Increased Visitation at Urban Water Sources by Bats and Raccoons: Implications for Cross-species Transmission of Rabies

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Abstract

We examined the potential for urban water sources to act as centers for rabies transmission from bats to mesocarnivores in the arid southwestern United States where free water is often limited. Because residential housing can act as den and roost sites for both mesocarnivores and bats, we also examined the effect of housing density on abundance. Using ultrasonic acoustic recorders to assess bat activity and camera traps to estimate mesocarnivore abundance, we compared 14 pairs of wet and dry locations over two years by surveying twice during the summer, once prior to summer monsoons and once during the monsoon season, when surface waters were more available. Number of calls for all bat species combined were greater at wet sites compared to dry sites and calls of two bat species often associated with rabies, big brown bat (*Eptesicus fuscus*) and silver-haired bat (*Lasionycteris noctivagans*), were recorded more at wet sites than dry sites in the monsoon season. In both years, raccoons (*Procyon lotor*) were photographed more often at wet sites while striped skunks (*Mephitis mephitis*) and gray foxes (*Urocyon cinereoargenteus*) were less likely to be detected at wet sites. Bat, fox and raccoon abundance was not associated with housing density while striped skunks showed a positive correlation with housing density. Higher abundance of bats at urban waters could increase potential for cross-species transmission of rabies from bats to mesocarnivores primarily for raccoons.

Introduction

The rabies virus is a zoonotic disease that threatens human health on a global scale. Although most human fatalities worldwide occur due to exposure from rabid dogs (World Health Organization, 2018), in North America, canine rabies has been nearly eliminated due to effective vaccination programs and the major rabies reservoirs are now bats and mesocarnivores (Gilbert, 2018; Velasco-Villa et al., 2017). Rabies virus variants typically circulate within reservoir populations, with cross-species transmission to non-reservoir species during outbreaks. Cross-species transmission typically leads to dead-end infections, but more rarely host-shift events may occur in which the virus adapts to the new host and is maintained in that host through time (Badrane and Tordo 2001). For example, three independent host-shifts occurred between 2001 and 2009 in Flagstaff, Arizona, when striped skunks (*Mephitis mephitis*) and gray foxes (*Urocyon cinereoargenteus*) became infected with a rabies variant associated with the big brown bat (*Eptesicus fuscus*) (Leslie et al. 2006, Kuzmin et al. 2012). Cross-species transmission is a concern because with every transmission, the rabies virus has an opportunity to adapt to a new reservoir host, potentially undermining rabies management programs and threatening public health (Wallace et al. 2014). Therefore, it is important to identify the environmental factors that may promote interspecies interactions and increase the potential for transmission between species.

One environmental factor that could affect the probability of cross-species interaction and disease transmission is creation of artificial waters in urban landscapes, especially in water-limited environments like the arid southwestern USA. Heightened levels of bat activity around surface waters are well documented (Anderson et al. 2006; Lisón and Calvo 2011) and likely driven both by need of bats to drink and higher levels of insect prey near water (Salvarina et al. 2018). Water availability is also critical for
lactation (Mclean and Speakman 1999), as demonstrated by the tendency of many species to establish maternity colonies near standing water (Walker et al. 2021, Adams and Thibault 2006). Free water is a fundamental requirement for terrestrial mammals as well (Leopold 1933, Nagy 2004) especially for wildlife in arid landscapes (Rosenstock et al. 1999). Proximity to water has been positively associated with occupancy and space use by mesocarnivores (Dias et al. 2019, Kluever et al. 2017) and anthropogenic water sources are commonly used to benefit wildlife in arid water-limited landscapes (Krausman et al. 2006).

In addition to water, both bats and mesocarnivores may respond to increasing density of human development. Mesocarnivores like skunks, foxes, and raccoons (Procyon lotor) often reach higher population densities in urban and suburban areas than in rural areas (Bateman and Fleming 2012) and often use anthropogenic food sources and structures as den sites (Hadidian et al. 1991, Theimer et al. 2017). Bat responses to urbanization and housing density vary but several species, including big brown bats, commonly roost in buildings (Agosta 2002; Neubaum et al. 2007, Fagan et al. 2018, Walker et al. 2021) while others may roost in large, non-native trees planted by humans in urban areas (Evelyn et al. 2004, Kubista and Bruckner 2015).

In this study, we examined the potential for artificial waters and housing density to influence the probability of cross-species interaction in a water-limited landscape. We predicted that bat activity and mesocarnivore detections would increase around artificial water sources and with increasing housing density.

**Study Areas**

Between 5 June and 5 August of 2018 and 2019 we collected bat acoustic recordings and trail camera images of mesocarnivores in three cities in northern Arizona USA: Williams, (35.2495°N, 112.1910° W), population = 3,200, elevation = 2061 meters; Flagstaff (35.1853°N, -111.6519°W), population = 73,964, elevation = 2105 meters; and Pinetop-Lakeside (34.1425°N, 109.9604°W), population = 4,433, elevation = 2073 meters. All three cities lie within extensive ponderosa pine (Pinus ponderosa) forests and experience a monsoonal rainfall pattern during summer, in which the month of June is typically dry while monsoonal rains begin in July.

In each city, we used a paired study design, with paired sites consisting of a dry and wet site. Dry sites lacked water bodies; no water sources were within 500 meters. Wet sites included artificial water bodies, ranging from 0.02 to 1.59 hectares in size. Within each pair, sites were ≥ 500 meters but ≤ 1000 meters apart. Nine pairs were located in and around Flagstaff, two pairs were in Williams and three pairs were in Pinetop. All fourteen pairs (28 sites) were located on golf courses within a suburban matrix. To control for the lack of tree canopy caused by the presence of water at wet sites, we chose dry sites that had open canopy areas of similar size.

**Methods**
To monitor bat activity, we deployed SM3BAT bioacoustics recorders (Wildlife Acoustics, Maynard, Massachusetts, USA) paired with an omnidirectional U1 ultrasonic microphone for six consecutive nights at each site. Each site was sampled once in June and once in July in both years to allow us to test for the effect of the monsoon (July) and pre-monsoon conditions (June). To account for spatial variation in bat activity, we moved our detectors every two nights, surveying each site at three survey points. The three survey points were spaced evenly around each site (Fig. 1). We mounted microphones to 12.7 mm conduit tubing measuring 2.5 meters tall. The recorders were programmed to initiate recording 30 minutes prior to sunset and cease recording 30 minutes after sunrise.

To monitor mesocarnivores, we deployed Reconyx HyperFire HC500 and Bushnell Trophy Cam HD trail cameras for 6 nights at each site concurrent with bat monitoring. We placed cameras in steel boxes mounted on trees approximately 0.5 meters above ground. During the 2018 field season, we used a single camera at each site, which we moved to a new location every two nights. During the 2019 field season, we used four cameras at each site and the cameras remained in a fixed position for all six nights.

To process bat recordings, we first used Sonobat Batch Scrubber 5.4 (Sonobat ™, Arcata, CA, USA) to eliminate any non-bat vocalizations and then used Sonobat version 3.2.1 U.S. West (SonoBat™, Arcata, CA, USA) to classify bat calls to species level when possible. Calls were defined by classifying an entire sequence (bat pass). Big brown and silver-haired bats (*Lasionycteris noctivagans*) are often associated with rabies virus in North America (Finnegan, et al. 2002) but their calls could not be distinguished from each other consistently due to similarities in call structure and frequency, so we considered them as a single category hereafter referred to as “Epfu/Lano calls. For all of our analysis, we utilized the recommended default Sonobat settings: setting maximum number of calls per file to 8, acceptable call quality to 0.80, acceptable quality to tally passes of 0.20, and a decision threshold of 0.90.

To process mesocarnivore data, we downloaded data from SD cards and recorded species, number of individuals, time of detection, and site. To minimize the chance of counting the same animals more than once, we considered all images of the same species photographed within 30 minutes of each other as the same individual. For images with multiple individuals of the same species, we counted each individual as a separate detection.

To estimate housing density around each site, we downloaded aerial imagery for the state of Arizona from the National Agriculture Imagery Program (NAIP) and used proximity tools in ArcMap 10.6.1 (ESRI, Redlands, CA) to create 1 km buffers around each study site. We determined housing density by counting the number of structures that we could distinguish within each buffer area. Garages and sheds that were separate from houses were counted as individual structures since they could be used for dens and roosts (Theimer et al. 2017).

**STATISTICAL ANALYSIS**

We compared activity (measured as rate of calls per hour) between wet and dry sites using 1) calls of all bats combined and 2) the subset of calls identified as either *Eptesicus fuscus* or *Lasionycteris*
*noctivagans* (Epfu/Lano). We used linear mixed modeling to predict activity of all bat species combined and Epfu/Lano separately. We considered “water”, “monsoon” and “housing density” to be fixed effects. “Water” was a categorical variable that indicated if a site had water present or not. “Monsoon” was a categorical variable that indicated if the data were collected in pre-monsoon (June) or monsoon conditions (July). “Housing density” was a numerical variable that reflected the number of structures within 1 km of each site. We included the random effects Site and Year since we expected site-to-site variation (e.g., bat populations are not evenly distributed throughout the landscape). Year accounted for some of the year-to-year variation in environmental conditions such as strength of the annual monsoon. Models were constructed and compared with Akaike information criterion (AIC) (Akaike 1973). Because distributions for both of our response variables were heavily skewed, consistent with a lack of homoscedasticity, we log-transformed our response variables. We then used pair-wise contrasts to test for differences between wet and dry sites in total bat calls or Epfu/Lano calls.

Mesocarnivore data were analyzed at the species level using logistic regression. We constructed separate models for skunks, raccoons, and foxes. The dependent variable for each model was converted into a discrete variable (0, 1) with detection equal to 1 and no detection equal to 0. The models were constructed with the independent variables: water, monsoon and housing density. Competing models were again compared based on Akaike information criterion. A pair-wise comparison between wet and dry sites allowed us to determine the probability for detection for each mesocarnivore species. All analysis were conducted with R (R Core Team. (2016). *R: A Language and Environment for Statistical Computing*. Vienna, Austria).

**Results**

We recorded 1,157,091 acoustic bat passes over 432 survey nights at 28 study sites. When pooled across all bat species, the mean call rate (± SE) at wet sites (388 ± 80) was significantly higher (*P* < 0.0001, α = 0.05) than dry sites (53 ± 11) (Figure 2). We did not detect an effect of monsoon (F = 0.49, *P* = 0.48) or housing density (F = 0.12, *P* = 0.73) (Table 1). Epfu/Lano calls accounted for 12% of all bat recordings. The number of Epfu/Lano passes recorded was significantly higher (*P* < 0.0002, α = 0.05) at wet sites (4.5 ± 2) than dry sites (1 ± 0.5) and activity for the Epfu/Lano group was higher during the monsoon (July) than during pre-monsoon conditions (June) (Table 1). The Epfu/Lano call rate during the monsoon (July) at wet sites was 6 ± 3 and 3 ± 2 at wet sites during Pre-monsoon. (June). We did not detect an effect of housing density on Epfu/Lano call rate (F = 0.04, *P* = 0.84).
Model summary statistics for all bat species model (top) and Epfu/Lano group (bottom). Water and housing density had a significant effect on skunk detections with the odds of detecting a skunk decreasing by 47% near water and increasing by 0.7% for each unit increase in housing density. Raccoons showed a positive response to water with the odds of detecting a raccoon increasing by 13% near water. Foxes showed a negative response to water with the odds of detecting a fox decreasing by 16% near water (Table 2, Fig. 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>P</th>
<th>Group</th>
<th>Variance</th>
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<td></td>
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Model summary statistics for final skunk model (top), fox model (middle) and raccoon model (bottom).

<table>
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</table>

Discussion

Our finding of higher bat activity around wet sites, for both all bat species combined and the Epfu/Lano subset, is consistent with previous studies examining bat responses to water (Loumassine et al, 2020; Monadjem and Reside 2008) and the responses of *Eptesicus fuscus* specifically (Li and Wilkins 2014, Gallo, et al. 2018). Bats primarily use water in two ways, as a drinking source and as foraging habitat (Campbell 2009). High energetic costs associated with flight (Voigt et al. 2010) and large wing membranes that result in higher surface area to volume ratios (Herreid and Schmidt-Neilsen 1966) make bats more susceptible to water loss through evaporation. Water may be especially important for females during lactation (Adams 2010) with lactating females visiting water 13 times as often as non-reproductive females in one study (Adams and Hayes 2008). In addition to meeting water demands, the increased availability of aquatic insect prey makes water sources valuable foraging grounds for
insectivorous bats (Salvarina, et al. 2018). Water availability may be especially important for bats in the water-limited landscape of the desert southwest USA (Loumassine, et al. 2020), and in these areas, artificial waters such as those on golf courses may be especially important.

Given the significant positive effect of water on bat activity, we were surprised that monsoon was not a significant predictor of total bat activity, as we expected bat use of our water sources would decline once monsoons increased water availability across the landscape, at least during our first study year (2018). The limited monsoon activity during our second (2019) field season (5.3 cm of rainfall compared to 25.1 cm during 2018 in Flagstaff), may be why we failed to see differences in total bat activity between pre-monsoon and monsoon conditions in that year. The higher call rates in the monsoon season for the Epfu/Lano group was also unexpected, though we hypothesize this could be due to either increased activity by lactating females or more activity from juvenile bats at that later time of year.

We also expected terrestrial mammals to show a positive relationship with artificial water sources, but found a positive association only for raccoons, the species most often detected by our camera traps. Although several studies indicated water is a limiting resource for raccoons (Gebrt 2003, Beasley and Rhodes 2010), striped skunks show variable responses to water. For example, distance to water was not a significant factor for striped skunks in den site selection in Canadian prairies (Lariviere et al. 2000). Baldwin et al. (2004) observed both positive and negative associations between water and skunk trap success in deciduous forests in Tennessee and Schneider et al. (2019) observed positive associations with water in urban North Dakota. As with striped skunks, gray foxes were detected more frequently at dry sites. Given that gray foxes may partition their use of anthropogenic water sources to reduce interspecific conflict (Atwood et al. 2011), the high raccoon activity around wet sites in our study may have reduced use at those sites by foxes, and potentially by skunks as well.

Housing density has been shown to interact with presence and abundance of both bats (Hale et al. 2012, Caryl et al. 2016) and mesocarnivores (Riley 2006, Cervinka et al. 2014), but we found no effect of housing density on bat activity, or on fox and raccoon occurrence. We speculated that increasing housing density would benefit mesocarnivores by providing den locations and opportunities to exploit anthropogenic food sources, but only striped skunks showed a positive relationship with housing density. Striped skunk abundance in suburban areas tends to be greater than rural areas (Bateman and Fleming 2012). Although housing density may reach levels that negatively impact skunks (Greenspan et al. 2018), our study sites were likely well below that threshold (SaLek et al. 2014). Although raccoons showed increased abundance with increasing housing densities in other studies (Riley et al. 1998, Ordenana et al. 2010, Gross et al. 2012), we did not find an effect, perhaps, because the response to water in the arid southwest overshadowed any effects of housing density. Likewise, gray foxes showed no response to housing density, although several studies have suggested that they are tolerant of urbanization (Harrison 1997, Riley 2006). Spatial overlaps with other mesocarnivores in urban areas appear to negatively affect gray foxes (Parsons et al. 2019), and the high incidences of raccoons in our suburban sites may be the reason fox detections were low.
Overall, our data indicate that artificial urban water sources, specifically those associated with golf courses, have the potential to increase the cross-species transmission of rabies between bats and mesocarnivores by increasing the number of bats attracted to artificial water sources. Notably, the positive response to artificial waters documented here was for bats with calls consistent with two bat species often associated with rabies in the United States, *Eptesicus fuscus* and *Lasionycteris noctivagans* (Finnegan, et al. 2002, Bonwitt et al. 2018). *Eptesicus fuscus* was also associated with repeated host shifts from bats to skunks and foxes in one of the cities we studied (Kuzmin et al. 2012). The presence of the two mesocarnivores important in that host-shift, striped skunks and gray foxes, both declined in the presence of artificial waters, so that the increase in bat activity at water sources may not increase the potential for bat-to-skunk and bat-to-fox transmission as much as for bat transmission to raccoons. Rabies in raccoons is not typically a concern in the southwestern USA, presumably because populations are much lower than those in urban and suburban areas of the eastern United States. However, if raccoon populations continue to grow as human-dominated landscapes spread in the southwestern United States, raccoons may become more important in rabies dynamics given the high levels of raccoon detections around artificial water sources on golf courses we documented. Overall, our results suggest that artificial waters, specifically those associated with golf courses, have the potential to increase cross-species transmission of rabies in arid, water-limited communities in the southwestern USA but that effect may be influenced by species-specific responses to water among mesocarnivores and the inter-specific interactions among those mesocarnivores.

**Declarations**

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**Author contributions:** Lias Hastings and Tad Theimer designed the study with input from Carol Chambers and Dave Bergman. Critical field equipment and support was furnished by Dave Bergman and Carol Chambers. Lias Hastings conducted the field work, data summary and analysis and wrote the initial draft with input from Tad Theimer and Carol Chambers. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**References**


Figures
Figure 1

A. Locations of the acoustic bat detectors (white triangles) and mesocarnivore cameras (white circles) placed at a wet site in 2018. A single camera was moved from one location to another every 2 nights. B. Locations at a wet site in 2019 when 4 cameras were placed in a fixed location for all 6 nights.
Figure 2

Variation in call rates across 14 pairs of wet (blue) and dry (red) sites at Flagstaff, Pinetop, and Williams, Arizona USA in June and July 2018-2019 for all bats combined (A) and big brown bat/silver-haired bat (*Eptesicus fuscus* / *Lasionycteris noctivagans*) subgroup (B). Sites are grouped by city, wet and dry pairs are plotted next to each other.
Figure 3

Main effects of models examining the relationship between the probability of mesocarnivore detection and presence of water and housing density for striped skunk (*Mephitis mephitis*) (A), raccoon (*Procyon lotor*) (B) and gray fox (*Urocyon cinereoargenteus*) (C) summarized across 14 pairs of wet and dry sites at Flagstaff, Pine Top, and Williams, Arizona, USA in June and July 2018-2019.