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December 22, 2017

Pesticide Registration Branch
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

RE: Comments on Department of Pesticide Regulation's Proposed Decision to Renew Rodenticide Registrations for 2018

To Whom It May Concern:

I am writing on behalf of Raptors Are the Solution and Project Coyote, both Projects of Earth Island Institute, to request that the Department of Pesticide Regulation (DPR) initiate reevaluation of rodenticide products containing the following active ingredients s as part of its proposed decision to renew pesticide product registrations for the year 2018.

(1) Brodifacoum
(2) Bromadiolone
(3) Difethialone
(4) Difenacoum
(5) Diphacinone
(6) Chlorophacinone
(7) Warfarin

As discussed more fully below and supported by the accompanying evidence, the continued use of anticoagulant rodenticides is likely to have significant impacts on wildlife health and the environment thereby triggering the requirements for reevaluation. *See* Food & Agriculture Code Sections 12824-12827; 3 Cal. Code of Reg. Sections 6220-6221; Public Resources Code Section 21080.5. Under DPR's CEQA certified regulatory program, DPR is required to make a finding, at the time of pesticide registration renewal, whether reevaluation is also warranted. 3 Cal. Code Reg. Sections 6215(c), 6253-6254.

A. LEGAL BACKGROUND

Pesticides used in California are registered both by the U.S. Environmental Protection Agency ("EPA") and DPR. Food & Ag. Code §12815. Through its registration powers, DPR has

authority to protect public health and safety and the environment, ensure proper labeling of pesticides and encourage less harmful alternatives to controlling pests. To protect the environment, DPR is given broad authority to deny, or cancel a registration for any pesticide that has been demonstrated to cause serious and uncontrollable adverse environmental impacts, even if the pesticide is registered under federal law. See Food & Ag. Code §§12824; 12825, 12827.5.

1. California's Pesticide Registration and Renewal Process

California's registration period for pesticide products is 12 months, at which time the registrant must apply for renewal. Food & Ag. Code §12817. Renewal is subject to the same evaluation criteria used for initial registration. Food & Ag. Code § 12824. Thus, the renewal evaluation is a discretionary decision by DPR as to whether a pesticide registration should be renewed for a year period based on the factors set forth in sections 12824 and 12825.

At the time of pesticide renewal, DPR must determine whether reevaluation of a pesticide registration is also appropriate. If DPR approves a renewal without reevaluation, the DPR director must make a "written finding that he or she has not received sufficient information necessitating reevaluation pursuant to sections 6220 and 6221." 3 Cal. Code Reg. § 6215(c.)

The criteria for whether a pesticide should be reevaluated are set forth at 3 Cal. Code Reg. Sections 6220 and 6221. Section 6220 provides:

The director may, at any time, evaluate a registered pesticide to carry out the provisions of Sections 12824, 12825, 12825.5 and 12827 of the Food and Agriculture Code. The Director shall investigate all reported episodes and information received by the Director that indicate a pesticide may have caused or is likely to cause, a significant adverse impact. If the Director finds from the investigation that a significant adverse impact has occurred or is likely to occur, the pesticide involved shall be reevaluated. 3 Cal. Code Reg. Section 6221 provides:

> The director shall also reevaluate a pesticide when certain factors have been found such as, but not limited to public or worker health hazard or other information suggesting a significant adverse risk.

In response to significant information submitted on DPR's proposed decision to renew pesticide registrations, DPR is required to consult with trustee agencies such as Fish and Game and the Regional Water Quality Control Boards with jurisdiction over affected resources, (3 Cal. Code Reg. Section 6252), investigate that significant information and review available, related information (3 Cal. Code Reg. Section 6220.) and respond to the public comments received in light of the information considered as part of DPR's ultimate determination. 3 Cal. Code Reg. Sections 6253-6254. If a pesticide is reevaluated, the director shall require submission of all data required for registration of a new pesticide by the EPA and by various administrative code provisions that are relevant to the focus on the reevaluation and has not been previously submitted to the department. 3 Cal. Code Reg. § 6222(a).

During the reevaluation process, the director shall determine if the pesticide should be classified

as a restricted material pursuant to Food & Agriculture Code § 14004.5. Section 14004.5 requires DPR to designate as "restricted materials" pesticides that present a danger of harming public health or the environment including where a pesticide presents a "hazard to the environment from drift onto streams, lakes and wildlife sanctuaries;" (§ 14004.5(d)); or "hazards relating to persistent residues in the soil resulting ultimately in contamination of the air, waterways, estuaries or lakes, with consequent damage to fish, wild birds and other wildlife. (§ 14004.5(e)). Subject to limited exceptions, operators proposing to apply such "restricted" pesticides must obtain a permit from the DPR, which limits uses to prevent potential injuries to the environment. F. & Ag. Code §§ 14005-14006.

2. Application of CEQA to Pesticide Regulation in California

Under the California Environmental Quality Act ("CEQA"), a state or local agency must initiate environmental review prior to carrying out or approving any discretionary project that may have a significant impact on the environment. (Pub. Res. Code § 21080(a.)) . If the agency finds that a project may have a significant impact, the agency must prepare an environmental impact report ("EIR"). (Pub. Res. Code § 21100(a) (state agencies). *Bozung v. Local Agency Formation Com*. (1975) 13 Cal. 3d 263, 277-279; An EIR provides the public and responsible government agencies with detailed information on the potential environmental consequences of an agency's proposed decision. *See e.g. No Oil, Inc. v. City of Los Angeles* (1974) 13 Cal. 3d 68, 81; *Sundstrom v. County of Mendocino* (1988) 202 Cal. App. 3d 296, 307.

CEQA provides a limited exemption from its EIR requirement for state agency regulatory programs whose written documentation containing environmental information serves as a functional equivalent of an EIR. Pub. Res. Code § 21080.5(a); *Sierra Club v. State Bd. of Forestry* (1994) 7 Cal. 4th 1215, 1229-1230; *Wildlife Alive v. Chickering* (1976) 18 Cal. 3d 190, 196; *Citizens for Non-Toxic Pest Control v. Department of Food & Agriculture* (1986) 187 Cal. App. 3d 1575, 1584.

California's pesticide regulatory program was certified as functionally equivalent on December 28, 1979. *See City of Sacramento v. State Water Resources Control Bd.* (1992) 2 Cal. App. 4th 960, 976-978; 14 Cal. Code Reg. § 15251(i).

As a functionally equivalent program, the pesticide registration process must still comply with the general policy goals of CEQA. *See* Pub. Res. Code § 21080.5(c); *Mountain Lion Foundation v. Fish & Game Commission, supra*, 16 Cal. 4th at 114; *Sierra Club v. State Board of Forestry, supra,* 7 Cal. 4th at pp. 1228, 1230-1231. This includes general CEQA directives that an agency consider the "cumulative impacts" of its project approvals, *EPIC v. Johnson* (1985) 170 Cal. App. 3d 604, 625, and provide timely and adequate responses to comments made by the public, *Id*. at 622; *Dunn-Edwards Corp. v. Southcoast Air Quality Management District* (1993) 19 Cal. App. 4th 519, 534). Further, to the extent that existing data suggests significant risk or indicates the potential for significant environmental impacts, DPR may not hide behind its own lack of complete data as a basis for not conducting the necessary environmental review in the form of reevaluation. *See* 3 Cal. Code Reg. Section 6222(a); *Sierra Club v. State Board of Forestry, supra,* 7 Cal. 4th at pp. 12134-1236; *Sundstrom v. County of*

Mendocino, supra, 202 Cal. App. 3d at 311.

B. EVIDENCE DEMONSTRATES THAT CONTINUING USE OF RODENTICIDES IN CALIFORNIA POSES A SIGNIFICANT RISK AND/OR IS LIKELY TO HAVE SIGNIFICANT CUMULATIVE IMPACTS ON WILDLIFE

If the Director finds from the investigation that a significant adverse impact has occurred or is likely to occur, the pesticide involved shall be reevaluated. *See* 3 Cal. Code Reg. § 6221.

The most recent data shows that rodenticide products containing active ingredients brodifacoum, bromadiolone, difethialone, difenacoum, diphacinone, chlorophacinone and warfarin continue to have significant adverse impacts to a wide range of wildlife species including species listed or candidates under the federal and state endangered species acts.

1. DPR's 2013 Risk Assessment and 2014 Regulatory Change Identified and Acknowledged Wildlife Hazard Posed by Rodenticides in California.

DPR has in the past acknowledged these adverse impacts, as part of its 2013 Second Generation Anticoagulant Rodenticide Assessment (2013 Risk Assessment), the scientific references and studies cited in which *we incorporate by reference* as part of these comments.

The 2013 Risk Assessment concluded:

> DPR analyzed wildlife incident and mortality data between 1995 and 2011, and rodenticide use and sales data between 2006 and 2010. The data indicate that exposure and toxicity to non-target wildlife from second generation anticoagulant rodenticides is a statewide problem. In addition, the data suggest that the problem exists in both urban and rural areas. Research data from various locations throughout California indicate that exposure is occurring in many taxa and in various ecosystems (urban, suburban, rural, and natural/wild areas). ....Of the 492 animals analyzed between 1995 and 2011, approximately 73% had residues of at least one second generation anticoagulant rodenticide ...The data also show that exposure of wildlife to second generation anticoagulant rodenticides can lead to sub-lethal effects. The sub-lethal effects reduce the fitness of wildlife at a time when wildlife are already meeting numerous challenges. Riley et al's (2007) study of bobcats is an example of the sub-lethal effects of rodenticides. The bobcats died due notoedric mange. Mange was not previously known as a significant pathogen in wild felids. However, exposure to rodenticides appears to have contributed to the disease process, and hence, the mortality of the bobcats.

2013 Risk Assessment, pp. 1-2. Based on the data reviewed, DPR found that "the use of second generation rodenticides *presents a hazard related to persistent residues in target animals resulting in impacts to non-target wildlife*." (emphasis added.)

The 2013 Risk Assessment states that "[w]hile the data show exposure, they do not link specific uses, or location of use of second generation anticoagulant rodenticide (i.e., indoors or

outdoors, homeowners or professionals) to exposure." Despite this lack of data, DPR determined that the banning of consumer applications of these rodenticides could potentially avoid the continued adverse effects on wildlife. Thus, on July 1, 2014, DPR adopted new regulations that restricted the purchase, possession, and use of rodenticide baits that contain the active ingredients brodifacoum, bromodialone, difenacoum, and difethialone. (The four widely used 2nd-generation anticoagulant rodenticides also known as "SGARs.")

The 2014 regulatory amendment limited the purchase, possession, and use of SGARs to certified pesticide applicators and those under their direct supervision. DPR's notices stated that it "adopted these regulations due to overwhelming evidence of wildlife weakened or killed by SGARs" but that "[o]ther categories of rodenticides—the 1st-generation anticoagulants, acute toxicants, and certain burrow fumigants—are still available to consumers." *See* Frequently Asked Questions about Rodents and Rodenticides, Department of Pesticide Regulation, Pest Management & Licensing Branch, 2014 ("Rodenticide FAQs")

At the time of this notice, DPR stated:

> DPR expects that trained certified applicators will exercise caution and fulfill their professional responsibilities when using SGARs and use them only when necessary. Once applicators are certified, they're required to take continuing education courses that include instruction about using rodenticides safely and only when necessary. *If DPR continues to receive reports of nontarget wildlife being adversely impacted by SGARs, further regulatory action may be considered.*

Rodenticide FAQs, p. 2.

2. Since the 2014 Regulatory Amendment, Wildlife Continue to be Harmed by Rodenticide Use in California.

Data collected from the Department of Fish and Wildlife since 2014 shows that since the adoption of the 2014 regulatory change, rodenticide contamination of wildlife in California has continued unabated, even increasing substantially for a number of both second and first generation rodenticides. In summary, the available data shows two trends.

First, contamination of wildlife from *second generation rodenticides* has remained at high levels, even increasing in many instances. This can be seen from data collected from the Department of Fish and Wildlife ("DFW") showing the following:

- documented rodenticide poisonings from **brodifacoum** has remained high with no significant change between 2013/2104 year prior to the regulatory change and the two years subsequent;

- documented rodenticide poisonings from **bromadiolone** has increased by approximately 10% in the two year period after the regulatory change;

● documented rodenticide poisonings from **difethialone** are three times as high in the two year period after the regulatory change as prior to the change;

● documented rodenticide poisonings from **difenacoum,** have also increased in the two year period after the regulatory change.

Second, the contamination of wildlife from *first generation rodenticides* has increased considerably, with data showing:

● documented rodenticide poisonings from **diphacinone** approximately four times as high in the two year period after the regulatory change as prior to the change;

● documented rodenticide poisonings from **chlorophacinone** from two to three times as high in the two year period after the regulatory change as prior to the change;

● documented rodenticide poisonings from **warfarin** approximately four times as high in the two year period after the regulatory change as prior to the change.

See Exhibits 1-3, attached hereto and Chart Below:

Rodenticide	Pre-reg total 2013-2014 deaths from bodies tested	Year 1 post reg (2014-2015) total deaths from bodies tested	Year 2 post reg (2015-2016) total deaths from bodies tested
Brodifacoum, 2nd gen	94	78	89
Bromadiolone, 2nd gen	59	52	69
Difethialone, 2nd gen	10	28	34
Difenacoum, 2nd gen	1.5	7.4	0
Diphacinone, 1st gen	13	50	47
Chlorophacinone, 1st gen	4.4	11	9.6
Warfarin, 1st gen	1.5	5.6	6.1

Third, the data also shows that wildlife may be contaminated with a variety of rodenticides, often a combination of first and second generation types. For example, virtually every mountain lion carcass examined in the year 2016 contained more than one rodenticide, with approximately half of the specimens positive for three to five different active ingredients. *See* Exhibit 2, attached. Similar figures exist for a host of other wildlife, from raptors including owls, hawks and peregrine falcons, to mammals including kit foxes, bobcats, coyotes and fishers. *See* Exhibit 3, attached. These results are corroborated by numerous other studies, including a recent WildCare study showing that over 76 percent of the wildlife they tested were positive for rodenticide exposure, meaning that many predatory wildlife are functionally living with anticoagulant toxins in their blood. *See* Exhibit 4, attached.

The data showing continued contamination of wildlife species despite the 2014 regulatory change constitutes new information that DPR must consider as part of its proposed decision to renew these pesticide registrations. DPR's Final Statement of Reasons adopting the 2014 regulations identifies the following comment:

> By continuing to allow certified applicators to use SGAR products, these active ingredients will continue to be present in the environment and affect nontarget wildlife as well as children and pets. Not only should consumer availability of the products be restricted, but *consider prohibiting the purchase and use of all SGAR products in California by cancelling, refusing to register or renew registration of products that contain SGAR active ingredients.*

See Final Statement of Reasons and Public Report, Department of Pesticide Regulation Title 3, Amending California Code of Regulations Amend Sections 6000 and 6400, and Adopting Section 6471 Designating Brodifacoum, Bromadiolone, Difenacoum, and Difethialone (Second Generation Anticoagulant Rodenticide Products) as Restricted Materials, Attachment A, p. 2. ("Attachment A.")

In response, DPR stated:

> DPR does not intend to ban SGARs at this time. The restricted materials designation will limit the purchase and use of SGARs to certified applicators and those under their direct supervision. DPR believes limiting the use of SGARs to trained applicators will reduce unintended exposures to nontarget wildlife. SGARs are only one of a number of tools that certified applicators may use for effective rodent control.

See Attachment A, p. 1.

The submitted data indicate that DPR's assumptions that the 2014 regulatory change making 2nd generation rodenticides restricted materials would reduce impacts on wildlife to insignificant levels is unfounded. This result was predictable given that the manner of use of the rodenticide – whether by the public or by a certified applicator - is unlikely to have any effect on whether such rodenticide ultimately ends up contaminating wildlife species that prey on the poisoned rodents. In sum, simply putting second generation anticoagulants into a restricted class (i.e., for use by pesticide companies only) has not prevented wildlife exposure and deaths. The pest control industry uses these poisons ubiquitously: when a rodent or other animal ingests these poisons and that animal in turn is consumed by a predatory animal like a hawk, owl, vulture, fox, fisher, bobcat, or mountain lion, it too can become sickened and/or die.

3. The Impacts to Wildlife from Rodenticide Use in California Requires Reevaluation.

The newest data demonstrates that first and second generation rodenticides are continuing to harm wildlife through indirect exposures, particularly through cumulative impacts caused by exposures to many types of rodenticides at the same time.

To the extent that more data are needed to determine the extent of contamination and the actual impacts of these pesticides based on an apparent increasing trend in use, those data must be collected as part of the reevaluation process. 3 Cal. Code Reg. 6222(a) provides DPR the

authority and legal obligation to fill data gaps relevant the significant risks raised by pesticide contamination, including consultation with trustee agencies such as the Department of Fish and Wildlife or the federal Fish and Wildlife Service.

These data should take into consideration the effect from the use of mixtures of two or more products in combination. 3 Cal. Code Reg. § 6192(c).

Reevaluation should also take into consider the substantial sublethal impacts that rodenticides are causing such as weakness, decreased fitness/increased vulnerability to other causes of mortality, reproductive impacts and birth defects such as shorter wings, tails, bones, and bills, neonatal transfer, internal bleeding, hemorrhaging of the heart, liver, kidney, lung, intestines, body wall, and bones, chronic anemia and mange, increased parasite and pathogen burdens, decreased resilience to environmental stressors, decreased food intake and decreased body weight. *See* Exhibit 5 (fact sheet on sublethal impacts), attached.

Reevaluation should also take into account new science published since DPR's last rodenticide evaluation process. Those include Vyas, et al. (American Midland Naturalist, 2017) showing that raptors are more likely to prey upon poisoned prey *See* Exhibit 6; Gabriel, et al. (PLOS One, 2015) showing a documented increase in mortality (57% increase) and exposure (6%) from pesticides in fishers in just the past three years, and also showing that exposure to multiple rodenticides significantly increased the likelihood of mortality from rodenticide poisoning. *See* Exhibit 7. Additionally, Poessel et al. (Journal of Wildlife Disease (2015) found brodifacoum and bromadiolone in very high concentrations in the livers of five coyotes and concluded that second generation anticoagulants are more likely to cause poisoning due to their persistence and accumulation in the liver. *See* Exhibit 8. Finally a recent study on bobcats shows that the primary threat to bobcat survival was diphacinone, a first-generation rodenticide. *See* Exhibit 9, Serieys, et al. 2015. Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study. *Ecotoxicology* 24: 844-862. (See also Exhibit 10, email from study author describing how prior testing was understating extent of contamination from 1st generation rodenticides.)

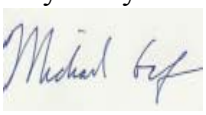
Reevaluation should also consider and evaluate viable alternatives to rodenticides be examined as part of this re-registration process. Such alternatives include exclusion and improved sanitation measures as well as the use of electronic rodent control devices such as The Raticator or the Rat Zapper. These products have been found to be very effective based on numerous reports we have received from schools, businesses, and other institutions that have switched from poison to traps. We also request that DPR evaluate the new, non-poisonous product ContraPest by Senestech that slows rat reproduction. This needs to be evaluated as an alternative since there have been very promising results with this compound in other states.

C. CONCLUSION

Raptors Are the Solution and Project Coyote request that DPR initiate reevaluation of rodenticide products containing the active ingredients brodifacoum, bromadiolone, difethialone, difenacoum, diphacinone, chlorophacinone and warfarin based on the continuing significant

adverse impacts to these pesticides are having on a wide range of wildlife species. As discussed above, to the extent that more data are needed to determine the extent of contamination and the actual impacts of these pesticides based on actual use in the field, that data must be collected as part of the reevaluation process.

Very Truly Yours,



Michael W. Graf

Final Comment Letter.doc

	Pre-Regs	Year 1	POST
brodifacoum	94.	78.	89.
bromadiolone	59.	52.	69.
difethiolone	10.	28.	34.
difenacoum	1.5	7.4	0.
diphacinone	13.	50.	47.
chlorophacinone	4.4	11.	9.6
warfarin	1.5	5.6	6.1
Total Cases	68	54	114
Bromethalin Cases	0	3	7

CDFW Mountain Lion database
Current as of 12/1/16

ACCESSION_I	DATE_DEATH	COUNTY	Brodifacoum	Bromadiolone	Difethialone	Difenacoum	Chlorophacinone	Diphacinone	Warfarin	Coumatetralyl
Z16-122	1/22/2016	El Dorado	0.087	Trace				Trace		
Z16-202	NA	NA	Trace				Trace			
Z16-217	1/7/2016	San Luis Obispo	0.058				0.51	0.25		
Z16-219	NA	NA	0.068					0.19		
Z16-222	NA	NA	Trace					0.23		
Z16-267	2/18/2016	Santa Barbara								
Z16-269	12/16/2015	Ventura	Trace	Trace				Trace		
Z16-270	1/15/2016	Los Angeles	Trace	Trace				Trace		
Z16-303	2/25/2016	Mono	Trace	Trace	Trace					
Z16-316	3/1/2016	Sonoma	0.42	Trace						
Z16-330	3/1/2016	Santa Cruz	0.66	0.22			Trace	0.28		
Z16-342	2/19/2016	Monterey	0.42	0.7	0.17			0.1		
Z16-344	3/14/2016	Butte	0.28	1.2				0.29		
Z16-407	2/16/2016	Modoc	Trace	Trace					Tace	
Z16-408	3/4/2016	Lassen	Trace	Trace					Trace	
Z16-409	3/16/2016	Modoc	0.063					Trace		
Z16-410	1/24/2016	Modoc	Trace	Trace					Trace	
Z16-427	3/22/2016	Mendocino	0.25	0.099	Trace			0.12	Trace	
Z16-429	3/31/2016	Santa Clara	0.37	0.44	Trace			0.06		
Z16-430	3/24/2016	Santa Cruz	0.34	0.11			Trace	0.11		
Z16-442	4/14/2016	Lake	Trace	Trace				Trace		
Z16-449	3/3/2016	Tuolumne	Trace	Trace						
Z16-450	2/25/2016	Tuolumne	Trace	Trace						
Z16-452	3/29/2016	Mariposa	0.12	Trace	Trace			0.071		
Z16-454	4/4/2016	San Mateo	0.057					0.1		
Z16-456	4/19/2016	Nevada	Trace							
Z16-469	4/5/2016	Shasta	Trace					0.11		
Z16-479	4/23/2016	Nevada	0.24	0.16	Trace					
Z16-521	4/24/2016	San Diego	Trace	0.078	Trace					
Z16-552	5/5/2016	El Dorado	0.1	0.14				0.05		
Z16-556	4/18/2016	San Mateo	0.13	0.19	Trace			0.13		
Z16-557	5/7/2016	El Dorado								
Z16-558	5/8/2016	Plumas								
Z16-574	2/19/2016	Tehama	Trace					0.07		
Z16-576	5/11/2016	Siskiyou	Trace					Trace		
Z16-577	4/28/2016	Siskiyou	Trace							
Z16-578	4/11/2016	Siskiyou	Trace	Trace				Trace		
Z16-583	5/17/2016	Nevada	0.39	Trace	Trace			0.057		
Z16-611	5/26/2016	Mendocino	Trace	0.12	Trace					
Z16-613	5/29/2016	Plumas	0.097	0.053				0.092		
Z16-614	5/30/2016	Plumas	0.49	0.056	Trace			0.32		
Z16-626	3/12/2016	San Luis Obispo	Trace	Trace	Trace		0.52	0.34		
Z16-627	11/24/2015	Santa Barbara	0.075	0.52	Trace			0.065		
Z16-628	11/24/2015	Santa Barbara	Trace	0.29				0.24		
Z16-629	2/17/2016	Monterey	0.097					0.44		
Z16-673	3/5/2016	Riverside								
Z16-674	NA	San Bernardino	0.93	0.26	Trace			0.11		
Z16-675	3/2/2016	Orange	0.16	0.84	Trace		Trace	0.23	Trace	
Z16-676	3/18/2016	San Diego	Trace	Trace				0.055		
Z16-756	7/12/2016	Tehama								
Z16-806	7/5/2016	Mendocino		Trace						
Z16-818	7/22/2016	Shasta	0.087	0.21				0.07		
Z16-843	7/14/2016	San Mateo	Trace	Trace	Trace			Trace		
Z16-846	7/26/2016	Santa Cruz	0.099	Trace				0.054		
Z16-878	7/5/2016	Amador	0.082	Trace				0.067		
Z16-880	2/25/2016	Calaveras	0.075	Trace						
Z16-881	4/13/2016	Amador	0.07	Trace			Trace	0.068		
Z16-884	4/5/2016	Calaveras	Trace							
Z16-886	8/3/2016	Amador	Trace	Trace						
Z16-895	8/9/2016	NA	Trace	Trace				Trace		
Z16-924	8/17/2016	Placer	Trace	Trace				Trace		
Z16-926	8/13/2016	Santa Clara	Trace		Trace					
Z16-931	8/19/2016	Yuba	0.35	0.21	Trace			0.1		
Z16-1018	9/14/2016	Modoc	Trace						Trace	
Z16-1069	9/8/2016	Shasta	Trace	Trace						
Z16-1118	9/29/2016	Placer	Trace	Trace						
Z16-1126	5/23/2016	Tuolumne	0.23	Trace						
Z16-1188	10/18/2016	Santa Cruz	0.068	0.15			0.48	Trace		

California Department of Fish and Wildlife Anticoagulant Rodenticide Exposure Cases 7/1/13 - present (compiled 12/27/16)
Wildlife Investigations Laboratory; Stella McMillin

Date	ID	County	Animal	2nd Generation ARs				1st Generation ARs			
				BROD	BROM	DIFETH	DIFEN	DIPH	CHLOR	WARF	OTHER FACTORS
PRE-REGULATION CHANGE											
7/9/2013	2713	Yolo	Red tailed hawk	trace*							
7/13/2013	2719	Kern	San Joaquin kit fox	0.096	0.43	trace					mange
7/17/2013	2706a-3	Contra Costa	Red shouldered hawk	0.52							
7/26/2013	2717	Sacramento	Coyote	0.071	trace			trace			
8/4/2013	2714	Alameda	Coopers hawk	trace	trace						trauma
8/8/2013	2722	Alameda	Turkey vulture	0.069	0.12	trace					
8/15/2013	2718	Monterey	Golden eagle	0.011							
8/22/2013	2706a-1	Contra Costa	Red shouldered hawk	0.39							
8/22/2013	2729	Butte	Mole	3.9							
9/1/2013	2720	Santa Barbara	Bobcat	0.4	0.69			trace			mange
9/9/2013	2702	Santa Barbara	Bobcat	0.016	0.18						Mange
9/12/2013	2706a-2	Contra Costa	Barn owl	0.017							
9/22/2013	2705	Ventura	Bobcat		0.26						Mange
10/19/2013	2708	Yolo	Barn owl	0.66							
10/19/2013	2716	Contra Costa	Red shouldered hawk	0.4							
10/21/2013	2721	Kern	San Joaquin kit fox	1.4							
10/24/2013	2745	Kern	San Joaquin kit fox		0.24						mange
10/25/2013	2746	Kern	San Joaquin kit fox	0.084	0.19	trace					mange
11/6/2013	2742	Kern	San Joaquin kit fox	0.027	0.3	trace					mange
11/11/2013	2728	Santa Barbara	Gray fox	0.46	trace			0.56			canine distemper virus (CDV)
11/18/2013	2741	Kern	San Joaquin kit fox	0.057	trace						trauma
11/20/2013	2739	San Diego	California spotted owl	trace	0.37			trace			bacterial infection
11/20/2013	2740	Kern	San Joaquin kit fox	0.25	0.14	trace	trace				
11/27/2013	2731	Ventura	Barn owl	0.16							
11/30/2013	2744	Placer	Gray fox	0.54	trace			trace			CDV
11/30/2013	2754	Kern	Desert kit fox	0.094	trace			trace			
12/11/2013	2736	Kern	San Joaquin kit fox	0.3	0.34						
12/13/2013	2737	Placer	Gray fox	trace	0.22						CDV
12/15/2013	2733	Sacramento	Striped skunk	trace							
12/31/2013	2743	Sacramento	Gray fox	0.71	0.31						CDV
1/2/2014	P2748	Alameda	Great horned owl	0.24	trace	trace					Sarcocystis
1/4/2014	P2751	Alameda	Gray fox	1.1	0.79						CDV
1/7/2014	P2788a	Los Angeles	Red tailed hawk	0.027							Pentobarbital

Date	ID	County	Animal	2nd Generation ARs				1st Generation ARs			
				BROD	BROM	DIFETH	DIFEN	DIPH	CHLOR	WARF	OTHER FACTORS
1/7/2014	P2788b	Los Angeles	Red tailed hawk	trace							Pentobarbital
1/15/2014	P2759	Alameda	Barn owl	0.72							Sarcocystis
1/15/2014	P2763a	Orange	Audubon cottontails	0.41							
1/15/2014	P2763b	Orange	Audubon cottontails	0.33							
1/15/2014	P2788c	Los Angeles	Red tailed hawk	trace							Pentobarbital
1/21/2014	P2757	Ventura	Western screech owl	0.46							
1/29/2014	P2753	Alameda	Barn owl	0.47							
2/2/2014	P2778	Kern	San Joaquin kit fox	0.095	0.18						Mange
2/6/2014	P2780	Alameda	Striped skunk	0.64	trace						Leptospirosis
2/11/2014	P2750	Sacramento	Geat horned owl	0.059	0.29						
2/24/2014	P2779	Nevada	Striped skunk	0.1							CDV
3/5/2014	P2765	Tulare	Gray fox	0.13					trace		CDV
3/8/2014	P2768	Sacramento	Gray squirrel	4.2							
3/10/2014	P2790	Alameda	Raccoon	trace	0.31						
3/15/2014	P2792	Santa Barbara	Black bear	0.14							Pneumonia
3/25/2014	P2760	Alameda	Turkey vulture	0.45							Lead, Aspergillosis
3/26/2014	P2775	Contra Costa	Cooper's hawk	0.027	trace						
3/31/2014	P2791	San Diego	Raccoon	0.32	0.31						CDV
3/31/2014	P2817	San Diego	Raccoon	0.32	0.311						CDV
4/18/2014	P2807	Kern	San Joaquin kit fox		trace						
4/25/2014	P2795	Kings	Beaver	0.013							
4/30/2014	P2811	Contra Costa	Great horned owl	0.36							trauma
5/6/2014	P2808	Humboldt	Humboldt marten	trace							predation
5/15/2014	P2798b	Kern	San Joaquin kit fox	0.13							
5/19/2014	P2801	Fresno	Gray fox	0.21	trace						CDV
5/19/2014	P2813	Ventura	Coyote	0.26	0.55	0.36					
5/22/2014	P2797	El Dorado	Coyote	trace	trace						
5/30/2014	P2902	Kern	San Joaquin kit fox	0.69	0.81						trauma
6/3/2014	P2836	Kern	San Joaquin kit fox	0.62	trace				trace		mange
6/24/2014	P2816	San Bernardino	Black bear		0.59						
0-1-YR POST-REGULATION CHANGE											
7/8/2014	P2825b	San Francisco	Gray squirrel		21						
7/8/2014	P2825a	San Francisco	Gray squirrel		5.4						
7/11/2014	P2818a	Santa Barbara	Coyote	0.26							
7/11/2014	P2818b	Santa Barbara	Coyote		0.069			trace			
8/22/2014	P2852	Santa Barbara	Bobcat	0.14	0.98	trace		trace			

Date	ID	County	Animal	2nd Generation ARs				1st Generation ARs			
				BROD	BROM	DIFETH	DIFEN	DIPH	CHLOR	WARF	OTHER FACTORS
9/3/2014	P2846a	Santa Cruz	Bobcat	0.12	trace			trace			mange
9/7/2014	P2843	Alameda	Raccoon	0.58							autolyzed
9/9/2014	P2846b	Santa Cruz	Bobcat	0.12	0.14			trace			trauma
10/6/2014	P2856	Monterey	Great horned owl	trace	trace						
10/26/2014	P2908	Ventura	Coyote	0.17	0.22	trace		1.3	trace		mange
11/4/2014	P2870	Los Angeles	Bobcat	trace	trace	trace		trace			
11/7/2014	P2853	Yolo	Red tailed hawk	trace							trauma
11/17/2014	P2855	Kern	San Joaquin kit fox	1.3	trace	trace	trace				
11/24/2014	P2865	Sacramento	Coyote	0.12	0.083			trace		trace	
12/4/2014	P2866	Santa Cruz	Bobcat	0.42	0.064			trace			trauma
12/8/2014	P2862	El Dorado	Coyote	0.2	0.39						mange
1/6/2015	P2917	Sacramento	Striped skunk	1.3			trace	trace			trauma
1/15/2015	P2901	Shasta	Gray fox	0.14				trace			CDV
1/25/2015	P2900	San Benito	Bobcat	0.13	0.13				trace		mercury, gunshot
2/4/2015	P2899	Trinity	Black bear						trace		Fungal hair loss
2/10/2015	P2910	Los Angeles	Great horned owl	0.37							
2/24/2015	P2915	El Dorado	Coyote	0.48	0.16	0.36		trace			trauma
3/2/2015	P2895	Contra Costa	Red shouldered hawk	0.44	trace						
3/2/2015	P2897a	Nevada	Striped skunk	0.74		trace	trace	trace			CDV
3/2/2015	P2897b	Nevada	Striped skunk	0.074							CDV
3/10/2015	P2906	Santa Barbara	Red shouldered hawk	0.51		trace		trace			
3/14/2015	P2896	Contra Costa	Great horned owl	0.32	0.07	trace					
3/25/2015	P2907	Santa Cruz	Peregrine falcon	trace							avian cholera
3/26/2015	P2909	Sacramento	Coyote	0.016	0.53	trace		trace			gunshot, hit by car
4/6/2015	P2936	Santa Cruz	Striped skunk	trace							parasitism
4/10/2015	P2916	Lake	Wild pig					trace			trauma
4/13/2015	P2918	San Francisco	Great horned owl	0.033	trace	trace					trauma
4/19/2015	P2912	Placer	Gray squirrel		trace						
4/24/2015	P2925	San Luis Obispo	Coyote					trace	trace		
5/2/2015	P2919	San Luis Obispo	Great horned owl		trace						
5/3/2015	P2933	Santa Cruz	Barn owl	0.02					trace		avian cholera
5/6/2015	P2921	Contra Costa	Great horned owl					trace			trauma
5/7/2015	P2924	Marin	Gray fox	trace				trace			septicemia
5/18/2015	P2937	Marin	Turkey vulture	0.38		trace	trace				
5/21/2015	P2944a	Nevada	Raccoon					trace			
5/24/2015	P2938	Alameda	Striped skunk	trace				trace			bromethalin intoxication
5/28/2015	P2935	Sacramento	Red shouldered hawk	trace							

Date	ID	County	Animal	2nd Generation ARs				1st Generation ARs			
				BROD	BROM	DIFETH	DIFEN	DIPH	CHLOR	WARF	OTHER FACTORS
6/2/2015	P2939	Santa Barbara	Bobcat	0.6	0.51	trace		trace			mange
6/9/2015	P2943	San Mateo	Raccoon	0.011				trace			bromethalin intoxication
6/15/2015	P2944b	Nevada	Raccoon	trace				trace			
6/20/2015	P2947	Monterey	Gray fox	0.018		0.76					
6/27/2015	P2967	Los Angeles	Great horned owl	0.02	0.32			trace			
1+-YR POST-REGULATION CHANGE											
7/3/2015	P2961	Ventura	Gray squirrel	trace	trace	6.2					
7/26/2015	P2794	Santa Barbara	Bobcat	0.096	trace	trace		0.085			
7/27/2015	P2957	Los Angeles	Great horned owl		0.31						
8/5/2015	P2980	Sacramento	Striped skunk	0.29	trace	trace					
8/6/2015	P2956b	Monterey	Raccoon	0.32	0.22						bromethalin intoxicatoni
8/6/2015	P2984a	Santa Cruz	Raccoon	0.059	1						CDV
8/16/2015	P2984b	Santa Cruz	Raccoon	trace				0.12			CDV
10/15/2015	P2983	Placer	Black bear	1.3	trace	trace		trace			
10/18/2015	P2982	Marin	Turkey vulture	0.57		trace					
11/16/2015	P3142	Sonoma	Striped skunk	0.13	0.26	trace					
11/18/2015	P3014	Marin	Fox Squirrel							trace	bromethalin intoxication
11/22/2015	P3015	Placer	Black bear	0.1	trace	0.1					trauma
12/24/2015	P2998	San Mateo	Gray fox	0.13	trace						bromethalin intoxication
12/29/2015	P3018	Ventura	Bobcat	0.096	trace	trace		0.085			caught in net
1/8/2016	P3034	Kern	San Joaquin kit fox	trace							hit by car
1/19/2016	P3031	Sacramento	Red fox	0.16	trace						mange
1/20/2016	P3007b	San Francisco	Raccoon	trace							bromethalin intoxication
1/26/2016	P3007a	San Francisco	Raccoon	trace							hit by car
2/6/2016	P3035	Santa Clara	Gray squirrel	trace	15	trace					
2/6/2016	P3033	Santa Clara	Striped skunk		0.39						CDV
2/10/2016	P3085	Butte	Fisher	Trace							hemothorax, hemoabdomen
2/20/2016	P3131	Trinity	Gray fox		2.1						CDV
2/29/2016	P3048	Fresno	Raccoon	trace	0.12						bromethalin intoxication
2/29/2016	P3130	Trinity	Gray fox	trace							CDV
3/11/2016	P3047	Fresno	Gray fox	0.45	0.23			0.063			bromethalin intoxication
3/16/2016	P3041	San Francisco	Great horned owl	0.16	trace	0.4					
4/20/2016	P3059	Placer	Raccoon	trace	1.2			0.087			bromethalin intoxication
4/22/2016	P3057	Santa Cruz	Gray fox	0.8	0.37	trace		0.18			
4/28/2016	P3058	Santa Cruz	Gray fox	trace		trace					
5/27/2016	P3084	Butte	Fisher	Trace							predation

Date	ID	County	Animal	2nd Generation ARs				1st Generation ARs			
				BROD	BROM	DIFETH	DIFEN	DIPH	CHLOR	WARF	OTHER FACTORS
6/22/2016	P3143	Los Angeles	Great horned owl	0.083	trace	0.46					
7/13/2016	P3080	San Bernadino	Desert kit fox		trace						drowning
7/21/2016	P3115	San Luis Obispo	Red shouldered hawk	trace	trace	0.56					
7/26/2016	P3086		Fisher	trace							trauma
7/29/2016	P3083	Butte	Fisher	trace							
8/4/2016	P3117	San Luis Obispo	San Joaquin kit fox	trace	0.4				trace		predation
8/14/2016	P3148	Los Angeles	Coyote	trace	0.29	trace		0.085			trauma
8/14/2016	P3149	Los Angeles	Coyote	trace				0.12			trauma
9/1/2016	P3150	San Luis Obispo	Striped skunk	2	0.075	trace					
9/5/2016	P3147	Contra Costa	Coyote	0.22	trace	trace		trace			trauma
10/7/2016	P3153	Santa Clara	Great horned owl	0.64							
10/9/2016	P3152	Placer	Great horned owl	0.09	0.48			0.12	trace		
10/17/2016	P3146	Orange	Coyote	0.51	0.58	0.13		0.099	trace		trauma
10/21/2016	P3155	Los Angeles	Red shouldered hawk			0.55					
10/25/2016	P3145	Orange	Coyote	trace	1.4	0.58		0.23			trauma
11/9/2016	P3154	Orange	Great horned owl	0.069	trace	0.43					

* trace = detected at a concentration below the reporting limit (0.02 ppm for chlorophacinone, diphacinone, and warfarin; 0.05 ppm for brodifacoum, bromadiolone, difethialone, difenacoum)

Database does not include mountain lion cases (listed in separate database)

Anticoagulant Rodenticide Exposure in Nontarget Wildlife, 2013-2014 Study Results and 2006-2014 Results

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2013-2014 Study Results

86% positive for exposure
5% toxicosis

95 submissions, 10 species

70 Mammals tested: Coyote, Gray Fox, Raccoon, Striped Skunk
74% of submissions, 93% positive, 2 toxicosis

22 Raptors tested: Barn Owl, Red-Shouldered Hawk, Red-Tailed Hawk, Turkey Vulture
23% of submissions, 68% positive, 3 toxicosis

3 Songbirds tested: Crow, Raven
2% of submissions, 67% positive, 0 toxicosis

Breakdown By Species, Positive for Exposure to Anticoagulant Rodenticides		Key: + = positive
Species	**Study Results, 2013-2014, 86% positive**	**All-Time Results, 2006-2014, 82.9% pos.**
Coyote	2 tested, 2+/2–100%	10 tested, 8+/10–80%
Gray Fox	20 tested, 18+/20–90%, **1/20 COD–5%**	43 tested, 39+/43–91%
Raccoon	33 tested, 30+/33–91%, **1/33 COD–3%**	64 tested, 57+/64–89%
Striped Skunk	15 tested, 15+/15–100%	44 tested, 42+/44–95%
Barn Owl	5 tested, 4+/5–80%, **2/5 COD–40%**	38 tested, 24+/38–63%
Great Horned Owl	None tested	22 tested, 20+/22–90%
Red-Shouldered Hawk	4 tested, 4+/4–100%, **1/4 COD–25%**	14 tested, 12+/14–86%
Red-Tailed Hawk	9 tested, 3+/9-33%	41 tested, 29+/41–70%
Turkey Vulture	4 tested, 4+/4–100%	13 tested, 11+/13–85%
Crow	2 tested, 1+/2–50%	14 tested, 11+/14–79%
Raven	1 tested, 1+/1–100%	2 tested, 2+/2–100%

Persistence of anticoagulants in liver (USEPA)

First generation (FGARs)	Second generation (SGARs)
Diphacinone = 90 days	Brodifacoum = 217 days
Warfarin = 35 days	Bromadiolone = 248 days
	Difethialone = 118 days

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

- Hemorrhaging beneath the skin and extensive bruising. Internal hemorrhaging in bones, body wall, heart, and elsewhere in the body. Possible heart failure.[1]
- Hemorrhaging of the heart, liver, kidney, lung, intestines, and muscles.[2]
- Increased vulnerability to other causes of death such as vehicular collisions and predation.[3]
- Chronic anemia, making animals more susceptible to diseases, including mange, and other stressors. [4]
- Reproductive impacts. Female sheep exposed to anticoagulants had more aborted or stillborn lambs (up to 50%); male sheep had lower sperm motility.[5]
- Decreased food intake[6] and decreased body weight.[7]
- Neonatal transfer to young kits. Decreased resilience to environmental stressors.[8] Fetuses more susceptible to brodifacoum toxicity than adults.[9]
- Increased parasite and pathogen burdens[10]
- Shorter wings, tails, bones, bills, and birth defects.[11]
- Rodents poisoned by anticoagulants are more likely to be eaten by predators.[12]

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Influence of Poisoned Prey on Foraging Behavior of Ferruginous Hawks

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Influence of Poisoned Prey on Foraging Behavior of Ferruginous Hawks

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ABSTRACT.— We recorded 19 visits by ferruginous hawks (*Buteo regalis*) over 6 d at two black–tailed prairie dog (*Cynomys ludovicianus*) subcolonies poisoned with the rodenticide Rozol® Prairie Dog Bait (0.005% chlorophacinone active ingredient) and at an adjacent untreated subcolony. Before Rozol® application ferruginous hawks foraged in the untreated and treated subcolonies but after Rozol® application predation by ferruginous hawks was only observed in the treated subcolonies. We suggest that ferruginous hawks' preference for hunting in the treated subcolonies after Rozol® application was influenced by the availability of easy-to-capture prey, presumably due to Rozol® poisoning. The energetically beneficial behavior of favoring substandard prey may increase raptor encounters with rodenticide exposed animals if prey vulnerability has resulted from poisoning.

INTRODUCTION

Foraging decisions are a compromise between the energetic costs of searching for, capturing, and subduing prey plus the risks of injury from the defending prey and the nutritional and energetic benefits derived from feeding on the prey. Therefore, raptors may preferentially take substandard prey because these animals display decreased vigilance, a poor ability to escape, and reduced defenses compared to healthy conspecifics (Hoogland *et al.*, 2006; Genovart *et al.*, 2010). This behavioral adaptation has allowed raptors to efficiently exploit food resources, but the beneficial behavior of favoring substandard or dead prey also can be a detriment to a raptor, *i.e.*, when prey vulnerability results from poisoning (Chesser, 1979; Hunt *et al.*, 1992; Elliott *et al.*, 1997).

Ferruginous hawks (*Buteo regalis*, FEHA) that migrate through and winter in central and southern plains in the United States and eastern Mexico prey on black-tailed prairie dogs (*Cynomys ludovicianus*, BTPD; Plumpton and Andersen, 1997; Bak *et al.*, 2001). Black-tailed prairie dogs, however, are considered to be an agricultural pest and BTPD eradications often are promoted and conducted by county, state, and federal agencies (Lamb *et al.*, 2006; Miller *et al.*, 2007). Two first generation anticoagulant rodenticide products, Rozol® Prairie Dog Bait (0.005% chlorophacinone active ingredient; 2-[(p-chlorophenyl) phenylacetyl]-1,3-indandione, hereafter Rozol®) and Kaput-D® Prairie Dog Bait (0.005% diphacinone active ingredient; 2-diphenylacetyl-1,3-indandione,) are registered for BTPD control October 1–March 15 in 10 states. First generation anticoagulant rodenticides disrupt blood clotting that can lead to hemorrhaging and death in vertebrates (Pelfrene, 2001). Sublethal adverse effects can occur within 48 h of exposure but mortality may occur $\geq$1 wk after lethal exposure (Whisson and Salmon, 2009; Rattner *et al.*, 2011). Consequently, poisoned BTPDs (active, impaired, moribund, and dead) are available as prey for raptors (Vyas *et al.*, 2012).

While conducting a larger study to determine the hazards of Rozol® to wildlife, we hypothesized ferruginous hawk behavior would follow predictions of foraging theory, and they would prefer to hunt BTPDs in Rozol® treated areas because of the availability of easy-to-capture (poisoned) prey.

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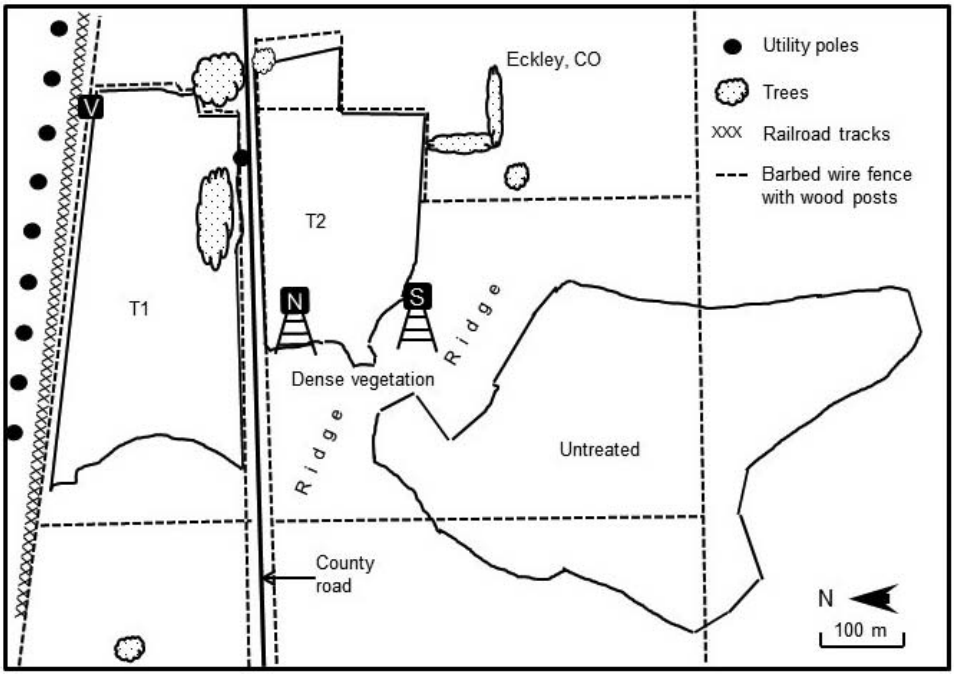

FIG. 1.—Map of the three black-tailed prairie dog (*Cynomys ludovicianus*) subcolonies at Eckley, Colorado, U.S.A. during January 2011. Subcolonies T1 and T2 were poisoned with Rozol® and the third colony (Untreated) did not receive Rozol® application. A ridge, dense vegetation, and a county road restricted black-tailed prairie dog movements among the subcolonies. Observations were conducted from N = north tower, S = south tower and V = vehicle

METHODS

STUDY SITE

The influence of Rozol® application on foraging behaviors of FEHAs was recorded at three subcolonies of a BTPD colony on a private pasture in Eckley, Colorado (40°6′45"N latitude and 102°29′22"W longitude; Fig. 1) in January–February, 2011. Two of the subcolonies (T1 and T2) were destined to be poisoned with Rozol®, and the third subcolony (untreated) did not receive Rozol® application. Subcolonies to be treated with Rozol® encompassed 16.3 ha and contained 1986 active BTPD burrows whereas the untreated subcolony was 16.8 ha and had 2032 active BTPD burrows.

SCAVENGER CARCASS-REMOVAL TRIAL

On the 4 d before Rozol® application and the 2 d of Rozol® application, a scavenger carcass-removal trial was conducted to document the loss of BTPD carcasses by scavengers from the three subcolonies. Twenty-two uncontaminated adult BTPD carcasses from the US Fish and Wildlife Service National Black-Footed Ferret Conservation Center, Wellington, Colorado were equally distributed to the treated and untreated subcolonies and four, eight, six, and four carcasses were randomly placed in the subcolonies on days 1–4 before Rozol®

application, respectively. Carcass locations were marked with a handheld GPS receiver (Garmin eTrex Summit® HC). On days 2–4 before Rozol® application and on the 2 d of Rozol® application, we conducted carcass searches to determine our carcass detection distance and scavenging activity. Our search patterns were alternated from north to south/south to north every other day to east to west/west to east. While conducting carcass searches, we opportunistically recorded FEHA activity in the subcolonies.

ROZOL® APPLICATION

Rozol® application was conducted on two consecutive days and hereafter, the 2 d are considered as day 0 of the study. Subcolonies T1 and T2 were poisoned with 112.6 kg of Rozol® according to the product label by certified pesticide applicators from the Yuma County Pest Control District as part of an ongoing BTPD control program. Black-tailed prairie dog movements among the subcolonies were restricted by a ridge, dense vegetation, and an unpaved, graded, secondary road, therefore the three subcolonies were considered independent of each other with respect to Rozol® treatment. The proximity of the treated and untreated subcolonies minimized spatiotemporal variability between the subcolonies and simplified determination of foraging preferences by the raptors.

DATA COLLECTION

Black-tailed prairie dog activity and FEHA foraging behavior were documented between 0930 and 1600 h on days 8–11 and 16 and 17 post Rozol® application. Observations were initiated 1 wk after Rozol® application based on the time course of adverse effects for chlorophacinone (Whisson and Salmon, 2009; Vyas *et al.*, 2012) and no observations were made on days 12–15 and after day 17 post application because of weather conditions that restricted above ground BTPD activity. Data were collected using binoculars (Nikon Monarch 3 8x42 ATB) and video cameras (Panasonic SDR-H80, JVC GZ-MG630, and Sony DCR-SR47) from two tower blinds (approximately 3.7 m high) and from a stationary vehicle at the northeast corner of the colony (Fig. 1). Three observers simultaneously monitored the three subcolonies to ensure visual coverage of the study area: one observer in each of the two towers and one observer in the stationary vehicle. Each tower provided a 360° view but the north tower primarily was used for observing the two treated subcolonies, whereas the south tower allowed scanning of the untreated subcolony. The vehicle facilitated observations on a small area in T1 that was blocked from view from the towers by trees. Observers in the tower blinds were rotated daily to reduce observer bias.

BLACK-TAILED PRAIRIE DOG ACTIVITY

The numbers of BTPDs active above ground in the three subcolonies were counted hourly from blinds to document their potential availability as prey for FEHAs. Changes in BTPD numbers over time in the subcolonies were analyzed by the Mann-Kendall test (https://www.researchgate.net/file.PostFileLoader.html?id=55bba3666225ff21e88b4569&assetKey=AS%3A273823084023809%401442295918401).

FERRUGINOUS HAWK FORAGING

Ferruginous hawk presence and duration of activity in the subcolonies were documented. Because FEHAs were not marked, after the FEHA had flown out of the three subcolonies and was out of our sight, it was not possible to reliably determine if the next conspecific that

was sighted was the same individual as the one observed earlier. Therefore, the FEHA count represents the number of FEHA visits to the colony. The amount of time a FEHA spent in the subcolonies (soaring, perched on trees and utility poles, and fence posts within and along the perimeter of the study area, perched on the ground at prey, and perched on the ground without prey) was documented from the first sighting to the last observation of that raptor. We used 'at prey' instead of the amount of a time a raptor spent consuming the prey because the raptors did not continuously feed while standing on the prey item and at times, other birds landed close to the bird that was in control of the food, challenging and displacing the feeding bird. Therefore, 'at prey' includes raptors feeding, standing over the prey, standing close to an unclaimed food item, or standing close to the raptor in control of the prey. We used 'perched on ground without prey' to describe FEHAs that perched on the ground where no above ground BTPD activity was observed within ~90 m of the hawks. Ferruginous hawks observed soaring in and out of the subcolonies without landing were allotted 1 min of time. The amount of time FEHAs spent in untreated and treated subcolonies was compared by the Tukey-Duckworth Procedure (http://www.ohio.edu/plantbio/staff/mccarthy/quantmet/lectures/Nonparm.pdf).

Predations by FEHAs were documented from the blinds and through discovery of preyed upon BTPDs during carcass searches conducted at the end of the daily observation period after the FEHAs had departed from the study area. Carcass searches involved walking transects approximately 4 m apart. Transect spacing was based on the mean distance of detection during the scavenger carcass-removal trial.

RESULTS

SCAVENGER CARCASS-REMOVAL TRIAL

Eighteen of 22 BTPD carcasses placed in the three subcolonies were removed during the night within 24 h of placement, presumably by mammalian scavengers. Two BTPD carcasses were removed within 48 hr of placement and two carcasses were not scavenged for at least 3 d. On day 2 before Rozol® application, two BTPD carcasses placed in the untreated subcolony and one placed in the treated colony were found partially scavenged before being removed by scavenger the following night. One of the carcasses in the untreated subcolony attracted a FEHA within 4 h of placement. Although we did not witness raptors at the two other partially scavenged BTPD carcasses, since raptors could not carry off the BTPD prey and fed at the site of carcass placement, the discovery of partially scavenged carcasses served as evidence of raptor foraging. We suspect FEHAs scavenged these two carcasses because five opportunistic sightings of FEHAs were recorded on that day. Opportunistic counts of FEHA ranged 1–5 sightings per day and the greatest number of FEHA sightings occurred on day 2 before Rozol® application. No other signs of predation or scavenging were observed during carcass removal trial. Ferruginous hawks were seen on all days during the scavenger carcass-removal trial and on the Rozol® application days.

BLACK-TAILED PRAIRIE DOG ACTIVITY

The numbers of BTPDs in the untreated subcolony showed no significant trend over time (Mann-Kendall 2-tailed $S = -7$; $P > 0.05$). However, a significant declining trend was detected in the numbers of BTPDs over time in the treated subcolonies (Mann-Kendall 2-tailed $S = -13$; $P < 0.05$).

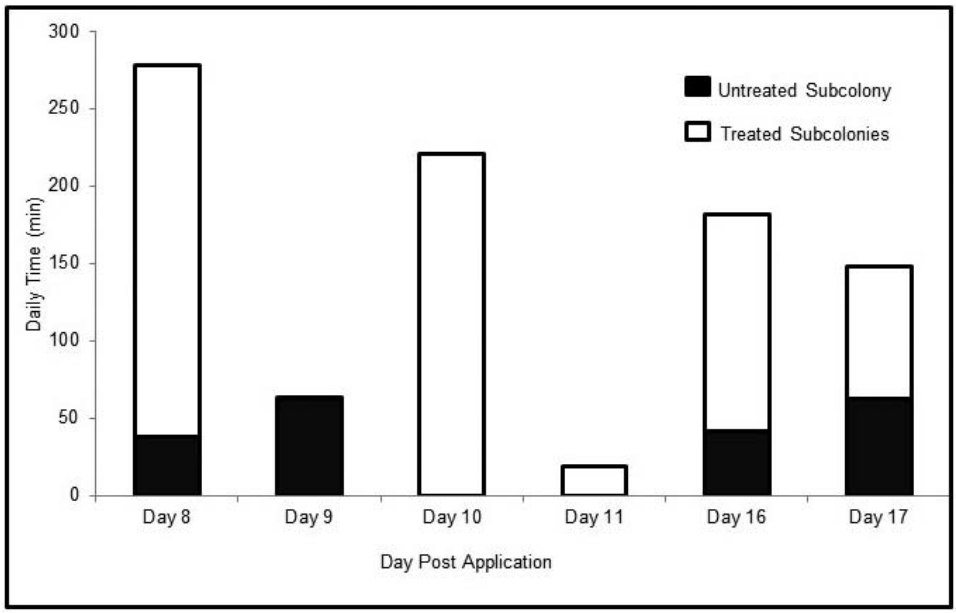

FIG. 2.—Amount of time that ferruginous hawks (*Buteo regalis*) spent in the untreated and Rozol® treated subcolonies of a black-tailed prairie dog (*Cynomys ludovicianus*) colony at Eckley, Colorado, U.S.A. during six observation days in January–February 2011

FERRUGINOUS HAWK FORAGING

We recorded 19 visits by FEHAs at the three subcolonies after Rozol® application. Ferruginous hawks spent 911 min in the three subcolonies over the six observation days: 203 min in the untreated subcolony and 708 min in the treated subcolonies (Fig. 2). The amounts of time FEHAs spent in the untreated and treated subcolonies were not significantly different (Tukey-Duckworth $C = 6$, $P > 0.05$). While in the treated subcolonies, FEHAs spent 310 min (daily range 0 min–194 min) at prey. Ferruginous hawks also perched on the ground without prey (no BTPDs active above ground observed within ~90 m of the FEHA) for 72 min (daily range 0 min–32 min) and 175 min (daily range 0 min–90 min) in the untreated and treated subcolonies, respectively.

Four predations by FEHAs were observed on days 10, 16 and 17 post application. Two predations occurred on day 10 post application and one predation was observed on days 16 and 17 post application. Predations were only observed in the treated subcolonies even though FEHAs spent time in the untreated subcolony. Ferruginous hawks spent time in the treated subcolonies on 6 d whereas they spent time in the untreated colony on 4 d. All preyed upon animals were BTPDs. No other evidence (*i.e.*, fur, blood, partial carcass) of predation or scavenging was found during carcass searches in the treated and untreated subcolonies. We also did not see failed hunting attempts by FEHAs. On days 10 and 16 post application, FEHAs that captured prey attracted two and one additional FEHAs, respectively. Three aggressions by FEHAs on conspecifics resulted in displacement of the feeding birds whereas two encounters were unsuccessful. Aggressive behaviors were similar to those described by Bechard and Schmutz (1995) and included lunging at a nearby bird, swooping

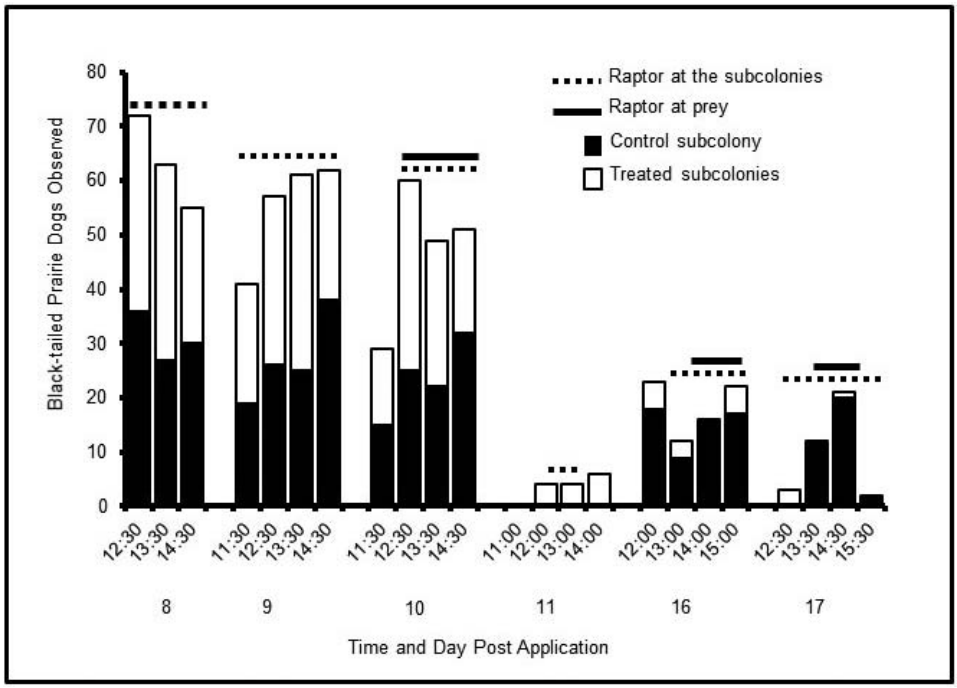

FIG. 3.—Number of black-tailed prairie dogs (*Cynomys ludovicianus*) observed above ground in the untreated and treated subcolonies after Rozol® application at Eckley, Colorado, U.S.A. during six observation days in January–February 2011. The dashed lines above the prairie dog counts show when at least one ferruginous hawk (*Buteo regalis*) was present in the untreated or treated subcolonies. The solid lines depict when at least one ferruginous hawk was at prey. Black-tailed prairie dogs were available to ferruginous hawks as prey in the untreated and treated subcolonies and ferruginous hawks were observed in all three colonies but all predations occurred in the treated subcolonies

low over a bird at prey, chasing the intruding bird on foot, snapping beaks, erecting feathers, lifting wings, and cupping wings to shelter the prey from other birds.

DISCUSSION

Foraging theory postulates that raptors should hunt such that they minimize their energy expenditure and maximize their net energy intake (Pyke *et al.*, 1977). The decision to forage in a particular area is based on prey availability and accessibility (Bechard, 1982; Preston, 1990) and the decision to prey on a particular animal is influenced by its vulnerability (Temple, 1987; Hoogland *et al.*, 2006). Black-tailed prairie dogs are large (700–1500 g, Hoogland and Foltz, 1982), colonial, diurnal rodents that provide FEHAs a concentrated food source in winter when other food is scarce. Black-tailed prairie dogs at our subcolonies were available as potential prey in the untreated and treated subcolonies while FEHAs foraged in the subcolonies (Fig. 3). A significant downward trend was detected in the numbers of BTPDs for the treated subcolonies over time but not for the numbers of BTPDs in the untreated subcolony. Because the proximity of the three subcolonies minimized

geographic and meteorological variations among the subcolonies, the decline in the numbers of BTPDs in treated subcolonies is attributed to chlorophacinone toxicity.

Surface BTPD availability is affected by meteorological factors including temperature, wind velocity, and precipitation (Tileston and Lechleitner, 1966; Lehmer *et al.*, 2003). Day 11 post application experienced fog and freezing rain and although no BTPDs were observed in the untreated subcolony, 14 BTPDs foraged above ground in the treated subcolonies. Captive BTPDs that were provided an ad libitum Rozol® diet suffered signs similar to diarrhea until the diet was supplemented with hay (Vyas, unpubl. data). The BTPDs in the treated subcolonies may have surfaced on day 11 post application to forage on vegetation despite unfavorable weather because of chlorophacinone's physiological effects.

Prey accessibility is affected by vegetation cover and perch availability. Visual obstruction of prey by vegetation in the three subcolonies was negligible because of grazing by BTPDs (Agnew *et al.*, 1986; Winter *et al.*, 2002) and plant winter dormancy and senescence. Elevated perch availability (trees, utility poles, fence posts) differed between the untreated and treated subcolonies. The two treated subcolonies had tall perches (telephone and utility poles and trees) along their perimeters and had ~2519 m of barbed wire fencing with wood fence posts. The control subcolony lacked tall perches and had ~597 m of the fencing (Fig. 1). However, FEHAs readily hunt perched on the ground or from soaring flight, therefore are not restricted in their foraging by a paucity of elevated perches (Wakeley, 1978; Janes, 1985; Bechard and Schmutz, 1995). During the scavenger carcass-removal trial, FEHAs foraged on the BTPD carcasses placed by us in the untreated subcolony despite the lack of trees and utility poles. After Rozol® application, FEHAs captured prey in the treated subcolonies from soaring flights and did not use the available elevated perches to initiate their hunts.

When more than one prey is available and accessible, raptors may select individuals based on their vulnerability (ease of capture) and the costs (energetics and risk of injury from the defending prey) of capturing healthy conspecifics (Temple, 1987; Ille, 1991; Taylor, 2009). Black-tailed prairie dogs in a Rozol® poisoned colony can exhibit a spectrum of vulnerabilities to predation, ranging from overtly healthy to dead animals for at least 4 wk after Rozol® application (Vyas *et al.*, 2012). Healthy BTPDs, when threatened, give alarm calls, run to their burrows, and if captured, attempt escape by biting the raptor's feet whereas Rozol® poisoned BTPDs are easily captured because of lethargy and reduced alertness (Vyas *et al.*, 2012).

Ferruginous hawks at our study site appeared to optimize their foraging before and after Rozol® application by taking easy-to-capture prey. During the scavenger carcass-removal trial, raptors fed only on the BTPD carcasses placed as part of the trial in the three subcolonies. No signs of predation were found although live BTPDs also were available above ground. After Rozol® application, FEHAs captured prey only in the treated subcolonies even though >80% of the above ground BTPDs in the three subcolonies were available in the untreated subcolony (Fig. 3). Additionally, after Rozol® application, FEHAs captured all prey in the treated subcolonies using high altitude soaring-flight, a hunting method used for vulnerable prey (Wakeley, 1978). Therefore, we suspect that after Rozol® application, FEHAs were drawn to the BTPDs in the Rozol® treated subcolonies because the animals were easy-to-capture, presumably due to Rozol® poisoning.

Before and after Rozol® application, FEHAs appeared to follow predictions of foraging theory by hunting easy-to-capture prey. Ferruginous hawks are designated as one of the Birds of Conservation Concern in the United States (U.S. Fish and Wildlife Service, 2008) and as Threatened in Canada (Committee on the Status of Endangered Wildlife in Canada, 2008).

Although our observations are limited by our small sample size on a single species at a single BTPD colony, our findings offer information towards conservation of FEHAs. First, the importance of BTPDs to FEHAs in winter, compounded with the FEHAs' preference for easy-to-capture prey, suggests a potentially greater risk of secondary poisoning for FEHAs if the prey vulnerability has resulted from poisoning. Second, in an effort to reduce the risks to raptors, the current Rozol® and Kaput-D® Prairie Dog Bait labels require rodenticide applicators to conduct follow-up visits to the poisoned colonies at one to two day intervals to remove above ground poisoned BTPDs (http://www.liphatech.com/uploads/files/pdf/US/Labels/Rozol/ENG_RZ_PrairieDogBait_Label.pdf; http://www.kaputproducts.com/wp-content/uploads/2013/07/72500-22-50lbKaput-D-Prairie-Dog-Label.pdf). However, many applicators consider the follow-up visits to be laborious and unrealistic (Vyas, 2013). Our observations of FEHAs hunting easy-to-capture prey by foraging in Rozol® treated areas supported the importance of the risk mitigation requirements on the pesticides' labels. Lastly, documentation of FEHAs' preference for vulnerable prey can improve rodenticide exposure encounter estimates in ecological risk assessments. An understanding of how foraging behavior modulates exposure to poisoned prey can aid risk assessments and guide hazard mitigation strategies.

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TENTH ANNIVERSARY

Patterns of Natural and Human-Caused Mortality Factors of a Rare Forest Carnivore, the Fisher (*Pekania pennanti*) in California

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Abstract

Wildlife populations of conservation concern are limited in distribution, population size and persistence by various factors, including mortality. The fisher (*Pekania pennanti*), a North American mid-sized carnivore whose range in the western Pacific United States has retracted considerably in the past century, was proposed for threatened status protection in late 2014 under the United States Endangered Species Act by the United States Fish and Wildlife Service in its West Coast Distinct Population Segment. We investigated mortality in 167 fishers from two genetically and geographically distinct sub-populations in California within this West Coast Distinct Population Segment using a combination of gross necropsy, histology, toxicology and molecular methods. Overall, predation (70%), natural disease (16%), toxicant poisoning (10%) and, less commonly, vehicular strike (2%) and other anthropogenic causes (2%) were causes of mortality observed. We documented both an increase in mortality to (57% increase) and exposure (6%) from pesticides in fishers in just the past three years, highlighting further that toxicants from marijuana cultivation still pose a threat. Additionally, exposure to multiple rodenticides significantly increased the likelihood of mortality from rodenticide poisoning. Poisoning was significantly more common in male than female fishers and was 7 times more likely than disease to kill males. Based on necropsy findings, suspected causes of mortality based on field evidence alone tended to underestimate the frequency of disease-related mortalities. This study is the first comprehensive investigation of mortality causes of fishers and provides essential information to assist in the conservation of this species.

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Introduction

Identifying the factors limiting imperiled wildlife populations requires an understanding of all influences affecting population growth and persistence. The geographic range of the fisher, (*Pekania pennanti*), a medium-sized mesocarnivore that inhabits northern North America, has contracted significantly over the past century [1, 2]. Several factors potentially explain this contraction, including trapping and habitat alteration associated with fire management and logging throughout the early 1900s [1–4].

Recent conservation efforts, such as reintroductions and forest restoration to improve habitat, have helped to increase the fisher's range from a range-wide low of 43% back to 68% of its historical range [1]. However, recent expansions were concentrated primarily in the central and eastern portions of the fisher's range. Fisher populations in the Pacific states (Washington, Oregon and California) currently occupy only 21% of their historic distribution in this region and have not expanded, even in some regions with ample available suitable habitat and limited forest fragmentation [1, 2]. Fishers were extirpated from the state of Washington and northern and central Oregon prior to reintroductions to these regions from northern and eastern populations [2–4]. Isolation and failure of population expansion in this portion of their range has prompted the United States Fish and Wildlife Service (USFWS) to deem these populations in these Pacific states a West Coast Distinct Population Segment (DPS) and propose them for listing under the US Endangered Species Act as a threatened species [4]. In 2015, the California Department of Fish and Wildlife listed the southern Sierra Nevada population of fishers, but not the northern California population, threatened under the California Endangered Species Act.

California contains two genetically and geographically distinct native populations of fishers within this DPS [2, 5–7]. The northern California population inhabits the coastal and southern Cascade mountain ranges and is the larger of two California populations. The southern Sierra Nevada population is considerably smaller, thought to contain approximately 300 individuals with fewer than 120 breeding females [2, 8].

The USFWS considers five potential limiting factors as merits for listing: 1) destruction or modification of the habitat or species' range; 2) overutilization for commercial, recreational, scientific or educational purposes; 3) disease or predation; 4) the inadequacy of existing regulatory mechanisms; or 5) other natural or manmade factors affecting its continued existence. Investigation into the frequencies of different causes of mortality can lend information to several of these concerns, most specifically factors 2, 3, and 5. Though several studies on western fisher populations have included descriptions of isolated cases of mortality for fishers [9–11], a systematic, large-scale investigation into cause-specific mortality as determined through full necropsies has not been conducted, specifically within the West Coast DPS [2, 12, 13]. Since 2004, several long-term studies of the California fisher populations have been initiated investigating demographics, habitat utilization, and mortality, and we took the opportunity to investigate fisher mortality across projects for a more comprehensive examination throughout California.

The objectives of the present study were to document causes of mortality in two distinct populations of California fishers using necropsy, histology, toxicology and molecular methods and to investigate demographic, temporal, spatial, and health-related patterns of the specific causes of mortality.

Methods

All procedures involving animals were approved by the University of California, Davis, Institutional Animal Care and Use Committee (Protocol No. 16551) and state scientific collecting and salvage permits issued by the California Department of Fish and Wildlife (#SC-7304). Permission to conduct research at Hoopa Tribal Lands was granted by the Hoopa Tribal Council and Chief Wildlife Forestry Branch manager. Permission to conduct this research on United States Forest Service lands was provided by the Pacific Southwest US Forest Service Research Station.

California Fisher Project areas and sampling

Fishers were collected through three long-term projects in California (Gabriel et al. 2012b), including one on the northern California population (Hoopa Valley Reservation Fisher Project, HVRFP) and two in the southern Sierra Nevada: the Sierra Nevada Adaptive Management Project (SNAMP), and the USFS Kings River Fisher Project (KRFP). The HVRFP project area was located in northwestern California on the Hoopa Reservation and adjacent private lands and federal United States Forest Service (USFS) public lands. The HVRFP personnel monitored fishers from the ground using telemetry approximately 1–2 times per week (J. Mark Higley, Hoopa Tribal biologist, personal communication). Both southern Sierra Nevada fisher projects were conducted on the Sierra National Forest in the northern and central portions of this population's range. Fishers from the SNAMP project were located 3–6 times per week via aerial telemetry (Rick Sweitzer, SNAMP biologist, personal communication), while the KRFP personnel located each fisher via ground telemetry 2–3 times per week (Craig Thompson, USFS biologist, personal communication).

In all projects, fishers were captured in box traps (model 207, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) modified with plywood cubby boxes to minimize environmental stressors [14, 15]. Each fisher was fitted with a VHF radio-collar and monitored via radio-telemetry. Radio-collars were equipped with activity or mortality sensors [16]. Inactivity on two consecutive location attempts separated by more than 24 hours or a single mortality signal from telemetry collars prompted attempts to recover carcasses as soon as was practical. When a fisher carcass was recovered, project biologists identified and recorded a suspected cause of mortality. Field based mortality determinations were constructed from evidence found at the immediate mortality site (predator tracks, nearby roadway, etc.) and the condition of the carcass (puncture wounds, cached carcass, etc.). Recovered fisher carcasses were stored in a -20°C freezer until further analysis. Fishers were subject to a complete necropsy performed by a board-certified veterinary pathologist specializing in wildlife at the University of California, Davis (UCD) Veterinary Medical Teaching Hospital or the California Animal Health and Food Safety Laboratory System (CAHFS) on the UCD campus. Additionally, any uncollared fishers that were collected opportunistically from the field within or near project areas were necropsied.

For each fisher carcass, age was determined by pulp-cavity closure or enumeration of cementum annuli of an upper premolar [17]. Fishers were classified as kits if they were altricial and dependent on mothers-milk for nourishment (roughly ≤10 weeks), juveniles if weaned and <12 months of age, sub-adults when between 12–24 months of age, and adults when ≥24 months of age [17].

Ancillary diagnostic testing was performed based on gross and histologic findings and consisted of molecular diagnostic tests to confirm a viral etiology [18], toxicological screening of selected tissues [12], forensic genetic tests of swabbed ante-mortem bite wounds to identify species of predators [19], and serology to determine exposure to three carnivore pathogens: canine distemper virus (CDV), canine parvovirus-2 (CPV), and *Toxoplasma gondii* [17].

Serological assessment and titer cutoffs were performed via indirect fluorescent-antibody (IFA) assays on uncoagulated blood collected by sterile cardiac puncture [17, 20, 21]. For both CDV and *T*. *gondii*, detection of both antibody isotype IgG, which persists for extended periods, and the short-duration antibody isotype IgM was used, while only detection of isotype IgG was used for CPV [22]. Isotype IgM was utilized for selected pathogen assays since recent or acute infections from these pathogens may predispose individuals to certain causes of mortality [23] [24, 25].

Predation was considered the cause of mortality if ante-mortem hemorrhage was observed and associated with bite, claw or talon wounds [19]. In addition, we followed up visible field signs of predation for which ante-mortem hemorrhaging could not be determined (e.g., due to consumption by the predator) with forensic DNA testing of tissue around putative bite wounds or tooth marks [19]. Mortalities were classified as “natural disease” if they exhibited clinically significant infectious (bacterial, viral, parasites, etc.) or non-infectious (malignant neoplasia, nutritional deficiency, etc.) factors that were considered by the pathologist to represent the primary cause of death [26–28]. Mortalities were classified as “poisoning” if an individual had acute clinically significant signs of toxicosis associated with toxicant exposure (e.g. carbamate, anticoagulant rodenticide). Fishers that died directly from anthropogenic factors (e.g. anesthesia, entrapment in human-structures) were classified as “human-caused.” Vehicular strike was considered to be the cause of death when carcasses were recovered on or near roads combined with evidence of blunt trauma. If a fisher carcass had insufficient tissues for a necropsy, severe autolysis or a lack of forensic evidence, its cause of death was classified as unknown.

Statistical analysis

Statistical analyses were performed using R studio version 0.98.507 and the “mlogit” package [29, 30, 31]. A kappa statistic for test agreement was calculated to assess the strength of test agreement between field biologist-suspected cause of death and necropsy-confirmed cause of death [32]. We used a multinomial logistic regression model to assess the effects of several variables on the relative frequencies of cause-specific mortality which consisted of “natural disease,” “poisoning,” “predation,” and “human-caused,” however, we excluded unknown causes. We pooled human-caused mortalities with those from vehicular strike as “vehicular/ human” for modeling due to small sample sizes and the common anthropogenic source of mortality. We used two different data sets for modeling. The first included 136 radio-collared and uncollared fisher mortalities documented between 2007 and 2014 for which cause of mortality was known. We built models using all possible combinations of 1–5 variables which included population, sex, age class, season and year. The second data set included 72 fisher mortalities pulled from the previous group for which exposure status for both anticoagulant rodenticides and the three pathogens was known; no demographic parameters were used for this model. Models were built using all possible combinations of 1–8 variables which included IgG and IgM titers to CDV and *T*. *gondii*, IgG titer to CPV, exposure to AR, and the total number of AR detected. The latter two variables were not used in the same model to avoid multicollinearity among variables.

We employed an information-theoretic approach to identify the most parsimonious models [33] relating demographic parameters and disease and toxicant exposure parameters to cause of mortality. We calculated the Akaike Information Criteria score corrected for small sample sizes (AICc;[33]) for each model and compared the scores among competing models. We considered as final models those with ΔAICc < 2. The model was built using the outcome categories “predation,” “poisoning” and “natural disease” as the reference groups in three separate analyses, resulting in odds ratios (OR) for “predation vs. disease,” “predation vs. poisoning,” “predation vs. human-caused,” “poisoning vs. disease,” “poisoning vs. human-caused” and “disease vs. human-caused.” Model coefficients were estimated using the maximum-likelihood method [34].

Results

A total of 167 fishers was collected for necropsy and/ or forensic examination from both California populations during 2007–2014 of which there were 105 adults (63%), 32 sub-adults (19%), 26 juveniles (16%) and 4 kits (2%). Males composed 44% (n = 73) and females 56% (n = 94) of all fisher mortalities. The necropsied population included 123 fishers (73%) which had adequate preservation and sufficient tissues for necropsy submittal. Of the remaining 44 fishers, 34 had suspected predator-inflicted wounds with insufficient tissues for necropsy and were submitted solely for molecular forensic examinations, while the remaining ten fisher carcasses (seven southern Sierra Nevada and three northern California) were too autolyzed for any examination.

Fifty-two (31%) of the fishers were from the northern California population while 115 (69%) were from the southern Sierra Nevada population (Table 1). Of the 163 adult, subadult or juvenile fishers (the four kits were excluded), 156 were radio-collared and seven were collected opportunistically, including three from northern California and four from the Sierra Nevada populations. Numbers of carcasses available for analysis were similar across years providing an approximately balanced multiannual data set (Table 1).

Characteristic		South Sierra (N = 115) n	South Sierra (N = 115) %	North CA (N = 52) n	North CA (N = 52) %
Sex					
	Male	52	45.2	21	40.4
	Female	63	54.8	31	59.6
Age Class					
	Kit	3	2.6	1	1.9
	Juvenile	25	21.7	1	1.9
	Sub-Adult	21	18.3	11	21.2
	Adult	66	57.4	39	75.0
Year of Death					
	2007	6	5.2	7	13.5
	2008	13	11.3	6	11.5
	2009	25	21.7	3	5.8
	2010	21	18.3	6	11.5
	2011	20	17.4	8	15.4
	2012	11	9.6	10	19.2
	2013	15	13.0	5	9.6
	2014	4	3.5	7	13.5
Season of Death					
	Spring	52	45.2	26	50.0
	Summer	26	22.6	9	17.3
	Fall	16	13.9	9	17.3
	Winter	21	18.3	8	15.4
Cause of Death					
	Predation	67	58.3	23	44.2
	Natural Disease	16	13.9	9	17.3
	Poisoning	6	5.2	7	13.5
	Vehicular Strike	7	6.1	3	5.8
	Human	1	0.9	1	1.9
	Undetermined	18	15.6	9	17.3

doi:10.1371/journal.pone.0140640.t001

Table 1. Comparison of sex, age class, year of death, season of death and necropsy-determined cause of mortality for 167 fishers (*Pekania pennanti*) from two isolated populations, southern Sierra Nevada (South Sierra) and northern California (North CA). These data include both collared and uncollared fishers of all age classes.

https://doi.org/10.1371/journal.pone.0140640.t001

Necropsy-determined causes of mortality

Confirmation of mortalities was based on necropsy and forensic examination and grouped into six categories based on our results: predation, natural disease, poisoning, vehicular strike, human-caused (other than vehicular strike) and unknown. We excluded fishers that were opportunistically collected due to vehicle strike (n = 7), kits recovered from dens (n = 4), and necropsied fishers whose cause of mortality was undetermined (n = 27) in order to more accurately represent the relative frequencies of different causes of mortality in the fisher populations. Of the 129 collared fishers for which cause of death was determined, predation was the highest contributing source of mortality (70%, n = 90), followed by natural disease (16%, n = 21), poisoning (10%, n = 13), vehicular strike (2%, n = 3) and human-caused (2%, n = 2) (Table 2, Fig 1).

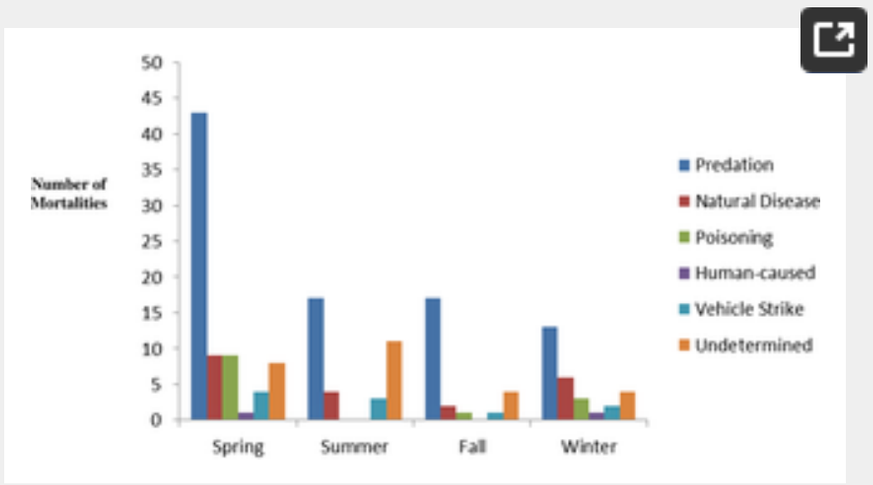


Fig 1. Contributions of each necropsy-determined cause of mortality confirmed by full necropsy and/ or forensic analysis over the seasons for California fisher (*Pekania pennanti*) populations in northern California and the southern Sierra Nevada.
Data were combined from 2007 to 2014 (n = 167).
https://doi.org/10.1371/journal.pone.0140640.g001

Characteristic		Total (n = 136) n	Predation (n = 90) n	Predation (n = 90) %	Disease (n = 21) n	Disease (n = 21) %	Poisoning (n = 13) n	Poisoning (n = 13) %	Vehicular/ Human (n = 12) n	Vehicular/ Human (n = 12) %
			Necropsy-determined Cause of Mortality							
Population										
	North Coast	42	23	54%	8	19%	7	17%	4	10%
	S. Sierra	94	67	71%	13	14%	6	6%	8	9%
Sex										
	Female	78	59	76%	11	14%	2	3%	6	8%
	Male	58	31	53%	10	17%	11	19%	6	10%
Age										
	Juvenile	22	17	77%	3	14%	1	5%	1	5%
	Sub-Adult	30	18	60%	7	23%	2	7%	3	10%
	Adult	84	55	65%	11	13%	10	12%	8	10%
Year										
	2007	12	8	67%	3	25%	0	0%	1	8%
	2008	17	9	53%	2	12%	1	6%	5	29%
	2009	25	12	48%	9	36%	1	4%	3	12%
	2010	19	15	79%	0	0%	3	16%	1	5%
	2011	22	19	86%	2	9%	0	0%	1	5%
	2012	17	10	59%	3	18%	3	18%	1	6%
	2013	17	12	70%	1	6%	4	24%	0	0%
	2014	7	5	71%	1	14%	1	14%	0	0%
Season										
	Spring	66	43	65%	9	14%	9	14%	5	8%
	Summer	24	17	71%	4	17%	0	0%	3	13%
	Fall	21	17	81%	2	10%	1	5%	1	5%
	Winter	25	13	52%	6	24%	3	12%	3	12%

doi:10.1371/journal.pone.0140640.t002

Table 2. Necropsy-determined cause-specific mortality frequencies for fishers (*Pekania pennanti*) by sex, age, year and season from fisher populations in northern California and southern Sierra Nevada.

Data were combined from 2007 to 2014 (n = 136). These data include 7 uncollared fishers discovered opportunistically dead due to vehicle strike so relative frequency of vehicle-related deaths may be overrepresented.
https://doi.org/10.1371/journal.pone.0140640.t002

Predation

Of the 90 fishers that died from predation, necropsy examination confirmed 58 predation events. The remaining 32 fishers had insufficient tissues for a full necropsy and were classified as predation events via molecular forensics and/ or ante-mortem hemorrhaging from wounds on remaining tissues. Specific predators of fishers could be determined for 67% (n = 60) of all predation events based on molecular forensic evidence; eight more were identified only to family, specifically Felidae. Of predators identified, bobcats (*Lynx rufus*, n = 27: 40%), mountain lions (*Puma concolor*, n = 26: 38%), unidentified Felidae (n = 8: 12%), coyotes (*Canis latrans*, n = 4: 6%), and domestic dogs (*Canis lupus familiaris*, n = 2: 3%) were confirmed predators of fishers while a single fisher (1%) was killed by a rattlesnake (*Crotalus oreganus oreganus*).

Natural disease

Of the 21 mortality events for collared fishers attributed to natural disease, 48% (n = 10) were attributed to bacterial infections, 28% (n = 6) to emaciation, 14% (n = 3) to viral infections, 5% (n = 1) to a protozoal infection and 5% (n = 1) to malignant neoplasia (cancer). Of the 10 bacterial infections, nine were associated with interstitial pneumonia or bronchopneumonia. Three of the four northern California mortalities due to bacterial infection had a concurrent, nematode parasitism of the lungs, which was not identified to genera. The nematode parasitism cases were associated with interstitial pneumonia with bacterial infiltrates, although this was not the proximate cause of mortality. Four of the six fisher mortalities due to bacterial infection in the southern Sierra Nevada had bacterial infiltrates associated with interstitial pneumonia, but contamination with mixed bacterial flora prevented identification. Two of these cases also involved an unknown lung nematode. The remaining two cases were septic with mixed bacterial flora, which may have resulted from cutaneous punctures with associated necrosis and bacterial infiltration consistent with predator bite wounds.

All six fishers that died due to starvation were severely emaciated with no pericardial, renal, mesentery or subcutaneous fat. All of these cases showed emaciation with no other detectable concurrent disease processes. For five of the six emaciation cases, the cause for emaciation was unknown. The remaining case was a female fisher with an acute complete fracture of the left mandible coupled with numerous canine, incisor and molar teeth fractures. The source of this acute trauma was unknown however predation, an illegal snare or vehicular strike, though presumptive, may have been the contributing cause due to the force required. In addition, two altricial kits were recovered from abandoned den sites and determined to have died of emaciation.

All three fishers that died of a viral etiology were infected with CDV as previously described [18]. We categorized one fisher as predation mortality that had a concurrent systemic CDV infection. The lesions caused by CDV were widespread and severe suggesting that they had a debilitating clinical effect, which facilitated the predation event. The mortality was hence classified as ‘predation’ rather than ‘disease’, though this fisher most likely would have succumbed to distemper due to the systemic infection. The sole mortality attributed to protozoal infection was due to a severe, non-suppurative, menigioencephalomyelitis caused by *T. gondii* as determined by immunohistochemistry. The only fisher that died from cancer had systemic lymphoma involving lymph nodes, liver and skin.

Poisoning

Thirteen fishers in the two populations died of toxicosis, all of which had trespass marijuana (*Cannabis sativa*) cultivation and associated toxicants within their home ranges. Anticoagulant rodenticides (ARs), which are toxicant compounds that inhibit the recycling of vitamin K1 leading to clotting and coagulation impairment, caused 11 fisher mortalities. In addition to detection of AR in these fishers’ livers, they exhibited coagulopathy and significant hemorrhage. Exposure to ARs alone did not constitute an AR toxicosis case. In addition to ARs, cholecalciferol, another rodenticide which causes hypercalcemia and has been found at several cultivation sites in the northern California project, was assumed to be the contributing cause of death in one male fisher from northern California. This fisher had multifocal mineralization in the aorta, testes and renal medulla. All other causes of hypercalcemia such as chronic renal failure and hyperparathyroidism were ruled out and cholecalciferol rodenticides were discovered near this fisher’s home range. The kidneys for this fisher were submitted for total vitamin D_3 assay (a measure to detect Vitamin D toxicosis) along with another kidney from a fisher that exhibited no mineralization in any tissues (Heartland assays LLC, Ames, Iowa, USA). Results demonstrated a 7.4 fold difference of total vitamin D_3 between the two samples (14.1 ng/g vs. 1.9 ng/g). Unfortunately, sample identifications during laboratory submission were not legible to lab staff, therefore correct assignment of results to the sample could not be completed with confidence. This fisher was also exposed to five different ARs, for a total of six different rodenticides it had consumed.

Another collared male fisher from northern California exhibited neurological signs including ataxia, lethargy and seizures before being euthanized (Permanent Video Link: https://www.youtube.com/watch?v=otognB4LdTY). This fisher was near an illegal marijuana cultivation site where bromethalin and carbamate insecticides, as well as numerous organophosphates, were found. However, no carbamates, organophosphates, illicit drugs, metaldehyde or bromethalin were detected in the stomach contents, liver, urine or kidney. In addition, we tested its bile for Anatoxin-a, but did not detect it in the sample. All other potential mechanisms for this fisher’s clinical signs were ruled out leading this case to be classified as suspected toxicosis.

Seven of the toxicosis cases were from the northern California population while the remaining 6 were from the southern Sierras (Table 2). Annual fisher mortality attributed to rodenticides varied with an average of 1.86 toxicosis cases each year (2007:0, 2008:1, 2009:1, 2010:3, 2011:0, 2012:3, 2013:4, 2014:1). Nine of the 13 toxicosis cases occurred in spring (March-June: 69%),

three in late winter (February: 23%) and one in fall (October: 8%). A total of 101 fishers had sufficient liver tissue to test for anticoagulant rodenticide exposure. Of these fishers, 86 (85%) were exposed to one or more ARs and had an average of 1.73 different AR compounds (range: 1–5, SD:0.91).

Vehicle strikes

All 10 fishers killed by vehicular strike were discovered on paved road systems with various speed limits for vehicles. Seven of these were uncollared and opportunistically collected, whereas three road killed fishers from the southern Sierra Nevada population had been radio-collared. Two additional fisher mortalities from the southern Sierras were originally suspected to be vehicular strikes due to the carcasses being discovered near or on a roadway but had no evidence of blunt force trauma, macerated muscles, comminuted fractures, torn viscera or ruptured blood vessels, all of which were observed in all of the 10 confirmed vehicular strike cases. These fishers were finally ruled as AR poisoning due to the significant pleural and abdominal cavity hemorrhaging, in addition to several ARs detected in tissues.

Anthropogenic causes

The two cases of human-caused mortalities were due to entrapment in man-made structures. A radio-collared female adult fisher at HVRFP died of dehydration when she was caught in a live trap that was inadvertently left operational between trapping sessions. The maximum duration over which the fisher could have been left in the live trap was five days. The second fisher was an uncollared, sub-adult male that was discovered in an air quality sampling tube at the KRFP study. This fisher's tissues were too autolyzed to perform a necropsy. A third fisher from the southern Sierras was initially suspected to have died from negative reaction from recalled ketamine. However upon necropsy, it was determined that this fisher was infected with CDV exhibiting clinical signs of disease but respiratory depression from anesthesia was the proximate factor which expedited inevitable death due to CDV infection [18].

Field-based vs. necropsy-confirmed causes of mortality

Of the 136 fisher carcasses for which cause of mortality was identified, field biologists reported cause of mortality as predation for 66% (n = 90) of fisher deaths, "unknown" for 12% (n = 16), disease for 8% (n = 11), vehicular strike for 9% (n = 12), other human-caused for 4% (n = 6) and drowning for 1% (n = 1) (Table 3). Three suspected human-caused mortalities were subclassified as "delayed negative anesthesia-reaction" and one was classified as a "VHF collar hanging". The kappa statistic for test agreement between biologist-determined cause of death and necropsy-confirmed cause of death was 0.5669, showing only moderate agreement. In contrast to the field-based suspected causes, pathological investigation indicated no mortalities were attributed to drowning, collar strangulation, or negative reactions to anesthesia. All of these mortalities were caused by disease. Disease was the mortality cause most underestimated by field biologists, while predation and vehicular strike were overestimated (Table 3).

			Necropsy Determined Causes of Mortality					
Field-based Suspected Cause of Mortality	Predation	Disease	Poisoning	Human	Vehicular	Drowning	Unknown	Total
Predation	**86**	3	1	0	0	0	0	90
Disease	0	**4**	5	0	0	0	0	9
Poisoning	0	0	**2**	0	0	0	0	2
Human	0	4	0	**2**	0	0	0	6
Vehicular	0	0	2	0	**10**	0	0	12
Drowning	0	1	0	0	0	**0**	0	1
Unknown	4	9	3	0	0	0	**0**	16
Total	90	21	13	2	10	0	0	**136**

Kappa = 0.5669.

doi:10.1371/journal.pone.0140640.t003

Table 3. Field-based causes of mortality determined by field evidence alone and necropsy determined causes of mortality for fishers (*Pekania pennanti*) within the two isolated populations, northern California and southern Sierra Nevada.
Data were combined from 2007 to 2012 (n = 136).
https://doi.org/10.1371/journal.pone.0140640.t003

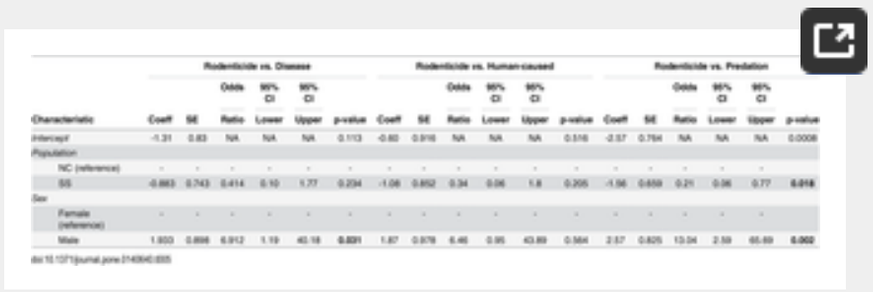
Multinomial logistic regression

The final fitted multinomial logistic regression model assessing the association of demographic data with cause of mortality identified sex and population as significant independent predictors of the cause of mortality (Table 4). No other variables were significant. In the comparison of poisoning vs. other causes of mortality, sex was the most significant predictor affecting cause of mortality (Table 5). Compared to females, males were approximately 7 times more likely to die of poisoning than natural disease (OR = 6.9, 95% CI: 1.19–40.18, p = 0.0313) and 13 times more likely to die of poisoning than predation (OR = 13.04, 95% CI: 2.59–65.69, p = 0.0019). Fishers from the northern California population were almost 5 times more likely than southern Sierra fishers to die of rodenticide than predation (odds ratio = 0.21, 95% CI: 0.06–0.77, p = 0.018).

Model	K	Log-likelihood	AICc	ΔAICc	w
SEX	6	-129.634	271.919	0.000	0.523
SEX + POPN	9	-126.433	272.294	0.375	0.433
NULL	3	-136.040	278.262	6.343	0.022

doi:10.1371/journal.pone.0140640.t004

Table 4. Performance statistics of three top models of demographic factors relating to ultimate cause of mortality for 136 fishers (*Pekania pennanti*) within the two isolated populations, northern California and southern Sierra Nevada.
The two factors in the final model were SEX (sex of the fisher) and POPN (population of fisher).
https://doi.org/10.1371/journal.pone.0140640.t004



	Rodenticide vs. Disease						Rodenticide vs. Human-caused						Rodenticide vs. Predation					
Characteristic	Coeff	SE	Odds Ratio	95% CI Lower	95% CI Upper	p-value	Coeff	SE	Odds Ratio	95% CI Lower	95% CI Upper	p-value	Coeff	SE	Odds Ratio	95% CI Lower	95% CI Upper	p-value
Intercept	-1.31	0.83	NA	NA	NA	0.113	-0.82	0.916	NA	NA	NA	0.516	-2.57	0.764	NA	NA	NA	0.0008
Population																		
NC (reference)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SS	-0.883	0.743	0.414	0.10	1.77	0.234	-1.06	0.852	0.34	0.06	1.8	0.205	-1.56	0.659	0.21	0.06	0.77	**0.018**
Sex																		
Female (reference)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Male	1.933	0.898	6.912	1.19	40.18	**0.031**	1.87	0.978	6.46	0.95	43.89	0.564	2.57	0.825	13.04	2.59	65.69	**0.002**

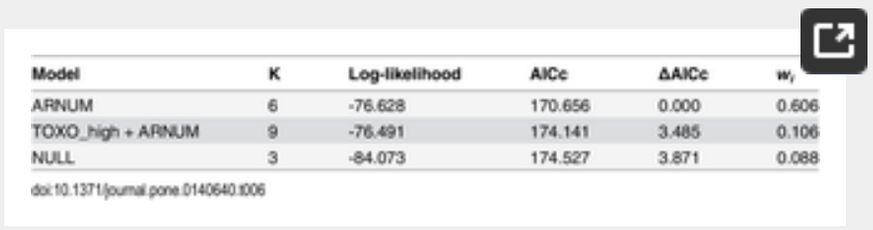
doi:10.1371/journal.pone.0140640.t005

Table 5. Results of a multinomial logistic regression in the final model indicating the effects of fisher (*Pekania pennanti*) sex and population on likelihood of mortality from a specific cause.

Significant variables in the model are bolded.

https://doi.org/10.1371/journal.pone.0140640.t005

The multinomial logistic regression model assessing the association between anticoagulant rodenticide or pathogenic exposure factors and causes of mortality indicated that the number of individual AR compounds to which a fisher was exposed was associated with cause of mortality (Table 6). For every additional AR type to which a fisher was exposed, its likelihood of dying of poisoning vs. disease increased almost 3-fold (OR = 2.75, 95% CI: 1.25–6.07, p = 0.0122) and of dying of poisoning versus predation increased approximately by 2.5 (OR = 2.56, 95% CI: 1.25–5.25, p = 0.0105) (Table 7). Fishers were almost 4 times as likely to die of poisoning vs. human-caused with each additional AR type to which they were exposed (OR = 3.75, 95% CI: 1.29–10.86, p = 0.0150). Exposure to the three pathogens was not a significant predictor of causes of mortality.



Model	K	Log-likelihood	AICc	ΔAICc	w_i
ARNUM	6	-76.628	170.656	0.000	0.606
TOXO_high + ARNUM	9	-76.491	174.141	3.485	0.106
NULL	3	-84.073	174.527	3.871	0.088

doi:10.1371/journal.pone.0140640.t006

Table 6. Performance statistics of three top models of pathogen and toxicant exposure factors relating to ultimate cause of mortality for 67 fishers (*Pekania pennanti*) within the two isolated populations, northern California and southern Sierra Nevada.

The one factor in the final model was ARNUM (number of different types of anticoagulant rodenticides to which the fisher was exposed). TOXO_high refers to exposure to *T*. *gondii* using the isotype IgM.

https://doi.org/10.1371/journal.pone.0140640.t006

	Rodenticide vs. Disease						Rodenticide vs. Human-caused						Rodenticide vs. Predation					
Characteristic	Coeff	SE	Odds Ratio	95%CI Lower	95%CI Upper	p-value	Coeff	SE	Odds Ratio	95%CI Lower	95%CI Upper	p-value	Coeff	SE	Odds Ratio	95%CI Lower	95%CI Upper	p-value
Intercept	-2.59	0.92	NA	NA	NA	**0.005**	-2.11	1.033	NA	NA	NA	**0.041**	-2.54	0.862	NA	NA	NA	**0.0008**
AR Number	1.01	0.404	2.75	1.25	6.07	**0.012**	1.32	0.543	3.75	1.29	10.86	**0.015**	0.94	0.367	2.56	1.25	5.25	**0.011**

doi:10.1371/journal.pone.0140640.t007

Table 7. Results of a multinomial logistic regression in the final model indicating the effects of the number of different types of anticoagulant rodenticides (AR) a fisher (*Pekania pennanti*) was exposed to on likelihood of mortality from a specific cause.

Significant variables in the model are bolded.

https://doi.org/10.1371/journal.pone.0140640.t007

Discussion

This study is the first to thoroughly describe necropsy-confirmed, cause-specific mortality of fishers in the West Coast DPS and our findings provide baseline information on the mortality factors potentially limiting fisher populations in other portions of this DPS [8, 35, 36, 37]. The most significant findings of our study were the relative importance of predation and poisoning as mortality factors and the apparent increase of pesticide poisoning frequency in a short span of time. Importantly, our finding that AR poisoning was a more likely cause of death than predation in the northern California population versus the southern Sierra Nevada signifies regional heterogeneity in anthropogenic influences in forest landscapes. Besides differences in likelihood of AR poisoning, we found little heterogeneity in most causes of mortality between the two study populations or among years supporting their generality in California fisher populations. This finding likely reflects similarities in habitat, prey utilization, and predator communities throughout the range of fishers in California [2, 3, 38]. A secondary finding was that field assessment of cause of death significantly underestimated the frequency of natural disease-related mortalities.

Our results confirmed earlier findings that predation was a significant mortality factor affecting fishers in California, causing the majority of all fisher deaths [19, 26, 35]. The addition of 28 new predation cases for this study did not change the frequencies of predation events by particular predator species for fishers from both populations determined in an earlier study [35]. Older studies suggested that predation was an insignificant mortality factor, thought primarily to affect vulnerable or reintroduced individuals [2, 13, 39]. In our study, females more frequently died from predation (relative to other causes) than males, possibly attributable to the smaller mass of females. Female fishers on average were half to two-thirds the mass of males [13], thus potentially increasing their susceptibility to a greater diversity of predators. Additionally, the importance of predation becomes more clear in light of recent findings suggesting that population size for fisher is heavily influenced by adult female survival [8]. It is unclear why these isolated California populations were subject to such high prevalence of predation relative to other populations, but further investigation of this finding is critical to the conservation of the California populations [8, 13, 39–41]. Furthermore, we do not know whether the

predation rates we observed for fishers are different from predation rates that fisher populations have suffered throughout their evolutionary history. However, recent research into the effects of habitat modification on likelihood of fisher predation does suggest that changes in habitat over the past century may be changing the rate of predation on female fishers by bobcats [42].

Although exposure to the protozoan *T*. *gondii* has been shown to predispose individuals to predation or vehicular strikes [23, 24, 43], we found no significant evidence of this relationship. However, many of the depredated fishers did not have any available blood to sample due to the predator consuming the heart or exposing the thoracic cavity leaving unsuitable samples for testing, resulting in a small sample size with which to detect such a relationship. Then again, all available brain tissue was tested for gliotic foci due to *T*. *gondii* and none were found.

Natural disease was the second-most frequent cause of mortality in our study. Kits died from disease more frequently than any other cause, likely since kits were den-bound and therefore less exposed to predators and humans. Bacterial infections accounted for the largest number of disease-related fisher deaths, and generally manifested as bacterial pneumonia. However, in no instance did we identify a single dominant bacterial pathogen but cultures yielded mixed flora in all cases. These results may be due to post-mortem autolysis and contamination of pathogenic bacteria by opportunistic species. Interestingly, two of the mortalities associated with bacterial infection also had full thickness, circular punctures in the skin suggestive of failed predation attempts resulting in a site for introduction of a bacterial infection. It should be noted that pulmonary viral infections that might have preceded and facilitated bacterial colonization could not be identified but cannot be ruled out.

The toxicosis cases discovered in this study signify an increase of this emerging threat for fishers in the West Coast DPS [12, 36]. Cultivation of marijuana and the associated use of toxicants have been recently documented in occupied fisher habitat [12, 36, 44]. In addition to the four fisher mortalities attributed to anticoagulant rodenticides by Gabriel et al. (2012), we documented nine additional pesticide toxicosis cases in the present study. The average incidence of toxicosis cases per year for the five year Gabriel et al. (2012) study spanning 2007–2011 was 5.6% (SE = 3.1%). However, in the final three years (2012–2014) of our study, we detected an increase in incidence per year to 18.7% (SE = 2.9%). Exposure also increased from 79% (46 of 58) to 85% (86 of 101) for the same two time periods [12]. This increase in cases and exposure could signify either an increase in the number of cultivation sites or area impacted or that cultivators are increasing the level of toxicants being dispersed within occupied fisher home ranges. In either case, this anthropogenic threat is of increasing concern.

Previous reports of cholecalciferol poisonings have not been reported in a remote forest dwelling carnivore. This type of toxicant has been promoted as an alternative to anticoagulant rodenticides due to the minimized risk for secondary poisonings [45]. Nevertheless, plant and animal based food flavorizers are often incorporated into rodenticides to enhance palatability to omnivorous rodents [12]. Because fishers are omnivorous [2], they could be susceptible to primary poisoning if they are attracted to these compounds when they are impregnated with flavorizers. In addition, the massive amount of rodenticide dispersed at some cultivation sites e.g>40 kg in some sites, which have cultivation footprints of typically less than 0.2ha [12, 46] likely pose a secondary risk of poisoning to fishers. Fishers may consume numerous prey that may have recently ingested these rodenticides, with the likely exception of cholecalciferol.

As was noted previously for a subset of cases, toxicosis deaths occurred primarily in the spring [12]. Additionally, males were more likely than females to die of poisoning relative to predation and other causes. This finding may be due to fewer predation events involving males than females [19, 35] or the higher prevalence of poison-related mortality in males. These trends could also be due to behavioral factors [28]. Female fishers in California increase their crepuscular and diurnal activity in spring to satisfy the additional energy requirements of lactation and care of weaned kits but typically within the confines of their established home-ranges. Male fishers may make extensive forays outside their normal home ranges in spring to search out females for mating opportunities [2, 13]. Marijuana cultivation coincides with the increased activity of fishers in early spring and frequently involves dispersal of large amounts of toxicants near occupied fisher home ranges [12, 36, 46]. Furthermore, survival of female fishers in one population was found to be influenced by the number of marijuana cultivation sites in the 95% fixed kernel home range [36].

The relationship between the number of ARs to which a fisher has been exposed and the increasing probability of death due to poisoning suggests that these pesticides may be acting additively or synergistically. However, little experimental data are available demonstrating exposure to multiple ARs increasing the risk of coagulopathy, [12, 36, 47, 48]. Our data suggest that coagulopathy risk increases significantly with each additional new AR compound exposure, though it’s possible this pattern is reflecting an additive relationship between AR number and cumulative level of exposure. However, potential synergistic mechanisms need to be addressed due to the significant amount of other pesticides, herbicides, molluscicides and fungicides documented at marijuana cultivation sites. Because fishers are exposed to > 1.7 different ARs on average, our concerns on the potential unknown mechanisms of deleterious effects of multiple ARs warrants further investigation [36, 46, 47].

Human-related mortalities were relatively rare, and although a small number were associated with research activities, such mortalities represented < 1% of the captured fishers. This figure is comparable to other studies [49]. Vehicle-related mortalities were also relatively rare with only three marked fishers suffering vehicle strikes, which represented < 2% of all mortalities. The higher number of uncollared fishers found killed in roadways suggests that roadkill may be a more local concern, associated with individual high-traffic corridors.

Field biologists did not always accurately identify general causes of disease. We found only a moderate correspondence between biologist-determined and necropsy-confirmed causes of death except for the detection of disease-related mortalities, which were significantly underestimated by initial field assessments. For example, the three fisher deaths attributed to CDV and many of the toxicosis cases were preliminarily attributed to other causes in the field. The underestimation of disease has been observed in other wildlife studies because gross observations in the field are inadequate to detect subtle signs of disease [18]. These findings fortify the need for full necropsies when studying causes of mortality, especially when knowledge of the frequencies of cause-specific mortality is required in managing or reducing the most significant limiting factors for fishers.

Although predation was often correctly identified by both field biologists and the pathologists, the incorporation of molecular forensic approaches coupled with traditional pathology allowed us to more definitively identify both predation events and predator species [19, 26, 35]. Predation is often implicated as the cause of mortality when field evidence such as tracks near or adjacent to the carcass, bite wounds, wound patterns or feces and/or hair near the carcass are found [9, 50–53]. However in our study, field observations misclassified 5 fishers as predation due to circumstantial predator evidence found near the carcass (e.g. tracks, scat). Field observations can be misleading, for example, bite wounds in soft tissue often change shape and size due to environmental factors [26, 54] (Linda Munson, University of California Davis, Personal Communications) and visual artifacts that resemble ante-mortem hemorrhaging can occur due to autolysis, scavengers consuming tissue and releasing non-clotted blood, or freezing and defrosting of a carcass.

Finally, we present mainly the proximate causes of mortality for fishers though there were a few cases where ultimate causes could be ascertained e.g. anesthesia related death but clinically infected with CDV. However, it would be difficult, if not impossible, to determine whether some of the predation mortalities were ultimately going to result in toxicosis. Many of the predation cases exhibited ante-mortem hemorrhaging that could have been due directly to predation or alternatively, AR exposure. Anticoagulant rodenticides have previously been shown to cause lethargy and weakness in exposed animals [12, 47], but teasing these two causes of death apart was not possible.

This study presents the first large assessment of cause-specific mortality frequencies in California fishers. We have identified predation and natural disease as the top two mortality factors. In addition, mortality from and exposure to toxicants appears to be on the rise and we have found exposure to multiple ARs increases probability of death from these compounds. Increases of additive mortality of only 10% can prevent fisher population expansion even in the presence of suitable habitat with no dispersal barriers [8]. Therefore, the high proportion of fisher mortality consisting of predation and disease may help explain the lack of growth and expansion of these populations to nearby suitable habitat. However, the growing number of toxicosis cases in fishers and the correlation of contributing mechanisms such as marijuana cultivation within fisher habitat suggest an emerging threat. Beyond direct poisoning, rodenticides have the potential to limit fitness through prey depletion and heightened competition between fishers and other carnivores. Future research should focus on the relationship between marijuana cultivation and associated rodenticide use and prey population cycles because carnivore population dynamics are often heavily influenced by fluctuations in prey base [55, 56].

Managing these threats should focus not only on the impacts on current fisher populations but also the reduction of threats that may be limiting expansion for future population growth. One recommendation is the complete removal of toxicants left at current and historicaltrespass marijuana grow sites. Most sites are not remediated, thus toxicants associated with these sites are a continuing threat. Furthermore, as female adult survival is notably important for population size and persistence in the southern Sierra Nevada population, forest managers should consider managing against habitat features that are conducive to interactions between fishers and their predators. Investigating these and other mechanisms for reducing mortality in California fishers within West coast DPS can be of assistance in effectively implementing policy or management options to potentially curb mortality rates in order to promote population recovery within California in addition to other fisher populations throughout the West Coast DPS.

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Author Contributions

Conceived and designed the experiments: MWG LWW GMW SMK PG MJ RP. Performed the experiments: MWG LWW GMW SMK PG MJ RP JMH SMM RAS CT KP RHB RNB. Analyzed the data: MWG GMW NS JEF. Contributed reagents/materials/analysis tools: MWG LWW GMW SMK PG MJ RP JMH SMM RAS CT KP RHB RNB NS JEF BNS. Wrote the paper: MWG LWW GMW SMK PG MJ RP JMH SMM RAS CT KP RHB RNB NS JEF DC BNS.

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Anticoagulant Rodenticide Exposure and Toxicosis in Coyotes in the Denver Metropolitan Area

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ABSTRACT: Anticoagulant rodenticides are widely used in urban areas to control rodent pests and are responsible for secondary poisoning in many nontarget wildlife species. We tested the livers of five coyotes (*Canis latrans*) in the Denver Metropolitan Area, Colorado, USA, for anticoagulant rodenticides. All five livers were positive for brodifacoum, with values ranging from 95 ppb to 320 ppb, and one liver was positive for bromadiolone, with a value of 885 ppb. Both of these rodenticides are second-generation anticoagulants, which are more potent and more likely to cause secondary poisoning than first-generation anticoagulants due to their accumulation and persistence in the liver. We concluded that exposure to these rodenticides may have caused the death of at least two of the five coyotes, and urban coyotes in our study area are commonly exposed to rodenticides.

Key words: Brodifacoum, bromadiolone, *Canis latrans*, poison, second-generation, toxicant, urban.

Anticoagulant rodenticides are used extensively throughout urban areas to control rodent populations (Hosea 2000; Watt et al. 2005). These compounds act by interrupting the normal synthesis of clotting factors in the liver once bleeding commences, resulting in fatal hemorrhaging (Eason and Spurr 1995; Eason et al. 2002). Second-generation anticoagulants (e.g., brodifacoum and bromadiolone) are more potent than first-generation anticoagulants (e.g., warfarin and chlorophacinone) because they can effectively poison a rodent after only a single dose (Eason and Spurr 1995; Berny et al. 2006). Second-generation compounds also have slower elimination times from the liver (Eason and Spurr 1995; Erickson and Urban 2004). This persistence in the liver can lead to secondary poisoning of nontarget wildlife (Stone et al. 1999; Hosea 2000; Elliott et al. 2014), including coyotes (*Canis latrans*) in urban areas (Hosea 2000; Riley et al. 2003; Gehrt and Riley 2010). We report finding anticoagulant rodenticides in urban coyotes residing in the Denver Metropolitan Area (DMA) of Colorado, USA.

We captured 32 coyotes in the DMA using padded leg-hold traps and snares and fitted them with global positioning system radio collars from April 2012 to May 2013 as part of an ecological study of urban coyotes. Research protocols were approved by the National Wildlife Research Center, Institutional Animal Care and Use Committee (QA-1972). We monitored study animals with radio telemetry from April 2012 to June 2014. Collars were equipped with mortality sensors that alerted us when a coyote died. Thirteen collared coyotes died during the study. When cause of death was unknown, the coyote was necropsied at the Colorado Division of Parks and Wildlife, Wildlife Health Laboratory (Fort Collins, Colorado). Liver samples were submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, Texas) to be screened for anticoagulant rodenticides using high-performance liquid chromatography. Brodifacoum, bromadiolone, chlorophacinone, difenacoum, difethialone, diphacinone, and warfarin were included in the screening. We began testing liver samples from deceased animals only after a coyote was found dead

TABLE 1. Values of anticoagulant rodenticides in coyote livers in the Denver Metropolitan Area, Colorado, USA, 2012–2013.

Coyote ID	Date	Brodifacoum (ppb)	Bromadiolone (ppb)[a]
01M	January 2013	150	N/A
17M	February 2013	95	N/A
24M	March 2013	176	N/A
21M	April 2013	320	885
Uncollared	April 2013	95	N/A

[a] N/A indicates the compound was not found in the coyote liver.

with sarcoptic mange because of the relationship between mange and rodenticide poisoning discovered in bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*) by Riley et al. (2007), Uzal et al. (2007), and Serieys et al. (2013). Thereafter, all necropsied coyotes were tested except for two coyotes that were too decomposed to obtain a valid liver sample. Hence, we only tested five coyote livers for rodenticide toxicosis. All five were positive for brodifacoum, with values ranging from 95 ppb to 320 ppb (Table 1). One coyote (the animal with the highest level of brodifacoum) also was positive for bromadiolone, with a value of 885 ppb (Table 1). No other compounds were found in the five liver samples.

Based on necropsy results, we concluded anticoagulant rodenticides contributed to the death of at least two of the five coyotes tested. The first case was a juvenile male (24M) found dead in open space, with no obvious external injuries or other signs of trauma. Upon necropsy, we found free blood in the abdominal cavity. A puncture wound was present on the left side of the body overlying the spleen but not penetrating the abdominal wall. The spleen was fractured and surrounded by clotted blood. We found no radiographic evidence of gunshot and no evidence of bite wounds. The interpretation for cause of death was acute severe hemorrhage, disproportionate to the amount of trauma observed. This coyote's liver was positive for brodifacoum (176 ppb; Table 1).

The second case was a juvenile male coyote (21 mo) found dead on a two-lane road, with minor evidence of skin tearing over the ventral neck and chest. Necropsy findings indicated additional moderate tearing of the muscle in the region overlying the thoracic inlet, although injuries did not penetrate the chest cavity. The chest was filled with blood. The interpretation for cause of death was severe acute hemorrhage, disproportionate to the mild to moderate trauma received from being hit by a vehicle. We suspected rodenticide toxicosis, and the liver was positive for brodifacoum and bromadiolone (Table 1).

In two additional cases, we found hemorrhage into body cavities with severe lesions to explain the hemorrhage, but also evidence of rodenticide exposure. An adult male coyote (01M) had severe lesions of sarcoptic mange, a gunshot through the chest from a pellet rifle, and free blood in the chest cavity. The liver was positive for brodifacoum (150 ppb; Table 1). A juvenile male coyote (17M) had severe crushing lesions to the head and body from being run over by a vehicle and free blood in the chest and abdomen. The liver was positive for brodifacoum (95 ppb; Table 1). One additional coyote (uncollared male) that we captured for our study was euthanized due to self-inflicted trap-related injuries, but the liver also was positive for brodifacoum (95 ppb; Table 1). Causes of death for nine collared coyotes that were not tested for rodenticide toxicosis-included vehicle collision (five coyotes), gunshot (one coyote), conflict resolution (one coyote removed from Denver International Airport), and undetermined (two coyotes).

Our findings suggest anticoagulant rodenticides likely contributed to at least two of the five mortalities, triggered by mild to moderate trauma resulting in fatal internal hemorrhaging. The detection of anticoagulant rodenticides in coyotes in the DMA indicates exposure to these poisons, either directly or secondarily. Because coyotes are omnivores, they could have ingested poisoned rodent bait (Hosea 2000). However, Elliot et al. (2014) determined that targeted rodents are more likely to provide the exposure pathway of anticoagulant rodenticides to secondary consumers. Small rodents are generally an important food source and the dominant animal prey for coyotes in urban areas (Morey et al. 2007; Lukasik and Alexander 2012), resulting in a high probability that repeated consumption of poisoned rodents leads to rodenticide toxicosis in urban coyotes.

The residue values of brodifacoum in our study coyotes were generally lower than those found in other coyote studies. The acute oral LD_{50} value of brodifacoum in dogs ranges from 250 ppb to 1,000 ppb (Stone et al. 1999). In a study conducted near Boston, Massachusetts, Way et al. (2006) found brodifacoum values of 733 ppb and 542 ppb in two coyotes that were presumably directly poisoned. Hosea (2000) identified values up to 500 ppb of brodifacoum in coyotes in California. Erickson and Urban (2004) described coyotes with values of brodifacoum up to 930 ppb. In our study, the two coyotes for which we interpreted exaggerated hemorrhage were also the two cases with the highest values of brodifacoum in their livers, although these values were still lower than the highest values found in other studies. The lower values are not surprising, however, considering both cases had readily observable mild to moderate trauma to initiate excessive bleeding. Nevertheless, our results indicated that poisoning at a lower level may be enough to contribute to fatal hemorrhaging in these carnivores.

Only one coyote was positive for bromadiolone. The acute oral LD_{50} value of bromadiolone in dogs ranges from 11,000 ppb to 15,000 ppb (Stone et al. 1999); the value in our study animal was 885 ppb. Both Erickson and Urban (2004) and Hosea (2000) reported values of bromadiolone in coyotes up to only 460 ppb. Our study coyote also was positive for brodifacoum, and other investigators also have identified coyotes with both of these rodenticides in liver tissue (Hosea 2000; Erickson and Urban 2004). Overall, brodifacoum appears to be more prevalent and of higher concern in the DMA than other rodenticides, although our results indicated that multiple toxicants may be in use throughout our study area.

In addition to the five coyotes in the DMA, we also tested the liver of another coyote carcass found in rural Colorado (Huerfano County) showing signs of hemorrhage. The most likely cause of death was trauma, but a definitive interpretation was limited by advanced decomposition. We found no evidence of any rodenticides in the liver, indicating that rodenticide toxicosis may not always occur in coyotes. To further understand the effects of anticoagulant rodenticides on coyotes, future studies should compare the values of these poisons in coyote livers across urban and rural systems.

Our findings are consistent with those of other studies that have determined anticoagulant rodenticides are contributing to mortality in urban wildlife (Hosea 2000; Riley et al. 2007). The exposure of all five tested coyotes to rodenticides, especially brodifacoum, indicates the ubiquity of these toxicants in the urban landscape and their ability to reach higher levels in the food chain (Riley et al. 2007). One coyote liver contained more than one rodenticide (both brodifacoum and bromadiolone), and multiple compounds have been found in wildlife species in other studies (Stone et al. 1999; Hosea 2000; Erickson and Urban 2004). The effects of

exposure to multiple anticoagulant rodenticides in urban wildlife species should be a focus of future research to increase our understanding of these toxicants and their population effects on urban carnivores.

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Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study

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Abstract Anticoagulant rodenticides (ARs) are increasingly recognized as a threat to nontarget wildlife. High exposure to ARs has been documented globally in nontarget predatory species and linked to the high prevalence of an ectoparasitic disease, notoedric mange. In southern California, mange associated with AR exposure has been the proximate cause of a bobcat (*Lynx rufus*) population decline. We measured AR exposure in bobcats from two areas in southern California, examining seasonal, demographic and spatial risk factors across landscapes including natural and urbanized areas. The long-term study included bobcats sampled over a 16-year period (1997–2012) and a wide geographic area. We sampled blood ($N = 206$) and liver ($N = 172$) to examine exposure ante- and post-mortem. We detected high exposure prevalence (89 %, liver; 39 %, blood) and for individuals with paired liver and blood data ($N = 64$), 92 % were exposed. Moreover, the animals with the most complete sampling were exposed most frequently to three or more compounds. Toxicant exposure was associated with commercial, residential, and agricultural development. Bobcats of both sexes and age classes were found to be at high risk of exposure, and we documented fetal transfer of multiple ARs. We found a strong association between certain levels of exposure (ppm), and between multiple AR exposure events, and notoedric mange. AR exposure was prevalent throughout both regions sampled and throughout the 16-year time period in the long-term study. ARs pose a substantial threat to bobcats, and likely other mammalian and avian predators, living at the urban-wildland interface.

Keywords Bobcats · Secondary poisoning · Anticoagulant rodenticides · Notoedric mange · Urbanization · Residential · Fetal transfer

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Introduction

Anticoagulant rodenticides (ARs) are toxicants increasingly recognized as a threat to nontarget wildlife (Erickson and Urban 2004; US EPA 2008; Elmeros et al. 2011; Gabriel et al. 2012; California Department of Pesticide Regulation 2013). As vitamin K antagonists, ARs interrupt the production of vitamin K-dependent blood clotting proteins, leading to the depletion of these proteins over a period of days inducing mortality by hemorrhage (Erickson

and Urban 2004). Comprised of two classes of compounds, ARs are the primary chemical method used worldwide for the control of rats and mice (Stone et al. 1999; Eason et al. 2002). First-generation ARs, including warfarin, diphacinone, and chlorophacinone, are more readily metabolized, have a shorter half-life in hepatic tissue (2 weeks to several months) (Eason et al. 2002), and must be consumed in multiple feedings to reach a lethal dose (Erickson and Urban 2004). Second-generation ARs include brodifacoum, bromadiolone, and difethialone, and were developed to target rodents with genetic resistance to warfarin (Hadler and Buckle 1992). Second-generation ARs have prolonged action and increased potency (Petterino and Paolo 2001), with hepatic half-lives ranging 6–12 months, and may persist in liver tissue for more than a year in some species (Eason et al. 2002). Both classes of compounds have delayed onset of action, and death from AR consumption can occur up to 10 days after ingestion (Cox and Smith 1992). Individual rodents may continue to accumulate the compounds over a period of days, increasing their attractiveness to predators as they become weakened by the toxicant, and are easier to capture (Cox and Smith 1992; Berny et al. 1997; Berny 2007). For predators that consume prey targeted with ARs, both acute and chronic secondary exposure to the toxicants can occur (Erickson and Urban 2004; Riley et al. 2007; Elmeros et al. 2011; Gabriel et al. 2012).

Exposure of nontarget wildlife to ARs has been documented for numerous predatory mammal and bird species (McDonald et al. 1998; Stone et al. 1999; Riley et al. 2003, 2007; McMillin et al. 2008; Walker et al. 2008; Elmeros et al. 2011). Detection rates for ARs can exceed 80–90 % in wildlife and are directly responsible for mortalities in many species including coyotes (*Canis latrans*, Riley et al. 2003), San Joaquin kit foxes (*Vulpes macrotis mutica,* McMillin et al. 2008), California fishers (*Martes pennanti,* Gabriel et al. 2012), mountain lions (*Puma concolor*; Riley et al. 2007), red kites (*Milvus milvus,* Berny and Gaillet 2008), barn owls (*Tyto alba*), barred owls (*Strix varia*) and great horned owls (*Bubo virginianus*) (Stone et al. 2003; Albert et al. 2009). Factors that lead to secondary exposure of nontarget species are complex (Eason et al. 2002; Shore et al. 2006) because exposure is related to the persistence of compounds, levels of usage, how and where the compounds are applied, and trophic ecology (Eason et al. 2002; Shore 2003; Erickson and Urban 2004; Shore et al. 2006). The accurate assessment of AR exposure in wildlife is difficult because studies often rely on post-mortem sampling of liver tissue from carcasses found opportunistically. This may lead to a bias towards detection of those compounds with the longest persistence in hepatic tissue and at lethal dosages, and an underestimation of the number of animals that are exposed to ARs.

In southern California, more than a decade of research by U.S. National Park Service biologists in and around Santa Monica Mountains National Recreation Area (SMMNRA), a national park bordering Los Angeles, has documented widespread AR exposure in multiple carnivore species. AR exposure was the second leading cause of mortality during a 9-year coyote study in which 83 % of individuals tested were exposed (Riley et al. 2003; Gehrt and Riley 2010). Approximately 90 % of mountain lions and bobcats (*Lynx rufus*) in the study area were also exposed (Riley et al. 2007; Beier et al. 2010). Using telemetry data on bobcats and mountain lions, AR toxicant load, or the concentration of AR residues detected, was positively associated with use of developed areas (Riley et al. 2007, 2010; Beier et al. 2010) suggesting that developed areas are a major source of AR contamination.

Although high rates of exposure were documented for bobcats in SMMNRA, death as a result of AR exposure was reported only once (Riley et al. 2010). However, secondary AR exposure at $\geq$0.05 ppm was significantly associated with death due to severe notoedric mange (*Notoedres cati*), an ectoparasitic disease (Riley et al. 2007). Further, a precipitous population decline and genetic bottleneck in bobcats occurred as a result of the mange outbreak from 2002 to 2006 (Riley et al. 2007; Serieys et al. 2014). Notoedric mange was previously reported only in isolated cases in free-ranging felids (Pence et al. 1982; Maehr et al. 1995; Pence et al. 1995), however, the disease may be increasing in bobcats across California (Serieys et al. 2013; Stephenson et al. 2013). To date, all bobcats with mange that have been tested were positive for ARs ($N = 19$, Riley et al. 2007; $N = 11$, Serieys et al. 2013). These correlative findings suggest that chronic, sublethal exposure to ARs may influence immune function in bobcats, increasing their susceptibility to mange infestation and decreasing anti-mite immune response (Riley et al. 2007; Serieys et al. 2013).

We investigated risk factors for exposure to ARs in bobcats from two areas in southern California: in the SMMNRA area northwest of Los Angeles, and in Orange County to the southeast. We used blood and liver to detect exposure to ARs across varied landscapes that included fragmented urban and protected natural areas. Liver samples were collected postmortem to evaluate exposure history of individuals. Blood samples were collected primarily during animal capture to evaluate recent exposure. We used multiple measures of AR exposure including prevalence of exposure to any AR, prevalence of exposure to specific ARs, the number of different compounds detected, and compound residue concentrations (toxicant load). We evaluated AR exposure from 1997 to 2012 as part of the long-term study at SMMNRA, and from 2006 to 2010 in Orange County. We assessed risk factors for exposure

including sex, age, season, and landscape characteristics, specifically proximity to residential, commercial, and other developed areas. Using a much larger number of samples collected over a longer period of time and from a greater geographic area than a previous study (Riley et al. 2007), we examined the potential association between ARs and notoedric mange by evaluating the association between mange and a range of residue concentrations and the number of compounds detected.

Methods

Study area and sample collection

Sampling primarily occurred in two areas (Fig. 1). In Los Angeles and Ventura Counties, samples were collected by NPS and University of California, Los Angeles (UCLA) biologists from 1997 to 2012 during an ongoing NPS bobcat ecology study in SMMNRA (Riley et al. 2003, 2006, 2007, 2010; Serieys et al. 2013; Serieys et al. 2014). The eastern boundary of SMMNRA is less than 10 km from downtown Los Angeles and the park encompasses both large regions of continuous protected habitat with minimal urban development, including state and national park lands, and highly fragmented areas with intense urban development. In the Orange County study area (OCSA), bobcats were sampled from 2006 to 2010 by the U.S. Geological Survey (USGS) across a network of public nature reserves within landscapes experiencing rapid urbanization and near the more protected Santa Ana Mountains (Lyren et al. 2006, 2008; Poessel et al. 2014). The Santa Ana Mountains straddle Riverside, Orange, and San Diego Counties but most of the samples (93 %) were collected in Orange County. Anthropogenic development across both study areas includes residential, commercial, and agricultural development, as wells as many "altered open" areas such as golf courses and landscaped parks (Table 1). Samples were also opportunistically collected in two additional areas north and south of our study areas in San Barbara ($N = 3$) and San Diego Counties ($N = 8$) when animals died in wildlife rehabilitation facilities or were reported dead by residents.

Bobcats were captured and handled as previously described (Riley et al. 2003, 2006, 2007; Serieys et al. 2013) with approval by the Office of Animal Research Oversight of UCLA (Protocol ARC#2007-167-12) and by the Colorado State University Animal Care and Use Committee (Protocol #11-2453A). Scientific collecting permits were authorized by the California Department of Fish and Wildlife (SC-9791). From 2000 to 2009, the majority of trapping efforts occurred from mid-October to mid-February, and thus collected during the non-breeding, wet season (November 1–April 30). Individuals were chemically immobilized with a mixture of ketamine HCl (10 mg/kg) and xylazine HCl (1 mg/kg) or ketamine HCl (5 mg/kg) and medetomidine HCl (0.1 mg/kg). We recorded age class, sex, weight, and morphological measurements (i.e., chest circumference, body length, tail length, ear length, head circumference, etc.). Individuals were classified as juveniles (<2 years) or adults (>2 years) based on body size, weight, tooth wear and eruption, and reproductive status (Riley et al. 2003, 2006). A subset of individuals were also radio-collared as part of the NPS and USGS studies (Riley et al. 2003, 2006, 2007; Poessel et al. 2014). To obtain serum samples, blood was centrifuged within 24 h of collection and serum was collected. All samples, including liver (see below), were transported from the site of collection to storage facilities on ice packs.

In both study areas, we obtained liver samples during necropsies from opportunistically found carcasses (e.g. road-kill) or from animals that died in rehabilitation centers (Table 2). In SMMNRA, when possible liver samples were also collected from radio-collared animals that died. For 20 individuals, blood and liver were simultaneously obtained postmortem (Table 2). The cause of mortality, collection date, sex, age class, and location found were recorded. All animals were visually inspected for clinical signs of notoedric mange that included severe dermatitis, alopecia, and lichenification of the skin. If clinical mange was observed, skin scrapings in the affected areas were performed to identify mite species as previously described (Riley et al. 2007; Serieys et al. 2013; Stephenson et al. 2013). To measure specific age, an upper canine tooth was extracted during necropsy to determine age in years based on cementum annuli (Matson's Laboratory LLC, Missoula, MT) (Crowe 1972). Capture and mortality locations were recorded using GPS devices. Blood, serum and liver were stored at −20 or −80 °C until tested. Anticoagulant rodenticide compounds are stable (Waddell et al. 2013) and so the length of time under refrigeration should not have affected the results.

Anticoagulant assessment

We assessed the presence and amount of warfarin, coumachlor, bromadiolone, brodifacoum, diphacinone, chlorophacinone, and difethialone in 2 g of liver tissue, 1 g of serum, or 2 g of whole blood by high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS/MS) (Riley et al. 2007; Ruder et al. 2011; Waddell et al. 2013). Samples were first screened for the presence of each AR by LC–MS/MS. Positive AR samples were then quantitated by HPLC using either UV diode array detection (diphacinone, chlorophacinone and difethialone) or fluorescence detection (warfarin, coumachlor, bromadiolone, and

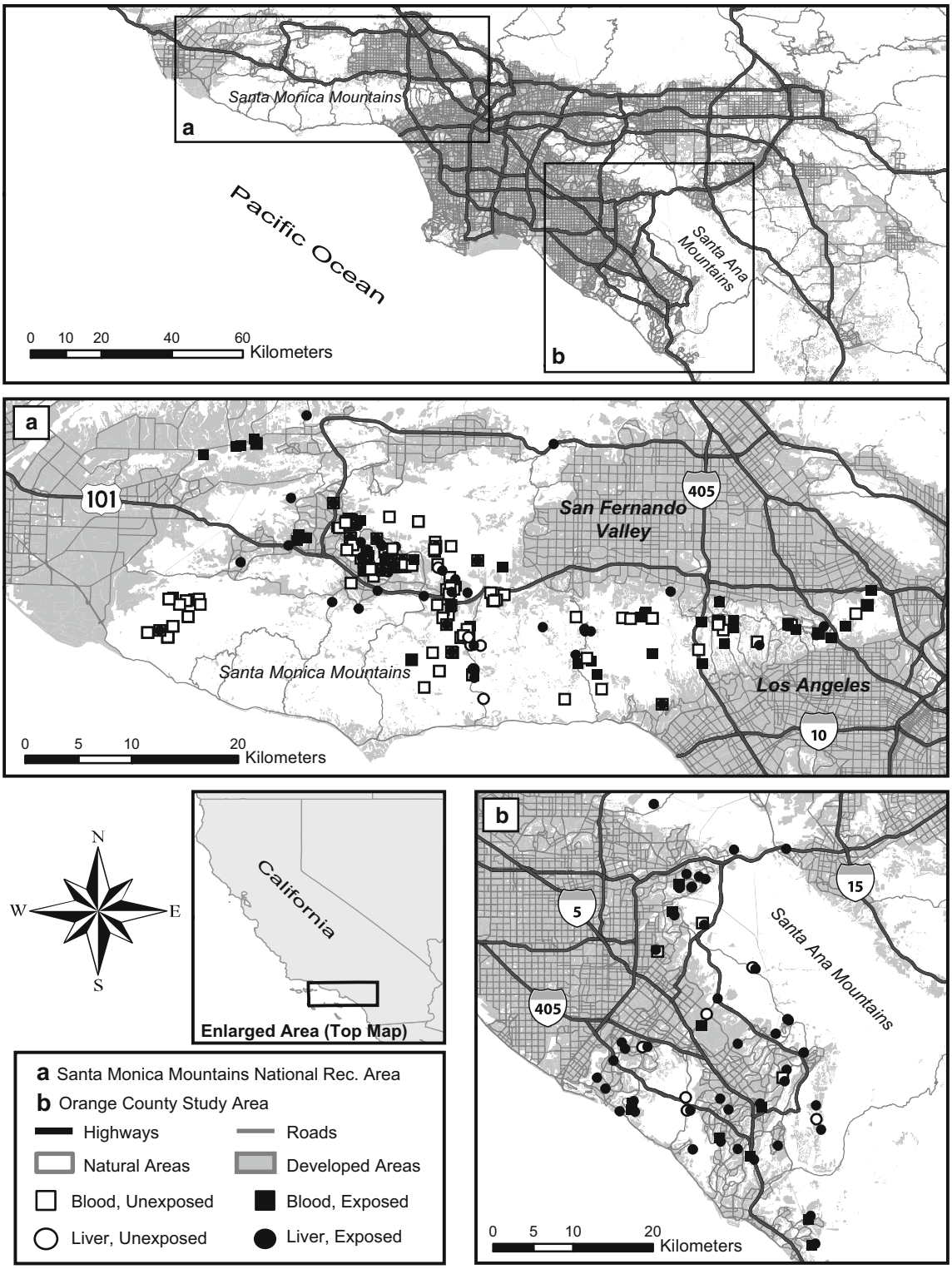

Fig. 1 Map of the study areas. **a** Santa Monica Mountains National Recreation Area (SMMNRA) and **b** Orange County Study Area (OCSA). Sampling locations and exposure results are shown. Blood sampling locations are represented with *squares* while liver sampling locations are represented with *circles*

brodifacoum). Limits of quantitation for these anticoagulants in liver tissue were 0.01 ppm for brodifacoum, 0.05 ppm for bromadiolone, warfarin, and coumachlor, and 0.25 ppm for chlorophacinone, diphacinone, and difethialone. Thirty-nine of 172 liver results were from Riley et al. (2007) (Table 2) and here we performed anticoagulant assessments using the same

Table 1 Classification of predictor land use variables used for analysis of dependent AR exposure measures

Broad classification	Specific land use tested in models	Percent of study areas			Percent of buffer zones		
		SMMNRA	OCSA	Mean	SMMNRA	OCSA	Mean
Agriculture	Crops, pastures orchards and vineyards	3.39	3.00	3.20	2.18	1.62	2.07
	Horse ranches	0.53	0.23	0.38	0.53	0.58	0.54
	Other agriculture	0.50	0.89	0.70	1.33	1.56	1.38
	Total agriculture	4.42	4.12	4.27	4.04	3.76	3.99
Commercial and industrial	Schools and religious	1.04	1.61	1.33	0.57	2.15	0.88
	Office and retail	1.29	2.89	2.09	1.18	1.20	1.18
	Mixed commercial and industrial	1.61	5.20	3.41	1.85	3.64	2.20
	Water facilities	0.34	0.57	0.46	0.49	3.79	1.13
	Total commercial and industrial	4.28	10.27	7.28	4.09	10.78	5.39
Residential	Multifamily/commercial high-density (>25 units/ha)	1.38	4.55	2.97	2.14	4.00	2.50
	Single-family high-density (5–10 units/ha)	14.80	17.04	15.92	10.76	7.58	10.14
	Single-family low-density (<5 units/ha)	5.63	1.96	3.80	3.90	8.22	4.74
	Total residential	21.81	23.55	22.68	16.80	19.80	17.38
Altered open space	Golf courses and cemeteries	1.02	1.75	1.39	0.55	2.67	0.96
	Other recreational/altered open space	0.61	1.43	1.02	0.53	1.86	0.79
	Total altered open space	1.63	3.18	2.41	1.08	4.53	1.75
Natural	Undeveloped natural	66.82	58.82	62.82	54.08	23.32	48.01

The percentage of each land use within a single polygon drawn around all bobcat buffer zones for each study area and the mean across both study areas is shown. Additionally, the mean value of each land use type across bobcat buffer zones for each study area and across all composite bobcat buffer zones is shown. The sum of land-use variables for each study area do not equal 100 % because some land-use types (e.g. open water, roads, railroads), comprising a mean of 0.55 % of the study areas, were not included in analyses

approach. In blood, limits of quantitation were 1 ppb for each compound with method detection limits ranging from 0.28 to 0.45 ppb. ARs that were determined to be positive by LC–MS/MS, but were below the limit of quantitation by HPLC, were defined as above the limit of detection (LOD) or "above LOD."

Finally, to make comparisons between AR exposure in bobcats, and the amount of toxicants applied where bobcats were sampled, we obtained data on reported use in Los Angeles, Orange, and Ventura Counties (measured in pounds) as posted in the California Department of Pesticide Regulation online database from 1997 to 2012 (http://www.cdpr.ca.gov/docs/pur/purmain.htm) for the four most commonly detected compounds. Records for Orange County were accessed only for the years for which we had samples from the study area (2006–2010). We averaged the pounds applied across the counties for each sample year (see Fig. 2c, Supplemental Fig. S1c).

Land use analysis

To evaluate the land use characteristics of surrounding landscape for all sampled bobcats, we created circular buffer zones with each capture or mortality location as the center. Each buffer zone was equal to the area of an average home range (95 % minimum convex polygon) for animals that have been radio-tracked in each study area (males: 5.2 km^2 SMMNRA; 5.6 km^2 OCSA; females: 2.3 km^2, SMMNRA; 3.2 km^2 OCSA) (Riley et al. 2010). Animals that were sampled in Santa Barbara and San Diego Counties were excluded from land use analysis because exact sampling locations were unavailable. We used the 2005 land use dataset provided by Southern California Association of Governments (SCAG, http://gisdata.scag.ca.gov/Pages/Home.aspx) with bobcat buffer zones in ArcGIS 10.1 (ESRI, Redlands, CA) to quantify land use types for each bobcat. Seventy-six land use types were included in bobcat buffer zones. These land use types were grouped into five general classes including: (1) agriculture; (2) commercial and industrial; (3) residential; (4) altered open areas such as landscaped parks, golf courses, and cemeteries; and (5) undeveloped natural areas (Table 1). We merged the 76 SCAG land use variables into 13 groups that were broadly characterized into five classes of land uses based on similarity and relevance to this study (Table 1, Supplemental Tables S1–S3). Using the five general classes of land use and the 13 specific variables, we used a total of 17 spatial predictor variables for analyses (Table 1). We quantified percent cover of each predictor variable in each buffer zone. To estimate percentage of

Table 2 Sample size and information

Sample type	Sample information	Total number
All	Total number of blood and liver samples	378 (individuals, $N = 304$)
	Paired blood and liver information	64 (Simultaneous collection postmortem, $N = 20$; blood collected at captures and liver collected postmortem, $N = 44$)
Blood	Total number	206 (individuals, $N = 195$; recaptures, $N = 11$)
	Type of blood collection event	Live captures, $N = 186$; postmortem, $N = 20$
	Total collected in SMMNRA	189 (LAC, $N = 88$; VC, $N = 101$)
	Total collected in OCSA	16
	Total collected outside of SMMNRA and OCSA	1 (SDC)
Liver[a]	Liver samples	172[b] (Independent samples used in analyses, $N = 169$)
	Total collected in SMMNRA	105 (LAC, $N = 39$; VC, $N = 56$, NA = 10)
	Total collected in OCSA	56 (OC, $N = 52$; RC, $N = 1$; SDC, $N = 3$)
	Total collected outside of SMMNRA and OCSA	11 (SBC and SDC: Rehab centers, $N = 9$; Reported dead, $N = 2$)
Spatial data	Available buffer zone data	Blood, $N = 196$; liver, $N = 121$
Mortalities[b]	Known mortality sources	172 (Mange, $N = 70$; Mange status unknown, $N = 16$; HBC, $N = 67$; Other, $N = 16$; NA = 17; Fetal, $N = 2$; Neonate, $N = 1$)
Mange	Number of cases during each season	Dry season, $N = 43$; Wet season, $N = 26$

SMMNRA Santa Monica Mountains National Recreation Area, *LAC* Los Angeles County, *VC* Ventura County, *OCSA* Orange County Study Area, *OC* Orange County, *SDC* San Diego County, *RC* Riverside County, *SBC* Santa Barbara County

[a] Twenty-three percent of these samples were also used in the Riley et al. (2007) study

[b] Anticoagulant data from three individuals were not used in analyses (fetuses, $N = 2$; neonate, $N = 1$)

each land use type within study areas, we created a single minimum convex polygon surrounding all buffer zones for each study area, and then calculated the percentage of each of the 17 land use variables within each study area's polygon (Table 1).

Data analysis

Descriptive statistics are presented as the mean, standard deviation, median, and range because all data were not normally distributed. Anticoagulant prevalence and 95 % confidence intervals for males, females, adults, and juveniles for wet (November 1–April 30) and dry (May 1–October 31) seasons were calculated separately for blood and liver samples. For prevalence calculations based on blood of recaptured animals, only the data from the most recent capture event was used. For spatial analyses using buffer zone data, we used only recaptures and post-mortem sampling that occurred a minimum of 4 months apart because ARs in blood are expected to decay by this time from an initial exposure (Eason et al. 2002; Erickson and Urban 2004; Vandenbroucke et al. 2008). Consequently, these successive samples of individuals are effectively independent measures of an exposure event, avoiding inflated values caused by multiple recaptures. For a subset of animals ($N = 64$), we had both liver and blood results (Table 2). For this group, we combined the AR residue data for both tissue types to calculate the anticoagulant exposure overall, and 95 % confidence intervals as well as range, mean and median number of compounds detected per individual.

We used 11 different measures of AR exposure for liver samples, and one measure for blood samples (Supplemental Table S4). For liver samples, we evaluated total exposure as presence or absence of any compound as well as individual exposure to each of the four most commonly observed individual compounds (brodifacoum, bromadiolone, diphacinone, and difethialone). We also measured the amount of AR exposure as the total residue concentration in parts per million (ppm) of all compounds detected ("total residues"), as well as separately for each of the four most commonly detected individual compounds. Finally, we used the total number of compounds detected (0–7). Using blood results, we evaluated total exposure only because the majority of detections for ARs in blood were diphacinone, and the total concentration of ARs was quantifiable for less than 10 % of samples tested (24 % of positive samples).

We evaluated risk factors for AR exposure using three types of generalized linear models (GLM). For presence/absence, we used a logistic regression to evaluate risk factors for total exposure measured using blood and liver,

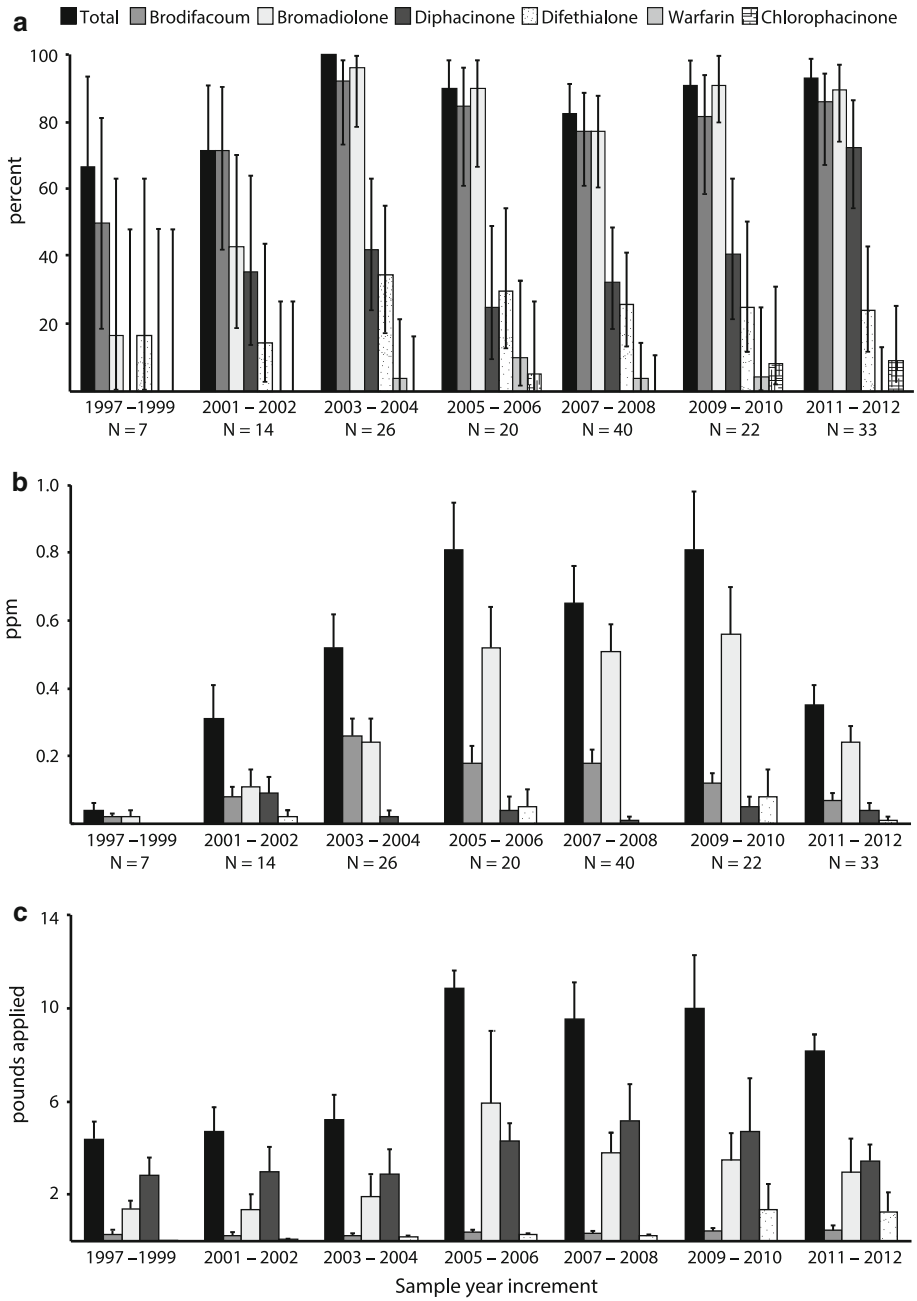

Fig. 2 AR data across 2–3 year time increments. **a** Exposure prevalence overall and by compound per 2–3 year increment. *Error bars* represent 95 % confidence intervals. **b** Concentrations detected per 2–3 year increments. *Error bars* represent standard errors. Warfarin, chlorophacinone, and coumachlor were rarely detected, and if so, were detected at above LOD levels (with the exception of chlorophacinone from 2006 to 2006 when 0.03 ppm was detected). Although lower concentrations of compounds were detected in 2011–2012, the difference, in comparisons with sample years from 2003 to 2010 was not significant. **c** Reported pounds of each compound applied per year increment in the three primary study area counties. *Error bars* represent standard errors. Los Angeles and Ventura Counties are represented across all years and Orange County data was included from 2006 to 2010

and separately, for exposure to brodifacoum, bromadiolone, diphacinone, and difethialone based on liver samples. We used a log-linear GLM to evaluate risk factors for the amount of exposure, both overall using total residues, and using residue concentrations for each of the four most commonly detected compounds in liver tissue. Two of 169 individuals from OCSA had outlier residue concentrations of greater than two standard deviations above the mean, one for difethialone and the other individual for bromadiolone. These individuals were excluded from concentration analyses for these specific compounds and for total residue analyses because preliminary analyses indicated that they dominated model results. We used a Poisson regression to evaluate risk factors for exposure to multiple compounds (0–7) for liver exposure data.

For each model type, we first performed univariate analyses to identify potential predictors, or risk factors, of exposure (Supplemental Table S4). We tested land use categories within each individual buffer zone, study area (SMMNRA, OCSA), sex (male, female), age class (adults $\geq$2; juveniles $<$2 years), age (in years), and season (wet, dry). For each age dataset, we performed separate analyses to avoid potential confounding effects. To evaluate the change in detection rates over time, animals were grouped

into 2–3 year increments depending on the number of animals sampled yearly such that in all time increments, $N \geq 7$ ($N = 23$; Fig. 2). Only four liver samples were collected during 1997–1999, so this time increment was excluded from temporal analyses.

Next, we performed multivariate GLMs to examine the influence of particular predictor variables on AR exposure while controlling for all other significant variables. Variables in the multivariate GLMs were selected by backward stepwise selection using Akaike's Information Criterion (AIC) for model selection. We report the strongest models with ΔAIC values ≤2 (Burnham and Anderson 2002). We report β, the standard error of β, and 95 % confidence intervals for β. A positive β indicates a positive association between the predictor and the exposure outcome, while a negative β indicates a negative association.

We also used logistic regression to examine anticoagulant exposure measures as predictors for notoedric mange. Our predictor variables for these analyses included the 11 anticoagulant exposure measures and the 17 land use predictors. Analyses were performed as above with univariate models followed by multivariate analyses. We also examined the association between notoedric mange and anticoagulant exposure using Fisher's exact tests to evaluate the number of compounds detected (≥2, ≥3, and ≥4) and the threshold value of total residues ≥0.05 ppm suggested by Riley et al. (2007). To further examine the potential relationship between mange and different levels of AR residues, we plotted the proportion of animals exposed to a range of anticoagulant residue concentrations, for animals with and without mange (Fig. 3). For animals with mange, we observed an increase in the proportion exposed to a residue range of 0.25–0.49 ppm. Consequently, we also used a Fisher's exact test to evaluate the association between mange and total residues ≥0.25 ppm. Next, we used a Kolmogorov–Smirnov test to evaluate the difference in the distribution of residue concentrations in bobcats that died with mange compared with those that died without mange. Finally, we used a Wilcoxon-rank sum test to evaluate the difference in median residue concentrations between the two groups.

Because commonly used methods of correction for multiple tests have been described as overly conservative with a higher probability of generating Type II errors in comparison with Type I errors (Moran 2003), we did not correct for multiple tests. Thus, all statistical tests were considered significant when $\alpha \leq 0.05$, but some of these may represent false positives. All statistical analyses were performed in the program R (R Development Core Team 2011).

When data were unavailable for sex (liver, $N = 18$; blood, $N = 2$), age class (liver, $N = 25$; blood, $N = 3$), year sampled (liver, $N = 7$), season sampled (liver, $N = 7$), or mange status ($N = 13$), AR results for those individuals were excluded from prevalence estimates and statistical analyses requiring these data. We also excluded exposure results from statistical analyses for livers from two fetuses (one from each study area), and a liver from a 1 day-old kitten because their exposure was likely not independent from that of their mother.

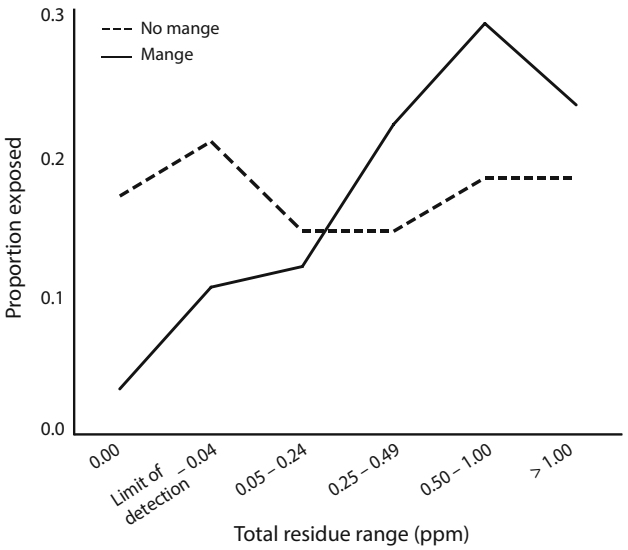

Fig. 3 The proportion of bobcats that died with and without severe mange when exposed to a range of total anticoagulant residues (ppm). The proportion of mange cases, compared with bobcats without mange, increases in the range of 0.25–0.49 ppm, and thus we investigated the relationship between mange and total residues ≥0.25 ppm. The limits of detection vary by compound. For brodifacoum and bromadiolone, the detection limits were 0.05 ppm, whereas the detection limits of chlorophacinone, diphacinone, and difethialone are 0.25 ppm

Results

Prevalence of exposure

Eighty-eight percent of liver samples had 1–5 AR compounds (Table 3; mean = 2.32, median = 2.00). The range of total residues detected in liver was 0.00–5.81 ppm (mean = 0.59, SD = 0.80, median = 0.40). The compounds most frequently detected were second-generation bromadiolone, brodifacoum, and difethialone, and first-generation diphacinone. Mean values for the four most commonly detected compounds were: brodifacoum, 0.14 ppm (SD = 0.20); bromadiolone, 0.38 ppm (SD = 0.55); difethialone, 0.04 ppm (SD = 0.31); diphacinone, 0.03 ppm (SD = 0.12). Brodifacoum and bromadiolone were the two most frequently detected ARs in liver samples (Fig. 4) and were detected approximately twice as frequently as difethialone or diphacinone. Warfarin and

Table 3 Proportion (Prop.) and 95 % confidence intervals of anticoagulant exposure across the study areas

Variable	Group	Liver			Blood		
		N	Prop.	95 % CI	*N*	Prop.	95 % CI
	All	169	0.88	0.82–0.92	195	0.39	0.32–0.46
Sex	Female	77	0.88	0.78–0.94	86	0.38	0.28–0.50
	Male	74	0.89	0.79–0.95	107	0.40	0.31–0.50
Age class	Adult	107	0.91	0.83–0.95	127	0.40	0.32–0.49
	Juvenile	37	0.86	0.70–0.95	65	0.37	0.26–0.50
Season	Wet	96	0.90	0.81–0.95	139	0.32	0.25–0.41
	Dry	66	0.89	0.81–0.94	56	0.55	0.42–0.68

Prevalence is partitioned by sample type, sex, age class, and season. When information on sex, age class, or season collected was not available, those data were not included in the proportion estimates, and so data partitioned by sex, age class, and season may not sum to the total number of blood or liver samples

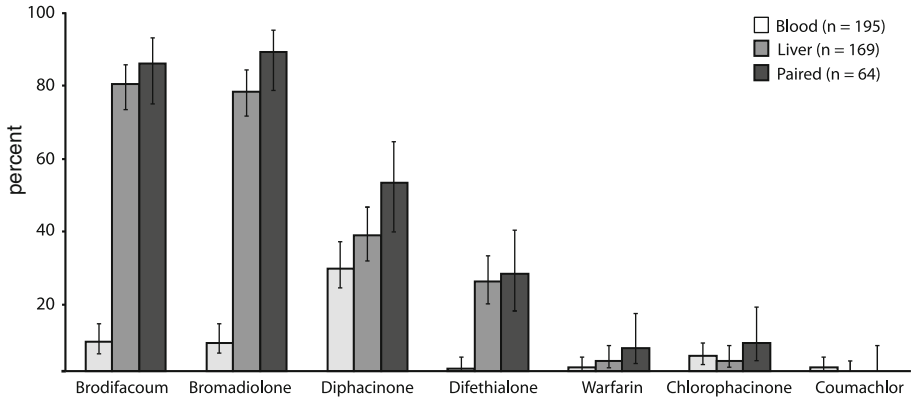

Fig. 4 Detection prevalence of each anticoagulant compound in the liver, blood, and for a subset of individuals, paired blood and liver tissue results are provided

chlorophacinone were rarely detected and coumachlor was not detected in liver samples. Seventy-seven percent of all bobcats and 87 % of those exposed showed the presence of ≥2 compounds in the liver.

In contrast, 39 % of blood samples tested positive for ARs (Table 3), most frequently to one compound (76 % of positives), but ranging from 0 to 4 compounds (mean = 0.53 compounds, median = 0.00). The total residues detected in blood ranged from 0 to 0.16 ppm (mean = 0.002, SD = 0.01, median = 0.00). Diphacinone, the most commonly detected compound in blood, was detected more than three times as frequently as brodifacoum or bromadiolone (Fig. 4). For animals with both blood and liver samples ($N = 64$), 92 % were exposed, most frequently to three or more compounds (median = 3.00, mean = 2.61, range 1–5).

Percent exposure was similar across sexes and age classes using liver or blood samples (Table 3). Sixty-six individuals were aged by cementum annuli (age range: 0–12 years). Fourteen individuals had age class data estimated during capture, and cementum annuli data collected postmortem. We used these paired data to test the accuracy of our age class estimations during captures and found we assigned correct age classes to 12 of 14 individuals. We did not detect a significant association between age and AR exposure measures.

Exposure did not vary by season when tested using liver samples (Table 3). In contrast, based on blood results, animals were significantly more likely to be exposed during the dry season [Odds ratio (OR) = 2.58] compared with the wet season (Tables 3, 4). Overall we detected 72 % more exposure in blood during the dry season than during the wet season with 32 % exposure detected during the wet season, and 55 % exposure detected during the dry season.

We examined exposure prevalence over time in liver samples and found exposure to exceed 67 % for all years, indicating high exposure prevalence throughout the study (Fig. 2a). Exposure rates varied for each of six compounds across sampling increments (Fig. 2a). Overall exposure was highest during 2003–2004 and 2011–2012. There was significantly less exposure overall and to bromadiolone in 2001–2002 compared with other years (Table 5; Fig. 2a). Diphacinone exposure was significantly greater in 2003–2004 and 2011–2012 compared with other time increments (Table 5; Fig. 2a). However, both total and

Table 4 Results of Fisher's exact tests for parameters that were significant during univariate GLM analyses

Sample type	Parameter	Comparison	Odds ratio	95 % confidence interval	*P*
Liver	Total residues ≥0.05 ppm	Severe mange versus no mange	4.00	1.67–10.48	<0.001
	Total residues ≥0.25 ppm	Severe mange versus no mange	3.16	1.51–6.84	<0.001
	Exposure to ≥2 AR compounds	Severe mange versus no mange	7.27	2.55–25.70	<0.001
	Exposure to ≥3 AR compounds	Severe mange versus no mange	2.11	1.06–4.23	0.023
	Exposure to ≥4 AR compounds	Severe mange versus no mange	3.98	1.54–11.26	0.002
Blood	Exposure detected	Dry season versus wet season	2.58	1.31–5.14	0.004
	Exposure detected	Capture event versus mortality	5.55	1.80–20.49	0.001
	Exposure detected	Capture event versus vehicle mortality	∞	1.00–∞	0.006

Table 5 Significant predictors of presence or absence of exposure in blood and liver

Outcome	Predictors of exposure		β	β SE	β 95 % CI	*P*
Total exposure (blood)	Dry season		0.95	0.32	0.31–1.56	0.003
	Crops, pastures, orchards and vineyards		4.85	2.08	0.98–9.21	0.015
	Horse ranches		88.75	36.10	21.90–166.11	0.011
	Other agriculture		15.46	7.29	1.63–30.67	0.029
	Water transfer and storage facilities		93.63	36.16	29.58–174.10	0.006
	Golf courses		15.69	7.75	0.50–30.88	0.043
	Multifamily high-density residential		9.47	3.56	2.49–16.44	0.008
	Single-family high-density residential		1.87	0.88	0.14–3.60	0.035
	Total residential		4.36	1.80	1.01–8.02	0.016
	Total commercial/industrial		4.42	1.84	0.81–8.02	0.016
	Total altered open		17.17	6.63	2.43–49.17	0.010
	Total residential		2.61	0.82	1.01–4.20	0.001
	Natural		−3.41	0.68	−4.74 to −2.09	<0.001
Total exposure (liver)	Single-family high-density residential		7.58	3.45	0.81–14.34	0.028
	Total residential		6.05	2.29	1.56–10.53	0.008
	Year (2011–2012 reference)	2001–2002	−2.72	1.18	−5.03 to 5.51	0.021
Brodifacoum exposure	Crops, pastures, orchards and vineyards		−5.62	2.67	−10.87 to −0.38	0.036
	Single-family high-density residential		6.19	2.36	1.56–10.82	0.009
	Total residential		6.68	1.90	2.95–10.41	<0.001
Bromadiolone exposure	Year (2011–2012 reference)	2001–2002	−1.54	0.67	−2.91 to −0.17	0.022
Diphacinone exposure	Single-family high-density residential		2.31	1.12	0.11–4.51	0.039
	Total residential		2.07	0.99	0.14–4.01	0.035
	Year (2011–2012 reference)	2001–2002	−1.46	0.70	−2.83 to −0.09	0.036
		2005–2006	−1.67	0.62	−2.98 to −0.52	0.005
		2007–2008	−1.30	0.48	−2.34 to −0.42	0.005
		2009–2010	−0.94	0.56	−2.26 to −0.01	0.048

Only results from statistically significant univariate analyses are shown

bromadiolone residue concentrations detected were greatest between 2005 and 2010, although the variation in residue concentrations across time was not significant (Fig. 2b). These years included samples from OCSA, where significantly greater bromadiolone residues were detected (Table 6; Fig. 5). Although the residue concentrations we detected in 2011–2012 were lower for all compounds the differences in overall exposure and residue concentrations were not significant. The apparent decrease in residue concentrations is the result of having OCSA samples, where bromadiolone residues were significantly higher for the years 2006–2010 (see below and Supplemental Fig. S1b). Further, the decrease in total and bromadiolone residues mirrors the County reports we compiled of the amount of rodenticide (in pounds) applied (Fig. 2c, Supplemental Fig. S1c). In blood samples, we did

Table 6 Significant predictors of AR residue concentrations, total compounds detected, notoedric mange, and exposure detected in blood at the time of capture versus mortality

Outcome	Predictor variables		β	β SE	β 95 % CI	*P*
Total concentration	Golf courses		5.88	1.01	3.90–7.85	<0.001
	Single-family high-density residential		1.24	0.46	0.34–2.13	0.008
	Total altered open		5.66	0.98	3.74–7.58	<0.001
	Total residential		1.31	0.44	0.44–2.17	0.004
	Natural		−1.20	0.35	−1.88 to −0.52	0.001
	Study area: OCSA		0.74	0.17	0.41–1.08	<0.001
Brodifacoum concentration	Office/retail		5.13	1.17	2.84–7.42	<0.001
	Golf courses		4.16	1.45	1.30–7.20	0.006
	Single-family high-density residential		1.31	0.54	0.25–2.37	0.017
	Total altered open		4.28	1.42	1.49–7.07	0.003
	Total residential		1.31	0.53	0.28–2.34	0.014
	Natural		−0.93	0.42	−1.75 to −0.11	0.029
	Study area: OCSA		0.58	0.22	0.11–0.96	0.014
Bromadiolone concentration	Mixed commercial/industrial		5.10	1.29	2.57–7.63	<0.001
	Golf courses		7.45	0.95	5.59–9.30	<0.001
	Multifamily high-density residential		1.58	0.76	0.09–3.08	0.040
	Single-family high-density residential		1.38	0.52	0.36–2.39	0.009
	Total commercial/industrial		1.43	0.57	0.31–2.55	0.014
	Total altered open		7.16	0.92	5.36–8.96	<0.001
	Total residential		1.38	0.51	0.38–2.39	0.008
	Natural		−1.45	0.40	−2.24 to −0.67	<0.001
	Study area: OCSA		1.03	0.21	0.61–1.45	<0.001
Diphacinone concentration	Mixed commercial/industrial		8.90	3.20	2.62–15.17	0.006
Total compounds	Single-family high-density residential		0.80	0.32	0.16–1.43	0.014
	Total residential		0.92	0.29	0.35–1.49	0.002
	Natural		−0.47	0.22	−0.90 to −0.03	0.036
	Year (2011–2012 reference)	2001–2002	−0.57	0.24	−1.04 to −0.10	0.018
Mange	Exposure		1.90	0.78	0.37–3.43	0.015
	Brodifacoum exposure		1.74	0.52	0.71–2.76	0.001
	Brodifacoum concentration		1.84	0.89	0.08–3.59	0.040
	Difethialone exposure		1.16	0.39	0.39–1.92	0.003
	Total compounds		0.56	0.15	0.26–0.85	<0.001
	Total residential		2.38	1.01	0.39–4.37	0.019
Mortality	Exposure (blood)		1.72	0.54	0.67–2.78	0.001

Only results from statistically significant univariate analyses are shown

not detect a trend of exposure prevalence across sampling years.

Two fetal bobcats were exposed to anticoagulant compounds. One animal was exposed to two compounds (brodifacoum and diphacinone) and the other was exposed to five compounds (brodifacoum, bromadiolone, diphacinone, difethialone, and chlorophacinone). For both fetuses, all compounds detected were above LOD but not quantifiable. The mother of the fetus with five compounds was also tested for exposure and had quantifiable levels of brodifacoum (0.32 ppm), bromadiolone (0.58 ppm) and was positive for difethialone, diphacinone, and chlorophacinone.

Spatial correlates of exposure

Exposure prevalence measured using liver tissue did not significantly differ between SMMNRA (89, 95 % CI 81–94; $N = 104$) and OCSA (84, 95 % CI 71–92; $N = 55$) (Fig. 5). The mean total residues were significantly greater in OSCA (Fig. 5; Table 6) even with two outliers removed (OCSA, 0.84 ppm; SMMNRA, 0.40 ppm). Brodifacoum and bromadiolone were each detected at significantly greater concentrations in liver tissue collected in OCSA (0.21 and 0.63 ppm) compared with SMMNRA (0.12 and 0.22 ppm) (Table 6).

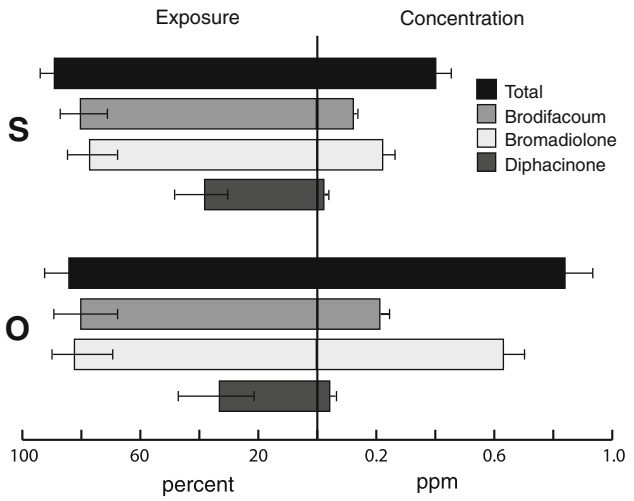

Fig. 5 *Left* Percent total exposure and exposure to individual compounds in SMMNRA (S) and OCSA (O). Bars represent 95 % confidence intervals. *Right* Total residue concentration and residue concentrations for each compound. *Bars* represent standard errors

Landscape variables were important predictors of exposure in both blood and liver samples (Tables 5, 6). Percent natural area in each individual buffer zone was negatively associated with multiple measures of exposure in blood and liver (Table 5). Golf courses and total altered open area were positively associated with exposure in blood (Table 5) and with total residues and the concentrations of bromadiolone and brodifacoum in liver (Table 6).

In terms of the non-residential urban and agricultural areas, all three more specific agricultural categories (Table 1, Supplementary Table S2) and total agricultural area were positively associated with exposure in blood (Table 5). However, brodifacoum exposure was negatively associated with the category comprised of open, active agriculture (crops, pastures, orchards, and vineyards; Table 5), and given that second-generation ARs are restricted for use indoors and within 100 m from human structures, this negative association is not surprising. Commercial and industrial areas were positively associated with bromadiolone and diphacinone concentrations in liver samples (Table 6). Water storage and transfer facilities and total commercial and industrial areas were positively associated with exposure in blood samples (Table 5). Office and retail area was positively associated with brodifacoum concentration in liver samples (Table 6).

Residential areas, by far the most common type of development in these study areas (22 %, SMMNRA and 23 %, OSCA), were frequently positively associated with AR values observed in both blood and liver samples (Tables 5, 6). In particular, single-family high-density residential area was among the most frequent land use type to have positive associations with anticoagulant exposure, and was significant for 8 of 11 anticoagulant exposure models tested (Tables 5, 6). In terms of broader measures, single-family high-density residential area was positively associated with overall exposure in blood and liver and the total number of compounds and total residues in liver samples. For specific compounds, single-family high-density residential was also positively associated with brodifacoum and diphacinone exposure and brodifacoum and bromadiolone concentrations in liver. Total residential area was also frequently important in univariate models. In terms of exposure, total residential area was associated with exposure in blood and liver and exposure to brodifacoum and diphacinone in liver. Total number of compounds, total residues, and liver concentrations of brodifacoum and bromadiolone were also positively associated with total residential area.

Multivariate models were significant for five measures of ARs in bobcats (Table 7). In terms of exposure, year and total residential area were important for diphacinone, and for total exposure, percent natural area (the reciprocal of percent development) was significant, along with season. For the amount of ARs detected in liver tissue, the best-fit model included golf courses, single-family high-density residential, and OCSA as the most important risk factors. For brodifacoum concentration detected in liver tissue, office and retail, single-family high-density residential, and total altered open space were the three most important predictors of residue load. Finally, mixed commercial and industrial, golf courses, single-family high-density residential, and OCSA were the most important predictors of total bromadiolone concentration in liver tissue.

Anticoagulants and notoedric mange

The median total residues for bobcats with mange was 0.52 ppm (mean = 0.65, SE = 0.06), while for bobcats that died without mange, the median total residues was 0.24 ppm (mean = 0.53, SE = 0.09), a significant difference ($W = 2141.00$, $P = 0.005$). The distribution of residue concentrations within the two groups also differed significantly ($D = 0.28$, $P = 0.004$). The median number of compounds observed was 3 (mean = 3.00) in bobcats with mange and 2 (mean = 2.00) for bobcats without mange. Sixty-four percent of bobcats without mange tested positive for ≥2 compounds, while 93 % of bobcats with mange tested positive for ≥2 compounds.

Severe mange was positively associated with anticoagulant exposure, brodifacoum exposure, difethialone exposure, brodifacoum concentration, and the total number of compounds detected. In terms of land use, mange was positively associated with total residential area, but this was the only significant land use predictor (Table 6). The mean total residential area in mange bobcat buffer zones was 32.2 % (SD = 18.61, median = 29.39) compared with a mean of 23.3 % for bobcats without mange

Table 7 Results of the best-supported statistically significant multivariate model analyses for anticoagulant exposure and mange

Outcome	Best-supported model	Predictor variables	β	β SE	β 95 % CI	*P*
Total exposure (blood)	Season + natural	Dry season	0.71	0.35	0.02–1.40	0.043
		Natural	−3.29	0.68	−4.62 to −1.95	<0.001
Diphacinone exposure	Total residential + year	Total residential	2.57	1.12	0.37–4.77	0.022
		2001–2002	−1.62	0.82	−3.23 to −0.02	0.048
		2003–2004	−1.42	0.69	−2.77 to −0.62	0.040
		2005–2006	−2.11	0.80	−3.68 to −0.55	0.008
		2007–2008	−1.78	0.65	−3.06 to −0.50	0.006
		2009–2010	−1.43	0.72	−2.84 to −0.01	0.048
Total concentration	Golf courses + single-family high-density residential + study area	Golf courses	3.91	1.06	1.84–5.98	<0.001
		Single-family high-density residential	0.99	0.43	0.15–1.82	0.022
		OCSA	0.69	0.20	0.30–1.07	0.001
Brodifacoum concentration	Office/retail + single-family high-density residential + total altered open	Office/retail	4.49	1.09	2.34–6.63	<0.001
		Single-family high-density residential	1.22	0.55	0.15–2.29	0.027
		Total altered open	3.88	1.55	0.85–6.91	0.013
Bromadiolone concentration	Mixed commercial/industrial + golf courses + single-family high-density residential + study area	Mixed commercial/industrial	3.48	0.99	1.55–5.42	0.001
		Golf courses	5.69	0.93	3.87–7.51	<0.001
		Single-family high-density residential	1.24	0.42	0.42–2.06	0.004
		OCSA	0.90	0.25	0.42–1.38	<0.001
Mange	Difethialone exposure + brodifacoum exposure	Brodifacoum exposure	1.54	0.53	0.49–2.58	0.004
		Difethialone exposure	0.93	0.40	0.14–1.72	0.021

(SD = 19.60, median = 19.05). In the multivariate model, after controlling for multiple AR parameters and land use, brodifacoum and difethialone exposure remained significant predictors of severe mange while land use was not (Table 6). We found a strongly significant association between mange and total residues ≥0.05 ppm and total residues ≥0.25 ppm (Fig. 3; Table 4). Bobcats that were exposed to ≥0.05 ppm were 4.0 times (95 % CI 1.67–10.48) more likely to die with severe notoedric mange than without, while those exposed to ≥0.25 ppm were 3.2 times (95 % CI 1.51–6.84) more likely to die with severe mange. Additionally, we observed a strong association between exposure to ≥2 compounds and severe mange (Table 4). Specifically, bobcats were 7.3 times (95 % CI 2.55–25.70) more likely to die with severe mange than without if they were exposed to 2 or more AR compounds. There were also significant associations between mange and exposure to ≥3 and ≥4 compounds (Table 4).

Anticoagulants and mortality

Anticoagulant exposure detected in blood was significantly more frequent in samples collected postmortem compared with samples collected antemortem (Tables 4, 6). In 75 % of blood samples collected postmortem ($N = 20$), we detected at least one AR compound. When blood samples collected at the time of mortality were excluded from blood AR prevalence estimates, we detected a 34 % exposure prevalence in blood samples collected at the time of animal capture ($N = 175$) compared with 39 % overall ($N = 195$). For blood samples collected at the time of mortality, ARs were detected in 77 % of bobcats that died of notoedric mange ($N = 13$), 100 % of bobcats that died of vehicle collision ($N = 5$), and one bobcat that died of starvation after a wildfire. Three bobcats that died of mange, one from a control action, and another that died of unknown cause did not have detectable ARs in their blood.

Discussion

We documented widespread exposure of bobcats to first- and second-generation ARs in two southern California areas. Bobcats are obligate carnivores that consume a wide range of small mammals (Anderson and Lovallo 2003) including mice, rats, and gophers (Fedriani et al. 2000;

Riley et al. 2010) that are frequent targets of pest control activities within SMMNRA (Morzillo and Mertig 2011a, b; Morzillo and Schwartz 2011; Bartos et al. 2012) and elsewhere (Morzillo and Mertig 2011b). Given that bobcats are obligate carnivores, it is very unlikely that they consume rodent baits directly. Thus, bobcat exposure to ARs is predominantly, if not entirely, secondary through prey consumption. Exposure rates and compounds detected varied considerably by sample type, but in individuals having blood and liver data (and therefore most comprehensively sampled), we detected an AR exposure rate of 92 % across the study areas, with animals most frequently exposed to three or more compounds. These findings are among the highest reported prevalence rates for AR exposure in a nontarget predatory species (e.g. Shore 2003; Fournier-Chambrillon et al. 2004; Riley et al. 2007; Walker et al. 2008; Gehrt and Riley 2010; Elmeros et al. 2011; Gabriel et al. 2012; Sánchez-Barbudo et al. 2012). Additionally, the combined liver and blood results indicate that exposure prevalence and exposure to certain compounds, specifically diphacinone, may be underestimated with liver samples alone (Fig. 4). We detected exposure to multiple AR compounds in two fetal bobcats, the first such cases, to our knowledge, reported for any wildlife species in a natural population. These data, including individuals caught multiple times more than 4 months apart, indicate multiple exposure events and suggest the potential for chronic exposure to ARs that can begin during prenatal development.

There are no toxicokinetic studies (the movement of toxic substances within the body) of ARs in wildlife, however, hepatic half-lives for ARs are reported across multiple species to be longer than plasma half-lives, particularly for second-generation ARs (Kamil 1987; Robben et al. 1998; Petterino and Paolo 2001; Vandenbroucke et al. 2008). The toxicokinetics of secondary AR exposure is more complex because the movement of the residues in both the primary and secondary consumer must be considered (Erickson and Urban 2004). Thus, we are limited in our ability to interpret bobcat AR exposure results with respect to dose and time since exposure using either blood or liver sample data. However, because we most frequently detect diphacinone in blood despite its shorter plasma half-life than second-generation ARs (Erickson and Urban 2004), diphacinone may be the compound that bobcats encounter most frequently in SMMNRA.

Risk factors for exposure

Exposure detected using liver tissue was high throughout the course of the 16-year study, ranging from 67 to 100 % for each 2- to 3-year time period, indicating high prevalence AR exposure in bobcats since at least 1997. Our samples indicated an increase in overall exposure both in prevalence and residue concentrations since 2002. We detected significant increases in total AR exposure, bromadiolone exposure, and total number of detected compounds. With the exception of diphacinone, overall exposure prevalence and exposure to individual compounds appears to have been relatively constant from 2003 to 2012. Total residues and bromadiolone residues were highest from 2005 to 2010, the time increments for which OCSA samples were available, which appears to reflect the degree of bromadiolone use in Orange County. Diphacinone exposure also increased in frequency from 1997 to 2012, reaching a high in 2011–2012. Despite this increase, the quantity applied in each county as reported to Department of Pesticide Regulation does not appear to have significantly changed over the course of the study (Fig. 2c, Supplemental Fig. S1c). Thus, increased diphacinone exposure may be the result of increased use of the compound in residential areas by homeowners and pest control companies that are not required to report amounts of ARs applied annually. In fact, single-family high-density and total residential area were important predictors of diphacinone exposure. Diphacinone is a first-generation compound and is considered to pose less risk to nontarget wildlife than the more toxic second-generation ARs (Erickson and Urban 2004), although first-generation ARs still pose a risk for toxic effects to wildlife, and secondary exposure can be a direct source of mortality for some species (Littrell 1988; Stone et al. 1999; Riley et al. 2003). Further, the degree to which there are additive or interactive effects between diphacinone and second generation ARs is unknown.

As measured in blood, we detected more than twice as much AR exposure during the dry season compared with the wet season. In southern California, the dry season coincides with peak rodent activity (Meserve 1976), and residents in the region are known to use ARs to target rat, mice, squirrel, and gopher populations (Morzillo and Schwartz 2011; Bartos et al. 2012). Although we detected no seasonal differences in exposure in liver samples, the long hepatic half-lives of second-generation ARs likely obscured our ability to detect seasonal differences. Additionally, because second-generation ARs may persist in small mammal species from 90 to 135 days after removal of poison baits, poisoned small mammals may remain a continuing source of exposure for predatory species long after the end of poisoning programs (Murphy et al. 1998; Sage et al. 2008).

Because an accumulated risk of exposure may occur with bobcat age, and female bobcats have smaller home ranges and are less likely to use urban areas compared with males (Riley et al. 2003, 2010), we expected to detect demographic differences in AR exposure prevalence and residue concentrations. However, neither age nor sex significantly influenced exposure in our study areas. Within

our study areas, the high prevalence of exposure may have diminished our ability to detect demographic differences. Further, the movement patterns and relatively high mobility of some rodent species may lead to AR exposure in even those individuals that avoid the use of urban areas (Riley et al. 2010). For example, wood mice (*Apodemus sylvaticus*) and house mice (*Mus domesticus*) were found exposed to multiple AR compounds in Northern Ireland even though they were sampled in agricultural areas where ARs were not in use (Tosh et al. 2012). Thus, movement of poisoned prey between areas may occur where AR control efforts differ (Tosh et al. 2012). The risk of secondary AR exposure in predatory species, therefore, may not be limited to areas where ARs are in use. As a result, even individuals that use urban areas less, such as female bobcats and not yet dispersed young animals, may still be at high risk of AR exposure.

Spatial predictors of exposure

The association between AR exposure and specific land use types likely reflects the degree of AR use in those areas. Previous studies have found an association between anthropogenic development and AR exposure in nontarget wildlife. For example, 95 % ($N = 74$) of wildlife carcasses sampled across California from 1994 to 1999 with exposure to ARs were reported to have been collected in areas with significant urban development (Hosea 2000). However, there was no specific information about the type and intensity of urban development where individuals were sampled. Other previous studies in these areas found a positive association between total AR concentrations and the percent of bobcat (Riley et al. 2007) and mountain lion (Beier et al. 2010) radio-telemetry locations in areas affected by anthropogenic development, including areas classified as altered open and areas of more intense urban development (e.g. composite residential, commercial, and industrial areas).

Single-family high-density residential (5–10 housing units/ha) and golf courses were among the most frequent risk factors for various measures of AR exposure, despite comprising a relatively small percentage of the study areas (15.9 and 1.4 %), suggesting their importance as a risk factor for AR exposure and toxicant loads. In a recent study in two southern California areas (SMMNRA, Bakersfield), residents in single-family high-density structures were the most likely to use ARs to control pest populations compared with those in multifamily or single-family low-density structures (Morzillo and Schwartz 2011). Residential AR use was highest in areas in close proximity to open areas, whether natural or altered open, compared with residential areas farther away from open spaces. Golf courses and other altered open spaces in the study areas are typically surrounded by, or very near to, single-family housing units. Of 21 golf courses in our study areas, 19 are bordered on at least 1 side by single-family high-density residential areas. Because residential AR use may be elevated in areas with altered open space in close proximity (Morzillo and Schwartz 2011), the association between AR exposure and altered open areas may also be the result of increased AR use in the single-family residential areas adjacent to golf courses. In OSCA, where bobcats had greater brodifacoum and bromadiolone residue loads, the mean percent of golf courses in bobcat buffer zones was nearly five times greater than in SMMNRA (0.6 vs. 2.7 %), potentially contributing to increased residue loads in OCSA. Although the residential and altered open types of urban development comprise a relatively small proportion (<25 %) of the study areas, Morzillo and Schwartz (2011) suggested a small degree of AR use in residential areas can lead to increased exposure risk for wildlife. Both bobcats (Riley et al. 2010) and coyotes (Gehrt and Riley 2010) have been observed to routinely utilize residential and altered open areas such as golf courses, increasing their probability of exposure to ARs if the compounds are regularly used there or nearby.

Although percent natural habitat was negatively associated with AR exposure and total residues, four bobcats whose buffer zones comprised 100 % natural habitat were found exposed to ARs. These data indicate that ARs may also affect wildlife living solely within protected park areas. Both of the individuals with bromadiolone residues were radio-collared during ongoing NPS research in SMMNRA, and their documented home ranges did not extend beyond protected park boundaries (Riley et al. NPS unpubl. data). Previous NPS research on coyote utilization of urban areas found that even animals with the lowest urban association died directly from AR toxicosis (Riley et al. 2003). A recent study on fishers (*Martes pennanti*), a remote forest carnivore in protected undeveloped parkland in northern California, found 79 % of fishers exposed to ARs and that four died directly of anticoagulant toxicosis (Gabriel et al. 2012). Gabriel et al. (2012) suggested illegal marijuana cultivation in remote areas could have been the source of ARs. Within SMMNRA, illegal marijuana cultivation also occurs, so this may also contribute to AR exposure for animals that reside entirely in protected park areas.

Consequences of exposure

Although the prevalence of AR exposure was very high at 92 %, AR exposure alone does not appear to be a significant source of direct mortality for bobcats. At present, there are few cases of AR toxicosis in bobcats documented in the literature. None of the bobcats in OCSA died directly

of anticoagulant toxicity, and in a broader study of poisoning cases of wildlife in California, Hosea (2002) observed clinical signs consistent with anticoagulant toxicosis in two bobcats, one of which was an individual from SMMNRA (Riley et al. 2007). In Marin County, a radio-collared bobcat died of anticoagulant toxicity; chlorophacinone was detected in the liver tissue (Riley 1999). AR exposure was suspected to have caused gastrointestinal bleeding in bobcats that died of severe notoedric mange and were exposed to ARs in several counties in California, though other signs of anticoagulant toxicity were absent (Serieys et al. 2013). Domestic cats are reported to be more tolerant of AR exposure than dog or rodents (Petterino and Paolo 2001; Erickson and Urban 2004). Whether this tolerance is similar for wild felids is unknown, but if so, it may account for the few cases of toxicosis detected. However, felid tolerance to low-grade AR exposure may increase their vulnerability to sublethal toxicosis, or affect their ability to respond to external stimuli such as predators and vehicles (see below).

In SMMRNA, secondary anticoagulant rodenticide exposure was associated with a population decline (Riley et al. 2007) and a genetic bottleneck (Serieys et al. 2014) that occurred due to notoedric mange. Mange and vehicle collisions are the primary sources of mortality for bobcats in our two southern California study areas (Riley et al. 2010). Notoedric mange is now documented in eight counties in northern and southern California. Across all of these areas, animals that died of mange were found to be exposed to ARs whenever tests were conducted (Serieys et al. 2013; Clifford, pers.comm.). Interestingly, 65 % of severe bobcat mange cases observed in our study areas during this 16-year period occurred during the dry season, coincident with increased AR exposure detected in blood samples. Sixty-nine of 70 bobcats that died with severe mange (covering >70 % of their body) were exposed to ARs. We detected a strong association between exposure to ≥2 compounds and notoedric mange. Detection of multiple compounds in a single individual suggests multiple exposure events since rodenticide baits sold in California are each formulated with a single compound. Thus, we suggest that a single anticoagulant exposure event itself may not increase bobcat susceptibility to mange, but rather repeated exposure events may be an important predictor of potential sublethal effects such as increased susceptibility to mange.

Severe mange in free-ranging wildlife and domestic animals is often associated with decreased immune competence (Pence and Ueckermann 2002). Humans that are immunocompromised are also more likely to suffer severe, crusted forms of mange due to infestation with a related mite, *Sarcoptes scabiei* (Walton et al. 2004; Roberts et al. 2005). The mode by which anticoagulant rodenticide exposure could compromise bobcat immunity is unknown, although recent studies in humans and laboratory animals have shown therapeutic doses of warfarin to have both immunostimulatory and suppressive effects when administered for ≤30 days (Kurohara et al. 2008; Belij et al. 2012; Popov et al. 2013). Laboratory experiments have shown that interactive effects between sublethal exposure to anticoagulants and other stressors can induce mortality. For laboratory subjects, sublethal anticoagulant doses produced 40–70 % mortality when combined with other stressors, such as frostbite (Jaques 1959). When stressed by shearing and captivity, sheep (*Oves aries*) required lower doses of the first-generation AR pindone to die as a result of anticoagulant toxicosis (Robinson et al. 2005). A potential interaction between the toxic effects of chlorophacinone and a bacterial pathogen, tularemia (*Francisella tularensis)* was described in common voles (*Microtus arvalis*, Vidal et al. 2009). Voles that were infected with *F.tularensis* died at lower doses of chlorophacinone than uninfected voles. Tularemia prevalence was also higher in areas treated with chlorophacinone, and the authors suggested that the AR field treatment may have also facilitated the spread of the disease in the affected vole population.

Sublethal AR exposure may also negatively affect individuals directly. In Denmark, Elemeros et al. (2011) found a negative association between anticoagulant exposure and body condition in weasels (*Mustela nivalis*) and stoats (*Mustela erminea*). A reduced escape response has been observed in rats dosed with ARs (Cox and Smith 1992), and if carnivores secondarily exposed to ARs have a similarly reduced response to threats, they may be more vulnerable to vehicle collisions or predation. Elmeros et al. (2011) found that for both stoats and weasels, those that were sampled after being trapped had significantly lower total AR residue concentrations than those sampled after vehicle collisions and predation events. Although we have a limited sample size ($N = 5$), all animals that died of vehicle collisions for which we collected blood post-mortem had detectable AR residues in their blood (compared with 34 % of captured animals). Thus we speculate that recent AR exposure events may increase bobcat vulnerability to vehicle collision but additional data are needed to test this hypothesis.

Bobcats with severe notoedric mange exhibit altered behavior increasing their susceptibility to other primary sources of mortality. For example, although bobcats are primarily nocturnal, especially in urban populations (Riley et al. 2003), we have observed bobcats with severe mange infestation frequently wandering in urban areas during daylight hours (Riley and Serieys unpubl.data). This shifted activity pattern may increase the risk of being struck by vehicles and vulnerability to other sources of mortality.

Though sample sizes are limited, our findings that AR transfers from mother to offspring suggests consequences

for reproduction in bobcats. Contaminant exposure that interferes with the reproductive success of wildlife populations can lead directly to population declines. We tested two bobcat fetuses, one from each study area and both were exposed to multiple AR compounds with one exposed to five compounds. Reproductive consequences associated with AR exposure in other species have included increased miscarriage, fetal toxicosis, fetal congenital deformities, and decreased sperm counts in humans (Ginsberg and Hirsh 1989), dogs (Munday and Thompson 2003), and sheep (Robinson et al. 2005). In humans, prenatal exposure to first-generation coumarin even at low, therapeutic doses has been associated with central nervous system abnormalities (Ginsberg and Hirsh 1989; Wesseling et al. 2001). Brodifacoum toxicosis was documented in neonatal puppies even though the mother was exposed 4 weeks prior to birth (Munday and Thompson 2003). AR exposure may be an important challenge for population viability in urban areas if chemical contamination causes detrimental effects on reproduction.

Conservation and management implications

Exposure of nontarget wildlife to ARs is increasingly recognized as a widespread conservation issue (Erickson and Urban 2004; US EPA 2008; California Department of Pesticide Regulation 2013) and numerous species have been exposed, sometimes causing direct mortalities (Scheuhammer 1987; Peakall 1992; Eason et al. 2002; Erickson and Urban 2004; Riley et al. 2007; Gabriel et al. 2012). Species that are exposed include federally listed endangered species such as San Joaquin kit foxes (McMillin et al. 2008), bald eagles (*Haliaeetus leucocephalus*, Stone et al. 2003; Salmon 2010), and the Northern spotted owl (*Strix occidentalis caurina*, Erickson and Urban 2004). Indirect mortalities associated with the poisons may also pose an important threat for wildlife populations, particularly those that are re-colonizing parts of their past range. For example, during a recent study of California fishers, which are candidates for protection under the US Endangered Species Act, a lactating female died of anticoagulant toxicosis, which most likely led indirectly to the death of her litter (Gabriel et al. 2012). For threatened populations, exposure to ARs may influence their reproductive success, lead to sublethal and lethal consequences and increase their vulnerability to other sources of mortality.

Although some U.S. States, such as California, are taking steps to increase regulation of the use and the availability of these poisons to consumers, the adequacy of these is unknown. Under current law, second-generation ARs are restricted to indoor use or within 30 m (100 ft) of buildings. In California, the Department of Pesticide Regulation has reduced that distance to a 17 m (50 ft) radius from buildings. However, Tosh et al. (2012) found no relationship between distance from buildings and residue concentrations in two species of mice reflecting the high mobility of the small mammals even after ingestion of ARs. They also detected a contaminated wood mouse (*Apodemus sylvaticus*) 110 m from a building where usage occurred and another 160 m from a building where no usage occurred (Tosh et al. 2012). In residential areas within SMMNRA, residents have reported off-label use of ARs, and use of second-generation ARs up to 100 m from buildings (Bartos et al. 2012). We have observed containers of second-generation ARs in natural areas behind homes at greater than 30 m from a building. Residents who use ARs have also reported continued use of the compounds although they were aware of the threat that the compounds posed to nontarget wildlife (Morzillo and Mertig 2011a). If wildlife are especially likely to be exposed to ARs due to use of these compounds in residential areas, then measures that address residential use of ARs may be particularly effective in mitigating ecological risks associated with these compounds.

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Gmail by Google

Kian Schulman <poisonfreemalibu@gmail.com>

Re: 4/17 LA Times story

1 message

Laurel Serieys <laurelserieys@gmail.com> Tue, Apr 22, 2014 at 8:22 AM
To: "Riley, Seth" <seth_riley@nps.gov>
Cc: Jan Dougall <jandougall@gmail.com>, Kate Kuykendall <kate_kuykendall@nps.gov>, Christy Brigham <christy_brigham@nps.gov>, "Moriarty, Joanne" <joanne_moriarty@nps.gov>, Kian Schulman <poisonfreemalibu@gmail.com>

Hi Jan,
I echo Seth's concerns about the use of first-generation anticoagulants as replacements for second-generation anticoagulants. I don't know if you've seen my website, but I have information on the website that could be useful: http://www.urbancarnivores.com/poisons/

However, for my research, we tested 195 bobcat blood samples for exposure to anticoagulants. 39% of animals were exposed, and diphacinone (a first-generation anticoagulant) was the most frequently detected compound. In 77% of blood samples in the 39% that we detected exposure, diphacinone was detected. In terms of other first-generation compounds, we also detected chlorophacinone and coumachlor in the blood samples. Diphacinone was detected 3 times as frequently as as second-generation compounds. Given our findings, we concluded that diphacinone, a first-generation compound, is probably the most frequent compound that bobcats are exposed to across our study areas, which included a significant number of samples from Ventura, Los Angeles, and Orange Counties, but also some samples from Santa Barbara and San Diego Counties.

During this study, we also tested 172 liver samples, and in those samples, we most frequently detected second-generation compounds brodifacoum and bromadiolone. We probably do not detect diphacinone in the liver as frequently because it has a significantly shorter half-life than either second-generation compound (up to several months for diphacinone vs. 6+ months for second-generation compounds).

Although we have not found an association between mange and first-generation anticoagulants, we have tested for an association only using results from anticoagulant testing using liver samples (we don't have enough blood samples from mangy animals to do the same testing using blood). And as mentioned above, we do not detect first-generation compounds as frequently in liver samples because they have much shorter half-lives than the second-generation compounds. We are among the first studies to use blood to test for anticoagulant exposure, and as far as I know, the only large-scale study to do this in wildlife. One of our significant findings using this method is that we learned we have been underestimating wildlife (or at least bobcat) exposure to first-generation anticoagulants by relying solely on liver samples to do the testing. In summary, we use liver samples to test for an association between mange and anticoagulants, and because we underestimate first-generation anticoagulant exposure when we test liver samples, a lack of association between mange and first-generation anticoagulants could potentially be driven by a bias in the shorter tissue half-life of first-generation compounds compared to second-generation compounds.

Overall, in terms of relationships between mange and anticoagulants, we did find evidence that multiple exposure events to anticoagulant may be the critical component in the development of severe mange. In bobcats with mange, we typically find higher residue concentrations and exposure to more different compounds compared with bobcats without mange, suggesting that multiple exposure events could be a critical factor. In fact, we find a strong association between bobcat exposure to 2 or more compounds, and mange- where bobcats that are exposure to 2 or more compounds are more than 7 times more likely to die of mange than other sources of mortality.

Another interesting note- we more frequently detected anticoagulant exposure in blood samples (and those detections were most frequently first-generation compounds) during the dry season (May- October). Bobcats are 2.6 times more likely to be exposed during the dry season, and we detect 55% more anticoagulant exposure during the dry season. Interestingly, we also 67% more mange cases during the dry season (which I speculate could be related to increased exposure to anticoagulants).

Finally, during some more recent literature research I've done, I discovered that diphacinone itself could potentially pose dangers aside from its effect as an anticoagulant. Similar to warfarin (or coumadin), it has been used therapeutically to prevent thrombosis in humans. But because a small, but significant, percentage of the human population who have used the drug develop a hypersensitive, immune-stimulated reaction, the drug is banned in the US. It is still used in Europe, however, but some papers have been published showing that human use of the drug can also result in suppression of certain types of immune-related cells and kidney failure. Whether these effects occur for animals that are exposed to diphacinone is unknown, but of potential concern.

I'm happy to answer any other questions you may have.
Best,
Laurel

-*- Check out my website and FB page! UrbanCarnivores.com and facebook.com/UrbanCarnivores -*-

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On Mon, Apr 21, 2014 at 6:08 PM, Riley, Seth <seth_riley@nps.gov> wrote:

> Hi Jan,
>
> No, first generation anticoagulant rodenticides can not "be used safely" from a wildlife perspective. They are still anticoagulant toxicants that are designed to, and do, kill wildlife. They are not as toxic or long-lasting as second generation ARs, but they are still poisons and still can affect wildlife. During our coyote study, we had animals that died directly from diphacinone poisoning, which is a first generation AR (and the one to which P22 has been exposed). I don't know where you got that idea about NPS thoughts on the matter, but I would certainly never claim that they could be used safely. It is true that they are definitely better than second generation ones.
>
> We don't really have much of an idea about whether P22 has been exposed to second generation ARs. There is no question that he has been exposed to first generation ones, specifically diphacinone and chlorophacinone. The more long-term and reliable test, particularly for second generation compounds, is to test the liver, which we can only do after death. I would be shocked if he was not exposed to second generation compounds, given where he lives, and that every mountain lion but one has been exposed to (generally multiple) compounds, and that we found exposure to ARs even in blood, which is rarer than in the liver.
>
> In terms of ARs and mange, we really don't know whether that interaction is specific to certain compounds or not, although I'm not sure why it would be. The second generation ones might be worse in terms of causing sublethal effects, just because they are more toxic in general. And the second generation ones are significantly associated with mange in bobcats, from my graduate student Laurel Serieys work at UCLA, in association with us. She has not found the same kind of association, statistically, with first generation compounds, but that is partly because of a lot less data on them, and I don't see why it couldn't happen with them as well (Laurel can also weigh in).
>
> Hope that's helpful, maybe that's too much information...
>
> Seth

On Thu, Apr 17, 2014 at 10:42 PM, Jan Dougall <jandougall@gmail.com> wrote:

> > Hi Seth,

> The 4/17 LA Times story said "P-22 was afflicted by two older "first generation" rat poisons." Were second generation rodenticides not detected? I was under the impression that pest control professionals and even NPS claim the first generation rodenticides could be used safely, even in this area.
>
> Can you clarify? I'd like to pass the information on to my HOA if 1st generations can result in mange like we saw on P-22. We've confirmed that 2nd generation rodenticides aren't used in our bait boxes and the HOA board believes what we have now is safe for wildlife.
>
> Thanks,
>
> Jan Dougall
> 27472 Country Glen Rd.
> Agoura Hills, CA 91301

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