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Anticoagulant rodenticides in *Strix* owls indicate widespread exposure in west coast forests

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ABSTRACT

Exposure of nontarget wildlife to anticoagulant rodenticides (AR) is a global conservation concern typically centered around urban or agricultural areas. Recently, however, the illegal use of ARs in remote forests of California, USA, has exposed sensitive predators, including the federally threatened northern spotted owl (*Strix occidentalis caurina*). We used congeneric barred owls (*S. varia*) as a sentinel species to investigate whether ARs pose a threat to spotted owls and other old-forest wildlife in northern regions of the Pacific Northwest. We analyzed the liver tissue from 40 barred owls collected in Oregon and Washington and confirmed exposure to ≥1 AR compounds in 48% of the owls examined. Brodifacoum, an extremely toxic second-generation AR, was the most common compound detected (89% of positive cases), followed by bromadiolone (11%), difethialone (11%), and warfarin (5%). Brodifacoum was also detected in one barred owl and one spotted owl opportunistically found dead (liver concentrations were 0.091 and 0.049 μg/g, respectively). We found no evidence that exposure varied with proximity to developed and agricultural areas, or among different study areas, age-classes, and sexes. Rather, exposure was ubiquitous, and the rates we observed in our study (38–64%) were similar to or greater than that reported previously for barred owls in California (40%). Together these studies indicate widespread contamination in forested landscapes used by spotted owls and other wildlife of conservation concern. Owls collected in older forests may have been exposed via illegal use of ARs, highlighting a mounting challenge for land managers and policy makers.

1. Introduction

Secondary poisoning of nontarget wildlife by anticoagulant rodenticides (ARs) is a global conservation concern. Food production, storage, and transport facilities throughout the world are often ringed by bait stations containing ARs to prevent damage to products and structures caused by rodents (Elliot et al., 2016, van den Brink et al. 2018). Indeed, most accounts of AR exposure in wildlife occur in or adjacent to agricultural, urban, or suburban settings where use of rodenticides is widespread (Erickson and Urban, 2004; Riley et al., 2007, López-Perea and Mateo, 2018). Recently, however, exposure within remote forest settings has been documented in northern California, USA, where the use of ARs associated with the illegal cultivation of marijuana (*Cannabis* spp.) has contaminated food webs of sensitive forest predators like fisher (*Pekania pennanti*), and the federally threatened northern spotted owl (*Strix occidentalis caurina*; Thompson et al., 2014; Gabriel et al., 2012, 2018; Franklin et al., 2018). Unregulated applications at marijuana cultivation sites have emerged as a primary source of ARs in forested landscapes of northern California (Gabriel et al., 2012, 2018), but information on AR exposure in northern spotted owls and other sensitive wildlife outside of California is lacking.

Northern spotted owls are an old-forest species of significant conservation concern. Despite nearly 30 years of protection under the Federal Endangered Species Act, populations have continued to decline because of loss of old-forest habitat and, more recently, competition with expanding populations of barred owls (*S. varia*; Wiens et al., 2014; Dugger et al., 2016; Lesmeister et al., 2018). Northern spotted owls are potentially at substantial risk to AR exposure as another conservation

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threat because their diet consists largely of small rodents and mammals, and they are most common in remote forests where illegal cultivation of marijuana often occurs (Gabriel et al., 2012, 2018; Franklin et al., 2018). As a wide-ranging, federally protected species with dwindling numbers, however, it is challenging to directly measure the level of threat ARs may pose. Studies in California have begun to address this issue by using ecologically similar barred owls as a surrogate species for inferring exposure in sympatric spotted owls (Gabriel et al., 2018), as these two species compete for space, habitat, and small mammal prey (Wiens et al., 2014).

The rapid expansion of barred owls into the Pacific Northwest has motivated conservationists, researchers, and land managers to develop broad-scale approaches to mitigate the negative effects of competition with barred owls on spotted owls (Long and Wolfe, 2019). As part of this effort, removal experiments were initiated to determine if reducing numbers of barred owls can benefit declining populations of spotted owls (Diller et al., 2014, 2016; Wiens et al., 2018, 2019). Barred owl removal experiments have been replicated in a variety of forest conditions occupied by spotted owls in the Pacific Northwest, which offers an opportunity to extend the initial work of Gabriel et al. (2018) to broader portions of the northern spotted owl's geographic range. This is relevant because clandestine marijuana operations also occur in forested landscapes of Oregon and Washington (Klassen and Anthony, 2019) and the illegal use of ARs at these sites may represent a widespread, albeit little studied, conservation concern to old-forest wildlife.

We investigated AR exposure in barred owls collected during removal experiments in Oregon and Washington during 2015–2017. Our objectives were to examine the prevalence of exposures relative to those recently reported for northern California by Gabriel et al. (2018), and to determine whether exposure was occurring across a larger portion of spotted owl's geographic range. We also investigated: 1) the extent of AR exposure within and among different study regions, and 2) possible intrinsic and extrinsic sources of variation in exposure (e.g., proximity to forest-urban interface, sex, age class, and territorial status of individuals). Our test sample included barred owls removed from breeding territories simultaneously co-occupied by spotted owls, which provided an indicator of the threat of exposure to spotted owls remaining in our study areas. We also analyzed ARs in livers of owls opportunistically found dead near our study areas during the same period.

2. Materials and methods

2.1. Study areas

We collected barred owls at one study area in central Washington, (Cle Elum) and two in western Oregon, USA (Coast Range and Union-Myrtle; Fig. 1). Study areas ranged in size from 580 to 780 km^2. Climate, topography, vegetation, and elevation varied considerably among these areas (Wiens et al., 2019). All study areas included mixtures of federal and private lands and had long-term (1990–2018) monitoring data on northern spotted owls. Private timberlands had restricted access by the public and contained mostly clear-cuts or second- and third-growth conifer and hardwood forests on a regular harvest schedule. The vegetation in all study areas was predominantly coniferous forest, but the age and species composition of forests varied among study areas depending upon climate and land-management history. The Cle Elum area was dominated by mixed conifer and ponderosa pine (*Pinus ponderosa*) forests with mixtures of Douglas-fir (*Pseudotsuga menziesii*) and grand fir (*Abies grandis*). In contrast, the Coast Range study area of western Oregon was dominated by coastal forests of western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*), and Douglas-fir. The Union-Myrtle area of southern Oregon was dominated by mixtures of Douglas-fir forest interspersed with incense cedar (*Calocedrus decurrens*). We did not have data on specific locations of marijuana cultivation sites in our study areas, but historical (National Drug Intelligence Center, 2007) and more recent reports (Klassen and Anthony, 2019) indicated widespread occurrence on public lands in each of our study regions.

2.2. Barred owl removal experiment and specimen collection

Barred owls were removed from habitats and territories historically used by spotted owls as part of a broad-scale experiment to determine if reducing populations of invasive barred owls can improve population trends of threatened spotted owls (Diller et al., 2016; Wiens et al., 2019). Barred owls identified during population surveys were removed using 12-gauge shotguns loaded with non-toxic shot (Diller et al., 2014; Wiens et al., 2019). Removals occurred primarily in the non-breeding season (Sep–Apr) to avoid collecting owls with dependent young. Lethal removal and scientific collection of barred owls was approved by the Institutional Animal Care and Use Committee at Oregon State University (Protocols 4728, 5067), and completed under Federal Fish and Wildlife Permit MB14305B-0 and Washington (HENSON 18-261) and Oregon (MB14305B-5) State Scientific Collection Permits.

We recorded the coordinates of the collection location, sex, and age of all individual barred owls. We determined sex of barred owls based on vocalizations and morphometric measurements and verified those determinations in the lab through examination of sex organs. We classified all barred owls as either adults (≥ 3 years old) or subadults (1–2 years old) based on the presence of distinctive juvenile (first-year) flight feathers and molt characteristics observed under ultraviolet light (Weidensaul et al., 2011; Wiens et al., 2018, 2019). In addition, we used site-specific detection histories from survey and removal activities to classify individual owls as either territorial residents (previously established owls collected at the beginning of the removal study) or new colonizers (owls collected at sites after the original occupants had been removed). Territorial residents were well-established owls (primarily adults) with home ranges averaging 450–700 ha in size (Wiens et al., 2014), whereas newly established colonizers (primarily subadults) were likely to have wide-ranging movements prior to collection, and thus may have a greater risk of exposure to ARs as they dispersed across urban or agricultural landscapes.

2.3. Tissue sampling and rodenticide analysis

We examined the frequency of exposure to ARs based on concentrations detected in the liver (Rattner et al., 2014; Gabriel et al., 2018). For tissue sampling we randomly selected a representative sample of 40 barred owls (18 female, 22 male) collected during removal experiments across all three study areas. Of the 40 owls, 23 were adults (≥ 3 yrs. old), 17 were subadults (1–2 yrs. old), and we classified 21 owls as territorial residents and 19 as new colonizers. Liver tissues from each owl were removed and homogenized in liquid nitrogen using a cryo-grinder (SPEX SamplePrep, Metuchen, New Jersey). We retained a 3-g aliquot sample for quantitative AR analysis (Reynolds, 1980). Liver tissue aliquots of owls were submitted to the California Animal Health and Food Safety Laboratory (CAHFS; Davis, California) for quantification of eight ARs, including 4 first-generation ARs (warfarin, diphacinone, chlorophacinone, and coumachlor), and 4 second-generation ARs (brodifacoum, bromadionlone, difethialone, and difenacoum). Contaminants were quantified by high-performance liquid chromatography-tandem mass spectrometry (Marek and Koskinen, 2007; Serieys et al., 2015). Quality control blanks all reported zero ARs detected and analytical spike recovery averaged 101.4 ± 3.5%.

We also screened tissues of 1 barred owl and 2 spotted owls opportunistically found dead near our study areas. Opportunistic recoveries were in fair to good postmortem condition and submitted for necropsy and liver tissue sampling to the Oregon State University Veterinary Diagnostics Lab (Corvallis, Oregon). Proximate cause of death was assessed based on clinical signs and gross necropsy as completed by a veterinary pathologist. Clinical criteria for postmortem

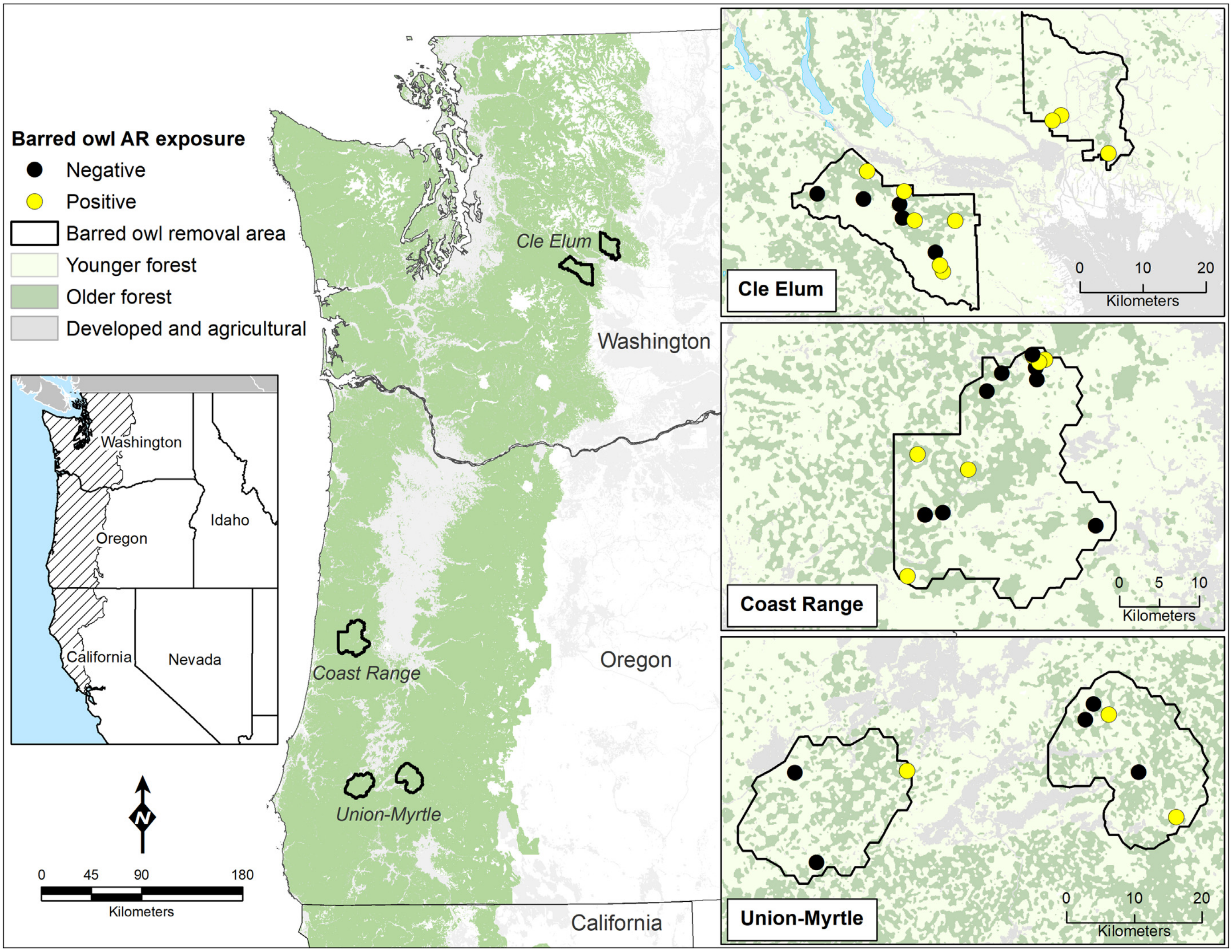

Fig. 1. Collection locations of barred owls that were screened for anticoagulant rodenticides (AR) in three study areas in Oregon and Washington, USA, during 2015–2017. The enlarged maps of each study area show individual collection locations relative to the spatial distribution of older forests used by spotted owls (from Davis et al., 2016) and developed and agricultural areas. The geographic range of the northern spotted owl is shown in inset on left side of figure.

diagnosis of AR toxicosis included extensive bruising over multiple regions of the body with no associated fractures, large amount of blood loss from a small wound, large amount of frank blood in the body cavity, and pallor of internal organs (Murray, 2018). Livers of opportunistically collected owls were analyzed for ARs at the Veterinary Diagnostic Laboratory at Michigan State University (East Lansing, Michigan). Across laboratories, MLOQ was $0.034 \pm 0.005\,\mu g/g$ wet weight (ww) for brodifacoum and $0.021 \pm 0.001\,\mu g/g$ ww for all other ARs. If an AR was detected in a sample, but below the MLOQ and ≥ the mean limit of detection (MLOD; the lowest concentration in a sample that could be detected but not necessarily quantified as an exact concentration), it was reported as a trace amount.

2.4. Analysis of exposure frequency

We used positive (i.e. trace amount or greater) and negative AR screening results from barred owls to assess possible differences in the frequency of exposure among study areas, sexes, and age classes. We predicted that recent colonizers, with presumably broad-ranging movements, would have higher exposure relative to well-established territorial residents. Previous studies of AR contamination in predatory birds show that species or individuals using areas within or adjacent to developed or agricultural environments tend to have greater exposure than those that use more natural landscapes (Christensen et al., 2012; Lohr, 2018). To examine this prediction, we used spatial data from the Gap Analysis Program (https://gapanalysis.usgs.gov, last accessed April 2019) to map the spatial distribution of developed and agricultural lands in our study areas (Fig. 1). We then recorded the linear distance (km) between the nearest developed and agricultural area (i.e. 30-m raster cell) and each individual owl's collection location.

We used generalized linear models with a binomial distribution and logit link function to examine the effects of study area, sex, age, residency status, and proximity to developed areas on the frequency of exposure. We only considered univariate models because of a limited sample size. We used information-theoretic methods (Burnham and Anderson, 2004) to rank models and determine which effects best explained variation in exposure. We included a null (intercept only) model to gauge the relative amount of support for alternative models considered and examined the degree to which 95% confidence intervals of slope coefficients overlapped zero to further evaluate support for main effects of interest. We were unable to examine differences among individuals in the concentration of AR's because only a few of our exposure levels were above trace levels. As a general indicator of exposure risk to the remaining spotted owls in our study areas, we calculated the linear distance between collection locations of barred owls and concurrently used activity centers of spotted owl pairs (i.e. a nest tree,

Table 1
Frequency of detection (%) of anticoagulant rodenticides (AR) in the liver tissue of barred owls collected in three study areas in Oregon and Washington, USA, 2015–2017.

Study area	Barred owls screened (F, M)	AR positive (% of owls screened)[a]	Brodifacoum positive (% of positive cases)
Cle Elum, Washington	14 (4, 10)	9 (64%)	9 (100%)
Coast Range, Oregon	18 (9, 9)	7 (39%)	5 (71%)
Union-Myrtle, Oregon	8 (5, 3)	3 (38%)	3 (100%)
Total	40 (18, 22)	19 (48%)	17 (90%)

[a] An analyte in the sample was detected but not necessarily quantified as an exact concentration.

adult with young, or mean coordinates of pair roosting locations). We used R (version 3.5.0) for all analyses. Exposure data for barred owls are available from the USGS ScienceBase-Catalog (https://doi.org/10.5066/P9S51J9K).

3. Results

We detected exposure to one or more AR compounds in 19 (48%) barred owls collected during removal experiments (Table 1, Fig. 1). Sixteen (40%) barred owls had a single detectable AR present (14 cases with brodifacoum, 1 case with bromadiolone), and 3 (8%) owls had 2 different types of ARs present (2 with brodifacoum and difethialone, 1 with brodifacoum and warfarin). Brodifacoum was the most common AR detected in barred owls (89% of positive cases), followed by bromadiolone (11%), difethialone (11%), and warfarin (5%). All detections of ARs were at a trace level (≤ MLOQ) with the exception of a single barred owl in the Oregon Coast Range with measurable quantities of difethialone (0.110 μg/g). The majority (77%) of barred owls that tested positive for exposure were collected during Fall and Winter (Sep–Feb).

None of the factors we examined (study area, sex, age, residency status, proximity to developed area) were significant predictors of exposure frequency among the barred owls we sampled. The best-supported candidate model of exposure was the null (intercept only) model, and 95% confidence intervals of regression slope coefficients for all main effects overlapped zero (Table 2). Median distance (km) from collection locations of positive barred owls and developed areas was 1.1 km (SD = 1.2, min = 0.2, max = 4.9 km; Fig. 2). Median distance between collection locations of positive barred owls and the nearest activity center concurrently used by a pair of spotted owls was 2.0 km (SD = 3.7, min = 0.1, max = 15.1 km).

We opportunistically collected and screened liver samples from 1 barred owl (subadult, F) and 2 adult (> 4 yr-old) spotted owls (M, F). The barred owl was found dead on 9 Nov 2017 near Mapleton, Oregon, and had been marked as a fledgling on Bainbridge Island, Washington, on 1 Jul 2016 (linear distance from recovery site = 435 km). Necropsy showed that the bird was in good body condition, and AR screening was positive for brodifacoum exposure (0.091 μg/g ww). Toxicosis from brodifacoum could not be confirmed in this case based on the criteria we used. The adult male spotted owl was found dead by field crews on 5 Oct 2017 in Douglas County, Oregon, after the bird had been observed acting sluggish the day before. Necropsy showed the bird was in poor body condition, and rodenticide testing was positive for exposure to brodifacoum (0.049 μg/g ww). Brodifacoum-related toxicosis was inconclusive because there were no apparent signs of bruising or internal hemorrhage that could be directly attributed to exposure. The female spotted owl was found dead in Douglas County, Oregon, on 11 Jun 2018. Proximate causes of death were inconclusive, and a liver sample tested negative for exposure to rodenticides.

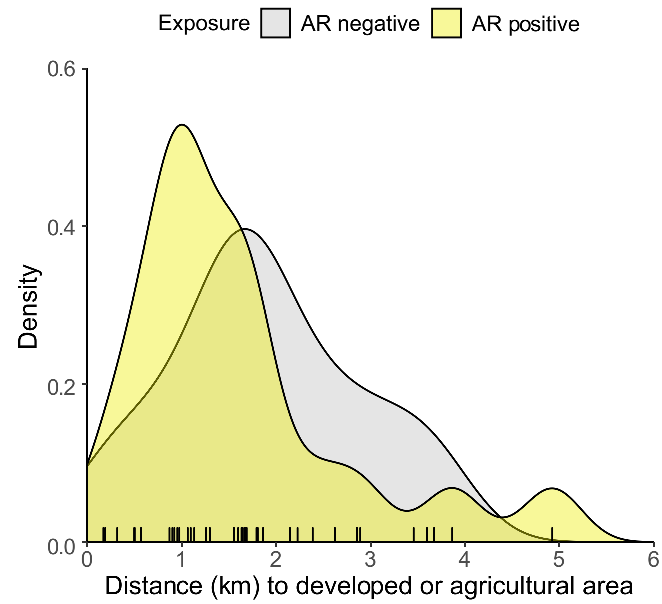

Fig. 2. Distribution of distances (km) between collection locations of barred owls with positive (n = 19) or negative (n = 21) detections of anticoagulant rodenticides (AR) and the nearest developed or agricultural area in Oregon and Washington, USA, 2015–2017.

Table 2
Coefficient estimates, standard errors (SE), and lower (LCI) and upper (UCI) 95% confidence intervals from univariate models used to examine variation in prevalence of anticoagulant rodenticides in barred owls in Washington and Oregon, USA, 2015–2017.

Model[a]	Estimate	SE	LCI	UCI	AIC_c[b]
Null (intercept only)	−0.100	0.317	−0.729	0.522	57.46
Dist. (km) to developed area	−0.254	0.304	−0.894	0.328	58.74
Age	−0.444	0.646	−1.740	0.816	58.98
Study area: Coast Range	−1.040	0.738	−2.552	0.377	58.99
Study area: Union-Myrtle	−1.099	0.919	−3.016	0.664	58.99
Residential status	−0.393	0.637	−1.664	0.853	59.07
Sex	−0.182	0.637	−1.446	1.072	59.38

[a] The reference category for age was subadult, for study area was Cle Elum, for residential status was colonizer, and for sex was male.
[b] Akaike's Information Criteria corrected for small sample size.

4. Discussion

We found that ARs were common in barred owls and likely pose a range-wide threat to the federally protected northern spotted owl and other old-forest wildlife in the Pacific Northwest. This was the first large scale study of secondary AR exposure in forest owls in Oregon and Washington, and we detected rodenticides in 48% of barred owls examined. These findings provide evidence that exposure was occurring in owl populations across much of the northern spotted owl's geographic range. Our study also represents the first confirmed case of rodenticide exposure in spotted owls in Oregon. Barred owls that tested positive were exposed to second generation ARs classified as extremely toxic (brodifacoum, difethialone) or moderately toxic (bromadiolone) to nontarget wildlife, and one barred owl was exposed to the slightly toxic first-generation AR warfarin (Erickson and Urban, 2004; U.S. EPA, 2011; Rattner et al., 2011). Our findings of high exposure rates in free-ranging barred owls collected in older forest types, often in close (< 2 km) proximity to spotted owls, confirmed that ARs have entered the diverse food web of these ecologically similar owl species. Most detections of ARs were below quantifiable levels, but we did find that exposure had the potential to occur at higher levels based on measurable quantities found in one spotted owl and one barred owl that were opportunistically found dead. The barred owls we sampled were primarily collected during the fall and winter, when any use of rodenticides would be expected to be very low. Given the long half-life of second-generation rodenticides we detected (> 150 days; Herring et al., 2017), owls with positive exposure could have been exposed to high concentrations several months prior to collection and only had trace amounts remaining in the liver upon collection.

Exposure in barred owls was ubiquitous relative to the environmental and individual factors we examined. Most (63%) barred owls that tested positive were collected 1–5 km from areas with legal applications of the rodenticides we detected, and we found no consistent trend between AR exposure and proximity to urban and agricultural landscapes. We also found no evidence of a difference in exposure between well-established residents (with presumably small home ranges) and newly established colonizers. Moreover, exposure did not vary with intrinsic traits of barred owls such as sex and age. Frequency of exposure to rodenticides was relatively high in our study, but within the range of estimates reported previously for barred owls (Table 3). With the exception of Gabriel et al. (2018), however, previous studies were often limited to injured or sick individuals submitted to rehabilitation facilities, or to salvaged specimens found haphazardly in areas with higher densities of humans. In contrast, our test sample of randomly sampled free-ranging barred owls was collected primarily in nonurban, mixed-conifer forests 50–250 yrs-old. Thus, our study circumvented many biases associated with previous studies of ARs in barred owls. Our findings parallel those recently reported in northern California, where rodenticides (brodifacoum and bromadiolone) were detected in 40% of barred owls and 70% of spotted owls examined (Gabriel et al., 2018, also see Franklin et al., 2018). Together, these studies and ours indicate that AR-contamination is an additional stressor and potential source of mortality to northern spotted owls and other old-forest wildlife of conservation concern.

4.1. Exposure pathways in older forests

Our study confirmed a high rate of exposure to ARs in barred owls occupying old-growth forests, but we were unable to determine if the source of exposure originated from legal or illegal applications. The second generation ARs we detected (brodifacoum and difethialone) have been classified as extremely toxic to predatory birds that feed on target or nontarget animals poisoned with bait (Erickson and Urban, 2004; Rattner et al., 2014). We detected trace amounts of these ARs in nearly all cases of positive exposure, with the exception of 3 owls that had measurable levels of either difethialone (1 barred owl) or brodifacoum (1 barred owl, 1 spotted owl). As in previous studies (Table 3), most (89%) barred owls we collected that tested positive were exposed to brodifacoum. Commercial use of second-generation ARs containing brodifacoum is widespread, but not legally permitted in areas > 31 m from man-made structures or agricultural containers (EPA.gov/rodenticides; accessed April 2019). Legal use of second-generation ARs to control rodents near the forest-urban interface could lead to exposure to nontarget wildlife because poisoned small mammals may disperse away from baiting sites near buildings to become available to predators and scavengers over much broader areas (Elmeros et al., 2019). Moreover, poisoned rodents may be more susceptible to being killed and consumed by avian predators (Vyas et al., 2012; Elliott et al., 2014). Thus, despite current restrictions on the commercial use of secondary-generation rodenticides, secondary exposure of forest owls may be an inevitable consequence of chemical rodent control in the locale of forested habitats.

Table 3
Previous studies that examined bioaccumulation of second-generation anticoagulant rodenticides (AR) in barred owls and northern spotted owls.

Species	Location	Individuals screened	AR positive (% of owls screened)	Brodifacoum positive (% of AR positive)	Mean brodifacoum exposure (μg/g ww)[b]	Source
Barred owl (*Strix varia*)	Washington, USA[a]	14	64%	100%	Trace	This study
	Oregon, USA[a]	26	38%	80%	Trace	This study
	Oregon, USA	1	100%	100%	0.091	This study
	California, USA[a]	84	40%	91%	0.056	Gabriel et al. 2018
	Southeastern USA	8	75%	100%	0.096	Weir et al. 2018
	Massachusetts, USA	24	88%	100%	0.041	Murray 2017
	Massachusetts, USA	40	75%	97%	0.004	Murray 2011
	Quebec, CA	3	33%	Not reported	Trace	Thomas et al. 2011
	British Columbia, CA	25	92%	74%	0.074	Albert et al. 2010
	New York, USA	2	100%	50%	0.040	Erickson & Urban 2004
	New York, USA	13	23%	Not reported	Trace	Stone et al. 2003
Northern spotted owl (*S. occidentalis caurina*)	Oregon, USA	2	50%	100%	0.049	This study
	California, USA	1	100%	100%	0.035	Franklin et al. 2018
	California, USA	10	70%	100%	Trace	Gabriel et al. 2018

[a] Based on free-ranging specimens collected during removal experiments from sites historically occupied by northern spotted owls.
[b] All reported exposure levels converted to μg/g to facilitate comparisons; Trace = an AR was detected in a sample, but below the mean limit of quantification (i.e. the lowest concentration of an analyte in a sample that could be detected but not necessarily quantified as an exact concentration).

Historically, rodenticides were used to control populations of forest rodents that can cause considerable economic damage to timber production by feeding on young replanted trees (Arjo and Bryson, 2007). The use of second-generation ARs in agricultural settings without human dwellings is not currently legally permitted unless they are incorporated in an Integrated Pest Management (IPM) strategy that minimizes rodenticide amounts in the environment. Forest mammals known to be problematic to timber production in our study areas include voles (*Muridea* spp.), pocket gophers (*Thomomys* spp.), Coast moles (*Scapanus orarius*), and mountain beaver (*Aplodontia rufa*; Arjo and Bryson, 2007). These mammal species are regularly identified in diets of northern spotted owls and barred owls, and both owl species are known to forage along forest edges or openings created by timber harvests (Hamer et al., 2001; Forsman et al., 2004; Wiens et al., 2014). The first-generation AR chlorophacinone is classified as a restricted-use product to control mountain beaver in forestry plantations in Oregon and Washington, in additional to agricultural areas in California. We did not detect this AR in our study and the level of use of this or other rodenticides during forestry operations was unavailable. As a consequence, we were unable to rule out forestry applications or IPM strategies as another possible source of exposure, which was a possibility given the extent of private lands managed for timber production in our study areas.

In northern California, AR exposure in barred owls, spotted owls, and fisher occurs through the thousands of illegal clandestine marijuana cultivation sites located on public and tribal lands (Thompson et al., 2014; Gabriel et al., 2012, 2018). Oftentimes, substantial amounts of AR (up to ~25 kg), including brodifacoum and bromadiolone, are found at illegal cultivation sites. Such sites are widespread throughout the range of the northern spotted owl and can contaminate food webs via bio accumulation. Outdoor cultivation sites may also provide foraging opportunities for owls by creating forest openings during growing operations (Franklin et al., 2018). In Oregon and Washington, legalization of marijuana has led to an exponential increase in numbers of grow sites; as of 2018 there were ~20,100 registered grow sites in Oregon alone (Oregon Liquor Control Commission, 2018). This rapid upsurge in licensed cultivation operations has unforeseen environmental and land-use implications (Owley, 2018). We did not have data on legal or illegal marijuana cultivation in our study areas, so were unable to evaluate links with AR exposure. We did find, however, that occurrence of ARs in owls collected in interior forests 1–5 km from potential legal application sites was high. Once settled on a breeding territory, barred owls generally remain within 0.5–1.5 km of their nesting sites throughout the year and spend the majority of their time foraging in older forest types (Singleton et al., 2010; Wiens et al., 2014). These lines of evidence suggest that barred owls sampled in our study rarely used urban areas with legal applications of ARs. Rather, our study suggests that illegal applications of ARs in the locale of old forests were a likely source of exposure. Increasing the sampling frequency for ARs relative to the spatial distribution of legal and illegal growing operations would permit a more detailed spatial and temporal analysis of associations between rodenticide exposure in nontarget wildlife and the cultivation of marijuana.

4.2. Threats to spotted owls and other old-forest wildlife

Secondary exposure of barred owls or spotted owls occurs exclusively through their diet, which can be highly variable over space and time. As generalist predators, barred owls prey upon a broad diversity of small mammal species that could be susceptible to AR applications in or near forested areas (Hamer et al., 2001; Wiens et al., 2014). Diets of barred owls also include invertebrate prey like earthworms, slugs, and ground beetles (Livezey et al., 2008; Wiens et al., 2014), all of which can feed directly on rodenticide bait and subsequently carry a considerable risk of secondary exposure to their predators (Elliott et al., 2014; Alomar et al., 2018). In contrast to barred owls, spotted owls are considered specialist predators that focus more heavily on arboreal and scansorial prey such as flying squirrels (*Glaucomys sabrinus*) and woodrats (*Neotoma* spp.). Although there are fine-scale differences in foraging tactics between spotted owls and barred owls, both species spend the majority of their time foraging in old conifer forests and rely on many of the same mammal prey for the bulk of their dietary biomass (Wiens et al., 2014). Despite the many uncertainties associated with the source or level of contamination in different prey species, our results did indicate a high level of exposure to second-generation ARs in barred owls, and likely northern spotted owls. This result supported the hypothesis that ARs pose an additional threat and stressor to populations of northern spotted owls across their geographic range (Gabriel et al., 2018; Franklin et al., 2018).

Population declines of spotted owls have been documented since the 1980s and are largely attributed to habitat loss and competition with barred owls (Dugger et al., 2016; Lesmeister et al., 2018), but the impacts of rodenticide exposure could easily go undetected. While there remains no direct evidence of population-level impacts to spotted owls that can be directly attributed to AR poisoning, cause-effect associations of chemical exposure leading to negative fitness consequences can be exceedingly difficult to detect. Moreover, AR exposure may be additive with other sources of mortality (Brakes and Smith, 2005). This is especially true in sparse populations known to be confronting a multitude of threats, as is the case with spotted owls (Lesmeister et al., 2018). The extent to which secondary poisonings may have demographic consequences for spotted owls or other predatory wildlife is likely to vary among species, populations, and the magnitude of lethal and sub-lethal effects (Gabriel et al., 2012, 2018). A sum concentration between 0.1 and 0.2 μg/g has been considered as a general threshold of AR poisoning in birds of prey (Newton et al., 1999), although these levels were based on barn owls (*Tyto alba*) and information is lacking for many species. Barred owls with measurable quantities of ARs were at or near this threshold level in our study (0.091–0.110 μg/g), and one spotted owl found dead had levels measured at 0.049 μg/g.

Our results add to a growing list of studies showing that second-generation ARs such as brodifacoum, bromadiolone and difethialone pose a persistent and widespread risk of exposure to old-forest wildlife. Our study and others (Rattner et al., 2014; Thompson et al., 2014; Gabriel et al., 2012) further demonstrate that large gaps remain in identifying pathways of AR exposure, species' sensitivity, effects of low-level exposure, consequences of sublethal effects, and extent of mortality to forest wildlife. The apparent frequency of exposure, and the uncertainties about the magnitude and drivers of lethal and sub-lethal poisoning, underscore the need for improved information on the prevalence of ARs in older forests designated as critical habitat for sensitive predators like spotted owls. Data are not yet available to fully interpret the ecological consequences of widespread AR-contamination in west-coast forests, but the potential threat for negative impacts has become evident. Remnant fragments of older forests are required to conserve biological diversity yet continue to be lost to severe wildfire, timber harvest, and expanding urbanization and agricultural development (Davis et al., 2016). Evidence of rodenticide exposure in these protected forests raises additional conservation concerns. The occurrence of unregulated use of ARs in or near older forests, in particular, has the potential to undermine the conservation benefits that these protected habitats provide. Indeed, such conservation benefits may be negated if ARs have become prevalent and are leading to widespread fitness consequences or mortality of threatened wildlife. Conservation planning and policy would benefit from a deeper understanding of the intensity and magnitude of threat that AR exposure poses to non-target forest wildlife, and how management actions might be focused to mitigate that threat.

Article impact statement

We detected anticoagulant rodenticides in 48% of barred owls sampled in protected older forests used by multiple species of conservation concern.

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Declaration of competing interest

The authors declare no conflicts of interest. This manuscript has been peer reviewed and approved for publication consistent with U.S. Geological Survey Fundamental Science Practices (http://pubs.usgs.gov/circ/1367/).

References

Albert, C.A., Wilson, L.K., Mineau, P., Trudeau, S., Elliott, J.E., 2010. Anticoagulant rodenticides in three owl species from western Canada, 1988–2003. Arch. Environ. Contam. Toxicol. 58, 451–459.

Alomar, H., Chabert, A., Coeurdassier, M., Vey, D., Berny, P., 2018. Accumulation of anticoagulant rodenticides (chlorophacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*. Sci. Total Environ. 610, 576–582.

Arjo, W.M., Bryson, D.T., 2007. Reducing non-target hazards of rodenticides in forest settings. In: USDA National Wildlife Research Center-Staff Publications (Ed.), Proceedings of the 12th Wildlife Damage Management Conference.

Brakes, C.R., Smith, R.H., 2005. Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. J. Appl. Ecol. 2, 118–128.

Burnham, K.P., Anderson, D.R., 2004. Model Selection and Multimodel Inference—A Practical Information-Theoretic Approach 2nd edition. Springer-Verlag, New York.

Christensen, T.K., Lassen, P., Elmeros, M., 2012. High exposure rates of anticoagulant rodenticides in predatory bird species in intensively managed landscapes in Denmark. Arch. Environ. Contam. Toxicol. 63, 437–444.

Davis, R.J., Hollen, B., Hobson, J., Gower, J.E., Keenum, D., 2016. Northwest Forest Plan—The First 20 Years (1994–2013): Status and Trends of Northern Spotted Owl Habitats. General Technical Report PNW-GTR-929. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon.

Diller, L.V., Dumbacher, J.P., Bosch, R.P., Bown, R.R., Gutiérrez, R.J., 2014. Removing Barred Owls from local areas—techniques and feasibility. Wildl. Soc. Bull. 38, 211–216.

Diller, L.V., Hamm, K.A., Early, D.A., Lamphear, D.W., Dugger, K.M., Yackulic, C.B., Schwarz, C.J., Carlson, P.C., McDonald, T.L., 2016. Demographic response of northern spotted owls to barred owl removal. J. Wildl. Manag. 80, 691–707.

Dugger, K.M., et al., 2016. The effects of habitat, climate, and barred owls on long-term demography of northern spotted owls. Condor 118, 57–116.

Elliot, J.E., Rattner, B.A., Shore, R.F., van den Brink, N.W., 2016. Paying the pipers: mitigating the impact of anticoagulant rodenticides on predators and scavengers. BioScience 66, 401–407.

Elliott, J.E., Hindmarch, S., Albert, C.A., Emery, J., Mineau, P., Maisonneuve, F., 2014. Exposure pathways of anticoagulant rodenticides to nontarget wildlife. Environ. Monit. Assess. 186, 895–906.

Elmeros, M., Bossi, R., Christensen, T.K., Kjær, L.J., Lassen, P., Topping, C.J., 2019. Exposure of non-target small mammals to anticoagulant rodenticide during chemical rodent control operations. Environ. Sci. Pollut. Res. 26, 6133–6140.

Erickson, W., Urban, D., 2004. Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach. United States Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substance, Washington, D.C., USA.

Forsman, E.D., Anthony, R.G., Meslow, E.C., Zabel, C.J., 2004. Diets and foraging behavior of northern spotted owls in Oregon. J. Raptor Res. 38, 214–230.

Franklin, A.B., et al., 2018. Grass is not always greener: rodenticide exposure of a threatened species near marijuana growing operations. BMC Res. Notes 11, 94.

Gabriel, M.W., Woods, L.W., Poppenga, R., Sweitzer, R.A., Thompson, C., Matthews, S.M., Higley, J.M., Keller, S.M., Purcell, K., Barrett, R.H., Wengert, G.M., Sacks, B.N., Cliffortd, D.L., 2012. Anticoagulant rodenticides on our public and community lands: spatial distribution of exposure and poisoning of a rare forest carnivore. PLoS One 7, e40163.

Gabriel, M.W., Diller, L.V., Dumbacher, J.P., Wengert, G.M., Higley, J.M., Poppenga, and R.H., Mendia, S. 2018. Exposure to rodenticides in northern spotted and barred owls on remote forest lands in northwestern California: evidence of food web contamination. Avian Conserv. Ecol. 13:2.

Hamer, T.E., Hays, D.L., Senger, C.M., Forsman, E.D., 2001. Diets or northern barred owls and northern spotted owls in an area of sympatry. J. Raptor Res. 35, 221–227.

Herring, G., Eagles-Smith, C.A., Buck, J., 2017. Characterizing golden eagle risk to lead and anti-coagulant rodenticide exposure. J. Raptor Res. 51, 273–292.

Klassen, M., Anthony, B.P., 2019. The effects of recreational cannabis legalization on forest management and conservation efforts in U.S. national forests in the Pacific Northwest. Ecol. Econ. 162, 39–48.

Lesmeister, D.B., Davis, R.J., Singleton, P.H., Wiens, J.D. 2018. Northern spotted owl habitat and populations: status and threats. In Spies, T.A., Stine, P.A., Gravenmier, R., Long, J.W., Reilly, M.J., technical editors. 2018. Synthesis of Science to Inform Land Management Within the Northwest Forest Plan Area. General Technical Report PNW-GTR-966. US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Oregon.

Livezey, K.B., Elderkin, M.F., Cott, P.A., Hobbs, J., Hudson, J.P., 2008. Barred Owls eating worms and slugs: the advantage in not being picky eaters. Northwest. Nat. 89, 185–190.

Lohr, M.T., 2018. Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. Sci. Total Environ. 643, 134–144.

Long, L.L., Wolfe, J.D., 2019. Review of the effects of barred owl on spotted owls. J. Wildl. Manag. 83, 1281–1296.

López-Perea, L., Mateo, R., 2018. Secondary exposure of anticoagulant rodenticides and effects in predators. In: van den Brink, N., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife. Springer Nature, Cham, Switzerland, pp. 159–193.

Marek, L.J., Koskinen, W.C., 2007. Multiresidue analysis of seven anticoagulant rodenticides by high-performance liquid chromatography/electrospray/mass spectrometry. J. Agric. Food Chem. 55, 571–576.

Murray, M., 2011. Anticoagulant rodenticide exposure and toxicosis in four species of birds of prey presented to a wildlife clinic in Massachusetts, 2006–2010. J. Zoo Wildl. Med. 42, 88–97.

Murray, M., 2017. Anticoagulant rodenticide exposure and toxicosis in four species of birds of prey in Massachusetts, USA, 2012–2016, in relation to use of rodenticides by pest management professionals. Ecotoxicology 26, 1041–1050.

Murray, M., 2018. Ante-mortem and post-mortem signs of anticoagulant rodenticide toxicosis in birds of prey. In: Van den Brink, N.W., Elliott, J., Shore, R., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife. Emerging Topics in Ecotoxicology (Principles, Approaches and Perspectives). 5 Springer, Cham, Switzerland.

National Drug Intelligence Center, 2007. Domestic Cannabis Cultivation Assessment. U.S. Department of Justice 48. https://www.justice.gov/archive/ndic/pubs22/22486/index.htm#Purpose, Accessed date: 1 August 2019.

Newton, I., Shore, R.F., Wyllie, I., Birks, J.D.S. and Dale, L. 1999. Empirical evidence of side effects of rodenticides on some predatory birds and mammals. In Cowan, D.P. & Feare. C.J., Advances in Vertebrate Pest Management, pp 347–367. Filander Verlag, Fürth.

Oregon Liquor Control Commission, 2018. Marijuana License Applications. June 29, 2017. http://www.oregon.gov/olcc/marijuana/Documents/mj_app_stats_by_county.pdf (accessed January 2018).

Owley, J. 2018. Unforeseen land uses: the effect of marijuana legalization on land conservation programs. University of California Davis Law Review 51:1673–1716.

Rattner, B.A., Scheuhammer, A.M. and Elliott, J.E. 2011. History of Wildlife Toxicology and the Interpretation of Contaminant Concentrations in Tissues. Environmental contaminants in wildlife, interpreting tissue concentrations. CRC, Boca Raton, FL, USA, pp. 9–44.

Rattner, B.A., Horak, K.E., Elliot, J.E., Shore, R.F., van den Brink, N.W., 2014. Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. Environ. Sci. Technol. 48, 8433–8445.

Reynolds, J.D., 1980. Extraction and identification of ten anticoagulant rodenticides from baits and stomach contents by high pressure liquid chromatography. In: Proc Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians. 23. pp. 187–193.

Riley, S.P., Bromley, R.H., Poppenga Uzal, F.A., Whited, L., Sauvajot, R.M., 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. J. Wildl. Manag. 71, 1874–1884.

Serieys, L.E.K., Armenta, T.C., Moriarty, J.G., Boydston, E.E., Lyren, L.M., Poppenga, R.H., Crooks, K.R., Wayne, R.K., Riley, S.P.D., 2015. Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study. Ecotoxicology 24, 844–862.

Singleton, P.H., Lehmkuhl, J.F., Gaines, W.L., Graham, S.A., 2010. Barred owl space use and habitat selection in the eastern Cascades, Washington. J. Wildl. Manag. 74, 285–294.

Stone, W.B., Okoniewski, J.C., Stedelin, J.R., 2003. Poisoning of wildlife with

anticoagulant rodenticides in New York. J. Wildl. Dis. 35, 187–193.
Thomas, P.J., Mineau, P., Shore, R.F., Champoux, L., Martin, P.A., Wilson, L.K., Fitzgerald, G., Elliott, J.E., 2011. Second generation anticoagulant rodenticides in predatory birds: probabilistic characterization of toxic liver concentrations and implications for predatory bird populations in Canada. Environ. Int. 37, 914–920.
Thompson, C., Sweitzer, R., Gabriel, M., Purcell, K., Barrett, R., Poppenga, R., 2014. Impacts of rodenticide and insecticide toxicants from marijuana cultivation sites on fisher survival rates in the Sierra National Forest, California. Conserv. Lett. 7, 91–102.
U.S. Environmental Protection Agency [EPA], 2011. Final Risk Mitigation Decision for Ten Rodenticides. US Environmental Protection Agency, Washington, DC. http://www.epa.gov/pesticides/reregistration/rodenticides/finalriskdecision.htm, Accessed date: 1 April 2019.
Van den Brink, N.W., Elliot, J.E., Shore, R.F., Rattner, B.A., 2018. Anticoagulant Rodenticides and Wildlife. Springer Nature, Cham, Switzerland.
Vyas, N.B., Hulse, C.S., Rice, C.P., 2012. Chlorophacinone residues in mammalian prey at a black-tailed prairie dog colony. Environ. Toxicol. Chem. 31, 2513–2516.
Weidensaul, C.S., Colvin, B.A., Brinker, D.R., Huy, J.S., 2011. Use of ultraviolet light as an aid in age classification of owls. Wilson J. Ornithol. 123, 373–377.
Weir, S.M., Thomas, J.F., Blauch, D.N., 2018. Investigating spatial patterns of mercury and rodenticide residues in raptors collected near the Charlotte, NC, USA, metropolitan area. Environ. Sci. Pollut. Res. 25, 33153–33163.
Wiens, J.D., Anthony, R.G., Forsman, E.D., 2014. Competitive interactions and resource partitioning between northern spotted owls and barred owls in western Oregon. Wildl. Monogr. 185, 1–50.
Wiens, J.D., Dugger, K.M., Lesmeister, D.B., Dilione, K.E., Simon, D.C., 2018. Effects of Experimental Removal of Barred Owls on Population Demography of Northern Spotted Owls in Washington and Oregon—2017 Progress Report: U.S. Geological Survey Open-File Report 2018–1086. 23 p.. https://doi.org/10.3133/ofr20181086.
Wiens, J.D., Dugger, K.M., Lesmeister, D.B., Dilione, K.E., Simon, D.C., 2019. Effects of Barred Owl (*Strix varia*) Removal on Population Demography of Northern Spotted Owls (*Strix occidentalis caurina*) in Washington and Oregon, 2015–18: U.S. Geological Survey Open-File Report 2019-1074. 17 p.,. https://doi.org/10.3133/ofr20191074.