Pathogens Associated with Fishers (*Martes pennanti*) and Sympatric Mesocarnivores in California

---Final Report submitted to the USFWS, Yreka, CA---

for USFWS Grant # 813335G021

Submitted: 13 May 2008

Contents

Chapter 1. Pathogens Associated with Fishers 2
Richard N. Brown,[1,2,7] Mourad W. Gabriel,[2,3] Greta M. Wengert,[2,4]
Sean Matthews,[5,6] J. Mark Higley,[6] and Janet E. Foley[7]

Chapter 2. Pathogens Associated with Mesocarnivores Sympatric with Fishers in Northwestern California 49
Mourad W. Gabriel,[2,3] Greta M. Wengert,[2,4] Janet E. Foley,[7]
J. Mark Higley,[6] Sean Matthews,[5,6] and Richard N. Brown[1,2,7]

Acknowledgements 74

Literature Cited 75

Appendix A. 91

Author Affiliations

[1]Department of Wildlife, Humboldt State University, Arcata, CA 95521
Phone: (707) 826-3320; Fax: (707) 826-4460; E-mail: RBrown@humboldt.edu

[2]Integral Ecology Research Center, 102 Larson Heights Rd., McKinleyville, CA 95519

[3]Graduate Group of Comparative Pathology, Department of Veterinary Medicine and Epidemiology, Davis, CA 95616 Phone: (707) 845-7847; E-mail: MWGabriel@ucdavis.edu

[4]Graduate Group of Ecology, Department of Veterinary Medicine and Epidemiology, Davis, CA 95616

[5]Wildlife Conservation Society, Hoopa, CA

[6]Wildlife Section, Hoopa Tribal Forestry, Hoopa, CA

[7]Center for Vector-borne Diseases and Department of Veterinary Medicine and Epidemiology, University of California, Davis, CA

Chapter 1. Pathogens Associated with Fishers

Richard N. Brown, Mourad W. Gabriel, Greta M. Wengert,
Sean Matthews, J. Mark Higley, and Janet E. Foley

ABSTRACT

We report disease exposures of fishers trapped on the Hoopa Valley Indian Reservation (HVIR), in northwestern California, near Redding, in north-central California, and along the west facing slopes of the southern Sierra Nevada Mountains. Samples from HVIR were collected from 76 individual fishers captured a total of 115 times between December 2004 and March 2007. Four (4%) of 99 serum samples from HVIR fishers had been exposed to West Nile virus (WNV), five (5%) of 98 fishers had been exposed to canine distemper virus (CDV), 28 (31%) of 90 had been exposed to canine parvovirus (CPV), four (4%) of 95 had been exposed to canine adenovirus (CAV2), five (5%) of 96 had been exposed to canine herpes virus (CHV), 24 (24%) of 102 had been exposed to *Borrelia burgdorferi* sensu lato, 60 (76%) of 79 had been exposed to *Anaplasma phagocytophilum*, 28 (51%) of 55 had been exposed to an agent similar to *Rickettsia rickettsii*, and 45 (58%) of 77 had been exposed to *Toxoplasma gondii*. None of 34 samples from HVIR fishers had detectable antibodies against *Yersinia pestis* and none of 35 had antibodies against *Bartonella* spp. DNA extracted from samples was amplified using polymerase chain reaction (PCR) and sequenced confirming current infections of CPV in 18 (18%) of 98 fecal samples and of *A. phagocytophilum* in three (4%) of 78 blood samples. Exposures of fishers to *T. gondii* were more common in female (69%)

than in male (49%) fishers (P = 0.054), but exposures to other pathogens did not vary significantly between males and females. All three fishers that were PCR-positive for *A. phagocytophilum* were female, but we found no significant differences between genders (P = 0.08). Exposures to CPV varied with age (P = 0.003); 20% of fishers aged 0-1 yr and 49% of fishers aged >2 yrs were exposed. Likewise, exposures to *A. phagocytophilum* varied with age (P = 0.01) with 68% of fishers aged 0-1 yr and 93% of fishers aged >2 yrs exposed. Other pathogen exposures did not vary with age or sex. Fifteen radio-collared fishers were found dead on the HVIR during this period. Two of the mortalities had been exposed to CDV, six to CPV, 10 to *A. phagocytophilum*, nine to *T. gondii*, and nine to three or more pathogens. However, there were no clear associations of co-exposures nor demonstrated association of pathogen exposure with mortality risk. Fishers trapped near Redding were less likely to be exposed to CPV (10% of 19; P = 0.052), *A. phagocytophilum* (25% of 19; P = 0.00006) and *B. burgdorferi* (5% of 19; P= 0.055) than were fishers from the HVIR. Additionally, 33 fecal samples collected from fishers in the southern Sierras were evaluated for CPV-DNA using PCR, of which none were positive for CPV, a significant difference (P = 0.013) compared to findings from the HVIR. Although little is known about diseases in fishers, many of the pathogens being evaluated cause morbidity or mortality in susceptible carnivores. We have demonstrated exposures to several serious pathogens and we believe ours to be the first report of exposures to West Nile virus in a mustelid. Several of the pathogens discussed commonly cause immunosuppression in other species, suggesting the potential for synergistic

effects of pathogen exposures. Several of the viruses can be transmitted via saliva, oculonasal discharges, or feces. Traps and handling equipment used to capture fishers and other mesocarnivores should be disinfected after each use to minimize risks of spreading viruses throughout fisher populations, and disease issues should be considered in any management strategy for fishers in the western states.

INTRODUCTION

Fishers (*Martes pennanti*) are mid-sized, forest-dwelling carnivores in the family Mustelidae. In western North America, the geographic distribution of the species historically included forests of southwestern Canada southward through the Cascade Range and coastal mountains along the Pacific Ocean, through northwestern California, and along the western slopes of the Sierra Nevada Mountains (Gibilisco 1994). However, forest management practices combined with the effects of over-trapping during the early 1900s resulted in low population densities throughout the western states of Washington, Oregon, and California and may have contributed to the isolation of the populations in the southern Sierra Nevada Mountains (United States Fish and Wildlife Service 2004). In 2004, the US Fish and Wildlife Service (USFWS) designated a “distinct population segment” (DPS) in California, Oregon, and Washington, that merited listing under the federal Endangered Species Act (United States Fish and Wildlife Service 2004). In the finding, the USFWS considered disease to be one of five

threats to fishers in the western region (United States Fish and Wildlife Service 2004).

Relatively little is known about the diseases of fishers. Lists of macroparasites found in fishers are available (Douglas and Strickland 1999, Powell 1993), but few references mention exposures to viruses or bacteria or the diseases that such pathogens cause (Philippa et al. 2004,Brown et al. 2006). The diseases most commonly reported in small and medium-sized wild mammals are those that cause widespread epizootics with noticeably high rates of mortality, such as plague (caused by *Yersinia pestis*) and distemper (caused by canine distemper virus; CDV). Other pathogens that cause chronic disease or that cause pathology synergistically with other pathogens often go unnoticed; animals that fail to thrive may be more likely to succumb to other mortality factors or may disappear and not be counted as disease-related mortalities (Cleaveland et al. 2002). Most important may be those pathogens that cause subclinical disease that affect fitness by reducing energy available for maintenance or growth, by decreasing mating success, or by causing reproductive failure (Addison et al. 1999, Cleaveland et al. 2002).

Controlled studies of exposures of fishers to pathogens are lacking, but by extrapolating from closely related species, some disease outcomes can be predicted. Pathogens known to cause severe disease in mustelids include rabies virus (Ruprecht et al. 2001), CDV (Williams 2001,Langlois 2005), parvoviruses (PV; especially those causing Aleutian disease of mink [ADV], mink virus enteritis [MEV], and feline panleukopenia virus [FPV]) (Barker and Parrish 2001, Langlois

2005), influenza viruses; corona viruses; *Brucella* spp.; *Y. pestis* (Williams et al. 1994), *Toxoplasma gondii* (the causative agent of toxoplasmosis) (Burns et al. 2003; Dietz et al. 1993, Frank 2001, Philippa et al. 2004), and the nematode *Trichinella spiralis* (Dick et al. 1986, Dick and Leonard 1979). In addition, West Nile virus (WNV), *Anaplasma phagocytophilum* (the agent causing granulocytic anaplasmosis) (Foley et al. 2004), *Borrelia burgdorferi* sensu lato (sl; meaning in the broad sense; members of this group cause Lyme borreliosis) (Brown and Burgess 2001, Foley et al. 2004, Lane et al. 2001, Lane et al. 2004), and *Bartonella* spp. (including several emerging zoonotic pathogens) (Chang et al. 1999, Chang et al. 2000, Gabriel et al. In Preparation) are of regional importance and their effects in fishers remain unknown. In addition, canine adenovirus (CAV2; a cause of infectious hepatitis) has been associated with mortality of striped skunks (*Mephitis mephitis*) as well as members of the family Canidae (Karstad et al. 1975, Woods 2001).

The current study was undertaken to (1) determine rates of exposure of fishers in northwestern California to pathogens, (2) characterize selected pathogens of fishers using molecular techniques, and (3) compare exposures of fishers from different populations to provide disease-related information relative to the conservation of fishers in the western DPS.

STUDY AREAS

Fishers were sampled from three study areas: blood and fecal samples were collected from fishers on the Hoopa Valley Indian Reservation (HVIR) in

northeastern Humboldt County, California. Blood samples were collected from a population near Redding, California, approximately 85-100 km east of the HVIR and fecal samples were analyzed from fishers from the western slopes of the southern Sierra Nevada Mountains.

The HVIR occupies approximately 362 km^2, with elevations ranging from 76 m to 1170 m. The landscape was a heterogeneous mix of habitats, but dominant forest species included Douglas fir (*Pseudotsuga menziesii*) and tan oak (*Lithocarpus densiflorus*) (Singer and Begg1975, Mayer and Laudenslayer 1988;). The HVIR fisher population sampled was thought to be a subset of a fairly continuous fisher population in northwestern California and southern Oregon (Zielinski et al. 1995).

Fishers also were sampled on Sierra Pacific Industries properties near Redding, California, from “Klamath mixed-conifer” forests with a predominance of ponderosa pine (*Pinus ponderosa*) and sugar pine (*Pinus lambertiana*) (Mayer and Laudenslayer 1988; Self and Kerns 2001). Other common forest trees included white fir *(Abies concolor),* incense cedar (*Calocedrus decurrens*), Jeffrey pine *(Pinus jeffreyi),* Douglas fir*,* California black oak *(Quercus kelloggii),* and western juniper *(Juniperus occidentalis).* These sites were dryer, ranged to higher elevations, and had more open habitat than sites near Hoopa (Mayer and Laudenslayer 1988, Self and Kerns 2001).

Fishers were sampled from the Sierra National Forest in the southern Sierra Nevada Mountains in typical “Sierra mixed-conifer” habitat at elevations ranging from 1,200 m to 2,133 m (Dr. Mark Jordan, University of California, Berkeley,

personal communication). Plant communities in this area ranged from mixed chaparral (*Adenostema* spp., *Cercocarpus* spp., *Arctostaphylos* spp., *Ceanothus* spp.) at the lower elevations, through montane hardwood forest dominated by canyon live oak (*Quercus chrysolepis*) and California black oak, to mixed conifer forest dominated by ponderosa pine, white fir, incense cedar, sugar pine (*Pinus lambertiana*), and giant sequoia (*Sequoiadendron giganteum*) (Zielinski et al. 1999).

METHODS

Capture, Handling, and Sampling

Fishers were trapped on the HVIR and at sites near Redding in wire mesh live traps (81 x 25 x 31cm) (Tomahawk Live Trap Company, Tomahawk, Wisconsin) baited with chicken during the months of December 2004 to April 2005, September 2005 to April 2006, and September 2006 to March 2007; traps were not set during Spring and early Summer to avoid capture of lactating females and young kits. Wooden nest boxes were attached to traps to provide security, reduce environmental stressors, and facilitate handling of trapped animals (Gabriel and Wengert 2005). Fishers were trapped and sampled near Redding from January to March 2006. Archived fisher fecal samples from animals trapped in the southern Sierras between October 1999 and August 2004 were saved frozen until sent to the University of California at Davis (UCD) for analysis.

Body weights of fishers trapped at Hoopa were measured to the nearest 0.2 kg, and fishers were handled using standard protocols, including the use of a handling cone with welded metal rods. Anesthesia was induced using 20mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and 0.5mg/kg diazepam (Hospira, Inc., Lake Forest, Illinois, USA). A brief physical exam was performed and anesthetized animals were monitored continuously throughout anesthesia. Passive integrated transponders (PIT tags) were injected subcutaneously, and numbered plastic tags were placed in both ear pinnae for future identification. The dentition was evaluated to allow field estimation of the age category of each animal and a first, upper premolar was removed for aging by cementum annuli (Matson's Laboratory, Milltown, Montana) (Poole et al. 1994). All fishers were released after recovery from anesthesia at their sites of capture.

Approximately 1-3 ml of whole blood was collected by venapuncture from fishers trapped at Hoopa and near Redding and stored frozen in ethylene diamine tetraacetic acid (EDTA) until shipped to the UCD for further evaluation. Blood was thawed and centrifuged to separate the plasma from the cells, and the plasma was serially diluted in 10 mM phosphate-buffered saline. All trapping and handling of fishers at Hoopa, and the reception of samples from collaborators, followed protocols approved by Humboldt State University's Institutional Animal Care and Use Committee.

Assays for Exposure to Pathogens

Plasma samples were assayed for presence of antibodies that bound to antigens of different pathogens by indirect immunofluorescence assays (IFAs). Antigen for the CDV IFA was from kidney cells of CDV-infected domestic ferrets (*Mustela furo*) (American Bioresearch, Milton, Tennessee). Positive controls were serum samples from domestic dogs (C*anis familiaris*) previously shown to have high, standardized titers, and negative controls were from dogs maintained at a specific pathogen-free (SPF) colony. Positive results were defined as those reacting at a dilution of ≥1:8. Antibodies specific to CPV, CAV2 and CHV were detected with commercially available antigen slides (Veterinary Medical Research and Development, Pullman, Washington). Positive controls for CPV, CAV2 and CHV tests were blood samples from dogs previously shown to have high standardized titers, and negative controls were from SPF colony dogs. Positive results were defined as those reacting at a dilution of ≥1:25. Exposure to WNV and *Toxoplasma gondii* was evaluated by the Animal Health Diagnostic Center (Cornell University, Ithaca, New York) using serum neutralization for WNV with positive results defined as those reacting at a dilution of ≥1:4, and indirect hemagglutination for *T. gondii* with positive results defined as those reacting at a dilution of ≥1:128. Antigen for the *A. phagocytophilum* IFA was from cells isolated from infected horse blood. Positive controls were serum samples from dogs of known infection history with high, standardized titers, and negative controls were from dogs from an SPF colony. Positive results were defined as those reacting strongly at a dilution of ≥1:25. Exposure to *Bartonella* spp. was

determined using antigen prepared from *B. clarridgeiae* and *B. henselae* and positive reactions were defined as those reacting at a dilution of ≥1:64. The IFA tests for exposure to *Borrelia burgdorferi* sl and *Rickettsia* sp. were conducted using commercial antigen slides (Protatek, St. Paul, Minnesota) with serum samples from domestic dogs with either known positive titers or known to have been unexposed, used as positive and negative controls, respectively; dilutions of 1:80 were used as the cutoff titers differentiating samples considered to be positive or negative for both pathogens. Exposures to *B. burgdorferi* were confirmed by Western blot. Tests for exposure to *Y. pestis* were performed by Dr. Bruno Chomel's lab (University of California, Davis) using a passive hemagglutination test to identify anti-F1 antibodies (IHA), and positive results were defined as those reacting at a dilution of ≥1:64.

Molecular Assays

DNA was extracted from whole blood using a Qiagen kit (Qiagen, Valencia, California) following manufacturer's instructions and from feces using a modified Boom method of silica extraction (Cheung et al. 1994). The polymerase chain reaction (PCR) was used to amplify DNA; thus identifying current infections of, rather than prior exposure to, a pathogen. TaqMan real-time PCR for the *A. phagocytophilum* p44 gene was performed as described in Drazenovich et al. (2006). Nested-PCR was performed as described in Hirasawa et al. (1994) for canine parvovirus, feline panleukopenia virus, and mink enteritis virus. Amplicons from the nested-PCR assay were cloned into a pCR2.1 vector using

the TOPO cloning kit (Invitrogen, Carlsbad, California) and amplified in TOP10F′-competent cells of *Escherichia coli* (Invitrogen, Carlsbad, California). The plasmid was purified using a Plasmid Miniprep kit (BioRad, Hercules, California) and the vector insert was sequenced with the plasmid M13f primer (Davis Sequencing, Davis, California) using the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, California). The sequences were determined to be from canine parvovirus by comparison with previously published sequences using the BLAST database search program (Altschul et al. 1990).

Statistics and Data Exclusion Rules

Co-exposures and prevalence of exposures in males vs. females, subadults vs. adults, and among seasons were assessed using Fisher's exact tests. Comparison of mean ages of male and female fishers was performed with a t-test for samples with unequal variance. Repeated sampling from individuals was included in the analyses of prevalence only when we considered the results to be independent to reduce potential errors associated with repeated sampling. Thus, data were excluded when (1) results followed a previously positive test result, or (2) when negative IFA samples were separated by less than nine months or negative PCR results were separated by less than six months. However, data from two repeated samples were included when negative results were followed by a positive result indicating recent infection. A nine-month interval was considered sufficient to allow exposures during the three seasons following collection of an initially negative sample. Lastly, the discounting of previously

positive samples insures that single exposures were not counted twice and that the prevalence reported was a minimum (i.e. we avoided inflation of our estimates of prevalence that could result from repeatedly reporting positive animals). Likewise, pairs of data for the analysis of co-exposure were included only when sampling occurred at an interval of greater than six or nine months (for PCR and IFA, respectively) or when one of the results changed from a negative to a positive. Total sample size varied among pathogens in the analyses of prevalence and between the analyses of prevalence and co-exposures because some laboratory tests were not performed or because of exclusion of individual results based on the criteria noted above.

RESULTS

Fishers from Hoopa

A total of 115 samples from 76 individual fishers trapped on the HVIR were included in analyses. The mean age of male fishers (1.8 years; N = 56) was less than the mean age of females (2.4 years; N = 49) (t stat = -1.56; p = 0.06) (Figure 1); ages of individual fishers sampled during multiple years were counted once per year.

Serologic evaluation of fisher plasma samples revealed that four (4%) of 99 had been exposed to WNV, five (5%) of 98 had been exposed to CDV, 28 (31%) of 90 had been exposed to CPV, four (4%) of 95 had been exposed to CAV2, five (5%) of 96 had been exposed to CHV, 28 (51%) of 55 had been exposed to a

Rickettsia sp. (either *R. rickettsii* or a species that causes cross reactions with this species), 24 (24%) of 102 had been exposed to *B. burgdorferi* sl, 60 (76%) of

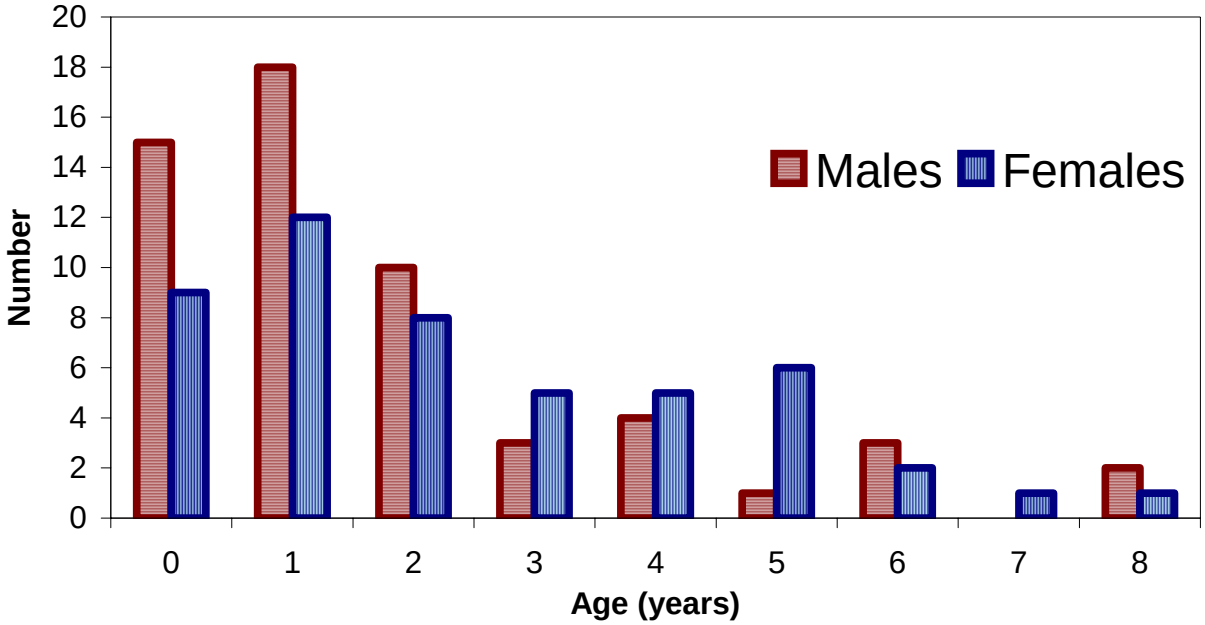


Figure 1. Age distribution, based on cementum annuli, of 56 male and 49 female fishers sampled on the Hoopa Valley Indian Reservation during December 2004 through March 2007.

79 had been exposed to *A. phagocytophilum*, 45 (58%) of 77 had been exposed to *T. gondii*, and none had been exposed to *Bartonella* spp. (N = 35) or *Y. pestis* (N = 34); data concerning fecally-transmitted viruses from the first 31 of these fishers were reported previously (Brown et al. 2006). All three DNA sequences amplified with primers specific to *A. phagocytophilum* matched previously reported sequences available for this pathogen on GenBank. Eighteen (18%) of 98 fecal samples were PCR-positive with primers specific to parvoviruses, and sequences of DNA amplicons were found to match CPV; no PCR-amplified DNA matched the sequences of any of the other parvoviruses.

A greater proportion of females (25 of 36) than males (20 of 41) were exposed to *T. gondii* (Fisher's exact test, P = 0.05). However, none of the other exposures to, or rates of infection by, pathogens differed significantly between males and females (Table 1-1).

Table 1-1. The proportion of male and female fishers (*Martes pennanti*) exposed to pathogens at the Hoopa Valley Indian Reservation, December 2004, through March 2007.

	Number (%) positive of No. Sampled		
Pathogens	Male Fishers	Female Fishers	P-value
West Nile Virus	2 (4%) of 53	2 (4%) of 46	P = 0.64
Canine Distemper Virus	2 (4%) of 52	3 (6%) of 46	P = 0.45
Canine Parvovirus	14 (29%) of 48	14 (33%) of 42	P = 0.45
Canine adenovirus-2	1 (2%) of 52	3 (7%) of 43	P = 0.25
Canine herpes virus	2 (4%) of 50	3 (6%) of 46	P = 0.67
Rickettisia spp.	16 (53%) of 30	12 (48%) of 25	P = 0.15
B. burgdorferi sl	11 (21%) of 53	13 (27%) of 49	P = 0.66
A. phagocytophilum	33 (77%) of 43	27 (75%) of 36	P = 0.45
Y. pestis	0 of 17	0 of 17	P = 1
Bartonella spp.	0 of 18	0 of 17	P = 1
T. gondii	20 (29%) of 41	25 (69%)of 36	P = 0.05 **
A. phagocytophilum – PCR*	0 of 43	3 (9%) of 35	P = 0.08 **
Parvovirus - PCR	11 (19%) of 59	7 (18%)of 39	P = 1

* PCR amplifies pathogen DNA, indicating current infections, whereas the other tests mentioned indicate prior exposure to the pathogen; ** statistical significance determined with Fisher's exact tests.

Exposure rates to two pathogens were higher in adult fishers than in juveniles and yearlings. Adult fishers (18 of 37) were more likely (P = 0.005) than subadults (10 of 51) to have had detectable levels of antibodies against CPV at the time of capture. Although a higher percentage of adults (24% of 42) were found to be actively shedding CPV in their feces than were subadults (14% of

56), this difference was not significant (P = 0.17). Likewise, the probability of exposure to *A. phagocytophilum* was greater (P = 0.02) for adults (26 of 28) than subadults (34 of 50). Nonetheless, all three (N = 52) PCR-positive results were obtained from samples from juveniles; no current infections of *A. phagocytophilum* were found in adults (N = 26). No other pathogen exposures were found to vary by fisher age.

Co-exposures were evaluated in a pair-wise fashion for each pair of potential exposures for each of the 115 samples (Table 1-2; with some data pairs excluded as described). We found few clear patterns of co-exposure. Fishers that were seropositive for CPV were more likely to be exposed to *A. phagocytophilum* than expected; 92% of 36 CPV-positive samples were also positive for *A. phagocytophilum* whereas only 76% of 63 samples negative for CPV were positive for *A. phagocytophilum* (P = 0.05). Fishers that were seropositive for CAV2 were more likely to be exposed to CHV than expected; 43% of seven CAV2-positive samples were also positive for CHV whereas only 4.5% of 88 samples negative for CAV2 were positive for CHV (P = 0.008). Fishers that were seropositive for *T. gondii* were more likely to be exposed to *B. burgdorferi* sl than expected; 43% of 47 *T. gondii*-positive samples were also

Table 1-2. Matrix of co-exposures. The numbers on the diagonal indicate the number of positive samples included in the analysis from 115 possible samples from fishers (*Martes pennanti*) trapped on the Hoopa Valley Indian Reservation, December 2004 through March 2007, for each pathogen listed. The numbers in other cells indicate the number of samples positive for exposure for both of two pathogens indicated along the borders.

Agents	**CDV [1]**	**CPV [2]**	**WNV [3]**	**CAV2 [4]**	**CHV [5]**	**T g [6]**	**R sp[7]**	**B b sl [8]**	**A p [9]**	**A p PCR[10]**
CDV	**5**	1	0	0	0	3	2	1	4	0
CPV		**28**	3	3	3	22	12	8	33	0
WNV			**4**	2	0	3	0	0	3	0
CAV2				**4**	3	4	2	0	7	0
CHV					**5**	3	3	0	9	0
T g						**45**	7	20	41	3
R sp							**28**	0	29	0
B b sl								**24**	17	2
A p									**60**	2
A p PCR										**3**

[1] canine distemper virus; [2] canine parvovirus; [3] West Nile virus; [4] canine adenovirus-2; [5] herpes virus reacting with canine herpesvirus; [6] *Toxoplasma gondii*; [7] *Rickettsia* sp. reacting to *R. rickettsia* antigen; [8] *Borrelia burgdorfier* sl; [9] *Anaplasma phagocytophilum*; [10] results of amplifying DNA of *Anaplasma phagocytophilum* using the polymerase chain reaction.

positive for *B. burgdorferi* sl whereas only 13% of 30 samples negative for *T. gondii* were positive for *B. burgdorferi* sl (P = 0.01). Lastly, the proportion of fishers that were seropositive for both *A. phagocytophilum* and *B. burgdorferi* sl (21% of 81 *A. phagocytophilum*-positive samples) was somewhat less than the proportion of fishers seropositive for only *B. burgdorferi* sl (44% of 18 *A. phagocytophilum*-negative samples were positive for *B. burgdorferi* sl), but this difference was not statistically significant (P = 0.07). Cumulative exposure histories indicated that seven (9%) of the 76 individual fishers sampled on the HVIR were exposed or infected with five pathogens at some point during the study; 20 (26%) were exposed to four pathogens; 21 (28%) were exposed to three pathogens; 16 (21%) were exposed to two pathogens; and 20 (26%) were exposed to only a single pathogen. A single fisher tested negative to four pathogens, but the volume of the sample was inadequate for other tests, and no fishers from which adequate blood volumes were obtained were free of evidence of prior exposures to all pathogens studied.

Fifteen radio-collared fishers were found dead during this study. Prior exposures of fishers found dead included 10 (67%) seropositive for *A. phagocytophilum,* nine (60%) seropositive for *T. gondii*, six (40%) seropositive for *B. burgdorferi* sl, six (40%) seropositive for *Rickettsia* sp., four (27%) seropositive for CPV, two (27%) PCR-positive for CPV, two (13%) seropositive for CDV, two (13%) seropositive for a CHV, one (7%) PCR-positive for *A. phagocytophilum*, one (7%) seropositive for CAV2, and none of the dead fishers were among those previously exposed to WNV. None of these prevalence values differed from

corresponding prevalences from the fishers that were not found dead; i.e. there was no single pathogen exposure clearly associated with the fisher mortalities at Hoopa.

Fishers Trapped near Redding

Nineteen fishers were sampled near Redding, California, by Steve Self (Sierra Pacific Industries) and Richard Callas (California Department of Fish and Game). Exposures to several pathogens differed between samples from these study areas and from fishers sampled on the HVIR (Table 1-3). Most importantly, fewer fishers from this group (2 of 19; 11%) had antibodies to CPV compared to 28 (31%) of fishers from Hoopa (P = 0.05). Likewise, the prevalences of tick-borne pathogens (*B. burgdorferi* sl, *A. phagocytophilum*, and *Rickettsia* sp.) were higher at Hoopa than in the fishers trapped near Redding (P = 0.05, 0.00009, and 0.02, respectively). The prevalence of WNV was numerically higher in the fishers trapped near Redding (3 of 19) than in fishers sampled at Hoopa (4 of 99), but this difference was not statistically significant (P = 0.08). In contrast to exposures of fishers at Hoopa, the fishers trapped near Redding did not show signs of prior exposure to CDV or CHV, but the small sample size makes these comparisons difficult to interpret.

Table 1-3. The proportion of fishers (*Martes pennanti*) exposed to pathogens from the Hoopa Valley Indian Reservation (HVIR) vs. fishers trapped near Redding, California, December 2004 through March 2007.

	Number (%) positive of No. Sampled		
Pathogens	Fishers from HVIR [1]	Fishers from near Redding	P-value
West Nile virus	4 (4%) of 99	3 (16%) of 19	P = 0.08
Canine distemper virus	5 (5%) of 98	0 of 19	P = 0.59
Canine parvovirus	28 (31%) of 90	2 (11%) of 19	P = 0.05 **
Canine adenovirus-2	4 (4%) of 95	1 (5%) of 19	P = 1
Canine hepatitis virus	5 (5%) of 96	0 of 19	P = 0.59
Rickettisia spp.	28 (51%) of 55	6 (32%) of 19	P = 0.02 **
B. burgdorferi sl	24 (24%) of 102	1 (5%) of 19	P = 0.05 **
A. phagocytophilum	60 (76%) of 79	5 (26%) of 19	P = 0.00009 **
Y. pestis	0 of 34	0 of 12	P = 1
Bartonella spp.	0 of 35	0 of 15	P = 1
T. gondii	45 (58%) of 77	6 (46%) of 13	P = 0.55

[1] Data on fecally-transmitted viruses from the first 31 of these fishers was reported previously (Brown et al., 2006); ** indicates statistical significance.

Fishers Trapped in the Southern Sierras

Fecal samples from 33 individual fishers trapped in the Sierra National Forest were analyzed. Interestingly, none of these samples tested PCR-positive for CPV DNA. Hoopa fishers were more likely (18 of 98) to be actively shedding CPV DNA in their feces than were fishers trapped in the Sierra National Forest (0 of 33; P = 0.006).

DISCUSSION

Fishers on the HVIR were found to be exposed to five viruses (WNV, CDV, CAV2, CHV, and CPV), three tick-borne bacteria (*B. burgdorferi* sl, *A. phagocytophilum*, and *Rickettsia* sp.), and the protist *T. gondii*; but not *Bartonella* spp. or *Y. pestis.*

Fishers trapped near Redding were exposed to many of the same pathogens as were those on the HVIR. For instance, four and 16 percent (respectively) of fishers sampled from the HVIR and from sites near Redding had been exposed to WNV. The somewhat (although not significantly) higher prevalence of exposure to WNV from sites near Redding may reflect higher rates of transmission in the northern Sacramento Valley than in the Klamath Mountains near Hoopa. Prevalences to several pathogens, including CPV and the tick-borne bacteria (*A. phagocytophilum*, *Rickettsia* sp. and *B. burgdorferi* sl), were lower in the fishers sampled near Redding than in those from the HVIR. Likewise, although no serum or plasma samples were available to compare serology of fishers from the southern Sierras with those from northern California, the PCR-prevalence of CPV in the fishers sampled from the southern Sierra Nevada Mountains was low compared to fishers from the HVIR. The fisher population in the southern Sierras is isolated from all other fisher populations by a broad gap extending approximately 420 km through the northern and central Sierra Nevada Mountains (Zielinski et al. 1995), and such isolation may influence risks of exposures to some pathogens.

The abundance of fishers on the HVIR appears to be greater than found previously in the Shasta-Trinity National Forest (Yaeger 2005; at sites west of the study areas near Redding), and higher densities of animals are expected to maintain higher prevalences of directly transmitted viruses such as CPV. However, we doubt that CPV cycles independently through fisher populations, but instead cycles through the multiple sympatric species of carnivores (see Chapter 2). The carnivore community on the HVIR is anecdotally quite diverse; commonly observed species include American black bears (*Ursus americanus*), gray foxes (*Urocyon cinereoargenteus*), ringtails (*Bassariscus astutus*), raccoons (*Procyon lotor*), striped skunks, western spotted skunks (*Spilogale gracilis*), fishers, bobcats (*Lynx rufus*), and cougars (*Puma concolor*). However, detailed comparisons of carnivore community diversity at the HVIR vs. the study areas near Redding are not available, and the details of community structure would be necessary to evaluate the role of community diversity in maintaining these pathogens.

Non-vaccinated domestic dogs might be a source of infection for wildlife. Domestic dogs are commonly encountered in the rural areas surrounding the HVIR, and they may be less common on large tracts of land owned or managed by timber agencies (including lands near Redding owned by Sierra Pacific Industries). If proximity to humans and their pets were responsible for the difference in prevalence between these sites then one would expect that prevalence in fishers found further from high densities of human habitation in the valley at Hoopa would be lower than in those fishers living closer to human

habitation, and this was not substantiated (see Chapter 2). Alternatively, higher exposures to CPV at Hoopa might indicate a random difference in fluctuating prevalences, and studies designed to compare community level exposures will be necessary to clarify the cause of high CPV prevalence at Hoopa.

Differences in exposures to tick-borne pathogens likely result from differences in the risk of transmission from ticks. The abundance and distribution of *Ixodes pacificus*, the primary tick vector of *A. phagocytophilum* and *B. burgdorferi* sl, has been well established in the inland regions of the north-coastal mountains of California (Lane et al. 2001, Lane et al. 2004, Brown et al. 2005), and patterns of vector density and distribution, as well as host community structure, likely influence the prevalence of exposures to these pathogens.

The sections that follow describe our results for each pathogen, disease signs observed in other species infected with each pathogen, exposures of these pathogens in populations of other carnivores, and perceived risks of transmission among fishers. We also discuss co-exposures, important potential differences among populations, and management implications of our work.

West Nile Virus

West Nile virus infects a broad range of birds and mammals, but mortality is most often seen in birds, horses, and people. Animals with disease show fever, muscle aches, lethargy, inappetence, diarrhea, vomiting, muscle stiffness, abdominal pain, blindness, head tremors, cardiac arrhythmias, coma, and death.

West Nile virus is transmitted mainly by ornithophilic mosquitoes (Hubalek and Halouzka 1999, Petersen and Roehrig 2001, Turell et al. 2001, Apperson et al. 2004), but some tick species also have been shown to be competent vectors (Lawrie et al. 2004, Hutcheson et al. 2005) and carnivores can become exposed during consumption of prey (Austgen et al. 2004, Kuno 2001). It is logical that species such as bats that feed on mosquitoes are exposed during feeding (Pilipski et al. 2004,Davis et al. 2005, DeKrey and Adams 2005,). Although many carnivores may be resistant, viremia and the ability to infect mosquito vectors has been documented in domestic cats, and cats have become infected through consumption of infected prey (Austgen et al. 2004). Thus, the fishers reported exposed in this report may have been exposed by mosquito bite or, perhaps, by feeding on infected birds.

Although WNV has not been documented in mustelids previously, exposures to the virus, or disease, have been verified in numerous other carnivores including: striped skunks (Centers for Disease Control and Prevention 2000); raccoons (Centers for Disease Control and Prevention 2000, Dietrich et al. 2005, Root et al. 2005); American black bears (Farajollahi et al. 2003); brown bears (*Ursus arctos*) (Madić et al. 1993); red pandas (*Ailurus fulgens*) (Ludwig et al. 2002); snow leopards (*Uncia uncia*) (Ludwig et al. 2002); a harbor seal (*Phoca vitulina*) (Del Piero et al. 2006); wolves (*Canis lupus*) (Lichtensteiger et al. 2003, Lanthier et al. 2004); domestic dogs (Blackburn et al. 1989, Komar et al. 2001, Lichtensteiger et al. 2003, Centers for Disease Control and Prevention 2005, Kile et al. 2005); and domestic cats (Komar et al. 2001, Centers for Disease Control

and Prevention 2003, Austgen et al. 2004, Kile et al. 2005,). Other wild mammals associated with WNV in the US include tree squirrels (*Sciurus niger* and *Sciurus carolinensis*) (Heinz-Taheny et al. 2004, Dietrich et al. 2005, Root et al. 2005), white-tailed deer (Odocoileus virginianus) (Farajollahi et al. 2004) and bats (*Eptesicus fuscus*; *Myotis lucifugus; Myotis septentrionalis*) (Pilipski et al. 2004, Davis et al. 2005, DeKrey and Adams 2005).

Few studies have reported population level exposures of wild carnivores to WNV. As examples, 6% of samples from a population of black bears in New Jersey (Farajollahi et al. 2003), 35% of a small sample of brown bears in Croatia (Madić et al. 1993), and 11% of gray foxes from the HVIR (Gabriel 2006), tested positive for exposure to WNV and our results are similar to these exposures from other species. To our knowledge, there is no documentation that WNV causes disease or death in any mustelid, including fishers. More work is necessary to determine the effects of WNV exposures in fishers and the likely route of exposures.

Canine Distemper Virus

Mustelids infected with CDV suffer from rash, often with a thickening of the muzzle and footpads, respiratory disease, immunosuppression, neurologic disease, and often death (Deem et al. 2000, Williams 2001, Langlois 2005). Survivors may suffer lingering effects from damage to organs long after the virus is eliminated from the host. Canine distemper virus causes disease in stone martens (*Martes foina*), black-footed ferrets (*Mustela nigripes*), mink (*Mustela*

vison), polecats (*Mustela putorius*), domestic ferrets, European badgers (*Meles meles*), weasels (*Mustela* spp.), striped skunks, domestic dogs, wolves, coyotes (*Canis latrans*), gray foxes, red foxes (*Vulpes vulpes*), African wild dogs (*Lycaon pictus*), raccoon dogs (*Nyctereutes procyonoides*), raccoons, coatis (*Nasua narica*), greater pandas (*Ailuropoda melanoleuca*), red pandas, African lions (*Panthera leo*), and probably many other carnivores (Addison et al. 1999, Deem et al. 2000, Williams 2001).

Although the host range is broad, susceptibility varies among species, with high rates of mortality in black-footed ferrets, gray foxes, and lesser pandas and some level of resistance in striped skunks and red foxes. Other carnivores, including black bears, develop antibodies when exposed to CDV but rarely suffer severe disease (Williams 2001). All species of mustelids probably are susceptible to severe clinical distemper (Deem et al. 2000), and this presumably includes fishers. Addison et al. (1999) stated that fishers are susceptible to CDV, but they did not present data concerning the severity of disease nor was a reference provided.

Exposure to CDV was evident in samples from five (5%) of 97 fishers from the HVIR, including positive samples from two male and three female fishers. Canine distemper virus is transmitted via respiratory droplets, contact with virus laden discharges from the eyes or nose, in urine, or via fecal material (Williams 2001, Langlois 2005). Although the virus does not persist for prolonged periods in the environment, it does persist as long as the contaminant remains fresh and moist. Thus, contamination of equipment used for trapping and handling of

fishers could provide a source of infection for subsequent captures (Brown et al. 2006).

Lack of antibody response (negative IFA) in individuals must be combined with other data to provide meaningful interpretations. Low levels of antibody response in a population can be interpreted as low levels of exposure to a pathogen, as too much time elapsed between exposure and sampling, as exposures too recent to have stimulated a detectable immune response, or as evidence that exposed animals have died prior to sampling (Delahay and Frolich 2000, Courtenay et al. 2001, Hanni et al. 2003). We know that CDV is present in the wildlife surrounding Hoopa because 24% of 80 black bears sampled were found to be exposed to CDV prior to 2001 (Brown et al. 2003). Also, exposure to CDV is common in unvaccinated dogs (R. N. Brown et al., unpublished data), and, anecdotally, because local veterinarians recognize distemper in domestic dogs throughout the region. The prevalence of exposures among fishers from the HVIR was lower than might be expected based on exposures of bears and unvaccinated dogs, but this lower prevalence remains difficult to interpret. If most fishers exposed to CDV die of acute disease, as reported from epizootics of black-footed ferrets (Williams et al. 1988, Williams and Thorne 1996, Greenacre 2003), then few living animals would be expected to show evidence of prior exposure. However, the relatively low prevalence might simply result from lower rates of exposure among fishers than among dogs or bears.

Additional work with CDV in fishers, as well as in the sympatric mesocarnivore community (see Chapter 2) and local domestic dogs, is needed to

allow interpretation of routes of exposure and risks to fishers. The lack of CDV in the populations near Redding may indicate that CDV was less prevalent in their local carnivore communities or that larger sample sizes would be necessary to evaluate the expectedly low seroprevalence.

Canine Adenovirus

Canine adenoviruses are highly contagious and can be shed in urine of apparently healthy animals. As disease develops, animals may shed virus in discharges from the eyes and nose, in vomitus, as well as in feces. This virus is easily transmitted via direct contact or by contact with contaminated inanimate objects (e.g. fomites) (Cabasso 1981, Woods 2001). Pathology caused by CAV2 infection of fishers has not been reported, but the potential for disease should not be ignored.

Five (4%) of 114 fisher samples had antibodies that bound to CAV2. Adenoviruses, including both CAV1 and CAV2, cause infectious hepatitis in domestic dogs and other susceptible carnivores, including mink, domestic ferrets, North American river otters (*Lontra canadensis*), skunks, raccoons, wolves, coyotes, red foxes, island foxes (*Urocyon littoralis*) and bears (Garcelon et al. 1992, Woods 2001). This virus can also cause encephalitis with inappetence, oculonasal discharge, vomiting, mucoid or bloody diarrhea, hyperexcitability, paralysis, depression, seizures, coma and death; the course of disease in striped skunks can be so short that no signs are observed before sudden death (Karstad et al. 1975, Cabasso 1981, Woods 2001). Other species, including mink, are

susceptible to canine adenoviruses, but they tend to develop mild, or at least non-fatal, clinical disease (Addison et al. 1999).

Our findings of 4% and 5% seroprevalence of populations of fishers from Hoopa and near Redding are comparable to a reported exposure of 4% of 28 fishers from British Columbia, Canada (Philippa et al. 2004). High seroprevalence of CAV1 and CAV2 has been reported from some populations of carnivores, including 50% of coyotes from Texas and 63% of striped skunks from Maryland (Jamison et al. 1973, Woods 2001). Although CAV2 may occur in many populations, actual disease has been reported rarely from wildlife and some authors believe that this virus has little impact on wildlife populations (Addison et al. 1999). Contamination of trapping and handling gear used in research or management of fishers could easily provide a source of infection for subsequently captured fishers (Brown et al. 2006).

Canine Herpesvirus

Canine herpesvirus causes respiratory disease, inappetence, abdominal pain, and lesions of the genitalia in juvenile and adult domestic dogs, reproductive failure and abortion in female dogs, and, rarely, a systemic hemorrhagic syndrome and death of pups less than four weeks of age (Gaskell and Willoughby 1999, Murphy et al. 1999).

Although it was originally thought that this virus was infectious only for the family Canidae (Gaskell and Willoughby 1999), CHV infects, survives, and replicates in fetal mink cells (McDonald and Lariviere 2001). Herpesviruses also

cause encephalitis and have been associated with pathology of the liver, spleen, and lung of skunks (Charlton et al. 1977, Diters and Nielsen 1978). Herpesvirus infections are exacerbated by physiologic and emotional stress in a wide range of species, and CHV (or related herpesviruses) would be expected to cause disease mainly in young, old, or otherwise immunocompromised animals.

Antibodies from five (5%) of 95 of the fisher samples from the HVIR reacted with CHV, and we've found no reports of exposures of fishers to herpesviruses for comparison in the literature. Herpesviruses typically are transmitted by direct, animal to animal contact (Gaskell and Willoughby 1999), but infectious animals may shed virus in oculonasal discharge, saliva or feces when the virus is actively causing signs of disease (Gaskell and Willoughby 1999). Although CHV is relatively fragile, infectious virus will persist for 1-2 days in moist fecal material (Pesaro et al. 1995). Therefore, there is some risk for researcher-enhanced transmission of CHV when trapping and handling mesocarnivores.

We have no evidence that CHV is causing serious disease in fishers, but seroprevalence in the fishers sampled at Hoopa suggests infection and replication of the virus in fisher cells. Most herpesviruses cause persistent latent infections and might be expected to cause disease synergistically in combination with other infectious organisms, if at all.

The potential exists for CHV to be transmitted via contaminated equipment used for trapping and handling, and we have not characterized the virus infecting fishers. Directed research would be necessary to clearly determine the risks of herpesvirus infections in fishers, but we have no reason to suggest that CHV be

considered a conservation concern for fisher populations in the western DPS (Brown et al. 2006).

Canine Parvovirus

Susceptibility of mustelid species to the feline parvovirus subgroup (including feline panleukopenia virus (FPV), CPV, mink enteritis virus (MEV), Aleutian disease of mink virus (ADV), and raccoon parvovirus (RPV)) probably depends on characteristics of specific strains of the viruses and species characteristics of the hosts. The most virulent parvoviruses in some mustelid species have been shown to be ADV and MEV (Barker and Parrish 2001; Langlois 2005). Canine parvovirus replicates in mink and ferret cells, and causes seroconversion, without causing severe disease in these species (Barker and Parrish 2001). Domestic ferrets appear to be susceptible only to disease caused by ADV and not those of other parvoviruses, and both North American river otters and domestic ferrets have been shown to be resistant to infection by CPV (Fox 1998, Kimber et al. 2000). In captive studies with striped skunks, CPV, FPV, and MEV failed to replicate (therefore causing no disease) and caused only minimal antibody response (Barker and Parrish 2001).

The disease manifestations caused by this group of parvoviruses result from infection and lysis (cell death) of rapidly multiplying cells; thus, many parvoviruses (including CPV, FPV, MEV and RPV) cause pathology in lymphoid tissues, tissues that produce blood cells, and the intestinal epithelium (Murphy et al. 1999). Disease syndromes observed in species other than fishers include

fever, reduction of white blood cells, immunosuppression, cerebellar hypoplasia (FPV) and hemorrhagic diarrhea (with or without vomiting) (Murphy et al. 1999,Barker and Parrish 2001, Langlois 2005). Inflammation and hemorrhaging of the intestinal epithelium of animals suffering from parvovirus enteritis often allows bacterial leakage into the blood vascular system, leading to sepsis, and endotoxic shock. The combination of weakness, dehydration from the diarrhea and endotoxic shock causes some animals to die before outward signs are apparent. Animals that survive acute infections typically are immune to further infections. Like distemper, disease caused by CPV should be expected to be most severe in young animals.

Fishers from the HVIR were commonly exposed (31% of 90) to CPV, and CPV-DNA was amplified from 19% (N=59) of the fisher fecal samples analyzed. A somewhat lower prevalence of exposure to CPV, two (11%) of 19, was detected in samples from fishers trapped near Redding, but a similar prevalence (18% of 39) was determined using PCR of DNA from fecal material from the two study areas. None of the fecal samples from fishers trapped previously in the southern Sierra Nevada Mountains were PCR positive to CPV. Although serology alone is insufficient to differentiate among related parvoviruses, PCR-amplification and sequencing of DNA from fisher scat proved that sequences evaluated were those of CPV, and no fishers were confirmed to have been infected with any other parvovirus. We interpreted these findings as suggesting that CPV was probably common in the carnivore communities on the HVIR and

near Redding, but we recognized that other parvoviruses might also have occurred in this region.

The prevalence of exposures to CPV was greater in adult fishers (49% of HVIR fishers $\geq$ 2 years) than in subadults (20% of HVIR fishers 0-1 year of age), and there are several possible explanations for this pattern. Older fishers may have simply had longer to accumulate exposures than have younger animals. Adult fishers may experience a higher rate of exposure to CPV than did subadult fishers, especially if exposures are generated from a single source (such as domestic dogs or gray foxes) to which subadult fishers might not be exposed. Lastly, disease caused by CPV may be more severe in juveniles, and adult fishers may have subclinical infections, as do adult mink and domestic ferrets. Assuming that survival of infection confers immunity, as it does in other carnivores, many adult fishers might be expected to have antibodies present in their blood but not to be actively shedding virus in their feces (i.e. PCR-negative).

The virulence of the different parvovirus strains, including CPV, for fishers remains unknown, and few of the fishers examined showed signs of bloody diarrhea, severe dehydration or sepsis during handling; two fishers at necropsy appeared to have signs consistent with hemorrhagic enteritis, but the results of histological examination remained equivocal (Gabriel et al. unpublished data). Nonetheless, the finding that 18% of individuals sampled were actively shedding virus in their feces at some point during the study suggests that CPV infections (with associated lysis of gut epithelium cells) occur commonly in the population of fishers at Hoopa. Canine parvovirus is spread via direct contact with feces or

other body fluids, but, unlike CDV, CAV2, CHV and WNV, CPV remains infectious for prolonged periods of time in the environment (Gaskell and Willoughby 1999, Murphy et al. 1999). Therefore, like CDV, CAV2, and possibly CHV, there is some potential for researcher enhanced transmission of CPV within and between fisher populations (Brown et al. 2006).

***Borrelia burgdorferi* sl**

Lyme borreliosis is caused by members of a group of related spirochetes referred to as *B. burgdorferi* sl, all of which are transmitted by ixodid ticks. In California, the most important vector is the western black-legged tick (*Ixodes pacificus*)(Brown et al. 2005).

There are 12 described genospecies within the *B. burgdorferi* sl group, and many strains within California and elsewhere that remain inadequately characterized (Brown and Burgess 2001, Brown et al. 2006). Moreover, these different genospecies or strains may all differ in the tendency to cause disease in different species of hosts. Domestic dogs, horses, humans and occasionally other species develop signs of disease related to infection by *B. burgdorferi* sl, but no wild populations of carnivores are reported to have been adversely impacted by this group of borreliae (Brown and Burgess 2001).

Although we can not predict exactly how individual fishers will be affected by infection by a given strain of spirochetes, we have no reason to suspect that fisher populations are threatened by these pathogens in California. However, carnivores can be very useful as sentinels of borrelial infections because many

species range widely and are exposed to relatively large numbers of potential vectors (Foley et al. 2004). Since our current analyses did not differentiate among the variety of strains and genospecies of borreliae present in California, we cannot interpret these findings beyond noting the prevalence of exposures in the different populations.

***Rickettsia* sp.**

The principal members of the spotted fever group of rickettsial pathogens include *Rickettsia rickettsii* (the cause of Rocky Mountain spotted fever), the *Rickettsia conorii*-complex (the cause of Mediterranean spotted fever and related infections), *Rickettsia sibirica* (the cause of North Asian tick typhus), and, *Rickettsia australis* (the cause of Queensland tick typhus). The Rocky Mountain wood tick (*Dermacentor andersoni*) transmits *R. rickettsii* throughout the Rocky Mountain regions of Canada and the US, in mountainous areas of the Great Basin, and in northeastern California along the east facing slopes of the northern Sierra Nevada Mountains (Sonenshine 1993, Brown et al. 2005). However, *D. andersoni* is not reported to occur within the geographic range of fishers in California. A related vector, *Dermacentor variabilis*, is an important vector of *R. ricketsii* in its eastern and southern range within the US. This species occurs at the sites sampled for this study, but it is not confirmed as a vector in the far western US. *Dermacentor occidentalis*, the Pacific Coast tick, is considered a potential vector of this agent in California (Philip et al. 1981, Lane et al. 1982), and it also occurs in each of the areas from which fishers were reported herein.

However, human cases of rickettsial disease are exceedingly rare throughout most of its distribution. Disease caused by *R. rickettsii* occurs in people and dogs (Kidd et al. 2006), but disease in other carnivores remains unreported.

We found serologic evidence of antibodies in fishers from the HVIR that react with *R. rickettsii*-antigen. Unfortunately, we can't confirm from our studies that the infecting agent is truly *R. rickettsii* because cross reactions are common within this group and because this group is rapidly expanding as people evaluate new sources of hosts and vectors. More work will be necessary to interpret our findings concerning this pathogen. We do note that serologic exposures were higher among fishers from the HVIR than among fishers near Redding (51% vs 32%; P = 0.02).

Anaplasma phagocytophilum

Sixty (76% of 79) of the fishers from the HVIR were exposed to *A. phagocytophilum* and this prevalence was significantly higher (P = 0.00009) than found in the fishers trapped near Redding (26% of 19). This difference is likely related to higher densities of the vector, *I. pacificus*, in the more mesic forests of the HVIR compared to the dryer forests surrounding the Sacramento Valley, which is near the Redding site (Foley et al. 2004). The difference in prevalences of exposure of adult and subadult fishers (93% and 68%, respectively) likely results from accumulated exposure to ticks through time.

Clinical manifestations of infections of *A. phagocytophilum* in horses, cats, and dogs include immunosuppression, fever, incoordination, swelling of the

ventral limbs, liver disease, and the reduction of white blood cells and thrombocytes (e.g. immunosuppression) (Madigan and Gribble 1987, Foley et al. 2003, Foley et al. 2004), but we have found no reference demonstrating clinical disease associated with infection of this bacterium in wild carnivores. Exposures of other carnivore populations to *A. phagocytophilum* include 46% of coyotes from central California (Pusterla et al. 2000), 17% of mountain lions from the Sierra Nevada mountains (Foley et al. 1999), 86% of black bears from the HVIR (Brown et al. 2003), and 50% of gray foxes from the HVIR (Gabriel 2006). In California, *A. phagocytophilum* is transmitted to wildlife, people, and domestic animals by the western black-legged tick as is *B. burgdorferi* sl (Lane et al. 2001, Holden et al. 2003, Foley et al. 2004, Lane et al. 2004), and carnivore populations exposed to large numbers of infected ticks are expected to have high exposures to these pathogens (Foley et al. 2004). Nymphs and adults of several species of ticks, including *I. pacificus*, have been removed from fishers captured at Hoopa (Brown et al., personal unpublished data). Disease caused by infection of fishers with *A. phagocytophilum* is unknown, and we expect outward signs of disease to be mild and of short-term duration with the potential complication of immunosuppression.

Yersinia pestis

Infamous as the agent that causes plague,*Y. pestis* is well established in California wildlife (Ruppanner et al. 1982, Clover et al. 1989, Chomel et al. 1994). This pathogen is usually associated with communities of rodents, some of which

die quickly (including prairie dogs, *Cynomys* spp., and ground squirrels, *Spermophilus* spp.) and some of which are capable of some level of resistance including some voles (*Microtus* spp.), some deer mice (*Peromyscus* spp.), marmots, (*Marmota* spp.), and gerbils, (*Rhombomys opimus* and *Meriones* spp.) (Gasper and Watson 2001, Gage and Kosoy 2005). Primates and many rodent species are susceptible to *Y. pestis* (Gasper and Watson 2001). Other mammals, including ungulates and most carnivores are resistant to *Y. pestis* (Gasper and Watson 2001), but domestic cats and black-footed ferrets are susceptible and may die (Williams et al. 1994, Williams et al. 1991, Gasper and Watson 2001, Gage and Kosoy 2005). Prevalence of exposures to *Y. pestis* have been reported from carnivores in northern California (Brown et al. 2003, Chomel et al. 1994, Hoar et al. 2003, Ruppanner et al. 1982, Zielinski 1984) and the susceptibility of some mustelids (Gage and Kosoy 2005, Gasper and Watson 2001, Williams et al. 1994, Williams et al. 1991) led us to evaluate exposures in the fisher populations associated with this study. Nonetheless, we did not detect exposures to *Y. pestis* in any of the sampled fishers. Until data suggest otherwise, plague should not be considered a significant threat to fisher populations in the western DPS.

***Bartonella* sp.**

Bartonella spp. occur commonly in California (Yamamoto et al. 1998, Chang et al. 1999, Hoar et al. 2003, MacDonald et al. 2004b, Riley et al. 2004, Beldomenico et al. 2005, Henn et al. 2005), prevalence was found to be high in

gray foxes trapped on the HVIR (Henn et al. 2007), and both exposure rates and *Bartonella*-associated disease have been found in wild mustelids (McDonald et al. 2001, McDonald and Lariviere 2001). However, none of 60 fishers sampled had been previously exposed to these bacteria. Unless future data suggest otherwise, these data do not suggest that *Bartonella* should be considered an immediate threat to fisher management or conservation.

Toxoplasma gondii

Toxoplasma gondii is a ubiquitous organism that utilizes both domestic and wild felids as its definitive hosts and many species of mammals (and even some birds) as its intermediate hosts. This species invades and multiplies in the cells lining the intestines of cats, from which oocysts are shed in the feces. Intermediate hosts do not shed oocysts in their feces, but rather develop intracellular tissue cysts in a variety of organs including skeletal muscle, pancreas, liver, heart, brain, eye, uterus, and placenta.

Intermediate hosts become infected either by exposure to sporulated oocysts, while feeding on tissue cysts infecting other intermediate hosts, or transplacentally prior to birth. Thus, carnivores become infected from a variety of sources and other serologic surveys have indicated that infections of *T. gondii* are common in carnivores (Dubey and Odening 2001). Although infections are common, most infections remain asymptomatic, with disease severity dependent upon the strain of the infecting parasite, the immune status of the host, and the age of the host; many species develop severe disease as young individuals prior

to development of immuno-competence, but are typically resistant if infected as adults (Dubey and Odening 2001).

Clinical toxoplasmosis is associated with the specific organ tissues infected in susceptible hosts that fail to immunologically control the development of tissue cysts. Thus, animals develop encephalitis, myocarditis, myositis, pancreatic necrosis, hepatitis, retinal inflammation, etc., depending on where the cysts localize within the individual. Infected animals may become anorexic, depressed, and suffer signs of pneumonia and meningoencephalitis (respiratory distress, disorientation, uncoordinated movements, paralysis). Infected mink and ferrets develop hind limb paralysis. Placental infections cause local edema and necrosis, and transmission to fetuses across the placenta allows uncontrolled multiplication due to the lack of an intact fetal immune system. Congenital infections are commonly associated with fetal deaths, abortions, stillbirths, neonatal deaths, and production of small, weak survivors (Dubey and Odening 2001, Frank 2001).

We report that 57% (51 of 90) of fisher plasma samples had detectable levels of antibodies reactive with *T. gondii* antigen and that exposures did not vary significantly between the populations sampled on the HVIR and near Redding. This compares to 18% of 28 fishers surveyed in British Columbia (Philippa et al. 2004), 41% of 379 fishers surveyed in Ontario (Tizard et al. 1976), and 41% (33 of 80) of black bears trapped at Hoopa (Brown et al. 2003). For comparison, examples of exposures in a few other (non-felid) mammalian carnivores include: 5 and 11% of martens (*Martes americana*) (Tizard et al. 1976,

Hurkova and Modry 2006); 100% of American mink (Tizard et al. 1976); 17 and 45% of northern river otters (Tocidlowski et al. 1997, Gaydos et al. 2007); 28 to 56% of striped skunks (Quinn et al. 1976, Tizard et al. 1976, Smith et al. 1992, Hill et al. 1998, Mitchell et al. 2006); 15 to100% of black bears (Quinn et al. 1976, Burridge et al. 1979, Chomel et al. 1995, Nutter et al. 1998, Zarnke et al. 2000); 15 to 100% of raccoons (Tizard et al. 1976, Dubey et al. 1992, Hill et al. 1998, Mitchell et al. 2006); 64 and 78% of coyotes (Quinn et al. 1976, Tizard et al. 1976); and 9% of wolves (Zarnke et al. 2000). However, since most infections remain asymptomatic, serologic results alone do not indicate population level risks. That said, several mustelid species and skunks have been shown to suffer severe disease and mortality associated with toxoplasmosis.

Black-footed ferrets were shown to be highly susceptible to *T. gondii* when a captive breeding colony at the Louisville Zoological Garden suffered severe disease and many deaths (Burns et al. 2003). Domestic ferrets and captive mink also have been shown to be highly susceptible to toxoplasmosis and both species transmit infections congenitally from infected females to their kits (Dietz et al. 1993, Fox 1998, Frank 2001, Thornton 1990). Frank (2001) reported that 1,976 (26%) of 7,800 captive female mink lost their litters either from abortion or neonatal mortality caused by *T. gondii*. Although a wild population might withstand such a high percentage of reproductive failure, *T. gondii* would be only one factor limiting fitness and the cumulative effects of multiple pressures might be expected to limit population growth.

The southern sea otter (*Enhydra lutris nereis*) provides another example of a mustelid that appears to be suffering significantly from toxoplasmosis. This subspecies of sea otters has failed to thrive and expand following the cessation of harvests, and several authors have recently suggested that such failure may result from epizootics of *T. gondii* (Miller et al. 2002, Hanni et al. 2003, Miller et al. 2004, Conrad et al. 2005,). In recent reports of infections in live and dead sea otters from California, Miller et al. (2002) reported 42% (49/116) seroprevalence for live otters and 62% (66/107) for dead otters and Conrad et al. (2005) reported that 38% of 257 live sea otters and 52% of 305 freshly dead sea otters were infected with *T. gondii.* Studies with southern sea otters has also identified a new strain, Type X, that appears to be more heterogeneous and virulent than the previously described terrestrial strains, Types I, II, and III (Cole et al. 2000, Miller et al. 2002, Conrad et al. 2005,).

At this point, we can only speculate about the potential disease caused by *T. gondii* in fishers. Our lack of association of age of fishers and seroprevalence as shown in other species (Quinn et al. 1976) suggests that fishers are either exposed very early in life or acquire infections congenitally. Congenital infections would be more likely to cause abortions, still births, or neonatal deaths than would infections acquired once the immune systems of the kits had fully developed. Future studies should attempt to molecularly characterize the infecting strains in fishers, assess neural pathology associated with protozoal cysts in animals that are necropsied, and describe the ecological patterns of disease transmission within fisher populations.

Sources of Mortality and Co-exposures

We did not identify clear associations between pathogen exposures and any of the fifteen radio-collared fishers found dead during this study. However, it should be noted that our ability to determine cause of death was compromised by the fact that most carcasses were recovered days after death and several were mutilated by predators or scavengers. We also had limited ability to evaluate fishers that died without radio-collars because we did not recover the carcasses. We did necropsy a few fishers in good enough condition to allow cellular examination (Gabriel et al., unpublished data). From these animals, we have identified several signs that appear highly suggestive of infectious disease (including severe hemorrhagic diarrhea, a perforated hemorrhagic intestine, protozoal cysts in the heart, and apparent high limb paralysis similar to descriptions of toxoplasmosis in mink). However, we have not yet found definitive evidence of severe disease caused by any specific pathogen (Gabriel et al., unpublished data). We noted that most fishers found dead were female, but this difference likely resulted from the study design of a parallel study in which most radio-collars were placed on females to monitor den sites and juvenile dispersal (Matthews et al., unpublished data).

Several of the pathogens discussed have the potential to cause immunosuppression, and disease may be most severe in hosts simultaneously infected with multiple pathogens (Diters and Nielsen 1978, Graham, 2002, Chen et al. 2005, Holden et al. 2005, Cho et al. 2006, Hofmann-Lehmann et al. 2004,

Davidar and Morton 2006). However, coinfection of some pathogens confers resistance rather than enhances severity (Gale et al. 1997, Furze et al. 2006); therefore, coinfection is a complicated issue and generalities may not always apply (Mosquera and Adler 1998, Foley et al. 2003, Lively 2005). Positive serology indicates exposure at some time in the past, and it does not necessarily indicate current infections. The most worrisome co-exposure reported is that of CPV and *A. phagocytophilum*, both of which cause immunosuppression in susceptible species. Immunosuppression would likely make fishers more susceptible to other pathogens, including potentially severe disease caused by CDV and toxoplasmosis. Co-exposure to CAV and CHV may indicate a focused source of infection to these pathogens such as provided by unvaccinated domestic dogs. Other co-exposure results, including those between *T. gondii* and *B. burgdorferi*, are difficult to explain.

Lack of co-exposure among the pathogens suggests that differences in routes of transmission led to a random pattern of exposures in the population, as might be predicted. Although the co-exposure of the pathogens mentioned above is interesting, the data do not appear to indicate clear risks for fisher populations. Co-exposure might be caused by similar routes of transmission or, indirectly, by exposure rates that vary with habitat or distance from humans, etc.

The sources of directly transmitted infections of fishers remain unknown, and the pathogens appear to be transmitted within the greater wildlife community (see Chapter 2). Domestic dogs also are a logical source of CDV, CAV, CDV, and CPV, and potentially represent the ultimate source of the fisher exposures

we've reported; unleashed dogs are very common in areas near houses, but less common in the backcountry of the HVIR (Gabriel 2006). If dogs are a proximate source of infections, then fishers living near humans should be more likely to be exposed than fishers with home ranges at greater distance from human habitation. Proximity to humans has been correlated with CPV infections in both spotted hyenas (*Crocuta crocuta*) sampled in the Masai Mara of Kenya and gray foxes living near parks in central California (Riley et al. 2004). However, if dogs represent only the ultimate source and transmission among other carnivores is high, then the association of exposures of fishers with people and dogs may not be obvious. Molecular analysis of a large number of samples from individually marked fishers and sympatric carnivores would be necessary to allow spatial analysis to help evaluate such patterns of abundance and distribution of pathogens within the carnivore community.

MANAGEMENT IMPLICATIONS

All animal populations suffer from infections of parasites and diseases, but the potential influence of disease to negatively impact populations increases in importance as populations become small and isolated. The pathogens discussed cause disease of unknown severity in fishers; we found few reports of exposures of fishers to pathogens in the literature, and no one has reported laboratory studies of the effects of these pathogens in fishers. At this time, severity can only be predicted based on extrapolation of disease in related species. Some disease agents, including West Nile virus, *Bartonella* spp., *Y. pestis, B.*

burgdorferi, and *A. phagocytophilum* are vector-borne and therefore risks associated with these pathogens will be difficult to manage.

Translocations of wildlife move pathogens along with their hosts. It is generally recommended that translocations be planned carefully and monitored throughout all phases to minimize risks associated with moving infected animals or moving naïve animals into areas in which epizootics are cycling (Nettles et al. 1980, Woodford and Rossiter 1993, Cunningham 1996, Kimber et al. 2000, Leighton 2002, Wobeser 2002,). Exposures of CDV in black-footed ferrets are managed by vaccination and risk monitoring, and vaccination of fishers to CDV, rabies virus, and feline panleukopenia virus (which would cross protect for CPV infections) should be considered during translocation events.

Some pathogens, including CDV, CAV2, CHV, and CPV are transmitted via feces or other body fluids, and CPV remains infectious for prolonged periods in the environment. Trapped animals often contaminate equipment used for trapping or handling. Unless trapping gear is cleaned and disinfected between captures, subsequently captured animals may be exposed to viruses shed from previous animals. Due to the documented morbidity and mortality of parvoviruses, distemper viruses, adenoviruses, and influenza viruses for related species of mustelids, all equipment in contact with trapped fishers should be cleaned thoroughly and sprayed with appropriate anti-viral disinfectants after each capture; all visible fecal material should be removed, surfaces should be wiped clean, and equipment should be generously sprayed with a disinfectant rated to kill parvoviruses. While this will not eliminate the potential for

researcher-facilitated transmission of these viruses, it should minimize the risks significantly.

Chapter 2. Pathogens Associated with Mesocarnivores Sympatric with Fishers

Mourad W. Gabriel, Greta M. Wengert, Janet E. Foley,
J. Mark Higley, Sean Matthews, and Richard N. Brown

ABSTRACT

Conservation efforts often require an understanding of the threats imposed by sympatric species on the species of concern, and transmission of disease among sympatric species should be considered in any management scenario for species conservation. Monitoring ecological communities allows for a logistically feasible assessment of pathogen prevalence than monitoring a sensitive or rare species. We investigated the prevalence of several pathogens within a mesocarnivore community that is sympatric with a fisher population within the West Coast Distinct Population Segment of fishers (*Martes pennanti*). A combined total of 63 gray foxes, ringtails, raccoons and spotted and striped skunks showed prior exposures to canine distemper virus (2%), canine parvovirus (30%), canine adenovirus (9%), West Nile virus (6%), *Anaplasma phagocytophilum* (50%), and *Toxoplasma gondii* (40%). No individuals were seropositive for canine herpesvirus. Of 20 individuals tested for active infections using the polymerase chain reaction to amplify pathogen DNA, 15% were found infected by *A. phagocytophilum*, and 19% were found to be actively shedding canine parvovirus. Exposure to *A. phagocytophilum* was higher in fishers than in other mesocarnivores ($p = 0.001$), but no other differences in exposures of fishers and sympatric mesocarnivores were found. Our results indicate that

fishers and their sympatric mesocarnivores share several pathogens in similar prevalence, suggesting a potential risk of disease transmission within this community. Additionally, we suggest that sympatric mesocarnivores be monitored to determine disease prevalence across the community and to help determine risks for fishers.

INTRODUCTION

The conservation of a species entails the understanding of many ecological factors, one of which involves health risks from infectious pathogens with the potential to limit populations (Scott 1988, Thorne and Williams 1988, Deem et al. 2001, Cleaveland et al. 2002, Mathews et al. 2006). Additionally, interactions between sympatric wild and domesticated animals may increase the risk of transmission of a pathogen within a sensitive species' established or potential range (Hudson and Greenman 1998, Deem et al. 2001, Macdonald et al. 2004, Mathews et al. 2006).

Monitoring common sympatric mesocarnivores that overlap or inhabit the same spatial niche as high-profile or sensitive species may provide an indication of ecosystem health and may also be necessary when monitoring a focal species with has low population numbers or is otherwise not feasible to investigate (Munson and Karesh 2002, Wobeser 2006). Unfortunately, research of many high-profile species or their sympatric community is not conducted until after an unexpected epidemic moves through a threatened population and creates a high

level of morbidity or mortality (Woodroffe 1999, Munson and Karesh 2002, Mathews et al. 2006)

In this chapter we compare the serological exposure and rates of infections of potentially limiting viral, bacterial, and protozoal pathogens in fishers and their sympatric mesocarnivores to determine whether information from sympatric communities might provide valuable information for fisher conservation efforts. We discuss the feasibility of proactive disease monitoring of the mesocarnivores sympatric with a subset of the West Coast fisher Distinct Population Segment (DPS).

STUDY AREA

Field sampling was conducted on the Hoopa Valley Indian Reservation (HVIR) in northeastern Humboldt County, northwestern California as described in Chapter 1. The valley floor and the outlying areas were distinguished as the valley and backcountry zones, respectively. Most human HVIR inhabitants reside in a residential zone near the valley floor (Figure 2-1).

Mid-sized to large mammals that inhabit the HVIR include Columbian black-tailed deer (*Odocoileus hemionus columbianus*), Roosevelt elk (*Cervus elaphus roosevelti*), American black bears (*Ursus americana*), gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), fishers, bobcats (*Lynx rufus*), mountain lions (*Felis concolor*), raccoons (*Procyon lotor*), ringtails (*Bassariscus astutus*), striped skunks (*Mephitis mephitis*), spotted skunks (*Spilogale gracilis*),

porcupines (*Erethizon dorsatum*), domestic cats (*Felis domesticus*), and domestic dogs (*Canis domesticus*) (M. W. Gabriel and J. M. Higley, personal observation).

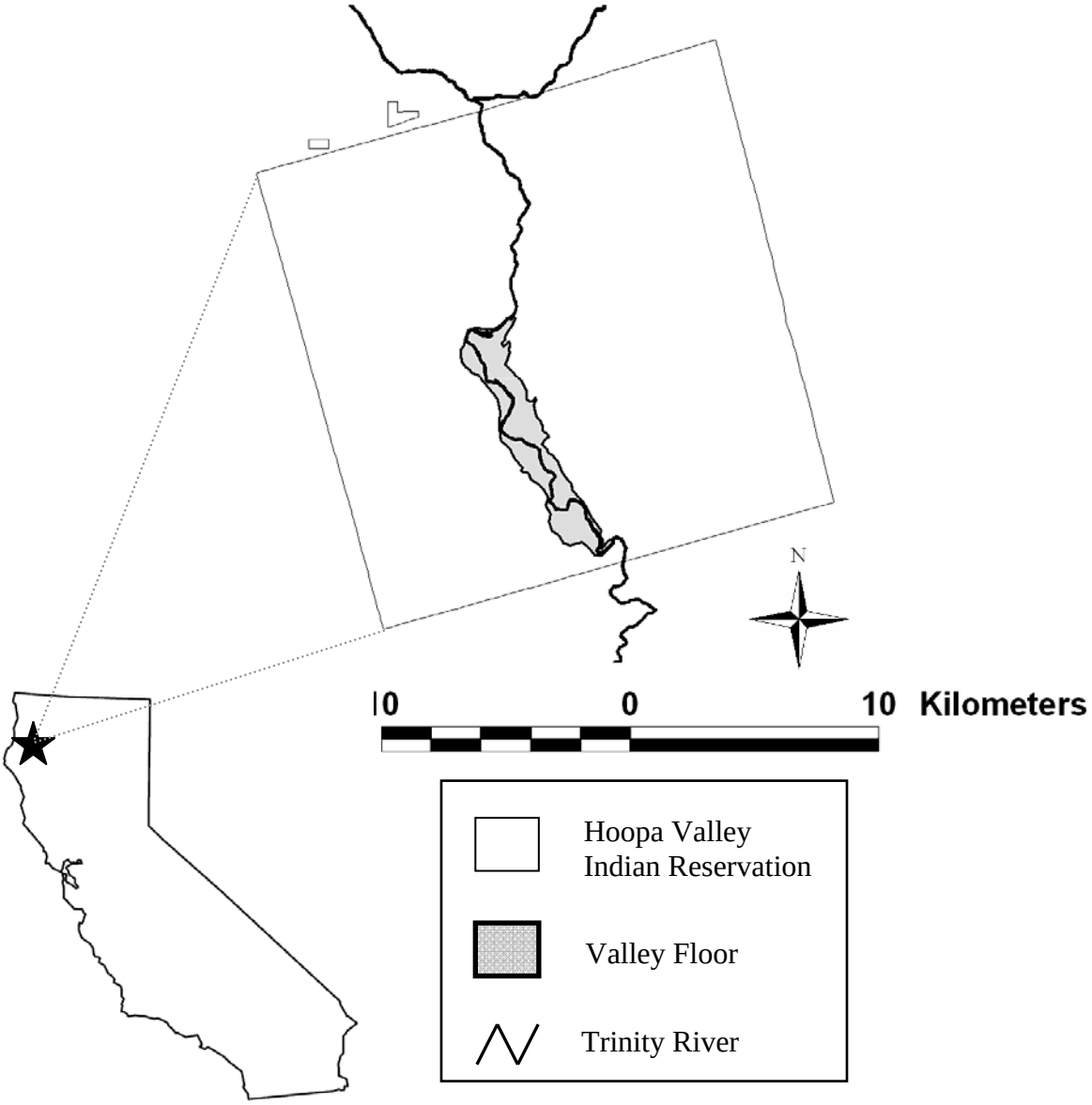


Figure 2-1. Hoopa Valley Indian Reservation, Humboldt County, California, USA, the study area for disease exposures in fishers and their sympatric mesocarnivores from February 2004 to February 2007.

METHODS

Capture, Handling, and Sampling

Five mesocarnivore species, including gray fox, ringtail, raccoon, striped skunk, and spotted skunk were opportunistically trapped from February 2004 through February 2007 in parallel with an ongoing fisher project at the HVIR. Carnivores were trapped with wire mesh live traps (81 x 25 x 31cm) (Tomahawk Live Trap Company, Tomahawk, Wisconsin) baited with chicken. Insulated wooden nest boxes were attached to traps to provide security, reduce environmental stressors, and facilitate handling of trapped animals (Wilbert 1992, Gabriel and Wengert 2005).

Captured foxes were moved to handling cones made of cloth mesh, weighed and then anesthetized with 20 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and 0.5mg/kg diazepam (Hospira, Inc., Lake Forest, Illinions, USA) via intramuscular injection. Ringtails were similarly processed but were intramuscularly injected with 25 mg/kg ketamine. Both species of skunks were lightly anesthetized with isoflurane- (Abbott Laboratories, Abbott Park, Illinois, USA) saturated cotton balls that were placed in the nest box ≥ 2 minutes prior to hand injection with 25 mg/kg ketamine to facilitate safe handling and reduce the likelihood that skunks would spray. Weights of raccoons were estimated to the nearest 0.2 kg before being moved to a handling cone made of welded metal rods and injected with a mixture of 25 mg/kg ketamine and 1.0 mg/kg diazepam.

Each anesthetized animal was maintained in lateral recumbency and a brief physical exam was performed. Passive integrated transponder (PIT) tags (Biomark Inc., Boise, Idaho, USA) were implanted subcutaneously for future identification of each individual, and relevant morphometrics were recorded. Animals were allowed to recover from anesthesia in the nest box of the traps in which they were caught, and were released at the site of capture upon full recovery.

Fecal samples were collected from each mesocarnivore using a fecal loop, a Dacron® swab (Fisher Scientific, Pittsburgh, PA, USA) or directly from the animal. Fecal samples were stored at -20°C until analysis. Approximately 1-3 ml of whole blood was collected by venapuncture and stored frozen in the anticoagulant ethylene diamine tetraacetic acid (EDTA) stored at -20°C until analysis. Blood was thawed and centrifuged to separate plasma from cells. Plasma samples were serially diluted in 10 mM phosphate-buffered saline (PBS; 138 mM NaCl, 2.7 mM KCl), then refrozen until thawed for analysis.

Assays for Exposure to Pathogens

Plasma samples were analyzed for presence of antibodies that bound to antigens of the different pathogens by indirect immunofluorescence assays (IFAs). Antigen for the canine distemper virus (CDV) IFA was from kidney cells of CDV-infected domestic ferrets (*Mustela furo*) (American Bioresearch, Milton, Tennessee, USA). Positive controls were serum samples from domestic dogs previously shown to have high, standardized titers, and negative controls were

from dogs maintained at a specific pathogen-free (SPF) colony. Positive results were defined as those reacting at a dilution of ≥ 1:8. Antibodies specific to canine parvovirus-2 (CPV), canine adenovirus (CAV-2) and canine herpesvirus (CHV) were detected with commercially available antigen slides (Veterinary Medical Research and Development, Pullman, Washington, USA). Positive controls for CPV, CAV2 and CHV tests were blood samples from dogs previously shown to have high, standardized titers, and negative controls were from SPF colony dogs. Positive results for these tests were defined as those reacting at a dilution of ≥1:25. Exposure to West Nile virus (WNV) was evaluated by the Animal Health Diagnostic Center (Cornell University, Ithaca, New York, USA) via serum neutralization and positive results were defined as those reacting at a dilution of ≥1:4. Antigen for the *Anaplasma phagocytophilum* IFA was from cells isolated from infected horse (*Equus callabus*) blood. Positive controls were serum samples from dogs of known infection history with high, standardized titers, and negative controls were from SPF colony dogs. Positive results were defined as those reacting at a dilution of ≥1:25. Antigen for *Toxoplasma gondii* was detected with indirect hemagglutination tests and positive results were defined as those reacting at a dilution of ≥1:64.

Molecular Assays

DNA was extracted from whole blood using a Qiagen kit (Qiagen, Valencia, California, USA) following manufacturer's recommendations and from feces using a modified Boom method of silica extraction (Cheung et al. 1994).

TaqMan real-time polymerase chain reaction (PCR) for the *A. phagocytophilum* p44 gene was performed as described previously (Drazenovich et al. 2006). A nested PCR was performed for CPV2 as described previously (Hirasawa et al. 1994). Amplicons from the nested-PCR assay were cloned into a pCR2.1 vector using the TOPO cloning kit (Invitrogen, Carlsbad, California, USA) and amplified in TOP10F′- competent cells of *Escherichia coli* (Invitrogen, Carlsbad, California, USA). The plasmid was purified using a Plasmid Miniprep kit (BioRad, Hercules, California, USA) and the vector insert was sequenced with the M13f primer (Davis Sequencing, Davis, California, USA) using the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA). The sequences were determined using the BLAST database search program and GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA) (Altschul et al. 1990).

Statistics and Data Exclusion Rules

Comparisons of exposures and active infections between fishers and all mesocarnivores as a single group, were assessed using two-tailed Fisher's exact tests of association with a cutoff of $p \leq 0.05$ using NCSS (Number Cruncher Statistical Software, Kaysville, UT, USA). Analyses included comparisons between fisher and all mesocarnivores as a single group, and between fishers and the group of mesocarnivores that were captured in the backcountry only. Data from recaptures were included in analyses involving seroprevalence except when individuals were resampled < 9 months after the initial capture or when the

first sample tested seropositive in order to reduce potential errors associated with repeated sampling of seropositive animals. The 9-month interval was considered sufficient to avoid counting repeated negative samples. All samples from which DNA was amplified to determine active infections were incorporated irrespective of sample date. In order to detect spatial patterns in disease prevalence among fishers and their sympatric mesocarnivores, animal locations, and disease exposures were spatially displayed using ArcMap 9.2 (ESRI, Redlands, California, USA).

All trapping and handling of mesocarnivores for this project followed protocols approved by Humboldt State University's Institutional Animal Care and Use Committee.

RESULTS

Capture Demographics

A total of 63 sympatric mesocarnivores were captured and processed February 2004 though February 2007. This total included 27 gray foxes, 16 ringtails, four raccoons, 10 striped skunks, and six spotted skunks. There were two male gray foxes and two male ringtails that were recaptured and processed twice each. No striped skunks, spotted skunks, or raccoons were processed more than once. Twenty-three percent of all sympatric mesocarnivores were captured within the valley floor of the reservation (all striped skunks, 11 gray foxes, and two raccoons); all other captures occurred in the backcountry.

Of the 27 gray foxes, 20 (74%) of 27 were male and seven (26%) of 27 were female. Eleven (69%) of 16 ringtails were male, four (25%) of 16 ringtails were female, and the sex of one ringtail was not recorded. Of the four raccoons, two were male and two were female. Of the 10 striped skunks eight (80%) were male and two (20%) were female. All six spotted skunks captured were males.

Pathogen Exposures

Serologic examination of plasma samples revealed that 2% (1 of 63) of animals sampled had been exposed to CDV, 30% (19 of 63) to CPV, 10% (6 of 63) to CAV2, 0% (0 of 63) to CHV, 6% (4 of 63) to WNV, 50% (32 of 63) to *A. phagocytophilum*, and 40% (8 of 20) to *T. gondii*. Exposures to pathogens within species were variable (Table 2-1).

Active Infections

From January 2006 to February 2007, a total of 47 fecal samples from 46 individual mesocarnivores were analyzed for CPV DNA. One gray fox was sampled twice within an approximately nine month interval. We amplified CPV DNA from 19% (9 of 38) of these samples using PCR. Of the nine positive samples, five were from gray foxes (N = 24), two were from ringtails (N = 12), one was from a raccoon (N = 3), and one was from a spotted skunk (N = 5); none of the three striped skunks sampled were PCR-positive for CPV. Sequences of DNA amplicons from fecal samples of the positive spotted skunk, the positive raccoon, and two positive gray foxes were found to match CPV, and no PCR-

Table 2-1. Results of serologic evaluation of plasma samples from gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), spotted skunk (*Spilogale gracilis*), striped skunk (*Mephitis mephitis*), and raccoon (*Procyon lotor*) trapped on the Hoopa Valley Indian Reservation, February 2004 – February 2007. Pathogens evaluated include canine parvovirus (CPV), canine distemper virus (CDV), canine herpes virus (CHV), canine adenovirus (CAV2), West Nile virus (WNV), *Anaplasma phagocytophilum* and *Toxoplasma gondii*.

	Gray Fox		Ringtail		Raccoon		Spotted Skunk		Striped Skunk	
Pathogen	N	No. (%) Positive	N	No. (%) Positive	N	No. (%) Positive	N	No. (%) Positive	N	No. (%) Positive
CDV	27	0	18	1 (6%)	4	0	5	0	10	0
CPV	28	12 (43%)	17	6 (35%)	4	1 (25%)	5	0	10	0
WNV	27	2 (7%)	18	1 (6%)	4	0	5	1 (20%)	10	0
CHV	27	0	18	0	4	0	5	0	10	0
CAV2	27	5 (19%)	18	1 (6%)	4	0	5	0	10	0
A. phagocytophilum	27	19 (70%)	18	4 (22%)	4	3 (75%)	5	0	10	0
T. gondii	11	6 (55%)	5	0	1	1 (100%)	2	1 (50%)	1	0

amplified DNA matched any of the other parvoviruses. The sample from the ringtail had insufficient DNA for sequencing. Temporal distribution of CPV active infections within sympatric mesocarnivores varied throughout the sampling period (Figure 2-2).

We PCR-amplified *A. phagocytophilum* DNA from 15% (3 of 20) of blood samples, including from one (N = 11) gray fox, one from a raccoon (N = 1), and one from striped skunk (N = 1). None of the two spotted skunks or the five ringtails sampled were PCR-positive for *A. phagocytophilum*.

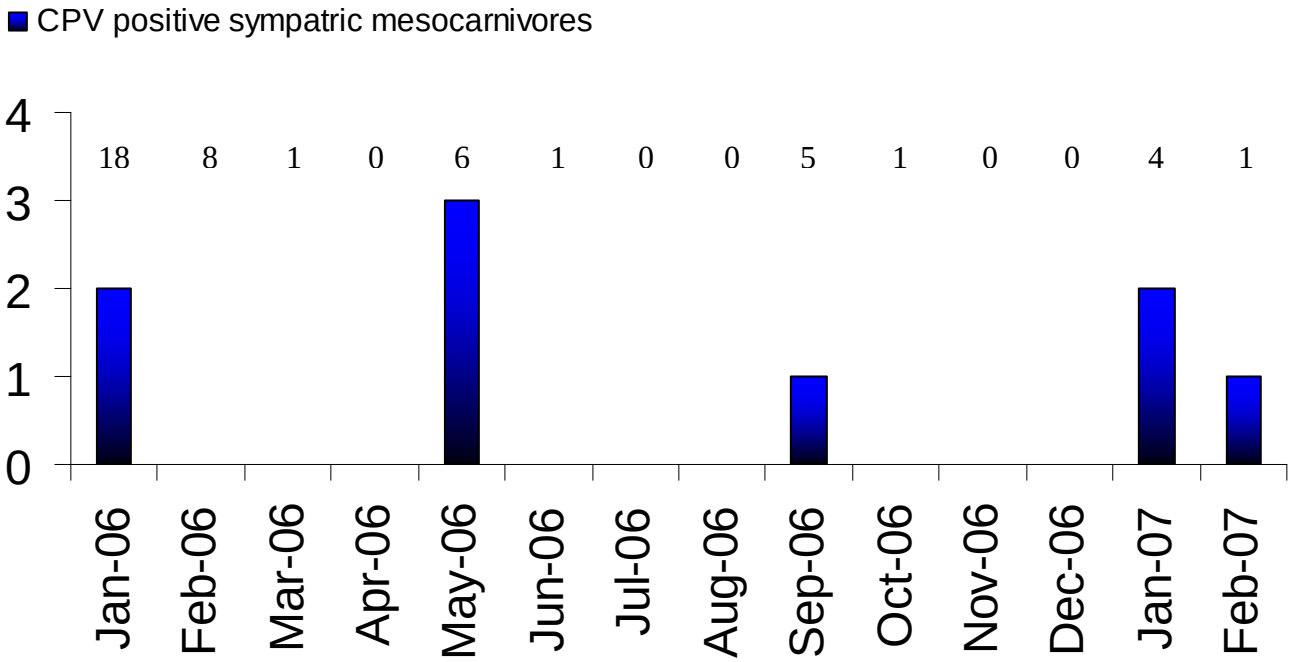


Figure 2-2. Temporal distribution of CPV active infections in gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), raccoon (*Procyon lotor*), spotted skunk (*Spilogale gracilis*), and striped skunk (*Mephitis mephitis*), trapped on the Hoopa Valley Indian Reservation, January 2006 – February 2007, and sympatric with a subset of the West Coast fisher DPS. Mesocarnivore species were combined and sample sizes are indicated above each month.

Comparison of Disease Exposures Between Fishers and Other Mesocarnivores

Comparison of pathogenic exposure between fishers and the group of sympatric mesocarnivores yielded few significant differences (Table 2-2). Fisher exposure to *A. phagocytophilum* was greater than in the other mesocarnivores when all mesocarnivores were considered, and also when only backcountry mesocarnivores were compared (Table 2-2). For all other pathogens, there was no significant difference in prevalence between fishers and other mesocarnivores (Table 2-2).

Fishers did not exhibit greater prevalence for active infections of either *A. phagocytophilum* or CPV than other mesocarnivores; this was true when all mesocarnivores were grouped together and also when only backcountry mesocarnivores were considered (Table 2-3). Active infection with CPV varied throughout the sampling period for both fishers and sympatric mesocarnivores (Figure 2-3).

Spatial clustering was apparent for only two pathogen-host groupings. Specifically, two individuals exposed to CDV, one fisher and one ringtail were sampled within 500m of each other on the east side of the Trinity River (Appendix A). All mesocarnivores other than fishers from which CPV DNA was amplified were captured either in the valley, or in a localized area in the southeast corner of HVIR; however, PCR-positive fishers were caught throughout the HVIR (Appendix A).

Table 2-2. Comparison of pathogen exposure determined from plasma samples from fisher (*Martes pennanti*) and the group of sympatric mesocarnivores including gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), raccoon (*Procyon lotor*), spotted skunk (*Spilogale gracilis*), and striped skunk (*Mephitis mephitis*), trapped on the Hoopa Valley Indian Reservation, February 2004 – February 2007. Pathogens evaluated include canine parvovirus (CPV), canine distemper virus (CDV), canine herpes virus (CHV), canine adenovirus (CAV2), West Nile virus (WNV), *Anaplasma phagocytophilum* (Ap) and *Toxoplasma gondii* (Tg). Comparisons were made between fishers and only the mesocarnivores trapped in the backcountry, and also between fishers and all mesocarnivores, regardless of capture location.

	Fisher		Backcountry Mesocarnivores			Fisher		All Mesocarnivores		
Pathogen	N	No. (%) Positive	N	No. (%) Positive	P	N	No. (%) Positive	N	No. (%) Positive	P
CDV	98	5 (5%)	41	1 (2%)	0.67	98	5 (5%)	64	1 (2%)	0.40
CPV	90	28 (31%)	41	10 (24%)	0.53	90	28 (31%)	64	19 (30%)	0.99
CHV	96	5 (5%)	41	0 (0%)	0.32	96	5 (5%)	64	0 (0%)	0.16
CAV2	95	4 (4%)	41	3 (7%)	0.43	95	4 (4%)	64	6 (9%)	0.20
WNV	99	4 (4%)	41	4 (10%)	0.23	99	4 (4%)	64	4 (6%)	0.71
Ap	79	60 (76%)	41	21 (51%)	0.008*	60	79 (76%)	64	32 (50%)	0.002*
Tg	77	45 (58%)	11	4 (36%)	0.20	77	45 (58%)	20	8 (40%)	0.21

* indicates statistical significance

Table 2-3. Comparison of polymerase chain reaction amplification (PCR) denoting active infection of canine parvovirus (CPV) and *Anaplasma phagocytophilum* (AP) determined from fecal samples from fisher (*Martes pennanti*) and the group of sympatric mesocarnivores including gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), raccoon (*Procyon lotor*), spotted skunk (*Spilogale gracilis*), and striped skunk (*Mephitis mephitis*), trapped on the Hoopa Valley Indian Reservation, February 2006 – February 2007. Comparisons were made between fishers and only the mesocarnivores trapped in the backcountry, and also between fishers and all mesocarnivores, regardless of capture location.

	Fisher		Backcountry Mesocarnivores			Fisher		All Mesocarnivores		
Pathogen	N	No. (%) Positive	N	No. (%) Positive	P	N	No. (%) Positive	N	No. (%) Positive	P
CPV	98	18 (18%)	32	4 (13%)	0.59	98	18 (18%)	47	9 (19%)	0.99
AP	78	3 (4%)	11	0 (0%)	0.99	78	3 (4%)	20	3 (15%)	0.10

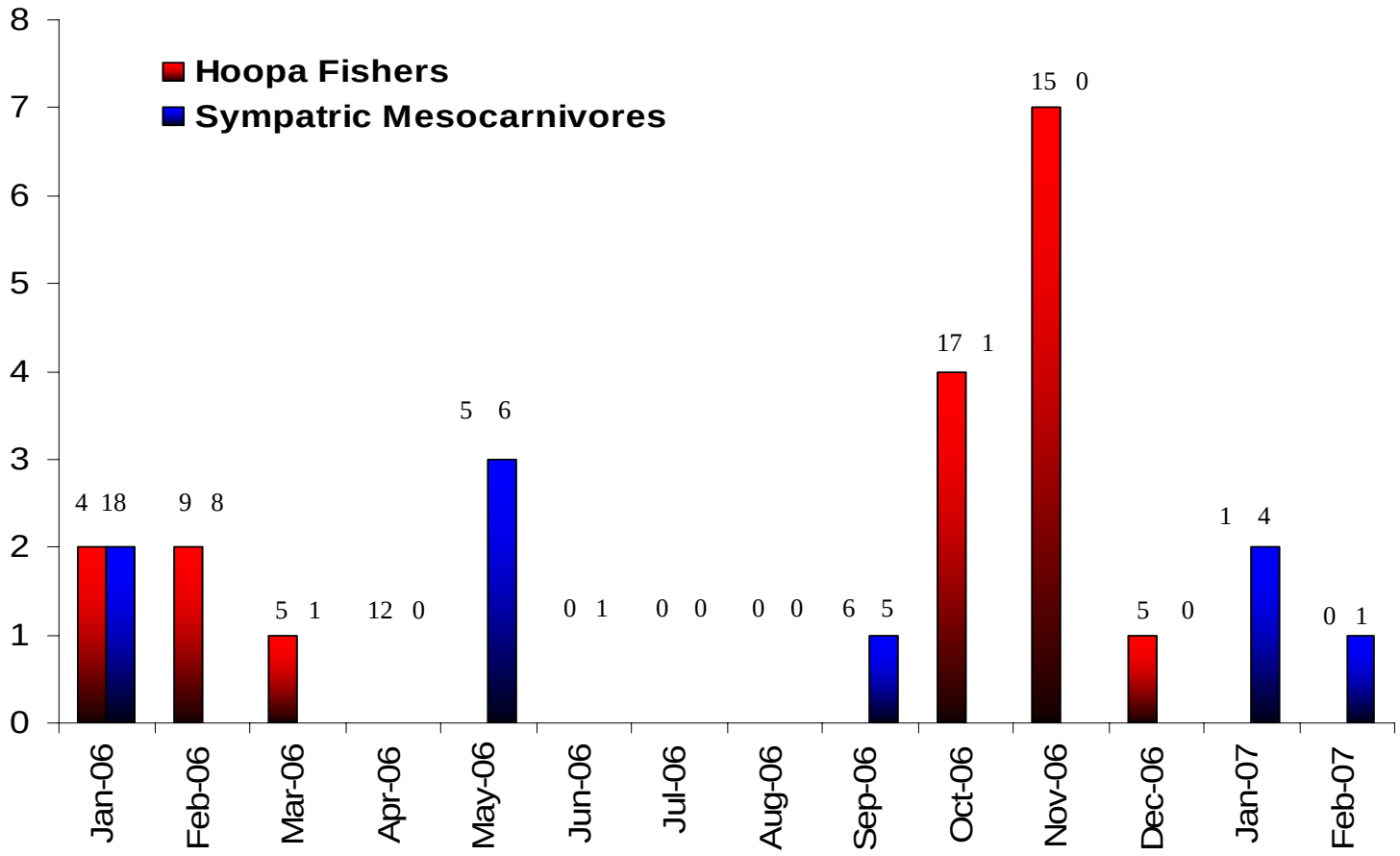


Figure 2-3. Temporal distribution of polymerase chain reaction amplification (PCR) denoting active infection of canine parvovirus (CPV) in fishers (*Martes pennanti*) versus sympatric mesocarnivores, gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), raccoon (*Procyon lotor*), spotted skunk (*Spilogale gracilis*), and striped skunk (*Mephitis mephitis*), trapped on the Hoopa Valley Indian Reservation, January 2006 – February 2007. Mesocarnivore species were combined and sample sizes are indicated above each month.

DISCUSSION

Canine Distemper

Canine distemper is a highly infectious disease, which affects all families of carnivores (Deem et al. 2000, Williams 2001). The transmission of CDV is primarily via aerosol or oral, respiratory, or oculonasal exudate fluids from either direct contact or possibly fomites previously visited by an actively infected individual (Deem et al. 2000, Williams 2001). CDV has variable incubation and clinical presentation periods that can range from one week to more than a month (Deem et al. 2000, Williams 2001)

Clinical signs of CDV are dependent on various abiotic and biotic factors which include strain type, environmental conditions, host species, immune status and age (Deem et al. 2000, Williams 2001). CDV causes 30-50% mortality among domestic canids, with 50%-70% of the individuals that survive serving as asymptomatic carriers (Deem et al. 2000). Gray foxes have been shown to be highly susceptible to CDV and develop clinical manifestations which include fever, vomiting, diarrhea, incoordination, oculonasal exudates, lack of fear from predators, tremors, ataxia, and depression (Deem et al. 2000, Williams 2001). It has been demonstrated that CDV is a significant mortality factor among some gray fox populations (Davidson et al. 1992b, Kelly and Sleeman 2003). The lack of antibody detection within this gray fox population could be due to at least two factors: that the sample size was too small to demonstrate exposure or, perhaps more likely, individual gray foxes rarely recover from CDV infections.

Among raccoons, natural CDV infections typically cause high mortality (Roscoe 1993, Deem et al. 2000, Williams 2001). Our sample size was small, but the lack of antibody detection within this species leads us to suspect that the same factors mentioned may be the reason. To date, there has been no documentation of the

exposure rates of CDV in spotted skunks, though striped skunks are suspected to be relatively resistant to CDV (Diters and Nielsen 1978).

Six mesocarnivores sampled during this study (five fishers and one ringtail) had antibodies to CDV, and this is the first documented report of CDV exposure in a ringtail. Assuming that ringtails have similar clinical manifestations as other procyonids, CDV may pose a threat to ringtail population health and other community species.

Highly susceptible mesocarnivores, including gray foxes, raccoons and possibly ringtails, may pose a spillover threat to vulnerable fisher populations by increasing the likelihood of transmission to fishers. Such risks are magnified if clinically ill animals manifest neural disease that reduce fear of predators (potentially fishers) (Gulland et al. 1995). It is interesting to note that the only two individuals on the east side of the Trinity River that were seropositive for CDV were trapped in close proximity to each other. Though no conclusions can be drawn from this occurrence, it does indicate a possible pattern to be investigated further for this directly transmitted pathogen.

Canine parvovirus

Canine parvovirus has been reported in numerous wild and domestic animals in North America since its emergence in the late 1970's (Kelly 1978, Steinel et al. 2001, Mccaw and Hoskins 2006). Antigenically and genetically-related parvoviruses include feline panleukopenia virus, canine parvovirus, mink enteritis virus, raccoon parvovirus, raccoon dog parvovirus, and blue fox parvovirus; all of which have genomic conservation of $\geq$98% (Berns et al. 2000).

Serology is inadequate to determine which parvovirus infected these animals because strain-specific identification of the infecting parvovirus would require virus

isolation and or sequencing of viral DNA from infected hosts. However, sequencing of four sympatric mesocarnivore CPV-positive fecal DNA samples from three different species (gray fox, raccoon, spotted skunk) matched CPV2 via BLAST and GenBank (Accession number AY742936). These sequences also matched the amplified canine parvovirus DNA from six PCR-positive fisher scats confirming all amplified parvoviral DNA was from CPV.

The disease caused by CPV manifests by lysis (cell rupture and death) of multiplying cells (Parrish 1995, Steinel et al. 2001, Mccaw and Hoskins 2006). Disease symptoms typically include fever, hemorrhagic diarrhea, vomiting, immunosuppression, and the reduction of white blood cells (Parrish 1995, Barker and Parrish 2001, Steinel et al. 2001, Mccaw and Hoskins 2006). Inflammation and hemorrhaging of the intestinal epithelium of animals suffering from parvovirus enteritis often allows bacterial leakage into the blood vascular system, leading to sepsis, and endotoxic shock. The combination of weakness and dehydration from the diarrhea and endotoxic shock causes some animals to die acutely before outward signs become apparent (Parrish 1995, Barker and Parrish 2001, Steinel et al. 2001, Mccaw and Hoskins 2006). Animals that survive acute infections typically have long-lasting and possibly life-long immunity to CPV (Steinel et al. 2001).

In our study, gray foxes, ringtails, and raccoons showed high seroprevalence for exposure to a parvovirus. Exposure of gray foxes, ringtails and raccoons to parvoviruses has been shown previously, and gray foxes and raccoons can develop severe clinical signs that reduce the fitness of infected individuals (Barker et al. 1983, Davidson et al. 1992a, Garcelon et al. 1992, Riley et al. 2004, Suzan and Ceballos 2005, Clifford et al. 2006). Spotted skunks also develop antibody titers to CPV (Suzan

and Ceballos 2005), suggesting that our negative serological results may result from small sample sizes. Striped skunks have been shown to develop low antibody titers without showing clinical signs, and infected striped skunks have not been shown to shed CPV in their feces (Barker et al. 1983, Barker and Parrish 2001).

Parvoviruses are resilient and can persist in the environment for months or even years under various conditions (Barker and Parrish 2001, Mccaw and Hoskins 2006). Nineteen percent of sympatric mesocarnivores at Hoopa were actively shedding CPV in their feces; suggesting that non-target captures in a fisher project may pose contamination risk to other species via contaminated handling material (Brown et al. 2006). Additionally, species sympatric with threatened species may serve as possible sources of spillover infections through feces left at latrines or marking sites, or even when preyed upon and their contaminated intestinal contents are consumed (Woodroffe 1999, Barker and Parrish 2001) .

Canine Herpesvirus

CHV causes a number of ailments in juvenile canids such as respiratory disease, inappetence, abdominal pain and possibly death, but within older domestic dogs the symptoms tend to be mild (Gaskell and Willoughby 1999). This virus not only infects canids but has been documented to infect skunks and mink (*Mustela vison*) (Diters and Nielsen 1978, Mcdonald and Lariviere 2001). None of the mesocarnivores captured at Hoopa had significant antibodies to CHV which again may result from small sample sizes.

Canine Adenovirus-2

Adenovirises cause infectious hepatitis in domestic dogs, gray foxes, skunks and raccoons (Garcelon et al. 1992, Woods 2001). The clinical signs of infection from CAV2 include hepatitis, encephalitis, oculonasal discharge, vomiting, mucoid or bloody diarrhea, paralysis, and death with a short clinical course or even sudden death (Woods 2001). This virus is highly contagious and can be shed via urine, feces, ocular, and nasal discharges from relatively healthy animals and can be transmitted via direct contact or fomites such as trapping and handling equipment (Woods 2001). Though no skunks or raccoons were seropositive for CAV2, striped skunks and raccoons have been shown to contract the virus and develop clinical manifestations to CAV2 infections, often resulting in death (Jamison et al. 1973, Karstad et al. 1975, Hamir et al. 1995).

West Nile Virus

The hosts of WNV include a wide range of species of birds and mammals (Marra et al. 2004). Clinical symptoms and signs of disease in people, horses, and birds include fever, muscle aches, lethargy, inappetence, diarrhea, vomiting, muscle stiffness, abdominal pain, blindness, head tremors, cardiac arrhythmias, coma, and death (Centers-for-Disease-Control-and-Prevention 2000, Marra et al. 2004). No information is available on the clinical manifestations in mammalian wildlife populations.

Striped skunks and raccoons are among the few species we tested for these diseases that have previously been documented to show WNV disease or exposure (Centers-for-Disease-Control-and-Prevention 2000, Dietrich et al. 2005, Bentler et al. 2007). This is the first report of exposure of gray foxes, ringtails and spotted skunks to WNV. Exposure to WNV as well as clinical disease have been demonstrated in canine

species, including wolves (*Canis lupus*) (Lichtensteiger et al. 2003, Lanthier et al. 2004), coyotes (Bischof and Rogers 2005) and domestic dogs (Blackburn et al. 1989, Lichtensteiger et al. 2003).

WNV is a vector-borne disease that is transmitted in North America by many species of mosquitoes (Marra et al. 2004). There is also evidence that carnivores can be exposed to WNV through the consumption of infected prey (Austgen et al. 2004, Kuno 2001). Therefore, prevention and management of WNV infections would be difficult. Additionally, potentially synergistic effects of WNV infections simultaneous with infections of other pathogens are unknown.

Anaplasma phagocytophilum

The area surrounding Hoopa has been shown to exhibit high levels of seroprevalence for *A. phagocytophilum* in its wildlife and domestic animal populations (Brown et al. 2003, Foley et al. 2004, Drazenovich et al. 2006, Gabriel 2006, Foley et al. 2007, Gabriel 2006). Domestic canids in nearby towns had a 50% seroprevalence while 52% of 54 gray foxes on the HVIR were shown to have been exposed to *A. phagocytophilum* in previous studies (Foley 2007, Gabriel et al. 2006) Clinical manifestations of granulocytic anaplasmosis in horses, cats, and dogs include fever, ataxia, ventral limb edema, icterus, and thrombocytopenia, all which facilitate immunosuppresion (Madigan and Gribble 1987, Foley et al. 2001, Foley et al. 2004). *Anaplasma phagocytophilum* is transmitted to domestic animals and wildlife by ticks of the family Ixodidae (Richter et al. 1996, Kramer et al. 1999, Foley et al. 2004). Known tick vectors include *Ixodes pacificus* (the western black-legged tick) in western North America (Richter et al. 1996, Nicholson 1998, Brown et al. 2005).

Gray foxes had levels of exposure to *A. phagocytophilum* similar to levels found during previous studies at Hoopa (Gabriel et al. 2006). This level of exposure indicates that the risk of contracting this tick-borne disease is high within the region, and also suggests that the related immunosuppression caused by *A. phagocytophilum* might exacerbate risks associated with coinfecting pathogens. Fishers showed a higher prevalence of *A. phagocytophilum* than the other mesocarnivores, even when only backcountry individuals were considered. This trend is not surprising since Gabriel et al. (2006) found backcountry foxes more likely to be *A. phagocytophilum*-positive than valley foxes. Prevalence of *A. phagocytophilum* may be greater in fishers due to their occurrence primarily within the backcountry.

Toxoplasma gondii

Toxoplasma gondii is a widespread zoonotic protozoan that parasitizes mesocarnivores with a range of outcomes that can result in asymptomatic infections or lameness, encephalitis with uncoordinated movements and head and body tremors, hepatitis, pneumonia, anorexia, blindness and death; importantly, *T. gondii* is also infamous for causing abortions, still births, and neonatal deaths of congenitally infected mustelid offspring (Dubey and Odening 2001, Burns et al. 2003, Jones et al. 2006). Oocysts of *T. gondii* are shed from infective felids, the only known definitive host for this parasite (Dubey and Odening 2001). Intermediate hosts acquire infections by either exposure to infective oocysts from felid feces or by consumption of prey items that contain *T. gondii* cysts in their tissues (Dubey and Odening 2001, Burns et al. 2003). All of the mesocarnivores sampled at Hoopa have been shown to serve as intermediate

hosts (Dubey and Odening 2001, Suzan and Ceballos 2005), but the development of clinical disease within these species is not fully understood.

Pathogenicity of *T. gondii* has been linked to the four genotypes that have been documented (Dubey and Odening 2001, Miller et al. 2004). Sea otters have demonstrated high mortality when infected with both genotypes II and X of *T. gondii* (Miller et al. 2004). To date it is not known which genotype of *T. gondii* is present in Hoopa or whether the *T. gondii* infections have a negative impact on fisher populations as they do in some other mustelid populations.

MANAGEMENT AND CONSERVATION IMPLICATIONS

Many of the pathogens assessed in this study are contracted via direct contact or from contaminated inanimate sources (fomites). These fomites could include trapping and handling equipment used by those of us studying and managing wildlife. Therefore, we recommend that researchers and managers disinfect all handling and trapping equipment between captures with an approved virocide and bacteriocide when conducting research on these mesocarnivore species.

Although there have been repeated recommendations in the scientific literature to monitor and gather baseline data of disease risk of threatened species or their sympatric community before health crises occur (Woodroffe 1999, Mathews et al. 2006), this is the first proactive demonstration to determine if sympatric species could possibly be utilized as sentinels of pathogenic risk to a species of concern in California. Our findings suggest that the sympatric species observed in this study have the same pathogenic exposure rates as fishers with the exception of *A. phagocytophilum*.

This study documents potential disease risks from sympatric mesocarnivores that may provide a reservoir for spillover and suppression of fisher populations. Wildlife managers considering the option of reintroduction may opt to screen the community of mesocarnivores at the release site in order to avoid releasing naïve animals into a high disease risk area. This precaution may reduce the likelihood of disease outbreaks in the newly released population, and increase the possibility of successful colonization and establishment of the translocated population (Mathews et al. 2006). We emphasize the potential benefits of monitoring carnivore species sympatric with fishers in Northern California in all assessments of risks from disease.

ACKNOWLEDGEMENTS FROM AUTHORS OF BOTH CHAPTERS

The authors thank the USFWS for supporting this research through grant #813335G021 to Integral Ecology Research Center. We thank MGW Biological for providing additional funding and support. We thank the Hoopa Valley Tribe and the Wildlife Conservation Society for facilitating all aspects of this work. We thank Steven Self (Sierra Pacific Industries, Redding, California), Richard Callas and Pete Figura (California Department of Fish and Game, Redding, California) for supplying samples collaboratively from the fisher populations near Redding, and Mark Jordan (University of California, Berkeley) for supplying fecal samples from fishers from the southern Sierras. Thanks also to Nikki Drazenovich at the University of California, Davis, Center for Vector-borne Diseases, for developing the appropriate assays.

LITERATURE CITED

Addison, E. M., I. K. Barker. and D. B. Hunter. 1999. Diseases and parasites of furbearers. *In* Wild furbearer management and conservation in North America (CD-ROM version), M. NOVAK, J. A. BAKER, M. E. OBBARD, AND B. MALLOCH, (eds.). Ministry of Natural Resources, Peterborough, Ontario, Canada. pp. 893-909

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410.

Apperson, C. S., H. K. Hassan, B. A. Harrison, H. M. Savage, S. E. Aspen, A. Farajollahi, W. Crans, T. J. Daniels, R. C. Falco, M. Benedict, M. Anderson, L. Mcmillen. and T. R. Unnasch. 2004. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. Vector-Borne and Zoonotic Diseases 4: 71-82.

Austgen, L. E., R. A. Bowen, M. L. Bunning, B. S. Davis, C. J. Mitchell. and G. J. Chang. 2004. Experimental infection of cats and dogs with West Nile virus. Emerging Infectious Diseases 10: 82-86.

Barker, I. K., R. C. Povey and D. R. Voigt. 1983. Response of mink, skunk, red fox and raccoon to inoculation with mink virus enteritis, feline panleukopenia and canine parvovirus and prevalence of antibody to parvovirus in wild carnivores in Ontario. Canadian Journal of Comparative Medicine 47: 188-197.

Barker, I. and C. R. Parrish. 2001. Parvovirus infections. *In* Infectious diseases of wild mammals, E. T. THORNE, E. S. WILLIAMS, AND I. K. BARKER, (eds.). Iowa State University Press, Ames, Iowa. pp. 131-147.

Beldomenico, P. M., B. B. Chomel, J. E. Foley, B. N. Sacks, C. J. Baldi, R. W. Kasten. and I. A. Gardner. 2005. Environmental factors associated with *Bartonella vinsonii* subsp berkhoffii seropositivity in free-ranging coyotes from northern California. Vector-Borne and Zoonotic Diseases 5: 110-119.

Bentler, K. T., J. S. Hall, J. J. Root, K. Klenk, B. Schmit, B. F. Blackwell, P. C. Ramey and L. Clark. 2007. Serologic evidence of West Nile virus exposure in North American mesopredators. American Journal of Tropical Medicine and Hygiene 76: 173-179.

Berns, K. I., M. Bergoin, M. Bloom, M. Lederman, N. Muzyczka, G. Siegl, J. Tal and P. Tattersall. 2000. Parvoviridae.*In* Virus taxonomy, classification and nomenclature of viruses Academic Press, Inc. , San Diego, Calif. pp. 311–323.

Bischof, R. and D. G. Rogers. 2005. Serologic Survey of Select Infectious Diseases in Coyotes and Raccoons in Nebraska. Journal of Wildlife Diseases 41: 787-791.

Blackburn, N. K., F. Reyers, W. L. Berry and A. J. Shepherd. 1989. Susceptibility of dogs to West Nile virus: a survey and pathogenicity trial. Journal of Comparative pathology 100: 59-66.

Brown, R. N.. and E. C. Burgess. 2001. Lyme borreliosis.*In* Infectious diseases of wild mammals, E. T. THORNE, E. S. WILLIAMS, AND I. K. BARKER, (eds.). Iowa State Press, Ames. pp. 435-454.

Brown, R. N., J. L. Sajecki, J. M. Higley and J. E. Foley. 2003. Exposure of American black bears to infectious disease agents in a managed Douglas-fir forest. Northwestern Naturalist 84: 95-96.

Brown, R. N., R. S. Lane and D. T. Dennis. 2005. Geographic distributions of tick-borne diseases and their vectors. *In* Tick-Borne Diseases of Humans, J. GOODMAN, D. T. DENNIS, AND D. SONENSHINE, (eds.). ASM Press, Washington, D.C. pp. 363-391.

Brown, R.N., M.W. Gabriel, G.M. Wengert., S. Matthews, J.M. Higley, and J.E. Foley. 2006. Fecally Transmitted Viruses Associated with Pacific Fishers (*Martes pennanti*) in Northwestern California. Transactions Western Section Wildlife Society.42: 40-46.

Brown, R. N., M. A. Peot. and R. S. Lane. 2006. Sylvatic maintenance of *Borrelia burgdorferi* (Spirochaetales) in northern California: untangling the web of transmission. Journal of Medical Entomology 43: 743-751.

Burridge, M. J., W. J. Bigler, D. J. Forrester. and J. M. Hennemann. 1979. Serologic survey for *Toxoplasma gondii* in wild animals in Florida. Journal of the American Veterinary Medical Association 175: 964-967.

Burns, R., E. S. Williams, D. O'toole and J. P. Dubey. 2003. *Toxoplasma gondii* infections in captive black-footed ferrets (*Mustela nigripes*), 1992-1998: clinical signs, serology, pathology, and prevention. Journal of Wildlife Diseases 39: 787-797.

Cabasso, V. J. 1981. Infectious canine hepatitis. *In* Infectious diseases of wild mammals, J. W. DAVIS, L. H. KARSTAD, AND D. O. TRAINER, (eds.). Iowa State University Press, Ames, Iowa. pp. 191-195

Centers for Disease Control and Prevention. 2000. West Nile virus activity--eastern United States, 2000. Journal of the American Medical Association 284: 3119-3120.

Centers for Disease Control and Prevention. 2003. West Nile virus activity--United States, November 13-19, 2003. Morbidity and Mortality Weekly Report 52: 1132.

Centers for Disease Control and Prevention. 2005. West Nile virus activity--United States, January 1-December 1, 2005. Morbidity and Mortality Weekly Report 54: 1253-1256.

Chang, C., K. Yamamoto, B. B. Chomel, R. W. Kasten, D. C. Simpson, C. R. Smith. and V. L. Kramer. 1999. Seroepidemiology of *Bartonella vinsonii* subsp. *berkhoffii* infection in California coyotes, 1994-1998. Emerging Infectious Diseases 5: 711-715.

Chang, C. C., R. W. Kasten, B. B. Chomel, D. C. Simpson, C. M. Hew, D. L. Kordick, R. Heller, Y. Piemont. and E. B. Breitschwerdt. 2000. Coyotes (*Canis latrans*) as the reservoir for a human pathogenic *Bartonella* sp.: molecular epidemiology of *Bartonella vinsonii* subsp. *berkhoffii* infection in coyotes from central coastal California. Journal of Clinical Microbiology 38: 4193-4200.

Charlton, K. M., G. C. Dulac, F. C. Thomas. and H. K. Mitchell. 1977. Necrotizing encephalitis in skunks caused by Herpes simplex virus. Canadian Journal of Comparative Medicine 41: 460-465.

Chen, C. C., S. Louie, B. Mccormick, W. A. Walker. and H. N. Shi. 2005. Concurrent infection with an intestinal helminth parasite impairs host resistance to enteric *Citrobacter rodentium* and enhances *Citrobacter*-induced colitis in mice. Infection and Immunity 73: 5468-5481.

Cheung, R. C., S. M. Matsui and H. B. Greenberg. 1994. Rapid and sensitive method for detection of hepatitis C virus RNA by using silica particles. Journal of Clinical Microbiology 32: 2593-2597.

Cho, J. G., S. A. Dee, J. Deen, A. Guedes, C. Trincado, E. Fano, Y. Jiang, K. Faaberg, J. E. Collins, M. P. Murtaugh. and H. S. Joo. 2006. Evaluation of the effects of animal age, concurrent bacterial infection, and pathogenicity of porcine reproductive and respiratory syndrome virus on virus concentration in pigs. American Journal of Veterinary Research 67: 489-493.

Chomel, B. B., M. T. Jay, C. R. Smith, P. H. Kass, C. P. Ryan. and L. R. Barrett. 1994. Serological surveillance of plague in dogs and cats, California, 1979-1991. Comparative Immunology, Microbiology, and Infectious Diseases 17: 111-123.

Chomel, B. B., R. L. Zarnke, R. W. Kasten, P. H. Kass. and E. Mendes. 1995. Serologic survey of *Toxoplasma gondii* in grizzly bears (*Ursus arctos*) and black bears (*Ursus americanus*), from Alaska, 1988 to 1991. Journal of Wildlife Diseases 31: 472-479.

Cleaveland, S., G. R. Hess, A. P. Dobson, M. K. Laurenson, H. I. Mccallum, M. G. Roberts and R. Woodroffe. 2002. The role of pathogens in biological conservation.*In* The ecology of wildlife diseases, P. J. HUDSON, A. RIZZOLI, B. T. GRENFELL, H. HEESTERBEEK, AND A. P. DOBSON, (eds.). Oxford University Press, New York, New York. pp. 139-150.

Clifford, D. L., J. A. K. Mazet, E. J. Dubovi, D. K. Garcelon, T. J. Coonan, P. A. Conrad and L. Munson. 2006. Pathogen exposure in endangered island fox (*Urocyon littoralis*) populations: implications for conservation management. Biological Conservation 131: 230-243.

Clover, J. R., T. D. Hofstra, B. G. Kuluris, M. T. Schroeder, B. C. Nelson, A. M. Barnes. and R. G. Botzler. 1989. Serologic evidence of *Yersinia pestis* infection in small mammals and bears from a temperate rainforest of north coastal California. Journal of Wildlife Diseases 25: 52-60.

Cole, R. A., D. S. Lindsay, D. K. Howe, C. L. Roderick, J. P. Dubey, N. J. Thomas. and L. A. Baeten. 2000. Biological and molecular characterizations of *Toxoplasma gondii* strains obtained from southern sea otters (*Enhydra lutris nereis*). Journal of Parasitology 86: 526-530.

Conrad, P. A., M. A. Miller, C. Kreuder, E. R. James, J. Mazet, H. Dabritz, D. A. Jessup, F. Gulland. and M. E. Grigg. 2005. Transmission of *Toxoplasma*: clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. International Journal for Parasitology 35: 1155-1168.

Courtenay, O., R. J. Quinnell. and W. S. Chalmers. 2001. Contact rates between wild and domestic canids: no evidence of parvovirus or canine distemper virus in crab-eating foxes. Veterinary Microbiology 81: 9-19.

Cunningham, A. A. 1996. Disease risks of wildlife translocations. Conservation Biology 10: 349-353.

Davidar, P.. and E. S. Morton. 2006. Are multiple infections more severe for purple martins (*Progne subis*) than single infections? The Auk 123: 141–147.

Davis, A., M. L. Bunning, P. Gordy, N. A. Panella, B. Blitvich. and R. A. Bowen. 2005. Experimental and natural infection of North American bats with West Nile virus. American Journal of Tropical Medicine and Hygiene 73: 467-469.

Davidson, W. R., M. J. Appel, G. L. Doster, O. E. Baker and J. F. Brown. 1992a. Diseases and parasites of red foxes, gray foxes, and coyotes from commercial sources selling to fox-chasing enclosures. Journal of Wildlife Diseases 28: 581-589.

Davidson, W. R., V. F. Nettles, L. E. Hayes, E. W. Howerth and C. E. Couvillion. 1992b. Diseases diagnosed in gray foxes (*Urocyon cinereoargenteus*) from the

southeastern United States. Journal of Wildlife Diseases 28: 28-33.

Dekrey, G. K.. and R. A. Adams. 2005. West Nile virus infection in wild bats of Colorado. *In* Annual Meeting of the American Association of Immunologists, San Diego, California. pp.

Deem, S. L., L. H. Spelman, R. A. Yates and R. J. Montali. 2000. Canine distemper in terrestrial carnivores: a review. Journal of Zoo and Wildlife Medicine 31: 441-451.

Deem, S. L., W. B. Karesh and W. Weisman. 2001. Putting theory into practice: wildlife health in conservation. Conservation Biology 15: 1224-1233.

Delahay, R.. and K. Frolich. 2000. Absence of antibodies against canine distemper virus in free-ranging populations of the Eurasian badger in Great Britain. Journal of Wildlife Diseases 36: 576-579.

Del Piero, F., D. W. Stremme, P. L. Habecker. and C. Cantile. 2006. West Nile Flavivirus Polioencephalomyelitis in a harbor seal (*Phoca vitulina*). Veterinary Pathology 43: 58-61.

Dick, T. A.. and R. D. Leonard. 1979. Helminth parasites of fisher *Martes pennanti* (Erxleben) from Manitoba, Canada. Journal of Wildlife Diseases 15: 409-412.

Dick, T. A., B. Kingscote, M. A. Strickland. and C. W. Douglas. 1986. Sylvatic trichinosis in Ontario, Canada. Journal of Wildlife Diseases 22: 42-47.

Dietrich, G., J. A. Montenieri, N. A. Panella, S. Langevin, S. E. Lasater, K. Klenk, J. C. Kile and N. Komar. 2005. Serologic evidence of West Nile virus infection in free-ranging mammals, Slidell, Louisiana, 2002. Vector-Borne and Zoonotic Diseases 5: 288-292.

Dietz, H. H., P. Henriksen, M. Lebech. and S. A. Henriksen. 1993. Experimental infection with *Toxoplasma gondii* in farmed mink (*Mustela vison* S.). Veterinary Parasitology 47: 1-7.

Diters, R. W. and S. W. Nielsen. 1978. Toxoplasmosis, distemper, and herpesvirus infection in a skunk (*Mephitis mephitis*). Journal of Wildlife Diseases 14: 132-133.

Douglas, C. W. and M. A. Strickland. 1999. Fisher. *In* Wild furbearer management and conservation in North America (CD-ROM version), M. NOVAK, J. A. BAKER, M. E. OBBARD, AND B. MALLOCH, (eds.). Ministry of Natural Resources, Peterborough, Ontario. pp. 510-529

Drazenovich, N., J. Foley and R. N. Brown. 2006. Use of real-time quantitative PCR targeting the msp2 protein gene to identify cryptic *Anaplasma phagocytophilum* infections in wildlife and domestic animals. Vector Borne and Zoonotic Diseases 6: 83-90.

Dubey, J. P., A. N. Hamir. and C. E. Rupprecht. 1990. Acute disseminated toxoplasmosis in a red fox (Vulpes vulpes). Journal of Wildlife Diseases 26: 286-290.

Dubey, J. P., A. N. Hamir, C. A. Hanlon. and C. E. Rupprecht. 1992. Prevalence of Toxoplasma gondii infection in raccoons. Journal of the American Veterinary Medical Association 200: 534-536.

Dubey, J. P. and K. Odening. 2001. Toxoplasmosis and related infections. *In* Parasitic Diseases of Wild Mammals, W. M. SAMUEL, M. J. PYBUS, AND A. A. KOCAN, (eds.). Iowa State University Press, Ames. pp. 478-519.

Farajollahi, A., N. A. Panella, P. Carr, W. Crans, K. Burguess. and N. Komar. 2003. Serologic evidence of West Nile virus infection in black bears (*Ursus americanus*) from New Jersey. Journal of Wildlife Diseases 39: 894-896.

Farajollahi, A., R. Gates, W. Crans. and N. Komar. 2004. Serologic evidence of West Nile virus and St. Louis encephalitis virus infections in white-tailed deer (*Odocoileus virginianus*) from New Jersey, 2001. Vector-Borne and Zoonotic Diseases 4: 379-383.

Foley, J. E., L. Crawford-Miksza, J. S. Dumler, C. Glaser, J. S. Chae, E. Yeh, D. Schnurr, R. Hood, W. Hunter. and J. E. Madigan. 1999. Human granulocytic ehrlichiosis in Northern California: two case descriptions with genetic analysis of the Ehrlichiae. Clinical Infectious Diseases 29: 388-392.

Foley, J. E., P. Foley and J. E. Madigan. 2001. The distribution of granulocytic ehrlichia seroreactive dogs in California. American Journal of Veterinary Research 62: 1599-1605.

Foley, J. E., P. Foley, R. N. Brown, R. S. Lane, J. S. Dumler. and J. E. Madigan. 2004. Ecology of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in the western United States. Journal of Vector Ecology 29: 41-50.

Foley, J. E., R.N. Brown, M. W. Gabriel, J. Henn, N. Drazenovich, R. Kasten, S. L. Green and B. Chomel. 2007. Spatial epidemiological analysis of tick-borne pathogen exposure in dogs in rural northern California coastal communities. The Veterinary Record 161: 653-657.

Fox, J. G. 1998. Biology and diseases of the ferret. Second edition. Williams and Wilkins, Baltimore, Maryland.

Frank, R. K. 2001. An outbreak of toxoplasmosis in farmed mink (*Mustela vison* S.). Journal of Veterinary Diagnostic Investigations 13: 245-249.

Furze, R. C., T. Hussell. and M. E. Selkirk. 2006. Amelioration of influenza-induced pathology in mice by coinfection with *Trichinella spiralis*. Infection and Immunity 74: 1924-1932.

Gabriel, M. W., J. Henn, J. E. Foley, R. N. Brown, R. Kasten, P. Foley. and B. Chomel. In Preparation Zoonotic *Bartonella* species in fleas collected on gray foxes (*Urocyon cinereoargenteus*) in Northern California.

Gabriel, M. W. 2006. Exposure to *Anaplasma phagocytophilum* and ticks in gray foxes (*Urocyon cinereoargenteus*) in northern Humboldt County, California. M.S. thesis. Humboldt State University, Arcata, California.

Gabriel, M. W. and G. M. Wengert. 2005. Assessment of the modification of stress-causing environmental factors in an improved nest box design for mesocarnivores. *In* Poster presented at the Annual Meeting of the Western Section of The Wildlife Society, Sacramento, California.

Gage, K. L.. and M. Y. Kosoy. 2005. Natural history of plague: perspectives from more than a century of research. Annual Review of Entomology 50: 505-528.

Gale, K. R., G. Leatch, C. M. Dimmock. and M. G. Gartside. 1997. Increased resistance to *Anaplasma marginale* infection in cattle chronically infected with *Theileria buffeli* (syn. *T. orientalis*). Veterinary Parasitology 69: 187-196.

Garcelon, D. K., R. K. Wayne and B. J. Gonzales. 1992. A serologic survey of the island fox (*Urocyon littoralis*) on the Channel Islands, California. Journal of Wildlife Diseases 28: 223-229.

Gaskell, R. and K. Willoughby. 1999. Herpesviruses of carnivores. Veterinary Microbiology 69: 73-88.

Gasper, P. W.. and R. P. Watson. 2001. Plague and yersiniosis.*In* Infectious Diseases of Wild Mammals, I. K. B. E. S. WILLIAMS, (ed.). Iowa State University Press, Ames, Iowa. pp. 313-329

Gaydos, J. K., P. A. Conrad, K. V. Gilardi, G. M. Blundell. and M. Ben-David. 2007. Does human proximity affect antibody prevalence in marine-foraging river otters (*Lontra canadensis*)? Journal of Wildlife Diseases 43: 116-123.

Gibilisco, C. J. 1994. Distributional dynamics of modern *Martes* in North America. *In* Martens, sables, and fishers: biology and conservation, S. W. BUSKIRK, A. S. HARESTAD, M. G. RAPHAEL, AND R. A. POWELL, (eds.). Cornell University Press, Ithaca, New York, USA. pp. 59-71.

Graham, A. L. 2002. When T-helper cells don't help: immunopathology during concomitant infection. Quarterly Review of Biology 77: 409-434.

Greenacre, C. B. 2003. Incidence of adverse events in ferrets vaccinated with distemper or rabies vaccine: 143 cases (1995-2001). Journal of the American Veterinary Medical Association 223: 663-665.

Gulland, F. M. D., B. T. Grenfell and A. P. Dobson. 1995. The impact of infectious diseases on wild animal populations-a review. *In* Ecology of Infectious Diseases in Natural Populations, B. T. GREENFELL, AND A. P. DOBSON, (eds.). Cambridge University Press, Cambridge. pp. 533.

Hamir, A. N., G. Moser, M. Kao, N. Raju and C. E. Rupprecht. 1995. Herpesvirus-like infection in a raccoon (*Procyon lotor*). Journal of Wildlife Diseases 31: 420-423.

Hanni, K. D., J. A. Mazet, F. M. Gulland, J. Estes, M. Staedler, M. J. Murray, M. Miller. and D. A. Jessup. 2003. Clinical pathology and assessment of pathogen exposure in southern and Alaskan sea otters. Journal of Wildlife Diseases 39: 837-850.

Heinz-Taheny, K. M., J. J. Andrews, M. J. Kinsel, A. P. Pessier, M. E. Pinkerton, K. Y. Lemberger, R. J. Novak, G. J. Dizikes, E. Edwards. and N. Komar. 2004. West Nile virus infection in free-ranging squirrels in Illinois. Journal of Veterinary Diagnotic Investigation 16: 186-190.

Henn, J. B., M. W. Gabriel, R. W. Kasten, R. N. Brown, J. H. Theis, J. E. Foley. and B. B. Chomel. 2007. Gray foxes (*Urocyon cinereoargenteus*) as a potential reservoir of a *Bartonella clarridgeiae*-like bacterium and domestic dogs as sentinels for zoonotic arthropod-borne pathogens in northern California. Journal of Clinical Microbiology In Press.

Henn, J. B., C.-H. Liu, R. W. Kasten, B. A. Vanhorn, L. A. Beckett, P. H. Kass. and B. B. Chomel. 2005. Seroprevalence of antibodies against *Bartonella* species and evaluation of risk factors and clinical signs associated with seropositivity in dogs. American Journal of Veterinary Research 66: 688-694.

Hill, R. E., Jr., J. J. Zimmerman, R. W. Wills, S. Patton. and W. R. Clark. 1998. Seroprevalence of antibodies against *Toxoplasma gondii* in free-ranging mammals in Iowa. Journal of Wildlife Diseases 34: 811-815.

Hirasawa, T., T. Kaneshige and K. Mikazuki. 1994. Sensitive detection of canine parvovirus DNA by the nested polymerase chain reaction. Veterinary Microbiology 41: 135-145.

Hoar, B. R., B. B. Chomel, D. L. Rolfe, C. C. Chang, C. L. Fritz, B. N. Sacks. and T. E. Carpenter. 2003. Spatial analysis of *Yersinia pestis* and *Bartonella vinsonii* subsp. berkhoffii seroprevalence in California coyotes (*Canis latrans*). Preventive Veterinary Medicine 56: 299-311.

Holden, K., J. T. Boothby, S. Anand. and R. F. Massung. 2003. Detection of *Borrelia burgdoferi, Ehrlichia chaffeensis*, and A*naplasma phagocytophilum* in ticks (Acari: Ixodidae) from a coastal region of California. Journal of Clinical Microbiology 40: 534-539.

Holden, K., E. Hodzic, S. Feng, K. J. Freet, R. B. Lefebvre. and S. W. Barthold. 2005. Coinfection with *Anaplasma phagocytophilum* alters *Borrelia burgdorferi* population distribution in C3H/HeN mice. Infection and Immunity 73: 3440-3444.

Hofmann-Lehmann, R., M. L. Meli, U. M. Dreher, E. Gonczi, P. Deplazes, U. Braun, M. Engels, J. Schupbach, K. Jorger, R. Thoma, C. Griot, K. D. C. Stark, B. Willi, J. Schmidt, K. M. Kocan. and H. Lutz. 2004. Concurrent infections with vector-borne pathogens associated with fatal hemolytic anemia in a cattle herd in Switzerland. Journal of Clinical Microbiology 42: 3775–3780.

Hudson, P. and J. Greenman. 1998. Competition mediated by parasites: biological and theoretical progress. Trends in Ecology & Evolution 13: 387-390.

Hubalek, Z.. and J. Halouzka. 1999. West Nile fever--a reemerging mosquito-borne viral disease in Europe. Emerging Infectious Diseases 5: 643-650.

Hurkova, L.. and D. Modry. 2006. PCR detection of *Neospora caninum*, *Toxoplasma gondii* and *Encephalitozoon cuniculi* in brains of wild carnivores. Veterinary Parasitology 137: 150-154.

Hutcheson, H. J., C. H. Gorham, C. Machain-Williams, M. A. Lorono-Pino, A. M. James, N. L. Marlenee, B. Winn, B. J. Beaty. and C. D. Blair. 2005. Experimental transmission of West Nile virus (Flaviviridae : Flavivirus) by *Carios capensis* ticks from North America. Vector-Borne and Zoonotic Diseases 5: 293-295.

Jamison, R. K., E. C. Lazar, L. N. Binn and A. D. Alexander. 1973. Survey for antibodies to canine viruses in selected wild mammals. Journal of Wildlife Diseases 9: 2-3.

Jones, Y. L., S. D. Fitzgerald, J. G. Sikarske, A. Murphy, N. Grosjean and M. Kiupel. 2006. Toxoplasmosis in a Free-ranging Mink. Journal of Wildlife Diseases 42: 865-869.

Karstad, L., R. Ramsden, T. J. Berry and L. N. Binn. 1975. Hepatitis in skunks caused by the virus of infectious canine hepatitis. Journal of Wildlife Diseases 11: 494-496.

Kelly, W. R. 1978. An enteric disease of dogs resembling feline panleucopaenia. Australian Veterinarian Journal 54: 593.

Kelly, T. R. and J. M. Sleeman. 2003. Morbidity and mortality of red foxes (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) admitted to the Wildlife Center of Virginia, 1993-2001. Journal of Wildlife Diseases 39: 467-469.

Kidd, L., B. Hegarty, D. Sexton and E. Breitschwerdt. 2006. Molecular characterization of *Rickettsia rickettsii* infecting dogs and people in North Carolina. Annals of the New York Academy of Sciences 1078:400-409.

Kile, J. C., N. A. Panella, N. Komar, C. C. Chow, A. Macneil, B. Robbins. and M. L. Bunning. 2005. Serologic survey of cats and dogs during an epidemic of West Nile virus infection in humans. Journal of the American Veterinary Medical Association 226: 1349-1353.

Kimber, K. R., G. V. Kollias. and E. J. Dubovi. 2000. Serologic survey of selected viral agents in recently captured wild North American river otters (*Lontra canadensis*). Journal of Zoo and Wildlife Medicine 31: 168-175.

Komar, N., N. A. Panella. and E. Boyce. 2001. Exposure of domestic mammals to West Nile virus during an outbreak of human encephalitis, New York City, 1999. Emerging Infectious Diseases 7: 736-738.

Kramer, V. L., M. P. Randolph, L. T. Hui, W. E. Irwin, A. G. Gutierrez and D. J. Vugia. 1999. Detection of the agents of human ehrlichioses in ixodid ticks from California. American Journal of Tropical Medicine and Hygiene 60: 62-65.

Kuno, G. 2001. Transmission of arboviruses without involvement of arthropod vectors. Acta Virologica 45: 139-150.

Lane, R. S., R. W. Emmons, V. Devlin, D. V. Dondero. and B. C. Nelson. 1982. Survey for evidence of Colorado tick fever virus outside of the known endemic area in California. Am. J. Trop. Med. Hyg. 31: 837-843.

Lane, R. S., J. E. Foley, L. Eisen, E. T. Lennette. and M. A. Peot. 2001. Acarologic risk of exposure to emerging tick-borne bacterial pathogens in a semirural community in northern California. Vector-Borne and Zoonotic Diseases 1: 197-210.

Lane, R. S., D. B. Steinlein. and J. Mun. 2004. Human behaviors elevating exposure to *Ixodes pacificus* (Acari: Ixodidae) nymphs and their associated bacterial zoonotic agents in a hardwood forest. Journal of Medical Entomology 41: 239-248.

Langlois, I. 2005. Viral diseases of ferrets. Veterinary Clinics of North America: Exotic Animal Practice 8: 139-160.

Lanthier, I., M. Hebert, D. Tremblay, J. Harel, A. D. Dallaire and C. Girard. 2004. Natural West Nile virus infection in a captive juvenile Arctic wolf (*Canis lupus*). Journal of Veterinary Diagnostic Investigation 16: 326-329.

Lawrie, C. H., N. Y. Uzcategui, E. A. Gould. and P. A. Nuttall. 2004. Ixodid and argasid tick species and West Nile virus. Emerging Infectious Diseases 10: 653-657.

Leighton, F. A. 2002. Health risk assessment of the translocation of wild animals. Review of Science and Technology 21: 187-195.

Lichtensteiger, C. A., K. Heinz-Taheny, T. S. Osborne, R. J. Novak, B. A. Lewis and M. L. Firth. 2003. West Nile virus encephalitis and myocarditis in wolf and dog. Emerging Infectious Diseases 9: 1303-1306.

Lively, C. M. 2005. Evolution of virulence: coinfection and propagule production in spore-producing parasites. Evolutionary Biology 5: 64.

Ludwig, G. V., P. P. Calle, J. A. Mangiafico, B. L. Raphael, D. K. Danner, J. A. Hile, T. L. Clippinger, J. F. Smith, R. A. Cook. and T. Mcnamara. 2002. An outbreak of West Nile virus in a New York City captive wildlife population. American Journal of Tropical Medicine and Hygiene 67: 67-75.

Macdonald, D. W., C. D. Buesching, P. Stopka, J. Henderson, S. A. Ellwood and S. E. Baker. 2004a. Encounters between two sympatric carnivores: red foxes (*Vulpes vulpes*) and European badgers (*Meles meles*). Journal of Zoology 263: 385-392.

Macdonald, K. A., B. B. Chomel, M. D. Kittleson, R. W. Kasten, W. P. Thomas. and P. Pesavento. 2004b. A prospective study of canine infective endocarditis in Northern California (1999-2001): emergence of *Bartonella* as a prevalent etiologic agent. Journal of Veterinary Internal Medicine 18: 56-64.

Madić, J., D. Huber. and B. Lugovic. 1993. Serologic survey for selected viral and rickettsial agents of brown bears (*Ursus arctos*) in Croatia. Journal of Wildlife Diseases 29: 572-576.

Madigan, J. E. and D. Gribble. 1987. Equine ehrlichiosis in northern California: 49 cases (1968-1981). Journal of the American Veterinary Medical Association 190: 445-448.

Marra, P. P., S. Griffing, C. Caffrey, A. M. Kilpatrick, R. G. Mclean, C. Brand, E. M. I. Saito, A. P. Dupuis, L. Kramer and R. J. Novak. 2004. West Nile virus and wildlife. BioScience 54: 393-402.

Mathews, F., D. Moro, R. Strachan, M. Gelling and N. Buller. 2006. Health surveillance in wildlife reintroductions. Biological Conservation 131: 338-347.

Mayer, K. E.. and W. F. Laudenslayer, (eds.) 1988. A guide to wildlife habitats of California. California Department of Forestry and Fire Protection, Sacramento, California. 166 pp.

Mccaw, D. L. and J. D. Hoskins. 2006. Canine viral enteritis. *In* Infectious diseases of the dog and cat, C. E. GREENE, (ed.). WB Saunders\ Elsevier Science, London,

UK. pp. 1424.

Mcdonald, R. A., M. J. Day. and R. J. Birtles. 2001. Histological evidence of disease in wild stoats (*Mustela erminea*) in England. Veterinary Record 149: 671-675.

Mcdonald, R. A.. and S. Lariviere. 2001. Diseases and pathogens of *Mustela* spp., with special reference to the biological control of introduced stoat *Mustela erminea* populations in New Zealand. Journal of the Royal Society of New Zealand 31: 721-744.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham. and P. A. Conrad. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology 32: 997-1006.

Miller, M. A., M. E. Grigg, C. Kreuder, E. R. James, A. C. Melli, P. R. Crosbie, D. A. Jessup, J. C. Boothroyd, D. Brownstein and P. A. Conrad. 2004. An unusual genotype of *Toxoplasma gondii* is common in California sea otters (*Enhydra lutris nereis*) and is a cause of mortality. International Journal for Parasitology 34: 275-284.

Mitchell, S. M., D. J. Richardson. and D. S. Lindsay. 2006. Prevalence of agglutinating antibodies to *Toxoplasma gondii* in striped skunks (*Mephitis mephitis*), opossums (*Didelphis virginiana*), and raccoons (*Procyon lotor*) from Connecticut. Journal of Parasitology 92: 664-665.

Mosquera, J.. and F. R. Adler. 1998. Evolution of virulence: a unified framework for coinfection and superinfection. Journal of Theoretical Biology 195: 293-313.

Munson, L. and W. B. Karesh. 2002. Disease monitoring for the conservation of terrestrial animals.*In* Conservation Medicine: ecological health in practice, R. S. O. A. AGUIRRE, GARY M. TABOR, CAROL HOUSE, MARY C. PEARL (ed.). Oxford University Press US, New York. pp. 95-103.

Murphy, F. A., E. P. Gibbs, M. C. Horzinek. and M. J. Studdert. 1999. Veterinary virology. Third edition. Academic Press, New York, New York.

Nettles, V. F., J. E. Pearson, G. A. Gustafson. and J. L. Blue. 1980. Parvovirus infection in translocated raccoons. Journal of the American Veterinary Medical Association 177: 787-789.

Nicholson, W. 1998. Epidemiology of human granulocytic ehrlichiosis, with special reference to the role of wild rodents and ixodid ticks as natural hosts of *Ehrlichia phagocytophila* sensu lato. PhD dissertation. Johns Hopkins University, Baltimore, MD.

Nutter, F. B., J. F. Levine, M. K. Stoskopf, H. R. Gamble. and J. P. Dubey. 1998. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in North Carolina black bears (*Ursus americanus*). Journal of Parasitology 84: 1048-1050.

Parrish, C. R. 1995. Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillieres Clinical Haematology 8: 57-71.

Petersen, L. R.. and J. T. Roehrig. 2001. West Nile virus: a reemerging global pathogen. Emerging Infectious Diseases 7: 611-614.

Philip, R. N., R. S. Lane. and E. A. Casper. 1981. Serotypes of tick-borne spotted fever group rickettsiae from western California. American Journal of Tropical Medicine and Hygiene 30: 722-727.

Philippa, J. D., F. A. Leighton, P. Y. Daoust, O. Nielsen, M. Pagliarulo, H. Schwantje, T. Shury, R. Van Herwijnen, B. E. Martina, T. Kuiken, M. W. Van De Bildt. and A. D. Osterhaus. 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. Veterinary Record 155: 135-140.

Pilipski, J. D., L. M. Pilipski. and L. S. Risley. 2004. West Nile virus antibodies in bats from New Jersey and New York. Journal of Wildlife Diseases 40: 335-337.

Poole, K. G., G. M. Matson, S. A. Strickland, A. J. Magoun, R. P. Graf. and L. M. Dix. 1994. Age and sex determination for American martens and fishers. *In* Martens, sables, and fishers: biology and conservation, S. W. BUSKIRK, A. S. HARESTAD, M. G. RAPHAEL, AND R. A. POWELL, (eds.). Cornell University Press, Ithaca, New York. pp. 204-223.

Pusterla, N., C. C. Chang, B. B. Chomel, J. S. Chae, J. E. Foley, E. Derock, V. L. Kramer, H. Lutz. and J. E. Madigan. 2000. Serologic and molecular evidence of *Ehrlichia* spp. in coyotes in California. Journal of Wildlife Diseases 36: 494-499.

Quinn, P. J., R. O. Ramsden. and D. H. Johnston. 1976. Toxoplasmosis: a serological survey in Ontario wildlife. Journal of Wildlife Diseases 12: 504-510.

Richter, P. J., Jr., R. B. Kimsey, J. E. Madigan, J. E. Barlough, J. S. Dumler and D. L. Brooks. 1996. *Ixodes pacificus* (Acari: Ixodidae) as a vector of *Ehrlichia equi* (Rickettsiales: Ehrlichieae). Journal of Medical Entomology 33: 1-5.

Riley, S. P., J. Foley and B. Chomel. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. Journal of Wildlife Diseases 40: 11-22.

Root, J. J., J. S. Hall, R. G. Mclean, N. L. Marlenee, B. J. Beaty, J. Gansowski. and L. Clark. 2005. Serologic evidence of exposure of wild mammals to flaviviruses in

the central and eastern United States. American Journal of Tropical Medicine and Hygiene 72: 622-630.

Roscoe, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. Journal of Wildlife Diseases 29: 390-395.

Ruppanner, R., D. A. Jessup, I. Ohishi, D. E. Behymer. and C. E. Franti. 1982. Serologic survey for certain zoonotic diseases in black bears in California. Journal of the American Veterinary Medical Association 181: 1288-1291.

Ruprecht, C., K. Stöhr. and C. Meredith. 2001. Rabies. *In* Infectious diseases of wild mammals, THORNE, E. S. WILLIAMS, AND I. K. BARKER, (eds.). Iowa State University Press, Ames, Iowa. pp. 3-36.

Scott, M. E. 1988. The impact of infection and disease on animal populations: implications for conservation biology. Conservation Biology 2: 40-56.

Self, S.. and S. J. Kerns. 2001. Pacific fisher use of a managed forest landscape in northern California. Sierra Pacific Industries, Redding, California, 32 pp.

Singer, B. C.. and E. L. Begg. 1975. Soil Survey, Hoopa Valley, California. Agricultural Extension Service of the University of California, Davis, California pp.

Sonenshine, D. E. 1993. Biology of Ticks. Oxford University Press, New York.

Steinel, A., C. R. Parrish, M. E. Bloom and U. Truyen. 2001. Parvovirus infections in wild carnivores. Journal of Wildlife Diseases 37: 594-607.

Suzan, G. and G. Ceballos. 2005. The role of feral mammals on wildlife infectious disease prevalence in two nature reserves within Mexico City limits. Journal of Zoo and Wildlife Medicine 36: 479-484.

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

Thornton, R. N. 1990. Toxoplasmosis in ferrets. New Zealand Veterinary Journal 38: 123.

Tizard, I. R., J. B. Billett. and R. O. Ramsden. 1976. The prevalence of antibodies against *Toxoplasma gondii* in some Ontario mammals. Journal of Wildlife Diseases 12: 322-325.

Tocidlowski, M. E., M. R. Lappin, P. W. Summer. and M. K. Stoskopf. 1997. Serologic survey for toxoplasmosis in river otters. Journal of Wildlife Diseases 33: 649-652.

Turell, M. J., M. R. Sardelis, D. J. Dohm. and M. L. O'guinn. 2001. Potential North American vectors of West Nile virus. Annals of the New York Academy of Sciences 951: 317-324.

United-States Fish and Wildlife-Service. 2004. 50-CFR Part 17 Endangered and threatened wildlife and plants; 12 month findings for a petition to list the west coast distinct population segment of the fisher (*Martes pennanti*); proposed rule. Federal Register 69: 18770-18792.

Wilbert, J. C. 1992. Spatial scale and seasonality of habitat selections by martins in southeastern Wyoming. Master's Thesis. University of Wyoming, Laramie, Wyoming.

Williams, E. S., E. T. Thorne, M. J. Appel. and D. W. Belitsky. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24: 385-398.

Williams, E. S., E. T. Thorne, J. Quan. and S. L. Anderson. 1991. Experimental infection of domestic ferrets (*Mustela putorius furo*) and Siberian polecats (*Mustela eversmanni*) with *Yersinia pestis*. Journal of Wildlife Diseases 27: 441-445.

Williams, E. S., K. Mills, D. R. Kwiatkowski, E. T. Thorne. and A. Boerger-Fields. 1994. Plague in a black-footed ferret (*Mustela nigripes*). Journal of Wildlife Diseases 30: 581-585.

Williams, E. S.. and E. T. Thorne. 1996. Infectious and parasitic diseases of captive carnivores, with special emphasis on the black-footed ferret (*Mustela nigripes*). Reviews of Science and Technology 15: 91-114.

Williams, E. S. 2001. Canine distemper. *In* Infectious diseases of wild mammals, E. T. THORNE, E. S. WILLIAMS, AND I. K. BARKER, (eds.). Iowa State University Press, Ames, Iowa. pp. 50-59.

Wobeser, G. 2002. Disease management strategies for wildlife. Review of Science and Technology 21: 159-178.

Wobeser, G. A. 2006. How disease is detected, described, and measured. *In* Essentials of Disease in Wild Animals, G. A. WOBESER, (ed.). Blackwell Publishing, Ames, Iowa. pp. 45-59..

Woodroffe, R. 1999. Managing disease threats to wild mammals. Animal Conservation 2: 185-193.

Woodford, M. H.. and P. B. Rossiter. 1993. Disease risks associated with wildlife translocation projects. Review of Science and Technology 12: 115-135.

Woods, L. W. 2001. Adenoviral diseases. *In* Infectious diseases of wild mammals, E. T.

THORNE, E. S. WILLIAMS, AND I. K. BARKER, (eds.). Iowa State University Press, Ames, Iowa. pp. 202-212.

Yaeger, J. S. 2005. Habitat at fisher resting sites in the Klamath province of northern California. MS thesis. Humboldt State University, Arcata.

Yamamoto, K., B. B. Chomel, L. J. Lowenstine, Y. Kikuchi, L. G. Phillips, B. C. Barr, P. K. Swift, K. R. Jones, S. P. D. Riley, R. W. Kasten, J. E. Foley. and N. C. Pedersen. 1998. *Bartonella henselae* antibody prevalence in free-ranging and captive wild felids from California. Journal of Wildlife Diseases 34: 56-63.

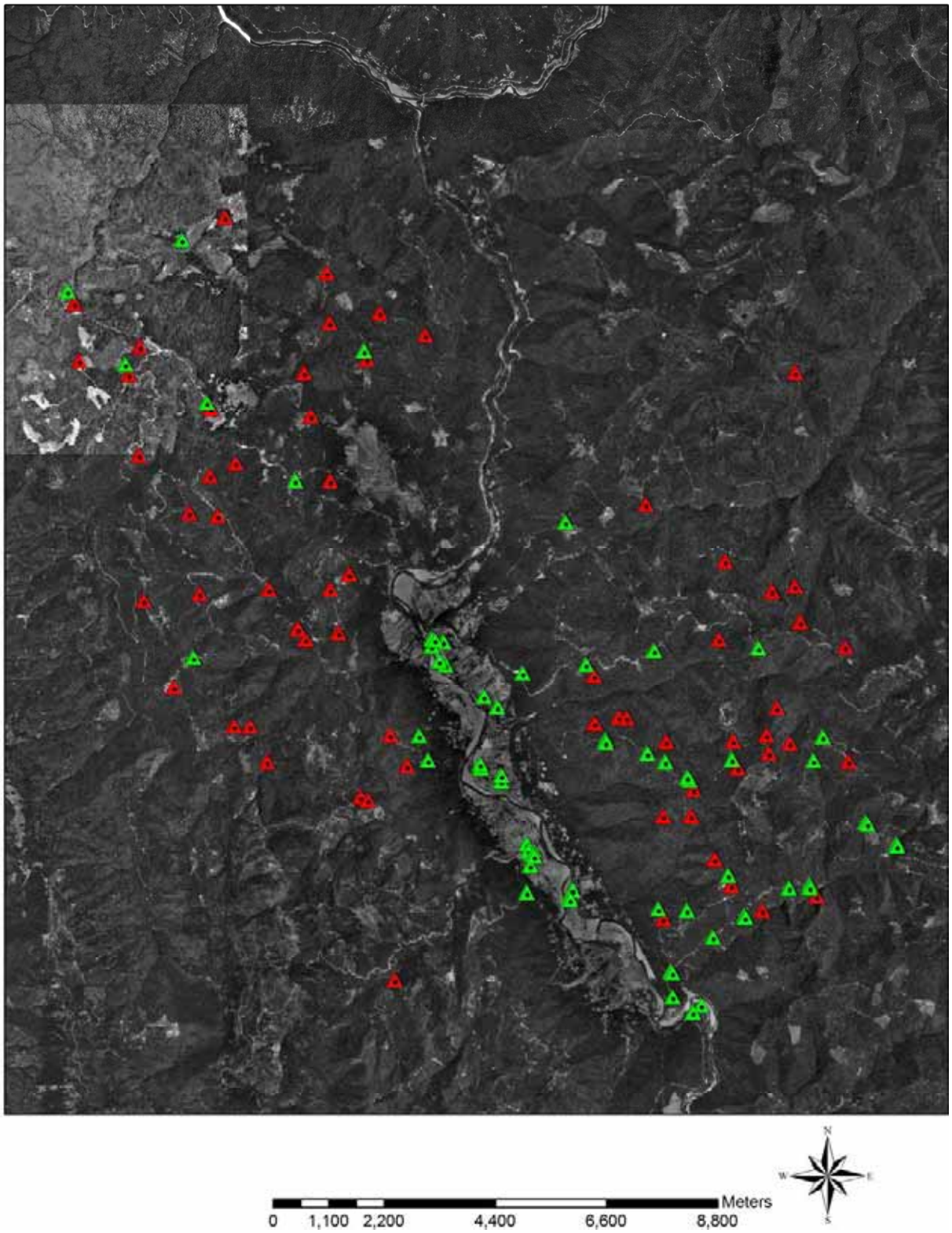
Zarnke, R. L., J. P. Dubey, O. C. Kwok. and J. M. Ver Hoef. 2000. Serologic survey for *Toxoplasma gondii* in selected wildlife species from Alaska. Journal of Wildlife Diseases 36: 219-224.

Zielinski, W. J. 1984. Plague in pine martens and the fleas associated with its occurrence. Great Basin Naturalist 41: 170-175.

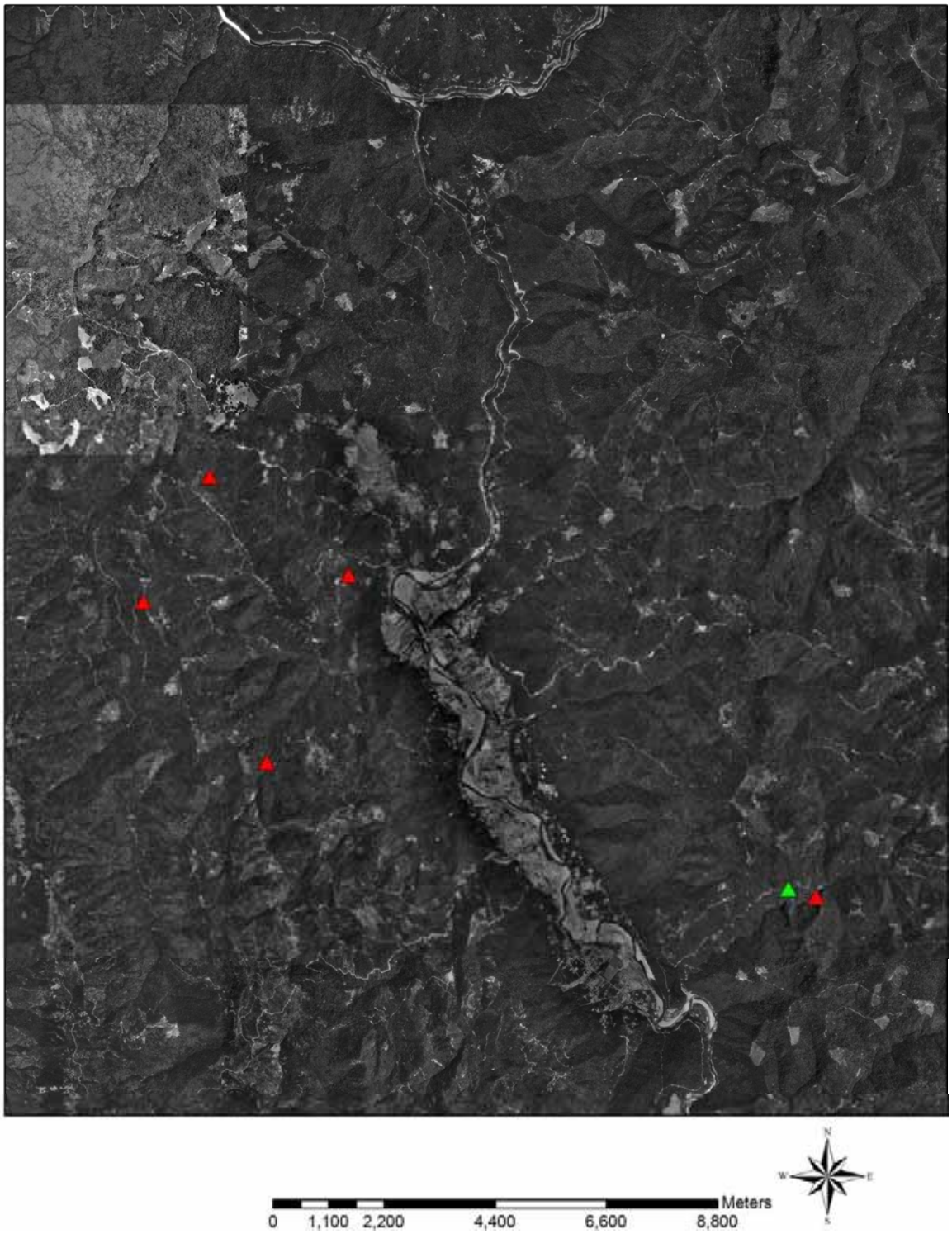
Zielinski, W. J., T. E. Kucera. and R. H. Barrett. 1995. Current distribution of the fisher, *Martes pennanti*, in California. California Fish and Game 81: 104-112.

Zielinski, W. J., N. P. Duncan, E. C. Farmer, R. L. Truex, A. P. Clevenger. and R. H. Barrett. 1999 Diet of fishers (*Martes pennanti*) at the southernmost extent of their range. Journal of Mammalogy 80: 961-971.

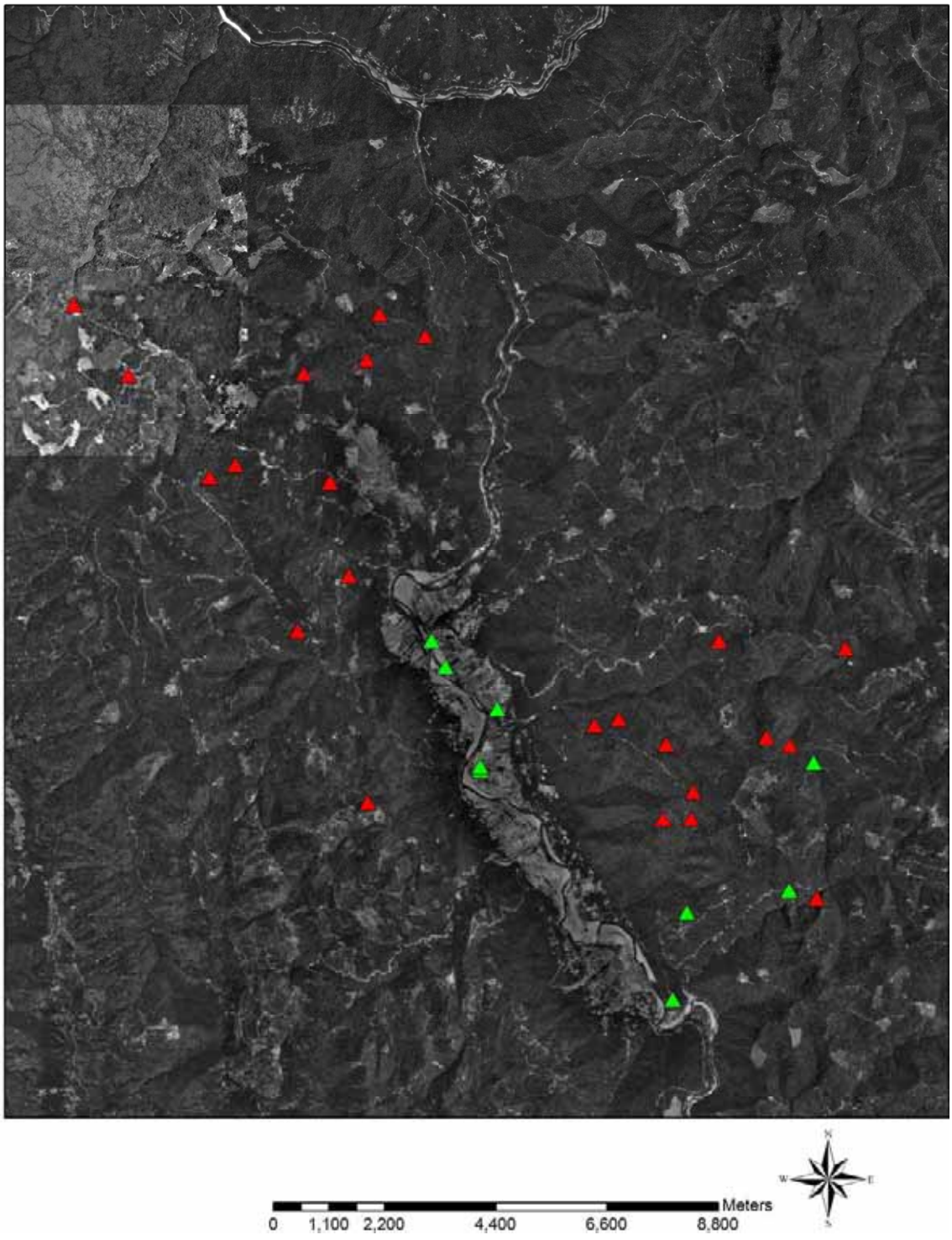
Appendix A. Spatial distribution of disease exposures among fishers (*Martes pennanti*) and sympatric mesocarnivores, gray foxes (*Urocyon cinereoargenteus*), ringtails (*Bassariscus astutus*), raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), and spotted skunks (*Spilogale gracilis*) trapped at Hoopa Valley Indian Reservation, February 2004 – February 2007.



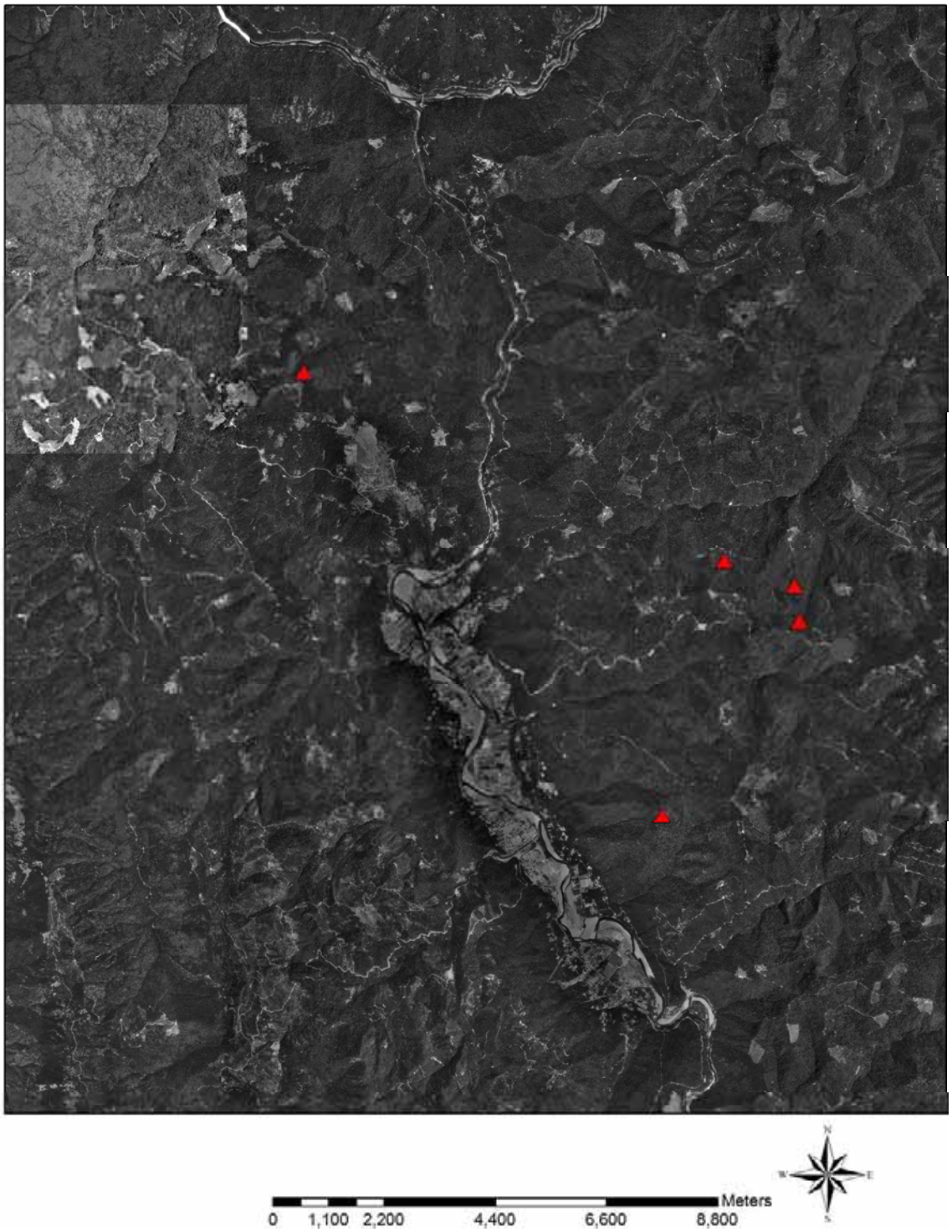
A-1. Spatial orientation of fishers (*Martes pennanti*, red triangles) and sympatric mesocarnivores (green triangles) that were captured and sampled for exposure to and active infections of various pathogens at Hoopa Valley Indian Reservation, February 2004 – February 2007.



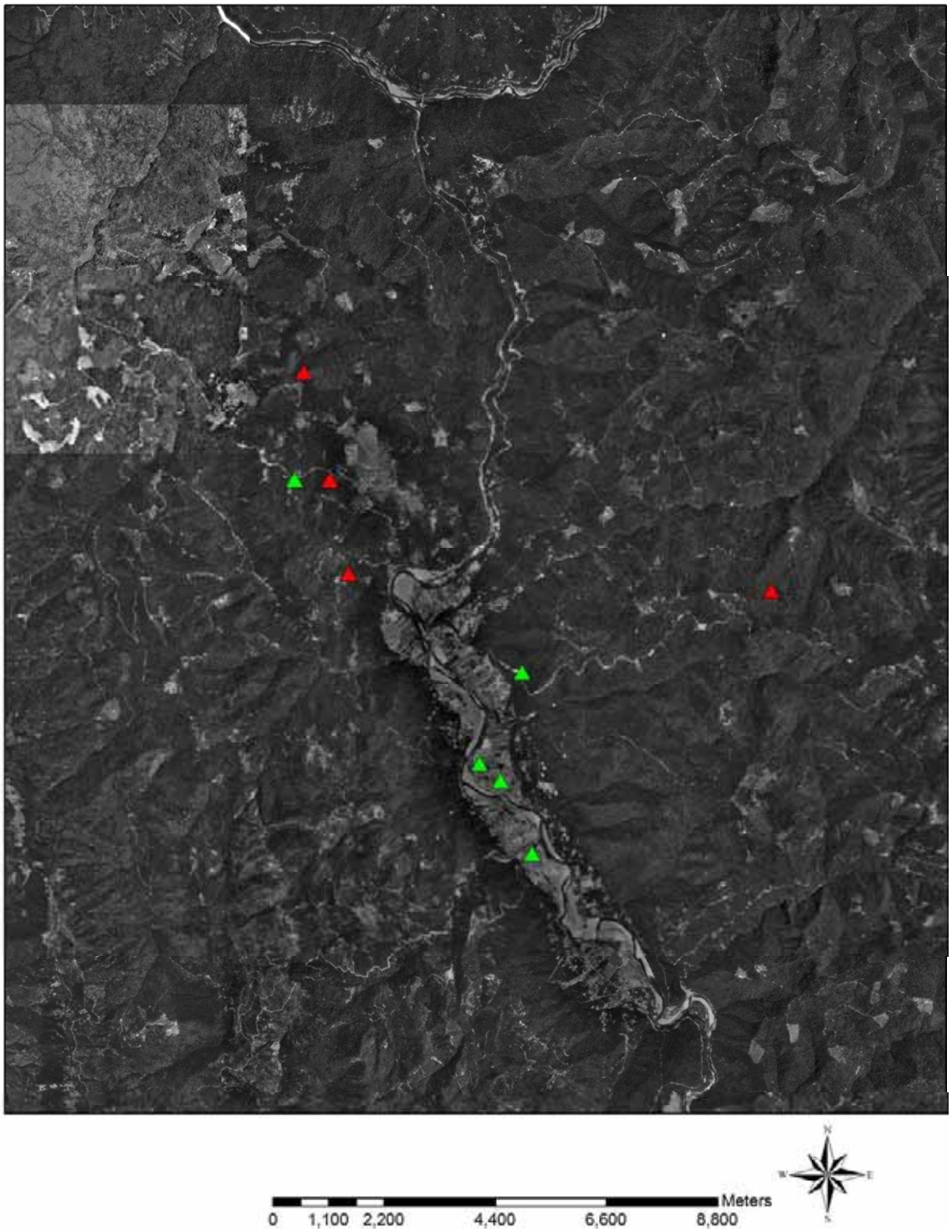
A-2. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=5) and sympatric mesocarnivores (green triangles, n=1) that were seropositive for canine distemper virus (CDV) at Hoopa Valley Indian Reservation, February 2004 – February 2007.



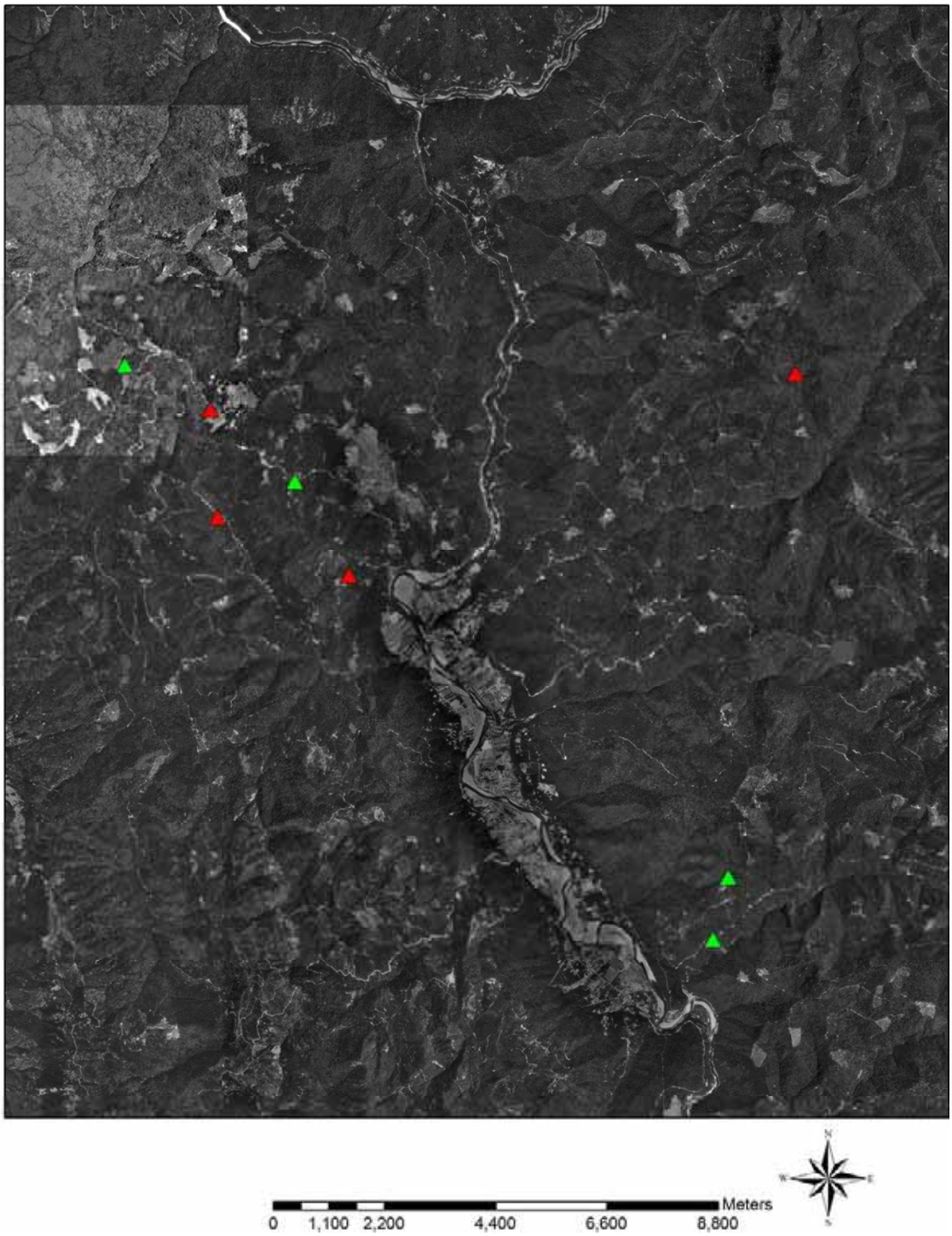
A-3. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=28) and sympatric mesocarnivores (green triangles, n=19) that were seropositive for canine parvovirus (CPV) at Hoopa Valley Indian Reservation, February 2004 – February 2007.



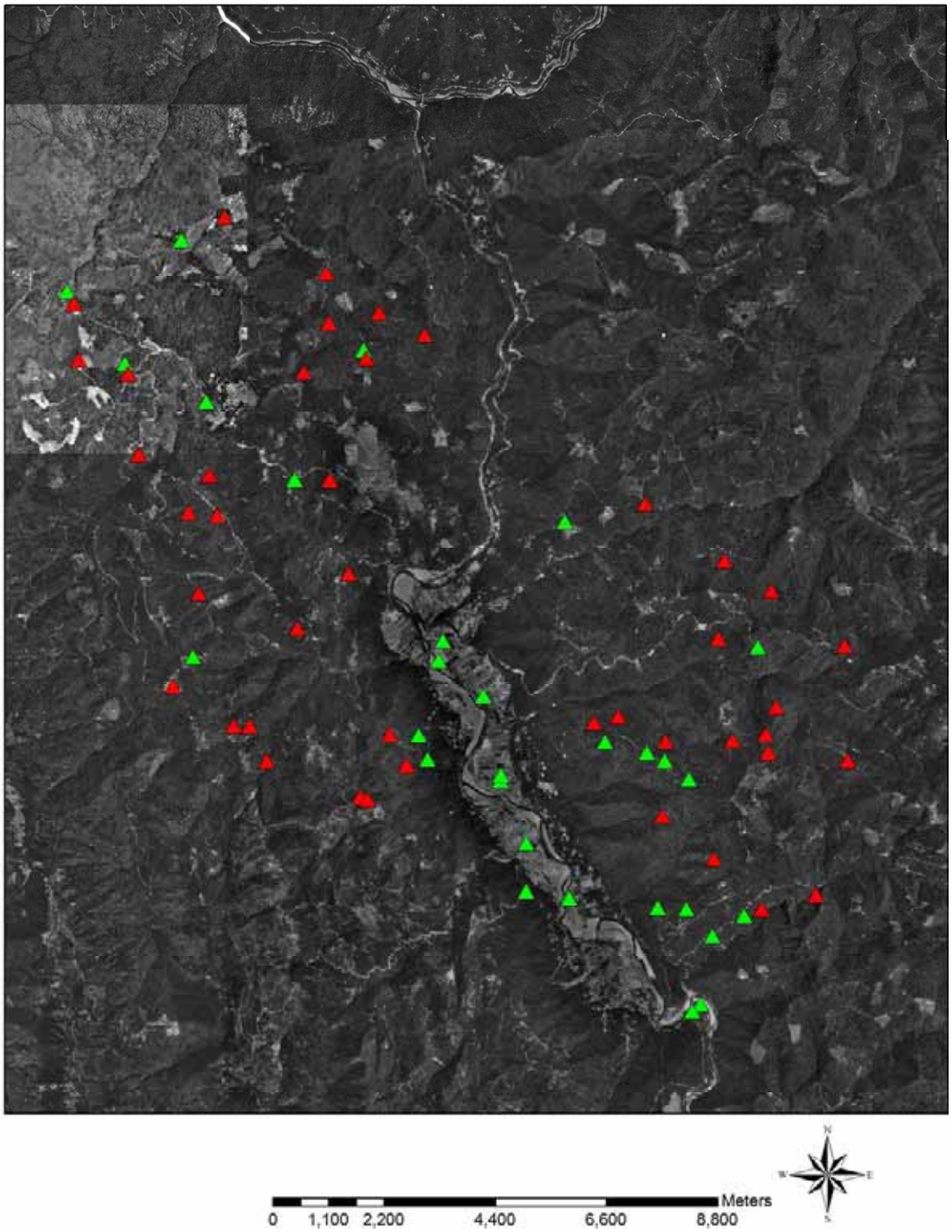
A-4. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=5) and sympatric mesocarnivores (green triangles, n=0) that were seropositive for canine herpesvirus (CHV) at Hoopa Valley Indian Reservation, February 2004 – February 2007.



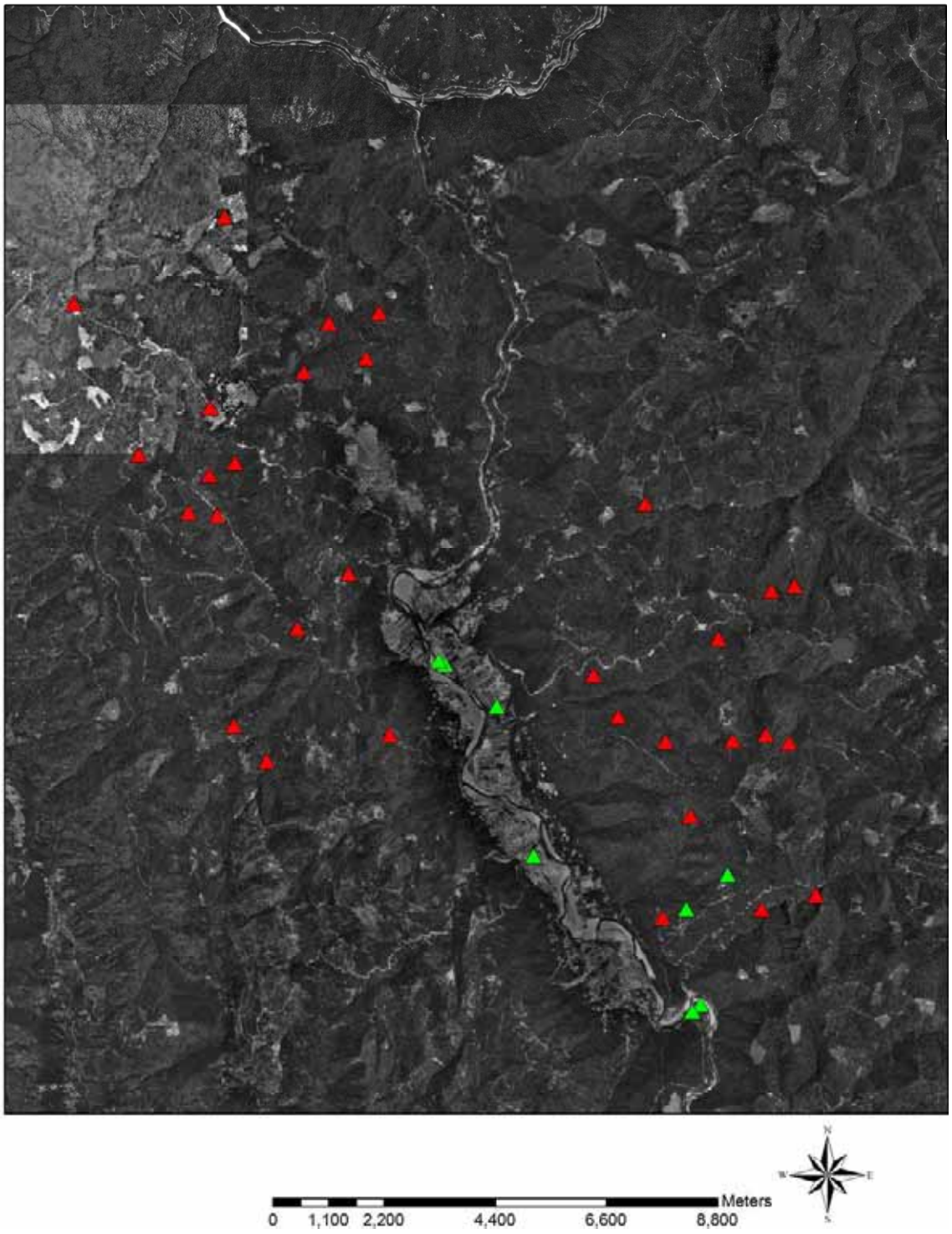
A-5. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=4) and sympatric mesocarnivores (green triangles, n=6) that were seropositive for canine adenovirus (CAV2) at Hoopa Valley Indian Reservation, February 2004 – February 2007.



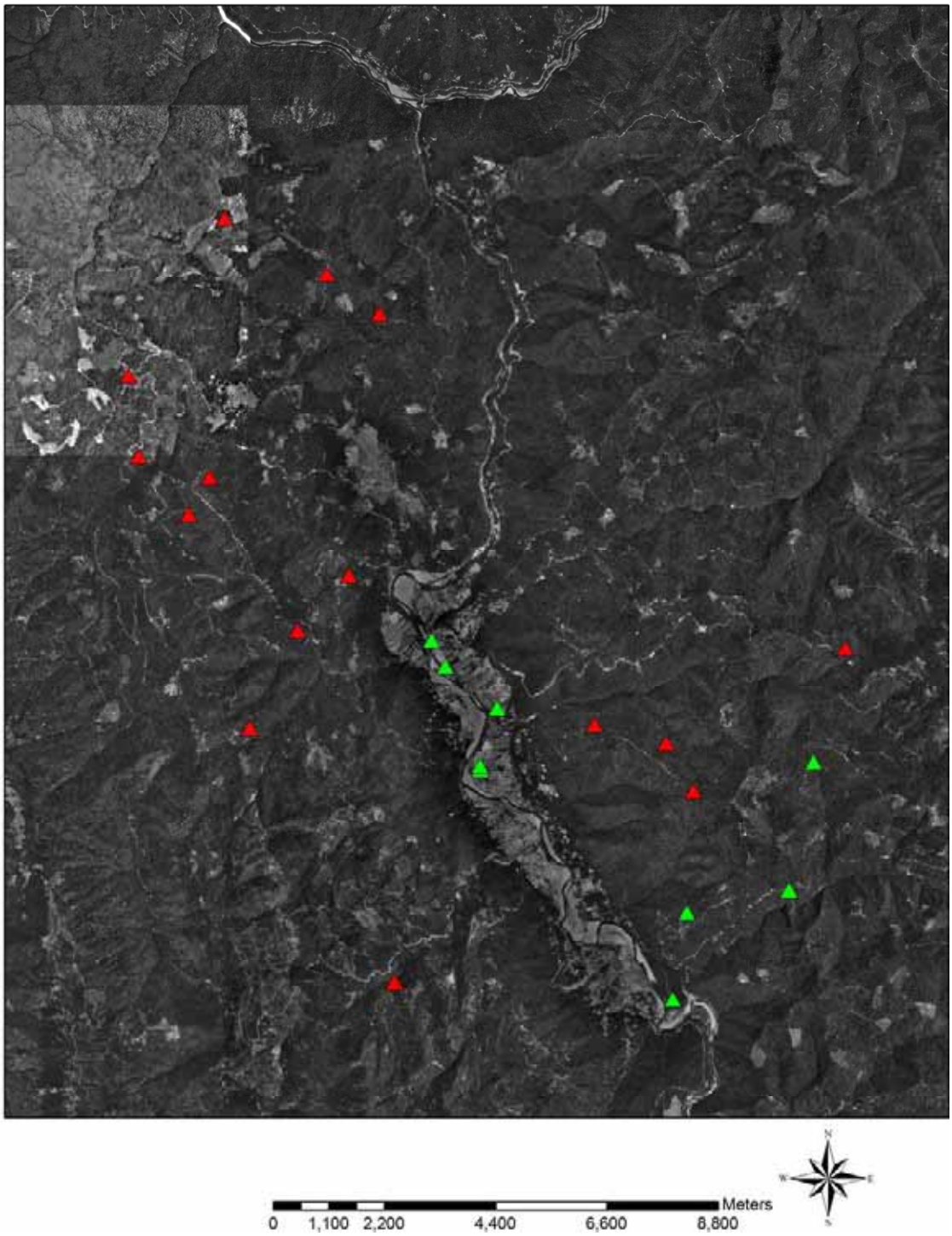
A-5. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=4) and sympatric mesocarnivores (green triangles, n=4) that were seropositive for West Nile virus (WNV) at Hoopa Valley Indian Reservation, February 2004 – February 2007.



A-6. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=78) and sympatric mesocarnivores (green triangles, n=32) that were seropositive for *Anaplasma phagocytophilum* at Hoopa Valley Indian Reservation, February 2004 – February 2007.



A-7. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=45) and sympatric mesocarnivores (green triangles, n=8) that were seropositive for *Toxoplasma gondii* at Hoopa Valley Indian Reservation, February 2004 – February 2007.



A-8. Spatial orientation of fishers (*Martes pennanti*, red triangles, n=18) and sympatric mesocarnivores (green triangles, n=9) that showed nested polymerase chain reaction active infection of canine parvovirus (CPV) at Hoopa Valley Indian Reservation, February 2004 – February 2007.