Ecology of Intraguild Predation on Fishers (*Martes pennanti)* in California

By

GRETA MAJOR WENGERT
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M.S. (Humboldt State University) 2001

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Approved:

_____
Benjamin N. Sacks, Chair

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Andrew Sih

_____
Robert J. Hijmans

Committee in Charge

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ABSTRACT

Intraguild predation, a common cause of mortality in populations of small to mid-sized carnivores, can have significant consequences at the population level. Understanding the roles of cause-specific mortality in the ecology of carnivores is essential for addressing the limiting factors that regulate their populations, especially for species of conservation concern. Populations of fisher (*Martes pennanti*), a mid-sized carnivore inhabiting North American coniferous and mixed coniferous forests, have been in decline in the Pacific states since the early 1900s and have not recovered. The role of intraguild predation as a limiting factor in fisher populations is poorly understood and the focus of this research.

In Chapter 1, I reviewed the variety of methods for the collection, storage, and analysis of biological samples and information for the study of cause-specific mortality and disease prevalence in carnivores while highlighting the importance of representative sampling infer the role of specific causes of mortality in population regulation. In Chapter 2, I describe the development of field-collection and molecular protocols for identifying species of fisher predators from DNA in saliva left on fisher carcasses. I tested the protocol on fisher carcasses suspected of having been killed by four different predators and successfully amplified and sequenced DNA from bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), coyote (*Canis latrans*), and domestic dog. I also confirmed that these protocols can identify other felid and canid predators of several other small North American carnivores.

In Chapter 3, I used these methods to study the prevalence and patterns of intraguild predation on fishers in two regions of California. Of 101 fisher carcasses recovered through telemetry studies, 62 (61%) deaths were attributed to predation. Combining our molecular

methods with full necropsies of fisher carcasses, I determined the species of predators responsible for killing fishers and found that bobcats, mountain lions, and coyotes were the primary predators of fishers in California. Bobcats killed only female fishers while mountain lions more frequently killed male than female fishers. I then used classification tree methods in association with our molecular results to identify the most discriminating physical characteristics of predated fisher carcasses for identifying predators and discovered that fisher sex and whether the skull suffered depressed fracture or nearly complete consumption classified predator species most accurately.

Finally, In Chapter 4, I used data from GPS-collared bobcats, the most frequent fisher predator, and VHF-collared fishers to investigate habitat use of bobcats in northwestern California and relate bobcat habitat selection and habitat composition in areas of fisher-bobcat overlap to risk of predation on fishers. I found that bobcats selected open (prairie and barren habitats) and brush habitats and selected against mature, older forest and young, closed-canopy forest types, and that areas of fisher-bobcat overlap were more likely to have greater proportions of open habitat than where fishers do not overlap with bobcats. Results supported the hypothesis that areas surrounding sites where fishers were killed by bobcats were characterized by higher proportions of open and brush habitats than areas surrounding those fishers' locations when they were alive. In summary, the results of these studies confirm that fishers are killed by different species of predators; that they are killed frequently by predators, and that bobcat presence and predation risk to fishers is heightened in open and brushy habitats. This information will help identify the most significant threats to fisher recovery and aid in developing effective conservation plans to address predation as a threat to the long-term persistence of fishers.

INTRODUCTION

Western populations of the fisher (*Martes pennanti*), a mid-sized carnivore in the Mustelidae family that inhabits mature coniferous and mixed coniferous forests, have been in decline since the early 1900s (Zielinski et al. 2005). In 2004, the west coast distinct population segment (DPS) of the fisher was listed as a candidate for the federal Endangered Species Act of 1973 (U.S. Fish and Wildlife Service 2004). The determination cited several threats to fishers that may have contributed to their decline, including habitat loss, commercial trapping, disease, and predation, and overuse for scientific or recreational reasons. Commercial trapping for fishers has been banned throughout the range of the DPS since 1946, and research into the habitat requirements and diseases of fishers has been ongoing for the past several decades. However, predation has not yet been the focus of any research efforts, leaving the severity of this threat unknown.

Intraguild predation among carnivores is common (Palomares and Caro 1999) and can limit and even threaten rare, declining, and endangered species or populations. In southern California, coyotes (*Canis latrans*) were the main cause of mortality for endangered San Joaquin kit foxes (*Vulpes macrotis mutica*, Cypher and Spencer 1998), and in certain areas of their range, African wild dogs (*Lycaon pictus*) face severe decline partly due to interference competition with lions (*Panthera leo*) and hyenas (*Crocuta crocuta*, Creel and Creel 1996). In some instances, invasions of novel predators pose an acute risk to small, isolated carnivore populations as in the case of golden eagle (*Aquila chrysaetos*) predation on island foxes (*Urocyon littoralis,* Roemer et al. 2001).

Until recently, healthy adult fishers were believed to be generally safe from predation except possibly in reintroduced populations (Powell and Zielinski 1994); however, most early empirical knowledge of fisher predation stems from eastern fisher populations where predator communities are vastly different and fishers are thought to be larger than in the west (Powell 1993). Some researchers in the western states have suspected fisher populations could be threatened by predation in disturbed habitats with less canopy cover and less forest structure (Buck et al. 1994). Buck et al. (1982, 1983) and Truex et al. (1998) were the first to document fisher mortality due to predation in California populations, though results were not always conclusive. Difficulties with recovering fisher carcasses from the field quickly enough for adequate necropsy and a lack of forensic tools to identify cause of death or responsible predator species likely contributed to a shortage of information regarding the ecology of predation and predation risk within fisher populations.

Developing an understanding of the relationship between fishers and sympatric predators and how this relationship might be mediated by habitat and forest management can foster more effective conservation of this imperiled species. Information gained through investigating intraguild predation on fishers at multiple spatial scales, from the individual fisher to fisher populations to ecology of the predators themselves, will advance our understanding of the threats facing California fishers. For this study, my objectives were to 1) present a detailed description of the tools and techniques available to study mortality and disease in carnivore populations, 2) develop a protocol using molecular methods for identifying predator species from forensic samples collected from predated fisher carcasses, 3) using molecular methods outlined in the protocol, identify the predators of fifty California fishers and couple these results with the most prominent pathologic findings associated with each carcass, 4) use these associations to build a

classification tree for identifying predators based on pathologic findings alone, and 5) investigate habitat use of bobcats (the main fisher predator) and how this relates to predation risk on fishers through a spatial tracking study of bobcats and fishers.

REFERENCES

Buck, S. 1982. Habitat utilization by fisher (*Martes pennanti)* near Big Bar, California. Thesis, Humboldt State University, Arcata, USA.

Buck, S., C. Mullis, and A. Mossman. 1983. Corral Bottom-Hayfork Bally fisher study. Final report to the U. S. Forest Service. Humboldt State University, Arcata, California, USA.

Buck, S., C. Mullis, A.S. Mossman, I. Show, and C. Coolahan. 1994. Habitat use by fishers in adjoining heavily and lightly harvested forests. Pages 368-376 *in* Buskirk, S.W., A.S. Harestad, M.G. Raphael, and R.A. Poweil, editors. Martens, sables, and fisher: biology and conservation. Comstock Publishing Assoc., Cornell Univ. Press, Ithaca, New York.

Creel, S., and N. M. Creel. 1996. Limitation of African wild dogs by competition with larger carnivores. Conservation Biology 10:526–538.

Cypher, B. L., and K. A. Spencer. 1998. Competitive interactions between coyotes and San Joaquin kit foxes. Journal of Mammalogy 79:204–214.

Palomares, F., and T. M. Caro. 1999. Interspecific killing among mammalian carnivores. American Naturalist 153:492–508.

Powell, R. A. 1993. The fisher: life history, ecology, and behavior. 2nd edition. University of Minnesota Press, Minneapolis.

Powell, R. A., and W. J. Zielinski. 1994. Fisher. *in* L. F. Ruggiero, K. B. Aubry, S. W. Buskirk, andW. J. Zielinski, editors. The scientific basis for conserving forest carnivores: American marten, fisher, lynx, and wolverine. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

Roemer, G. W., T. J. Coonan, D. K. Garcelon, J. Bascompte, and L. Laughrin. 2001. Feral pigs facilitate hyperpredation by golden eagles and indirectly cause the decline of the island fox. Animal Conservation 4:307–318.

Truex, R. L., W. J. Zielinski, R. T. Golightly, R. L. Barrett, and S. M. Wisely. 1998. A meta-analysis of regional variation in fisher morphology, demography, and habitat ecology in California. United States Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station, Arcata, California. Unpublished draft report submitted to California Department of Fish and Game.

United States Fish and Wildlife Service. 2004. 50 CFR part 17 Endangered and threatened wildlife and plants; 12 month finding for a petition to list the west coast distinct population segment of the fisher (*Martes pennanti*); proposed rule Federal Register.

Zielinski, W.J., R.L. Truex, F.V. Schlexer, L.A. Campbell, C. Carroll. 2005. Historic and contemporary distributions of carnivores in forests of the Sierra Nevada, California, USA. Journal of Biogeography 32:1385–1407.

CHAPTER 1

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Chapter 13 INVESTIGATING CAUSE-SPECIFIC MORTALITY AND DISEASES IN CARNIVORES: TOOLS AND TECHNIQUES

Greta M. Wengert

Mourad W. Gabriel

Deana L. Clifford

Abstract.— Understanding the roles of cause-specific mortality and underlying factors in the ecology of carnivores is essential to fully comprehend the scope of limiting factors that regulate their populations. Appreciating how disease influences mortality rates and fecundity also informs biologists and managers of the threats that carnivore populations may face. Here, we provide a variety of tools and techniques for the collection, storage, and analysis of biological samples and information for the study of cause-specific mortality and disease prevalence in carnivores while highlighting the importance of representative sampling in order to make sound inferences about disease and specific causes of mortality in population regulation. We offer ways to incorporate these data into epizootiological studies and modeling efforts to make predictions about disease transmission through populations and the effects of disease management actions. Finally, we describe and compare methods employed by wildlife managers and health professionals to control disease in carnivore populations.

Conservation of carnivores entails an understanding of ecology and life-history requirements of species, which is essential for identifying the factors limiting or threatening populations. Because population growth and persistence relate to mortality and fecundity, identification of threats requires thorough characterization of cause-specific mortality or causes of low fecundity, along with representative sampling to ensure an unbiased perspective on the relative importance of specific causes. Disease, as a potential cause of mortality or influence on reproductive success, can be easy to overlook as a contributing cause of population decline. For this reason, a working knowledge of disease processes at individual and population levels and basic methods to study these processes is vital to biologists and managers involved in carnivore conservation. In this chapter, we describe several tools and techniques for collecting biological data and samples to study cause-specific mortality and the presence of pathogens and disease in carnivores, and emphasize the necessity for thoroughness and representativeness. We offer a variety of ways to analyze mortality and disease data through epizootiological studies and modeling programs. Lastly, we present a variety of commonly used disease intervention programs for the prevention or control of detrimental pathogens in carnivore populations.

13.1 DETERMINING CAUSES OF MORTALITY IN CARNIVORES

Studies of sources of mortality should accomplish two objectives: thorough understanding of the direct and indirect reasons for the death of each individual, and the relative frequency and importance of each contributing cause of mortality to the population as a whole. The latter requires representative sampling, a sufficiently large sample size, and knowledge of whether sources are additive or compensatory, in addition to accomplishment of the first objective. Here we focus on the techniques for finding, recovering, and analyzing carcasses effectively, in itself a

challenging task, but stress that this information translates to effective management only if causes of mortality are evaluated in terms of their effects at the population level.

13.1.1 Locating Dead Animals to Determine Cause-specific Mortality

Continuous monitoring, or at least daily monitoring, of transmitter-tagged animals is the most effective technique for locating dead animals quickly enough to determine the cause of death and understand predisposing factors. Such monitoring, however, requires extensive live-trapping (Ch 5) to outfit many animals with transmitters, and is expensive, requiring extensive man-hours of ground telemetry, frequent flights to locate animals, or expensive satellite- transmitter systems that report at least daily.

Most wildlife transmitters with a VHF telemetry component and Argos telemetry packages can be equipped with mortality sensors (Ch 8). Sensors cause VHF units to pulse at different rates, indicating whether an animal has moved or not for a pre-set period of time, allowing a researcher to locate a dead animal reasonably quickly. Alternatively, some researchers use activity sensors if studying activity patterns is a research goal. Though not as effective as mortality sensors, repeated "inactive" signals suggest possible mortality, prompting a researcher to locate an animal and verify death vs extended inactivity.

Alternatively, nonprobability sampling, or convenience sampling, is used commonly to find dead animals. Unfortunately, such opportunistic sampling with no *a priori* sampling design is characterized by large sampling error and commonly misrepresents the relative importance of causes of mortality in the population (Nusser et al. 2008). Thus, if determining cause-specific mortality is a main objective of a carnivore research program, a sampling design is mandatory.

Direct interaction with personnel of public agencies, including transportation departments, state and national parks, and game management agencies, can provide a wealth of information on the locations or final dispositions of carcasses found by their personnel (Knight et al. 1988). Animal removal services or depredation programs also provide carcasses and information on locations of carcasses. Additionally, local wildlife rehabilitation centers and veterinary clinics may generate information from the public on morbidity and mortality of wild carnivores. Regularly scheduled contact with agency and other pertinent personnel may yield samples with less bias than completely opportunistic sampling.

For research on road mortality, schedule systematic road-kill surveys. Surveys of equal, or representative, lengths along roads of different classes throughout a study area allow inference about the rates of road-induced mortality on roads of different classes by individuals of different age classes and sex (if age and sex data are collected; (Clarke et al. 1998)).

13.1.2 Handling Dead Animals and Important Precautions

Approximately 75% of emerging human infectious diseases originate in animals (Taylor and Latham 2001). Therefore, researchers should be familiar with the common routes of disease transmission and with the clinical signs of pathogens that can be passed between animals and people (zoonotic pathogens) before starting research. Accordingly, when handling carcasses, the following basic precautions should be observed to minimize risk of researcher exposure to diverse zoonotic pathogens, including some that are quite debilitating and sometimes fatal, like rabies. First and foremost, wear personal protective equipment when handling dead and live animals. Gloves (latex, nitrile, or vinyl) provide protection from most biological agents and some chemical agents. Masks prevent the transmission of airborne zoonotic pathogens (Wong et

al. 2009), especially important when performing field necropsies. Double-bag a carcass with plastic bags that are thoroughly sealed, airtight and well-labelled. If possible, keep the carcass on ice, or at least cool, until it can be refrigerated. If it cannot be sampled or necropsied within 24-36 hours, freeze it. Store carcasses and tissues in an area that is not used by humans or domestic animals; especially avoid freezers and refrigerators used to store food. Researchers should also carry an easily accessible informational card advising medical personnel that zoonotic diseases should be considered in differential diagnoses, in case a researcher can not do so him- or herself.

Whenever possible, tag a limb of the animal with relevant information; at a minimum include the date and time, location where the carcass was collected (latitude and longitude, if possible), the collector's name and contact information, and other important reference information (i.e. project name, affiliations, etc.). (Munson 2006) detailed the safest, most effective methods for collecting, storing, and shipping carcasses for necropsy and diagnostic testing. Also consult www.nwhc.usgs.gov/mortality_events/shipping_instructions.doc.

13.1.3 Field Data Collection at Mortality Sites

In human forensics cases, thoroughly documenting the details of the scene is essential. Likewise, when investigating the cause of mortality in wildlife, follow strict protocols to investigate a mortality site. Upon discovery of a deceased animal, leave the carcass and site undisturbed until fully photographed. Key characteristics to record include whether the carcass was cached by a predator; whether the carcass was intact, dismembered, or partially consumed; whether the carcass was dragged; and other notable features of the site. For example, the position or location of a carcass may suggest whether the animal secured itself in a sheltered

location due to extreme morbidity before death. If interested in linking habitat features to mortality risk, especially in the case of predation (Hebblewhite et al. 2005; Kunkel and Pletscher 2000), record information on habitat and terrain in detail. Write complete directions to reach the site, flag the location to facilitate return to the site, if necessary, and record the location with a GPS unit.

13.1.4 The Clinical Necropsy

More often than not, the reason for death of an animal is not obvious at first examination, and multiple factors may have interacted to cause death. To understand the underlying factors that contribute to mortality, both at the individual and population levels, conduct clinical necropsies. Ideally, field biologists or wildlife managers can collaborate with veterinary pathologists experienced with wildlife to conduct systematic, thorough necropsies. Because we strongly suggest that carnivore researchers studying mortality make every attempt to have carcasses investigated in a pathology laboratory, we highlight fundamental components of a necropsy in order to inform researchers observing or assisting with necropsy.

Hemorrhage is easily identified during necropsy and is used to determine whether injuries occurred ante- or post-mortem. Photograph all areas of hemorrhage such that the pathologist, if not present, can later characterize the nature of the hemorrhage. In addition to gross examination of the tissues, the pathologist collects samples from all major organs for in-depth histological investigation; lesions at the cellular level may be associated with an animal's death or morbidity prior to death. Occasionally, immunohistochemistry, a method that detects antigens within cells, is employed by the pathologist to confirm or rule-out infection by certain pathogens. Staining techniques (e.g. gram staining, acid-fast staining) also help detect and identify pathogens.

Clinical necropsies also allow biopsy of tissues for diagnoses of non-infectious diseases such as cancer, which can be critical for the health of some species (island fox, *Urocyon littoralis*, (Vickers et al. 2007); Tasmanian devil, *Sarcophilus harrisii* (McCallum et al. 2007)). Pathologists typically work with toxicologists, who screen for abnormal levels of heavy metals and presence of toxins within tissues, such as anticoagulant rodenticides or other pesticides. Gross necropsy is also an opportune time to remove a tooth for cementum annuli analysis to estimate the age of the animal (Ch 7).

13.1.5 When Clinical Necropsies Just Aren't Feasible – A Quick Guide to Field Necropsy

Sometimes, logistics or budgets simply don't allow for a full clinical necropsy by a wildlife pathologist. Whether financial constraints or remote field sites prohibit a full necropsy, field biologists can perform field necropsies. Obtain training from a wildlife pathologist to ensure safe and thorough necropsy procedures. Photograph and sample all tissues for later lab analysis and secure collaboration with a pathology laboratory beforehand to receive and analyze tissue samples. Split each tissue sample between formalin and plastic whirlpacks on ice, then freeze the whirlpacks, as soon as possible. Collect blood, nasal, and ocular exudates, ectoparasites and fecal samples. Document and record all collections and abnormal observations (write on a necropsy form or voice-record). Consult and use the online manual of necropsy methods (Munson 2006): http://www.vetmed.ucdavis.edu/whc/pdfs/necropsy.pdf. Box 1 contains a minimal list of tools and supplies needed for a field necropsy.

13.1.6 Field and Laboratory Investigation of Intraguild Predation

Intraguild predation in carnivore communities can be a frequent cause of mortality (Mills and Gorman 1997; Moehrenschlager et al. 2007; Ralls and White 1995; Thompson and Gese 2007). When intraguild predation is suspected, photograph the immediate surroundings and all bite wounds or obvious injuries to the dead animal. Measurements can be taken of the bite wounds, though only punctures in bone can be truly diagnostic for idenitifying predator species (Lyver 2000).

Molecular analyses are proving quite useful for determining predator species. Until recently, these methods were restricted to identifying livestock predators (Sundqvist et al. 2008; C. L. Williams et al. 2003), but in research on fishers (*Martes pennanti*) and American martens (*M. americana*), intraguild predators have been determined through sampling saliva around bite wounds and extracting DNA (Wengert et al., unpublished data). Rub sterile, polyester-tipped swabs within bite wounds and clip fur surrounding wounds. Store these samples in airtight vials, and freeze at or below -20 °C. Arrange collaboration with a genetics laboratory ahead of time, so that the laboratory personnel can determine the most appropriate genetic protocols for a particular project. For example, if the researcher is studying intraguild predation on small carnivores, such as weasels or small foxes, a variety of predators ranging from raptors to bears must be considered.

A somewhat less accurate method for identifying predators is use of molecular techniques on feces left at carcasses (Ernest et al. 2002; Onorato et al. 2006). Though this evidence is often only circumstantial, it can infer predator identity when coupled with other information from the predation event. Molecular material from the feces can provide the animal's individual identity (Ch 4) (Ernest et al. 2002), sex (Ch 4) (Blejwas et al. 2006), diet (Ch 11), and potentially information on the predator's health (Ch 12).

Remote cameras can be set to identify the predator of a smaller carnivores that returns to a carcass or cache site (Ch 4; John Erb, Minnesota Department of Natural Resources, personal communication).

13.2 STUDYING DISEASE AND PATHOGEN CYCLES IN CARNIVORES

Disease can lead to mortality of individuals and to population reduction or regulation (McCallum et al. 2007; Randall et al. 2006; Thorne and Williams 1988). Certain pathogens affecting carnivores, like rabies and canine distemper, are extremely virulent and have caused dramatic population decreases and local extinctions (Laurenson et al. 1998; Roelke-Parker et al. 1996; Timm et al. 2009). On the other hand, disease impacts may be subtle, not causing mortality or obvious clinical signs, yet still affect reproductive success or the ability of a carnivore to secure enough food for itself and its offspring, or potentially making individuals vulnerable to other forms of mortality.

13.2.1 Detection of Disease, Infection, and Pathogen Exposure

Handling live-trapped carnivores provides a perfect opportunity to collect biological samples, including blood, exudates, feces, and parasites, for disease screening and assessment. Even if health and disease are not the primary focus of a study, archiving these samples for future use can reduce the need to resample animals to obtain health information, alleviating potential negative impacts to a population from additional handling (Ch 7; (Botzler and Armstrong-Buck 1985)). Obtain technical instruction from qualified researchers or veterinarians to avoid complications that could arise from collecting and handling animals and biological samples improperly. Table 1 provides information about sampling techniques.

Photo documentation - Photograph a carnivore under anesthesia to create a baseline reference of visual characteristics that might vary with changes in health over time. Pelage quality can deteriorate due to mite infestations (mange) or with emaciation linked to disease. Tooth wear may indicate excessive biting due to ectoparasite infestation or a coarse diet. Wounds or other external abnormalities should be reassessed if the animal is captured again. Photographs also document conditions of animals noted in one region but not elsewhere.

Blood - Blood can provide critical clues to an animal's health status (Ch 12). Antibodies in blood can be used to determine past pathogen exposure or potentially, active infection. Fluctuations in glucose and urea levels may indicate disease. Abnormal white blood cell counts often indicate response to viral, bacterial, and parasitic infections. Information on collection, storage and pertinent diagnostic tests is summarized in Table 1.

Feces - Feces can harbor bacteria, endoparasites and their ova, various environmentally resistant viruses, and some labile viruses. Collect feces using a swab within the rectum of an anesthetized animal or opportunistically collecting a scat. Feces collected for genetic or diet studies can also be used to screen for pathogens or parasites. Collection and storage methods for feces are shown in Table 1.

Exudates - Certain pathogens are transmitted by ocular, nasal and oral exudates. Canine herpesvirus, canine distemper virus, and influenza virus are often shed within ocular-nasal exudates, which can be tested for their presence (E. S. Williams and Barker 2001). Since many of these viruses are extremely labile, collect samples properly to maximize chances of detecting infected animals (Table 1).

Urine - Though uncommon, certain pathogens are shed in urine. *Leptospira* species of bacteria are shed by this route, and all carnivores can be either maintenance or accidental hosts

for *Leptospira* spp. Manually express the bladder and collect urine mid-stream to avoid contaminating the sample with traces of feces or other materials near the opening of the urinary tract. If working with trained wildlife veterinarians, they can obtain a sterile urine sample by cystocentesis (collection of urine with needle and syringe).

Ectoparasites and Endoparasites - Collecting ectoparasites and endoparasites (Table 1) provides information regarding vector-borne pathogens that may infect carnivores, such as plague (*Yersinia pestis*) and heartworm (*Dirofilaria immitis*). Many parasites themselves cause disease in carnivores, such as mange caused by mite infestation or infections with helminthes or protozoans. Parasites also provide information on the life history traits of the focal carnivore, such as habitat associations of parasites in turn providing clues to habitats the carnivore may have visited. Many endoparasites require specific intermediate hosts that are eaten by carnivores, thereby providing insight to a carnivore's diet.

Rapid Diagnostic Testing for Disease - Several rapid diagnostic tests (RDT) are available for detecting pathogens of domestic animals, including heartworm, distemper virus, parvovirus and rabies. Although these tests are convenient, rapid diagnostic tests that have been developed for domestic animal use cannot simply be transferred for use in wildlife (Stallknecht 2007). First, validate any rapid diagnostic test to be used on wild carnivores against an acceptable gold standard test to determine its effectiveness with the focal species (Stallknecht 2007). Recent evaluation of a parvovirus RDT on fisher and gray fox (*Urocyon cinereoargenteus*) fecal samples demonstrated that the test failed to detect any parvovirus infections, while the conventional parvovirus PCR readily detected the virus in many of the same samples (Gabriel et al. 2010).

Disinfection Considerations - Many pathogens that infect carnivores are generalists, affecting members of many sympatric carnivore species. Furthermore, many of these pathogens are stabile in the environment (e.g. parvoviruses). Steps can be taken by researchers and managers to avoid spreading disease inadvertently via indirect monitoring equipment, like cameras, scent stations, and track-plates, in addition to live-traps and handling equipment. After every new animal contacts the equipment, remove all fecal material and visible exudates and then disinfect the equipment (always wear personal protective equipment). Many disinfectants are suitable for neutralizing most pathogens of concern, but safety and application methods determine which are most appropriate. Sodium hypochlorite, or bleach, is a common choice of disinfectant and is readily available and inexpensive. Use a dilution of 1:32 up to 1:10 to cover the contaminated surface and let it remain there for at least 10 minutes. Though hazardous if ingested or inhaled, it is very effective in neutralizing even highly resistant viruses (Gilman 2004). Quaternary ammonium compounds are available from veterinary and tack and feed supply stores (e.g. Roccal-D®, Parvo-sol®, Spectrasol®). This group may not be particularly effective for neutralizing resistant viruses (Eleraky et al. 2002; Kennedy et al. 1995), but should be effective for removing many pathogenic bacteria and some labile viruses. Potassium peroxymonosulfate, a relatively new class of disinfectant marketed as Virkon-S® or Trifectant®, is effective for neutralizing a wide array of pathogens including resistant viruses. This disinfectant has low toxicity and few corrosive or irritating properties.

Indirect Animal Sampling - Direct sampling of live animals is the most sensitive and reliable technique for diagnosing disease, detecting infection, or documenting prior exposure to pathogens. However, constraints to capturing and processing multiple individuals, especially rare, elusive, or trap-shy carnivores, often preclude direct sampling. Two alternatives to direct

animal sampling are collecting feces for pathogen detection and sampling opportunistically-found carcasses.

When collected with an *a priori* systematic sampling design, fecal sampling can provide prevalence data for fecally-shed pathogens that are known to infect many carnivore species, such as parvoviruses, coronaviruses, many helminthes and protozoans, and potentially canine distemper virus (Ballmann-Acton and Elaine 2009). Scrape or cut the outside of feces to ensure that the mucous, gastrointestinal epithelium, and virons lining the epithelium are included in the sample. This method also ensures that genomic DNA from the focal animal is sampled, allowing accurate species identification (Ch 4). Store samples in 95-100% ethanol to fix the pathogen's DNA or RNA and the focal carnivore's DNA. Sampling design should accommodate the likelihood of sampling individuals multiple times. Individual identities can often be verified using fecal DNA. Depending on research objectives, this allows the researcher to use only samples from different individuals to avoid pseudoreplication, or to track clearing of infections and shedding cycles within the same individuals over time.

Carnivore carcasses provide a wealth of information on the health status of a population. As mentioned earlier, working with agency personnel and trappers or hunters can generate a sample of carcasses greater than can be achieved by researcher-based collection alone. Collect blood directly from the right heart ventricle using a sterile syringe. Take care not to puncture the heart prior to drawing blood as blood within the heart is the most sterile and keeping the heart intact ensures blood sterility. Collect all other samples similarly to methods described earlier in this chapter for live animals and in Table 1.

13.2.2 Epizootiology in Carnivore Populations

Epizootiology is the study of population-level patterns of disease in animals. It assesses disease risk in a population, correlating "risk factors" such as animal characteristics (e.g. sex, age, habits) and environmental variables to pathogen exposure, infection and transmission. Knowledge of baseline information on population health is essential to assess disease risk accurately and understand whether pathogens occur at an "enzootic" rate (expected and relatively low, constant rate) or "epizootic" rate (elevated rate that is unexpected due to its temporal patterns, spatial patterns or frequency (Wobeser 2007)).

Incorporating Age Structure - Pathogens affect some age classes more than others; thus understanding the age structure of a population in relation to disease dynamics is integral for characterizing disease risk and past epizootics. For example, a population missing a particular cohort may have experienced an epizootic of a pathogen that selectively affects juveniles when that cohort was young. When this evidence is supported by high prevalence of exposure to that particular pathogen in older cohorts, one can more conclusively infer the epizootic history of the population.

Incorporating Fecundity - Pathogens affect carnivore reproductive success through many mechanisms including abortions and reduced survival of offspring. Although challenging to obtain, data on pregnancy rates, neonatal loss, and survival of juveniles allows inferences about pathogen exposure and fecundity. Pregnancy status can be assessed in free-ranging carnivores (Ch 12) by detecting relaxin hormone in blood, serum, plasma, or urine (Bauman et al. 2008; Carlson and Gese 2007; De Haas van Dorsser et al. 2007), detecting fecal progesterone in induced ovulators (Brown 1997), and using ultrasound (Clifford et al. 2007; McNay et al. 2006). For carnivores with known dens, young can be counted directly or less invasively by using small "Peeper" cameras (e.g. Sandpiper Technologies, Manteca, CA). If examination of pre-weaning

young is possible, collect samples for disease testing and mark young uniquely for later identification (Ch 8) and correlation of neonate survival with pathogen exposure. Collect and test feces at dens for diseases and parasites of weaned offspring.

If pregnancy can be documented and fetuses counted via ultrasound, and pregnant females tracked via telemetry, remote cameras can be placed near dens to document emergence of the young, thereby providing an index of perinatal mortality (Clifford et al. 2007). Given sufficient sample sizes, perinatal mortality together with pathogen exposure histories of females allow inference regarding effects of disease on reproductive performance of females. Long-term demographic data combined with disease prevalence or pathogen exposure rates can reveal otherwise undetectable impacts of disease. Thirty years of demographic data on wolves (*Canis lupus*) in Minnesota showed that pup survival decreased dramatically after the appearance of canine parvovirus in the population, lowering annual population growth rates (Mech et al. 2008). Long-term data sets of placental scarring or other pregnancy stages with corresponding disease data can be collected from animals that are harvested.

Estimating Contact Rates - The contact rate, or rate at which a disease is transmitted throughout a population, depends on 1) how often an animal capable of contracting the disease contacts an infected animal or infectious material, and 2) how likely that contact is to result in disease transmission. Proximity data-loggers affixed to animal collars estimate contact rate by recording the number and specific times of contact with another collar, estimating the probability and frequency of contacts (Böhm et al. 2009; Hamede et al. 2009). Users can program the distance between collars required to log as a "contact."

From home ranges calculated as utilization distributions (use raster format), researchers can calculate the probabilities of two individuals being in their area of home range overlap at the

same time, and can test for avoidance or attraction by comparing actual use of the overlap to that predicted for random use (Ch 9). These probabilities can be used as a proxy for contact rate and predict risk of pathogen spread throughout populations of different densities (Kauhala and Holmala 2006; White et al. 1995).

At natural or human-made sites where animals gather, such as watering holes, large animal carcasses, supplemental feeding stations, or latrine sites, remote cameras can provide data on interactions and log contacts between conspecifics as well as among species (Macdonald et al. 2004). These data can be used to calculate nightly contact rates (Totton et al. 2002). When direct contact between individuals is not necessary for pathogen transmission (as in the case of parvoviruses, coronaviruses, and many nematodes and protozoans), simple contact between an individual and an infected animal's feces may be considered an effective contact in the disease sense, and these interactions could be well-documented and quantified at latrines using cameras (Page et al. 1999).

Finally, another indicator of contact between individuals is multi-species latrines and areas where many animals leave scats for marking. Especially where overmarking occurs, these areas can be used to develop an index of contact rate for fecally-shed pathogens. Documenting presence of scats from domestic animals in these areas also helps assessment of spillover risk, especially from dogs and feral cats, whose scats should be collected and tested for pathogen presence.

13.2.3 Modeling Techniques in Disease Ecology

Mathematical modeling contributes to the understanding of researchers and managers of disease transmission dynamics within and among carnivore populations, and assists in building

hypotheses for the occurrence, spatial spread and population-level impacts of disease. SIR models, a form of "compartment model," are commonly used to model disease dynamics and epidemics. In these models, individuals move among compartments depending on whether they are susceptible to (*S*), infected with (*I*), or recovered from (*R*) a disease (Abbey 1952). Differential equations derived from contact rate and disease prevalence data estimate the rates at which individuals move among the *S-I-R* compartments. Population demographic parameters, density dependence and stochasticity can be included. Spatial modeling approaches including nearest neighbor, moving window analyses (Alexander and Boyle 1996), simulation models (Deal et al. 2000), and diffusion models (Adjemian et al. 2007; Moore 1999) can be integrated with or "added onto" an SIR framework. The result is spatially explicit predictions (hypotheses) for rates and patterns of disease spread that also can be used to evaluate the effects of different disease control actions. Stochastic simulation models combined with disease, spatial, and demographic data from field studies have been used to assess risk of disease spillover from domestic animals to wildlife (Clifford et al. 2009) and to examine disease dynamics in a multi-host carnivore community (Craft 2008).

Spatial scan statistics use models to perform both geographical and time surveillance on disease occurrence data to detect and locate spatial and temporal clustering of disease. These analyses can help predict "hotspots" of disease indicating greater pathogenic risk, and may be important for selection of the safest and most appropriate locations for carnivore reintroductions or vaccination programs. Incorporating temporal data into the model may define seasonal cycles of disease or periods of greater pathogenic risk. Examples of software for use in spatial scan statistics are highlighted in Table 2.

Frequency and resultant mortality rates of disease can also be incorporated into population viability analysis (PVA) models developed to assess extinction risk (Lacy 2000). This approach was used to examine the risk of quasi-extinction from canine distemper virus for endangered island foxes (Kohlmann et al. 2005). More complex epidemiological disease dynamic models have been embedded into population viability models to evaluate rabies vaccination strategies needed to prevent critically low post-outbreak population densities for African wild dogs (*Lycaon pictus*, (Vial et al. 2006) and Ethiopian wolves (*Canis simiensis*, (Haydon et al. 2002)), and to examine the effects of periodic canine distemper outbreaks on the persistence of the Ethiopian wolf population.

For PVA models to be truly useful for carnivore conservation, the hypotheses they generate (usually called "predictions") should be tested with independent data (Powell et al., in press). Barring independent tests, models must be built using the best-available disease, demographic and spatial data, and incorporate uncertainty in their predictions to account for both environmental stochasticity and the limitations of the data. The extinction risk generated by these models is best used as an index. A non-exhaustive list of mathematical, spatial, and PVA modeling software and their features is provided in Table 2.

13.3. PREVENTION AND CONTROL OF DISEASE

Disease is a natural component of carnivore ecology. Species co-evolve with pathogens adapting to new pathogens, developing resistance to new strains of old pathogens as they re-emerge, and undergoing population fluctuations that modify the pathogen cycles they experience. Human induced changes may increase the frequency of disease epizootics in carnivores by introducing exotic pathogens, new strains of pathogens, and toxins to new areas;

by altering disease cycles through changing ecological communities and reducing ecosystem function; and by driving wildlife populations to low numbers so that they are vulnerable to stochastic processes like disease. When humans recognize the risks posed by disease cycles to threatened species, we must decide whether intervention is warranted, feasible, and morally justified. Our decisions will be constrained by financial conditions, stakeholder opinions, odds of success, the logistical ability to implement an intervention, and often, whether the disease threatens humans or domestic animals.

13.3.1 Intervention Options: Removing the Causative Factor

In some rare cases, the agent causing disease can be removed. This option is usually available only for diseases caused by toxic agents such as anticoagulant rodenticides (Fournier-Chambrillon et al. 2004) (Riley et al. 2007) and heavy metals (Laskowski 1991). Although the ideal option is removal of a toxic agent, this management action is rife with legal, political, ethical, financial, and cultural issues, which often delay or prevent removal the agent. This lengthy and complex process is typified by the years needed to ban lead ammunition throughout the range of the California condor (*Gymnogyps californianus*), (Title 14 California Code of Regulations, section 475). In the end, despite clear biological information, most decisions are based on sociological, political and economic issues.

13.3.2 Intervention Options: Manipulating the Host Population

The cycle of infectious disease depends on a range of host population characteristics that influence the intensity, infection rate, and duration of an epizootic. Demographic and population parameters such as density, vital rates, and social systems influence how a disease behaves in a

population, and therefore, changes in these parameters alter the course of the disease. Public health specialists and wildlife managers have used attributes of pathogen cycles to develop management techniques for wildlife diseases; managing the affected host population is usually more feasible than attempting to eradicate a pathogen. First, managers must understand the ecology of the pathogen and the host within the area of interest. They must know whether the pathogen is a specialist or generalist, whether it circulates through a community of many different species, and whether it has vectors and intermediate hosts.

Treating Individuals - One way to reduce disease transmission is to reduce the number of susceptible or infected individuals under a threshold density below which the pathogen cannot persist. Treating infected individuals through focal animal treatment (e.g. treating bobcats, *Lynx rufus*, with ivermectin for infestions with notoedric mange (Riley et al. 2007)) or mass distribution of medicines via food bait (e.g. antihelmintics in red foxes, *Vulpes vulpes*, against the tapeworm *Echinococcus multilocularis*, (Hegglin et al. 2003)) directly reduces the number of infected animals and inherently reduces the infection rate.

Culling – Through a somewhat controversial method to control disease, managers can reduce contact rates and disease prevalence by reducing a dense host carnivore population, or "culling." Three approaches to culling can be effective: culling only infected individuals, culling randomly over a large area simply to reduce population density, and culling in a specific area to create a barrier to pathogen spread (e.g. a *cordon sanitaire*). The second approach has been attempted numerous times throughout the world with mixed success, often directed towards carnivores because of the zoonotic threat of rabies (Irsara et al. 1982; Rosatte 1988). Local depopulation to create a barrier to disease spread has shown some success in rabies control (Gunson et al. 1978). When employing any culling program for disease control, choosing the correct target species or

group of species is paramount, as is understanding potential indirect effects of depopulation, such as opening territories and inducing immigration of infected individuals from adjacent areas (Woodroffe et al. 2006).

Vaccination - Through vaccination, managers reduce the number of susceptible hosts by switching them to the equivalent of "recovered." A number of new vaccines developed for domestic animals have been validated for wild carnivores and used in control programs, conservation programs, and carnivore reintroductions. Effective and safe vaccines are those that, 1) do not produce disease in the host, 2) provide long-term immunity, 3) protect the species of concern against all strains or varieties of the pathogen, 4) cannot revert to virulence, and 5) allow one to distinguish between individuals with vaccine-induced immunity and natural immunity (Wobeser 2007). Managers and biologists should first consult with a wildlife veterinarian to determine the safest, most effective, appropriate vaccine for the focal species.

Broad-scale vaccination using widely broadcast, vaccine-laden baits is regularly implemented to control rabies in carnivores throughout the world (Capello et al. 2010; Niin et al. 2008; Slate et al. 2005; Sterner et al. 2009; Stöhr and Meslin 1996). These programs have met with mixed, though generally positive, success after all costs are accounted (Sterner et al. 2009). Appropriate baits must be chosen for the target species and are typically distributed throughout a large geographic area by hand or by aircraft. Oral rabies vaccine packages contain a biomarker (often tetracycline) that allows biologists to assess whether a particular individual has consumed the bait and vaccine, thus allowing the percentage of the population that was vaccinated to be estimated. Targetting a particular host carnivore is difficult with broadcast baits, sometimes requiring that more baits be used than needed just for the target carnivore species.

Another approach to vaccination is Trap-Vaccinate-Release (TVR), where animals are live-trapped, vaccinated typically by intramuscular or subcutaneous injection, and released at the capture site (Rosatte et al. 1992). TVR programs can target specific carnivore species that may not readily consume oral baits, release non-target species for which the vaccines are not suitable, and use vaccines that have not been developed in an orally-administered form, such as vaccines for distemper virus and parvoviruses. TVR programs can be integrated into ongoing trapping and monitoring programs, allowing for initial and follow-up blood samples to determine pathogen exposure prior to and post-vaccination. Survival of vaccinated individuals can be assessed through radio collaring or mark-recapture methods.

The costs of TVR programs are usually substantially higher than mass-distributions of oral vaccines, but high bait costs and unforeseen legal obstacles of oral bait distribution may make TVR more desirable to managers. Some programs combine the two methods of mass-immunization of wildlife to achieve the greatest coverage of a focal population(s) (Sterner et al. 2009). Vaccination programs for free-ranging carnivores should be adaptively designed with a monitoring component to assess the effectiveness of the program, examine costs, and reassess the need to continue the program.

13.3.3 Intervention Options: Manipulating Sympatric Species including Domestic Animals

The objective of many mass-vaccination efforts is to minimize disease risk to humans and domestic animals. In most of North America and Europe, many domestic animals are protected from common infectious diseases by mandatory immunizations. In many other regions of the world, however, domestic dog populations are immense, largely unvaccinated, and pose a constant threat of disease spillover to nearby carnivore populations. To control a pathogen in a

threatened population, one might need to manage a common, sympatric species, which might be a domestic animal population.

When domestic carnivores pose a serious disease threat to wild carnivores, as in the case for the Ethiopian wolf (Randall et al. 2006), domestic dog vaccination is an essential part of the disease control program. Data on a domestic dog population size and the contact rates between dogs, other wildlife, and the focal species can be incorporated into SIR-type models to estimate the minimum proportion of the domestic dog population, or a sympatric wildlife population, that must be vaccinated in order to reduce spillover risk to focal species.

13.3.4 Intervention Options: Addressing Human Activities

Though few, if any, pathogens of humans directly threaten wild carnivore populations, human behavior and activities with domestic dogs and cats, often pose threats to carnivore populations. An effective system encouraging dog vaccination for critical pathogens can reduce disease threats to endangered carnivores (Randall et al. 2006), if everything goes as planned. Preventing contact between domestic animals and wild carnivores can also reduce disease threats to wildlife (Laurenson et al. 1997), again only when pet owners are responsible.

Humans inadvertently affect wild carnivore populations by artificially inflating the densities via supplemental feeding of wildlife with pet food left within the reaches of wild carnivores. Human encroach wildlife habitats and relegate diminishing carnivore populations to small, isolated habitat patches, increasing the likelihood of contact among infected individuals, and, thereby, intensifying the risk of infection throughout these populations. Elevated risks make the likelihood of catastrophic epizootics more probable in the short-term, especially for urban carnivores that are notorious for perpetuating disease cycles, like raccoons (*Procyon lotor*) and

striped skunks (*Mephitis mephitis*). Altering the behavior of humans is a daunting task. Developing creative solutions to these real problems must be a priority, nonetheless, in the conservation and management of wild carnivores.

Literature Cited

Abbey, H. (1952). An examination of the Reed-Frost theory of epidemics. *Human Biology; An International Record of Research,* 24, 201-33.

Adjemian, J.Z., Foley, P., Gage, K.L., and Foley, J.E. (2007). Initiation and spread of traveling waves of plague, Yersinia pestis, in the western United States. *The American Journal of Tropical Medicine and Hygiene,* 76, 365-75.

Alexander, F.E. and Boyle, P. (1996), *Methods for Investigating Localized Clustering of Disease*; International Agency for Research on Cancer.

Ballmann-Acton, B. and Elaine, A. (2009). Evaluation of Noninvasive Molecular Monitoring for Fecal Pathogens Among Free-ranging Carnivores. (North Carolina State University).

Bauman, J.E., Clifford, D.L., and Asa, C.S. (2008). Pregnancy diagnosis in wild canids using a commercially available relaxin assay. *Zoo Biology,* 27, 406-13.

Blejwas, K.M., Williams, C.L., Shin, G.T., McCullough, D.R., and Jaeger, M.M. (2006). Salivary DNA evidence convicts breeding male coyotes of killing sheep. *Journal of Wildlife Management,* 70, 1087-93.

Böhm, M., Hutchings, M.R., and White, P.C.L. (2009). Contact networks in a wildlife-livestock host community: identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. *PloS ONE,* 4, 1-12.

Botzler, RG and Armstrong-Buck, SB (1985). Ethical considerations in research on wildlife diseases. *Journal of Wildlife Diseases,* 21, 341-45.

Brown, J.L. (1997). Fecal steroid profiles in black-footed ferrets exposed to natural photoperiod. *The Journal of Wildlife Management,* 61, 1428-36.

Capello, K., Mulatti, P., Comin, A., et al. (2010). Impact of emergency oral rabies vaccination of foxes in northeastern Italy, 28 December 2009-20 January 2010: preliminary evaluation. *Euro Surveillance,* 15, 1-4.

Carlson, D.A. and Gese, E.M. (2007). Relaxin as a diagnostic tool for pregnancy in the coyote (*Canis latrans*). *Animal Reproduction Science,* 101, 304-12.

Clarke, G.P., White, P.C.L., and Harris, S. (1998). Effects of roads on badger *Meles meles* populations in south-west England. *Biological Conservation,* 86, 117-24.

Clifford, D.L., Woodroffe, R., Garcelon, D.K., Timm, S.F., and Mazet, J.A.K. (2007). Using pregnancy rates and perinatal mortality to evaluate the success of recovery strategies for endangered island foxes. *Animal Conservation,* 10, 442-51.

Clifford, D.L., Schumaker, B.A., Stephenson, T.R., et al. (2009). Assessing disease risk at the wildlife-livestock interface: A study of Sierra Nevada bighorn sheep. *Biological Conservation,* 142, 2559-68.

Craft, M.E. (2008). Predicting Disease Dynamics in African Lion Populations. (University of Minnesota).

De Haas van Dorsser, FJ, Lasano, S., and Steinetz, BG (2007). Pregnancy diagnosis in cats using a rapid, bench top kit to detect relaxin in urine. *Reproduction in Domestic Animals,* 42, 111-12.

Deal, B., Farello, C., Lancaster, M., Kompare, T., and Hannon, B. (2000). A dynamic model of the spatial spread of an infectious disease: the case of fox rabies in Illinois. *Environmental Modeling and Assessment,* 5, 47-62.

Eleraky, N.Z., Potgieter, L.N.D., and Kennedy, M.A. (2002). Virucidal efficacy of four new disinfectants. *Journal of the American Animal Hospital Association,* 38, 231-34.

Ernest, H.B., Rubin, E.S., and Boyce, W.M. (2002). Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. *Journal of Wildlife Management,* 66, 75-85.

Fournier-Chambrillon, C., Berny, P.J., Coiffier, O., et al. (2004). Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: implications for conservation of European mink (*Mustela lutreola*). *Journal of Wildlife Diseases,* 40, 688-95.

Gabriel, M.W., Wengert, G.M., Matthews, S.M., et al. (2010). Effectiveness of rapid diagnostic tests to assess pathogens of fishers (*Martes pennanti*) and gray foxes (*Urocyon cinereoargenteus*). *Journal of Wildlife Diseases,* 46, 966-70.

Gilman, N. (2004), Sanitation in the animal shelter, In L. Miller and S. Zawistowski (eds.), *Shelter Medicine for Veterinarians and Staff* (Blackwell Publishing), 67-78.

Gunson, JR, Dorward, W.J., and Schowalter, D.B. (1978). An evaluation of rabies control in skunks in Alberta. *The Canadian Veterinary Journal,* 19, 214-20.

Hamede, R.K., Bashford, J., McCallum, H., and Jones, M. (2009). Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. *Ecology Letters,* 12, 1147-57.

Haydon, DT, Laurenson, MK, and Sillero Zubiri, C. (2002). Integrating epidemiology into population viability analysis: managing the risk posed by rabies and canine distemper to the Ethiopian wolf. *Conservation Biology,* 16, 1372-85.

Hebblewhite, M., Merrill, E.H., and McDonald, T.L. (2005). Spatial decomposition of predation risk using resource selection functions: an example in a wolf-elk predator-prey system. *Oikos,* 111, 101-11.

Hegglin, D., Ward, P.I., and Deplazes, P. (2003). Anthelmintic baiting of foxes against urban contamination with *Echinococcus multilocularis*. *Emerging infectious diseases,* 9, 1266-72.

Irsara, A., Ruatti, A., Gagliardi, G., Orfei, Z., Bellani, L., and Mantovani, A. (1982). Control of wildlife rabies in North-Eastern Italy. *Comparative Immunology, Microbiology and Infectious Diseases,* 5, 327-35.

Kauhala, K. and Holmala, K. (2006). Contact rate and risk of rabies spread between medium-sized carnivores in southeast Finland. *Annales Zoologici Fennici,* 43, 348-57.

Kennedy, M.A., Mellon, V.S., Caldwell, G., and Potgieter, LN (1995). Virucidal efficacy of the newer quaternary ammonium compounds. *Journal of the American Animal Hospital Association,* 31, 254-58.

Knight, R.R., Blanchard, B.M., and Eberhardt, L.L. (1988). Mortality patterns and population sinks for Yellowstone grizzly bears, 1973-1985. *Wildlife Society Bulletin,* 16, 121-25.

Kohlmann, S.G., Schmidt, G.A., and Garcelon, D.K. (2005). A population viability analysis for the island fox on Santa Catalina Island, California. *Ecological Modelling,* 183, 77-94.

Kunkel, K.E. and Pletscher, D.H. (2000). Habitat factors affecting vulnerability of moose to predation by wolves in southeastern British Columbia. *Canadian Journal of Zoology,* 78, 150-57.

Lacy, R.C. (2000). Structure of the VORTEX simulation model for population viability analysis. *Ecological Bulletins,* 48, 191-203.

Laskowski, R. (1991). Are the top carnivores endangered by heavy metal biomagnification? *Oikos,* 60, 387-90.

Laurenson, K., Shiferaw, F., and Sillero-Zubiri, C. (1997), Disease, domestic dogs and the Ethiopian wolf: the current situation, *C. Sillero-Zubiri, DW MacDonald and the IUCN/SSC Canid Specialist Group. The Ethiopian wolf—status survey and conservation action plan. IUCN, Gland, Switzerland*, 32-42.

Laurenson, K., Sillero-Zubiri, C., Thompson, H., Shiferaw, F., Thirgood, S., and Malcolm, J. (1998). Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. *Animal Conservation,* 1, 273-80.

Lyver, P.O.B. (2000). Identifying mammalian predators from bite marks: a tool for focusing wildlife protection. *Mammal Review,* 30, 31-44.

Macdonald, D.W., Buesching, C.D., Stopka, P., Henderson, J., Ellwood, S.A., and Baker, S.E. (2004). Encounters between two sympatric carnivores: red foxes (*Vulpes vulpes*) and European badgers (*Meles meles*). *Journal of Zoology,* 263, 385-92.

McCallum, H., Tompkins, D.M., Jones, M., et al. (2007). Distribution and impacts of Tasmanian devil facial tumor disease. *EcoHealth,* 4, 318-25.

McNay, M.E., Stephenson, T.R., and Dale, B.W. (2006). Diagnosing pregnancy, in utero litter size, and fetal growth with ultrasound in wild, free-ranging wolves. *Journal of Mammalogy,* 87, 85-92.

Mech, L.D., Goyal, S.M., Paul, W.J., and Newton, W.E. (2008). Demographic effects of canine parvovirus on a free-ranging wolf population over 30 years. *Journal of Wildlife Diseases,* 44, 824-36.

Mills, M.G.L. and Gorman, M.L. (1997). Factors affecting the density and distribution of wild dogs in the Kruger National Park. *Conservation Biology,* 11, 1397-406.

Moehrenschlager, A., List, R., and Macdonald, D.W. (2007). Escaping intraguild predation: Mexican kit foxes survive while coyotes and golden eagles kill Canadian swift foxes. *Journal of Mammalogy,* 88, 1029-39.

Moore, D.A. (1999). Spatial diffusion of raccoon rabies in Pennsylvania, USA. *Preventive Veterinary Medicine,* 40, 19-32.

Munson, L. (2006), *Necropsy of Wild Animals*; Wildlife Health Center, School of Veterinary Medicine, University of California, Davis.

Niin, E., Laine, M., Guiot, A.L., Demerson, J.M., and Cliquet, F. (2008). Rabies in Estonia: Situation before and after the first campaigns of oral vaccination of wildlife with SAG2 vaccine bait. *Vaccine,* 26, 3556-65.

Nusser, S.M., Clark, W.R., Otis, D.L., and Huang, L. (2008). Sampling considerations for disease surveillance in wildlife populations. *Journal of Wildlife Management,* 72, 52-60.

Onorato, D., White, C., Zager, P., and Waits, L. P. (2006). Detection of predator presence at elk mortality sites using mtDNA analysis of hair and scat samples. *Wildlife Society Bulletin,* 34, 815-20.

Page, L.K., Swihart, R.K., and Kazacos, K.R. (1999). Implications of raccoon latrines in the epizootiology of baylisascariasis. *Journal of Wildlife Diseases,* 35, 474-80.

Ralls, K. and White, P.J. (1995). Predation on San Joaquin kit foxes by larger canids. *Journal of Mammalogy,* 76, 723-29.

Randall, DA, Marino, J., Haydon, DT, et al. (2006). An integrated disease management strategy for the control of rabies in Ethiopian wolves. *Biological Conservation,* 131, 151-62.

Riley, S.P.D., Bromley, C., Poppenga, R.H., Uzal, F.A., Whited, L., and Sauvajot, R.M. (2007). Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. *Journal of Wildlife Management,* 71, 1874-84.

Roelke-Parker, M.E., Munson, L., Packer, C., et al. (1996). A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature,* 379, 441-45.

Rosatte, R.C. (1988). Rabies in Canada—history, epidemiology and control. *The Canadian Veterinary Journal,* 29, 362-65.

Rosatte, R.C., Power, M.J., MacInnes, CD, and Campbell, J.B. (1992). Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. *Journal of Wildlife Diseases,* 28, 562-71.

Slate, D., Rupprecht, C.E., Rooney, J.A., Donovan, D., Lein, D.H., and Chipman, R.B. (2005). Status of oral rabies vaccination in wild carnivores in the United States. *Virus Research,* 111, 68-76.

Stallknecht, D.E. (2007). Impediments to wildlife disease surveillance, research, and diagnostics. *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission,* 315, 445-61.

Sterner, R.T., Meltzer, M.I., Shwiff, S.A., and Slate, D. (2009). Tactics and economics of wildlife oral rabies vaccination, Canada and the United States. *Emerging Infectious Diseases,* 15, 1176-84.

Stöhr, K. and Meslin, F.M. (1996). Progress and setbacks in the oral immunisation of foxes against rabies in Europe. *Veterinary Record,* 139, 32-35.

Sundqvist, A. K., Ellegren, H., and Vilà, C. (2008). Wolf or dog? Genetic identification of predators from saliva collected around bite wounds on prey. *Conservation Genetics,* 9, 1275-79.

Taylor, L.H. and Latham, S.M. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* 356, 983-89.

Thompson, C.M. and Gese, E.M. (2007). Food webs and intraguild predation: community interactions of a native mesocarnivore. *Ecology,* 88, 334-46.

Thorne, E.T. and Williams, E.S. (1988). Disease and endangered species: the black-footed ferret as a recent example. *Conservation Biology,* 2, 66-74.

Timm, S.F., Munson, L., Summers, B.A., et al. (2009). A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). *Journal of Wildlife Diseases,* 45, 333-43.

Totton, S.C., Tinline, R.R., Rosatte, R.C., and Bigler, L.L. (2002). Contact rates of raccoons (*Procyon lotor*) at a communal feeding site in rural eastern Ontario. *Journal of Wildlife Diseases,* 38, 313-19.

Vial, F., Cleaveland, S., Rasmussen, G., and Haydon, D.T. (2006). Development of vaccination strategies for the management of rabies in African wild dogs. *Biological Conservation,* 131, 180-92.

Vickers, T.W., Munson, L., and Garcelon, D.K. (2007), 'Unusually high prevalence of ceruminous gland carcinomas in Channel Island foxes on Santa Catalina Island,

California', (Abstract Presented at the 2007 Wildlife Disease Association Meeting, Estes Park, Colorado USA).
White, P. C. L., Harris, S., and Smith, G. C. (1995). Fox contact behaviour and rabies spread: a model for the estimation of contact probabilities between urban foxes at different population densities and its implications for rabies control in Britain. *Journal of Applied Ecology,* 32, 693-706.
Williams, C. L., Blejwas, K., Johnston, J. J., and Jaeger, M. M. (2003). A coyote in sheep's clothing: predator identification from saliva. *Wildlife Society Bulletin,* 31, 926-32.
Williams, E.S. and Barker, I.K. (2001), *Infectious Diseases of Wild Mammals*; Wiley-Blackwell: Ames.
Wobeser, G.A. (2007), *Disease in Wild Animals: Investigation and Management*; Springer Verlag: Berlin, Germany.
Wong, D., Wild, M.A., Walburger, M.A., et al. (2009). Primary pneumonic plague contracted from a mountain lion carcass. *Clinical Infectious Diseases,* 49, 33-38.
Woodroffe, R., Donnelly, C.A., Cox, DR, et al. (2006). Effects of culling on badger *Meles meles* spatial organization: implications for the control of bovine tuberculosis. *Journal of Applied Ecology,* 43, 1-10.

Box 1. Minimum list of supplies and equipment a researcher should have available during field necropsy (Munson 2006)

Personal Protective Equipment
Gloves (rubber, latex, or nitrile)
Rubber boots or plastic foot protectors
Scrubs or coveralls
Mask (to cover mouth and nose)
Eye protection

Necropsy Equipment and Supplies
Camera
Field notebook and/ or necropsy forms
Water-proof writing utensils
Labeling tape or tags
Measuring tape
Sharp knife and sharpening tool
Scalpels and razor blades
Scissors
Forceps
Ax or hatchet
Bone saw
Sterile syringes and needles
Blood tubes (red-tops for centrifugation and purple-top EDTA)
Sterile polyester swabs
Rigid plastic containers with airtight lids for samples in formalin
Sterile airtight vials for samples (swabs, ectoparasites)
Plastic bags (zip-lock or whirl-pack)
Ice coolers and ice packs
Leak-proof, break-proof containers

Fixatives and Disinfectants
10% buffered formalin
95 – 100% ethanol for fecal and exudate swabs
70% ethanol for parasites
Disinfectants (10% bleach, quaternary ammonium compounds)
Alcohol lamp or gas burner for sterilizing instruments

Table 1. Biological sample collection purposes and techniques for storage and transport in the study of carnivore disease. Also consult with collaborating laboratory for lab-specific preferences on storage and shipping.

Sample Type	*Purpose*	*Container Type*	*Storage (ST=short-term; LT=long-term)*	*Transport*
Blood: Serum or Plasma	Determine antibody presence and titers; biochemistry panels to detect differences in selected markers	Red-top tube or sterile container with no anticoagulant	Let clot for 20 minutes, centrifuge after clot forms, transfer supernatant, discard red cell clot. Avoid direct sunlight. ST: < 48 hours, refrigerate LT: > 48 hours, -20 to -80C freezer	Use coolers with ice to transport.
Blood: Whole Blood	Assess health parameters, such as inflammation, anemia; determine pathogen presence, antibody presence and titers	Purple-top tube with an anticoagulant (e.g. EDTA)	Refrigerate, do not freeze before certain selected tests. Avoid direct sunlight. ST: < 48 hours, refrigerate. LT: > 48 hours, -20 to -80C freezer	Use coolers with ice to transport.
Feces (Fresh or Old)	Molecular detection of non-labile viruses (parvoviruses, etc.) and some endoparasites and their ova	Clearly labeled airtight plastic container; if rectal swab is used, also store in airtight plastic container	ST: Store in 95-100% ETOH and refrigerate. Avoid direct sunlight. LT: Store in 95-100% ETOH and freeze in -20 to -80C freezer.	Use coolers with ice to transport.
Feces (Fresh Only)	Detection of endoparasites and their ova; analysis of fecal hormones	Clearly labeled airtight plastic container	ST: Refrigerate and keep cool for analysis ≤ 2 hours. Avoid direct sunlight. LT: If parasites must be stored, 5 – 10% buffered formalin to fix ova.	Use coolers with ice to transport.
Exudates (nasal, ocular, wounds)	Molecular detection of viruses shed in exudates; bacterial culture	Synthetic-tipped swabs placed in an airtight plastic container containing either guanidine-thyocyanate solution, 95-100% molecular grade ethanol, or a commercially-produced RNA/DNA preservative	ST and LT: Freeze in -20 to -80C freezer. Avoid direct sunlight. For bacterial culture, refrigerate and send to lab as soon as possible.	Use coolers with ice to transport.
Urine	Detection of pathogens shed in urine	Clearly labeled airtight plastic container	Neutralize urine with phosphate buffered saline solution. ST: ≤ 24 hours, refrigerate LT: > 24 hours, freeze at -20 to -80C	Use coolers with ice to transport.
Ectoparasites (ticks, fleas, mites, etc.)	Species determination of parasites and possible pathogens they vector	Clearly labeled airtight plastic container	ST and LT: (ticks, fleas, lice): ≥ 70% ETOH and 5% glycerol ST and LT: (mites): 87 parts 70% ethanol, 5 parts glycerin, 8 parts glacial acetic acid	Keep in a cool dry place; do not freeze.
Endoparasites (flukes, nematodes, tapeworms, etc.)	Species determination of parasite and possible pathogens they harbor	Clearly labeled airtight plastic container	ST and LT: 92 parts 70% ethanol, 3 parts 40% formaldehyde, 5 parts glycerin	Keep in a cool dry place; do not freeze.

Table 2. Computer software available for disease modeling, spatial analysis, and disease risk assessment. Freeware programs are marked with an asterisk (*).

Name	Application	Website & Manufacturer
@RISK	Disease risk analysis tool for Microsoft Excel using Monte Carlo simulations	http://www.palisade.com/risk/ Palisade Corp., Ithaca, NY USA
Precision Tree	Performs decision analysis in Microsoft Excel using decision tree and influence diagrams	http://www.palisade.com/precisiontree/ Palisade Corp., Ithaca, NY USA
STELLA	Icon-based and graphically oriented systems modeling and simulation software	http://www.iseesystems.com/softwares/Education/StellaSoftware.aspx Isee Systems Inc., Lebanon, NH USA
Vensim	Graphical development, analysis and packaging program for dynamic feedback models	http://www.vensim.com/software.html Ventana Systems Inc., Harvard, MA USA
AnyLogic	Simulation modeling software for systems dynamics, discrete-event and agent-based models	http://www.xjtek.com/anylogic/why_anylogic/ XJ Technologies, Hampton, NJ USA
VORTEX*	Population viability analysis	http://www.vortex9.org/vortex.html Bob Lacy, Dept. of Conservation Science, Chicago Zoological Society, Brookfield, IL, USA
Model Builder*	Graphical tool for designing, simulating and analyzing ordinary differential equation mathematical models	http://sourceforge.net/projects/model-builder/
SaTScan*	Spatial, temporal, space-time scan statistical program to detect spatial and temporal clusters of disease	http://www.satscan.org Martin Kulldorff, Harvard Medical School, 133 Brookline Avenue, 6th Floor, Boston, MA USA
FlexScan*	Spatial scan statistical program to detect spatial clusters of disease; allows users to define spatial connections among data	http://www.niph.go.jp/soshiki/gijutsu/download/flexscan (Tango and Takahashi 2005)

CHAPTER 2

Molecular Techniques for Identifying Intraguild Predators of Fishers and other North American Small Carnivores

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GRETA M. WENGERT,[1,2] *Canid Diversity and Conservation Unit, Veterinary Genetics Laboratory, University of California Davis, Davis, CA 95616, USA*

MOURAD W. GABRIEL, *Integral Ecology Research Center, McKinleyville, CA 95519, USA; and Canid Diversity and Conservation Unit, Veterinary Genetics Laboratory, University of California Davis, Davis, CA 95616, USA*

JANET E. FOLEY, *Veterinary Medicine and Epidemiology, University of California Davis, Davis, CA 95616, USA*

TERI KUN, *Forensics Unit, Veterinary Genetics Laboratory, University of California Davis, Davis, CA 95616, USA*

BENJAMIN N. SACKS, *Canid Diversity and Conservation Unit, Veterinary Genetics Laboratory, University of California Davis, Davis, CA 95616, USA*

[1] *E-mail: gmwengert@ucdavis.edu*

[2] *Present address: Integral Ecology Research Center, 102 Larson Heights Road, McKinleyville, CA 95519, USA*

ABSTRACT Identifying predators of threatened and endangered species is important for understanding and reducing the impacts of predation. Visible evidence collected from a carcass alone is often insufficient to accurately identify predator species. The DNA from the predator left

on the carcass allows for a definitive identification of predator species associated with the carcass, but DNA can be difficult to isolate independently from the prey. We developed field collection and molecular protocols for amplifying canid and felid predator DNA from saliva on fisher (*Martes pennanti*) carcasses without amplifying fisher DNA itself. We tested the protocol on fisher carcasses suspected of having been killed by a bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), coyote (*Canis latrans*), and domestic dog. We successfully amplified and sequenced DNA from these 4 predator species, confirming predation by them on fishers. We confirmed that these protocols can also identify other felid and canid predators of several other small North American carnivores.

KEY WORDS fisher, forensic, intraguild predation, *Martes pennanti,* polymerase chain reaction, predator.

Determining predators of threatened or endangered species is essential to conservation efforts (Ratz et al. 1999, Ernest et al. 2002, Benson et al. 2010). Identification of predator species based solely on physical evidence visible at kill sites is difficult, even for seasoned field ecologists acutely familiar with their focal species (Larivière 1999, Rosas-Rosas et al. 2008). Poor carcass or environmental conditions and similarities in attack pattern by different species can hinder accurate identification of predators (Williams and Johnston 2004). For example, bite wound size and position are often used to identify predator species (Yom-Tov et al. 1995, Lyver 2000), but environmental conditions or autolytic state of carcasses often promote morphological changes and loss of skin structure, resulting in alteration of wound size, spacing, and shape, and ambiguity and inaccuracy in identifying predators (L. Munson, University of California Davis

School of Veterinary Medicine, personal communication).

Technological advances have added to the tools available for identification of predators. For example, remote cameras have been used to identify nest predators (Williams and Wood 2002). Scats have been used to identify predators both from prey remains in scats (Neale et al. 1998, Sundqvist et al. 2008) and from predator DNA from scats left at a carcass (Ernest et al. 2002). However, scat-based methods are not ideal for distinguishing scavenging from predation. The DNA swabbed from wounds confirmed to be antemortem because of associated hemorrhage provides a direct means of determining predator species and has been used, for example, to identify individual predators of livestock (Blejwas et al. 2006). The considerable phylogenetic divergence between carnivores and most prey species reflected in mitochondrial DNA facilitates specific amplification of carnivore DNA, avoiding contamination by the prey DNA. This approach is more difficult for the identification of predators in the same taxonomic order as prey (i.e., Carnivora), and therefore, has not yet been used to study intraguild predation.

The recent designation of the fisher (*Martes pennanti*) as a candidate for listing under the U.S. Endangered Species Act has stimulated a flurry of research on this species in California, providing opportunities to investigate cases of suspected intraguild predation in northern California and in the southern Sierra Nevada mountains. We developed field collection and genetic protocols for the collection and analysis of forensic data and samples from carcasses of fishers to assist in the determination of species responsible for intraguild predation.

STUDY AREA

We investigated fisher carcasses from 2 California fisher research projects, one in the southern Sierra Nevada Mountains (Kings River Fisher Project) and one in northwestern California (Hoopa Valley Indian Reservation Fisher Project). Elevations within the Kings River Fisher

Project ranged from 1,100 m to 2,282 m and dominant forest types included montane hardwood conifer, mixed conifer, and ponderosa pine with small patches of montane chapparal, barren rock, and wet meadows. The Hoopa Valley Indian Reservation fisher project was located about 50 km northeast of Eureka, California, where elevations ranged from 98 m to 1,170 m. The dominant vegetation types are Douglas-fir (*Pseudotsuga menziesii*) and montane hardwood conifer, and meadows occur sparsely throughout the project area.

METHODS

Field Protocol

Four fisher carcasses were recovered ≤3 days after detection of a very high frequency mortality signal by field crews between Spring 2007 and Winter 2012. Because predation was suspected as the cause of death, we photographed all visible bite wounds and location of the carcass, and recorded if and where carcasses were cached. We measured distance between canine tooth punctures. We collected any non-fisher hairs on or near the carcass. One fisher was suspected of having been killed by a bobcat (*Lynx rufus*) because of putative bobcat hairs found on the carcass; another was suspected of having been killed by a coyote (*Canis latrans*) because of a recent coyote sighting in the area; the third fisher was suspected of having been killed by a mountain lion (*Puma concolor*) because a fresh mountain lion scat was found near the carcass; and the last fisher was suspected of having been killed by a domestic dog due to dog tracks near the carcass. In an effort to obtain DNA from predator saliva (including shed epithelial cells), we collected 2 types of forensic samples: 1) synthetic-tipped swabs rubbed aggressively within bite wounds, and 2) matted fisher fur clipped (to avoid hair roots) within 1 cm of any bite wounds. Swabs were stored dry in 1.5 mL or 2.0 mL air-tight plastic vials and frozen at –20° C until further analysis.

Molecular Analyses

To extract DNA from the bite wound swabs and matted fur samples, 250–400 μL of 1× phosphate-buffered saline solution was first added to each vial containing the swab or fur sample, and the vial was gently vortexed for 60–90 seconds. We then used a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) to extract DNA from 200 μL of this solution according to the manufacturer's protocol for blood samples. To extract DNA from predator hairs left on the carcass, hair follicles were digested overnight following manufacturer's instructions for tissue extraction in the DNeasy Blood and Tissue Kit (QIAGEN).

We chose primer pairs developed by the Forensics Unit of the University of California Davis Veterinary Genetics Laboratory that were family-specific for Felidae (Felid HV1A, Felid HV1B2) and Canidae (Canid HV1A, Canid HV1C) that would amplify variable regions of the mitochondrial genome in Hypervariable region I of the D loop, allowing us to produce sequences to differentiate species within each family (Table 1). Alignments of the primers with sequences of these 4 predator species indicated they were family-specific (Fig. 1). These D-loop fragments ranged from 200 base pairs to 300 base pairs (bp) for felids and from 300 bp to 400 bp for canids. We conducted polymerase chain reactions (PCR) in 25 μL reactions, which included 3 μL of DNA template, 1 U *Taq* polymerase (Titanium Taq; Clontech, Mountainview, CA), 6 μL of 5× reaction buffer (with MgCl), 1.2 mM of total DNTPs, and primers (i.e., felid or canid) at 0.7 μM concentration. Reactions were conducted with an initial denaturation step of 1 minute at 95° C, followed by 36 cycles of 20 seconds of denaturation at 95° C, 30 seconds of annealing at 55° C (felid) or 51° C (canid), and 40 seconds of extension at 72°C, and lastly, a final extension of 10 minutes at 72° C.

We electrophoresed the PCR products on a 1.0% agarose gel with GelStar (Lonza Group

Limited, Basel, Switzerland) as a nucleic acid stain and visualized them using a Dark Reader non-ultraviolet transilluminator (Clare Chemical Research, Inc., Dolores, CO). We excised 1–2 of the strongest gel bands from each carcass in the range of 200–300 bp for felids and 300–400 bp for canids and gel-extracted them using the Qiagen Gel Extraction kit (QIAGEN) according to the manufacturer's instructions. We sequenced the 5' to 3' DNA strand PCR products using Big Dye Terminator cycle-sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3730 DNA Analyzer (Applied Biosystems). Sequences were aligned using RidomTraceEdit (Ridom GmbH, Würzberg, Germany) and cross-referenced on GenBank using the basic local alignment search tool to determine closest match to published species sequences.

Tests of Protocol Specificity

To verify that the primers would not amplify DNA from non-target carnivore species (especially fishers) and to determine other North American felids and canids detectable by the corresponding primers, we conducted PCR on DNA extracted from blood from several other North American carnivores. Test species included coyote, domestic dog, gray wolf (*C. lupus*), red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), bobcat, mountain lion, Canada lynx (*Lynx canadensis*), fisher, marten (*M. americana*), black bear (*Ursus americanus*), raccoon (*Procyon lotor*), ringtail (*Bassariscus astutus*), striped skunk (*Mephitis mephitis*), and western spotted skunk (*Spilogale gracilis*). The PCR reactions were conducted on 2 DNA samples from each of these species according to the methods outlined above.

RESULTS

We sampled wounds on each carcass from injuries confirmed to have occurred before death (antemortem) by the presence of subcutaneous hemorrhaging, which was verified through gross necropsy and histology. For the fisher suspected to have been killed by a bobcat, a non-fisher

hair, 5 swabs, and 1 matted fur sample from wounds on the fisher yielded DNA fragments of approximately 200 bp that were amplified using the felid primers but not the canid primers (Table 2). The non-fisher hair sample and one swab sample sequenced matched a bobcat sequence with 100% homology (GenBank no. GQ979707.3). For the fisher suspected to have been killed by a mountain lion, 4 swabs and 1 matted fur sample from wounds on the fisher yielded DNA fragments of approximately 260 bp that were amplified using the felid primers but not the canid primers (Table 2). Both a swab and matted fur sample were sequenced and matched a mountain lion sequence with 98% homology (GenBank no. JN999997.1). For the fisher suspected to have been killed by a coyote, 3 swabs and 1 matted fur sample from wounds on the fisher yielded DNA fragments of approximately 380 bp that were amplified using the canid primers but not felid primers (Table 2). The matted fur sample was sequenced and it most closely matched a coyote sequence, including 97% homology (Genbank no. FJ213925.2). For the fisher suspected to have been killed by a dog, 2 swabs from wounds on the fisher yielded DNA fragments of approximately 400 bp that were amplified using the canid primers but not felid primers (Table 2). Both sequences most closely matched a dog sequence with 100% homology (Genbank no. HE687017.1).

In tests of known carnivore DNA samples, the felid primers amplified an approximately 200-bp DNA fragment from bobcat and 260-bp DNA fragments from mountain lion and Canada lynx, but did not amplify DNA from any of the other species tested. The canid primers amplified an approximately 380-bp DNA fragment from coyote and 400-bp DNA fragments from dog and wolf, but only weakly amplified red fox and gray fox. The canid primers also amplified an approximately 270-bp DNA fragment from ringtail (Genbank no. KC427988) and weakly amplified an approximately 270-bp DNA fragment from one striped skunk DNA sample,

although this product was not sequenceable. Neither primer set amplified DNA from fisher, American marten, raccoon, bear, or western spotted skunk.

DISCUSSION

We developed a protocol for genetic analysis of fisher carcasses that enabled determination of fisher predators from both Felidae and Canidae. Our results show that DNA left on the carcass from saliva of the predator can be amplified through PCR and differentiated from DNA of the fisher. Previous predation forensics work focused on wildlife predators of livestock and was successful in identifying individual coyotes (Williams et al. 2003, Blejwas et al. 2006) and dogs or wolves (Sundqvist et al. 2008) responsible for killing sheep. Our research builds on this foundation by extending the approach to intraguild predators of smaller carnivores. The greater similarity in mitochondrial sequences among species within the Carnivora order than between orders (e.g., Carnivora vs. Ruminantia) required us to choose different primers for the 2 primary carnivore families most likely to prey on fishers to achieve the necessary specificity. Nevertheless, this protocol was successful in differentially amplifying felid and canid DNA from saliva left on the 4 fisher carcasses.

Additionally, tests of this protocol on other carnivore species demonstrated their potential utility for identification of other felid and canid predators, including Canada lynx and wolves. These species have been suspected of killing fishers and other small carnivores across North America (Apps 1999, White et al. 2002). However, poor success of the canid primers in amplifying DNA from the 2 fox species suggest they would not be as useful in identifying fox predators of smaller carnivores. Although we found no documentation of intraguild predation from gray foxes, red foxes have killed smaller carnivores such as American marten (Thompson 1994) and European pine marten (*Martes martes;* Lindström et al. 1995).

Lack of amplification of DNA from American marten, raccoon, and western spotted skunk using both primer pairs suggests that they would be effective in differentiating predator DNA from carcasses of these species in addition to fisher. Correspondingly, bobcats and coyotes have been suspected of killing American martens (Bull and Heater 2001), coyotes of killing raccoons (Kamler and Gipson 2004), and dogs of killing eastern spotted skunks (*Spilogale putorius;* Crabb 1948). Our protocol could, therefore, be used to assess predation by canids and felids on these small carnivore species. Unfortunately, our canid primers amplified ringtail and weakly amplified striped skunk DNA, which indicated the need for further optimization before using our approach on these species.

MANAGEMENT IMPLICATIONS

Given the potential significance of intraguild predation in the population dynamics and life history of small to mid-sized carnivores (Palomares and Caro 1999), methods to identify predator species are essential. Without definitive knowledge of predator species, quantitative estimates of impacts due to specific predators cannot be addressed appropriately through conservation and management programs. The protocol described here provides an accurate and effective way to identify felid and canid predators of fishers and other small carnivores in North America, such that field researchers and wildlife managers can correctly estimate the predator-specific impacts of predation on small and mid-sized carnivore populations and take appropriate management and conservation actions.

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LITERATURE CITED

Apps, C. D. 1999. Space-use, diet, demographics, and topographic associations of lynx in the southern Canadian Rocky Mountains: a study. Pages 351–371 *in* K. B. A. L.F. Ruggiero, S. W. Buskirk, G. M. Koehler, C. J. Krebs, K. S. McKelvey, and J. R. Squire, editor. Ecology and conservation of lynx in the United States. U.S. Department of Agriculture, General Technical Report GTR7 RMRS-30WWW, Rocky Mountain Research Station, Fort Collins, Colorado, USA.

Benson, T. J., J. D. Brown, and J. C. Bednarz. 2010. Identifying predators clarifies predictors of nest success in a temperate passerine. Journal of Animal Ecology 79:225–234.

Blejwas, K. M., C. L. Williams, G. T. Shin, D. R. McCullough, and M. M. Jaeger. 2006. Salivary DNA evidence convicts breeding male coyotes of killing sheep. Journal of Wildlife Management 70:1087–1093.

Bull, E. L., and T. W. Heater. 2001. Survival, causes of mortality, and reproduction in the American marten in northeastern Oregon. Northwestern Naturalist 82:1–6.

Crabb, W. D. 1948. The ecology and management of the prairie spotted skunk in Iowa.

Ecological Monographs 18:201–232.

Ernest, H. B., E. S. Rubin, and W. M. Boyce. 2002. Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. Journal of Wildlife Management 66:75–85.

Kamler, J. F., and P. S. Gipson. 2004. Survival and cause-specific mortality among furbearers in a protected area. American Midland Naturalist 151:27–34.

Larivière, S. 1999. Reasons why predators cannot be inferred from nest remains. The Condor 101:718–721.

Lindström, E. R., S. M. Brainerd, J. Helldin, and K. Overskaug. 1995. Pine marten–red fox interactions: a case of intraguild predation? Annales Zoologici Fennici 32:123–130.

Lyver, P. O. B. 2000. Identifying mammalian predators from bite marks: a tool for focusing wildlife protection. Mammal Review 30:31–44.

Neale, J. C. C., B. N. Sacks, M. M. Jaeger, and D. R. McCullough. 1998. A comparison of bobcat and coyote predation on lambs in north-coastal California. Journal of Wildlife Management:700–706.

Palomares, F., and T. M. Caro. 1999. Interspecific killing among mammalian carnivores. American Naturalist 153:492–508.

Ratz, H., H. Moller, and D. Fletcher. 1999. Predator identification from bite marks on penguin and albatross chicks. Marine Ornithology 27:149–156.

Rosas-Rosas, O. C., L. C. Bender, and R. Valdez. 2008. Jaguar and puma predation on cattle calves in northeastern Sonora, Mexico. Rangeland Ecology & Management 61:554–560.

Sundqvist, A. K., H. Ellegren, and C. Vilà. 2008. Wolf or dog? Genetic identification of predators from saliva collected around bite wounds on prey. Conservation Genetics 9:1275–1279.

Thompson, I. D. 1994. Marten populations in uncut and logged boreal forests in Ontario. Journal of Wildlife Management 58:272–280.

White, K. S., H. N. Golden, K. J. Hundertmark, and G. R. Lee. 2002. Predation by wolves, *Canis lupus,* on wolverines, *Gulo gulo,* and an American Marten, *Martes americana,* in Alaska. Canadian Field Naturalist 116:132–134.

Williams, C. L., K. Blejwas, J. J. Johnston, and M. M. Jaeger. 2003. A coyote in sheep's clothing: predator identification from saliva. Wildlife Society Bulletin 31:926–932.

Williams, C. L., and J. J. Johnston. 2004. Using genetic analyses to identify predators. Sheep and Goat Research Journal 19:85–88.

Williams, G. E., and P. B. Wood. 2002. Are traditional methods of determining nest predators and nest fates reliable? An experiment with wood thrushes (*Hylocichla mustelina*) using miniature video cameras. The Auk 119:1126–1132.

Yom-Tov, Y., S. Ashkenazi, and O. Viner. 1995. Cattle predation by the golden jackal *Canis aureus* in the Golan Heights, Israel. Biological Conservation 73:19–22.

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Figure 1. Alignments of 4 predator species' partial sequences (bobcat, *Lynx rufus;* mountain lion, *Puma concolor;* domestic dog; and coyote, *Canis latrans*) from the D-loop region of the mitochondrial genome with the 4 primers used to identify predators of fisher (*Martes pennanti*) between Spring 2007 and Winter 2012 at the Hoopa Valley Indian Reservation Fisher Project, Hoopa, CA and Kings River Fisher Project in the Sierra Nevadas, CA. Periods indicate the same nucleotide as the reference sequence at the top of each alignment, and dashes represent deletions.

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bobcat          CAAGGAAGAA GCAACAGCCC CACTATCAGC ACCCAAAGCT GAAATTCTTT CTTAAACTAT TCCTTGCCA
mountain lion   .......... .......... .......... .......... .......... .......... .........
fisher          .......... ....T....T .G.C...... .......... ..C.....AA ..-....... ...C..A.C
domestic dog    .......... ..TCTT..T. ...C...... .......... ........-. .......... ...C..A..
coyote          .......... ..TCTT..T. ...C...... .......... ........-. .......... ...C..ATG
Felid-HV1a                            C CACTATCAGC ACCCAAAGC

bobcat          ATCG--TGCA TTAATTGCCA GTCCCCATGA ATATTAAGC- -ATGTACA-- -GTAGTTTAT ATATATTACA
mountain lion   .......... ....C..... .......... ......G... .......... .......... ..........
fisher          T-.AAA..GG AC.TC.CGAT .GA.TA.... C..A.C...C C...AT..CA CA..A..GTG G.G.CA.G..
coyote          CG.AAA..GG AC.TC.CGAT .GA.TA.... C..A.C...C C...AT..CA CA..AC.GTG G.G.CA.G..
domestic dog    CG.AAA..GG AC.TC.CGAT .GA.TA.... C..A.C...C C...AT..CA CA..AC.GTG G.G.CA.G..
Felid-HV1b2                           A GTCCCCATGA ATATTAAGC. .ATG

domestic dog    CTGAGATTCT T-CTTAAACT ATTCCCTGAC ACCCCTACAT TCATATATTG AATCACCCCT ACTGTGCTAT
coyote          ....A..... .......... .......... .......... .......... .......... ..........
fisher          ....C..... AA..-..... .......... -.TATC...C CTTA..TCAT .TATTTAATA ..ATCT.ATG
bobcat          ....A..... .T........ .....T..-. -.AAT---.C C..A.ACCA- --A.C..A.A ...T.CAC.A
mountain lion   ....A..... .T........ .....T..-. -.AAT---.C CAGA.ACCA- --A.C..A.A ...TCCA.GA
Canid-HV1A                              CCCTGAC ACCCCTACAT TCA

domestic dog    ATCTCGATGG ACTAATGACT AATCAGCCCA TGATCACACA TAACTGTGGT GTCATGCATC TGGTATCTTT
coyote          .......... .......... .......... .......... .......... .........T ..........
fisher          .......... .......... .......... .......... .......... .........T ......T...
bobcat          CAG..----- -.CC....A. .T.A...--. ..TA..---G ..GT.TATA. A.AT.A...A A..C..AC.A
mountain lion   TG..A.T--- C.CC....A. .T.AG..--. ..TA..---G ..GT.TATA. A.AT.A...A A.AC..AC.A
Canid-HV1c                              TCAGCCCA TGATCACACA TAA
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Table 1. Primers used to identify intraguild predator species of fishers (*Martes pennanti*) using DNA from bite wounds on carcasses between Spring 2007 and Winter 2012 at the Hoopa Valley Indian Reservation Fisher Project, Hoopa, CA and Kings River Fisher Project in the Sierra Nevadas, CA.

Primer	Sequence
Felid HV1A	CCACTATCAGCACCCAAAGC
Felid HV1B2	TTATGTGTGATCATGGGCTGA
Canid HV1A	CCCTGACACCCCTACATTCA
Canid HV1C	TTATGTGTGATCATGGGCTGA

Table 2. Number of swab and matted fur samples taken from 4 fisher (*Martes pennanti*) carcasses suspected of being killed by bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), coyote (*Canis latrans*), and domestic dog between Spring 2007 and Winter 2012 at the Hoopa Valley Indian Reservation Fisher Project, Hoopa, CA and Kings River Fisher Project in the Sierra Nevadas, CA; and number of samples with successful predator DNA amplification through polymerase chain reaction. NA indicates that no samples of this type were tested through polymerase chain reaction and, therefore, no results were obtained.

Predator species	No. swabs collected and tested	No. swabs yielding predator DNA	No. matted fur samples collected and tested	No. matted fur samples yielding predator DNA	No. non-fisher hairs tested	No. non-fisher hairs yielding predator DNA
Bobcat	5	5[a]	3	1	1	1[a]
Mountain lion	4	4[a]	1	1[a]	0	NA
Coyote	4	3	1	1[a]	0	NA
Domestic dog	2	2[b]	0	NA	0	NA

[a] One DNA sample of this type was successfully sequenced and the predator species was confirmed when cross-referenced on GenBank.

[b] Two DNA samples of this type were successfully sequenced and the predator species was confirmed when cross-referenced on GenBank.

Chapter 3

Intraguild Predation on Fishers in California: Patterns of Predation by Three Larger Carnivores

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GRETA M. WENGERT[1], *University of California Davis, Veterinary Genetics Laboratory, Davis, CA 95616, USA and Integral Ecology Research Center, Blue Lake, CA 95525, USA*

MOURAD W. GABRIEL, *Integral Ecology Research Center, Blue Lake, CA 95525, USA*

SEAN M. MATTHEWS, *Wildlife Conservation Society, Hoopa, CA 95546, USA*

J. MARK HIGLEY, *Wildlife Department, Hoopa Tribal Forestry, Hoopa, CA 95546, USA*

RICK A. SWEITZER, *Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA*

CRAIG M. THOMPSON, *USDA Forest Service, Pacific Southwest Research Station, 2081 E. Sierra Avenue, Fresno, CA 93710, USA*

KATHRYN L. PURCELL, *USDA Forest Service, Pacific Southwest Research Station, 2081 E. Sierra Avenue, Fresno, CA 93710, USA*

REGINALD H. BARRETT, *Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA*

LESLIE W. WOODS, *California Animal Health and Food Safety Laboratory System, Davis, California 95616, USA*

REBECCA E. GREEN, *USDA Forest Service, Pacific Southwest Research Station, 2081 E. Sierra Avenue, Fresno, CA 93710, USA*

[1] gmwengert@ucdavis.edu

STEFAN M. KELLER, *Department of Pathology, Microbiology and Immunology, University of California, One Shields Avenue, Davis, California 95616, USA*

PATRICIA M. GAFFNEY, *Department of Pathology, Microbiology and Immunology, University of California, One Shields Avenue, Davis, California 95616, USA*

MEGAN JONES, *Department of Pathology, Microbiology and Immunology, University of California, One Shields Avenue, Davis, California 95616, USA*

BENJAMIN N. SACKS, *University of California Davis, Veterinary Genetics Laboratory, Davis, CA 95616, USA*

ABSTRACT Intraguild predation is common among carnivores and can have population-level effects on imperiled species. Rates of intraguild predation can differ between sexes, among age classes, and among populations of the intraguild prey species. The fisher (*Martes pennanti*) is a rare forest carnivore in western North America and a candidate for listing under the United States Endangered Species Act. Intraguild predation is poorly understood in fishers and a potential threat to existing western populations. We studied the prevalence and patterns of intraguild predation on fishers in the southern Sierra Nevada mountains and Coastal Range of California. We recovered 101 (59 female, 42 male) fisher carcasses, 62 (61%) for which death was attributed to predation. We collected forensic evidence and samples from the carcasses and predation sites, conducted full necropsies when possible, and used molecular methods to determine species of predators responsible for killing fishers. We found that bobcats (*Lynx rufus*, $n = 25$ fisher mortalities), mountain lions (*Puma concolor*, $n = 20$), and coyotes (*Canis latrans, n* = 4) were the predators of fishers in our study areas. Bobcats killed only female fishers while mountain lions more frequently killed male than female fishers, confirming our hypothesis that

female fishers would suffer predation from smaller predators than would male fishers. Coyotes were a rare predator of fishers. We identified that fisher sex and whether the skull suffered depressed fracture or nearly complete consumption were the most discriminating physical characteristics of predation events for identifying predators.

KEY WORDS bobcat, *Canis latrans*, coyote, fisher, intraguild predation, *Lynx rufus*, *Martes pennanti*, mountain lion, predator, *Puma concolor*

Intraguild predation has been documented regularly in nature, frequently among the larger carnivorous mammals (Palomares and Caro 1999, Janssen et al. 2007, Vance-Chalcraft et al. 2007). This interaction can be the consequence of intense competition for resources resulting in interference competition, or simply opportunistic predation by a larger carnivore on a smaller competitor (Polis et al. 1989). Regardless of the underlying causes, the population-level effects of intraguild predation can be substantial (Creel and Creel 1996, White and Garrott 1997, Roemer et al. 2001, Berger and Gese 2007). It can regulate populations directly but also indirectly affect population dynamics by forcing intraguild prey into marginal habitat to escape predation (Polis and Holt 1992, Mills and Gorman 1997). Intraguild predation has reduced abundance or altered distributions of several sensitive carnivore species throughout the world, including San Joaquin kit fox (*Vulpes macrotis mutica*; Cypher and Spencer 1998); African wild dog (*Lycaon pictus,* Creel and Creel 1996); swift fox (*Vulpes velox,* Thompson and Gese 2007); and Channel Island fox (*Urocyon littoralis,* Roemer et al. 2001).

Sexes and age classes within a species can experience dissimilar vulnerabilities to different intraguild predator species (Polis and Holt 1992). Rates of intraguild predation can also fluctuate temporally as intensity of competition for shared resources changes with seasonal diets (Koehler and Hornocker 1991). Understanding the drivers of intraguild predation, the

demographic and seasonal patterns in predation rates, and the species of predators responsible is essential for conservation efforts to mitigate the population-level effects in a vulnerable population.

The fisher (*Martes pennanti*) is a mid-sized carnivore in the family Mustelidae that inhabits coniferous and mixed hardwood-coniferous forests of the western and eastern United States and southern Canada (Powell 1981). In California, the fisher historically ranged throughout the mixed coniferous forests of the northwest mountains, through the Cascade Range of north-central California, and south throughout most of the Sierra Nevada mountain range. Since the early 1900s the fisher's range in California has contracted, resulting in two isolated populations, one in the Coastal Range and Klamath mountains and a much smaller population in the southern Sierra Nevada mountains, estimated at fewer than 300 adult fishers (Spencer et al. 2011). In 2004, fisher populations in the western United States were deemed a candidate for the United States Endangered Species Act of 1973, with the determination that listing was "warranted but precluded" (United States Fish and Wildlife_Service 2004). Among the poorly understood threats to fishers is predation (United States Fish and Wildlife Service 2004). Though intraguild predation on fishers was known to occur occasionally and presumably limited to otherwise vulnerable individuals (Powell 1993, Powell and Zielinski 1994), until this study, the frequency of intraguild predation on healthy adult fishers and its significance in the mortality rates of resident fisher populations was unknown.

In this study, we determined the prevalence of intraguild predation on fishers, used molecular analysis to identify predators of fishers, and conducted complete necropsies to identify wounding patterns that could help discriminate among predator species. We analyzed the predation frequencies by different predator species in relation to study area, sex, age class, and

physical characteristics of the fisher carcasses and predation events to determine differential predation risks and characteristics from different predators. We tested the following hypotheses: 1) female fishers experience higher predation rates than males due to sexual size dimorphism between the fisher sexes; 2) female fishers experience predation from smaller species of predators than males due to their smaller size; and 3) predator species of fishers are distinguishable by physical characteristics of the predation event.

STUDY AREAS

We recovered deceased radio-collared fishers from 3 California research projects between 2007 and 2011. Two were in the southern Sierra Nevada mountains on the Sierra National Forest, the Sierra Nevada Adaptive Management Project (SNAMP) just south of Yosemite National Park and the Kings River Fisher Project (KRFP) south of SNAMP (Fig. 1). The third project was in northwestern California on the Hoopa Valley Indian Reservation (HVRFP, Fig. 1).

Elevation within SNAMP ranges from 1000 m to 1850 m and dominant habitat types included Sierran mixed conifer, montane hardwood conifer, and ponderosa pine (Mayer and Laudenslayer 1988). Elevation within the KRFP ranges from 1100 m to 2282 m and dominant forest types included montane hardwood conifer, Sierran mixed conifer, and ponderosa pine (*Pinus ponderosa*, Mayer and Laudenslayer 1988). Both the SNAMP and KRFP project areas included small patches of montane chapparal, barren rock, and wet meadows.

The HVRFP project was located within the Klamath physiographic province (Kuchler 1977) of northern California, about 50 km northeast of Eureka, California. Elevation ranges from 98 m to 1170 m. The dominant habitat types were Douglas-fir (*Pseudotsuga menziesii*) and

montane hardwood conifer. Meadows occurred sparsely throughout the HVRFP project area. Mid-sized to large carnivores potentially able to kill fishers within the 3 project areas included bobcat (*Lynx rufus*), coyote (*Canis latrans*), domestic dog, mountain lion (*Puma concolor*), black bear (*Ursus americanus*), great-horned owl (*Bubo virginianus*), barred owl (*Strix varia*, at HVRFP only), great gray owl (*S. nebulosa*, at SNAMP and KRFP only), northern goshawk (*Accipiter gentilis*), red-tailed hawk (*Buteo jamaicensis*), and golden eagle (*Aquila chrysaetos*).

METHODS

Field Data Collection

Fishers were radio-collared and tracked at SNAMP (R. Sweitzer and R. Barrett, unpublished data), KRFP (Thompson et al. 2011), and HVRFP (Matthews et al. 2011) with primary goals unrelated to this research. Collars were equipped with mortality or activity sensors, allowing us to detect fisher mortalities and recover carcasses 1–7 days after death in most cases. In all cases, the mortality site was photographed in detail. We collected data and samples following Wengert et al. (2013) when predation was suspected as the cause of death (e.g. obvious punctures, partial consumption). We recorded information on the characteristics of the predation event including patterns of consumption and evidence of caching or burying. Samples included swabs of visible bite wounds, clipped (to avoid fisher DNA in root bulbs) fur from near the bite wounds, swabs of the claws and teeth, and non-fisher hairs left on or near the carcass (Wengert et al. 2013). Carcasses were double-bagged in plastic bags, labeled, and transported back to the field offices where they were frozen in a -20°C freezer until being shipped to University of California, Davis for further analysis.

Pathology

We performed necropsies on all available fisher carcasses either at the University of California Davis, Veterinary Medical Teaching Hospital or California Animal Health and Food Safety Laboratory, Davis, CA. When possible, we determined cause of death for each fisher. When predation was determined to be the cause of death, all lesions attributed to predation were described in detail. We identified the presence or absence of the following lesions on each carcass: depressed skull fractures, full-thickness or subcutaneous-only skull punctures, cervical trauma or fracture, full-thickness or subcutaneous-only punctures in thoracic area or abdominal areas, lacerations in intercostal muscles, and punctures or lesions in extremities. We noted whether the lesions had associated hemorrhage and edema, which indicated ante-mortem wounds likely inflicted by the predator, to distinguish the wounds from scavenging. In 14 cases, too few remains were present to identify hemorrhage at wound sites, so only molecular analyses were conducted in these cases. Age-classes of the fishers at time of death was estimated as either adult (>2 yr of age), subadult (1 – 2 yr of age), and juvenile (<1 yr of age) based either on tooth wear or cementum annuli counts.

Molecular Analyses

We processed forensic samples and analyzed them for predator (either felid or canid) DNA according to the methods of Wengert et al. (2013). Because multiple polymerase chain reaction (PCR) products were occasionally obtained when the products were visualized on an agarose gel, we gel-excised the appropriately sized fragment (200 – 300bp for felids and 400 for canids) and extracted DNA using Qiagen Qiaquick Gel Extraction kit according to the manufacturer's instructions. The PCR products were sequenced, then aligned using RidomTraceEdit (Ridom GmbH, Würzberg, Germany). We cross-referenced the sequences on GenBank using Basic

Local Alignment Search Tool (BLAST) to match them to the most closely aligned sequence to identify species of predator DNA.

Statistical Analyses

We used Chi-square tests of independence or Fisher exact tests, as appropriate (Zar 1999) to determine whether certain fisher attributes or predation characteristics were more or less frequently associated with particular predator species. All analyses were conducted using program R version 2.14.1, (http://www.r-project.org, accessed 4 June 2012).

We used Random Forest (RF; Breiman 2001) to evaluate the discriminatory power of predation characteristics to identify fisher predators based on physical evidence alone. Predation characteristics considered included: 1) sex of the fisher, 2) presence of depressed fracture or near complete consumption of skull, 3) presence of focal skull punctures, 4) whether the skull was fully intact, 5) caching of the carcass, and 6) consumption of any part of the carcass. Random Forest is based on the construction of classification and regression trees (CART; Breiman 1984). CART is a nonparametric procedure that predicts group membership and provides a description of the basis for the classification and a means to test the significance of the classification (Breiman 1984, De'ath and Fabricius 2000). CART methods recursively partition training data into dichotomous groups. Data sorting is governed by the selection of a predictor variable that maximizes homogeneity in the split groups, as measured by a Gini index (Segurado and Araujo 2004, Garzon et al. 2006, Chan and Paelinckx 2008). Successive partitioning produces a classification tree, whose nodes determine a set of variables that predict group membership.

Random Forest improves the strength of single classification trees by generating an ensemble or "forest" of CART models each fitted with varying bootstrapped subsamples of the original data set (an approach referred to as bootstrap aggregation or "bagging"). Approximately

63% of the original observations are selected randomly and with replacement as a training dataset to grow each tree. Observations in the original data set that do not occur in a bootstrap sample are called out-of-bag observations. In addition, RF selects and uses random subsamples of the available predictor variables for partitioning the training data within individual classification trees, and therefore it controls for correlation among predictors and overfitting (Garzon et al. 2006, Cutler et al. 2007, Buechling and Tobalske 2011). The algorithm classifies each observation by majority vote of the individual trees. Estimates of classification error are calculated from the out-of-bag observations and are used to quantify the relative importance of predictor variables. The measure of variable importance computes the total decrease in node impurities (Gini index) for each variable given by the splitting of the variable. We generated RF models using forest sizes of 1,000 trees in the *R* package randomForest (Liaw and Wiener 2002).

RESULTS

From 2007 through 2011, we captured, radio-collared and tracked 188 fishers for which fate was known. We recovered and analyzed 101 radio-collared fisher carcasses (59 female, 42 male) for cause-specific mortality, of which 62 deaths (61%) were attributed to predation, either through necropsy or circumstantial forensic information. Forty-three (73%) female deaths and 19 (45%) male deaths were due to predation. We were able to amplify predator DNA from 50 (81%) of these predated carcasses (35 females and 15 males), including DNA from bobcat (n = 25 carcasses), mountain lion ($n = 20$), coyote ($n = 4$) and one carcass with both bobcat and lion DNA. No other carcasses had DNA from more than one predator species. All fishers were classified as either adult or subadult at the time of death, except three juvenile male fishers and two juvenile female fishers at KRFP, and one juvenile female and one juvenile male fisher at

SNAMP. Fisher predation was attributed to all three predator species at SNAMP and KRFP, but only bobcat and mountain lions at HVRFP (Table 1). Because there were only four coyote-killed fishers throughout the three study areas, we did not include them in statistical analyses. We also did not include the fisher carcass with both bobcat and lion DNA because we were uncertain which species was the predator. Fishers were killed by bobcats and mountain lions at similar relative frequencies across the three study areas ($\chi^2 = 4.758$, df = 2, $P = 0.116$), allowing us to pool data across study areas for further analyses.

All 25 fishers killed by bobcats were female, whereas only 7 of the 20 (35%) fishers killed by mountain lions were female (Table 1). Female fishers were more frequently killed by bobcats than by mountain lions, and male fishers were more frequently killed by mountain lions than by bobcats ($\chi^2 = 19.797$, df = 1, $P \leq 0.001$, Table 1). Coyotes killed 2 male and 2 female fishers. Predation frequency by lions of juvenile vs. adult and subadult fishers did not differ between fisher sexes ($\chi^2 = 0.073$, df = 1, $P = 0.613$). We did not evaluate predation frequency of juveniles between the sexes for bobcats because bobcats did not kill any male fishers. Thirty-seven (76%) of the 49 predated fishers for which we identified the predator were partially or mostly consumed, including 22 of 25 (88%) bobcat-killed fishers, 18 of 20 (90%) mountain lion-killed fishers, but none of the 4 coyote-killed fishers (Table 2). Bobcats and mountain lions consumed the fishers with similar frequencies ($\chi^2 = 0.0703$, df = 1, $P = 1.000$). Six of the fishers (12%) were cached in some manner, including 1 mountain lion kill, 1 coyote kill, and 2 bobcat kills discovered under snow or snow mixed with duff, and 2 carcasses, cached by coyotes, buried almost fully under soil (Table 2).

Thirty-six fisher carcasses exhibited skull trauma, either focal punctures ($n = 10$), depressed (i.e., crushed-bone) skull fractures ($n = 16$), or consumption of most of the skull,

leaving only partial jaw bones ($n = 8$), or consumption of the entire skull ($n = 2$). Mountain lions more frequently inflicted depressed skull fractures or left only small pieces of the skull or no skull remaining ($\chi^2 = 19.90$, df = 1, $P \leq 0.001$) than bobcats (Table 2). Thirteen of 25 (52%) bobcat-killed fishers had fully intact skulls. Of these, cardiac, lung, and tracheal punctures were the apparent causes of death. No mountain lion-killed or coyote-killed fishers had fully intact skulls. Bobcats left the skull fully intact more frequently than mountain lions ($\chi^2 = 12.20$, df = 1, $P \leq 0.001$). Though fisher carcasses killed by all three predator species exhibited trauma in the thoracic and abdominal regions, only coyote-killed fishers showed massive hemothorax and intercostal muscle tearing without external punctures in the skin (n=3).

Random Forest analysis indicated that fisher sex and whether the skull suffered depressed fracture and/or nearly complete consumption the fisher's skull were the two most discriminating factors in predicting predator species based on physical characteristics (Fig. 2) and the model was accurate 81.6% of the time. Results from the Random Forest confusion matrix indicated that mountain lion predation was overestimated (estimated at 26 while confirmed for 20), bobcat predation was underestimated (estimated at 20 while confirmed for 25), and coyote predation was underestimated (estimated at 3 while confirmed for 4, Table 3). The most common misidentification of predator using the Random Forest analysis was the six cases in which the predicted predator was mountain lion, but the confirmed predator was bobcat.

DISCUSSION

Predation composed 62% of all fisher deaths we investigated. The proportion of fisher mortalities caused by predation was higher than reported previously both in California (Buck 1982) and British Columbia (Weir and Corbould 2008). Powell and Zielinski (1994) suspected

that significant rates of predation of healthy adults would occur mainly in translocated fisher populations. In a study of reintroduced fishers translocated from Minnesota to northwest Montana, over half of the fisher deaths were attributed to predation (Roy 1991). Likewise in our study, over half of the fisher deaths were due to predation; however, ours were native fishers. Our study clearly indicates native populations, including adult fishers, are also susceptible to high rates of mortality from predation.

The species we identified as predators of fishers (mountain lions, bobcats, and coyotes) differ from those suspected of killing fishers in prior California accounts (Grinnell et al. 1937, Buck 1982, Buck et al. 1983) and in other areas throughout their range (Roy 1991, Krohn et al. 1994, Vashon et al. 2002, Weir and Corbould 2008). Bobcats were the most frequent fisher predator in our study, but not previously reported to prey on fishers. Conversely, we found coyotes to be a rare predator of fisher, whereas coyote was the most frequently cited predator of fisher in the literature (Buck 1982, Roy 1991, Krohn et al. 1994, Aubry and Raley 2006). Similarities between our study and previous accounts of predation on fishers are limited to those studies identifying mountain lions as fisher predators (Grinnell et al. 1937, Aubry and Raley 2006). Other predators suspected of fisher predation in other regions with different predator communities include wolverine (*Gulo gulo*) and Canada lynx (*Lynx canadensis*, Roy 1991, Weir and Corbould 2008), golden eagle (*Aquila chrysaetos*, Roy 1991), great-horned owl (*Bubo virginianus*, Buck et al. 1983) and other fishers (Buck 1982, Weir and Corbould 2008).

This is the first study to use DNA to verify predators and differentiate intraguild predator species, and in our Random Forest analysis the most common misidentification of bobcat-killed fishers was mountain lion based on depressed skull fractures in some bobcat-killed fisher carcasses. For these reasons, it is possible that some of the kills attributed to lions in previous

studies were in fact by bobcats, which seems especially plausible with respect to previous fisher studies in California. Differences in findings with studies from other locations with different predator communities, however, are not surprising, such as those finding wolverine or lynx as predators of fisher (Roy 1991, Weir and Corbould 2008). Furthermore, fishers in California are thought to be smaller than in other areas of their range, ostensibly making them more vulnerable to predation by smaller predators (Powell 1993). However, average bobcat size may also be smaller within the fisher project areas, so heightened susceptibility to predation by bobcats due to smaller fisher size may not necessarily be significant (G.M. Wengert, unpublished data).

Other factors could explain differences in predator species observed to prey on fisher in different times and places. Dissimilar relative densities of predators or predator-fisher ratios may also explain differences in species-specific predation rates across projects and between previous studies and ours. In a study of gray wolf (*Canis lupus*) predation on moose (*Alces alces*), the best predictor of kill rates was the ratio of moose density to wolf density (ratio-dependent predation) (Vucetich et al. 2002). Furthermore, when prey suffer predation from multiple species, as in the case of fishers at all of our project sites, nonlinear predation rates can occur due to competition or intraguild predation among predator species resulting in risk reduction to fishers (Sih et al. 1998). Unfortunately, at this time we have no data regarding densities or relative abundance of the three predators at each study area. Finally, different habitat types and features at the three projects might subject fishers to greater risk of predation by a particular predator species with, for example, a greater penchant for hunting or traveling within certain habitat features, as shown in other canid and felid predator communities (Brown and Litvaitis 1995, Murray et al. 1995, Karanth and Sunquist 2000).

Predators of twelve carcasses we investigated could not be identified by the molecular approach, either because of insufficient DNA, advanced autolysis of the carcass, or because the predator species was not canid or felid. None of the predated fishers in our study displayed forensic characteristics consistent with killing by another fisher (Weir and Corbould 2008) or raptor predation, such as degloving ("stripping" skin inside out along bone) or symmetrical talon marks (Coonan et al. 2005). Using Random Forest for prediction, a majority of the twelve carcasses with unidentified predators were predicted as bobcat predation ($n = 7$), with 4 predicted as mountain lion predation, and 1 as coyote predation (Table 2). Though our Random Forest analysis underestimated the contribution of coyotes to fisher predation rates, we suggest that using the molecular approach may also underestimate rates of coyote predation due to their tendency to leave the carcass unconsumed and presumably with less salivary DNA.

We found that female fishers were more likely to be killed by bobcats and male fishers were more likely to be killed by mountain lions. Similarly, in a reintroduction of fishers in Montana, Roy (1991) found that mountain lions only killed male fishers ($n = 3$), although coyotes apparently killed both male and female fishers. Also in that study, two female fishers were killed by golden eagle and Canada lynx (Roy 1991). Their findings were similar to Weir and Courbould (2008) and Buck (1982) who determined Canada lynx and raptor, respectively, to be responsible for female fisher deaths. It is probable that this dichotomy in sex-specific predation by different predator species stems from the pronounced sexual size dimorphism between male and female fishers. An average male fisher in California, at 3.5 – 4.5kg, falls at the high end of the typical size range for bobcat prey whereas female fishers at 2.0 – 2.5kg are well within typical prey size range for bobcats (Anderson and Lovallo 2003).

Mountain lions typically take larger prey than bobcats do (Leopold and Krausman 1986, Koehler and Hornocker 1991). Perhaps the significantly lower energetic value of a female fisher contributes to this trend, although diet of mountain lions in the southern Sierra Nevada can include a large percentage of small mammal prey (Neal et al. 1987) and recent work has shown that small-bodied prey are probably much more frequent in California mountain lion diet than typically reported (L.M. Elbroch, pers. comm.). Furthermore, diet does not always follow optimal diet theory predictions regarding energetic value vs. handling time, especially for highly mobile prey like fishers (Sih and Christensen 2001). Though our results indicating greater likelihood of female fisher predation by bobcats rather than other predator species were significant, we noted an emerging trend in the last two years of our study at KRFP of more frequent predation on female fishers by mountain lions. Of the six female fishers killed by lions, five occurred in late 2010 through 2011. Though this trend may relate to changing relative predator densities in this project area, we also must consider the possibility of behavioral syndromes (Sih et al. 2004) in which individual lions may begin to favor or target prey of female fisher size. This type of repeat behavior has been documented in individual lions repeatedly killing bighorn sheep (*Ovis canadensis*, Ernest et al. 2002), "specialist" lions in Patagonia (Elbroch and Wittmer 2013), and in this study, in which we have documented 2 bobcats each responsible for the predation of multiple fishers (G.M. Wengert, unpublished data).

Regardless of the reason for these differences, the implications of female fisher predation occurring mostly by bobcats are significant for fisher population dynamics, especially when considering that over 70% (19 of 25) of female predation deaths by bobcats occurred between late March through July, the period when fisher kits are completely dependent on their mothers for survival (J.M. Higley, pers. comm.; Powell 1993). Interestingly, this is also a time when

female fishers concentrate their home ranges and areas of activity around den sites (S.M. Matthews, R.A. Sweitzer, unpublished data), so it is not likely increased space use during the denning season that predisposes them to bobcat predation.

Coyotes did not consume any fisher carcasses but did cache three of four fisher carcasses. However, it is possible that coyotes cached the carcasses with the intent to consume the prey at a later time had field biologists not removed the fisher carcasses from the cache sites. Bobcats and mountain lions seldom cached the fisher carcasses (2 of 25 and 1 of 20, respectively). That both bobcats and mountain lions consumed fishers suggests they were killed as prey rather than competitors. However, in the one instance that the mountain lion killed but did not eat the fisher, the carcass was found by a recently killed deer carcass suggesting that the fisher was killed for competitive reasons while investigating or consuming the lion's cache. Finally, in several cases, there were too few remains to detect hemorrhage which would verify predation rather than scavenging. However, we believe a majority of these cases were direct predation because, 1) we found DNA from more than one predator species (suggesting one of them scavenged) on only one carcass, 2) in 2 of these cases, the remains were cached, a behavior typically done for prey that is killed, and 3) in about half these cases, the carcass was reached within a day of death leaving little time for a would-be scavenger to find and consume the carcass. Furthermore, if bobcats were scavenging female fisher carcasses, they also would likely scavenge male fisher carcasses for which we found no evidence.

MANAGEMENT IMPLICATIONS

Our results show high prevalence of predation as a mortality source for fishers. In their evaluation of the status of the southern Sierra Nevada fisher population, Spencer et al. (2011)

proposed that even minor decreases in survival rates of this fisher population could prevent the population from expanding northward into currently unoccupied portions of their former range. As such, because predation is the most common cause of mortality of fisher in these study areas, it is possible that fluctuations in fisher predation could constitute increases in mortality sufficient for population limitation. Furthermore, as predator populations change, perhaps due to changes in forest cover (Smallwood 1994, Litvaitis et al. 2006), predation rates on fishers will likely respond with as yet, uncertain consequences. In the context of our results coupled with the work of Spencer et al. (2011), concern for fisher populations is warranted. Most importantly, our findings highlight the heretofore unknown and potentially critical importance of bobcat predation on female fishers, especially during the denning season.

Management of fisher habitats and conservation plans should take into consideration predation risks to fishers by bobcats as well as mountain lions and other predators. The high percentage of fisher mortality caused by predation, particularly from bobcats and mountain lions, and the apparent potential of such mortality to influence fisher population dynamics demonstrates the importance of further study of fisher, bobcat and mountain lion habitat use and selection, especially in relation to habitat manipulation by humans. Future studies of predation on fishers should describe habitat at predation sites and if possible, interaction sites between fishers and their two main predators, bobcats and mountain lions. Using these data, a model of habitat types that may heighten the risk of fisher predation can assist biologists and land managers in figuring ways to alleviate predation risk through habitat management.

We also suggest that our results can be used to build a dichotomous key based on the predation characteristics most discriminatory in identifying predator species, an example of which we have provided (Fig. 3). A simple, yet field-tested key would be indispensable to on-

the-ground managers and researchers of fishers in the west, and preclude the need to use molecular approaches to identify predators with a significant amount of correctness. However, until this key can be tested for accuracy with independent data from future predation events at these study sites or others throughout the west, we encourage fisher researchers and managers use our Random Forest model to assist in predator identification (see Tables S1 and S2, available online at www.onlinelibrary.wiley.com).

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LITERATURE CITED

Anderson, E., and M. Lovallo. 2003. Bobcat and Lynx. Pages 758–786 *in* Wild Mammals of North America: Biology, Management, and Economics. Johns Hopkins Press, Baltimore, Maryland.

Aubry, K. B., and C. M. Raley. 2006. Ecological characteristics of fishers (*Martes pennanti*) in the southern Oregon Cascade Range. U. S. Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

Berger, K. M., and E. M. Gese. 2007. Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76:1075–1085.

Breiman, L. 1984. Classification and Regression Trees. Chapman & Hall/CRC, New York, New York, USA.

Breiman, L. 2001. Random forests. Machine Learning 45: 5–32.

Brown, A. L., and J. A. Litvaitis. 1995. Habitat features associated with predation of New England cottontails: what scale is appropriate? Canadian Journal of Zoology 73:1005–1011.

Buck, S. 1982. Habitat utilization by fisher (*Martes pennanti)* near Big Bar, California. Thesis, Humboldt State University, Arcata, USA.

Buck, S., C. Mullis, and A. Mossman. 1983. Corral Bottom-Hayfork Bally fisher study. Final report to the U. S. Forest Service. Humboldt State University, Arcata, California, USA.

Buechling, A., and C. Tobalske. 2011. Predictive habitat modeling of rare plant species in Pacific Northwest forests. Western Journal of Applied Forestry 26:71–81.

Chan, J. C. W., and D. Paelinckx. 2008. Evaluation of Random Forest and Adaboost tree-based ensemble classification and spectral band selection for ecotope mapping using airborne hyperspectral imagery. Remote Sensing of Environment 112:2999–3011.

Coonan, T. J., C. A. Schwemm, G. W. Roemer, D. K. Garcelon, L. Munson, and C. A. Jones. 2005. Decline of an island fox subspecies to near extinction. The Southwestern Naturalist 50:32–41.

Creel, S., and N. M. Creel. 1996. Limitation of African wild dogs by competition with larger carnivores. Conservation Biology 10:526–538.

Cutler, D. R., T. C. Edwards Jr, K. H. Beard, A. Cutler, K. T. Hess, J. Gibson, and J. J. Lawler. 2007. Random forests for classification in ecology. Ecology 88:2783–2792.

Cypher, B. L., and K. A. Spencer. 1998. Competitive interactions between coyotes and San Joaquin kit foxes. Journal of Mammalogy 79:204–214.

De'ath, G., and K. E. Fabricius. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81:3178–3192.

Elbroch, L.M. and H.U. Wittmer. 2013. The effects of puma prey selection and specialization on less abundant prey in Patagonia. Journal of Mammalogy 94:000-000.

Ernest, H. B., E. S. Rubin, and W. M. Boyce. 2002. Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. Journal of Wildlife Management 66:75–85.

Garzon, M. B., R. Blazek, M. Neteler, R. S. Dios, H. S. Ollero, and C. Furlanello. 2006. Predicting habitat suitability with machine learning models: The potential area of *Pinus sylvestris* L. in the Iberian Peninsula. Ecological Modelling 197:383–393.

Grinnell, J., J. S. Dixon, and J. M. Linsdale. 1937. Fur-bearing mammals of California: their natural history, systematic status, and relations to man. University of California Press, Berkeley, California, USA.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. Van Der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88:2713–2719.

Karanth, K. U., and M. E. Sunquist. 2000. Behavioural correlates of predation by tiger (*Panthera tigris*), leopard (*Panthera pardus*) and dhole (*Cuon alpinus*) in Nagarahole, India. Journal of Zoology 250:255–265.

Koehler, G. M., and M. G. Hornocker. 1991. Seasonal resource use among mountain lions, bobcats, and coyotes. Journal of Mammalogy 72:391–396.

Krohn, W., S. Arthur, and T. Paragi. 1994. Mortality and vulnerability of a heavily trapped fisher population. Pages 137-146 *in* S. W. Buskirk, A.S. Harestad, M.G. Raphael, R.A. Powell, editors. Martens, sables and fishers: biology and conservation. Comstock Pub. Associates, Ithaca, New York, USA.

Leopold, B. D., and P. R. Krausman. 1986. Diets of 3 predators in Big Bend National Park, Texas. The Journal of wildlife management 50:290–295.

Liaw, A., and M. Wiener. 2002. Classification and Regression by randomForest. R news 2:18–22.

Litvaitis, J., J. Tash, and C. Stevens. 2006. The rise and fall of bobcat populations in New Hampshire: relevance of historical harvests to understanding current patterns of abundance and distribution. Biological Conservation 128:517–528.

Matthews, S. M., J. Mark Higley, J. Scott Yaeger, and T. K. Fuller. 2011. Density of fishers and the efficacy of relative abundance indices and small-scale occupancy estimation to detect

a population decline on the Hoopa Valley Indian Reservation, California. Wildlife Society Bulletin 35:69–75.

Mills, M. G. L., and M. L. Gorman. 1997. Factors affecting the density and distribution of wild dogs in the Kruger National Park. Conservation Biology 11:1397–1406.

Murray, D. L., S. Boutin, M. O'Donoghue, and V. O. Nams. 1995. Hunting behaviour of a sympatric felid and canid in relation to vegetative cover. Animal Behaviour 50:1203–1210.

Neal, D. L., G. N. Steger, and R. C. Bertram. 1987. Mountain lions: preliminary findings on home-range use and density in the central Sierra Nevada. U. S. Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, USA.

Palomares, F., and T. M. Caro. 1999. Interspecific killing among mammalian carnivores. American Naturalist 153:492–508.

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology & Evolution 7:151–154.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297–330.

Powell, R. A. 1981. *Martes pennanti*. Mammalian Species 156:1–6.

_____. 1993. The fisher: life history, ecology, and behavior, 2nd ed. University of Minnesota Press, Minneapolis, Minnesota, USA.

Powell, R. A., and W. J. Zielinski. 1994. Fisher. *in* L. F. Ruggiero, K. B. Aubry, S. W. Buskirk, andW. J. Zielinski, editors. The scientific basis for conserving forest carnivores:

American marten, fisher, lynx, and wolverine. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

Roemer, G. W., T. J. Coonan, D. K. Garcelon, J. Bascompte, and L. Laughrin. 2001. Feral pigs facilitate hyperpredation by golden eagles and indirectly cause the decline of the island fox. Animal Conservation 4:307–318.

Roy, K. D. 1991. Ecology of reintroduced fishers in the Cabinet Mountains of northwest Montana. Thesis, University of Montana, Missoula, USA.

Segurado, P., and M. B. Araujo. 2004. An evaluation of methods for modelling species distributions. Journal of Biogeography 31:1555–1568.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372–378.

Sih, A., and B. Christensen. 2001. Optimal diet theory: when does it work, and when and why does it fail? Animal Behaviour 61:379–390.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution 13:350–355.

Smallwood, K. S. 1994. Trends in California mountain lion populations. The Southwestern Naturalist 39:67–72.

Spencer, W., H. Rustigian-Romsos, J. Strittholt, R. Scheller, W. Zielinski, and R. Truex. 2011. Using occupancy and population models to assess habitat conservation opportunities for an isolated carnivore population. Biological Conservation 144:788–803.

Thompson, C. M., and E. M. Gese. 2007. Food webs and intraguild predation: community interactions of a native mesocarnivore. Ecology 88:334–346.

Thompson, C. M., W. J. Zielinski, and K. L. Purcell. 2011. Evaluating management risks using landscape trajectory analysis: a case study of California fisher. The Journal of Wildlife Management 75:1164–1176.

United States Fish and Wildlife Service. 2004. 50 CFR part 17 Endangered and threatened wildlife and plants; 12 month finding for a petition to list the west coast distinct population segment of the fisher (*Martes pennanti*); proposed rule Federal Register.

Vance-Chalcraft, H. D., J. A. Rosenheim, J. R. Vonesh, C. W. Osenberg, and A. Sih. 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88:2689–2696.

Vashon, J., A. Vashon, and S. Crowley. 2002. Partnership for Lynx Conservation in Maine December 2001–December 2002 Field Report. Maine Department of Inland Fisheries and Wildlife, Maine, USA.

Vucetich, J. A., R. O. Peterson, and C. L. Schaefer. 2002. The effect of prey and predator densities on wolf predation. Ecology 83:3003–3013.

Weir, R., and F. Corbould. 2008. Ecology of fishers in the sub-boreal forests of north-central British Columbia: Final Report. Peace/Williston Fish and Wildl. Compensation Program Report No. 315.

Wengert, G.M., M.W. Gabriel, J.E. Foley, T. Kun, B.N. Sacks. 2013. Field and molecular techniques for identifying intraguild predators of fishers and other North American small carnivores. Wildlife Society Bulletin 37:659-663.

White, P., and R. A. Garrott. 1997. Factors regulating kit fox populations. Canadian Journal of Zoology 75:1982–1988.

Zar, J. H. 1999. Biostatistical analysis. Volume 564. Prentice Hall, Upper Saddle River, New Jersey, USA.

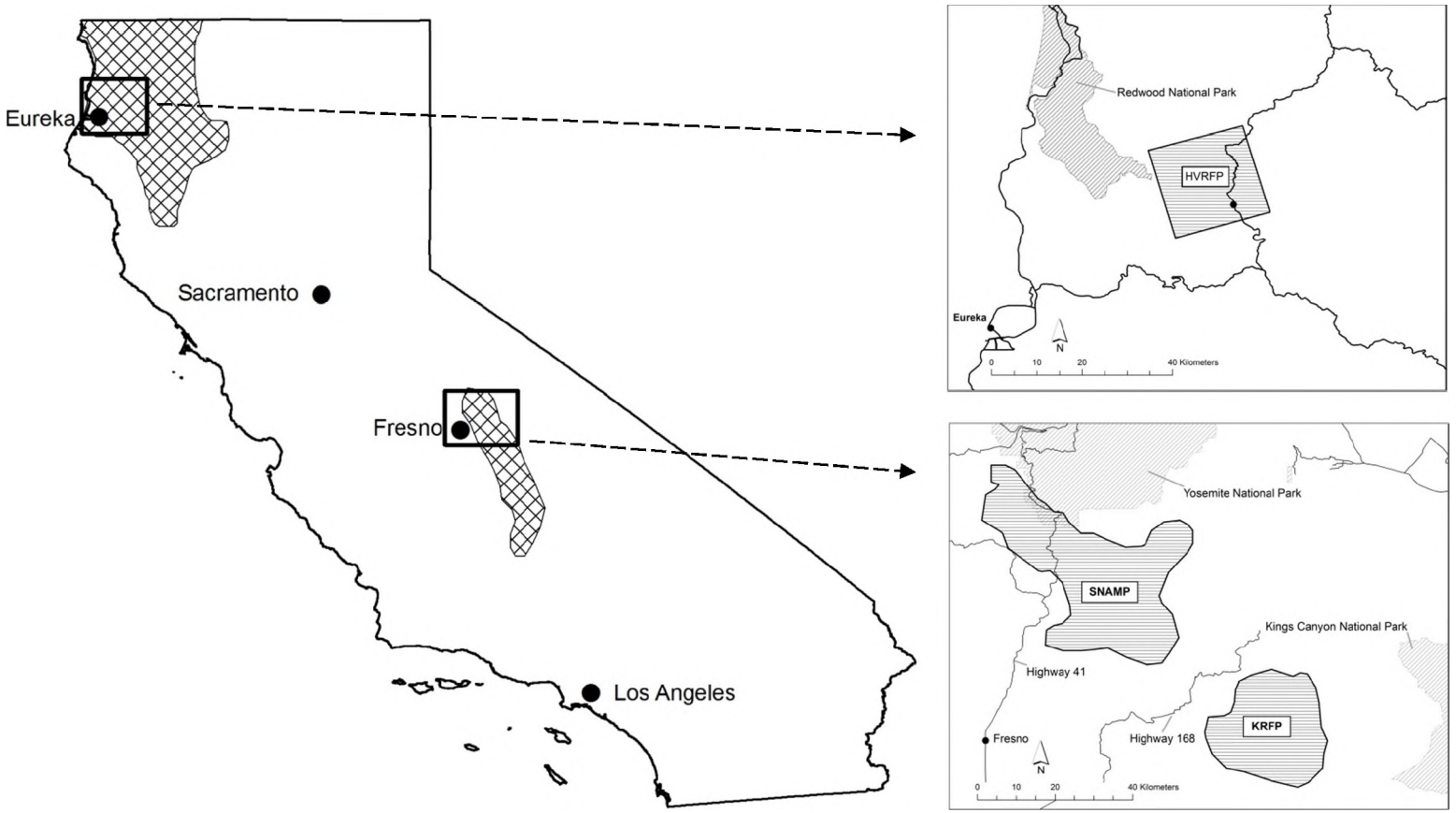

Figure 1. Map of locations of the Hoopa Valley Indian Reservation Fisher Project (HVRFP), Sierra Nevada Adaptive Management Project (SNAMP), and Kings River Fisher Project (KRFP) where we identified predators of fishers (*Martes pennanti*) between 2007 – 2011.

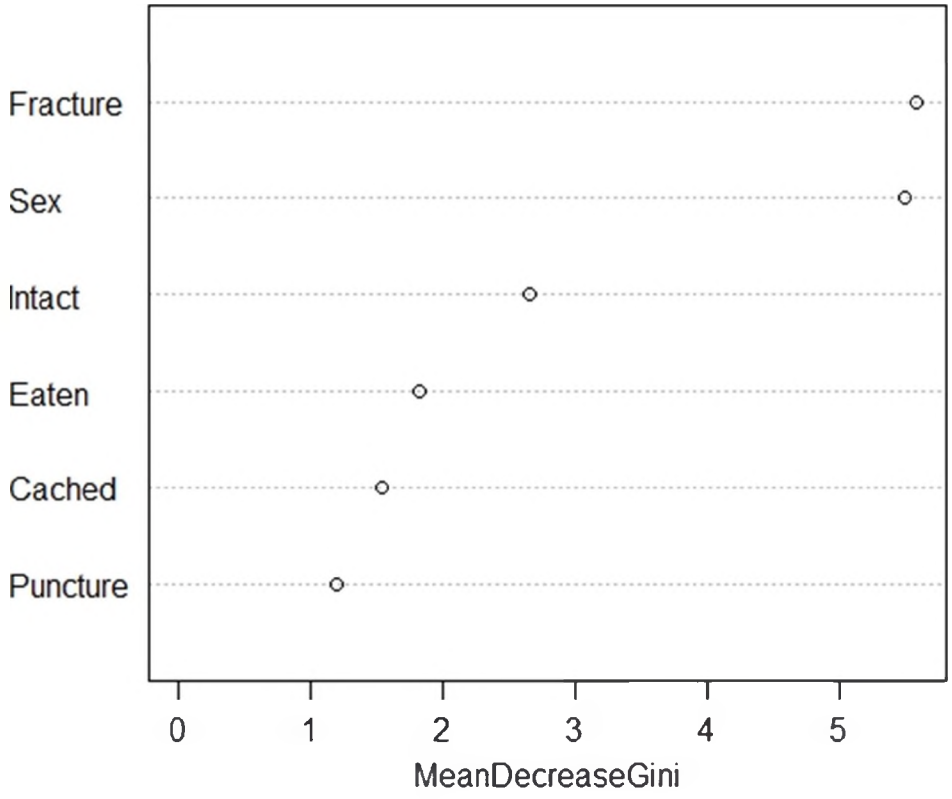

Figure 2. Results of Random Forest analysis indicating sex of fisher (*Martes pennanti*) and depressed fracture of the skull are the most discriminating factors for predicting predator species of fishers. The mean decrease in Gini coefficient measures how much each variable contributes to the accuracy of the Random Forest model.

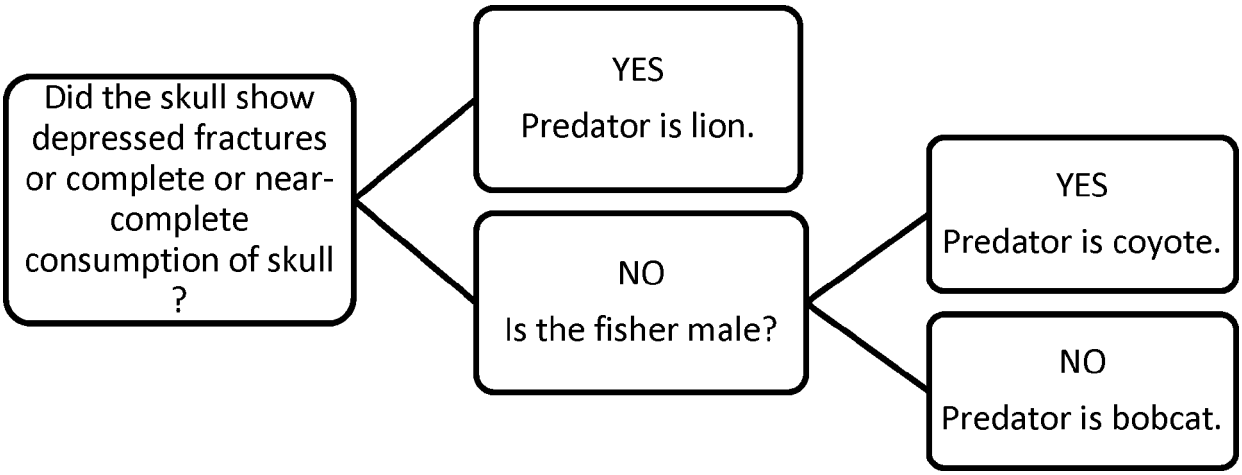

Figure 3. Dichotomous key to determine species of predator of fisher (*Martes pennanti*) from field and necropsy evidence.

Table 1. Distribution of predated fishers (*Martes pennanti*) and predator species across three fisher research projects in California: Sierra Nevada Adaptive Management Project (SNAMP), Kings River Fisher Project (KRFP), and Hoopa Valley Reservation Fisher Project (HVRFP) from 2007 – 2011. Numbers in parentheses represent juvenile fishers included in that sample.

Project	Bobcat	Mountain Lion	Coyote	Total
SNAMP				
Female	12(1)	1	1	14
Male	0	6(1)	1	7
KRFP				
Female	5(1)	6(1)	1	12
Male	0	4(3)	1	5
HVRFP				
Female	8	0	0	8
Male	0	3	0	3
Total	25	20	4	49

Table 2. Field and pathologic characteristics of fishers (*Martes pennanti*) killed by bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), or coyote (*Canis latrans*) across three fisher research projects in California from 2007 – 2011. Also shown are characteristics of 12 fishers (Mape51 – Mape62) killed by unknown predator species and suspected predator species determined through Random Forest model.

Predator Species/ Fisher	Fisher Sex	Number Consumed	Number Cached	Depressed Skull Fracture or Skull Mostly/ Fully Consumed	Focal Punctures in Skull	Intact Skull	Random Forest-Predicted Predator Species
Bobcat (*n*=25)		22 (88%)	2 (8%)	6 (24%)	5 (20%)	13 (52%)	
Mountain Lion (*n*=20)		18 (90%)	1 (5%)	19 (95%)	1 (5%)	0	
Coyote (*n*=4)		0	3 (75%)	1 (25%)	3 (75%)	0	
Mape51	F	YES	NO	YES	NO	NO	Mountain lion
Mape52	F	NO	NO	NO	NO	YES	Bobcat
Mape53	F	NO	YES	NO	NO	YES	Bobcat
Mape54	F	YES	NO	NO	NO	YES	Bobcat
Mape55	F	NO	YES	NO	NO	YES	Bobcat
Mape56	M	NO	NO	NO	NO	NO	Mountain Lion
Mape57	M	NO	YES	NO	YES	NO	Coyote
Mape58	M	NO	NO	NO	YES	NO	Mountain Lion
Mape59	M	NO	NO	NO	YES	NO	Mountain Lion
Mape60	F	YES	NO	NO	NO	YES	Bobcat
Mape61	F	YES	NO	NO	NO	YES	Bobcat
Mape62	F	YES	YES	NO	NO	YES	Bobcat

Table 3. Confusion matrix from the Random Forest analysis using vote totals of the association of pathologic characteristics of predated fisher (*Martes pennanti*) carcasses and predator species (bobcat, *Lynx rufus*, mountain lion, *Puma concolor*, or coyote, *Canis latrans*) responsible for 49 predated fisher carcasses in three fisher study areas in California 2007 – 2011.

	Predicted			
Observed	Bobcat	Coyote	Mountain Lion	Class Correct Classification Rate
Bobcat	19	0	6	0.76
Coyote	1	2	1	0.50
Mountain Lion	0	1	19	0.95

CHAPTER 4:

Habitat Selection of Bobcats in a Mixed-Coniferous Forest and Relationship to Predation Risk to Fishers

ABSTRACT

Bobcats (*Lynx rufus*) are the main predators of fishers (*Martes pennanti*) in California, though it has been suggested that certain habitat types may expose fishers to higher predation risk than other types. Very little is known about bobcat habitat selection in mixed coniferous and coniferous forest types in California, and even less is known about how bobcat habitat use relates to the risk of bobcat predation of fishers. We investigated habitat use of bobcats in mixed coniferous forests in northern California and created a model of bobcat habitat selection based on locations of GPS-collared bobcats. We conducted this study simultaneously to an ongoing project examining fisher habitat use in order to relate bobcat habitat selection to fisher habitat use within areas of fisher-bobcat overlap. We used the results of these two investigations to form a hypothesis about habitat types most associated with areas surrounding sites where fishers were killed by bobcats vs. habitat types at those fishers' locations when they were alive. We found that bobcats selected open (mainly characterized by prairie and barren habitats) and brush habitats and selected against mature, older forest and young, closed-canopy forest types. Areas where bobcats overlapped fisher home ranges were characterized by more open habitats and lower density of non-driveable roads (overgrown and old, logging roads) than areas of fisher home ranges where bobcats did not overlap. Based on these results, we tested the hypothesis that areas surrounding sites where fishers were killed by bobcats were characterized by higher proportions of open and brush habitats than were areas surrounding those fishers' locations when they were alive, and the results of this test supported our hypothesis. Our results for bobcat habitat selection

analyses differed from most investigations of bobcat habitat use in California and other areas throughout North America. However, this study was novel in its investigation of bobcat habitat use in mixed coniferous forest dominated habitats; therefore, our results are especially relevant to an understanding of habitat-mediated predation risk to fishers in their preferred habitats.

INTRODUCTION

Many carnivores are subject to the risks of predation by larger predators (Palomares and Caro 1999). Such intraguild predation can threaten individuals, populations, or entire species (Creel and Creel 1996, Cypher and Spencer 1998, Coonan et al. 2005). The underlying causes of interspecific killing among carnivores vary, but probably relate most often to competition (Polis et al. 1989).

Frequency of interspecific killing among carnivores likely depends on habitat (Janssen et al. 2007). Animals make decisions based on trade-offs between high-quality prey habitat and safe habitats, such that survival from predation might be higher but obtaining enough resources to survive and reproduce becomes more challenging (Sergio et al. 2007, Thompson and Gese 2007). Alternatively, smaller carnivores may use habitats frequently used by their predators but choose microhabitat features that afford them some protection from being discovered and killed by their predators (Viota et al. 2012). Rarely, smaller carnivores may become vulnerable to predation by larger predators when the predators venture into the habitats of the smaller carnivore; this phenomenon is exemplified by predation on island foxes (*Urocyon littoralis*) by golden eagle (*Aquila chrysaetos*) following their incursion to the Channel Islands of California (Roemer et al. 2002).

For fishers (*Martes pennanti*), a rare forest carnivore and candidate for listing in the western United States under the federal Endangered Species Act, the greatest single source of mortality throughout California is predation by bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*, G.M. Wengert, Chapter 3). Bobcats were responsible for 54% of all female fisher mortality (G.M. Wengert, Chapter 3). Given conservation concern over fishers in the western states and our recent discovery of the importance of bobcat predation, we initiated research on bobcat habitat use in a study area where an ongoing fisher research project provided comparative data. This coupling of research efforts provided a valuable opportunity to monitor closely both species within the same project area and habitat types.

Our objectives were to 1) describe second-order habitat selection (an animal's selection of its home range from that available across the landscape) and third-order habitat selection (an animal's selected habitats within its home range, Johnson 1980) by bobcats, 2) identify habitat types and landscape features associated with fisher and bobcat overlap, and 3) based on results from Objectives 1 and 2, develop and test predictions about habitat features associated with locations where fishers were killed by bobcats.

We predicted that roads and manmade linear features (overgrown logging roads, edges between contrasting habitat types) would be heavily used by bobcats and therefore associated with high overlap between fishers and bobcats. Many studies have found elevated predation rates on nesting birds at habitat edges (Hartley and Hunter 1998) and frequent use of linear features by predators (James and Stuart-Smith 2000, Silver et al. 2004, Frey and Conover 2006, Courbin et al. 2009, Ruell and Crooks 2009). We also predicted that fishers would be most vulnerable to predation by bobcats when in habitats typically used by bobcats. To test these predictions, we

captured bobcats, fit them with telemetry collars, and compared their use locations with random locations, fisher habitat use, and fisher predation sites in terms of habitat classes or features.

STUDY AREA

The Hoopa Valley Indian Reservation (HVIR) is located within the Klamath physiographic province (Kuchler 1977) of northern California, about 50 km northeast of Eureka, California. The HVIR is approximately 362.5 km^2 and is bisected by the Trinity River in a north-south direction. Elevations vary between 98 m and 152 m above sea level in the valley along the river, and up to 1170 m in the mountainous sections of HVIR. The mountainous areas are dominated by mixed coniferous forest with some scattered areas of meadow and brushfield. The dominant vegetation type is Douglas-fir (*Pseudotsuga menziesii*) forest, including a midstory dominated by hardwood trees including tanoak (*Notholithocarpus densiflorus*), madrone (*Arbutus menziesii*), and canyon live oak (*Quercus chrysolepis*). Pure hardwood stands occur sparsely throughout HVIR. At the highest elevations, pine (*Pinus* spp.) and white fir (*Abies concolor*) replaces the Douglas-fir community. Other mid-sized to large carnivores inhabiting the project area included coyote (*Canis latrans*), mountain lion (*Puma concolor*), and black bear (*Ursus americanus*).

A mix of mature and early seral-stage forest exists across HVIR due to past and current timber harvests. From 1960 to the late 1980s, 30% of the reservation was harvested, averaging over 500 ha of cut per year. Since 1994 harvest has been implemented under the Hoopa Tribe's Forest Management Plan which includes timber harvesting, pre-commercial thinning, early release, and cultural burning. Most recent logging was implemented using regeneration methods

with green tree and snag retention on small (<10 ha) modified clearcuts and a minor amount of commercial thinning, single tree and group selection.

METHODS

All procedures involving animals were reviewed and approved by the University of California, Davis, Animal Care and Use Committee (Protocol No. 16551) and the Humboldt State University Institutional Animal Care and Use Committee (Protocol 04104.W.42.A). We captured bobcats between October 2010 and March 29, 2012 using Nos. 1 ½ and 1 ¾ Soft-catch foot-hold traps (Oneida Victor® Inc., Euclid, OH) and cage-traps (Homesteader Deluxe 42D, Tru-catch Traps, Belle Fourche, SD) baited with live ring-necked doves (*Streptopelia capicola*), or visual and scent lures with no live bait during the months of August through March. Traps were placed opportunistically in areas with recent bobcat sign or sightings prioritized, and/ or target areas where several fishers were already being monitored.

We immobilized trapped bobcats with an intramuscular injection of 10 mg/kg ketamine hydrochloride and 2 mg/kg xylazine. Each anesthetized bobcat was maintained in lateral recumbency in order to stimulate blood circulation and avoid adverse post-capture effects. A brief physical exam was performed to identify injuries. Vital rates of anesthetized animals were monitored continuously throughout anesthesia. We inserted a passive integrated transponder (PIT tag, 134.2 kHz Super Tag, Sterile, Biomark, Inc., Boise, ID, USA) into each bobcat for permanent identification, took biological samples for genetic and health analyses, took morphometric measurements, and attached to each bobcat one of three types of tracking collars: Quantum 4000 Enhanced Medium GPS Collar with VHF beacons (Telemetry Solutions, Concord, California, USA), Tellus Ultralight GPS collars with VHF beacons (Followit AB,

Stockholm, Sweden), and VHF-collars (Holohil Systems Ltd., Carp, Ontario, Canada). All GPS collars were scheduled to attempt a GPS fix every 4 hours; in addition, they attempted a fix every two hours on every fourteenth day. Fisher capture, monitoring, and data analysis were as described elsewhere (Higley and Matthews 2007).

In addition to GPS location data from bobcats, we monitored bobcats at least once per week with the use of TR4 (Telonics, Mesa, AZ, USA) or R1000 (Communications Specialists, Inc., Orange, CA, USA) handheld telemetry receivers and 4-element RA-14 antennas (Telonics, Mesa, AZ, USA). Locations were taken from systematic points, with azimuths on a single animal being taken within a 20-minute period and with angles >20° of each other to reduce error when bobcats were active. Three or more of these locations were used to estimate locations of animals through triangulation. Triangulations were plotted using the Andrews-M estimator in the computer program L.O.A.S (Ecological Software Solutions, Inc., Sacramento, California). We used bobcat obtained through telemetry for analyses if observer confidence level for a triangulation was at least level 3 (on an observer designated scale of 1 – 5 where 1 = location not used for any analyses, 2 = animal is likely in watershed, 3 = use location for home range, 4 = use location for habitat analysis, and 5 = animal is likely within 20m of location). We used bobcat GPS fix locations for analyses if HDOP, an index of location error, was 6 or less. Fisher locations were subject to the same guidelines (Higley and Matthews 2007, unpublished data).

Home Range and Core Area Analysis. We used fixed kernel density (FK; Worton 1989) methods to calculate home ranges for all bobcats with ≥100 locations (Powell 2000, Börger et al. 2006). We calculated FK home ranges using ad hoc smoothing parameters (had hoc) designed to prevent over- or under-smoothing (Berger and Gese 2007). This method involved choosing the smallest increment of the reference bandwidth (href) that resulted in a contiguous 95% fixed

kernel home range polygon that contained no lacuna (i.e., had hoc = 0.9 * href, 0.8 * href, etc.; J.G. Kie, Idaho State University, Pocatello, Idaho, personal communication). FK home ranges were estimated using the Home Range Tools extension (HRT; Rogers et al. 2007) available for ArcMap 9.3 (Environmental Systems Research Institute, Redlands, California, USA). To estimate the core area for each bobcat, we computed the sizes of 19 FK isopleths using had hoc containing from 5-95% of the observations in increments of 5% (Seaman and Powell 1990, Bingham and Noon 1997). We used these isopleths in regression analyses to identify the home range isopleth in which use exceeded that expected under a null model of a uniform distribution of locations (Bingham and Noon 1997).

Bobcat Habitat Selection. For all habitat analyses, we used ArcGIS 9.3 (ESRI, Redlands, CA) and a habitat map extending across the study area (Hoopa Valley Indian Reservation buffered by 3200m). The habitat map was developed by the Hoopa Tribal Forestry Department and attempted to stratify HVIR lands into polygons of different habitats types > 3 acres. The map originally consisted of 3570 unique types consisting of different values for plant species, canopy height and canopy density classes for up to four canopy layers within a single type. These types were grouped to present a more manageable number of unique habitat types. For this project, adjustments were made to create a group of seven habitat strata likely to be most relevant to an analysis of bobcat and fisher habitat use (Table 1).

We estimated second-order and third-order habitat selection for bobcats (Johnson 1980). We estimated second-order habitat selection for both 95% FK and core areas of each bobcat using compositional analysis (Aebischer et al. 1993) with the Adehabitat package (Calenge 2006) which compares habitat use to availability. We calculated the percent of each bobcat's

95% FK and core area comprised by the different habitat strata and compared them to availability of habitat types across the study area (the Reservation buffered by 3200 m) (Table 1).

We estimated third-order habitat selection three ways. First, we used compositional analysis to compare the percent of locations for each bobcat that fell into a particular habitat strata type to the percent of each bobcat's corresponding home range comprised by the different habitat strata types. Second, we calculated individual habitat selection indices for each bobcat using a standard resource selection index [(Observed – Expected)/√Expected]. Observed values were the proportion of all bobcat locations falling within a particular habitat types and expected values were the proportions of that habitat type composing that bobcat's home range. Index values fell between -1 and +1, where negative values represent selection against a habitat type, and positive values represent selection for a habitat type.

Third, we used a case-control logistic regression approach. We used 3525 bobcat locations from the seven collared bobcats to represent "used" locations. We then created random points throughout each bobcat's 95% fixed kernel equal to the number of "used" points for each individual bobcat to represent "available" habitat using Hawth's Tools for ArcGIS 9 (Beyer 2004). We coded each bobcat location and each random point according to habitat type (STRATA), forest cover (FOR/NONFOR), management scheme (UNMAN, CABLE, TRACTOR), distance to road (both drivable, DRVROAD and non-drivable, NODRVROAD), and distance to nearest edge (edge between any two habitat strata, EDGE, and edge between forest and non-forest types, HRDEDGE) from each location (Table 1). All spatial analyses were completed using ArcGIS 9.3 (ESRI, Redlands, CA).

We used generalized linear mixed models logistic regression fit by the Laplace approximation (Bolker et al. 2009). We included individual bobcat as a random effect to block

for individual bobcat differences in habitat use, unbalanced sample sizes among bobcats, and differences in availability of habitats for each bobcat (Gillies 2006). We created dummy variables for the habitat strata, forest, and management scheme categorical variables. We checked for multicollinearity among our variables using variance inflation factors (VIF), and did not use variables in the same models when VIF $\geq$ 10.

Some controversy has surrounded the use of logistic regression to build resource selection functions when available habitats or locations are included as part of the binary response variable (Keating and Cherry 2004). Concerns are that available habitats may be "contaminated" with actual used locations, biasing estimates of probability of use. However, more recent analyses of this problem, including numerous simulations demonstrating this issue, suggest that the bias is negligible (Desrochers et al. 2010), except in the most extreme cases where dense aggregation of animals use a small number of strongly selected resource units (Johnson et al. 2006). Finally, the biases apply to quantitative estimates of probability of presence, but not qualitative assessment of habitat ranking, which was our objective in this case (Phillips and Elith 2013).

Habitat in Areas of Fisher and Bobcat Overlap. To describe habitats where fishers and bobcats would be more likely to encounter each other, we analyzed habitat features in areas of overlap between the two species. We defined overlap as regions that fell within both the 95% fixed kernels for individual fishers (Higley et al. 2013) and within one or more core areas of bobcats that were tracked during the same period. We used only female fishers because no males were documented to have been killed by bobcats in California (Chapter 3). We quantified composition of these overlap and non-overlap areas as described above.

We conducted univariate statistics for analysis of habitat variables between regions of overlap and non-overlap for fishers and bobcats using paired *t*-tests. We then used generalized linear mixed models logistic regression fit by the Laplace approximation (Bolker et al. 2009). We included individual as a random effect to block for individual fisher differences in habitat use and unbalanced sample sizes among fishers (Gillies 2006). We checked for multicollinearity among our variables using variance inflation factors (VIF), and did not use variables in the same models when VIF $\geq$ 10.

Spatial Predation Risk Using Predation Sites. We recovered deceased fishers from the field, conducted necropsies when possible, and determined predator species using forensic molecular analysis (Chapter 2, Wengert et al. 2013) or classification and regression methods to assess the predator species based on physical characteristics of the predation event (Chapter 3). We used the results of the bobcat habitat selection analyses and bobcat-fisher habitat overlap analysis to choose the habitat variables most associated with bobcat presence to form hypotheses about habitats associated with fisher predation sites. We coded all sites where fishers were killed by bobcats and all available telemetry locations for each of these predated fishers with these habitat variables and performed a *t*-test for each to test our predictions about differences in habitat variables between predation sites and live-fisher locations.

Statistical Analysis. We employed an information-theoretic approach to identify the most parsimonious models (Burnham and Anderson 1998) in our third-order habitat selection analysis and fisher-bobcat overlap analysis. We calculated the Akaike Information Criteria score corrected for small sample sizes (AICc; Burnham and Anderson 1998) for each model and compared the scores among competing models. Our list of competing models included those with ΔAICc < 2. We estimated model-averaged parameters (coefficients, unconditional 95%

confidence intervals, odds-ratios) for each variable in a best predictive model (Burnham and Anderson 1998). All statistical analyses were conducted using R version 3.0.0 (R Core Development Team 2010).

RESULTS

Seven bobcats (4 male, 3 female) were captured 9 times between February 6, 2011 and February 14, 2012. All three female bobcats and three of the male bobcats were equipped with a Quantum 4000 Enhanced Medium GPS Collar with a VHF beacon (Telemetry Solutions, Concord, California, USA). One male bobcat was equipped with a VHF-collar (Holohil Systems Ltd., Carp, Ontario, Canada) for a full year, then re-captured and equipped with a Tellus Ultralight GPS collar with a VHF beacon (Followit AB, Stockholm, Sweden). We monitored individual bobcats between 2 – 20 months and GPS collar fix rate ranged from 35.6% to 73.8% of all attempts. We obtained between 141 and 953 independent and usable locations (meeting our criteria for inclusion based on error) from each of the seven bobcats.

Bobcat Home Range and Core Area. Bobcat 95% fixed kernels (FK) ranged from 11.1 km^2 to 18.3 km^2 with a mean of 14.4 km^2 (3.6 km^2 SD) for females (Figure 1) and 13.7 km^2 to 92.0 km^2 with a mean of 57.6 km^2 (35.2 km^2 SD) for males (Figure 2, Table 2). Bobcat core areas were either 60% (n=6) or 70% (n=1) fixed kernels and ranged from 3.1 km^2 to 4.1 km^2 with a mean of 3.9 km^2 (0.2 km^2 SD) for females and 4.6 km^2 to 25.3 km^2 with a mean of 17.2 km^2 (9.4 km^2 SD) for males (Table 2). Although fixed kernels and core areas were larger for males than females, these differences were not statistically significant at the alpha = 0.05 level (P=0.090) and (P=0.067), respectively.

Bobcat Habitat Selection. Proportions of 95% FK and core areas composed by different habitat types were on average very similar to the composition of the study area overall (Table 3, Figure 3). However, the compositional analysis indicated a marginally significant selection of certain habitat types by bobcats (Wilk's $\lambda = 0.00158$, $P=0.058$). This analysis indicated bobcats selected for YFM, BRUSH, YCCF, and OPEN, and selected against MOF, OPSHRB, and TOW at the home range scale. For the core areas, selection for any particular habitat type was not significant (Wilk's $\lambda = 0.06887$, $P=0.609$), although OPEN habitats were selected over MOF in core areas.

For third-order habitat selection, compositional analysis indicated a strong selection for OPEN, BRUSH, and OPSHRB habitat types, which collectively represented non-forested habitats, and selection against MOF, YFM, and YCCF, the forested habitats (Wilk's $\lambda = 0.000086$, $P=0.024$). The habitat type TOW was neither selected for or against in relation to the other habitat types. Habitat selection indices were strongly positive for OPEN for five of seven bobcats, positive for BRUSH for five of seven bobcats, and negative for MOF and YFM for six of seven bobcats (Figure 4). All bobcats selected against YCCF (Figure 4).

There were differences in some variables between used and available bobcat locations, most notably in BRUSH, MOF, OPEN and YCCF (Table 4). Habitat strata, distance to any type of road, and distance to any type of edge were included in the model that best predicted bobcat third-order habitat selection (Tables 5 and 6). The only other competing model included habitat strata, distance to any type of road, and hard edge specifically (Table 5). Likelihood of bobcat occurrence increased in OPEN (odds ratio [OR] = 2.9068) and BRUSH (OR = 1.8453), while it decreased in MOF (OR = 0.3653), YCCF (OR = 0.3815), and YFM (OR = 0.4404)(Table 6).

Habitat in Areas of Fisher and Bobcat Overlap. We had fifteen fishers whose home ranges overlapped at least one bobcat's core area. Average habitat composition was similar between these overlap areas and portions of fisher home ranges not overlapping bobcat cores (Table 7). The best model describing overlap of fisher kernels and bobcat core areas included density of non-driveable road (NODRVROAD), proportion consisting of young, multi-storied forest (YFM), and proportion consisting of open habitats (OPEN, Tables 8 and 9). Decrease in NODRVROAD density and increase in amount of open habitats significantly increased the odds of fisher and bobcat overlap (Table 9).

Spatial Predation Risk. We attributed eleven fisher deaths to bobcat predation (Chapter 3, Figure 5). Based on the previous analyses of bobcat habitat selection, which consistently indicated OPEN and BRUSH habitats were selected for by bobcats, we predicted that fisher predation sites would be closer than expected by chance to these habitats. Indeed, these predation sites tended to be closer to the edge of OPEN or BRUSH habitats (average = 212.3 m, SE = 37.2 m) than live-fisher locations (average = 339.9m, SE = 7.5; $t = 3.0128$, df = 9.835, $P = 0.006645$).

DISCUSSION

Bobcat home range and core area sizes that we found in our study were similar to those reported by other studies throughout the United States (Ferguson et al. 2009), but large relative to those reported by others for California (Neale and Sacks 2001; Riley et al. 2003; Riley 2006), suggesting relatively low bobcat densities for the region. Male bobcat home ranges are typically larger than female home ranges (Ferguson et al. 2009). Although we found no significant difference between female and male home range sizes, our sample size was small and individual

variability high, with one male bobcat exhibiting an atypically small home range (Table 2), suggesting a lack of statistical power. We found that bobcats selected against mature, older forest at the home range scale (95% fixed kernels), which was unexpected because other studies have found home ranges to be composed of more forest habitat than that found in surrounding areas (Tucker et al. 2008) or more lowland forest and wetlands and less upland forest and open fields than in surrounding areas (Preuss and Gehring 2007).

Very few studies have investigated bobcat habitat use where coniferous or mixed-coniferous forests are the dominant habitat type (but see Witmer and deCalesta 1986). However, bobcat habitat-use studies in regions with somewhat similar dominant habitat types show some similarities, but many differences to our study (Litvaitis and Harrison 1989, Chamberlain et al. 2003, Pruess and Gehring 2007, Tucker et al. 2008). We observed 1.5 times as many used locations than random locations in brush habitats. In Vermont, bobcats also more frequently used brush or scrub/ shrub habitat than in less-frequently used areas of their home ranges (Donovan et al. 2011). However, in that study and in others (Chamberlain et al. 2003, Preuss and Gehring 2007), field or open habitats were selected against. In contrast, we found that 3.5 times as many bobcat locations were in open habitats than were randomly selected available locations. The only other study in which bobcats showed preference for open habitats was in agricultural areas of Mississippi (Conner and Leopold 1993). Bobcats in other studies also selected forested habitats over other types (Chamberlain et al. 2003, Preuss and Gehring 2008, Tucker et al. 2008, Donovan et al. 2011), but in this study, forested habitats were selected against. Of most studies on bobcat habitat selection, habitat conditions in the study by Litvaitis and Harrison (1989) in Maine are likely most similar to that found in our study area. Both in that study as well as this

study, bobcats were located more frequently in hardwood forest (in our study area, oak (*Quercus* spp.) woodland) than in random locations.

The differences between bobcat habitat use observed in our study and that observed in other studies could stem from whether the studies occur in areas of bobcat exploitation, differing distributions of prey abundance or diversity, and possibly dissimilar competitor densities. Neale and Sacks (2001) found slight avoidance by bobcats of coyotes (*Canis latrans*) during coyote breeding seasons and bobcats avoided coyote core areas in south-central Florida (Thornton et al. 2004) which may manifest as differences in use-frequency of certain habitat types. Anecdotal records of coyotes throughout our study area are infrequent and suggest low population density (J.M. Higley, personal communication) which could in turn promote more frequent use by bobcats of typical coyote preferred habitat (open areas, Person and Hirth 1991, or recent clear-cuts, Witmer and deCalesta 1986).

Although roads and edge were selected as a variable in our top models, we also failed to find a biologically meaningful relationship between bobcat presence and these variables, as the odds ratios were nearly equal to 1 (Table 6). Our predictions were not supported by our analysis, which contrasted with evidence for predator use of roads in the literature and our frequent direct observations of bobcats traveling by road. It seems plausible that bobcats use roads primarily for travel and, therefore, do not spend much time on them, which would reduce our ability to detect them on roads. The HVIR has a high density of developed and logging roads due to its strong dependence on the timber industry. Thus, it is also possible that most locations throughout a bobcat's home range, whether used or unused, are inherently close to some kind of road, thereby obscuring any proclivity for bobcat frequency on roads.

Predation by bobcats is the leading cause of mortality for female fishers throughout California (Chapter 3), although very little is known about the spatial or competitive relationships between these species. Researchers in Wisconsin investigated the effects of a translocated fisher population on the resident bobcat population with the expectation that fishers would have a negative impact on bobcat reproductive rates, kitten and adult survival, body condition, and population density due to resource competition (Gilbert 2000). They found that in bobcat-inhabited areas to which fishers were translocated and maintained high densities, bobcat kitten survival and population growth were lower, suggesting some level of competition between the species and possibly intraguild predation by fishers on bobcat kittens (Gilbert 2000). We do not know whether fishers prey on bobcat kittens in our study area, but do know that bobcats have killed at least eight adult female fishers (Chapter 3) and likely several more (Higley and Matthews 2007). Unfortunately, there is no information from the Wisconsin study or any others regarding the shared habitat use of bobcats and fishers or a description of risky habitat heightening chances of encounter between them.

It is well-understood that habitat structure affects the frequency of encounters between intraguild predators and their intraguild prey (Janssen et al. 2007). Among the many routes by which habitat structure might influence intraguild predation, two are particularly relevant at our study area. First, vegetation or structural elements in forests may provide escape cover or concealment for prey, even when predator and prey are in close proximity (Litvaitis et al. 1985, Godbout and Ouellet 2010). Alternatively, vegetation structure may hinder escape if prey rely on visually detecting predators early enough to enable a quick retreat to safe areas, such as underground burrows (Schooley et al. 1996, Thompson and Gese 2007). Second, intraguild prey may simply avoid areas or habitats used frequently by intraguild predators for hunting or

traveling (Sergio et al. 2007, Thompson and Gese 2007). When individuals do use these high-risk habitats, probability of encounter and subsequent attack and predation is augmented (Hebblewhite et al. 2005, Kunkel and Pletscher 2000).

When we looked at fisher-bobcat habitat overlap, we found that fishers were less likely to overlap with bobcats in areas with typical habitat for fishers in our study area (forest with dense canopy, Zielinski et al. 2012) such as young, closed canopy forest (YFM), but more likely to overlap in habitats typical for bobcats in our study area (open areas) suggesting that fishers are at risk when they use less common fisher habitats, rather than when bobcats venture into typical fisher habitat. Older, complex forest is typically characterized by structure, and in the mixed coniferous forests of northwestern California, this characteristic stems from downed woody debris, tree malformations, snags, and large, highly structured individual trees (Franklin et al. 1981). We found that fisher-bobcat overlap areas consisted of a lower proportion of YFM, a forest type that also exhibits similar structure. This is consistent with Janssen et al. (2007) who found that habitat structure diminishes impacts to intraguild prey. This finding is not surprising because young, multistoried Douglas-fir forests also are characteristically structured in that they are comprised of multiple canopy layers, brushy openings created by tree mortality, varying levels of shrub understory, and patches of ground cover. These attributes likely provide cover from predation for forest species and represent habitat types not selected by bobcats in our study area. Interestingly, mature and older forest (MOF) was not significantly different between overlap and non-overlap areas for fishers and bobcats.

One landscape feature of fisher-bobcat overlap areas that was surprisingly less likely to be associated with fisher-bobcat overlap areas than areas where fisher do not overlap with bobcats is non-driveable roads. We expected areas with bobcats to have greater densities of

roads, either driveable or non-driveable, and areas where fishers did not overlap with bobcats to have fewer roads. Why we found the opposite result is unclear, but perhaps could stem from a tendency for overgrown, unused logging roads to be associated with much older harvested stands that have become young, multistoried forest (YFM), a habitat type selected against by bobcats, and likely selected for by fisher (Zielinski et al. 2012). In support of this, YFM was also found in higher proportions in areas where fishers did not overlap with bobcats.

We found that proximity to open and brushy habitats heightened the risk of predation by bobcats on fishers. Clearly, bobcats frequent open habitats so the closer to these habitat types that fishers venture, the greater risks they take of encountering bobcats. In addition, brushy habitats may harbor more prey that is shared by both bobcats and fishers, such as dusky-footed woodrats (*Neotoma fuscipes,* K.E. Weller, unpublished data, Golightly et al. 2006) which are found in highest abundance in brushy, poletimber stands in the forests of northwestern California (Sakai and Noon 1993). Leporids, specifically brush rabbits (*Sylvilagus bachmani*) also a common prey item in bobcat diet in our study area (K.E. Weller, unpublished data), require brushy areas (Chapman 1974), and may also attract bobcats to this habitat type. Furthermore, our third-order habitat selection results indicated bobcats selected for brushy habitats, second only to open areas. When fishers use these habitat types they don't frequently visit, they may expose themselves to greater risk of bobcat predation.

Intraguild prey using habitats similar to their predators often avoid encounters by using shared habitats at different times of day or seasons. Data from bobcats and coyotes in Oregon showed that bobcats used the same types of habitats as coyotes, but their main periods of activity differed from coyotes (Witmer and deCalesta 1986) and smaller carnivores evaded interaction with coyotes by avoiding areas at specific times (Crooks and Soulé 1999). Our spatial co-

occurrence data were not temporally explicit and do not represent simultaneous locations of bobcats and fishers. Therefore, we could not determine whether fishers use areas more likely inhabited by bobcats only at times of day when bobcats are less active, and thus less likely to be present. Furthermore, even if fishers and bobcats co-occur in certain habitat types, other habitat characteristics present at encounter sites may protect fishers from predation risk. For instance, high-risk habitats that increase likelihood of encounter might also feature protective structures such as downed logs or thick brush, thereby reducing risk of predation. Although we were able to investigate predation site characteristics at the stand scale, future studies should incorporate more fine-scale analyses of predation sites to identify microhabitat features that may afford fishers protective cover from predation by bobcats.

LITERATURE CITED

Aebischer, N.J., P.A. Robertson and R.E. Kenward. 1993. Compositional analysis of habitat use from animal radio-tracking data. Ecology 74:1313-1325.

Aubry, K.B. and C.M. Raley. 2006. Ecological characteristics of fishers (*Pekania pennanti*) in the Southern Oregon Cascade Range. Unpublished report, U.S. Forest Service, Pacific Northwest Research Station, Olympia, Washington. 31 pp.

Berger, K.M. and E.M. Gese. 2007. Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76: 1075-1085.

Beyer, H. L. 2004. Hawth's Analysis Tools for ArcGIS. Available at http://www.spatialecology.com/htools.

Bingham, B. B., and B. R. Noon. 1997. Mitigation of habitat "take": application to habitat conservation planning. Conservation Biology 11:127–139.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution 24:127–135.

Burnham, K. P., and D. R. Anderson. 1998. Model Selection and Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York, New York, USA.

Calenge, C. 2006. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. Ecological Modelling 197: 516-619.

Chamberlain, M.J., B.D. Leopold, and L.M. Conner. 2003. Space use, movements and habitat selection of adult bobcats (Lynx rufus) in central Mississippi. American Midland Naturalist 149: 395-405.

Constible, J.M., M.J. Chamberlain, and B.D. Leopold. 2006. Relationships between landscape pattern and space use of three mammalian carnivores in central Mississippi. American Midland Naturalist 155: 352-362.

Chapman, J.A. 1974. *Sylvilagus bachmani*. Mammalian Species 34: 1-4.

Conner, L.M. and B.D. Leopold. 1993. Habitat characteristics of bobcat core use areas in Mississippi. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 47:53-61.

Coonan, T.J., C. A. Schwemm, G.W. Roemer, D.K. Garcelon, and L. Munson. 2005. Decline of an island fox subspecies to near extinction. The Southwestern Naturalist 50: 32-41.

Courbin, N., D. Fortin, C. Dussault, and R. Courtois. 2009. Landscape management for woodland caribou: the protection of forest blocks influences wolf-caribou co-occurrence. Landscape Ecology 24: 1375-1388.

Creel, S. and N.M. Creel. 1996. Limitation of African wild dogs by competition with larger carnivores. Conservation Biology 10: 526-538.

Crooks, K. R. and M.E. Soulé. 1999. Mesopredator release and avifaunal extinctions in a fragmented system. Nature 400: 563-566.

Cypher, B. L., and K. A. Spencer. 1998. Competitive interactions between coyotes and San Joaquin kit foxes. Journal of Mammalogy 79:204–214.

Desrochers, A., E.J.B. McIntire, S.G. Cumming, J. Nowak, and S. Sharma. 2010. False negatives—A false problem in studies of habitat selection? Ideas in Ecology and Evolution 3: 20-25.

Donovan, T.M., M. Freeman, H. Abouelezz, K. Royar, A. Howard, and R. Mickey. 2011. Quantifying home range habitat requirements for bobcats (Lynx rufus) in Vermont, USA. Biological Conservation 144: 2799-2809.

Ferguson, A.W., N.A. Currit, and F.W. Weckerly. 2009. Isometric scaling in home-range size of male and female bobcats (Lynx rufus). Canadian Journal of Zoology 87: 1052-1060.

Franklin, J.F., K. Cromack, Jr., W. Denison, A. McKee, C. Maser, J. Sedell, F. Swanson, and G. Juday. 1981. Ecological characteristics of old-growth Douglas-fir forests. USDA For. Serv. Gen. Tech. Rep. PNW-118, 48 p. Pac. Northwest For. and Range Exp. Stn., Portland, OR.

Frey, S.N. and M.R. Conover. 2006. Habitat use by meso-predators in a corridor environment. Journal of Wildlife Management 70: 1111-1118.

Gilbert, J.H. 2000. Impacts of reestablished fishers on bobcat populations in Wisconsin. PhD Dissertation, University of Wisconsin-Madison.

Gillies, C. S., Hebblewhite, M., Nielsen, S., Krawchuk, M. A., Aldridge, C. L., Frair, J. L., Saher, D. J., Stevens, C. E. and Jerde, C. L. 2006. Application of random effects to the study of resource selection by animals. Journal of Animal Ecology 75:887–898.

Godbout, G. and J. Ouellet. 2010. Fine-scale habitat selection of American marten at the southern fringe of the boreal forest. Ecoscience 17: 175-185.

Golightly, R.T., T.F. Penland, W. J. Zielinski, and J.M. Higley. 2006. Fisher diet in the Klamath Province. Final Report to U.S. Fish and Wildlife Service, Contract #113334J025.

Hartley, M.J. and M.L. Hunter. 1998. A meta-analysis of forest cover, edge effects, and artificial nest predation rates. Conservation Biology 12: 465-469.

Hebblewhite, M., E H. Merrill, and T.L. McDonald. 2005. Spatial decomposition of predation risk using resource selection functions: an example in a wolf-elk predator-prey system. Oikos 111: 101–111.

Higley, J.M., G.M. Wengert, S.M. Matthews, and K. Rennie. 2013. Bobcat ecology and relationship with and influence on fisher survival on the Hoopa Valley Indian Reservation, California. Final Report USFWS Tribal Wildlife Grant, U-29-NA-1.

Higley, J.M. and S.M. Matthews. 2007. Fisher habitat use and population monitoring on the Hoopa Valley Reservation, California. Final Report USFWS Tribal Wildlife Grant, U-12-NA-1.

James, A.R.C. and A.K. Stuart-Smith. 2000. Distribution of caribou and wolves in relation to linear corridors. Journal of Wildlife Management 64: 154–159.

Janssen, A., M.W. Sabelis, S. Magalhães, M. Montserrat, and T. van der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88:2713–2719.

Johnson, C.J., S.E. Nielsen, E.H. Merrill, T.L. McDonald, and M.S. Boyce. 2006. Resource selection functions based on use-availability data: theoretical motivation and evaluation methods. Journal of Wildlife Management 70:347–357.

Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61: 65–71.

Keating, K.A. and S. Cherry. 2004. Use and interpretation of logistic regression in habitat-selection studies. Journal of Wildlife Management 68:774–789.

Kuchler, A. W. 1977. Appendix: the map of natural vegetation of California. Pages 909-938 in M.G. Barbour and J. Major, eds. Terrestrial vegetation of California, John Wiley and Sons, New York, USA.

Kunkel, K.E. and D.H. Pletscher. 2000. Habitat factors affecting vulnerability of moose to predation by wolves in southeastern British Columbia. Canadian Journal of Zoology 78: 150–157.

Litvaitis, J.A. and D.J. Harrison. 1989. Bobcat-coyote niche relationships during a period of coyote population increase. Canadian Journal of Zoology 67: 1180–1188.

Litvaitis, J.A., J.A. Sherburne and J.A. Bissonette. 1985. Influence of understory characteristics on snowshoe hare habitat use and density. Journal of Wildlife Management 49: 866-873.

Neale, J.C.C. and B.N. Sacks. 2001. Food habits and space use of gray foxes in relation to sympatric coyotes and bobcats. Canadian Journal of Zoology 79: 1794–1800.

Palomares, F. and T.M. Caro. 1999. Interspecific killing among mammalian carnivores. The American Naturalist 153: 492–508.

Person, D.K. and D.H. Hirth. 1991. Home range and habitat use of coyotes in a farm region of Vermont. Journal of Wildlife Management 55: 433-441.

Phillips, S.J. and J. Elith. 2013. On estimating probability of presence from use–availability or presence–background data. Ecology 94:1409 – 1419.

Polis, G.A., C.A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecological Systems 20: 297-330.

Preuss, T.S. and T.M. Gehring. 2007. Landscape Analysis of Bobcat Habitat in the Northern Lower Peninsula of Michigan. Journal of Wildlife Management 71: 2699-2706.

R Core Development Team. 2010. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org.

Roemer, G.W., C.J. Donlan, and F. Courchamp. 2002. Golden eagles, feral pigs, and insular carnivores: How exotic species turn native predators into prey. Proceedings of the National Academy of Sciences 99: 791-796.

Ruell, E.W., S.P.D. Riley, M.R. Douglas, J.P. Pollinger, and K.R. Crooks. 2009. Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture–recapture sampling. Journal of Mammalogy 90: 129-135.

Sakai, H.F. and B.R. Noon. 1993. Dusky-footed woodrat abundance in different-aged forests in northwestern California. Journal of Wildlife Management 57: 373-382.

Schooley, R.L., P.B. Sharpe, and B. Van Horne. 1996. Can shrub cover increase predation risk for a desert rodent? Canadian Journal of Zoology 74: 157-163.

Sergio, F., L. Marchesi, P. Pedrini, and V. Penteriani. 2007. Coexistence of a generalist owl with its intraguild predator: distance-sensitive or habitat-mediated avoidance? Animal Behaviour 74: 1607-1616.

Silver, S.C., L.E.T. Ostro, L.K. Marsh, L. Maffei, A.J. Noss, M.J. Kelly, R.B. Wallace, H. Gómez and G. Ayala. 2004. The use of camera traps for estimating jaguar *Panthera onca* abundance and density using capture/recapture analysis. Oryx 38: 148-154.

Thompson, C.M. and E.M. Gese. 2007. Food webs and intraguild predation: community interactions of a native mesocarnivore. Ecology 88: 334-336.

Thornton, D.H., M.E. Sunquist, and M.B. Main. 2004. Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. Journal of Mammalogy 85: 973-982.

Tucker, S.A., W.R. Clark, and T.E. Gosselink. 2008. Space Use and Habitat Selection by Bobcats in the Fragmented Landscape of South-Central Iowa. Journal of Wildlife Management 72: 1114-1124.

Viota, M., A. Rodríguez, J.V. López-Bao, and F. Palomares. 2012. Shift in microhabitat use as a mechanism allowing the coexistence of victim and killer carnivore predators. Open Journal of Ecology 2: 115- 120.

Vucetich, J. A., R. O. Peterson, and C. L. Schaefer. 2002. The effect of prey and predator densities on wolf predation. Ecology 83:3003–3013.

Wengert, G.M., M.G. Gabrie, J.E. Foley, T. Kun, and B.N. Sacks. 2013. Field and molecular techniques for identifying intraguild predators of fishers and other North American small carnivores. Wildlife Society Bulletin 37:659-663.

Witmer, G. W. and D.S. deCalesta. 1986. Resource use by unexploited sympatric bobcats and coyotes in Oregon. Canadian Journal of Zoology 64: 2333-2338.

Yaeger, J. S. 2005. Habitat at fisher resting sites in the Klamath Province of northern California. Master's Thesis, Humboldt State University, Arcata, California, USA.

Zielinski, W.J., J.R. Dunk, and A.N. Gray. 2012. Estimating habitat value using forest inventory data: The fisher (*Pekania pennanti*) in northwestern California. Forest Ecology and Management 275: 35-42.

Table 1. Descriptions of habitat variables included in analyses of bobcat (*Lynx rufus*) habitat selection, predation site habitat analysis, and fisher (*Pekania pennanti*) overlap with bobcat habitat at Hoopa Valley Indian Reservation, February 6, 2011 to October 31, 2012.

Variable	Description
Habitat Strata	Categorical variable with seven habitat types below
MOF	Mature and older Forest
YFM	Young multistoried forest
YCCF	Young closed-canopy forest
TOW	True oak woodland
BRUSH	Dense brush
OPSHRB	Open canopy, low to moderately dense shrub/pole tree cover
OPEN	Prairie, landslide, urban industrial
Management	Categorical variable with three types below
UNMAN	Unmanaged Forest
CABLE	Managed Forest Logged with Yarder
TRACTOR	Logged with Tractors, makes a network of skid roads of varying density
Forest	Categorical variable with two types below
NONFOR	Lumps BRUSH, OPEN and OPSHRB together to represent non-forest cover
FOR	Lumps MOF,YFM<YCCF and TOW together to represent forest cover
EDGE	Any edge between two habitat strata types
HRDEDGE	Any edge between FOR and NONFOR
ROAD	Total Road density
DRVROAD	Drivable Road density
NODRVROAD	Non-drivable (brushed over) road density

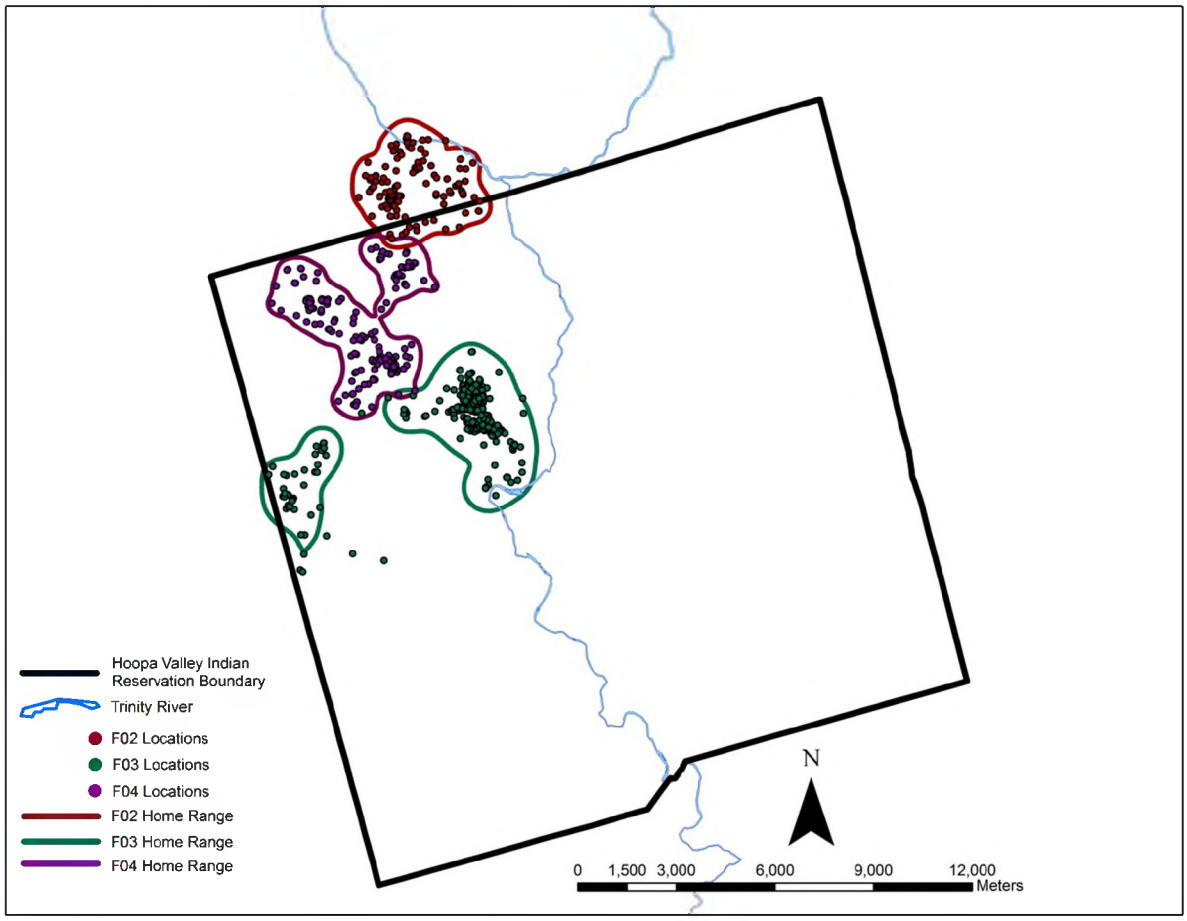

Figure 1. Map of the study area, Hoopa Valley Indian Reservation in north Coastal California showing 95% fixed kernel home ranges of three female bobcats (*Lynx rufus*) affixed with GPS collars and individual bobcat locations within the home ranges, at Hoopa Valley Indian Reservation, February 6, 2011 to October 31, 2012.

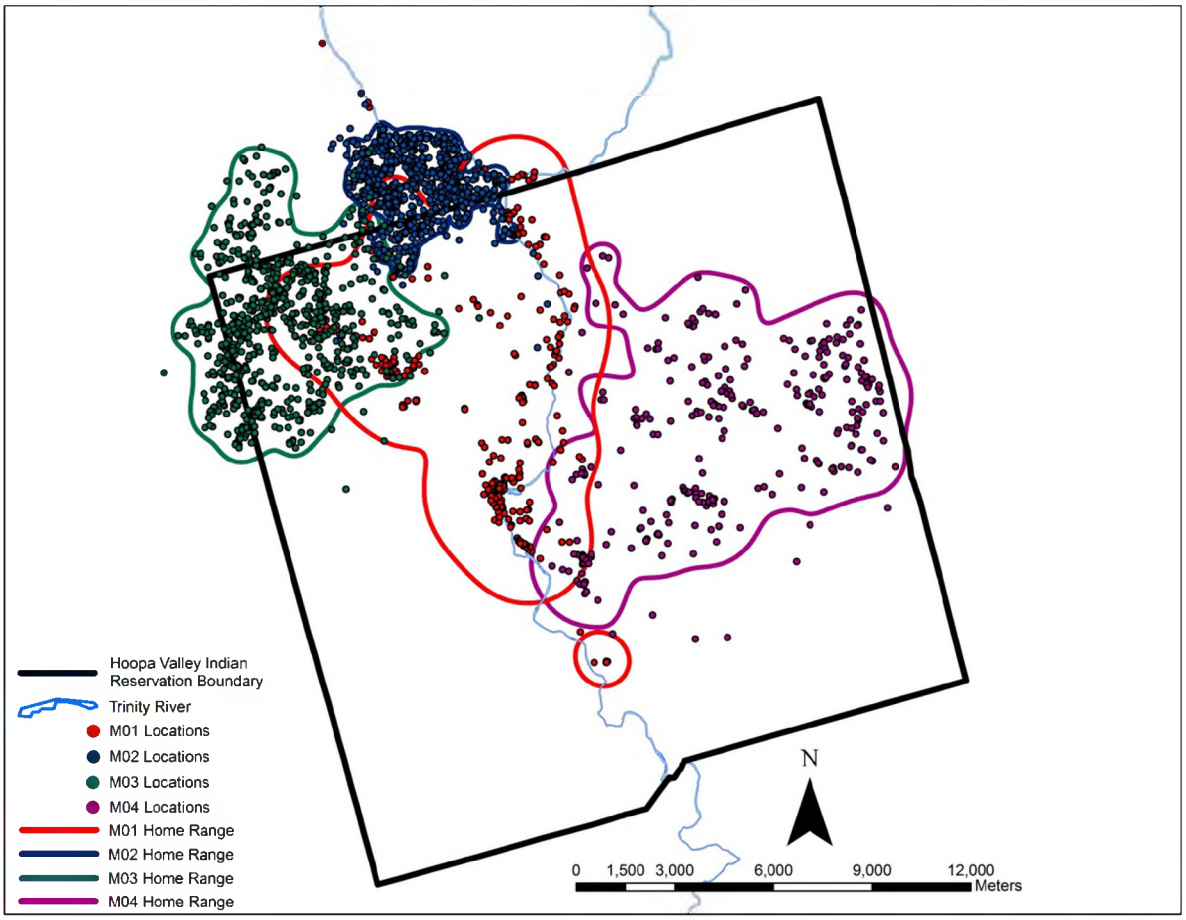

Figure 2. Map of the study area, Hoopa Valley Indian Reservation in north Coastal California showing 95% fixed kernel home ranges of four male bobcats (*Lynx rufus*) affixed with GPS collars and individual bobcat locations within the home ranges, at Hoopa Valley Indian Reservation, February 6, 2011 to October 31, 2012.

Table 2. Bobcat (*Lynx rufus*) 95% fixed kernel home ranges, core area percents of home range, and core areas at Hoopa Valley Indian Reservation, February 6, 2011 to October 31, 2012. Numbers of locations used in each analysis are shown in parentheses.

Bobcat	95 %Fixed Kernel (km^2)	Core Area %	Core Area (km^2)
F02 (137)	11.1	60	3.7
F03 (452)	18.3	70	4.1
F04 (215)	13.7	60	3.9
M01 (445)	92.0	60	23.4
M02 (953)	13.7	60	4.6
M03 (916)	45.7	60	15.4
M04 (413)	79.1	60	25.3

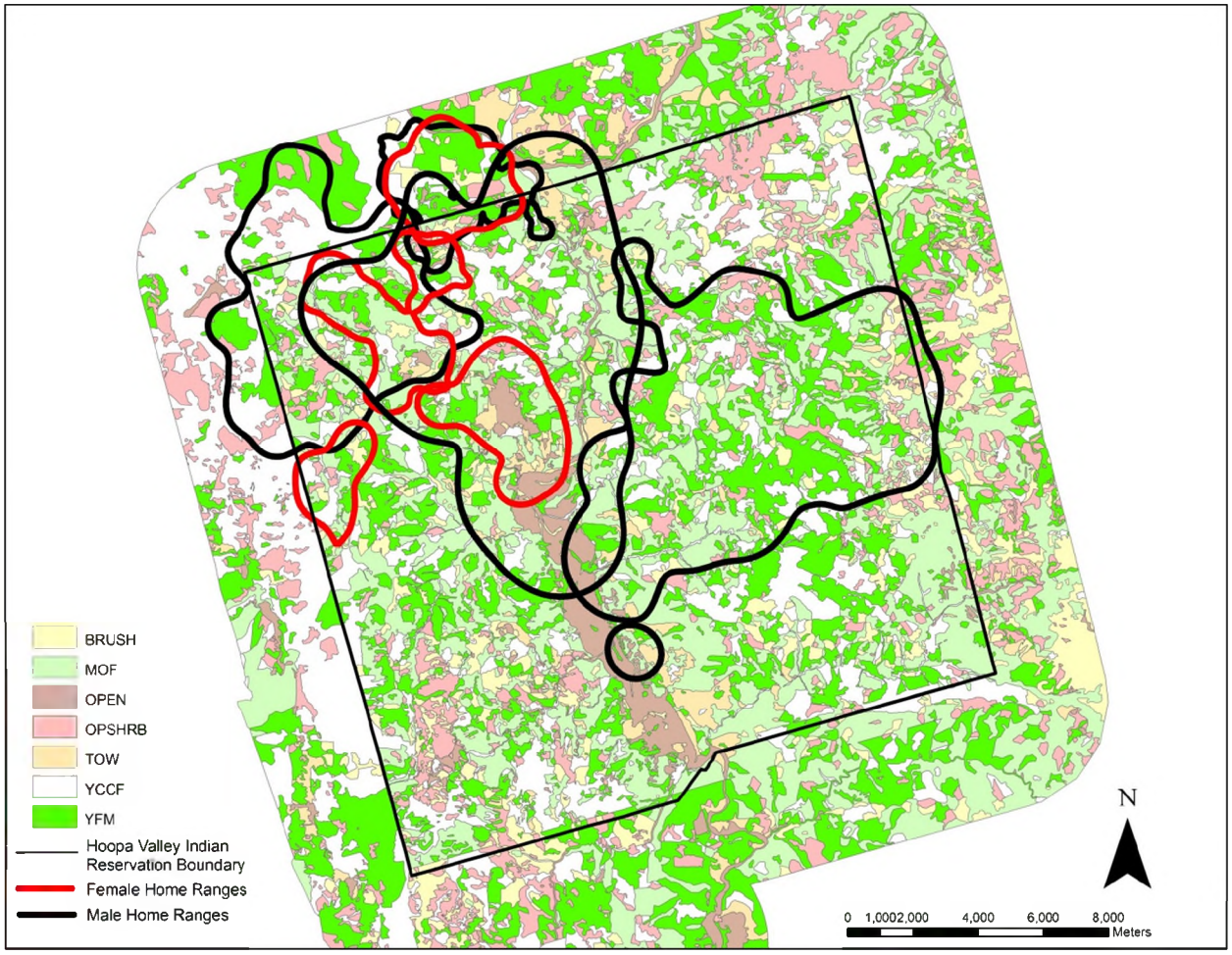

Figure 3. Distribution of three female and four male bobcat (*Lynx rufus*) 95% fixed kernel home ranges across different habitat types at Hoopa Valley Indian Reservation, February 6, 2011 to October 31, 2012. BRUSH = brush habitats, MOF = mature and older forest, OPEN = prairie, landslide and urban habitats, OPSHRB = open canopy, low to moderately dense shrub/pole tree cover, TOW = true oak woodland, YCCF = young, closed canopy forest, and YFM = young, multistoried forest.

Table 3. Average and standard errors of proportions of 95% fixed kernel and core home ranges for seven bobcats (*Lynx rufus*) consisting of the different habitat strata and overall study area composition of habitat strata at Hoopa Valley Indian Reservation, February 6, 2011 and October 31, 2012.

	95% Fixed Kernel		Core Fixed Kernel		Study Area
Parameter	Mean	SE	Mean	SE	
MOF	0.242	0.044	0.196	0.050	0.254
YFM	0.276	0.037	0.273	0.035	0.254
YCCF	0.231	0.030	0.193	0.037	0.223
BRUSH	0.087	0.009	0.115	0.027	0.083
OPSHRB	0.076	0.012	0.064	0.018	0.109
OPEN	0.058	0.020	0.110	0.047	0.044
TOW	0.030	0.011	0.049	0.019	0.033

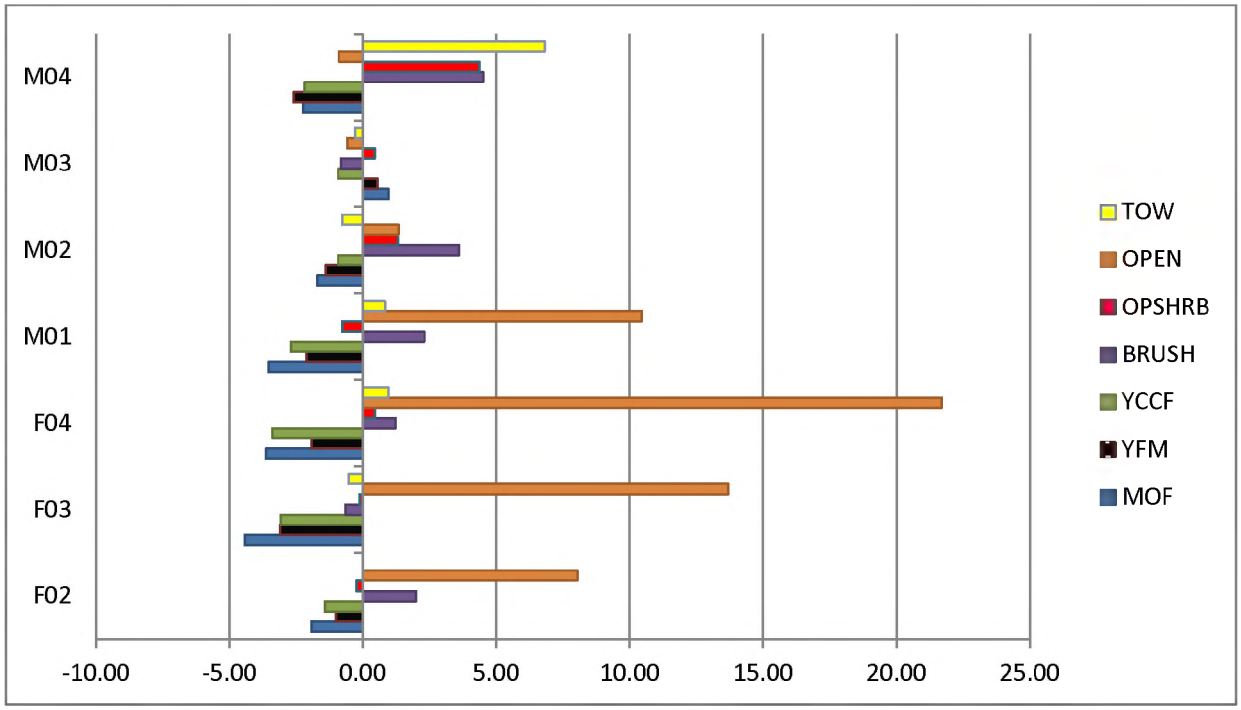

Figure 4. Individual bobcat (*n*=7) habitat selection indices for seven habitat strata types within each bobcat's 95% fixed kernel home range at Hoopa Valley Indian Reservation, Hoopa, CA, built with data collected from GPS and VHF collars on seven bobcats between February 6, 2011 and October 31, 2012.

Table 4. Average proportion of total locations in different habitat strata and averages and standard errors of other habitat variables at "used" locations of GPS-collared bobcats (*Lynx rufus*) and randomly created locations (included as "available" locations in habitat selection analyses) throughout bobcat 95% fixed kernel home ranges at Hoopa Valley Indian Reservation, 2010 – 2012.

	Used		Available	
	Average proportion or distance (m)	SE	Average proportion or distance (m)	SE
Habitat Strata				
BRUSH	0.140	0.027	0.087	0.009
MOF	0.121	0.032	0.252	0.044
OPSHRB	0.099	0.025	0.080	0.015
YCCF	0.138	0.040	0.226	0.030
OPEN	0.260	0.100	0.059	0.023
TOW	0.048	0.020	0.030	0.012
YFM	0.194	0.044	0.265	0.033
FOR	0.501	0.077	0.774	0.024
NONFOR	0.499	0.077	0.226	0.024
Distance to EDGE (m)	61.29	3.79	67.78	4.70
Distance to HRDEDGE (m)	137.81	18.93	155.71	12.46
Distance to ROAD (m)	105.63	8.64	132.69	5.58
Distance to DRVROAD (m)	173.72	27.52	232.11	25.15
Distance to NODRVROAD (m)	474.72	111.99	400.35	70.86

Table 5. Model performance statistics of three top models of habitat selection by bobcats (*Lynx rufus*) at Hoopa Valley Indian Reservation, Hoopa, CA, built with location data collected from GPS and VHF collars on seven bobcats between February 6, 2011 and October 31, 2012. STRATA represents a categorical variable consisting of seven habitat types. Other variables include distance to nearest road (ROAD, m), distance to nearest edge between habitat types (EDGE, m), distance to nearest edge between forest and non-forest habitats (HRDEDGE, m), and distance to nearest driveable road (DRVROAD, m). Individual bobcat was included as a random effect in all models tested.

Model	K	Log-likelihood	AICc	ΔAICc	w_i
STRATA + ROAD + EDGE	10	-4561.093	9142.217	0.0000	0.4649
STRATA + ROAD + HRDEDGE	10	-4561.258	9142.548	0.3316	0.3939
STRATA + DRVROAD + HRDEDGE	10	-4562.562	9145.155	2.9377	0.1070

Table 6. Model-averaged estimates of coefficients (β), standard errors, odds ratios and results of significance tests for the top model of habitat selection by bobcats (*Lynx rufus*) at Hoopa Valley Indian Reservation, Hoopa, CA, built with location data collected from GPS and VHF collars on seven bobcats between February 6, 2011 and October 31, 2012. Shown are seven habitat categories within the STRATA categorical variable, and distance to nearest road (ROAD, m), distance to nearest edge between any two habitat types (EDGE, m), and distance to nearest edge between forest and non-forest habitats (HRDEDGE, m).

Parameter	β	SE	Odds Ratio	Wald Statistic	*P*
STRATA					
Intercept (BRUSH)	0.6127	0.1365	1.8453	4.489	< 0.001
MOF	-1.0069	0.1036	0.3653	9.723	< 0.001
OPEN	1.0670	0.1280	2.9068	8.338	< 0.001
OPSHRB	-0.3481	0.1091	0.7060	3.191	0.0014
TOW	0.0298	0.1588	1.0303	0.188	0.8510
YCCF	-0.9636	0.1160	0.3815	8.303	< 0.001
YFM	-0.8200	0.1025	0.4404	8.002	< 0.001
ROAD	-0.0148	0.0026	0.9853	5.671	< 0.001
EDGE	-0.1285	0.0039	0.9872	3.267	0.0011
HRDEDGE	0.0056	0.0017	1.0056	3.230	0.0012

Table 7. Means and standard errors of habitat variables comprising regions of overlap between fisher (*Pekania pennanti*) 95% fixed kernels and bobcats (*Lynx rufus*) core areas, and regions of fisher kernels that don't overlap with a bobcat at Hoopa Valley Indian Reservation, between February 6, 2011 and October 31, 2012. Also shown are the results of paired t-tests between habitat composition (%) of the region of a fisher's home range where it overlaps with a bobcat core and the region where it does not overlap with a bobcat core.

	Overlap Regions		Non-overlap Regions		*P*
	Mean	SE	Mean	SE	
Habitat Strata					
%MOF	39.11	4.51	34.98	3.79	0.271
%YCCF	18.78	4.60	20.76	3.39	0.565
%YFM	20.36	2.34	26.88	3.38	0.096
%OPSHRB	1.93	0.47	5.04	1.20	0.017
%OPEN	2.79	0.95	1.42	0.53	0.150
%TOW	4.13	1.14	3.05	1.02	0.374
%BRUSH	12.89	4.62	7.88	1.04	0.290
%FOR	82.39	4.32	85.66	1.83	0.505
%NONFOR	17.61	4.32	14.34	1.83	0.505
ROAD density (km/km^2)	2.53	0.27	2.97	0.25	0.326
DRVROAD density (km/km^2)	2.15	0.29	1.80	0.18	0.369
NODRVROAD density (km/km^2)	0.38	0.13	1.17	0.16	0.005
EDGE density (km/km^2)	7.48	0.26	7.82	0.85	0.743
HRDEDGE density (km/km^2)	2.68	0.38	2.76	0.45	0.871

Table 8. Model performance statistics of three top models of kernel overlap of bobcats (*Lynx rufus*) and fishers (*Pekania pennanti*) at Hoopa Valley Indian Reservation, Hoopa, CA, between February 6, 2011 and October 31, 2012. Individual fisher was included as a random effect in all models tested.

Model	K	Log-likelihood	AICc	ΔAICc	w_i
NODRVROAD + YFM + OPEN	5	-8.5340	29.5681	0.0000	0.3639
NODRVROAD + OPEN	4	-10.2292	30.0585	0.4904	0.2848
NODRVROAD + YFM + OPEN + MOF	6	-8.3314	32.3149	2.7468	0.0922

Table 9. Model-averaged estimates of coefficients (β), standard errors, odds ratios and results of significance tests for the top model of kernel overlap of bobcats (*Lynx rufus*) and fishers (*Pekania pennanti*) at Hoopa Valley Indian Reservation, Hoopa, CA, between February 6, 2011 and October 31, 2012.

Parameter	β	SE	Odds Ratio	Wald Statistic	*P*
Intercept	3.9261	2.7141	50.7102	1.447	0.1480
NODRVROAD	-5.0732	1.9413	0.0063	2.613	0.0090
YFM	-0.1256	0.0786	0.8819	1.599	0.1098
OPEN	0.7366	0.3245	2.0889	2.270	0.0232

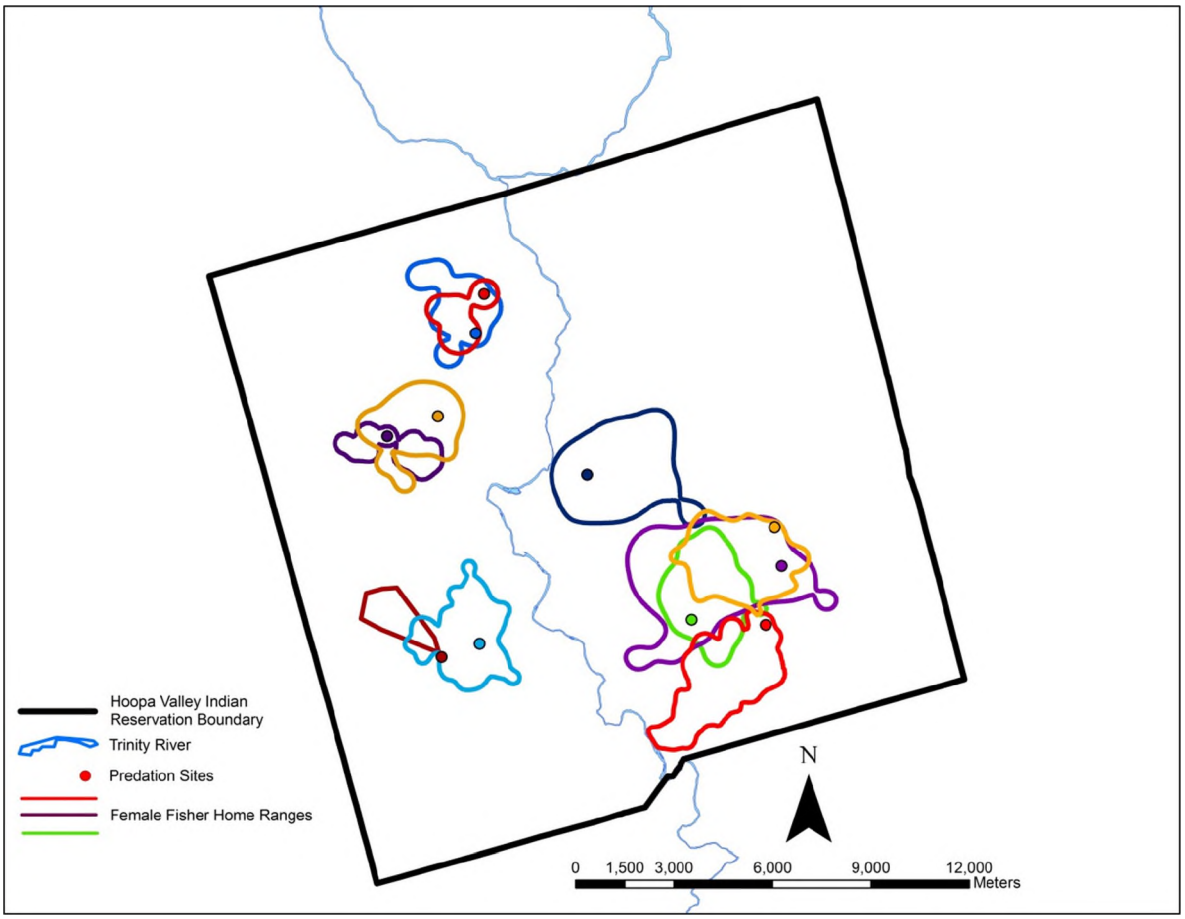

Figure 5. Map of the study area, Hoopa Valley Indian Reservation in north Coastal California showing 95% fixed kernel home ranges of eleven female fishers (*Pekania pennanti*, Higley et al. 2013) that were killed by bobcats (*Lynx rufus*), and their predation sites between April 28, 2005 and April 17, 2013. Each fisher's predation site is the same color as its home range.