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Spatial modelling of non-target exposure to anticoagulant rodenticides can inform mitigation options in two boreal predators inhabiting areas with intensive oil and gas development

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ABSTRACT

Intensive industrial development occurs in the ecologically significant boreal forest, including oil and gas development in northern Alberta, Canada. This forest is home to many highly-valued animal species including fisher (*Pekania pennanti*; formerly *Martes pennanti*) and American marten (*Martes americana*). Second-generation anticoagulant rodenticides (SGARs) are commonly used near human infrastructure in developed areas to control and reduce damage from rodent pests. High body burdens of SGARs in rodent prey pose risks of secondary poisoning for fisher and marten that readily consume rodents. The objective of this research was to determine if fisher and marten living in anthropogenically-disturbed areas of northern Alberta showed evidence of SGAR exposure. Fisher and marten carcasses were collected from the region, aged, sexed, and liver samples were analysed for rodenticides using liquid-chromatography mass spectrometry (LCMS). SGARs were found in the livers of non-target fisher and marten. As SGARs were found in the livers of fisher with sufficient frequency for complete statistical analysis, analyses including ANOVA, linear regression, and spatial cluster analyses were used to assess spatial patterns exhibited by fisher exposure frequencies against potential explanatory variables such as boreal anthropogenic disturbances and land cover classes. Additionally, companies operating in the region were surveyed to identify their current rodent control measures in an effort to verify the results of the spatial analyses. This is the first study to demonstrate non-target SGAR exposure of fisher and marten in Canada. Exposure frequency in fisher exhibited clustering, which showed the strongest relationships to factors including total boreal disturbances, number of oil sands mines, and broadleaf forest cover. The spatial methods used in this paper provide tools to develop local interventions for mitigation and conservation efforts.

1. Introduction

Alberta, Canada, is rich in natural resources, found primarily in the northern half of the province. This region contains 100% of the oil sands deposits in the province and approximately 30% of its conventional oil and natural gas production, 28% of its total farm area and 86% of its forests (Nichols Applied Management, 2012). This abundance of natural resources has been vital to the growth and development of the region, which has resulted in an economy focused on resource extraction and production. In 2011, 17% of Alberta's total $241 billion Gross Domestic Product (GDP) was produced in northern Alberta, 56% of which was attributed to the mining, oil and gas sectors (Nichols Applied Management, 2012).

A large portion of Alberta's boreal forest is also located in the northern part of the province. This ecosystem is home to many *Mustelidae* family members, including North American river otter (*Lontra canadensis*), mink (*Neovison vison*; formerly *Mustela vision*), American marten (*Martes americana*), and fisher (*Pekania pennanti*; formerly *Martes pennanti*). Fisher and marten are habitat-specialized carnivores that favor old-growth coniferous forest stands with complex vertical and horizontal structures, and a dense canopy cover that provides adequate resting, denning, and feeding sites (Carroll et al., 1999; Cheveau et al., 2013; Davis et al., 2007; Schwartz et al., 2013; Zielinski, 2014); they avoid open and fragmented landscapes. Their preferred food includes small to medium-sized mammals and birds, as well as carrion (Powell, 1993). Historically, both species exhibited wide distribution throughout North America (Graham and Graham, 1994). However, fisher populations have sharply declined due to

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overharvesting and habitat destruction from logging and development projects (Powell, 1993). Recently, conservation efforts aimed at restricting trapping seasons, imposing harvest quotas, and implementing recovery and re-introduction programs (Proulx and Dickson, 2014; Proulx and Genereux, 2009). As a result, fisher populations have increased but never reaching previously documented levels (Gibilisco, 1994). Marten and fisher are especially sensitive to anthropogenic disturbances that cause direct mortality, or indirect effects through the loss or degradation of suitable habitat (Aubry and Lewis, 2003; Cheveau et al., 2013; Stinson and Lewis, 1998). Therefore, these species should be sensitive indicators of ecosystem function in the boreal region.

A relatively large part of the Northern Alberta human population is non-permanent, involved in the exploratory drilling and construction phases of oil sands projects as well as similar activities for conventional oil and gas and forestry (Nichols Applied Management, 2012). With recent increases in natural resource extraction activities in this region, facilities capable of housing thousands of transitory workers have been needed, each producing typical waste capable of attracting rodent pests. Industrial and other developments, such as mining, oil and gas, forestry and agriculture, also require pest management strategies to rid infrastructure of rodents and prevent damage. Classic rodenticides (or first-generation compounds- FGARs) and more recently, second-generation anticoagulant rodenticides (SGARs) have been widely used worldwide. Most companies regularly deploy rodenticide baits in a prophylactic manner (Elliott et al., 2016). Rodenticide sales and use data are difficult to obtain but recent estimates suggest expenditures of hundreds of millions of dollars annually in the United States and European countries (Rattner et al., 2014).

Introduced to address resistance to FGARs, SGARs have been surrounded by controversy (Elliott et al., 2016). SGARs differ from their first-generation counterparts in that they are more acutely toxic and are more persistent in vertebrate livers (Erickson and Urban, 2004; Newton et al., 1999; Parmar et al., 1987; Stone et al., 1999). Greater acute toxicity increases the potential for primary poisoning amongst non-target species and longer tissue half-lives of SGARs enhances the potential for bioaccumulation in non-target predators, which may increase the risk of secondary poisoning. Furthermore, the latency of death after consuming a lethal dose of SGARs is approximately 5–7 days (Cox and Smith, 1992; Gabriel et al., 2012; Macdonald and Service, 2007) and, during this time, continued ingestions of SGARs by prey species can cause body burdens to exceed LD50 or even the LD100 dose for predators. Poisoned animals may remain available for capture by predators for an extended period after exposure (Gabriel et al., 2012). Additionally, poisoned rodents exhibit an altered state of behaviour. Spending more time in open areas in a lethargic state may further predispose them to predation or scavenging (Cox and Smith, 1992; Macdonald and Service, 2007).

Non-target exposures to SGARs in mustelids has been well-documented (Birks, 1998; Eason et al., 2002; Elmeros et al., 2011; Fournier-Chambrillon et al., 2004; Gabriel et al., 2012, 2015; McDonald et al., 1998; Quinn et al., 2012; Ruiz-Suárez et al., 2016; Shore et al., 2003, 2015; Thompson et al., 2013). For example, fisher are victims of secondary poisoning with subsequent population level impacts (Gabriel et al., 2012, 2015; Thompson et al., 2013). To investigate how targeted mitigation of this impact could be implemented, we conducted a landscape level analysis of relationships between fisher SGAR exposure and landscape-level variables. We examined spatially-explicit models that can scale individual effects up to population levels while accounting for spatial variation in exposure rates as a function of various landscape characteristics (Köhler and Triebskorn, 2013; Schmolke et al., 2010). We used the spatial models to help predict fisher exposure to environmental contaminants such as SGARs over the landscape. Our objective was to determine whether areas with higher natural resource industrial activity (i.e. areas with denser human populations) would increase exposure of fisher and marten populations to SGARs. We predicted an increased likelihood of exposure to rodenticides in non-target wildlife inhabiting areas of higher industrial development.

2. Materials and methods

2.1. Wildlife collections

We analysed the carcasses of fisher and marten that had been trapped under permit for the commercial fur trade. Carcasses were provided by Northern Alberta commercial trappers recruited through the Alberta Trappers Association (Westlock, Alberta). All animals were trapped following the Alberta Code for Responsible Trapping and the *Agreement on International Humane Trapping Standards* (AIHTS). Carcasses were stored at − 20 °C until wildlife post-mortem evaluations and necropsies were conducted at the wildlife diagnostic laboratory of Alberta Environment and Parks (Fish and Wildlife, Edmonton, Alberta). Gross necropsies were undertaken on each fisher and marten carcass. Livers were dissected and stored in chemically-cleaned amber glass jars at − 20 °C and were sent to the National Wildlife Research Center (NWRC, Environment and Climate Change Canada, Ottawa, Ontario). Liver samples were homogenized following NWRC operating protocol (#SOP-TP-PROC-07F). An aliquot of approximately 1.0 g was submitted for rodenticide analysis at NWRC by liquid-chromatography mass spectrometry (LCMS).

Fisher were aged with the assistance of Alberta Environment and Parks (Bonnyville, Alberta). The ratio of pulp cavity width (mm) to tooth width (mm; percent pulp) from canine teeth was determined by radiography and each individual was assigned to juvenile or adult age class (Poole et al., 1994).

2.2. Chemical analysis

2.2.1. Liver extraction

Chemical analysis followed NWRC method #MET-ROD-02A (see Albert et al., 2010). In brief, a liver homogenate subsample was spiked with an internal standard, extracted with acetonitrile, centrifuged and the supernatant was evaporated to dryness, reconstituted in methanol, filtered and injected onto LCMS (Agilent 1200 HPLC system, Agilent Technologies, CA, USA).

2.2.2. LCMS analysis of target rodenticide compounds

FGARs including pindone, warfarin, diphacinone and chlorphacinone and SGARs such as brodifacoum, bromadiolone, and difethialone were detected by mass spectrometry using an AB Sciex API 5000 Triple Quadropole Mass Spectrometer with the TurboSpray ion source in negative polarity using MRM (multiple reaction monitoring) scan type. Quality assurance and control methods included the use of quantification rodenticide standards (obtained from Sigma Aldrich, ChemService, and CDN) to generate calibration curves at 6 concentrations ranging from 0.25 to 10 ng/mL. Data were corrected for % recovery of each internal standard. Three methanol blanks were injected at the beginning and end of each set of samples and before and after the calibration standards to monitor cross-contamination. Additionally, sample blanks were analysed with each set of 9 samples, and duplicate/triplicate extractions were made of one random sample per set of 9 livers.

Since there is no certified reference material containing rodenticides, a positive quality control liver pool (from various raptor species) was prepared at NWRC. This in-house reference material contains brodifacoum and bromadiolone, and a sample was included with every set of extractions, and analysed along with the samples to monitor day to day variability.

2.3. Company surveys

Because of the difficulty in obtaining rodenticide sales or use data, northern Alberta industries were surveyed to determine their rodenticide

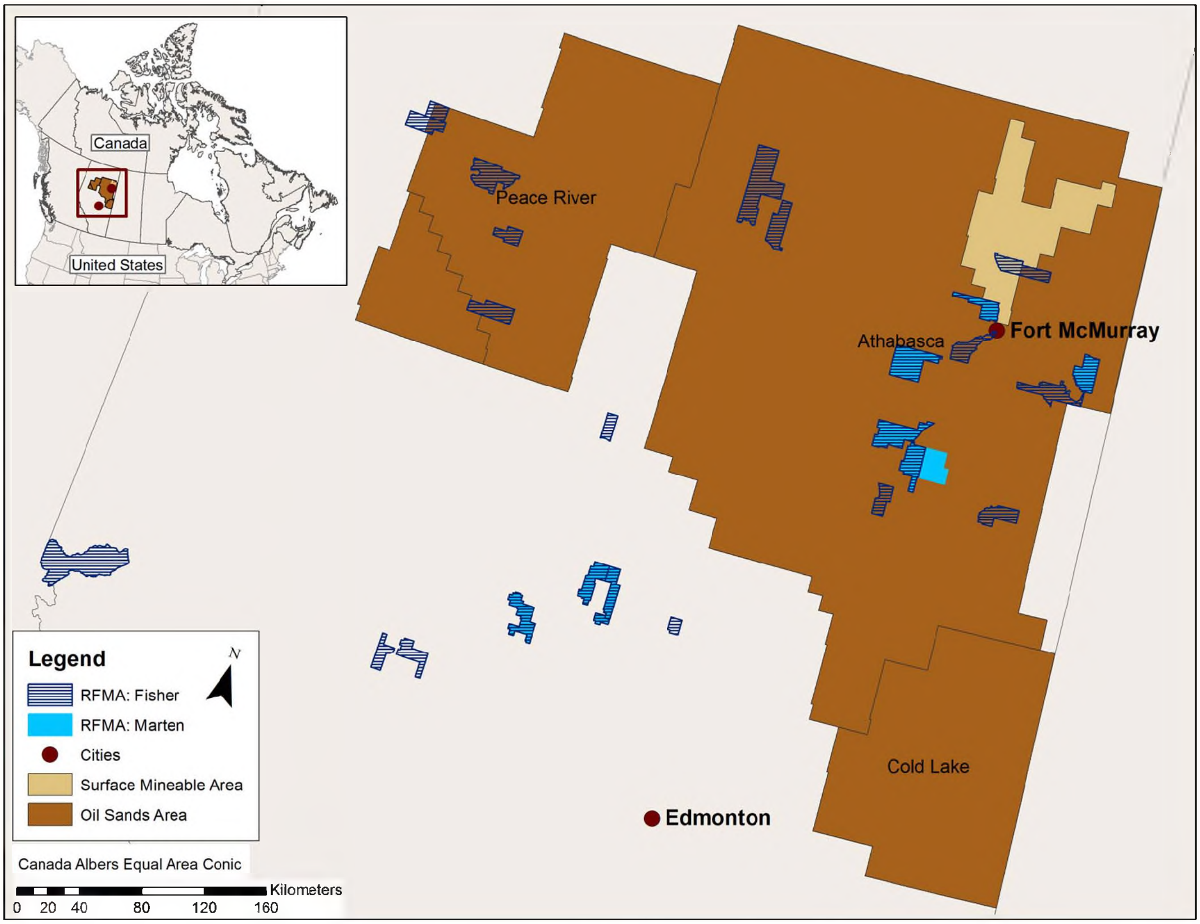

Fig. 1. Sampled trap lines (or RFMA: Registered Fur Management Area) in relation to oil sands deposits (Peace River, Athabasca, and Cold Lake) in northern Alberta.

use practices through Canada's Oil Sands Innovation Alliance (COSIA; Calgary, Alberta). A semi-structured questionnaire was distributed to oil sands and forestry companies. Many oil companies also operate regional conventional oil and gas facilities. Information on rodent control methods, active ingredients and amount used, commercial formulations employed and areas treated was requested.

2.4. Data analysis

2.4.1. Statistical analysis

Total rodenticide residue levels were compared between sites, age class, and sex in marten and fisher using a non-parametric Kruskall-Wallis H test due to violation of parametric model assumptions. Further, the independence of age, sex and treatment groups were assessed using contingency tables and Fisher's Test. Treatment group differences were tested using Kruskall-Wallis H test. Groups were based on the location of the trap line producing the animal. The control group was located outside of oil sands areas and "treatment" group include three oil and gas development areas in northern Alberta (Mineable Oil Sands [MOS], Athabasca in situ Area [ATH], and the Peace River deposit [PRD]).

2.4.2. Fisher spatial modelling

Environmental data for the spatial model was obtained from Alberta Biodiversity Monitoring Institute's land cover data, the boreal ecosystem anthropogenic disturbance layers (Pasher et al., 2013), and the registered fur management area (RFMA) spatial layer (Alberta Environment and Parks, 2015). Data was extracted in ArcDesktop 10.3.1 and 10.4 and analysed in R 3.0.1.

A total of 34 independent variables pertaining to land cover and anthropogenic disturbance variables were mapped and quantified (see Table A1). These included percent of the RFMA (trap line) covered by various land covers, the number of disturbances, and the area covered by various disturbances inside the RFMA. Additionally, land cover inside a 1 km buffer (typical fisher home range) was also quantified and modelled in order to determine if optimal fisher habitat near disturbances increased fisher exposure to rodenticides. Disturbances included agriculture (land cleared for crops, and related infrastructure such as barns and storage silos), mines (oil sands upgrader facilities, worker camps, mining infrastructure), oil and gas disturbances (compressor stations, staging areas, pump stations), settlements (cities, towns, and indigenous reserves), and well sites (often equipped with pump jacks, well heads, and other infrastructure).

The dependent variable used for the spatial modelling was the frequency of exposure to SGARs in fisher (i.e. - the number of animals with detectable SGAR residues divided by the sampled population of the trap line). The proportion was used to avoid pseudoreplication as fisher were geolocated to a RFMA and were not independent experimental units. The frequency of exposure to SGARs in fisher was tested for global and local clustering using the global Moran's I and local Getis and Ord's G* statistics. The tests were specified using inversely weighted spatial relationships. A general linear model was used to determine which landscape variables (independent variable) could explain the greatest amount of variance in the fisher's exposure to SGARs (dependent variable).

Variables for the regression model were selected using a Pearson's moment correlation to avoid multicollinearity. When two independent variables had a correlation coefficient > 0.7 or less than -0.7, one variable was removed. The selected variables were regressed against the proportion of fisher exposed to SGARs. Using an ordinary least squares linear regression, non-normal raw data were log10 transformed to meet model assumptions. Due to the low sample size, a forward variable selection method was used, regressing the independent variable with the highest correlation with the dependent variable first. The remaining variables were added one at a time until an added variable became insignificant. In variable selection, the traditional 95% confidence interval ($p \leq 0.05$) was relaxed to a 90% confidence interval ($p \leq 0.10$) due to the nature of environmental data in a complex heterogeneous landscape (Suter, 1996). The model was forced through zero (no intercept) as rodenticides are not naturally occurring in the environment. The Akaike Information Criterion (AICc) was used to further validate the selected model (by comparing its value to the null model value). The residuals of the final model were tested for homoscedasticity (Breush-Pagan test), normality (Shapiro-Wilk test), linearity (Reset test), and lack of serial autocorrelation (Durbin-Watson test). The selected model was compared with the null model using an ANOVA to confirm model selection.

3. Results

3.1. Rodenticide residues in fisher

Out of 93 *Mustelidae* liver samples analysed for rodenticide residues, the greatest proportion (63/93 or 68%) were fisher. The fisher were collected from 27 different RFMAs (Fig. 1). Fewer animals were available for collection in the mineable oil sands treatment group (MOS – Table 1) as the area has fewer active RFMAs due to more intensive industrial/anthropogenic disturbance (i.e. open pit mines).

Four fisher could not be aged, of the remainder, 51% were juveniles (32/63) and 43% (27/63) were adults. The sex ratio was 46% (29/63) female, and 54% (34/63) male. Of all fisher sampled, 24% (15/63) tested positive for SGARs. No first generation compounds were detected.

Fisher and marten with non-detectable SGAR residue levels were classed as zero in the dataset and included in all mean computations. Bromadiolone was the most frequent and abundant SGAR detected in livers, present in 87% of positive cases. Brodifacoum was detected twice while a single fisher liver had both bromadiolone and difethialone. The combined levels of SGARs in this animal were the highest (0.44 μg/g w.w.). Compound specific trends in SGAR residue levels in each treatment group are presented in Table 1 for only those animals that tested positive for SGARs, and for the complete dataset (including zeros). There were no statistical differences in SGAR residue concentrations between juvenile and adult fisher ($p = 0.84$) or between males and females ($p = 0.07$). Further there was no difference in the proportions of samples in each group (Fig. A1) for age by treatment ($p = 0.83$) and sex by treatment ($p = 0.23$). However, SGAR residue levels were significantly different between treatment groups (see Table 1 and Fig. A2; $p = 0.008$). Fisher from the MOS had significantly higher hepatic total SGAR burdens, followed by fisher collected in the Peace River deposit,

Table 1
Summary statistics of rodenticide residue levels in fisher collected on 27 different trap lines, binned in four treatment groups (Control = 10 trap lines; mineable oil sands [MOS] = 2 trap lines; Peace River deposit [PRD] = 5 trap lines; and Athabasca In Situ area [ATH] = 10 trap lines). Sample sizes (n) of exposed animals (Exp.) and the complete dataset (including animals with no detectable rodenticides; [All]) is presented for bromadiolone (BD), difethialone (DF), brodifacoum (BF), and sum of all SGARs detected. Mean, median, standard deviation (Std), Min, Max and the interquartile ranges (IQR) for 25%, 50% and 75% are presented. Note the zero-inflated data in the complete dataset.

Treatment		Rodenticide residues							
		BD exp.	BD all	DF exp.	DF all	BF exp.	BF all	Sum exp.	Sum all
Control	n	3	20	0	20	1	20	4	20
(10)	Mean	0.016	0.002	–	–	0.002	0.000	0.013	0.002
	Median	0.008	0.000	–	–	0.002	0.000	0.005	0.000
	Std	0.019	0.008	–	–		0.000	0.017	0.008
	Min	0.003	0.000	–	–	0.002	0.000	0.002	0.000
	25%	0.005	0.000	–	–	0.002	0.000	0.003	0.000
	50%	0.008	0.000	–	–	0.002	0.000	0.005	0.000
	75%	0.023	0.000	–	–	0.002	0.000	0.015	0.000
	Max	0.037	0.037	–	–	0.002	0.002	0.037	0.037
MOS	n	3	5	1	5	0	5	3	5
(2)	Mean	0.164	0.098	0.265	0.053	–	–	0.252	0.151
	Median	0.173	0.129	0.265	0.000	–	–	0.188	0.129
	Std	0.030	0.092		0.119	–	–	0.164	0.180
	Min	0.129	0.000	0.265	0.000	–	–	0.129	0.000
	25%	0.151	0.000	0.265	0.000	–	–	0.159	0.000
	50%	0.173	0.129	0.265	0.000	–	–	0.188	0.129
	75%	0.181	0.173	0.265	0.000	–	–	0.313	0.188
	Max	0.188	0.188	0.265	0.265	–	–	0.439	0.439
PRD	n	4	8	0	8	0	8	4	8
(5)	Mean	0.046	0.023	–	–	–	–	0.046	0.023
	Median	0.043	0.001	–	–	–	–	0.043	0.001
	Std	0.043	0.038	–	–	–	–	0.043	0.038
	Min	0.003	0.000	–	–	–	–	0.003	0.000
	25%	0.015	0.000	–	–	–	–	0.015	0.000
	50%	0.043	0.001	–	–	–	–	0.043	0.001
	75%	0.074	0.031	–	–	–	–	0.074	0.031
	Max	0.097	0.097	–	–	–	–	0.097	0.097
ATH	n	3	30	0	30	1	30	4	30
(10)	Mean	0.005	0.000	–	–	0.009	0.000	0.006	0.001
	Median	0.005	0.000	–	–	0.009	0.000	0.006	0.000
	Std	0.003	0.002	–	–		0.002	0.003	0.002
	Min	0.002	0.000	–	–	0.009	0.000	0.002	0.000
	25%	0.004	0.000	–	–	0.009	0.000	0.004	0.000
	50%	0.005	0.000	–	–	0.009	0.000	0.006	0.000
	75%	0.006	0.000	–	–	0.009	0.000	0.008	0.000
	Max	0.008	0.008	–	–	0.009	0.009	0.009	0.009

control locations, and Athabasca in situ areas.

3.2. Rodenticide residues in American marten

Only 30 marten were analysed for rodenticide residues which were collected from 10 different RFMAs (Fig. 1); no marten were collected from RFMAs in the Peace River deposit. Given the lower sample size, these samples were not aged. The collected individuals included equal numbers (n = 15) of males and females.

Ten percent of marten (3/30) tested positive for SGARs; no FGARs were detected. These data also violated all statistical assumptions and the extremely low sample size prevented any meaningful analysis. The purpose of these collections was to evaluate if rodenticide exposure could be detected in a similar species, occupying the same ecological niche. The three positive animals were obtained from a collection of 11 marten sampled in the MOS (Fig. 1). Total SGAR residue levels in all marten averaged 0.018 ± 0.031 μg/g w.w., much lower than in fisher. Bromadiolone and difethialone were the detected compounds. One marten tested positive for both compounds, one had detectable levels of difethialone only, and the other bromadiolone only.

3.3. Spatial analysis

RFMAs at control locations and in the Peace River deposit were most impacted by agricultural disturbances (Fig. 2). Athabasca in situ RFMAs (in the Athabasca and Cold Lake Deposits) were most impacted by well sites as this resource extraction process relies on underground extractions coupled to a network of exploratory/drill well sites. MOS RFMAs were most affected by mining disturbances and associated infrastructure (Fig. 2). This area also had more settlements than other study areas.

Using the land cover data, on average, RFMAs outside oil sands deposits (n = 10; control treatment group) in northern Alberta were only 0.005% disturbed by any anthropogenic disturbance. Forestry cut blocks and linear features such as roads, pipelines, seismic lines, etc., were not considered for this analysis as fisher avoid open areas (Cheveau et al., 2013; Powell, 1994; Schwartz et al., 2013). Additionally, rodenticides are likely not being deployed in such areas with limited human activity or infrastructure.

RFMAs located in the Athabasca in situ area (n = 10; ATH treatment group) were only 0.002% disturbed. Two mineable oil sands area RFMAs (MOS treatment group) were on average 0.02% disturbed. Conversely, RFMAs from the Peace River deposit (n = 5; Peace River treatment group) were most disturbed at 0.07%. There were no statistically significant differences in percentage of disturbed trap line surface area by any anthropogenic disturbances (Kruskall-Wallis test, p = 0.17).

3.4. Spatial models

Global cluster analysis assesses the degree of spatial autocorrelation (spatial self-similarity) over the whole study area. This method

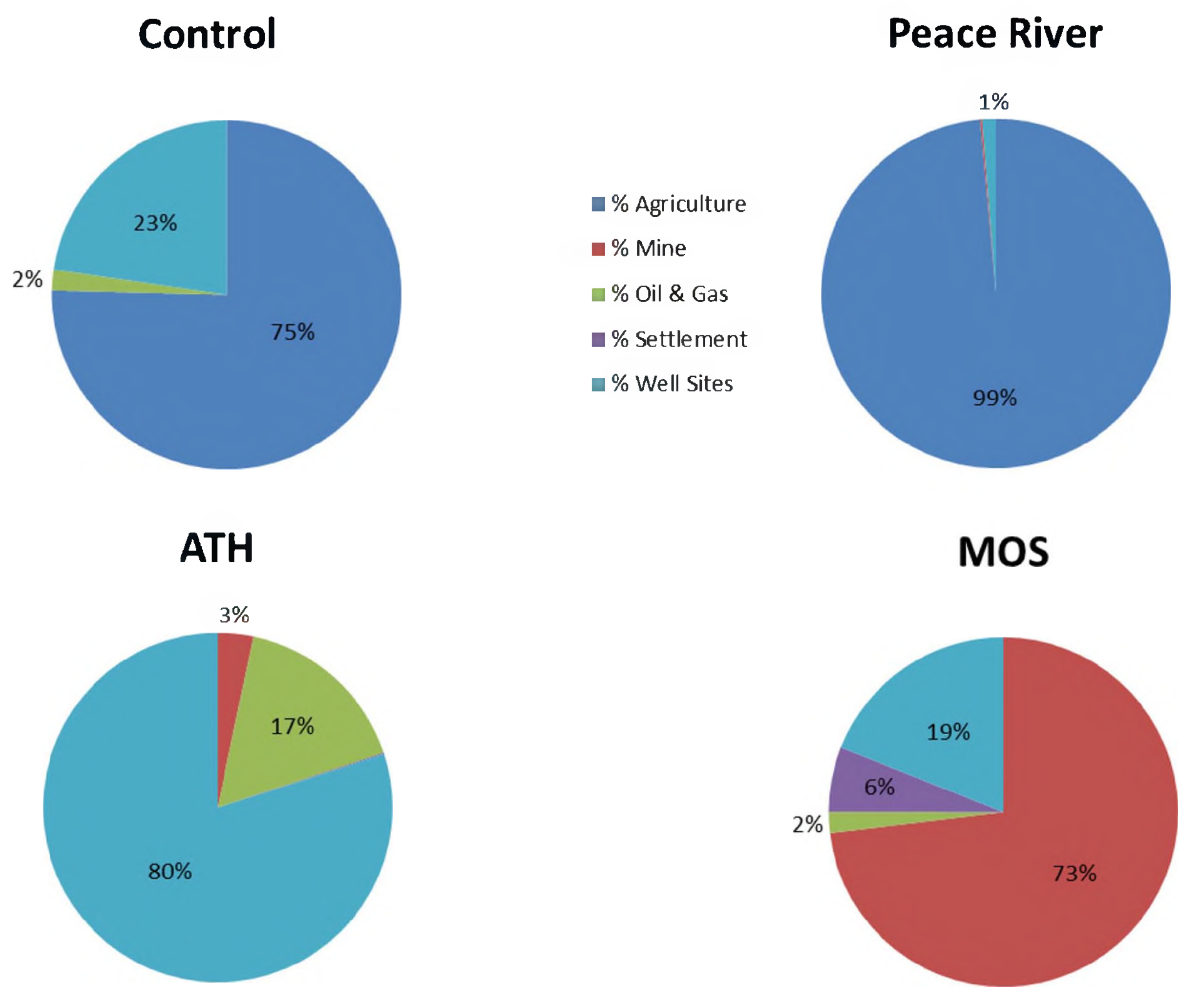

Fig. 2. Average percentage disturbance type on trap lines in northern Alberta, including areas outside oil sands deposits (Control), Peace River deposit, Athabasca and Cold Lake deposits (Athabasca in situ area; ATH) and mineable oil sands (MOS). The overall % of total disturbance in any treatment area was very small (< 0.1%).

Table 2
Final linear regression model, where the dependent variable is the frequency of fishers exposed to SGARs. **D_tot_perc** = Percentage of land covered by all disturbance classes (agriculture, mine, oil and gas, well, and settlement), **D_Mine_n** = Number of mine disturbances within the RFMA, **LC_BrdFor_perc** = % RFMA covered by broadleaf forest. Please refer to Table A1 for description of additional variables.

	Estimate	Std. error	T value	Pr(> |t|)	Partial R^2
D_tot_perc	0.222	0.082	2.7	0.013	0.248
D_Mine_n	0.125	0.067	1.88	0.073	0.138
LC_BrdFor_perc	0.034	0.018	1.96	0.063	0.148
Residual standard error	0.209	R^2	0.66	Model AICc	− 0.51
Degrees of freedom	22	Adj R^2	0.61	Null AICc	6.7
$F_{(3,22)}$	13.92	p-Value	2.63e-05		

produces one spatial autocorrelation metric for the entire study area as it assumes the spatial autocorrelation is homogenous over the surface. Local cluster analysis assesses local patterns of spatial associations based on a defined neighbourhood distance (*d*). Local methods allow for the identification of anisotropic spatial autocorrelation over a study area which will identify localized clusters of high values (hot spots) and low values (cold spots) (Getis and Ord, 1992; Anselin, 1995).

While the sample size was small (n = 27 RFMAs), exploratory results of both the global and local cluster analysis indicated that there was slight clustering with respect to the frequency of fisher exposure to rodenticides. A summary of these results can be seen in Fig. A3. The statistical significance of these results approached the 95% confidence interval (global p = 0.08, local p = 0.056), thus there was less than a 10% chance that these results could be due to random chance.

The results of the linear model demonstrated the percentage of the trap line area covered by all disturbance types (i.e. agricultural, mine, oil and gas, well, and settlement) to be the strongest predictor of the frequency of SGAR poisoning in fisher. Significant in the model were the number of oil sands mines, and the percentage of the trap line covered by broadleaf forest (Table 2). This global linear model was statistically significant (p = $2.63e^{-05}$), had a statistically smaller residual sum of squares value than the null model (ANOVA, p = 0.0006), and lower AICc than the null model (null AICc = 6.73, selected model AICc = − 0.51). Overall, the selected model could explain 61% of the variance (R^2 = 0.61). Due to the small sample size, the results were confirmed using bootstrapping with 1000 iterations. There was no difference in variable significance and influence between the bootstrapped and non-bootstrapped models.

The residuals of the model were homoscedastic (BP = 1.228, p-value = 0.5412) and did not demonstrate any issues with spatial (serial) autocorrelation (DW = 2.0171, p = 0.5597). Thus, there was no need for geographically weighted regression models. While the dependent variable (exposure frequency) was spatially autocorrelated at both the global and local level, it is likely that correct model specification via variable inclusion was able to account for this in the model. Similarly, other research has found that the inclusion of the correct independent variables can account for the spatial processes observed in the dependent variable, alleviating the need for a spatial regression and providing a more parsimonious model (Bertazzon et al., 2015). This is further supported by Fig. 3 demonstrating that the exposure frequency to SGARs in fisher is higher in closer proximity to denser boreal forest disturbance features (D_tot_perc in the model). Conversely, the lower exposure frequencies are located in proximity to lesser disturbed areas. This gives further support to the variable specification in the general linear regression model.

3.5. Company surveys

A total of 25 companies voluntarily responded to the questionnaire. Information on rodenticide amounts used (including product formulation) and exact locations deployed were not provided. However, the answers submitted support our results. Fewer than half of companies surveyed reported using more than one targeted rodent control measure (Table A2). The majority of companies also used rodent control products with bromadiolone as the active ingredient. Half of companies who answered this question reported using rodenticides outdoors. Other modes of mechanical rodent control such as snap traps and live traps were also reported but not as commonly as chemical strategies. One company reported the use of house cats at a facility to control rodent populations.

4. Discussion

4.1. Underestimation of exposure

Our study is the first in Canada to report non-target exposure of fisher and marten to anticoagulant rodenticides. As mentioned previously, mustelids have been identified as non-target exposure victims in various other studies. Exposure to highly toxic and persistent SGARs results in the inhibition of key enzymes in the vitamin K cycle such as vitamin K_{1-2-3} epoxide reductase. This inhibition leads to clotting and coagulation impairment, and eventual death through the onset of spontaneous or accidental haemorrhaging after trauma (Watt et al., 2005). It is probable that the actual frequency of exposure is greater as individuals that would have died from exposure or that had reduced fitness would not have been sampled (by trapping). This is further supported by Gabriel et al. (2015) who demonstrated an underestimation of the frequency of exposure in radio-collared fisher that died out of sight.

Furthermore, risk to fisher populations could be greater through exposure to sub-lethal levels of SGARs. Compromised clotting functions could turn a minor wound into a lethal injury (Erickson and Urban, 2004). As fisher actively pursue both terrestrial and arboreal prey (Powell, 1993), these high risk pursuits could result in lethal injuries to a predator that would have otherwise survived in the absence of rodenticides.

4.2. SGAR levels in fisher and marten

In our study, bromadiolone levels ranged from trace levels to 0.19 μg/g w.w. Some fisher presented with two different hepatic SGAR compounds (most often brodifacoum or difethialone + bromadiolone) with total rodenticide burden levels approaching 0.44 μg/g w.w. Even if marten seem to have lower frequency of exposure, and lower SGAR residue levels, the low sample size prevents confidence in any interspecies differences in exposure conclusions. However, we did confirm exposure to rodenticides in a new furbearing species. Thus, in agreement with Gabriel et al. (2012), there could be significant risks to marten as well as fisher.

However, unlike Gabriel et al. (2012) that found almost 80% of all California fisher sampled tested positive for SGARs, our study confirmed exposure in roughly 25% of sampled individuals. Our results are likely a reflection of the relative levels of environmental stress exerted on fisher populations in northern Alberta. The province of Alberta has a population density of 5.7 individuals/km^2 while the state of California has a population density of 92.6 individuals/km^2. Even with fisher living near large-scale oil and gas infrastructure projects in northern Alberta (such as in the mineable oil sands area), the lower human population density likely does not require as intensive of a rodent control program. Additionally, differences in climate and rodent population densities in both jurisdictions likely influence the choice of rodent control strategies and the resulting non-target exposures.

4.3. Possible routes of exposure

Both marten and fisher were detected with compounds that are restricted to indoor use in Canada (i.e. difethialone and brodifacoum;

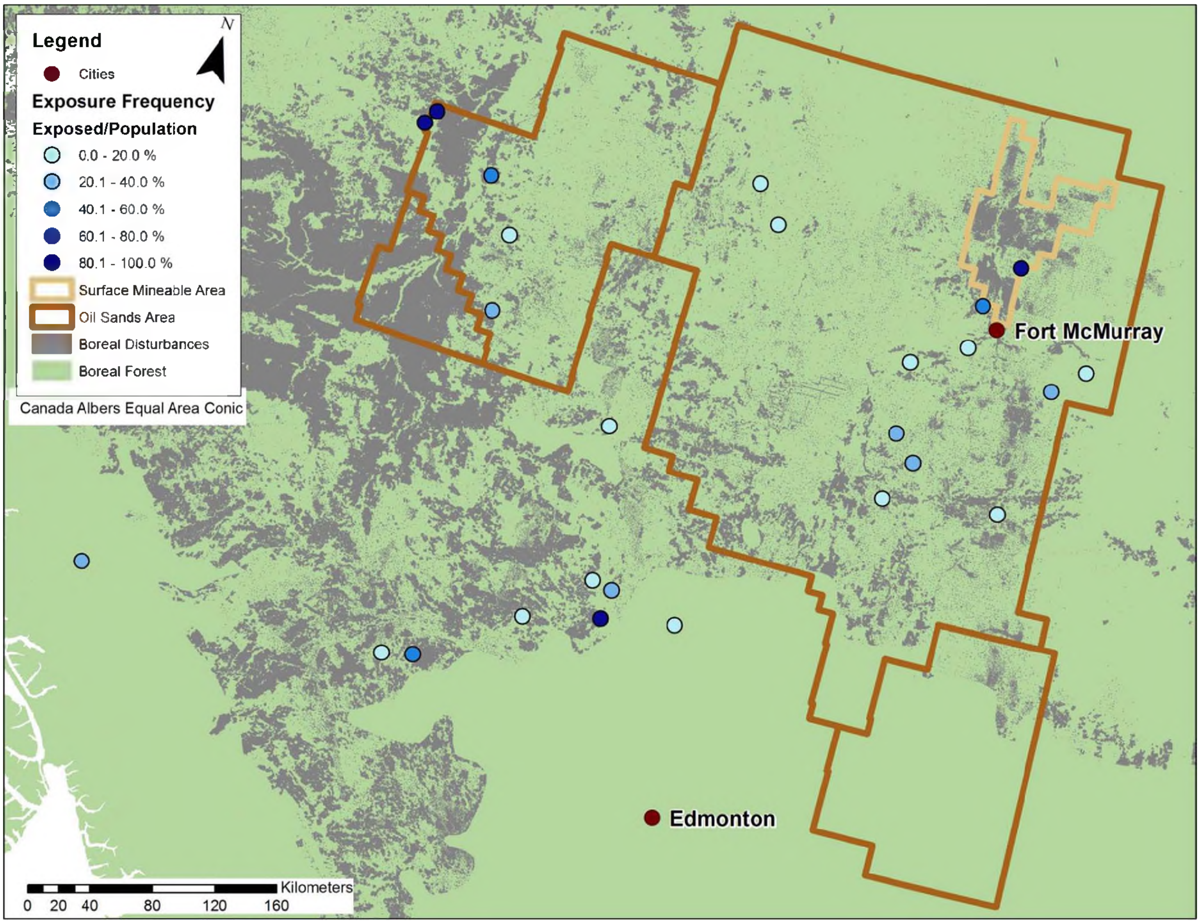

Fig. 3. Map showing exposure frequency of fishers on trap lines (centroid represented by blue circles) in relation to oil sands deposits and Northern Alberta Boreal forest disturbances. Human encampments were not available as a standalone geospatial product, but included in the Boreal disturbance spatial layer.

Health Canada, 2013). Although detected at low levels, our results demonstrate that use restrictions alone are not enough to completely eliminate non-target exposures to SGARs in mustelids (as previously suggested by Shore et al. (2003)). Assuming these compounds are being used legally by trained commercial applicators, poisoned rodents are likely moving in and out of doors, increasing the probability of being consumed.

In fact, rodents, a main prey item to fisher and marten (Lofroth et al., 2010; Powell, 1993), are known to survive for up to two weeks after poisoning before dying (Macdonald and Service, 2007). Therefore, the exposure of fisher and marten through a secondary exposure pathway (i.e. through the consumption of poisoned prey items such as rodents) is more likely than exposure through direct routes as was suggested by Gabriel et al. (2012). Fisher and marten usually tend to avoid open areas (Cheveau et al., 2013; Powell, 1994; Schwartz et al., 2013), and are generally secretive and elusive creatures (Powell, 1993). They are unlikely to visit busy industrial areas where these baits are typically deployed. Additionally, if used outdoors, the bait is required to be stored in tamper proof bait stations to limit non-target exposure (as per label directions).

4.4. Statistical and spatial modelling of SGAR exposure

The novelty of our study lies in the use and evaluation of spatially explicit methods to examine relationships between our measures of fisher exposure to SGARs and land use and cover. The variable that had the most influence on the model was the total anthropogenic disturbance footprint (expressed as the percentage of land covered by all disturbance types at a RFMA). This supports a conclusion of widespread use of SGARs for rodent control.

The MOS region had consistently higher exposure frequencies to SGARs with correspondingly higher hepatic residue levels in fisher. This area shows the highest level of disturbance (Fig. 2) with a higher frequency of open pit mines, bitumen upgrading facilities, and all associated support infrastructure (including worker camps). This could further explain the statistical significance of the mining disturbance in our model. Hepatic SGAR burdens in fisher collected the Peace River deposit area were second highest (Table 1). RFMAs in the Peace River deposit are located in agricultural areas. The differences in exposure frequencies and residue levels between both areas are a reflection of the human population in both regions, and higher densities of infrastructure in oil and gas areas relative to agricultural areas, where most of the disturbance is crop land typically not treated with rodenticides. The percentage of the trap line area covered by broadleaf forest was also significant. Fisher and marten are usually both associated with mature coniferous forest stands that provide adequate denning, resting, breeding and feeding sites (Carroll et al., 1999; Cheveau et al., 2013; Davis et al., 2007; Schwartz et al., 2013; Zielinski, 2014).

Understanding the habitat relationships of fisher and marten prey may be an important element of understanding their exposure to SGARs. White-footed mice (*Peromyscus leucopus*), deer mice (*P. maniculatus*), red-backed voles (*Clethrionomys gapperi*) and meadow voles (*Microtus pennsylvanicus*) are the most common mice species consumed by fisher, and are generally the most common rodents found in fisher habitat (Zielinski and Kucera, 1998). The most troublesome and economically detrimental of the species found in Alberta are the house mouse (*Mus musculus*), the white-footed mouse, and the meadow vole (Government of Alberta, Agriculture and Forestry, 2002). Given this overlap, future work should focus on quantifying SGAR exposure in rodent prey and fisher in broadleaf and coniferous forest stands near anthropogenic disturbances. Prey habitat use as well as susceptibility to exposure to SGARs is important and should be investigated as demonstrated by a Richardson's ground squirrel (*Urocitellus richardsonii*) poisoning campaign in the Canadian prairies that resulted in impacts to predator populations (Proulx and Mackenzie, 2012; Proulx, 2010).

4.5. Conservation implications

Conservation of important keystone species such as marten and fisher is important for ecosystem integrity and resilience as top predators in sensitive and complex ecosystems promote species richness (Sergio et al., 2008; Zielinski, 2014). Further, they are linked to multiple ecosystem components at the spatial, temporal, and biological levels, and are not only good indicator species, but could also help managers meet biodiversity targets in predator-centered conservation strategies.

Sound and cost-effective mitigation actions are required to address anthropogenic stressors that affect mustelids but are easily mitigated. A sensitivity analysis in Sweitzer et al. (2015) revealed that subtle changes in fisher survival were a stronger determinant of population growth than even fecundity. This demonstrates regulatory importance of reducing the number of fisher deaths caused by exposure to SGARs. Improving survival by 10% could change the growth trajectory for the local and regional fisher population from negative to positive (Sweitzer et al., 2015). Thus, an easily implementable mitigation action is the discontinuation of the use of SGARs. Based on this research, mitigation efforts should be focused on areas with higher population densities near prime fisher and marten habitat, specifically those around resource extraction activities in northern Alberta.

Surveys of companies operating in the region by Canada's Oil Sands Innovation Alliance (COSIA) revealed that fewer than half of responding companies employ more than one targeted rodent control strategy, with bromadiolone being the chemical of choice. We recommend that mitigation efforts should include an integrated pest management (IPM) approach to decrease non-target exposures. In an IPM program for rodent control, a variety of management tools may be used in an integrated approach to manage pest populations (Brenner et al., 1998). Interventions are ideally of the least toxic nature (including the use of less toxic compounds such as diphacinone), incorporating whenever possible the use of biological, genetic, cultural, or mechanical control measures, including effective handling and removal of waste and exclusion measures to avoid infestations. Monitoring fisher habitat and prey populations are essential components of an IPM program. Spatial modelling tools such as the ones used in the present study could help industry create localized interventions leading to more impactful and cost-effective interventions.

5. Conclusions

Spatial modelling of rodenticide exposures allowed us to relate non-target mammal exposure to rodenticides to land use and land cover variables, proxies for anthropogenic disturbance measures. The study demonstrated the utility of geographic information systems for providing information on the spatial component of environmental contamination and wildlife exposure, and to help predict the risk of exposure to other species. Spatial analytical tools can be used in conjunction with traditional statistical methods to gain a better understanding of population level patterns of exposure and how these patterns may vary with influencing factors, such as total disturbed land. Through the identification of factors influencing the observed patterns, localized interventions or mitigation actions can be recommended. Through collaboration in this study, COSIA companies have already followed the very easily implementable action of restricting the use of bromadiolone. Continued monitoring and modelling of SGAR exposure frequencies in non-target mammals could help demonstrate the environmental benefits from implementing the recommended mitigation actions in other areas of intensive resource extraction. Further, these methods could extend to other applications in many areas of conservation biology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2017.06.005.

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