



# 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 vison*), 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 Triebkorn, 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  $-20^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$  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 Quadrupole 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

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