Impacts of environmental conditions on fleas in black-tailed prairie dog burrows

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ABSTRACT: Sylvatic plague, caused by the bacterium *Yersinia pestis* and transmitted by fleas, occurs in prairie dogs of the western United States. Outbreaks can devastate prairie dog communities, often causing nearly 100% mortality. Three competent flea vectors, prairie dog specialists *Oropsylla hirsuta* and *O. tuberculata,* and generalist *Pulex simulans*, are found on prairie dogs and in their burrows. Fleas are affected by climate, which varies across the range of black-tailed prairie dogs (*Cynomys ludovicianus*), but these effects may be ameliorated somewhat due to the burrowing habits of prairie dogs. Our goal was to assess how temperature and precipitation affect off-host flea abundance and whether relative flea abundance varied across the range of black-tailed prairie dogs. Flea abundance was measured by swabbing 300 prairie dog burrows at six widely distributed sites in early and late summer of 2016 and 2017. Relative abundance of flea species varied among sites and sampling sessions. Flea abundance and prevalence increased with monthly mean high temperature and declined with higher winter precipitation. Predicted climate change in North America will likely influence flea abundance and distribution, thereby impacting plague dynamics in prairie dog colonies. ***Journal of Vector Ecology* 45 (2): 356-365. 2020.**

Keyword Index: Plague, prairie dogs, fleas, climate, environment, burrows.

INTRODUCTION

Sylvatic plague, caused by the bacterium *Yersinia pestis*, is a flea-borne disease of wildlife that can also affect humans. It has become endemic in the western United States, where it is devastating to prairie dog (*Cynomys spp.*) populations (Gage and Kosoy 2005). Prairie dog mortality during plague outbreaks is high (>90%) and often leads to local extirpation (Hoogland 1995, Cully and Williams 2001). Many other species directly or indirectly depend on prairie dogs, and although their status as keystone species has been debated, their extirpation can have cascading effects through the grassland ecosystem (Miller et al. 1994, Stapp 1998, Kotliar et al. 2006, Eads and Biggins 2015).

Fleas can drive plague outbreaks on prairie dog colonies. Models suggest that fleas are responsible for ~70% of transmission that occurs during plague events (Richgels et al. 2016). Vector control methods, such as applying insecticides to prairie dog burrows, can halt outbreaks, providing strong evidence for the importance of fleas in plague transmission (Seery et al. 2003, Tripp et al. 2016). Flea abundance on individual prairie dogs are influenced by a variety of factors, including prairie dog age, body condition, and environmental conditions, such as temperature and precipitation (Eads et al. 2016, Russell et al. 2018). However, little is known about how environmental conditions affect the abundance of off-host fleas that occur in prairie dog burrows. Like most insects, fleas are sensitive to the temperature and relative humidity of their environment (Krasnov 2008), especially during development which occurs in underground burrows. Development rates of immature fleas typically increase as temperature gets higher, and survival is dependent on sufficient humidity to avoid desiccation (Krasnov et al. 2001a, 2001b, Kreppel et al. 2016).

Prairie dog burrows can moderate the extreme effects of aboveground weather conditions and thus provide conditions that are more favorable for flea development and population dynamics.

Three flea species are commonly associated with black-tailed prairie dogs: *Oropsylla hirsuta* and *O. tuberculata* are often referred to as "prairie dog fleas" because they are rarely found on other species of hosts, while *Pulex simulans* is considered a host generalist. These fleas are often combined when discussing plague transmission, but differences among the species are evident. While all three species are competent vectors of plague (Burroughs 1947), transmission efficiency varies among species (Wilder et al. 2008). *Oropsylla tuberculata* is roughly three times more efficient at transmitting *Y. pestis* than *O. hirsuta*, and the exact transmission efficiency of *P. simulans* is unknown. The two *Oropsylla* species also have different seasonal peaks in abundance. *Oropsylla tuberculata* is most abundant in the early spring, while *O. hirsuta* becomes dominant through the summer and early fall (Salkeld and Stapp 2008). Several studies have examined the relationship between environmental conditions and flea abundance on prairie dog colonies. In New Mexico, Eads et al. (2016) observed that the abundance of *O. hirsuta* and *P. simulans* on black-tailed prairie dogs increased dramatically during a year with a severe drought. As the drought ended, *P. simulans* abundance declined to pre-drought levels while *O. hirsuta* abundance remained elevated, indicating differing responses to environmental conditions. On black-tailed prairie dog colonies in Montana and South Dakota, increased winter precipitation was associated with increased abundance of *O. hirsuta* and a decline in *P. simulans* (Russell et al. 2018). Because different flea species have different competence as plague vectors, these shifts in flea abundance and community

composition due to environmental factors could impact plague dynamics.

Black-tailed prairie dogs have a wide distribution, ranging from Canada to Mexico and from the foothills of the Rocky Mountains eastward through the short and mixed grass prairies (Hoogland 1995). Climate changes dramatically across this range, and these environmental differences may also affect the abundance and seasonal peaks of different flea species. Because fleas are critical for plague transmission, understanding how the environment acts on different species is important for understanding plague dynamics.

To assess the relationship between ambient climate and flea populations, we conducted field studies across a wide latitudinal gradient, allowing us to survey a range of environmental conditions. We sampled six sites over two years and two seasonal time points to assess how flea abundance in burrows varied both geographically and temporally. We measured patterns of flea abundance in black-tailed prairie dog colonies by burrow swabbing. We predicted that *O. tuberculata* would be more common earlier in the year, while *O. hirsuta* would dominate later in the summer, as suggested by previous studies (Salkeld and Stapp 2008, Mize and Britten 2016). We tested the hypotheses that 1) relative abundance of different flea species varies among sites across the black-tailed prairie dog range, and 2) total flea abundance (all species) would be higher when summer temperature is high and winter precipitation is low. Our results provide insights on how future climate change may affect flea populations and plague dynamics.

MATERIALS AND METHODS

We studied six sites that were broadly distributed across the range of black-tailed prairie dogs within the United States (Figure 1): Charles M. Russell National Wildlife Refuge (MT) in Phillips County, MT, Theodore Roosevelt National Park (ND) in Billings County, ND, Buffalo Gap National Grassland (BG) in Pennington County, SD, Lower Brule Indian Reservation (LB) in Lyman County South Dakota, Pueblo Chemical Depot (CO) in Pueblo County, CO, and public lands surrounding Roswell (NM) in Chaves County, NM during 2017 only. We selected three black-tailed prairie dog colonies at each site. Each colony was sampled during two sessions, once during May or June (early session), and once in August (late session) in 2016 and 2017, except where noted. ND was added to the study during the summer of 2016 and so was only sampled during the late session that year.

We swabbed one hundred burrows per colony to collect fleas. Burrows were swabbed by attaching a 15 cm by 15 cm piece of white flannel to a 4.5 m plumbing snake and pushing the snake as far as possible down the burrow. We left swabs in the burrow for 30 s before slowly pulling them out and placing them in a zip-lock bag. Each bag was labeled with the GPS coordinates of the burrow, burrow status (active or inactive), and the depth of the swab in the burrow. Burrows were classified as "active" if there were signs of fresh digging, scat, or if we observed prairie dogs using the burrow; otherwise burrows were considered "inactive." Swabs

were frozen to kill fleas, and dead fleas were then placed in tubes filled with 1 ml of 70% ethanol until later laboratory identification. We identified fleas to species using a dissecting microscope according to keys described by Hubbard (1947) and Furman and Catts (1982), with revisions by Stark (1970) and Lewis (2002). If a flea could not be identified to species, it was labeled as "unknown" (seven of 1,776 fleas).

We modeled the relative abundance of each flea species (calculated as a proportion of the number of that species/ total fleas collected) in a generalized linear model with a binomial distribution using the glm function from package stats in R (R Core Team 2018, http://www.r-project.org). Relative abundance was modeled as a function of site, sampling session, and year. We used backwards selection to select the most parsimonious model. We used a Cochran-Mantel-Haenszel test from the R package "stats" to assess if there was a consistent relationship of relative abundance among flea species and session across sites. To compare the relative abundance of each flea species among sites, we calculated a 95% confidence interval using the Clopper-Pearson exact interval.

We evaluated absolute flea abundance (total number of fleas found in each burrow) in a generalized linear mixed model with a negative binomial distribution using the glmer. nb function from package lme4 (Bates et al. 2015) in R. Flea abundance was modeled as a response to monthly mean high temperature, winter precipitation, sampling session (early or late), and year, as well as the interactions between year and winter precipitation, year and monthly mean high temperature, session and winter precipitation, and session and monthly mean high temperature. We calculated monthly mean high temperature (mean of the daily high temperatures) for the nearest NOAA National Centers for Environmental Information weather station for each site and month during which burrow swabbing occurred. Similarly, we calculated winter precipitation as the sum of the precipitation received during the December, January, and February preceding burrow sampling. Depth (meters) and burrow status (active or inactive) were included as fixed effects because previous studies reported fleas are more likely to be collected from active burrows and swabs inserted deeper into burrows (Eads 2017). Colony was nested within site to account for the lack of independence among colonies at the same site, and both were included as random effects. We used backwards selection to determine the optimal model for flea abundance.

We also modeled the prevalence of infested burrows (burrows with at least one flea of any species = 1, burrows with no fleas = 0) in a generalized linear mixed model with a binomial distribution using the glmer function from package lme4 (Bates et al. 2015) in R. We used the same explanatory variables and assessments for these models as we did when modeling flea abundance.

We used the ΔAIC to compare different models for abundance and prevalence of fleas in burrows. If ΔAIC for a given model is <2 from the lowest scoring model, we considered it well supported by the data in addition to the lowest scoring model (Burnham and Anderson 1998). We evaluated the model goodness of fit by calculating the ratio
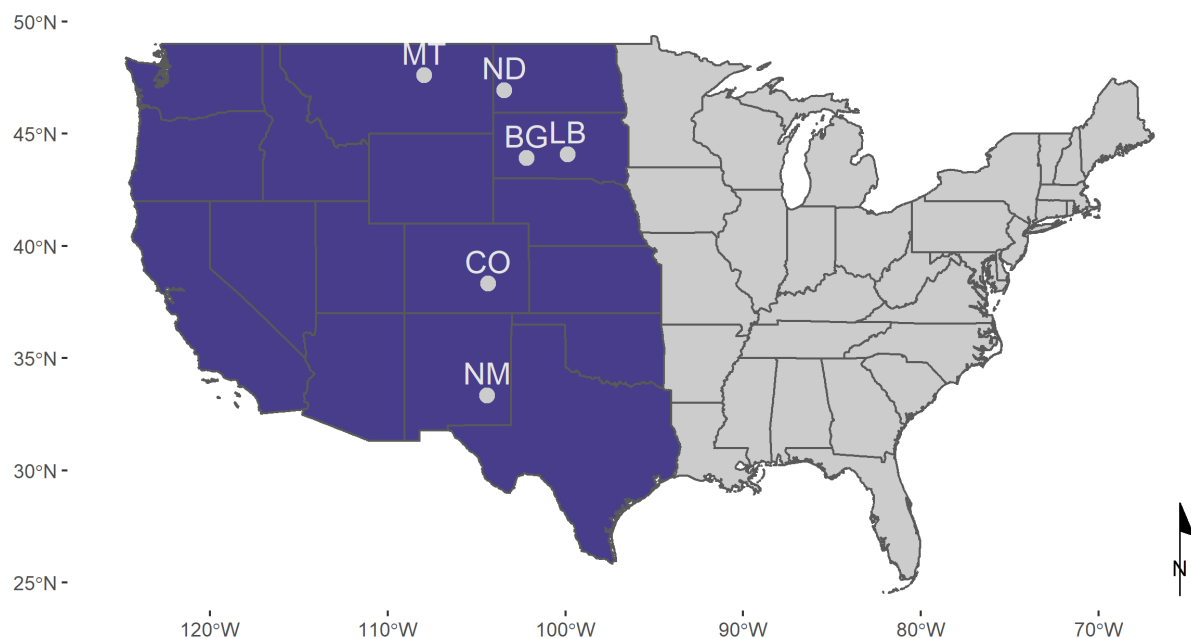
Figure 1. Plague is endemic in the western United States (purple states). Six field sites (labeled in gray) were chosen in six U.S. states (Montana, North Dakota, South Dakota, Colorado, and New Mexico). Sites in Montana, North Dakota, and Colorado were sampled during 2016 and 2017. The site in New Mexico was sampled during 2017 only.
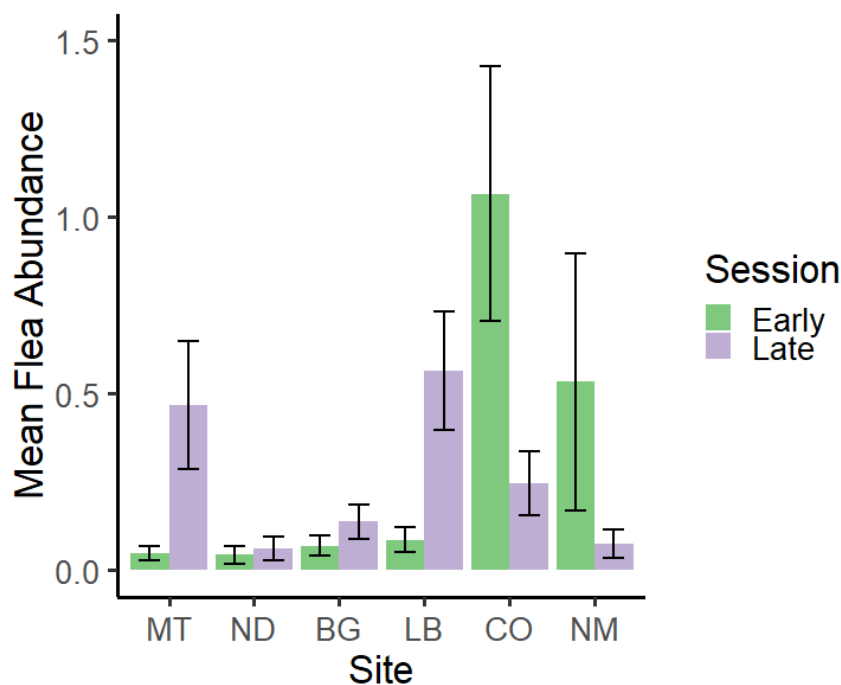

Figure 2. Mean flea abundance (total number of fleas collected / number of burrows sampled) with error bars representing 95% confidence intervals at six sites in the U.S. states of Montana (Charles M. Russell National Wildlife Refuge, MT; n = 1197), North Dakota (Theodore Roosevelt National Park, ND; n = 901), South Dakota (Buffalo Gap National Grassland, BG; n = 1206, and Lower Brue Indian Reservation, LB; n = 1201), Colorado (Pueblo Chemical Depot, CO; n = 1112) and New Mexico (Pecos District, Bureau of Land Management, NM; n = 544) during an early sampling session (late May – June) and a late sampling session (August) in the summers of 2016 and 2017. Mean flea abundance increased from the early sampling session to the late sampling session at the northern sites (MT, ND, BG, and LB) and declined at the southern sites (CO and NM).

of the residual deviance divided by the model degrees of freedom. We considered a model to adequately fit if that value was <2. We report the quantitative effect of predictor variables using either risk ratios (negative binomial models of abundance) or odds ratios (binomial models of prevalence). We note that both types of models have inherently non-linear responses to predictor variables.

RESULTS

We swabbed 6,161 burrows during the summers of 2016 and 2017. From these, we collected 1,756 fleas from 619 burrows. *Oropsylla hirsuta* accounted for 91% (95% CI 89% - 92%) of the fleas we collected, followed by *P. simulans* (7%, 95% CI 6% - 8%) and *O. tuberculata* (1%, 95% CI 0.6% - 1.5%) (Table 1). The proportion of active burrows exceeded 90% at all sites except CO, where it was 83%. Patterns of flea abundance (total number of fleas collected) varied by region and sampling session. In both 2016 and 2017, flea abundance decreased from the early to the late sampling session at southern sites, while flea abundance increased from early to late sampling at northern sites (Figure 2).

Relative Flea Abundance

Patterns of relative abundance varied among flea species. The relative abundance of *O. hirsuta* exceeded 90% and was similar at all sites, except MT, where it was 73% (Table 2).The generalized linear mixed model found no significant change in relative abundance between years (Odds Ratio = 1.17, 95% CI 0.77 – 1.78). There was a significant decline between sampling sessions (Odds Ratio = 0.56, 95% CI 0.35 – 0.91), although this change was not consistent across sites (Cochran-Mantel-Haenszel common odds ratio = 0.68, 95% CI 0.45 – 1.01). *Oropsylla tuberculata* was found at every site except NM, was always less abundant than other flea species, and varied among sites (Table 3). Its relative abundance consistently declined from the early to the late session across the sites (Cochran-Mantel-Haenszel common odds ratio = 0.12, 95% CI 0.04 – 0.36) and from 2016 to 2017 (Odds ratio = 0.28, 95% CI 0.09 – 0.91). *Pulex simulans* was only found at MT, CO, and NM and was less abundant than *O. hirsuta* but more abundant than *O. tuberculata* (Table 4). Its relative abundance increased from the early to the late session (Cochran-Mantel-Haenszel common odds ratio = 2.67, 95% CI 1.53 – 4.66) and did not change significantly between years (Odds Ratio = 1.12, 95% CI 0.69 – 1.85).

Overall Flea Abundance

The best model for overall flea abundance included the fixed effects session, year, monthly mean high temperature, winter precipitation, the interactions between session and winter precipitation and between year and monthly mean high temperature, depth, and activity status, as well as the random effects of colony nested within site (AIC =5620, K = 11). Fleas were more abundant in the late session than the early session (Risk Ratio = 2.01, 95% CI 1.59 – 2.54), in 2017 than 2016 (Risk Ratio = 9.47, 95% CI 6.06 – 14.79) and in active burrows than inactive burrows (Risk Ratio = 1.89, 95% CI 1.25 – 2.82).

Flea abundance increased by 64% with every additional meter of depth that swabs were inserted into the burrow (Risk Ratio = 1.64, 95% CI 1.43 – 1.88). Flea abundance increased by 78% (Risk Ratio = 1.78, 95% CI 1.53 – 2.07) for every 1° C increase in monthly mean high temperature in 2016, and by 111% (Risk Ratio = 2.11 95% CI 1.82 – 2.45) for every 1° C increase in monthly mean high temperature in 2017 (Figure 3a). The effect of winter precipitation on flea abundance changed between the early and late session. In the early session, flea abundance significantly decreased by 51% (Risk Ratio = 0.49, 95% CI 0.37 – 0.64) for every 1 cm increase in winter precipitation. In the late session, flea abundance declined at a rate of 23% (Risk Ratio = 0.77, 95% CI 0.58 – 1.02) for every 1 cm increase in winter precipitation (Figure 3b); however this decline was not significant.

Prevalence

The best model for the prevalence of flea-infested burrows included the fixed effects session, year, monthly mean high temperature, winter precipitation, depth, activity status, interactions between year and winter precipitation and session and winter precipitation, as well as the random effects of colony nested within site (AIC = 3660, K = 10). The odds of finding an infested burrow were higher in 2017 (Odds Ratio = 6.03, 95% CI 3.74 – 9.72) and the late session (Odds Ratio = 1.79, 95% CI 1.49 – 2.16) compared to 2016 and the early session, respectively. The prevalence of flea infested burrows was higher among active burrows than inactive burrows (Odds Ratio = 3.00, 95% CI 1.93 – 4.68) and increased by 45% for each additional meter that swabs were inserted into the burrow (Odds Ratio = 1.45, 95% CI 1.30 – 1.62). Higher monthly mean high temperatures increased prevalence by 73% (Odds Ratio = 1.73, 95% CI 1.54 – 1.95) for every 1° C increase (Figure 4a). During 2016, the prevalence of infested burrows in the early session declined by 64% (Odds Ratio = 0.36, 95% CI 0.24 – 0.55) for every additional cm of winter precipitation and by 46% (Odds Ratio = 0.54, 95% CI 0.36 – 0.81) in the late session. In 2017, prevalence of infested burrows declined by 52% (Odds Ratio = 0.48, 95% CI 0.36 – 0.63) for every additional cm of winter precipitation during the early session and by 28% (Odds Ratio = 0.72, 95% CI 0.56 – 0.93) during the late session (Figure 4b).

DISCUSSION

Oropsylla hirsuta was the most abundant species of flea found during our study, comprising 91% of fleas collected, followed by *P. simulans* and *O. tuberculata*. These species also dominated burrow swab samples in studies conducted on black-tailed prairie dog colonies in the northern Great Plains (Mize and Britten 2016), Montana (Holmes et al. 2006), Kansas (Cully et al. 2000), and Colorado (Seery et al. 2003, Stevenson et al. 2003, Salkeld and Stapp 2008). We detected few *O. tuberculata* and its relative abundance declined from the early to the late sampling session. Others have shown that *O. tuberculata* abundance in burrows was high in March and declined through the summer (Salkeld and Stapp 2008, Mize and Britten 2016), so our early sampling session (May or June)

Table 1. Number of different flea species collected at study sites during early and late sampling sessions in 2016 and 2017 at the Charles M. Russell National Wildlife Refuge, Montana (MT), Theodore Roosevelt National Park, North Dakota (ND), Buffalo Gap National Grassland, South Dakota (BG), Lower Brule Sioux Reservation, South Dakota (LB), Pueblo Chemical Depot, CO (CO), and BLM land near Roswell, New Mexico (NM).

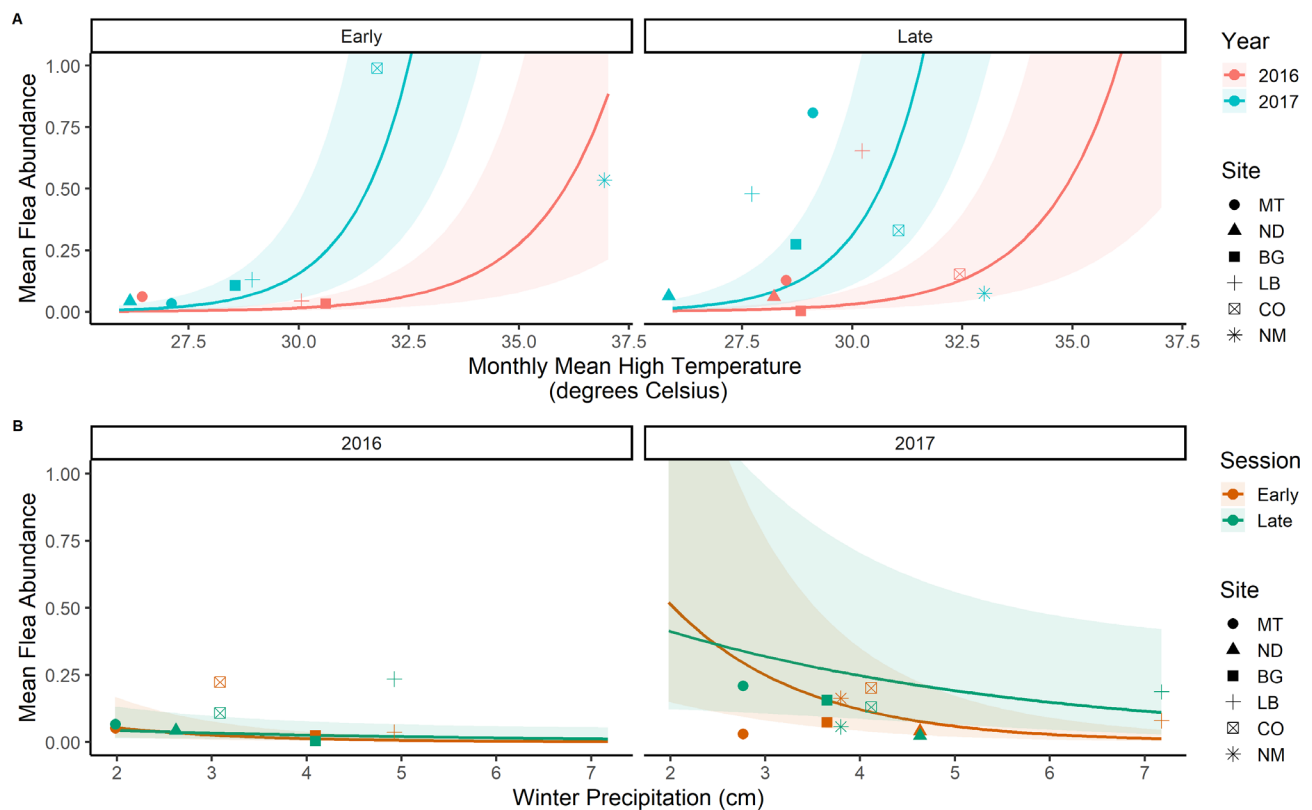| Site	Year	Session	Date	*Oropsylla hirsuta*	*Oropsylla tuberculata*	*Pulex simulans*	*Thrassis fotus*	*Polygenis gwyni*	Unknown	Session Totals	Yearly Totals
MT	2016	Early	May 24-29	7	9	2	0	0	0	18	56
		Late	August 2-4	37	0	1	0	0	0	38	
	2017	Early	June 17-19	9	1	0	0	0	0	10	253
		Late	August 20-21	173	0	70	0	0	0	243	
ND	2016	Late	August 13-14	18	0	0	0	0	0	18	18
	2017	Early	June 14-15	9	3	0	1	0	0	13	32
		Late	August 17-18	19	0	0	0	0	0	19	
BG	2016	Early	June 14-15	10	0	0	0	0	0	10	11
		Late	August 5-6	1	0	0	0	0	0	1	
	2017	Early	June 10-11	30	2	0	0	0	0	32	114
		Late	August 14-15	80	0	0	1	0	1	82	
LB	2016	Early	June 19-20	13	0	0	0	0	0	13	205
		Late	August 8-9	192	0	0	0	0	0	192	
	2017	Early	June 7-8	38	1	0	0	0	0	39	185
		Late	August 23-24	146	0	0	0	0	0	146	
CO	2016	Early	June 9-10	271	0	18	1	0	0	290	331
		Late	August 22-26	28	2	6	0	0	5	41	
	2017	Early	May 25-26	279	0	11	0	0	0	290	389
		Late	August 7-8	87	0	6	5	0	1	99	
NM	2017	Early	June 1-3	130	0	7	2	2	0	141	162
		Late	August 10-11	15	0	5	1	0	0	21	
				1,592	18	126	11	2	7	1,756	1,756

Figure 3. **A)** Mean flea abundance per prairie dog burrow increased with monthly mean high temperature at different rates in 2016 and 2017. In 2016, flea abundance increased by a rate of 1.79 for every 1° C increase in temperature, while in 2017 abundance increased by a rate of 2.11. In both years, mean flea abundance was higher in the late session. **B)** The relationship between flea abundance and winter precipitation changed between the early and the late sampling sessions. In the early session, flea abundance declined at a rate of 0.49 for every additional cm increase in precipitation. In the late session, there was no significant effect of winter precipitation on abundance. Mean flea abundance was higher in 2017 than 2016 during both the early and late sessions. NM was only sampled in 2017, and ND was not sampled during the early session in 2016.

was likely too late to capture peak abundance. The relative abundance of *P. simulans* increased through the summer at the three sites where we detected this species (MT, CO, and NM). Our trend was similar to on-host abundance reported by Eads et al. (2015) on black-tailed prairie dogs in New Mexico, and Tripp et al. (2009) found that *P. simulans* on-host abundance peaked in the late fall. Because of its small size, *P. simulans* can be challenging to detect (Eads et al. 2015), so the actual relative abundance may be higher than we report here.

Differences in the total abundance, and potentially relative abundance, of flea species in burrows could be an important factor for plague dynamics in prairie dog colonies. Flea species have different levels of competence as plague vectors (Eskey 1938, Burroughs 1947, Wilder et al. 2008). In laboratory studies, *O. tuberculata* was roughly three times more effective at transmitting plague than *O. hirsuta* (Wilder et al. 2008), so the timing and severity of plague outbreaks could be influenced by the species of flea that is most abundant. Though its exact transmission efficiency remains unknown, the abundance of *P. simulans* could also impact plague dynamics. *Pulex simulans* abundance could have an additional impact because they are host generalists, while *O. hirsuta* and *O. tuberculata* are prairie dog specialists. In particular, *P. simulans* is commonly found on mammalian

carnivores, so this flea species could act as a vector in moving plague to new areas.

We were more likely to collect fleas from active burrows than inactive burrows and from swabs inserted to a greater depth. Our findings are similar to other studies (Salkeld and Stapp 2008, Eads 2017), and it is not surprising that fleas are more likely to be found in active burrows, as they require a host for feeding (Krasnov 2008). Questing fleas may be more abundant deeper in burrows because fleas prefer cooler, more humid conditions (Seery et al. 2003). Fleas are sensitive to desiccation (Krasnov et al. 2001a), and burrows can provide a refuge from hot, dry ambient conditions (Longanecker and Burroughs 1952, Hall and Myers 1978, Bennett et al. 1988, Shenbrot et al. 2002). However, higher flea abundance on deeper swabs could also be a result of proximity to prairie dog nests. *Oropsylla montana*, a flea of California ground squirrels (*Otospermophilus beecheyi*), was two times more abundant in the nests of its hosts than on-hosts (Eskey and Haas 1940). If *O. hirsuta* and *O. tuberculata* have similar patterns on prairie dogs, fleas may be more abundant deeper in burrows independent of the outside temperature.

Our study provides a unique assessment of how environmental conditions at different geographic sites impact off-host fleas. We found that temperature and precipitation are
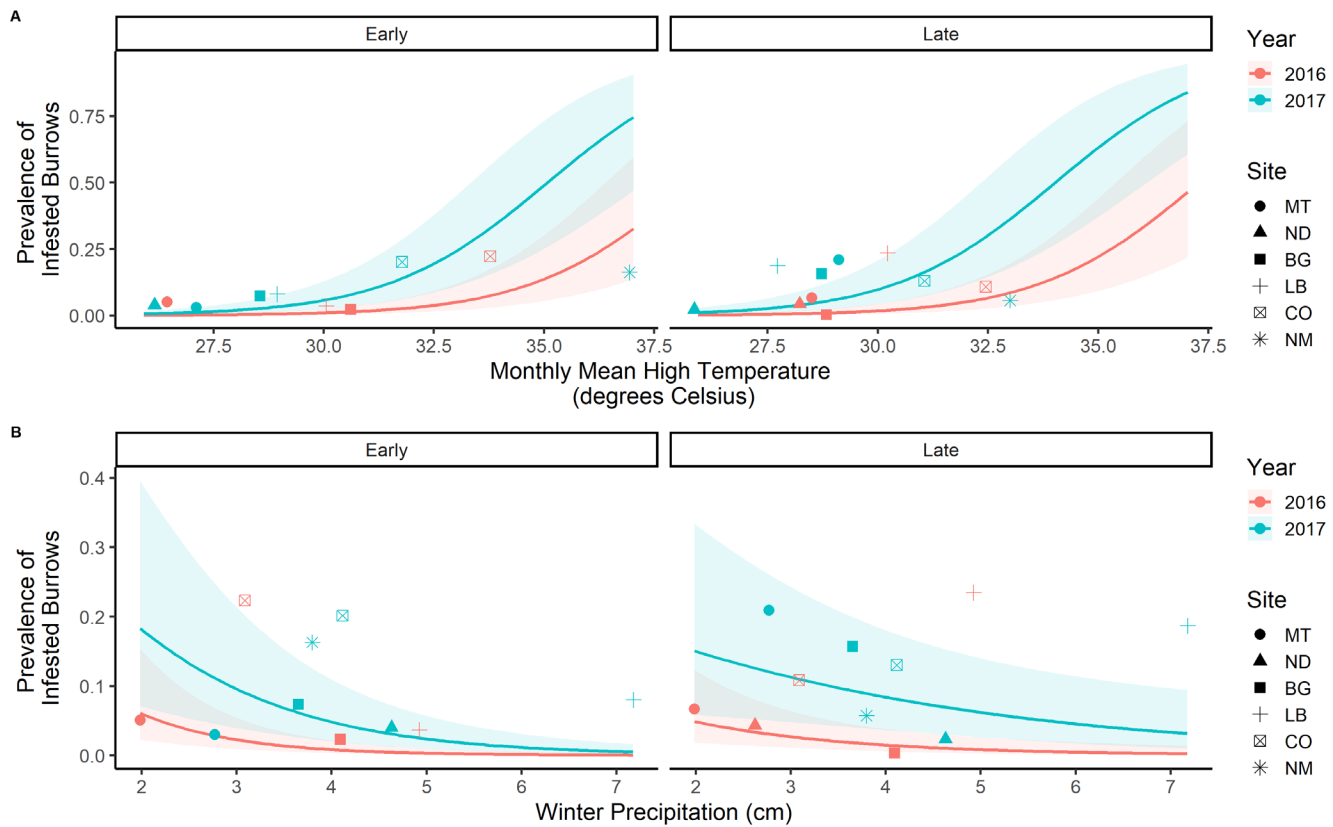
Figure 4. **A)** The likelihood of a prairie dog burrow being infested with fleas increases by 1.73 with every additional 1° C increase in monthly mean high temperature. The relationship between prevalence and temperature remained constant across session and year, though prevalence of flea infested prairie dog burrows was higher in 2017 than 2016 and higher in the early session than the late session. **B)** The relationship between the likelihood of a burrow being infested with fleas and winter precipitation changes between sessions and years. In the 2016 early session, the likelihood of a burrow being infested declines by 64% for every additional cm of winter precipitation. In the 2016 late session, prevalence of infested burrows declines by 46% for every additional cm of winter precipitation. In 2017, the prevalence of infested burrows declines by 53% in the early session and by 28% in the late session for every additional cm of winter precipitation. In both sessions, flea infested burrows were more prevalent in 2017 than 2016. NM was only sampled in 2017, and ND was not sampled during the early session in 2016.

Table 2. Relative abundance (proportion) of *Oropsylla hirsuta* was higher than other species at every site sampled. The proportion of *O. hirsuta* and 95% confidence intervals are shown for each site. Sites are grouped (A-D) if their 95% confidence interval contain the point estimate for another site, indicating that the relative abundance is similar among sites. *Oropsylla hirsuta* relative abundance is lower at MT (group A) intermediate at NM, ND, CO, and BG (groups B and D), and highest at LB (group C).

Site	Proportion of *O. hirsuta*	95% Confidence Interval	Group
MT	0.731	0.678 - 0.780	A
ND	0.920	0.808 - 0.978	B,D
BG	0.968	0.920 - 0.991	B
LB	0.997	0.986 – 1.000	C
CO	0.924	0.902 - 0.942	B,D
NM	0.895	0.837 - 0.938	D

Table 3. Relative abundance (proportion) of *Oropsylla tuberculata* was lower than other species at every site sampled. The proportion of *O. tuberculata* and 95% confidence intervals are shown for each site. Sites are grouped (A-B) if their 95% confidence interval contain the point estimate for another site, indicating that the relative abundance is similar among sites. *Oropsylla tuberculata* abundance is highest at MT and ND (group A) and lower at BG, LB, CO, and NM (group B).

Site	Proportion of *O. tuberculata*	95% Confidence Interval	Group
MT	0.032	0.016 - 0.059	A
ND	0.060	0.013 - 0.165	A
BG	0.016	0.002 - 0.057	A,B
LB	0.003	0.000 - 0.014	B
CO	0.003	0.000 - 0.010	B
NM	0.000	0.000 - 0.023	B

Table 4. *Pulex simulans* was only detected at MT, CO and NM during our sampling periods. At these sites, relative abundance (proportion) of *P. simulans* was lower than *Oropsylla hirsuta* and higher than *O. tuberculata*. The proportion of *P. simulans* and 95% confidence intervals are shown for each site. Sites are grouped (A-C) if their 95% confidence interval contain the point estimate for another site, indicating that the relative abundance is similar among sites. Estimated *P. simulans* abundance was highest at MT (group A), lower at CO and NM (group C), and lowest at ND, BG, and LB (group B).

Site	Proportion of *P. simulans*	95% Confidence Interval	Group
MT	0.236	0.190 - 0.288	A
ND	0.000	0.000 - 0.071	B,C
BG	0.000	0.000 - 0.029	B
LB	0.000	0.000 - 0.009	B
CO	0.057	0.041 - 0.076	C
NM	0.074	0.039 - 0.126	C

related to off-host flea populations in black-tailed prairie dog colonies across a wide latitudinal gradient. At six sites in five U.S. states, flea abundance and prevalence of infested burrows increased with monthly mean high temperature and declined with increased winter precipitation. Salkeld and Stapp (2008) similarly observed that *O. hirsuta* prevalence in black-tailed prairie dog burrows in northeastern Colorado increased with mean monthly temperature. During a single year at multiple sites, Russell et al. (2018) found on-host flea abundance on black-tailed prairie dogs increased through the season (with increasing degree days) and declined with increasing winter precipitation. Studies at single sites have also found that fleas on black-tailed prairie dogs increased in New Mexico during droughts (Eads et al. 2016) and in South Dakota following winters with below average precipitation (Eads and Hoogland 2016). Further research on the impact of large-scale climate variables on both on- and off-host flea abundance is needed, but our results concur with previous studies indicating flea abundance in black-tailed prairie dog colonies increases with temperature and declines with increased precipitation.

Flea loads increase both in burrows (Salkeld and Stapp 2008) and on prairie dogs (Tripp et al. 2009) during plague outbreaks, most likely due to fleas leaving dying hosts and concentrating on remaining hosts and in burrows (Biggins and Eads 2019). Individual fleas may be inefficient transmitters of plague, so increased numbers of fleas on hosts or in burrows may be necessary for plague to transition from low levels of mortality that are difficult to detect to a large outbreak that causes widespread prairie dog mortality (Biggins and Eads 2019). Plague outbreaks among prairie dogs in Colorado were more likely during years following abnormally dry years (Eads and Hoogland 2017). Our results indicate that reduced winter precipitation and elevated monthly mean high temperatures are positively related to off-host flea abundance and prevalence in prairie dog burrows. Because insect development rates increase with temperature, higher temperatures could lead to faster growth of flea populations

and an increase in abundance and prevalence (Kreppel et al. 2016). Temperatures in the Plains states are projected to warm by 1.9-3.6° C by 2085 (Kunkel et al. 2013a, 2013b). Based on our results, off-host flea abundance could increase between 186% and 502% under a change of that magnitude, and prevalence of infested burrows could increase between 169% and 437%. In addition, hot and dry conditions during droughts could also lead to declines in prairie dog body condition and reduced defenses against parasitism, which leads to higher on-hosts flea loads (Eads et al. 2016). Once flea:host ratios are sufficiently elevated, plague transmission could reach levels that trigger a die-off. As prairie dogs nest in burrows, the relevant temperatures for flea development are the prairie dog burrow microclimates. An important step for predicting how plague dynamics may change throughout the century is to determine how temperature and relative humidity in prairie dog burrows impact prairie dog flea survival and development rates.

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