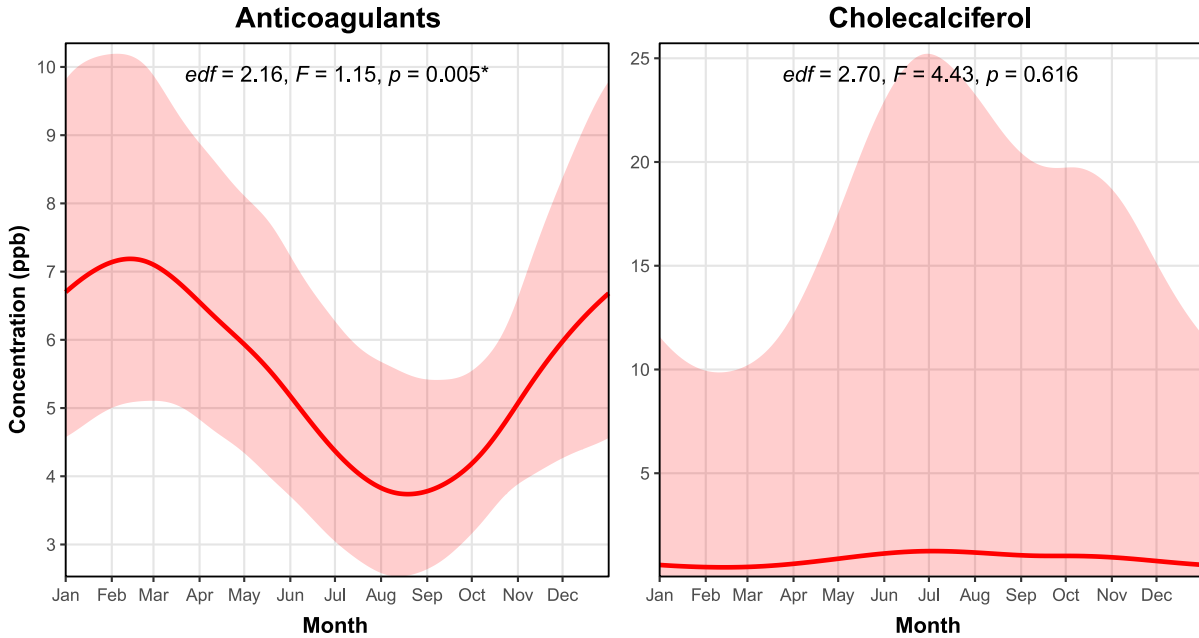


Figure S6. Seasonal patterns in rodenticide concentrations for Barred Owls (*Strix varia*). Left: anticoagulant rodenticides (ARs). Right: cholecalciferol. Curves are generalized additive models (GAMs) of concentration (ppb) across day of year (DOY) using cyclic cubic splines; a random intercept for year accounts for interannual differences. Predictions (lines) are population-level smooths with 95% CIs (shaded). Concentration models include zeros (non-detections). Panel annotations report edf , test statistic (F), and p -value for the seasonal smooth.

SUPPLEMENTARY REFERENCES

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- Yadav, A.P., Goel, V., Vig, N., Banerjee, S., Singh, R., Tripathi, M., 2021. Simultaneous determination of vitamin D2 and D3 in milk: Agilent 1290 Infinity II LC with Agilent 6470 LC/TQ system in food fortification (Application note No. 5994–3275EN). Agilent Technologies, Inc., Santa Clara, CA.