EXPOSURE TO *ANAPLASMA PHAGOCYTOPHILUM* AND TICKS IN GRAY FOXES (*UROCYON CINEREOARGENTEUS*) IN NORTHERN HUMBOLDT COUNTY, CALIFORNIA

by

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By

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ABSTRACT

Exposure to *Anaplasma phagocytophilum* and ticks in gray foxes (*Urocyon cinereoargenteus*) in northern Humboldt County, California.

Mourad Wisam Gabriel

Granulocytic anaplasmosis is an emerging zoonotic tick-borne disease with over 1300 human cases documented in the US since 2002. Although granulocytic anaplasmosis has been documented in numerous wildlife species, gray foxes (*Urocyon cinereoargenteus*) have not been evaluated previously for exposure to this agent. During a 16 month period in 2003-2004 within the Hoopa Valley Indian Reservation, Humboldt County, California, 54 individual gray foxes were live-trapped, of which 16 individual foxes later were recaptured.

Four tick species were found on the foxes: *Ixodes pacificus* adults and nymphs, *Ixodes texanus* adults and nymphs, *D. variabilis* adults, and a *D. occidentalis* adult. The number of *I. pacificus* adults found on captured foxes differed ($p < 0.001$) among the seasons with winter having the highest number per fox ($\bar{X}$ 8.4 ± 0.97, n= 9) compared to fall ($\bar{X}$ 0.27 ± 0.62, n = 22) and summer ($\bar{X}$ 0.71 ± 0.59, n = 24). The number of *I. pacificus* removed from foxes in spring ($\bar{X}$ 6.1 ± 0.77, n = 15) did not differ significantly than those removed in winter. Foxes in the backcountry had significantly more ($p < 0.001$) *I. pacificus* ($\bar{X}$ 4.4 ± 0.54, n=30) than urban caught foxes ($\bar{X}$ 1.5 ± 0.46, n= 40). The number of *I. texanus* nymphs found on captured foxes differed ($p < 0.05$) among the seasons with spring having the highest number per fox ($\bar{X}$ 6.9 ±1.75, n=15), compared to

fall ($\bar{X}$ 0.59 ± 1.44, n = 22), winter ($\bar{X}$ 0.67 ± 2.26, n = 9) and summer ($\bar{X}$ 0.83 ± 1.38, n = 24).

Twenty eight of the 54 (52%) gray foxes were *Anaplasma phagocytophilum* seropositive by indirect immunoflorescent antibody assay (IFA). There was a significant ($p < 0.001$) decreasing trend in seroprevalence from summer to winter. Also, foxes trapped in areas outside human residential boundaries were more likely ($p < 0.05$) to be seropositive (16 of 23; 70%) than foxes trapped within the human residential boundaries (12 of 31; 39%).

Twenty-eight of 90 (31%) dogs from the reservation were seropositive for antibodies against *A. phagocytophilum* by IFA. This seroprevalence was significantly lower ($p < 0.05$) in dogs compared to backcountry foxes.

Six of 70 (9%) foxes were infected with *A. phagocytophilum* at the time of capture as determined by polymerase chain reaction amplification and sequencing of *A. phagocytophilum* DNA. I propose that gray foxes may serve as competent wildlife sentinels of *A. phagocytophilum*; I also recommend further studies to determine the abiotic or biotic factors contributing towards the differences in exposure observed between the seasons and areas.

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INTRODUCTION

Granulocytic anaplasmosis, also known as human granulocytic ehrlichiosis is an emerging tick-borne zoonotic disease in North America (Walker and Dumler 1996, Fang et al. 2002, Foley et al. 2004). The causal agent of granulocytic anaplasmosis, *Anaplasma phagocytophilum* is a gram-negative, polymorphic, obligate intracellular parasite belonging to the group of rickettsia bacteria (Walker and Dumler 1996, Dumler et al. 2001). Recently *A. phagocytophilum* was taxonomically reorganized from *Ehrlichia phagocytophila* and *Ehrlichia equi*, both of which cross-react serologically and have identical morphological characteristics, as well as several other previously unnamed human granulocytic ehrlichiosis agents (Dumler et al. 2001, Bakken and Dumler 2001).

Within vertebrate hosts *A. phagocytophilum* is phagocytized by neutrophils that serve as the main target cells (Walker and Dumler 1996, Dumler et al. 2001). The rickettesie replicate within the neutrophils (Chen et al. 1994, Dumler et al. 2001) and inhibit the phagosome-lysosome fusion within these leukocytes (Walker and Dumler 1996, Dumler et al. 2001).

Clinical manifestations of the disease in horses, cats, and dogs, include fever, ataxia, ventral limb edema, icterus, and thrombocytopenia (Madigan and Gribble 1987, Foley et al. 2001, Foley et al. 2003). Human clinical signs include fever, chills, malaise, myalgias, headaches, nausea, vomiting, cough, confusion, and rashes (Bakken et al. 1994). The case fatality rate of humans is approximately 5%, but 56% of victims endure

a hospitalizing febrile course of approximately one to two weeks in children and three to 11 weeks in adults (Bakken et al. 1994, Bakken et al. 1996, Walker and Dumler 1996).

In 1994, the first report of granulocytic anaplasmosis was seen in a cluster of twelve human cases in the midwestern United States; in all cases, inclusion bodies were observed within the cytoplasm of peripheral neutrophils (Bakken et al. 1994). Since 1994, more than 1300 cases have been documented in the United States, with a 137% increase between 1999 and 2002 (Centers for Disease Control 2004). To date, only eight confirmed cases of human anaplasmosis have been documented in California (USA), including two cases in southern Humboldt County in 1998 (Foley et al. 1999, 2004). However, granulocytic anaplasmosis is well documented within numerous domestic and wildlife species in California (Foley et al. 2004).

Anaplasma phagocytophilum is transmitted to humans, domestic animals and wildlife by ticks of the family Ixodidae (Richter et al. 1996, Kramer et al. 1999). Known tick vectors include *Ixodes pacificus* (the western black-legged tick) in western North America (Richter et al. 1996, Nicholson et al. 1999, Vredevoe et al. 1999), *Ixodes scapularis* (the black-legged tick) in eastern North America (Pancholi et al. 1995), and *Ixodes ricinus* in Europe (Grzeszczuk et al. 2004). In California, *I. pacificus* parasitizes a wide variety of vertebrates including reptiles and birds, as well as small, medium, and large-sized mammals (Furman and Loomis 1984) and occurs in 55 of the 58 counties at elevations ranging from sea level to approximately 2,150 m. It is not known whether other ixodid species in California also are vectors for this disease agent (Nicholson et al. 1999, Foley et al. 2004).

It is still uncertain what the primary vertebrate reservoir hosts of *A. phagocytophilum* are among wildlife; however, dusky-footed woodrats (*Neotoma fuscipes*) have been implicated as a natural reservoir of *A. phagocytophilum* in California (Nicholson et al. 1999, Foley et al. 2002). Several species of carnivores have had a high seroprevalence of this agent in California, including 46% in coyotes (*Canis latrans*) (Pusterla et al. 2000), 17% in mountain lions (*Felis concolor*) (Foley et al. 1999), and 93% in American black bears (*Ursus americanus*) (Brown 2005, personal communication). I propose that this high seroprevalence combined with a low success in amplifying *A. phagocytophilum* DNA from their blood, is evidence that carnivores may be more efficient as sentinels than as reservoirs of *A. phagocytophilum* (Foley et al. 1999). Carnivores also may be good sentinels due to their larger home ranges, longer life spans, and increased exposure to *I. pacificus*, when compared to the dusky-footed woodrat (Foley et al. 1999).

I propose that gray foxes (*Urocyon cinereoargenteus*) may be useful as wildlife sentinels of *A. phagocytophilum*. Gray foxes are common and can occur at high spatial densities in California (Trapp and Hallberg 1975) as well as other areas in their range within North America (Fritzell and Haroldson 1982). They also completely overlap the known *A. phagocytophilum* human case distribution (Fritzell and Haroldson 1982, Centers for Disease Control 2004) in the United States. Foxes could be exposed to pathogens and various stages of infected ixodid ticks from prey items such as woodrats (Vestal 1938, Ingles 1965, Cashier et al. 2002).

Gray foxes may serve as sentinel species in determining whether *A. phagocytophilum* is present and may be a zoonotic risk to its human co-inhabitants because of their common occurrence in human-inhabited areas and documented incidences of living in human-made structures (Hoff et al. 1974, Harrison 1997, Neale and Sacks 2001).

Human risk of contracting granulocytic anaplasmosis also could be increased by movement of infected ticks into close human proximity by domesticated animals or wildlife. Domestic dogs (*Canis familaris*) in rural areas within northern California commonly are exposed to *I. pacificus* and their pathogens and could increase the human risk to *A. phagocytophilum* by carrying infected ticks into human-inhabited areas (Foley et al. 2001). Foley et al. (2001) found an 8.7% seroprevalence for *A. phagocytophilum* among domestic dogs in California and a seroprevalence of 47% in dogs from Humboldt County. Layfield and Guilfoile (2002) reported that all of 21 *I. scapularis* found polymerase chain reaction (PCR)-positive for *A. phagocytophilum* were collected from free-ranging domestic dogs.

If gray foxes contribute to transferring *A. phagocytophilum* from wildlife to domestic animals or humans, one would expect to find *A. phagocytophilum*-infected ticks on foxes and a high prevalence of foxes exposed to *A. phagocytophilum*. In addition, if foxes are a significant source of infected ticks for dogs, then dogs exposed to foxes also should be exposed to *A. phagocytophilum*. Evaluation of this issue could clarify the role of foxes as potential reservoirs or sentinels of *A. phagocytophilum* and potential sources

of *A. phagocytophilum* to domestic dogs, humans, and wildlife, as well as provide insight on the relationship between foxes and dogs in the epidemiology of the parasite.

My objectives were: 1) to compare species and number of ticks on foxes by season of capture, capture area, and sex of foxes, 2) to compare prevalence of antibodies to *A. phagocytophilum* in foxes by season of capture, capture area, and sex of foxes, 3) to compare antibody prevalence to *A. phagocytophilum* among local domestic dogs to antibody prevalences among foxes of both capture areas, and 4) to compare the prevalence of active *A. phagocytophilum* infection in gray foxes by season of capture, capture area, and sex of foxes.

STUDY AREA

Field sampling was conducted on the Hoopa Valley Indian Reservation in northeastern Humboldt County, northwestern California (Zone 10, 443624E, 4544450N, UTM NAD 83 datum, Figure 1). The reservation is a square of approximately 19 km on a side with an area of 362.5 km^2 (Singer and Begg 1975, Hoopa Valley Indian Reservation 2004). I distinguished two zones within the Hoopa Valley Indian Reservation for this study as the urban zone and the backcountry zone. The urban zone included the valley floor as it follows the Trinity River over 16.4 km in a south-north direction with elevations varying between 76 m and 152 m above sea level (Singer and Begg 1975, Figure 1). This zone comprised the majority of human residential areas of the Hoopa Valley Indian Reservation as defined in the Hoopa Tribal Forestry Management Plan (Higley 2004, personal communication; Figure 1). The backcountry zone included the remaining area of the Hoopa Valley Indian Reservation with elevations ranging from 152-1170 m; this area is managed for its natural resources and cultural preservation.

The annual mean high and low temperatures in the Hoopa Valley Indian Reservation urban zone at an elevation of 98 m are 7° C and 21° C, respectively, with an annual overall mean of 14° C (National Oceanic and Atmospheric Administration 2004). The annual mean backcountry temperature at an elevation of 518 m elevation is 11° C (Hoopa Valley Indian Reservation 2004). The annual mean precipitation within the Hoopa Valley Indian Reservation at an elevation of 98 m is 1460 mm and mean snowfall

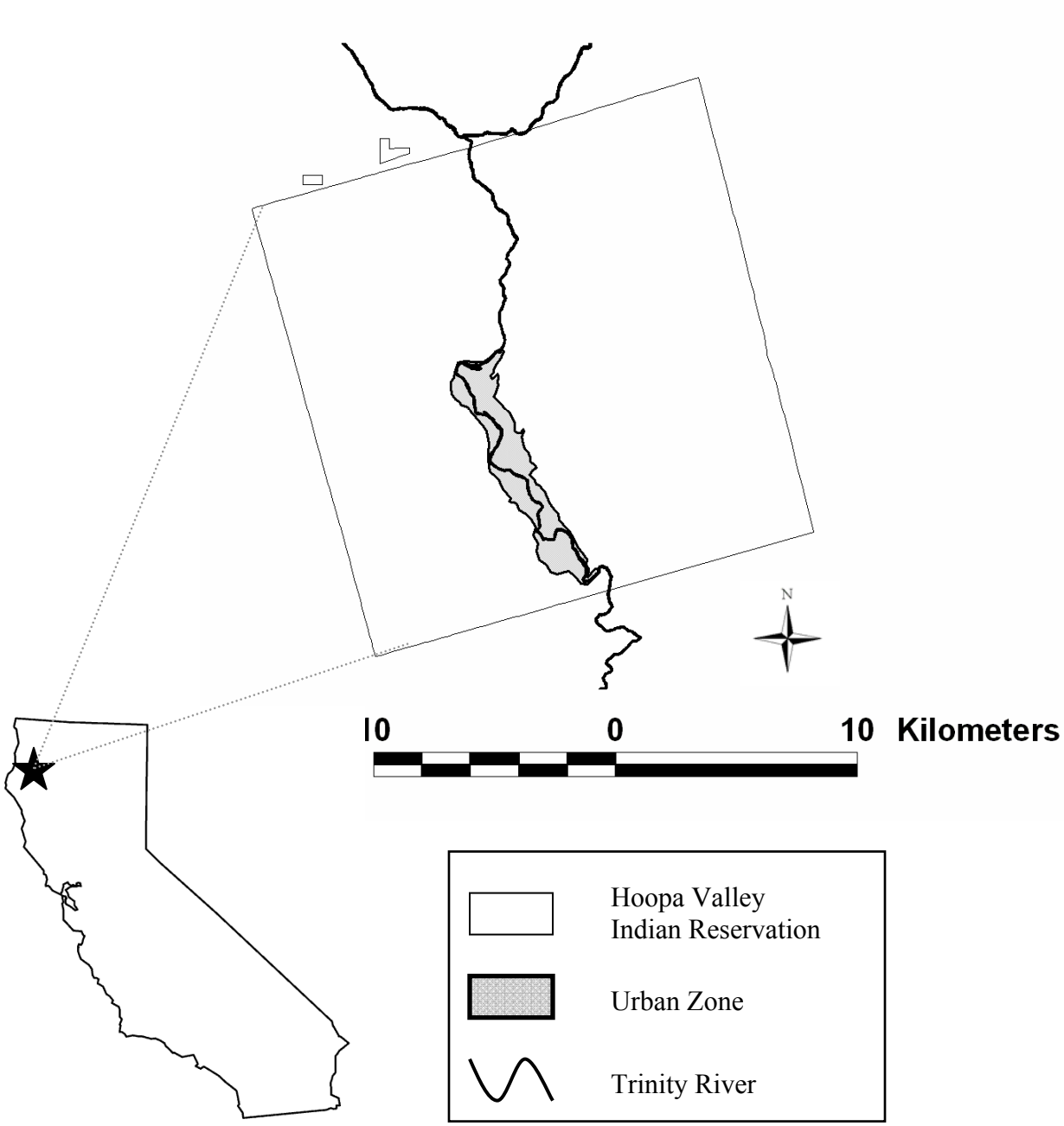

Figure 1. Map of the Hoopa Valley Indian Reservation, Humboldt County, California.

is 10 mm (National Oceanic and Atmospheric Administration 2004). The dominant vegetation in the urban zone includes California foothill pine (*Pinus sabiniana*), western redbud (*Cercis occidentalis*), California blackberry (*Rubus ursinus*), California wild grape (*Vitis californica*), Pacific madrone (*Arbutus menziesii*), manzanita (*Arctostaphylos*) and annual grasses (Singer and Begg 1975). Vegetation of the outlying upland areas of the urban zone and backcountry zone includes Douglas-fir (*Pseudotsuga menziesii*), tanoak (*Lithocarpus densiflorus*), Pacific madrone, white fir (*Abies concolor*), big-leaf maple (*Acer macrophyllum*), incense cedar (*Calocedrus decurrens*), Port Orford-cedar (*Cupressus lawsoniana*), golden chinquapin (*Chrysolepis chrysophylla*), Jeffrey pine (*Pinus jeffreyii*), sugar pine (*Pinus lambertiana*), western white pine (*Pinus monticolia*), knobcone pine (*Pinus attenuata*), ponderosa pine (*Pinus ponderosa*), Pacific yew (*Taxus brevifolia*), Pacific dogwood (*Cornus nuttallii*), willow (*Salix* spp.), California blackberry (*Rubus ursinus*), manzanita, and annual grasses (Singer and Begg 1975, Matthews 2002). Mid-sized to large mammals inhabiting the Hoopa Valley Indian Reservation include gray foxes, coyotes, American black bears, Pacific fishers (*Martes pennanti*), bobcats (*Lynx rufus*), mountain lions, raccoons (*Procyon lotor*), ringtails (*Bassariscus astutus*), porcupines (*Erethizon dorsatum*), black-tailed deer (*Odocoileus hemionus*), Roosevelt elk (*Cervus elaphus roosevelti*), domestic dogs, feral domestic cats (*Felis domesticus*), domestic horses (*Equus caballus*) and cattle (*Bos taurus*) (Higley 2004, personal communication).

METHODS

All methods were approved by Humboldt State University InstitutionalAnimal Care and Use Committee (IACUC), IACUC approval number 02/03/04.W.60.A.

Gray foxes were live trapped between 3 June 2003 and 10 October 2004 in 81 cm x 25 cm x 31 cm, model 108 Tomahawk traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) with wooden boxes, "nest box", attached to the rear of the Tomahawk trap (Wilbert 1992) that had modifications which included water resistant fiberglass reinforced insulation paneling (Kemlite Corporation, Joliet, Illinois, USA) for which proprietary concentrations of polyester/styrene copolymer and inorganic compounds inhibit bacterial and fungi propagation; the nest box also had larger entrance and exit openings, openings on the sides at each end to confine and restrict animal movement through aluminum slides, an increase in structural integrity, and reduction of overall weight (Gabriel and Wengert 2005; Figure 2). Traps were placed opportunistically on the forest floor near gray fox sign, such as scats or tracks. Traps usually were placed at least 30 m from roads and parallel to large downed woody debris or placed in grass or in brushy and shrubby areas. All trap interiors were lined with locally available vegetative material and the trap exterior was covered with a burlap sack and vegetation to hide the trap and reduce hypothermia in trapped animals.

Gray fox urine and gland paste (Murray Lure and Trapping Supplies, Elizabeth, West Virginia, USA) derived from equal portions of male and female samples to reduce

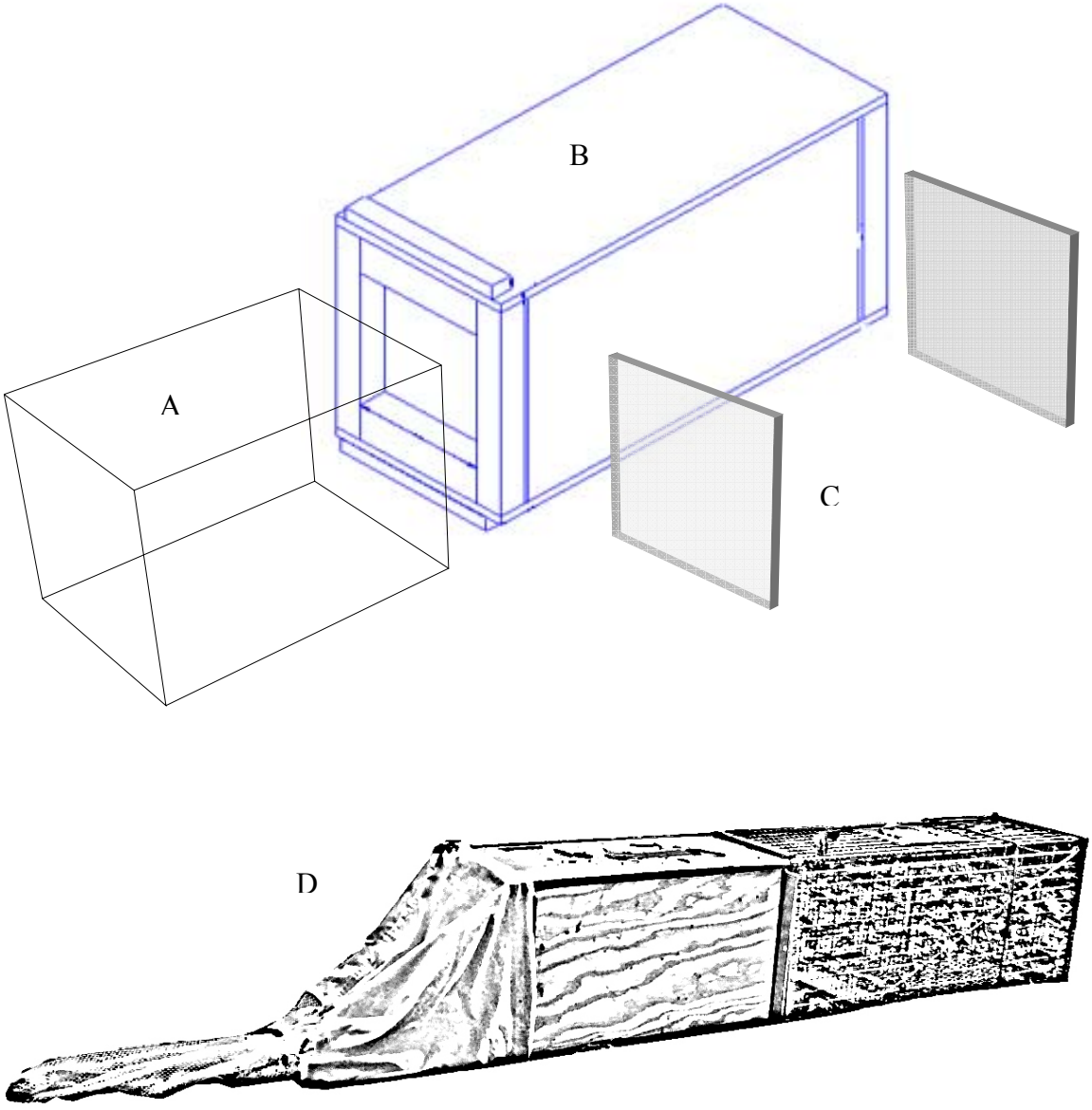

Figure 2. Live trap (A) with placement of nest box (B) and aluminum slides (C) placed in the front and rear of the nest box to confine the captured subject. Handling net (D) placed on the end of nest box.

possible trap bias towards one sex were used to attract gray foxes. Fresh chicken meat was used as bait to lure individuals into the traps. Traps were checked ≤ 12 hours from initial set time. If a gray fox was caught, an aluminum slide was placed in the front of the nest box to confine the fox within the nest box (Figure 2). A handling net composed of thick canvas material tapering down to a cone of fine mesh was attached to the rear section of the nest box (Figure 2) and the rear aluminum slide was removed to allow the fox to enter the handling net.

A circular buffer with an area of 129 ha was projected around every capture location to account for the maximum expected homerange size (Fritzell and Haroldson 1982). A fox was considered to be interactive with residential areas if any part of the buffer overlapped the urban zone and the capture area of such an animal was labeled "urban". A fox whose homerange buffer did not overlap with any part of the urban zone was classified as residing in the "backcountry". Foxes captured in both areas would have been labeled as "multiple use" foxes.

Each captured fox was weighed and then anesthetized with 20 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and 4 mg/kg xylazine (Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) via intramuscular injection. A brief physical examination was performed monitoring rectal temperature, respiration rate, jaw tone, pupil position, and jaw capillary refill time were monitored at regular intervals. Each fox was placed in lateral recumbency in order to stimulate blood circulation and avoid adverse post-capture effects (Nielsen 1999). A small amount of sterile eye

lubricant (Phamaderm, Mellville, New York, USA) was placed within each eye and a blindfold was applied to prevent ocular damage from direct sunlight.

Blood was collected from a femoral, jugular or cephalic vein and stored in 4-ml non-heparinized sterile tubes for removal of serum and a 3-ml sterile heparinized tubes for isolation of pathogens. Blood was kept at 4 -12° C until centrifuged later the same day. Each fox received a uniquely numbered modified Roto® ear tag (Premier 1 Supplies, Washington, Iowa,) in each ear for future identification. Ear tag sites were scrubbed with Long's® Antiseptic Skin Cleanser Solution (Longs Drugs, Walnut Creek, California) and a small amount of Kirkland® Signature Topical Antibiotic (Alpharma USPD Inc., Baltimore, Maryland) was placed on the tag prior to placement in the ear to further reduce the risk of infection.

A thorough systematic exam (≥ 5 min) of the fur on each individual fox was conducted. All fleas and ticks observed were collected and stored in 70% ethanol with 5% glycerol for later identification. Morphologic data collected for each fox included left ear length, left hind foot, body length, tail length and total length (Ingles 1965).

After all samples and data were collected, 0.125mg/ml of yohimbine (Lloyd laboratories, Shenandoah, Iowa), an antagonist to xylazine, was administered intravenously ≥ 40 min post-injection of the xylazine. Each fox was then placed back into the nest box to allow a rapid and safe recovery. All foxes were released at their capture location when they exhibited normal responses to stimuli and regained all locomotor skills. However, foxes recaptured within 4 weeks of sampling and those estimated to be < 3 months of age were released immediately.

Several animal safety techniques were used in the event of adverse weather conditions. For example, a central processing tent shelter (International E-Z, Inc. Riverside, California) was erected, and the tailgate of a pickup-truck was lined with an insulating foam pad to help reduce body temperature loss. To further prevent hypothermia, an electric warming pad, powered by an inverter within the vehicle was placed under the fox, and reusable sodium nitrate warming packs were placed in key core areas for immediate heat. In the case of hyperthermic reactions, foxes either were iced with water or wetted down with isopropyl alcohol to lower body temperatures. All instruments and work areas were sprayed and scrubbed with Benz-all® (Xttrium Laboratories, Chicago, Illinois, USA), a hard surface bacteriocide/virucide, to ensure a sanitized processing area.

Serum samples from domestic dogs were collected 29 to 31 March 2004, during an annual spay and neuter clinic conducted by the University of California, Davis, Rural Area Veterinarian Services (Rural Area Veterinarian Services, Davis California) on the Hoopa Valley Indian Reservation. For each dog, blood was collected through a femoral, jugular, or cephalic vein and stored in vacutainer tubes as described for fox samples. No attempt was made to collect ectoparasites from dogs.

Adult and nymphal stages of ticks removed from foxes were identified microscopically using keys provided in Furman and Loomis (1984) and confirmed by Richard N. Brown, Department of Wildlife, Humboldt State University. Larval stage ticks were not identified or further analyzed.

Serology was conducted on fox and dog sera using an indirect immunoflorescent antibody assay (IFA) (Dumler et al. 1995). Plasma was separated from whole blood by centrifugation at 200 x g for 10 min, diluted in phosphate buffered saline (PBS) at 1:25, applied to commercial *A. phagocytophilum* antigen slides (Protatek International, Saint Paul, Minnesota), and incubated at 37° C in a humid environment for 30 min. Slides then were washed three times in PBS and incubated with goat anti-dog secondary antibody (Kirkegaard & Perry Labratories Inc., Gaithersburg, Maryland) that had been diluted in PBS at 1:30. Slides were washed three additional times and, during the third wash, two drops of Eriochrome T-Black (Lab Safety Supply, Janesville, Wisconsin) were added. Positive and negative controls were included in each run (Drazenovich 2005, personal communication)

All DNA was extracted from 200 µl of whole blood using the Dneasy Tissue Kit, (Qiagen, Valencia, California) according to manufacturer's instructions. A polymerase chain reaction (PCR) assay (Pusterla et al. 1999) was completed by obtaining the complete sequence of the *msp2* gene from GenBank, (National Center for Biotechnology Information 2005, accession number AY151054). Primers and probes were designed using the Primer Express software program (Applied Biosystems, Foster City, California). The predicted specificity of the primers and probe were verified as specific for *A. phagocytophilum* by use of the BLAST database search program (Altschul et al. 1990) and excluded all bacteria other than *A. phagocytophilum.* Oligonucleotide primers and probe were synthesized by Invitrogen (Carlsbad, California) and MWG Biotech (High Point, North Carolina, USA), respectively. The fluorescent reporter dye at the 5′

end of the TaqMan probe (939p- TTAAGGACAACATGCTTGTAGCTATGGAA-GGCA) was 6-carboxy-fluorescein; the quencher at the 3′ end was 6-carboxy-tetramethyl-rhodamine. Primers 903f (5′-AGTTTGACTGGAACACACCT- GATC-3′) and 1024r (5′-CTCGTAACCAATCTCAAGCTCAAC-3′) amplified a 122 base-pair fragment of the *msp2* gene. Amplification was performed with an ABI 7700 Prism Sequence Detector (Applied Biosystems, Foster City, California) and the products were analyzed with Applied Science ABI 7700 Sequence Detection System software (Applied Biosystems, Foster City, California). Each 12-µl reaction contained undiluted Taqman Universal Master Mix (Applied Biosystems, Foster City, California), 2 nmol each primer, 400 pmol probe, and 1 µl DNA. The thermocycling conditions consisted of 50° C for 2 min, 95° C for 10 min, and 40 cycles at 95° C for 15 sec, followed by 60° C for 1 min. Samples were considered positive if they had a cycle threshold value < 40, meaning that after 40 cycles of PCR there was no signal. (Drazenovich 2005, personal communication). Only gray fox whole blood was analyzed for *A. phagocytophilum* DNA.

Seasons were designated as spring (20 March to 20 June), summer (21 June to 21 September), fall (22 September to 20 December), and winter (21 December to 19 March). Data collected in different years but during the same season were combined for analyses of seasonal variation. In cases where months of different years were combined, weather variability during all months were within the Western Regional Climate Center (2005) pre-determined normal averages of precipitation, minimum temperature and maximum temperature.

All data were screened for normality before analyses and all statistical analyses were conducted using Number Cruncher Statistical Software (NCSS© 2001); non-parametric tests were used to analyze non-normally distributed data.

A Chi-squared test for independence analysis was used to test for differences in prevalence of antibodies between the capture areas, sexes, and seasons. An Armitage test was used to analyze for trends in proportions of seropositive foxes throughout the seasons. Recaptured foxes found seropositive during any previous capture were classified seropositive due to the long duration of antibodies in other species shown in recent studies (Bakken et al. 1996, Foley et al. 2002). Each fox capture, regardless of whether it was sampled previously, was considered an independent data point for all PCR analysis since positive PCR results indicate a current infection within the individual (Walker and Dumler 1997).

No PCR assays were performed on domestic dog samples. A Chi-squared analysis test for independence was used to test for differences in prevalence of antibodies between foxes and domestic dog. A Fisher's exact test was used to determine which individuals was more likely to be seropositive due to a small number of species sampled. No data from recaptured foxes were used in the analysis; only first time captures were used due to the duration of antibodies.

The weights of individual foxes captured more than once were combined and their mean was included as one weight per fox. A Mann Whitney U-Test was used to assess differences between male and female weights and morphometric measures (Zar 1999).

A general linear model analysis-of-variance (ANOVA) was used to test for differences in numbers of ticks among the seasons, the capture areas and the sexes (Zar 1999). Pair-wise contrast among seasons was performed with the Tukey-Kramer adjustment for multiple comparisons (Zar 1999).

RESULTS

Traps were set for 1522 trap-nights, with a mean (± SE) of 108.7 (± 70.7) trap-nights per month between 3 June 2003 and 10 October 2004 (Table 1). Trapping effort was distributed equally between the backcountry and urban regions (Figure 3) with 761 trap-nights per region, 70 foxes were handled and sampled and 23 were released without sampling resulting in 93 fox captures (Figure 3); 54 individual foxes were sampled of which 10 foxes were recaptured on an additional 16 occasions. Males accounted for 61% (33 of 54) of the foxes captured while females accounted for 39% (21 of 54). Seven of the recaptured foxes were female and three were male. Five of the recaptured foxes were recaught only once, four were recaught twice and one was recaught three times (Appendix A).

Of the 54 new captures, 31 were urban and 23 were backcountry foxes. No recaptured foxes were taken in both urban and backcountry set traps; therefore no "multiple use" foxes were sampled. Females comprised 45% (14 of 31) of the captured urban foxes and 30% (7 of 23) of the backcountry foxes. Males accounted for 55% (17 of 31) of urban and 70% (16 of 23) of the backcountry captures. Based on a Chi-squared test for independence, the sex of foxes captured did not differ between the areas (X^2 = 1.20, df = 1, p = 0.27).

There were seven known mortalities for ear-tagged individuals during the study. Two mortalities occurred with foxes under anesthesia. The remaining five foxes were

Table 1. Distribution of 1522 trap-nights for gray fox (*Urocyon cinereoargenteus*) captures on the Hoopa Valley Indian Reservation between 3 June 2003 and 10 October 2004.

Month	Year	Trap nights	
		Urban	Backcountry
	2003		
June		18	18
July		62	62
August		39	38
September		57	56
October		86	86
November		62	62
December		34	35
	2004		
January		0	0
February		97	97
March		133	134
April		78	78
May		42	42
June		30	30
July		18	18
August		0	0
September		0	0
October		5	5
Total trap nights		761	761

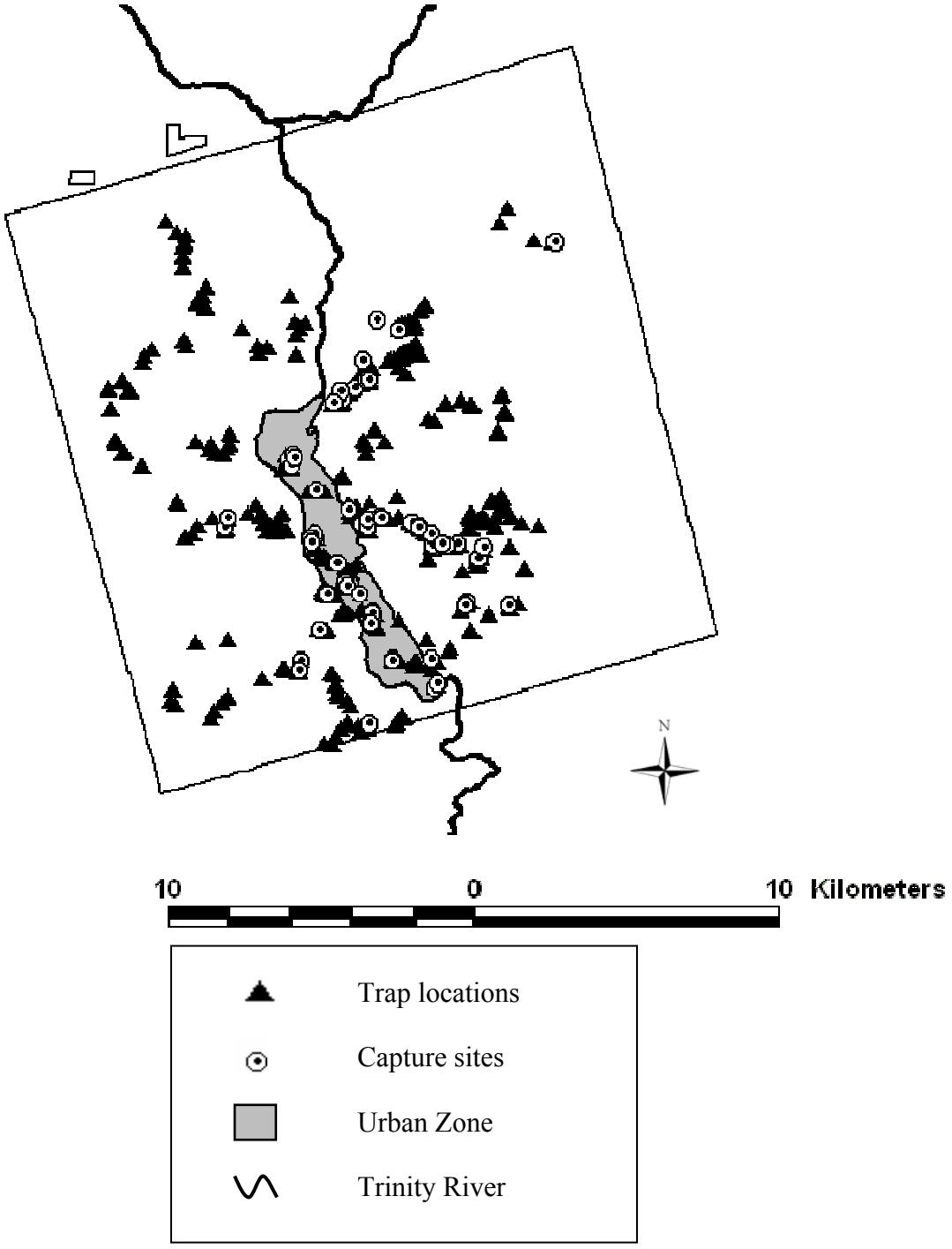

Figure 3. Map of trap locations and gray fox (*Urocyon cinereoargenteus*) captures from 3 June 2003 to 13 October 2004 within the Hoopa Valley Indian Reservation, Humboldt County, California. Each trap location is representative of ≥ 2 trap placements.

found dead later on the study area. On necropsy, four individuals had visually noticeable major lacerations, compound factures, hemorrhaging throughout the body, and severe trauma throughout all of the viscera; these four likely were hit by vehicles. The final fox was found near a group of domestic dogs; it had several superficial and deep puncture wounds on the skull and along the dorsal midline section. Domestic dog predation was suspected to be the cause of death.

Male foxes were significantly larger than female foxes based on body weight ($p = 0.02$), hind foot length ($p = 0.05$), and length without tail ($p = 0.01$) (Table 2). Among recaptured foxes the greatest weight changes were an increase of 1.3 kg in a female between September 2003 and December 2003, and a decrease of 0.6 kg in a male between July 2003 and April 2004. There was no consistent difference ($Z=0.79$, $df = 12$, $p>0.05$) in weight change between recaptured males and females.

A total of 500 ticks were recovered from the 70 processed foxes, with 47 larval ticks, 441 intact nymphs and adults and an additional 12 unidentifiable ticks damaged during their removal. I collected 191 adult *I. pacificus*, with 101 females and 90 males, six adult *I. texanus* females, 98 adult *Dermacentor variabilis* of which 51 were females and 47 were males, and one *Dermacentor occidentalis* male. I also recovered 143 *I. texanus* nymphs and two *I. pacificus* nymphs.

Prevalence of ticks found on all gray fox captures was 47% (33 of 70) *I. pacificus* adults, 2.9% (2 of 70) *I. pacificus* nymphs, 8.6% (6 of 70) *I. texanus* adults, 24% (17 of 70) *I. texanus* nymphs, 37% (26 of 70) *D. variabilis* adults, and 1.4% (1 of 70) *D. occidentalis* adults (Table 3).

Table 2. Morphometric measures of adult and sub-adult (≥ 3 months) male and female gray foxes (*Urocyon cinereoargenteus*) caught on the Hoopa Valley Indian Reservation, Humboldt County, California, 3 June 2003 to 10 October 2004. Differences between males and females based on a Mann-Whitney U test.

	Male			Female			Mann-Whitney U-Test	
	n	mean	SE	n	mean	SE	Z	P
Weight (kg)	33	3.49	0.09	21	3.20	0.13	-2.33	0.02
Left hind foot (mm)	32	120.2	1.25	20	118.1	1.34	-1.97	0.05
Left ear notch (mm)	32	71.0	0.73	20	70.6	1.06	0.02	0.98
Total body length (mm)	32	910	21.0	20	908	14.0	-1.37	0.17
Tail length (mm)	32	333	9.0	20	338	6.3	0.49	0.62
Body length without tail (mm)	32	576	13.0	20	570	9.0	-2.50	0.01

Table 3. Prevalence of tick species and life stages found on gray foxes (*Urocyon cinereoargenteus)* from 3 June 2003 to 10 October 2004.

Month	Total foxes Sampled (n)	Foxes with *Ixodes pacificus* (n)		Foxes with *Ixodes texanus* (n)		Foxes with *Dermacentor variabilis* (n)	
		Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
Jun 2003	2	0	2	0	0	0	2
Jul 2003	14	0	4	4	2	0	12
Aug 2003	2	0	0	0	0	0	2
Sept 2003	8	0	0	0	0	0	1
Oct 2003	8	0	1	1	0	0	0
Nov 2003	2	0	1	1	0	0	0
Dec 2003	4	0	2	2	0	0	0
Jan 2004	0	0	0	0	0	0	0
Feb 2004	3	0	3	1	1	0	0
Mar 2004	6	0	6	0	0	0	0
Apr 2004	5	1	5	1	0	0	3
May 2004	7	0	5	5	3	0	2
Jun 2004	3	1	3	2	0	0	3
Jul 2004	2	0	1	0	0	0	1
Aug 2004	0	0	0	0	0	0	0
Sept 2004	0	0	0	0	0	0	0
Oct 2004	4	0	0	0	0	0	0
Totals	70	2	33	17	6	0	26

[a] One *Dermacentor occidentalis* adult male was recovered from an urban female fox during July 2003.

Based on an three-way ANOVA the number of *I. pacificus* adults found on captured foxes varied among the seasons ($F = 28.17$, $df = 3$, $p < 0.001$, $n = 70$) as well for the areas of capture ($F = 17.57$, $df = 1$, $p < 0.001$, $n = 70$). Based on a Fisher's Least Significant Difference Multiple Comparison Test, the mean (± SE) number of *I. pacificus* for all foxes was significantly ($p < 0.05$) greater in winter (8.4 ± 0.97, $n = 9$) compared to fall (0.27 ± 0.62, $n = 22$) and summer (0.71 ± 0.59, $n = 24$) but not in spring (6.1 ± 0.77, $n = 15$) (Table 4). Foxes in the backcountry had a significantly ($p < 0.001$) higher mean number of *I. pacificus* (4.4 ± 0.54, $n = 30$) than foxes in the urban zone (1.5 ± 0.46, $n = 40$). There was no difference in the number of *I. pacificus* adults found on captured male or female foxes ($F = 0.59$, $df = 1$, $p = 0.44$). All two-way and three-way interactions were significant (Appendix B).

Based on an ANOVA, the number of *I. texanus* nymphs found on captured foxes varied by season ($F = 3.06$, $df = 3$, $p = 0.035$). The mean (± SE) number of *I. texanus* nymphs for all foxes was significantly ($p < 0.05$) greater in spring (6.9 ± 1.75, $n = 15$) compared to fall (0.59 ± 1.44, $n = 22$), winter (0.67 ± 2.26, $n = 9$) and summer (0.83 ± 1.38, $n = 24$) (Table 4). There was no difference in the number of *I. texanus* nymphs found on captured foxes between the areas of capture ($F = 0.86$, $df = 1$, $p > 0.05$), or sexes ($F = 0.68$, $df = 1$, $p > 0.05$). None of the two-way or three-way interactions were significant (Appendix C).

The number of *D. variabilis* adults found on captured foxes varied among the seasons ($F = 4.10$, $df = 3$, $p = 0.010$). There was no difference in the number of *D. variabilis* adults found on captured foxes between the areas of capture ($F = 0.15$, $df = 1$, p

Table 4. Means and standard errors of tick species removed from gray foxes (*Urocyon cinereoargenteus*) among seasons and tick stage on the Hoopa Valley Indian Reservation, Humboldt County, California during 3 June 2003 to 10 October 2004. NA denotes that no ticks of that species were removed during a specific season. Total numbers of ticks of a species and stage removed during a season (n) are indicated below tick means and standard errors.

Tick species	Season							
	Winter (n = 9)		Spring (n = 15)		Summer (n = 24)		Fall (n = 22)	
	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult
Ixodes texanus	0.66 ± 2.27 (n = 6)	0.11 ± 0.11 (n = 1)	6.93 ± 1.76 (n = 104)	0.20 ± 0.11 (n = 3)	0.83 ± 1.39 (n = 20)	0.08 ± 0.05 (n = 2)	0.59 ± 1.44 (n = 13)	NA
Ixodes pacificus	NA	8.44 ± 0.98 (n = 76)	0.06 ± 0.06 (n = 1)	6.13 ± 0.75 (n = 92)	0.04 ± 0.04 (n = 1)	0.71 ± 0.60 (n = 17)	NA	0.27 ± 0.63 (n = 6)
Dermacentor occindentalis	NA	NA	NA	NA	NA	0.04 ± 0.04 (n = 1)	NA	NA
Dermacentor variabilis	NA	NA	NA	2.20 ± 0.66 (n = 33)	NA	2.67 ± 0.52 (n = 64)	NA	0.05 ± 0.55 (n = 1)

= 0.70), or the sexes (F = 0.61, df = 1, p = 0.44). None of the two-way or three-way interactions were significant (Appendix D). The mean (± SE) number of *D. variabilis* for all foxes was significantly ($p < 0.05$) greater in summer (2.7 ±0.52, n = 24)compared to winter (0 ± 0.85, n = 9) and fall (0.05 ± 0.55, n = 22), but not spring (2.2 ± 0.66, n = 15) (Table 4).

Of the six *I. texanus* adults found on foxes, four were found on urban foxes, two female and one male, and two *I. texanus* were found on backcountry foxes, one male and one female. Three of the *I. texanus* adults were found on male foxes while three were found on female foxes. The two *I. pacificus* nymphs were found on one male and one female backcountry captured foxes, respectively. One *D. occidentalis* was found in this study on an urban female fox in July 2003.

Of the 70 fox captures, including recaptures, 36 were seropositive for antibodies to *A. phagocytophilum* (Appendix E). Based on a Chi-squared test for independence the antibodies to *A. phagocytophilum* in foxes were most prevalent in the spring and least prevalent in the fall ($X^2 = 12.54$, df = 3, p = 0.0057) (Table 5). Based on the Armitage test for trend in proportions, there was a decreasing trend in proportions of seropositive foxes from the summer to winter (Z=-2.87, df = 3, p = 0.002) (Table 5).

Based on a Chi-squared test for independence, backcountry foxes (16 of 23, 70%) had higher seroprevalence to *A.phagocytophilum* than foxes captured in urban areas (12 of 31, 39%) ($X^2 = 5.04$, df = 1, p = 0.024). Five of 14 (36%) urban female foxes were seropositive and 5 out of 7 (71%) backcountry female foxes were seropositive; this

Table 5. Seroprevalence of gray foxes (*Urocyon cinereoargenteus*) captured on the Hoopa Valley Indian Reservation, Humboldt County, California during 3 June 2003 to 13 October 2004 against *Anaplasma phagocytophilum* based on indirect immunoflorescent antibody test (IFA), as well as *A. phagocytophilum* DNA through polymerase chain reaction (PCR) throughout the seasons.

Season	IFA		PCR	
	Number tested	Number positive (%)	Number tested	Number positive (%)
Spring	15	11 (73)	15	1 (7)
Summer	24	16 (67)	24	3 (13)
Fall	22	5 (28)	22	2 (9)
Winter	9	4 (44)	9	0 (0)
Total	70	36 (51)	70	6 (9)

difference between the two groups was not significant ($X^2 = 2.39$, df = 1, p = 0.12). Seven of 17 (41%) urban male foxes were seropositive, and 11 of 16 (69%) backcountry male foxes were seropositive; this difference between the two groups also was not significant ($X^2 = 2.53$, df = 1, p = 0.11). Of all captured foxes, 10 of 27 (37%) females were seropositive and 18 of 33 (55%) males were seropositive. Based on a Chi-squared test for independence, this difference was not significant ($X^2 = 0.25$, df = 1, p = 0.62). All foxes seropositive for exposure to *A. phagocytophilum* upon initial capture were seropositive at all subsequent recaptures (Appendix A).

Twenty-eight of the 90 (31%) dogs sampled at Hoopa Valley Indian Reservation during the Rural Area Veterinarian Services clinic were seropositive for antibodies against *A. phagocytophilum*. Backcountry foxes were more likely (16 of 23; 70%) to be seropositive than domestic dogs ($X^2 = 11.39$, df = 1, p < 0.01). However, there was no difference in the prevalence of antibodies against *A. phagocytophilum* between domestic dogs and urban foxes (12 of 31; 39%) ($X^2 = 0.60$, df = 1, p = 0.44).

Six of the 70 (9%) foxes sampled were PCR-positive for *A. phagocytophilum* (Appendix E). Four of 30 (13%) urban foxes were PCR-positive, with one female and three male foxes. Two of the 34 (6%) backcountry foxes were PCR-positive, with one female and one male fox. Differences between the seasons ($X^2 = 1.39$, df = 3, p = 0.70), areas ($X^2 = 0.86$, df = 1, p = 0.35), or sexes ($X^2 = 0.88$, df= 1, p = 0.77), were not significant. Based on an Armitage Test for trends in proportions there was no trend in proportions of PCR positive foxes from the spring season to winter season (Z = -0.47, df = 3, p >0.05).

There was no difference in *A. phagocytophilum* PCR-prevalence between urban captured males (3 of 17) and backcountry captured males (1 of 16) ($X^2 = 1.00$, df = 1, p = 0.31) or between urban captured females (1/14) and backcountry captured females (1/7) ($X^2 = 0.16$, df = 1, p = 0.68). None of the recaptured foxes (n =16) were PCR-positive at any subsequent recaptures (Appendix A).

DISCUSSION

The morphometric measures of gray foxes in this population were within the range of those reported from other studies (Fritzell and Haroldson 1982). The significant difference in weight between the sexes was expected, but both sexes were at the lower end of the recognized range (3 to 7 kg) of published weights. These findings could be influenced by food resource availability, differences in age of captured individuals or clinical manifestations from pathogen/parasite exposures that were not investigated in this study.

Prior reports of seasonal variation among ectoparasite species prevalences or intensities among gray foxes were not found. Most accounts have reported incidental data on ectoparasites encountered in unrelated projects, focused on general ectoparasites of a mammalian community, or centered on host associations for a particular tick species (Eads and Menzies 1950, Wilson and Baker 1972, Furman and Loomis 1984). Adults of three of the four tick species found in this study (*D. variabilis*, *D. occidentalis*, and *I. pacificus*) have been found on gray foxes within California (Furman and Loomis 1984); prior reports of *I. texanus* adults in foxes were not found.

The seasonal distribution of *I. pacificus* adults in this study varied from other studies (Padgett and Lane 2001). Typical life cycles for *I. pacificus* adults on the north coast of California have a peak emergence during the fall and winter months with a 90% mortality of the adult cohort by late June (Padgett and Lane 2001). However, I found

adults throughout all seasons; thus this known vector for *A. phagocytophilum* is possibly present on foxes throughout the year.

While most observations of *I. pacificus* on foxes occurred during the winter months, this extension of non-replete *I. pacificus* into June and July might be influenced by several abiotic factors. Survivability and questing of *I. pacificus* is positively correlated with relative humidity (Loye and Lane 1988, Peavey and Lane 1996, Perret et al. 2004). The western edge of Hoopa Valley Indian Reservation is located only 26 km from the Pacific Ocean and is subject to occasional coastal fog during the early summer months. Hoopa Valley Indian Reservation also receives river fog which is generated by the Trinity River corridor that bisects the study area, which may increase relative humidity.

Another potential abiotic factor in *I. pacificus* emergence is that the Hoopa Valley Indian Reservation is surrounded by perennial streams and creeks as well as a large amount of springs throughout the area. This, in conjunction with Hoopa Valley Indian Reservation forestry riparian buffers, may lead to multiple corridors that have an increase in relative humidity.

Therefore, the life cycle of this species on the Hoopa Valley Indian Reservation should be investigated further to determine the factors influencing this year-round presence of *I. pacificus* on foxes.

Several environmental factors may contribute to the difference in numbers of *I. pacificus* adults found on gray foxes between the two zones. *I. pacificus* adult tick density is positively correlated with high brush density, presence of both uphill and

downhill slopes, and trail systems (Li et al. 2000). Immature *I. pacificus* were found in high numbers on various rodent species throughout forest stands of different types in the backcountry zone at the Hoopa Valley Indian Reservation (Whitaker 2003), which could also contribute to the higher *I. pacificus* adults found in the backcountry foxes in this study. *Ixodes pacificus* nymphs are more likely to infest western-fence lizards (*Sceloporus occidentalis*) in woodland than in grassland habitats (Eisen and Eisen 1999). The urban zone is dominated by grasses and forbs (Singer and Begg, 1975) while the backcountry zone is dominated with brush and trees (Matthews 2000); this may be one reason for the differences in *I. pacificus* numbers between the two zones. The differences in other factors such as habitat and numbers of adult ticks flagged between the zones needs to be investigated further.

The seasonal effect differed between the sexes and the areas as indicated by the significant interactions (Appendix B). I propose that the difference in the number of *I. pacificus* adults between back country and urban foxes most likely is driven by the high prevalence of *I. pacificus* on backcountry foxes only during the winter season (Appendix F).

The difference in the number of *I. pacificus* adults between sexes is likely driven by the disparity in high prevalence of *I. pacificus* between the sexes that occurred only in the spring season (Appendix G). The three-way interaction between season, area and sex indicates that the effect of each of the variables influenced the other.

It was surprising to find only two *I. pacificus* nymphs during this study since the study overlapped peak emergence identified as late spring to late summer (Padget and

Lane 2001, Cashier et al. 2002). However, this is likely because *I. pacificus* nymphs normally feed on small vertebrate hosts such as rodents and lizards (Furman and Loomis 1984, Cashier et al. 2002); thus, these two immature *I. pacificus* may have been incidental findings.

Ixodes texanus has been observed on gray foxes in several states within the U.S. (Darsie and Anastos 1957), but has not been documented on gray foxes in California or as far north as Hoopa Valley Indian Reservation. The finding of an *I. texanus* on a gray fox in California is both a host and geographic extension for this species. *Ixodes texanus* is commonly known as a "mustelid tick" or a "raccoon tick" due to its close association with these species (Darsie and Anastos 1957). Within this study, the six adult *I. texanus* adults corresponded to other observations of finding only small numbers and only females (Cooley and Kohls 1945, Ouellette et al. 1997). *Ixodes texanus* males have been recovered from vertebrates in previous studies, but have not been extensively documented (Cooley and Kohls 1945, Darsie and Anastos 1957, Furman and Loomis 1984). It would be of value to determine if the sexes of this species are vertebrate host-specific.

The finding of *I. texanus* nymphs on west coast study sites has not been documented previously. Within the eastern seaboard of the United States, nymphs can be found throughout the seasons on various host with their peak emergence between April and June (Ouellette et. al 1997, Kollars and Oliver 2003). My findings were similar.

The *D. variabilis* adults found on this study correspond with other reports of vertebrate hosts and seasonal activity (Furman and Loomis 1984). The one occurrence of

a *D. occidentalis* in July was typical of other findings within California (Furman and Loomis 1984). However, the numbers were considerably lower then expected based on the numbers of *D. occidentalis* found on black bears on the Hoopa Valley Indian Reservation in previous years (Brown 2005, personal communication)

The finding of *A. phagocytophilum* antibodies among foxes establishes the presence of this parasite among the Hoopa Valley Indian Reservation foxes. The observed prevalence (50%) was similar to the prevalence (46%) reported from coyotes sampled in California (Pusterla et al. 2000).

Seasonal patterns in fox with *A. phagocytophilum* seropositivity were correspond to seasonal patterns in *I. pacificus* intensities except that the antibodies peaked approximately 3 months after *I. pacificus* peak activity on foxes. These parallel patterns are congruent with the delayed response of antibodies to *A. phagocytophilum* observed in exposed individuals of other species such as cats and humans, with documented delays ranging from 3 to 4 weeks (Bakken et al. 2002, Foley et al. 2003). This pattern appears consistent with *I. pacificus* being the implicated vector for *A. phagocytophilum* on the Hoopa Valley Indian Reservation.

There have been no studies comparing exposure to *A. phagocytophilum* in different habitat types. However, the significantly higher prevalence of fox antibodies against *A. phagocytophilum* in backcountry foxes compared to the urban foxes could be due to several factors. Brown (2005, personal communication) determined that 93% of black bears sampled within Hoopa Valley Indian Reservation had antibodies to *A. phagocytophilum.* These omnivorous generalists were sampled in both urban and

backcountry areas in Hoopa Valley Indian Reservation and have much larger home ranges than gray foxes. This overlap in zone use among black bears may mask differences in exposure rates between the zones. None of the foxes caught in one area of capture were caught in any other area throughout the capture effort; therefore, gray foxes, with their relatively small home ranges, may be a more appropriate species to use in comparing the risks of exposure to *A. phagocytophilum* between the two areas.

The estimated home range used as a buffer (129 ha) in this study may actually be a liberal estimate; in comparison, Fuller (1978) found female gray foxes in the Sacramento Valley to have a mean home range of 122 ha, Kodani (1996) found southern California female gray foxes to have a 110 ha home range and males with a 71 ha, and Matthews (2000) found females to have a 58 ha home range and males a 54 ha in southern California. The longest distance a fox was recaptured in my study from any one of its previous captures was 1237 m (≥ 120 ha home range), with the shortest distance of 142 m (≥ 1.6 ha home range) and a mean of 613 m (SE ± 139.0 m, n = 16; ≥ 29.5 ha home range). The lack of cross over between urban and backcountry zone captures and differences in *A. phagocytophilum* exposure between the two areas warrants further investigation.

A possible biotic factor contributing to the higher antibody prevalence among backcountry foxes compared to urban foxes is the difference *I. pacificus* intensities between the areas of capture. Since *I. pacificus* is the implicated vector in the western states and their intensities were significantly higher in the backcountry foxes, the increase of *I. pacificus* numbers within the backcountry may increase the risk of exposure in these

foxes to *A. phagocytophilum*. In the future, I would propose an assessment in the differences in *I. pacificus* populations of questing ticks between the two areas as well as PCR differences of *A. phagocytophilum* prevalence within these ticks.

Woodrats may play a role as a reservoir host based on their long duration infections of *A. phagocytophilum* (Castro et al. 2001, Foley et al. 2002). Variation in the local numbers or diversity of incompetent and competent vertebrate reservoirs for ticks may contribute to differences in gray fox exposures to *A. phagocytophilum* (Ostfeld and Keesing 2000). The "dilution effect" theory suggest that when competent reservoir hosts (i.e. woodrats) are dominant in the community, disease risks are more pronounced in comparison to communities that are species rich with incompetent hosts that "dilute " the disease risk (Schmidt and Ostfeld 2001). Differences in woodrat population numbers or other rodent hosts between the two sites on the Hoopa Valley Indian Reservation may affect such a dilution effect and thus contribute towards the differences in exposures observed.

The prevalence of seropositive urban caught foxes (39%) was similar to the overall percentage of domestic dogs (31%). Based on personal observation, care of domestic dogs varies considerably within the Hoopa Valley Indian Reservation. Many owners leash and confine their pets within their property boundaries while others allow their pets to roam freely. Currently there is no tribal law that restricts roaming of domestic dogs within the Hoopa Valley Indian Reservation (Higley 2004, personal communication). Dogs commonly were seen roaming unrestrained within the urban zone

while dogs were seen on only two occasions during the duration of the study in the areas of the backcountry.

It is likely that they would have a similar exposure risk to *A. phagocytophilum* since most domestic dogs and urban caught foxes appearing restricted to the urban zone. This similarity may be enhanced by interactions of urban foxes and domestic dogs. Foley et al. (in press) found that 10 of 20 (50%) domestic dogs were seropositive to *A. phagocytophilum* in rural communities adjacent to the Hoopa Valley Indian Reservation.

This antibody prevalence among domestic dogs on the Hoopa Valley Indian Reservation (31%) is relatively high compared to other dogs sampled in previous studies within the state (8.7%, Foley et al. 2001), but yet within the range of dogs sampled in Humboldt County (18% to 47% , Foley et al. 2001, Foley et al. in press). The communities studied by Foley et al.(in press) have no clear delineation between urban and backcountry zones and may actually comprise an intermediate zone that has characteristics of both. The differences in exposure between groups of strictly feral and free roaming dogs, and urban captured foxes within the Hoopa Valley Indian Reservation would be a better assessment to see if domestic dogs and foxes truly differ in their antibody prevalences.

The season with the highest intensities of *I. pacificus* adults on gray foxes (winter) had no PCR-positive foxes. For *I. pacificus* adults to act as the contributing vector of *A. phagocytophilum* to foxes there should be an evident relationship between PCR-positivity in foxes and *I. pacificus* numbers.

In conclusion, future studies should encompass the abiotic differences between the two zones and whether this plays into the differences in exposure to both ixodid ticks and *A. phagocytophilum* among gray foxes of the two areas. Ixodid ticks flagged between the two areas should be assessed to determine which species of non-replete ticks are positive for *A. phagocytophilum*. The life history of *I. texanus* and their possible role as a vector of *A. phagocytophilum* or maintaining an enzootic transmission cycle should be studied. Finally, clinical manifestations of gray foxes exposed to *A. phagocytophilum* warrants further investigation.

MANAGEMENT IMPLICATIONS

There are few publications regarding exposure of gray foxes to *A. phagocytophilum*. However, based on my data, I propose that since gray foxes occur in human-inhabited areas, and have been documented to use human-made structures (Hoff et al. 1974, Harrison 1997, Neale and Sacks 2001), gray foxes should be considered as a potential sentinel species within northwestern California.

Antibody prevalence of foxes, dogs and other species in northwestern California indicate that these species have been exposed to vectors of *A. phagocytophilum*. Therefore, individuals that reside or spend time in an outdoor setting have to be diligent in preventing or reducing tick exposure in order to reduce possible transmission.

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Appendix A. Occurrence of *Anaplasma phagocytophilum* among recaptured gray foxes (*Urocyon cinereoargenteus*) on the Hoopa Valley Indian Reservation, Humboldt County, California, from 3 June 2003 to 10 October 2004, with their results through indirect immunoflorescent antibody assay (IFA) and polymerase chain reaction assay (PCR). Numbers in parenthesis designates which buffer wash cycle a positive reaction occurred with the PCR test.

Fox Number	Sex	Area	Initial Capture	IFA	PCR	Second Capture	IFA	PCR	Third Capture	IFA	PCR	Fourth Capture	IFA	PCR
008	Female	Backcountry	7/10/2003	+	-	10/15/03	+	-						
006	Male	Backcountry	7/3/2003	+	-	2/8/2004	+	-	4/9/2004	+	-	5/15/2004	+	-
018	Female	Backcountry	10/10/2003	+	+ (33)	3/5/2004	+	-						
001	Female	Backcountry	6/23/2003	+	-	4/29/2004	+	-	5/14/2004	+	-			
007	Male	Urban	7/6/2003	-	-	10/15/2003	-	-	4/9/2004	-	-			
017	Female	Urban	9/27/2003	-	-	12/9/2003	-	-						
009W	Female	Urban	9/28/2003	-	-	2/22/2004	-	-						
013	Female	Urban	8/22/2003	-	-	5/20/2004	+	-	10/10/2004	+	-			
012	Female	Urban	7/30/2003	-	-	5/21/2004	-	-	7/3/2004	-	-			
011	Male	Urban	7/29/2003	-	-	10/10/2004	-	-						

Appendix B. Results of three-way ANOVA analyzing the effects of season, area of capture, and fox sex on the prevalence of *Ixodes pacificus* adults on gray foxes (*Urocyon cinereoargenteus*) caught on the Hoopa Valley Indian Reservation, Humboldt County, California, 3 June 2003 to 10 October 2004.

Variables	Degrees of Freedom	F-Ratio	Probability value
Season (A)	3	28.17	< 0.01
Area (B)	1	17.57	< 0.01
Sex (C)	1	0.59	0.44
AB	3	16.14	< 0.01
AC	3	8.01	< 0.01
BC	1	8.2	< 0.01
ABC	3	6.93	< 0.01

Appendix C. Results of three-way ANOVA analyzing the effects of season, area of capture, and fox sex on the prevalence of *Ixodes texanus* nymphs on gray foxes (*Urocyon cinereoargenteus*) caught on the Hoopa Valley Indian Reservation, Humboldt County, California, 3 June 2003 to 10 October 2004.

Variables	Degrees of Freedom	F-Ratio	Probability value
Season (A)	3	3.06	0.03
Area (B)	1	0.86	0.35
Sex (C)	1	0.68	0.41
AB	3	1.09	0.36
AC	3	0.46	0.71
BC	1	0.46	0.49
ABC	3	0.91	0.44

Appendix D. Results of three-way ANOVA analyzing the effects of season, area of capture, and fox sex on the prevalence of *Dermacentor variabilis* adults on gray foxes (*Urocyon cinereoargenteus*) caught on the Hoopa Valley Indian Reservation, Humboldt County, California, 3 June 2003 to 10 October 2004.

Variables	Degrees of Freedom	F-Ratio	Probability value
Season (A)	3	4.10	0.01
Area (B)	1	0.15	0.70
Sex (C)	1	0.61	0.43
AB	3	0.66	0.58
AC	3	0.23	0.87
BC	1	0.30	0.58
ABC	3	0.17	0.91

Appendix E. Prevalence of antibodies towards *Anaplasma phagocytophilum* through Immunoflorescent antibody test (IFA) and *Anaplasma phagocytophilum* DNA through polymerase chain reaction (PCR) throughout the months in gray foxes (*Urocyon cinereoargenteus*) caught on the Hoopa Valley Indian Reservation, Humboldt County, California, from 3 June 2003 to 10 October 2004.

Month	IFA		PCR	
	Number tested	Number positive (%)	Number tested	Number positive (%)
June 2003	2	2 (100)	2	0 (0)
July 2003	14	8 (57)	14	1 (7)
August 2003	2	1 (50)	2	1 (50)
September 2003	8	5 (63)	8	1 (13)
October 2003	8	3 (38)	8	1(13)
November 2003	2	0 (0)	2	0 (0)
December 2003	4	0 (0)	4	1 (25)
January 2004	0	0 (0)	0	0 (0)
February 2004	3	1 (33)	3	0 (0)
March 2004	6	3 (50)	6	0 (0)
April 2004	5	4 (80)	5	0 (0)
May 2004	7	5 (72)	7	0 (0)
June 2004	3	2 (67)	3	1 (33)
July 2004	2	1 (50)	2	0 (0)
August 2004	0	0 (0)	0	0 (0)
September 2004	0	0 (0)	0	0 (0)
October 2004	4	1 (25)	4	0 (0)
Totals	70	36 (51)	70	6 (9)

Appendix F. Means and standard errors of *Ixodes pacificus* removed from gray foxes (*Urocyon cinereoargenteus*) among seasons and areas on the Hoopa Valley Indian Reservation, Humboldt County, California during 3 June 2003 to 10 October 2004.

Location of Capture	Season			
	Winter	Spring	Summer	Fall
Backcountry	11.33 ± 1.19	5.88 ± 1.04	1.50 ± 0.93	0.17 ± 1.20
Urban	2.67 ± 1.69	6.43 ± 1.11	0.14 ± 0.78	0.31 ± 0.73

Appendix G. Means and standard errors of *Ixodes pacificus* removed from gray foxes (*Urocyon cinereoargenteus*) among seasons and sexes on the Hoopa Valley Indian Reservation, Humboldt County, California during 3 June 2003 to 10 October 2004.

Sex	Season			
	Winter	Spring	Summer	Fall
Male	7.83 ± 1.20	7.89 ± 0.98	0.62 ± 0.81	0.55 ± 0.88
Female	9.67 ± 1.69	3.50 ± 1.19	0.82 ± 0.88	0.00 ± 0.88