Burrowing owls as potential phoretic hosts of ground squirrel fleas during a plague epizootic

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ABSTRACT: During the course of a plague epizootic, decimation of rodent host populations may result in the transfer of fleas to alternate or phoretic hosts, including to sympatric raptors that prey on rodents. We studied flea abundance and flea species assemblages on burrowing owls (*Athene cunicularia hypugaea*) in southwestern Idaho before (2012 – 2014), during (2015 – 2016), and after (2017) an epizootic of plague in Piute ground squirrels (*Urocitellis mollis*). We examined (1) if a larger proportion of burrowing owl nests contained fleas, (2) the likelihood that owls within a high flea abundance class increased, and (3) if owls harbored ground squirrel fleas during the epizootic. Using a flea abundance index assigned to 1,184 owls from 236 nests, the proportion of nests and the likelihood that owls had high flea abundance decreased rather than increased during epizootic years. Moreover, of 3,538 collected fleas from owls at 143 nests, no fleas were species that Piute ground squirrels typically harbor. Instead, *Pulex irritans* was the predominant flea collected in all study years (> 99%). Thus, although raptors may play a role in plague, there was no evidence that the die-off of ground squirrels resulted in higher flea intensity in burrowing owls or that they served as frequent accidental or phoretic hosts for ground squirrel fleas that could potentially be infectious with *Yersinia pestis*. ***Journal of Vector Ecology* 46 (1): 48-56. 2021.**

Keyword Index: *Pulex irritans*, plague, epizootic, burrowing owl, Piute ground squirrel, phoretic host, Idaho.

INTRODUCTION

Plague is broadly distributed around the world and maintained generally by wild rodents and their fleas in natural foci (Biggins and Kosoy 2001, Gage and Kosoy 2005). First introduced into the United States in 1900, this zoonotic disease is now endemic in its western portions. The etiological agent is the gram-negative bacterium *Yersinia pestis,* and although other routes of transmission are possible, hematophagous fleas are the main vectors of *Y. pestis*. Outbreaks of plague (epizootics) occur in colonial mammals including prairie dogs (*Cynomys* sp.) and ground squirrels (Stapp et al. 2009, Tripp et al. 2009, Salkeld et al. 2010, Jones et al. 2011, Brinkerhoff et al. 2011), during which colonies of some species may be rapidly and completely decimated (Lechleitner et al. 1968, Rayor 1985, Cully et al. 1997, Pauli et al. 2006). As an epizootic progresses and fleas move to alternative hosts (Stapp et al. 2009), the rate of disease transmission may be affected. Moreover, when hosts parasitized by fleas succumb to plague, remaining fleas must seek out alternative hosts. Thus, a plague epizootic in rodent populations increases the likelihood of infection in domesticated animals, humans, and other less typical host species (Kugeler et al. 2015).

Although mammals and especially rodents are susceptible to plague, relationships among *Y. pestis*, plague, and birds remain largely unclear. Birds are typically not subject to infection, but they are believed to be among the possible agents for spreading plague among rodent populations (Stenseth et al. 2008, Gage 2012). Gage (2012) explains that in the course of capturing, killing, and feeding on rodents, birds of prey especially may acquire fleas that are carried to new sites where the fleas leave their accidental host and find new rodent hosts. During the course of a plague epizootic, decimation of flea host populations may also result in increased transfer of fleas to alternative hosts; consequently, epizootics may result in fleas from typical rodent hosts on birds of prey, which may then serve as phoretic hosts, i.e., physically hosting and transporting fleas without necessarily being parasitized.

We studied flea prevalence, intensity, and assemblages of a bird of prey that commonly occupies commensal rodent colonies. Western burrowing owls (*Athene cunicularia hypugaea*, hereafter burrowing owls) are small, ground-dwelling owls of western North America. This owl species has the potential to play a role in plague dynamics because its geographic range overlaps areas with endemic plague. Burrowing owls nest widely in prairie dog colonies (Desmond et al. 2000, Restani et al. 2001, Lantz et al. 2007, Alverson and Dinsmore 2014) and elsewhere inhabit the burrows of other fossorial mammals with frequent exposure to *Y. pestis* (e.g., American badgers, *Taxidea taxus*, Messick et al. 1983). Burrowing owls also hunt rodent prey, including species that are susceptible to plague (Moulton et al. 2005), harbor fleas (Smith and Belthoff 2001a), and undergo widespread migratory (e.g., Holroyd et al. 2010) and other movements that, in theory, could disperse fleas among plague regions (Navock et al. 2019).

Perhaps because of their association with fossorial mammal burrows, burrowing owls often harbor fleas, although the propensity appears to vary geographically (Belthoff et al. 2015). One prevalent flea on burrowing owls is *Pulex irritans* (Siphonaptera: Family Pulicidae; Lewis et al. 1988). Despite a wider geographic range for both burrowing owls and this species of flea, *P. irritans* has adapted to burrowing owls mainly in the Pacific Northwest of the U.S.A. (Lewis et al. 1988). Belthoff et al. (2015) found no fleas on adult burrowing owls in South Dakota, a single flea from 80 adult owls trapped in Colorado, and generally plentiful fleas on adult and nestlings in Oregon, Washington, and Idaho, where 99.4% fleas were *P. irritans*. Migratory populations of burrowing owls in Idaho and Oregon hosted almost exclusively *P. irritans* that exhibited isotopic signatures reflective of breeding grounds or nearby migratory stopover areas, suggesting that owls acquired fleas upon arrival in the breeding areas or had transported fleas only short distances (Navock et al. 2019). Rather than simply using burrowing owls as phoretic hosts, *P. irritans* feeds on burrowing owls and mates while on them (Graham et al. 2016). *P. irritans* can transmit Y. pestis (Blanc and Baltazard 1941, Ratovonjato et al. 2014), so their association and that of other potential fleas with burrowing owls in colonies of commensal rodents is germane to plague dynamics.

Our aim was to examine the frequency with which burrowing owls acted as possible accidental, alternative, or phoretic hosts for flea species more typical of ground squirrels during an epizootic of plague that afflicted ground squirrels in southern Idaho in 2015 and 2016. To do so, we assessed flea abundance on burrowing owls and compared flea assemblages before, during, and after the epizootic under the notion that fleas that more commonly parasitize ground squirrels may pervade burrowing owls during the die-off of their typical hosts. Burrowing owls are common hosts for *P. irritans*, which do not regularly parasitize ground squirrels in our study area (Steenhof et al. 2006), and the proportion of fleas on burrowing owls that are *Pulex* is typically often close to 0.99. We therefore posited that if burrowing owls acted as accidental or phoretic hosts of ground squirrel fleas during an epizootic, there would be appreciable increases in the proportion of owl nests with fleas, increased frequency of owls in the highest flea abundance class, changes in flea species richness on owls, and flea species more typical of ground squirrels more commonly found on burrowing owls during/after years of the epizootic.

MATERIALS AND METHODS

Study site and burrowing owl population

We studied flea assemblages on burrowing owls captured during six successive breeding seasons (2012 to 2017) within the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in portions of Ada and Elmore counties, ID (43.19083° N, 116.150277° W). See Belthoff and King (2002), Belthoff and Smith (2003), and Riding and Belthoff (2015) for descriptions of land cover, topography, and climate. There are no prairie dogs in the study area, so owls nest in abandoned American badger burrows or commonly in artificial burrows installed by researchers (Smith and Belthoff 2001b, Belthoff and King 2002). There are >300 locations in the NCA in which artificial burrows have been installed, often in clusters of two or three underground burrows (Belthoff and Smith 2003, Welty et al. 2012 for maps of portions of the study areas) in which ~40 to 60 pairs of burrowing owls nest each year. Piute ground squirrels (*Urocitellis mollis*) occur in most, if not all, areas where burrowing owls nest in the study area (J. Belthoff, pers. observ.). Although fleas occur on owls that nest in both artificial and natural burrows (Smith and Belthoff 2001a, Belthoff et al. 2015), most of the nests where we captured owls to assess flea prevalence and intensity were in artificial burrows. All but one flea collection were made from owls associated with an artificial burrow nest when captured; the exception was an adult male burrowing owl captured on 11 April 2013 at the entrance to a natural burrow nest. However, even when nesting in artificial burrows, individuals within a family of owls use nearby natural burrows (i.e., those dug by badgers) for cover, roosting, and to cache prey (personal observation). The additional burrows nesting adult owls and their offspring use are a regular feature of burrowing owl breeding biology and are referred to as satellite burrows (Smith and Belthoff 2001b). Thus, a burrowing owl nesting site generally includes the nest burrow (i.e., where eggs are laid and incubated) and nearby (within 5 m in the case of artificial burrows) satellite burrows, where either young owls or adults may be located before the young fledge and ultimately disperse. Ground squirrels also find the artificial burrows attractive and frequently colonize them for nesting/cover.

While breeding, burrowing owls lay eight to 12 eggs that females incubate for ~22 d (Conway et al. 2012), and nestlings remain in nests for ~1 mo while also making increased use of nearby burrows (natural and artificial) for roosting as they age. Nestlings gradually disperse from the vicinity of their nest as summer progresses (King and Belthoff 2001), although they remain associated with animal burrows, and adults and nestlings initiate southward migration in late summer or early autumn. Although many of the artificial burrow sites we studied are occupied by breeding owls in multiple years (Belthoff and Smith 2003), between reproductive years many owls re-pair and relocate to different nest locations (especially females), as the NCA population has among the highest breeding dispersal rates (~80%) for burrowing owls throughout their range (Riding and Belthoff 2018). Owls breeding in the NCA winter predominantly in southern California or Mexico (Navock et al. 2019), although a small number of individuals may remain in breeding grounds for the winter.

Antibody surveillance focused on American badgers indicates that plague persists in the study area primarily in enzootic form (Messick et al. 1983). However, during 2015 and 2016, there was an epizootic involving die-offs of Piute ground squirrels in areas between Boise (Ada County) and Mountain Home (Elmore County), including in our NCA study area (Idaho Department of Health and Welfare, Division of Public Health, Idaho Department of Fish and Game, and

Central District Health Department public notices dated 22 May 2015 and 27 May 2016). The epizootic, confirmed via tissue testing by state wildlife and public health agencies, resulted in obvious clusters of ground squirrel carcasses above ground combined with presumptive underground mortality of squirrels. The epizootic also ultimately infected five domestic cats (four of which died) locally and one pet dog that recovered, but there were no human cases.

Owl capture

We routinely monitored nesting attempts at artificial and natural burrow locations and captured adult owls and their offspring in each breeding season between 2012 and 2017. While working with owls in the field, investigators typically wore long sleeves and pants and regularly applied insect repellent containing >30% DEET following Boise State University animal care and institutional biosafety procedures. During the epizootic, nitrile gloves were used to handle all owls. Adult burrowing owls were captured using rectangular wire traps (15 cm wide x 14 cm high x 46 cm long) equipped with hardware cloth one-way doors at both ends that we placed at burrow entrances. These traps were used frequently in combination with a portable mp3 player (SanDisk Clip Jam, Western Digital Corporation, San Jose, CA) and a 9 x 4 cm cylindrical Wi-Fi speaker (MakeTheOne, Portland, OR), or similar, that broadcasted the owl's primary call (*coo-coo* call, modified from an audio file originally obtained from Cornell Laboratory of Ornithology, allaboutbirds.org). Nestlings were captured typically when about two to four weeks of age by hand from artificial burrow nest chambers or nearby satellite burrows after excavation of chamber lids, or from outside of burrows near the tunnel entrances. All newly captured owls were banded with a numbered U.S. Geological Survey metal leg band for identification and measured, weighed, and scored on a flea abundance index (see below). Adult female burrowing owls are recognizable from brood patches they develop during the breeding season; otherwise their plumage is similar to adult males. Only females incubate the eggs, while males provision females with prey. Nestlings are monomorphic in plumage so their sex class is typically unknown at the time of capture. After processing, captured owls were released at original capture locations by returning them to the mouth of the burrow from which they were captured.

Flea abundance index on owls

For each captured owl, we estimated flea prevalence and intensity based on the approximate number of fleas seen on the owl and on our hands during handling, similar to how Rendell and Verbeek (1996) counted hematophagous lice. Flea abundance was approximated using an ordinal index as none (no fleas seen), low (one to four fleas), medium (five to nine fleas), or high (≥ 10 fleas) following previous research of burrowing owl ectoparasites in the Morley Nelson Snake River Birds of Prey NCA (Riding and Belthoff 2015). We considered a burrowing owl nest to have fleas if one or more individuals captured from that nest had one or more fleas. We used flea index values to estimate proportion of nests with and without fleas and examined the likelihood that owls were in a derived 'high abundance class' to help understand changes in flea abundance on owls before, during, and after the epizootic (see Statistical Analysis below).

Flea collection and identification

Fleas were also collected opportunistically in April to July in 2012 through 2017, primarily from the plumage of captured owls, identified to species, and then used to help characterize flea species assemblages before, during, and after the epizootic. These collections occurred on a subset of the owls described above for which the standardized flea abundance index was assigned to all. With the exception of three owl nest sites in 2013 at which fleas were collected using a flannel cloth placed in the nest chamber because eggs rather than nestlings were present, we collected fleas from adult or nestling owls with tweezers or an aspirator. Sometimes, flea capture included a white flannel cloth beneath the owls that helped entangle for capture fleas that jumped from an owl. Fleas were stored frozen (-20° C; in 2012 and 2013) or mixed with 70% ethanol in the field (2014 through 2017).

Importantly, unlike the systematic assignment of flea abundance as described above, which was standardized and comparable among years, flea collection effort and approach differed between and among individual owls, owl nest sites, and study years, so collections were not sufficiently standardized for comparison of flea abundance between years. For instance, there was no attempt to collect all fleas from individual owls to represent an actual count, collected fleas were often pooled from all owls at a nest site rather than sorted by individuals, so actual number of fleas per individual owl is not known and, in one year (2017), fleas were only collected from up to three nestlings in a brood rather than from all individuals. For these and related reasons, we used collected fleas only to assess possible changes in flea species richness and flea species assemblage among years for which we believe they represent a sufficient random sample.

Fleas were taxonomically identified where possible under a dissecting microscope and at times using a standard clearing procedure to lighten the flea for morphological evaluation (Hastriter and Whiting 2003, Whiting et al. 2008). Nearly all fleas collected from burrowing owls (see results) were *Pulex* sp. Morphological features allow researchers to distinguish male *Pulex* fleas as *P. irritans* or *P. simulans* (a closely related congener more typical of occurrence on prairie dogs than *P. irritans*). There are currently no known morphological features in female *Pulex* fleas to distinguish between these two species, so we considered all female *Pulex* to be *P. irritans,* as all of the males present were *P. irritans*. Previous studies using molecular tools supported this approach to identification of *P. irritans* females (Belthoff et al. 2015).

Ground squirrel fleas

The best available data on Piute ground squirrel fleas for comparison with burrowing owl flea assemblages come from Steenhof et al. (2006) working in our same study area. In 2003, these investigators collected 905 fleas from Piute ground squirrels that included: *Oropsylla tuberculata* (n = 614, 67.8%), *Thrassis francisi* (n = 282, 31.2%), *Rhadinopsylla sectilis* (n = 8, 0.9%), and *Rhadinopsylla fraterna* (n = 1, < 0.1%). As there have been no more recent collections, our assumption is that ground squirrel flea assemblages approximated this complement of species and these proportions during the years of our study. Of note is that *O. tuberculata*, the most abundant flea species on Piute ground squirrels in the previous collections, has been involved in plague epizootics elsewhere (Friggens et al. 2010).

Statistical analysis

To examine changes in relative flea intensity on burrowing owls that may have resulted from the plague epizootic, we chose to dichotomize the flea abundance index by assigning owls with few or no fleas to a 'low flea class' and those with higher fleas (medium or high) to the derived 'high flea class.' We believe these two classes helped minimize the number of owls incorrectly placed into one of the four index levels because of a small number of fleas being misestimated, for example. Further, we reasoned that increases in flea numbers on burrowing owls accompanying the die-off of ground squirrels from plague should be reflected in an increase in the likelihood of owls being in the high flea class. We used generalized linear mixed models to assess the potential effect of year (categorical, fixed effect) on the likelihood of individuals expressing the high flea class (binomial distribution, logit link), while including a nest burrow variable as a random effect to account for observations of multiple individuals of a family at nest sites and sites used more than once among years. Using linear contrasts, we examined the hypothesis that the odds of being in the high flea class increased during epizootic years by comparing (1) the years before and during the epizootic, and (2) the years during and after the epizootic. We report contrast parameter estimates, odds ratios (OR), 95% confidence bounds on the OR, and results of null hypothesis tests that the parameter estimates = 0. We also conducted the GLMM analysis twice: once including all owls at a nest site (adults and nestlings) and separately including nestlings only. Inferences for both approaches were the same for comparisons between the pre-epizootic and epizootic years, but the comparison between epizootic and post-epizootic years was significant only in nestlings (see Results).

We characterized flea species assemblages in burrowing owls from collections made in 2012 – 2014 (pre-epizootic period), 2015 – 2016 (during the epizootic of plague in ground squirrels), and after the epizootic in 2017. We tabulated number of fleas collected, the percentage of fleas that were *Pulex irritans* and non-*Pulex,* and the number and percentage of fleas that were more representative of the assemblage typical of Piute ground squirrels. Using a Fisher's Exact Test, we evaluated the null hypothesis that the probability of non-*Pulex* fleas, because these species would be more characteristic of ground squirrels, was similar in the years before the plague epizootic compared with the time period during/after. Ultimately, there were so few ground squirrel fleas noted on burrowing owls that a statistical comparison of species richness before and during/after the epizootic was not warranted. All statistical analyses were conducted using JMP Pro v.15.2 or SAS v.9.4 (SAS Institute, Cary, NC, U.S.A.), means ± 1 SD are presented throughout except where noted, and effects were considered significant when alpha < 0.05.

RESULTS

Nests with and without fleas

Burrowing owls were captured at 236 nests (n = 98 different artificial or natural burrow locations) in 2012 – 2017 and scored for flea abundance. Most nests had some individuals (adults and/or nestlings) for which at least one flea

Table 1. Number of adult female, adult male, and nestling burrowing owls whose derived flea abundance class was low (≤ 4) or high (≥ 5) at owl nest sites in three years before an epizootic of plague in Piute ground squirrels (2012 – 2014), two years during the epizootic (2015 – 2016, shaded), and after (2017) the epizootic in southwestern Idaho, U.S.A.

Year	Adult Female		Adult Male		Nestlings		No. Owl Nests[1]	No. Owls Scored/Nest (mean ± SD)
	Low	High	Low	High	Low	High		
2012	23	0	3	0	106	111	36	6.8 ± 2.9
2013	25	1	15	5	43	61	38	3.8 ± 2.2
2014	13	0	18	1	45	34	21	5.3 ± 3.4
2015	14	1	24	1	152	28	38	5.7 ± 2.9
2016	37	1	21	1	196	73	58	5.6 ± 2.7
2017	28	1	33	2	41	26	45	3.0 ± 2.3

[1] Includes seven natural burrow nests and 229 artificial burrow nests.

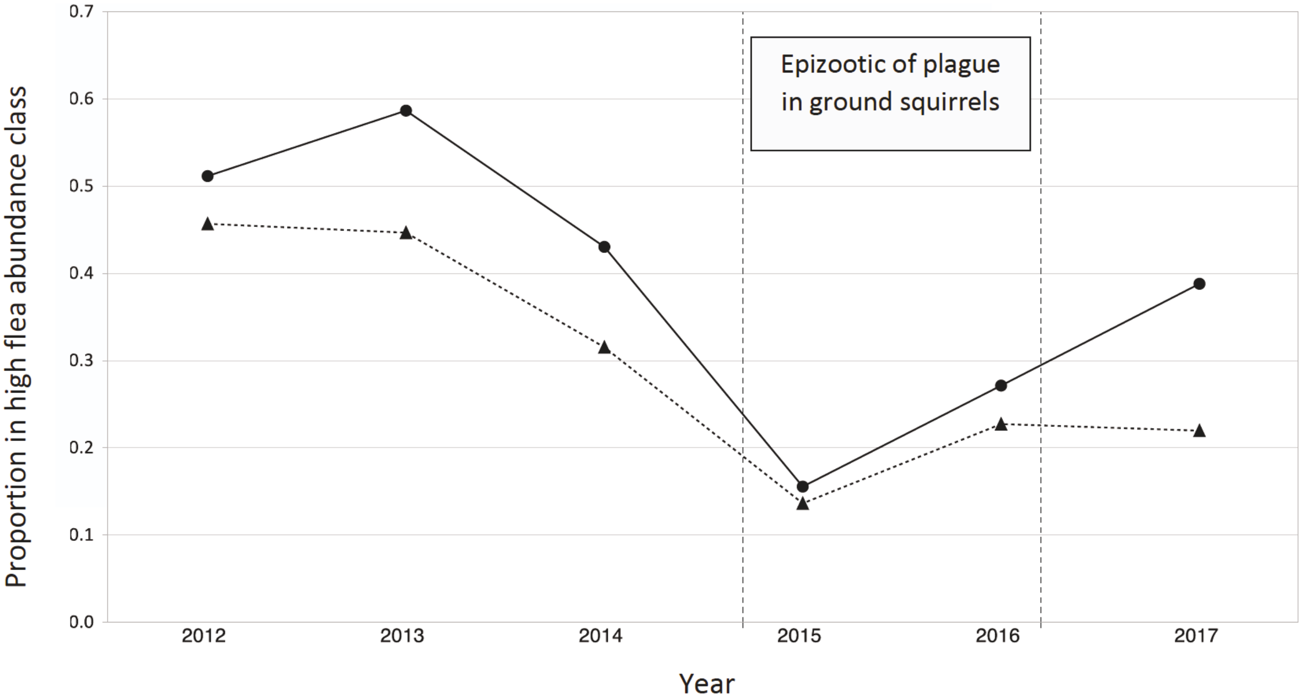

Figure 1. Proportion of individuals with fleas in the highest abundance class in burrowing owls captured before, during, and after an epizootic of plague in Piute ground squirrels in southern Idaho, U.S.A. Data are for all individuals (adults and nestlings; triangles and dashed line) and for nestlings only (circles and solid line; sample sizes are in Table 1). The odds of burrowing owls being in the high flea class was significantly greater in the pre-epizootic years (2012 – 2014).

Table 2. Number of fleas collected from burrowing owls captured at nest sites in three years (2012 − 2014) before an epizootic of plague in Piute ground squirrels, in two years during the epizootic (2015 − 2016, shaded), and one year after (2017) the epizootic in southwestern Idaho, U.S.A.

Year	Dates	Nest Sites	Fleas Collected	Range of Fleas per Nest Site
2012	29 May – 30 Jun	24	774	1 – 199
2013	11 Apr – 21 Jun	29[1]	1,686[2]	1 – 282
2014	5 Jun – 17 Jun	8	92	1 – 27
2015	30 May – 6 Jul	17	198	1 – 40
2016	5 May – 22 Jun	43	630	1 – 53
2017	1 Apr – 29 Jun	24	149	1 – 18

[1] includes one natural burrow nest site.

[2] includes n = 11, 30, and 38 fleas collected using flannel placed in nest chambers with owl eggs rather than collected directly from owls.

was observed, such that the proportion of nests in each year with fleas was generally high (0.94, 0.74, 0.76, 0.71, 0.81, and 0.60 in 2012 – 2017, respectively). Mean annual proportion of nests with at least one flea before the epizootic was 0.81 ± 0.11 (n = 3) and 0.76 ± 0.07 during the epizootic (n = 2). The proportion of nests with at least one flea was lowest in the year after the epizootic.

Owl flea abundance

The flea abundance index was available for 1,184 burrowing owls captured in 2012 – 2017, including 144 adult females, 124 adult males, and 916 nestlings (n = 236 owl nests) from both artificial and natural burrow nest sites (Table 1). Of these, 2.8% of females, 8.1% of adult males, and 36.4% of nestlings had flea index values in the derived 'high flea class' (Table 1).

There was a significant effect of Year on the odds of owls being in the high flea class both when assessing all owls at a nest site (binomial logistic regression: $F_{5,134} = 23.37$, $p < 0.001$; Figure 1) and nestlings only ($F_{5,92} = 24.15$, $p < 0.001$; Figure 1). The odds of being in the higher flea class were 7.4 times greater in the pre-epizootic years (estimate = 2.0 ± 0.22 [SE], $t_{134} = 8.97$, $p < 0.001$; Lower and Upper 95% confidence bounds of OR = 4.8 and 11.6, respectively) when including all owls at a nest site. This comparison was similarly judged as significant when nestlings only were considered (estimate

Table 3. Number and identity of fleas (annual number and percentage) collected from burrowing owls at nest sites in southwestern Idaho before (2012 — 2014), during (2015 —2016, shaded), and after (2017) an epizootic of plague affecting Piute ground squirrels to assess the possibility that burrowing owls acted as phoretic or accidental hosts for ground squirrel fleas during the epizootic.

Year	Fleas collected	Number of *Pulex irritans* (Annual %)	Number of Non-*Pulex irritans* (Annual %)	Number of Piute ground squirrel fleas[1] (Annual %)
2012	774	773 (99.9)	1[2] (0.1)	0 (0)
2013	1,686[3]	1679[3] (99.6)	7[4] (0.4)	≤1[5] (0.0)
2014	92	92 (100)	0 (0)	0 (0)
2015	198	198 (100)	0 (0)	0 (0)
2016	630	629 (99.8)	1[6] (0.2)	0 (0)
2017	149	149 (100)	0 (0)	0 (0)

[1] Based on species composition of fleas on Piute ground squirrels identified by Steenhof et al. (2006) for our study area.

[2] *Hoplospyllus anomalus* (n = 1).

[3] Includes 10 *Pulex irritans* collected from an adult male at a natural burrow and 79 *P. irritans* collected from three different owl nests with eggs using flannel.

[4] *Aetheca wagneri* (n = 5), one unknown flea for which the four species flea found on Piute ground squirrel fleas were excluded, and one flea only identifiable as non-*Pulex*.

[5] Up to one flea if the non-identifiable flea above was one of four species characteristic of Piute ground squirrels; otherwise n = 0.

[6] *Hoplospyllus anomalus* (n = 1).

= 2.9 ± 0.29 [SE], OR confidence bounds = 10.5 and 33.6, t_{92} = 10.02, $p < 0.0001$). There was no difference in the odds of being in the high flea class between the epizootic years and the year after (t_{134} = -0.26, p = 0.794) for all owls at a nest site, but odds of being in the higher flea class were significantly greater (OR = 2.7) in the year after the epizootic (estimate = 1.0 ± 0.46 [SE], OR confidence bounds = 1.1 and 6.6, t_{92} = 2.10, p = 0.038) when considering only nestlings.

Flea species assemblage

There were 3,538 fleas collected from 143 burrowing owl nest sites (24 ± 12 nest sites per year) during 2012 – 2017, with an average of 590 ± 609 fleas per year (Table 2). The three flea collections performed in 2013 using flannel placed inside the nest chamber accounted for 79 of the collected fleas, and the one adult male captured at a natural burrow in 2013 yielded ten fleas (Table 2).

In all study years, > 99% of collected fleas were identified as *P. irritans* (Table 3), including all fleas collected using flannel inside owl nests and all fleas on the adult male captured at a natural burrow. Only nine individual fleas (0.2%) were identified as non-*Pulex* (Table 3); these included *Aetheca wagneri* (Family Ceratophyllidae, n = 5) and *Hoplospyllus anomalus* (Family Pulicidae, n = 2, Table 3). The other two non-*Pulex* fleas had anatomical morphology for which the four species of Piute ground squirrel fleas could be excluded or for which identification was only possible as non-*Pulex* (Table 3).

The proportion of non-*Pulex* fleas on burrowing owls did not increase during/after the epizootic (proportion of non-*Pulex* fleas before = 0.003, proportion during/after = 0.001; Fisher's Exact Test, p = 0.46; Table 3). Irrespective of time period, essentially none of the flea species typical of Piute ground squirrels was collected from burrowing owls during the six-year study (Table 3).

DISCUSSION

The extent of transport of *Y. pestis*-infected fleas from one rodent population to another is important for understanding the dynamics of plague (Salkeld et al. 2016, Maestas and Britten 2017). In prairie dog colonies, for instance, the movement of infected prairie dogs, or the movement of infectious fleas on prairie dogs, can account for a large portion of plague spread and persistence (Stapp et al. 2004). *Y. pestis* dispersal may also be explained by the wider-ranging behavior of carnivores or other plague resistant species that carry infected fleas (Girard et al. 2004, Snäll et al. 2009, George et al. 2013). Stapp et al. (2009) speculate that grasshopper mice (*Onychomys leucogaster*) also help spread *Y. pestis* during epizootics in prairie dog colonies through their ability to survive infection, harbor prairie dog fleas (*Oropsylla hirsuta*), and transport infected fleas among burrows (see also Salkeld et al. 2016).

Burrowing owls are common inhabitants of prairie dog colonies and other settings with colonial rodents that experience enzootic plague and epizootic outbreaks, such as the one we observed in southern Idaho involving Piute ground squirrels. However, there was no evidence that owl flea intensity increased or that owls served as phoretic hosts for ground squirrel fleas. Instead, we observed that the odds of being in the highest flea class were significantly greater in the years *before* the ground squirrel die-off and, at least for nestling owls, significantly higher again following the plague years. Proportion of nests with any fleas also, on average, tended to decrease during the epizootic. Thus, the pattern of change of the odds of burrowing owls being in the highest flea abundance class was seemingly the opposite of that expected if ground squirrel fleas moved in large numbers to burrowing owls after the death of their more typical hosts.

If the declines in owl flea prevalence and apparent intensity were related to the epizootic, one potential explanation is that species other than ground squirrels were also affected by the epizootic, either directly or indirectly. For example, if sympatric canids (coyotes, foxes) or mustelids such as badgers or weasels, some of which regularly harbor *P. irritans* (Dobler and Pfeffer 2011 and references therein), suffered ill effects of plague, their abundance or activity could have dropped. This would have resulted in fewer interactions and ultimately lower rates of transference of *P. irritans* to burrowing owls. Also, perhaps these species of predators frequented the plague areas less often because there were fewer ground squirrels on which to prey following die-offs. A different possibility not necessarily directly related to the epizootic might be that weather or some other environmental factor changed during 2015 – 2016 to make it less conducive for *P. irritans* to proliferate or survive during the time periods of owl capture. Previous studies have reported annual variation in flea abundance on burrowing owls (Riding and Belthoff 2015), but factors driving any patterns in this variability remain largely unexplored.

Rucker (1909) noted that burrowing owls were constant companions of the "ground squirrel," occupying the same burrows, and suggested that they may play an important role in dissemination of an epizootic of plague, perhaps by flying from burrow to burrow while carrying infected fleas. Jellison (1939) later recovered 109 live rodent fleas of six species from a burrowing owl nest in a plague area near Dillon, MT, a result that suggests the potential for burrowing owls as phoretic hosts for infectious fleas if those flea species colonized owls. However, in a large sample of owls captured from nests within the plague area we studied, ground squirrel fleas were essentially not detected on burrowing owls (or in the nests with eggs that were examined via burrow swabs), so they did not increase in proportion on owls during the epizootic years. It is possible that ground squirrel fleas may have remained on owls for only short durations, which meant our potential to detect them was lowered, but we believe the absence of typical ground squirrel fleas in the samples we collected from owls is more likely evidence of the lack of substantial movements to owls.

The two most prevalent fleas on Piute ground squirrels in southwestern Idaho are reported to be *Oropsylla tuberculata* and *Thrassis francisi*, which together comprise 98% of the fleas present (Steenhof et al. 2006). During the epizootic of plague that we studied, however, these and other flea species characteristic of Piute ground squirrels (Steenhof et al. 2006) were not collected from burrowing owls or from nests. The absence of ground squirrel fleas on burrowing owls was apparent before, during, and after the epizootic. Instead, the predominant flea species collected from burrowing owls was *Pulex irritans*, which is a species that was not recorded on the ground squirrels (Steenhof et al. 2006).

Nonetheless, *Pulex irritans* is a competent plague vector (Burroughs 1947, Eisen and Gage 2012, Ratovonjato et al. 2014), but the only apparent record of burrowing owls harboring plague-infected fleas is from Wheeler et al. (1941) who collected 70 sticktight fleas, *Echidnophaga gallinacea*, from a burrowing owl following an epizootic in California. These fleas were mass inoculated into a test guinea pig and proved to be infected with plague organisms. Wheeler et al. (1941) concluded that this was the first record of a bird hosting plague-infected parasites and the first demonstration of natural plague infection in this species of flea. No evidence of *Y. pestis* was detected in a previous study of *P. irritans* collected from burrowing owls (Belthoff et al. 2015).

Bird-facilitated dispersal of potential vectors of disease is of particular interest because it can have rapid and far-reaching consequences for disease dynamics and for human public health (Hubálek 2004, Altizer et al. 2011, Viana et al. 2016). For example, migratory birds carrying ticks spread the pathogens that cause Lyme and other diseases (Palomar et al. 2012, Cohen et al. 2015). Similarly, wild birds have an important theoretical role in plague, but one that is less well understood than in many other disease systems. Our results documented the absence of *en masse* accumulation of ground squirrel fleas during an outbreak of plague by a sympatric species of raptor and indicated that burrowing owls did not regularly become phoretic or accidental hosts for these species of fleas in this epizootic.

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