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INTERNAL STRUCTURAL COVER AND LEDGES
FACILITATE THE USE OF LARGE UNDERPASSES
BY MULTIPLE WILDLIFE SPECIES AND GROUPS**

September 2022

**Nevada Department of Transportation
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Carson City, NV 89712**

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| <p>16. Abstract</p> <p>We conducted a 2-year Before-After Control-Impact (BACI) study in 8 large wildlife road underpasses connecting upland habitats of San Diego County. After a single year of monitoring using highly sensitive passive infrared cameras, internal structure/cover treatments of repeating cinderblock rock piles were added to one-side within 4 of the underpasses and all passages were monitored for an additional year. Our objectives were to: 1) determine if wildlife species were currently using or avoiding these wildlife underpasses, and 2) to determine the effectiveness of adding cover structures within underpasses to enhance their use by smaller vertebrate species and groups. Because many underpasses also contained narrow ledges along the interior walls, we were also able to evaluate the use of these ledges by small animal species. Overall, we analyzed responses of 13 focal groups: lizard, mouse, rat, snake, squirrel, rabbit, roadrunner, skunk, raccoon, fox, bobcat, coyote, and deer.</p> <p>Prior to placement of any underpass treatments, model estimates for the relative activity of most animal groups were not significantly different within vs. outside the underpasses. However, in comparison to exterior habitat, mice and bobcats were significantly more active within the underpasses potentially indicating preference, while activity of rabbits and roadrunners were significantly lower within the underpasses potentially indicating avoidance. Although not significant, activity within underpasses was also substantially lower than exterior for snakes, squirrels, and fox. Mice activity was significantly higher on ledges and they were often documented appearing to prey on invertebrates floors of the underpasses.</p> <p>Addition of structure/cover treatments were associated with significant increases in use by mice, rats, rabbits, fox, and coyotes and substantial, but not significant, increase in activity of snakes and roadrunners. Internal structures such as the cinderblock rock piles in our study as well as the boulders and downed logs recommended in many guidance documents appear to provide an inexpensive way to increase underpass use by a wide variety of species. Our results indicate that responses may be associated with trophic interactions, such as bottom-up and top-down effects. Internal structural cover can enhance habitat value by shielding moisture which in turn can support more diverse microbial communities that are often the base of food chains. Internal cover also offers protection for invertebrates and small animal species. Positive responses of small animals and their predators in our study (mice, rats, rabbits, snakes, roadrunners, fox, coyote) may provide supportive evidence for bottom-up effects as a result of increased resources. However,</p> | | |

increased use by large predators, such as coyote, may have resulted in top-down effects from predation and competition pressure such as decreased use of underpasses by medium sized prey species (i.e., skunks) and intra-guild competitors, such as bobcats.

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Wildlife passage, reptiles, small mammals, large mammals, permeability, enhancement, passage design

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Internal Structural Cover and Ledges Facilitate the Use of Large Underpasses by Multiple Wildlife Species and Groups



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Introduction

Roads of different sizes, substrates, and traffic volumes can inhibit the movement of large mammals, small mammals and herpetofauna. If a road creates an impermeable barrier to animal movement, populations can become isolated or fragmented. Fragmented populations are more vulnerable to local extinctions and other negative effects from demographic and environmental stochasticity, as well as from increased inbreeding and genetic drift (see reviews by Trombulak and Frissel 2000, Forman et al. 2003, Fahrig and Rytwinski 2009, Taylor and Goldingay 2010, van der Ree et al. 2015b).

Large underpasses are intended to facilitate safe wildlife movement between natural areas transected by major roads. To date, most studies have focused on large animal movement through these types of road underpasses, particularly movement of large carnivores and ungulates (see review by Denneboom et al. 2021). In many instances, these large animals may be considered “umbrella species,” indicating the concurrent use areas by a wide variety of smaller animals. Currently, there are few studies that address whether large underpasses facilitate the connectivity of smaller vertebrates across roads (Mata et al. 2005, Denneboom et al. 2021).

Animals have been postulated to respond to roads based on perceived risk (Jacobson et al. 2016), and therefore, may also use or avoid open underpasses based upon perceived risk. Although there is little supporting data, many guidance documents currently recommend that structure and cover, such as boulders and downed wood, be added within large underpasses to increase their use by smaller animals (e.g., Clevenger and Huijser 2011, Ascensão et al. 2015, Smith et al. 2015, Gunson et al. 2016, Huijser and Gunson 2019, Langton and Clevenger 2021). Theoretically, these would better simulate their natural habitat and provide hiding places or safe havens for small animals that are wary of predators. However, it is possible that addition of internal structure may deter species if they perceive an increased threat from predators potentially hiding within or behind the structures. Therefore, studies on large underpass use and responses of species to internal structure/cover are needed to better understand and inform construction and retrofitting of these passages.

We conducted a 2-year Before-After Control-Impact (BACI) study in 8 large wildlife road underpasses connecting upland habitats of San Diego County. After a single year of monitoring using passive infrared cameras, internal structures were added to half of the underpasses and all passages were monitored for an additional year. Our objectives were to: 1) determine if wildlife species are

currently using or avoiding these wildlife underpasses, and 2) to determine the effectiveness of adding cover structures within underpasses to enhance their use by smaller vertebrate species and groups. Because many underpasses also contained narrow ledges along the interior walls, we were also able to evaluate the use of these ledges by small animal species.

Methods

We selected a Before-After Control-Impact (BACI) design (McDonald et al. 2000) to investigate the effectiveness of adding structures to underpasses to enhance small vertebrate use. A pre-treatment sampling period was conducted in 2012 to acquire data to establish baseline conditions and relative activity of species and species groups within and outside of 8 large wildlife underpasses. Next, the treatment was applied to half of the underpasses and then a second sampling period was conducted in 2013. During each sampling period, animal use was monitored within the underpasses using motion detection cameras specially configured to detect small and large animals. We also sampled adjacent habitat outside the underpass to assess differences in animal activity.

Study Sites

We considered only large underpasses in coastal San Diego County with no roads or water courses passing through them for the study. We selected 8 underpasses that satisfied these criteria and connected natural upland habitats (Figure 1, Table 1). A three-letter Site ID identifies each sample unit (Figure 1, Table 1). The three underpasses at Valley Center Road (VCS, VCN, and VCM) are constructed completely of concrete. During our initial reconnaissance, we observed evidence of small mammals, bats, and granite spiny lizards in the seams between the concrete sections and in the drainage pipes in the concrete. The Valley Center underpasses also have concrete ledges on each side. In addition, VCS and VCM have a shallow concrete drainage ditch running along the east side of the underpass interior. The two underpasses at Carmel County Road (CCS and CCN) are right next to each other and are constructed entirely of corrugated metal. CCS has a recreation path through the underpass whereas CCN does not. The Sorrento Valley Road (SVR) underpass is also constructed entirely of corrugated metal. The two southern-most underpasses, Scripps Poway Parkway (SPP) and California State Highway 52 (HFT) are also the longest. SPP is constructed of corrugated metal covered with spraycrete. It has light tubes for illumination and wooded structures mounted to the ceiling in which birds can nest as well as bat boxes attached to the wooden structures. HFT is

constructed of concrete side walls and a corrugated metal upper section. The concrete side walls provide ledges on which small animals could move. HFT also has openings to add illumination.

The eight study sites are associated with three complexes of conserved lands. The three Valley Center Road sites are associated with Daley Ranch Open Space Preserve/City of Escondido Open Space. The two Carmel County Road underpasses and the Sorrento Valley Road underpass are associated with Los Penasquitos Canyon and surrounding conserved lands. Finally, the Scripps Poway Parkway and Highway 52 sites are associated with a relatively large area of open space that includes Mission Trails Regional Park, Marine Corps Air Station Miramar, and Sycamore Canyon.

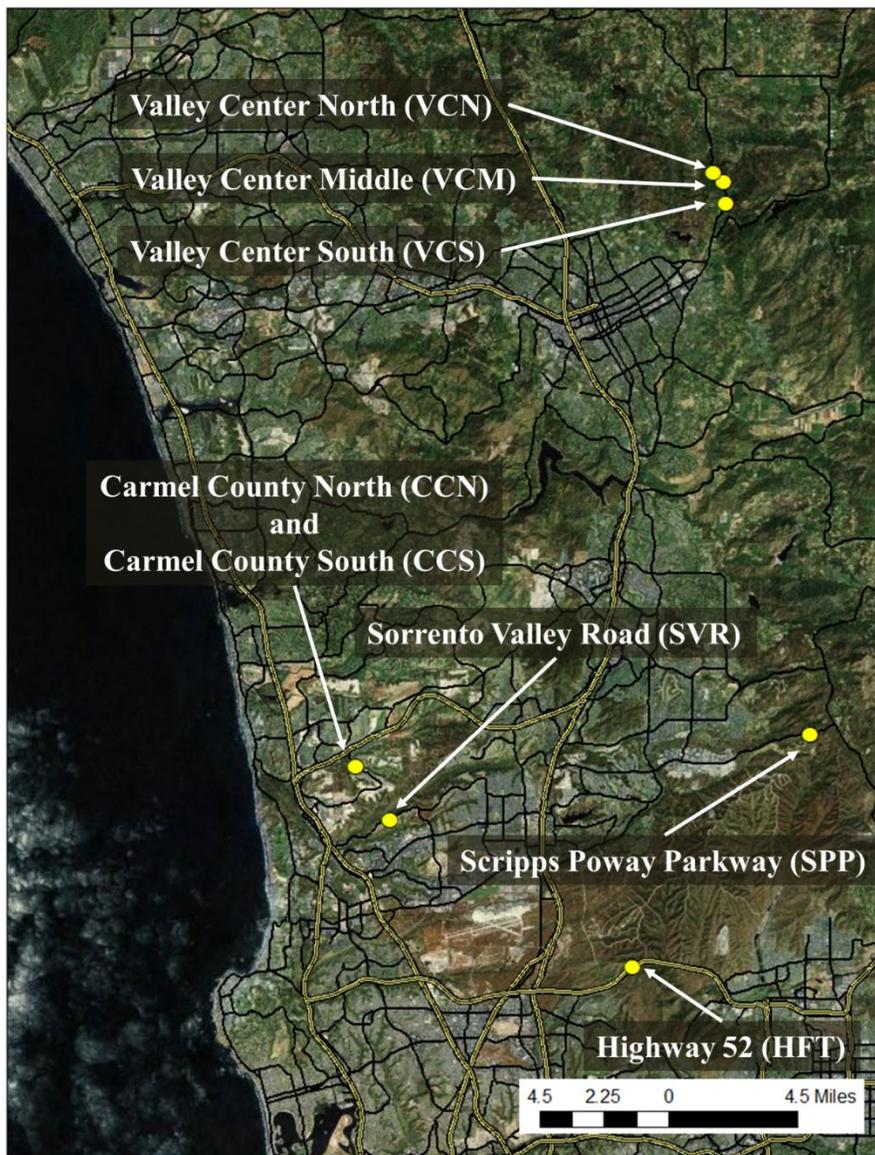


Figure 1: Locations of the eight underpasses selected for study in coastal San Diego County.

Table 1: Underpasses and their attributes. Each of the eight study sites is assigned a three-letter Site ID. The number of cameras at each site is given under # Cam and the number of treatment structures placed in the underpass in January 2013 is given under # Struct.

| Site (Site ID) | Longitude (deg) | Latitude (deg) | Length (m) | Width (m) | Azimuth (deg) | Group | # Cam | # Struct |
|---------------------------------|-----------------|----------------|------------|-----------|---------------|---------|-------|----------|
| Valley Center North (VCN) | -117.0303 | 33.1871 | 34 | 4.5 | 34 | Control | 4 | NA |
| Valley Center Middle (VCM) | -117.0249 | 33.1833 | 45 | 4.5 | 31 | Treat | 4 | 12 |
| Valley Center South (VCS) | -117.02401 | 33.1743 | 37 | 4.5 | 255 | Control | 4 | NA |
| Carmel Country Road North (CCN) | -117.21028 | 32.9368 | 51 | 9 | 256 | Treat | 3 | 13 |
| Carmel Country Road South (CCS) | -117.21028 | 32.9368 | 51 | 9 | 256 | Control | 2 | NA |
| Sorrento Valley Road (SVR) | -117.19281 | 32.9143 | 46 | 6 | 330 | Treat | 3 | 12 |
| Scripps Poway Parkway (SPP) | -116.9814 | 32.9501 | 62 | 9 | 11 | Control | 3 | NA |
| Highway 52 (HFT) | -117.07096 | 32.8517 | 87 | 5 | 315 | Treat | 4 | 18 |

Field Study

General locations of camera placements are shown in Figure 2. We used Reconyx PC800 HyperFire™ Professional cameras housed in steel security boxes (Figures 3A, 3B). These cameras feature semi-covert infrared flashes that make no noise. To increase detection probability for small animals, the manufacturer increased the trigger sensitivity and set the focal length to 5 feet (although the cameras had considerable depth of field, so that a large portion of the image was typically in focus). To sample small and large animal use inside the underpasses, we placed two interior cameras at ground level in each underpass. Interior cameras were placed on each wall at opposite ends within the underpass (7.5 meters from the underpass opening) and mounted approximately 4 cm above ground level at a 10-degree angle from the wall toward the central axis of the underpass (Figures 2, 3C). We used a wooden bracket to control the orientation of the camera and toggle bolts or drop-in anchors and carriage bolts for mounting, depending on the underpass construction (Appendix 1). If the underpass had concrete ledges, we also added a camera to one of the ledges (Figures 2, 3D). We mounted a ledge camera on one ledge at the midpoint of the underpass, aimed directly along the ledge, and approximately 10 cm above the ledge so that small vertebrates could pass under it without obstruction. We added exterior cameras (as funding permitted) near one opening of each underpass to observe

species using areas outside the underpass. We placed exterior cameras about 4 cm above ground level perpendicular to a trail or wing fence (Figure 2). We mounted exterior cameras to a metal post driven at least 0.5 meters into the ground with carriage bolts or existing wing fencing posts with U bolts (Figure 3E, Appendix 1).

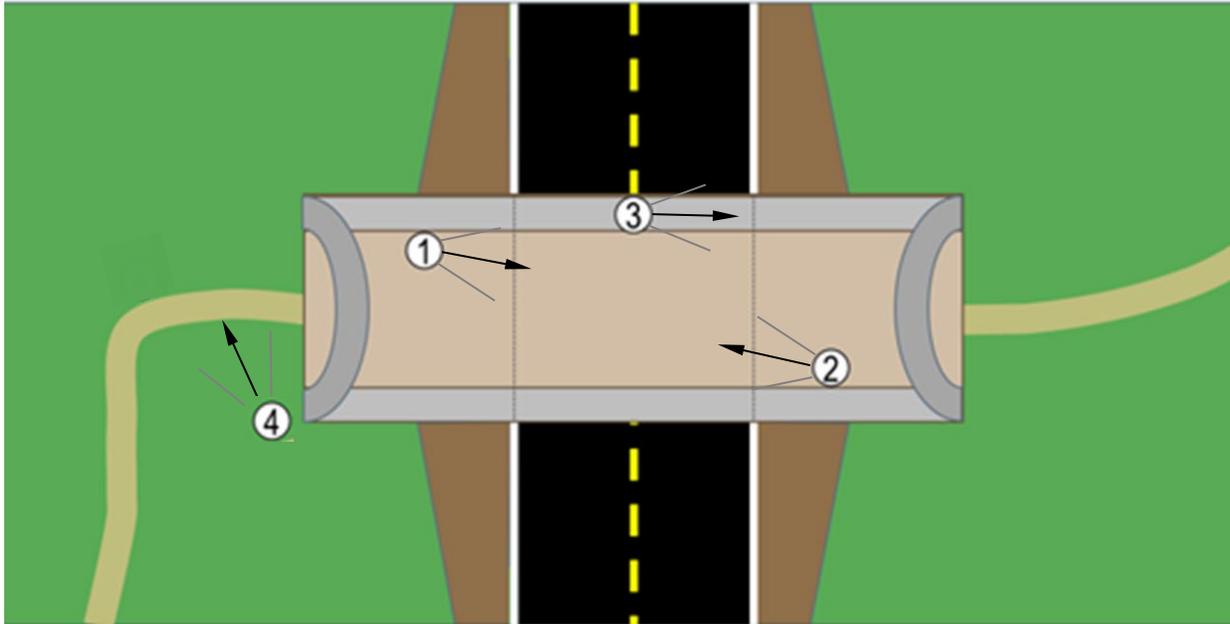


Figure 2: General placement of cameras in the underpass interior ① & ②, on ledges ③, and exterior ④.

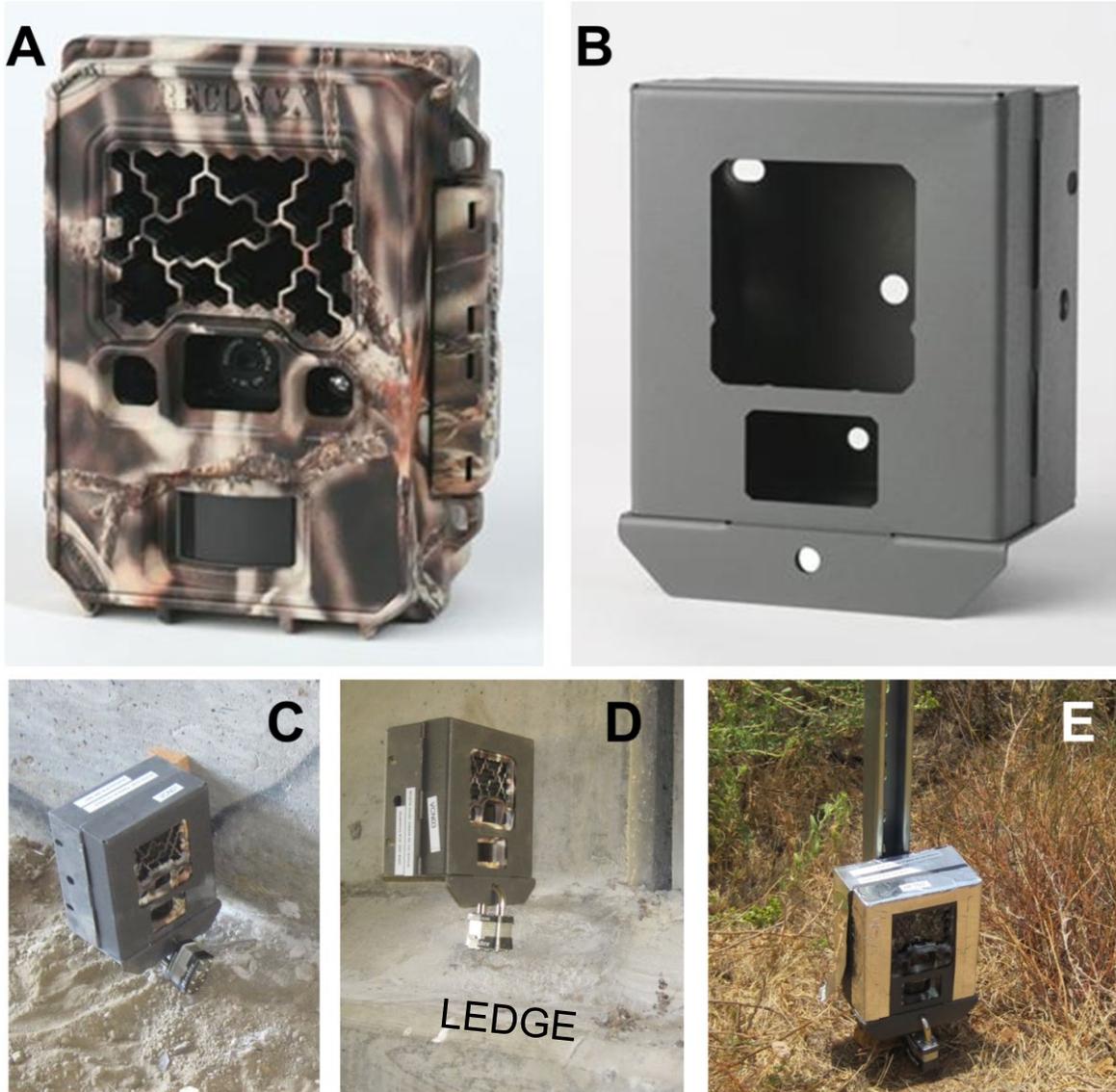


Figure 3: Cameras and installation. The camera model we used, the Reconnyx HyperFire PC800, is shown in A. The camera security box is shown in B. We placed cameras in the underpass interior at ground level (C), on concrete ledges inside the underpass (D), and at exterior locations at ground level (E).

In 2012 and 2013, we operated the cameras continuously during summer months when small mammals and reptiles were most active. We set the cameras to take both motion-detection and time-lapse images at five-minute intervals. Time-lapse images provide an alternative way to observe species independent of the motion detection mechanism. We checked the cameras every two weeks. During camera checks, we replaced the batteries, exchanged the memory cards, cleaned the camera lenses, and

corrected any other problems that arose. The increased sensitivity of the cameras required to detect small vertebrates exacerbated issues related to continual triggering of the motion detector in some exterior cameras. At some locations, 8GB or 16GB memory cards were completely filled with images in 2 to 4 days, halting any further image collection until the camera was checked. As a result, we switched some exterior cameras to time lapse mode only. When we removed the cameras at the end of the 2012 season, we left the security boxes in place so that we could reinstall the same camera at the same position with the same height and orientation.

In January of 2013, we added small structures to the treatment underpasses. We randomly assigned four underpasses to the treatment group and four underpasses to the control group (Table 1). Due to permit constraints, we did not use natural materials for the structures within the underpasses. Each structure consisted of 4 concrete cinder blocks stacked on top of two half-meter sections of black PVC pipe (Figure 4A). The structures were placed at five-meter intervals along the entire length of one side of the treatment underpasses (Figure 4B), intended to allow small vertebrates to move from one structure to the next, thereby facilitating movement through the underpass. We only placed structures on one wall of the treatment underpasses. The purpose of this design was to facilitate movement of species with both open and closed microhabitat preferences. Furthermore, the structures were very small compared to the lengths and widths of the underpasses, so they would not impede the movement of humans or any animals. Since there were two interior cameras at ground level on each wall of each underpass, one of the ground-level interior cameras was on the cover side (i.e., side with our structures) and the other was on the open side of the treatment underpasses. We allowed over two months for animals to habituate to the treatment before subsequent post-treatment monitoring (late March to late September 2013).

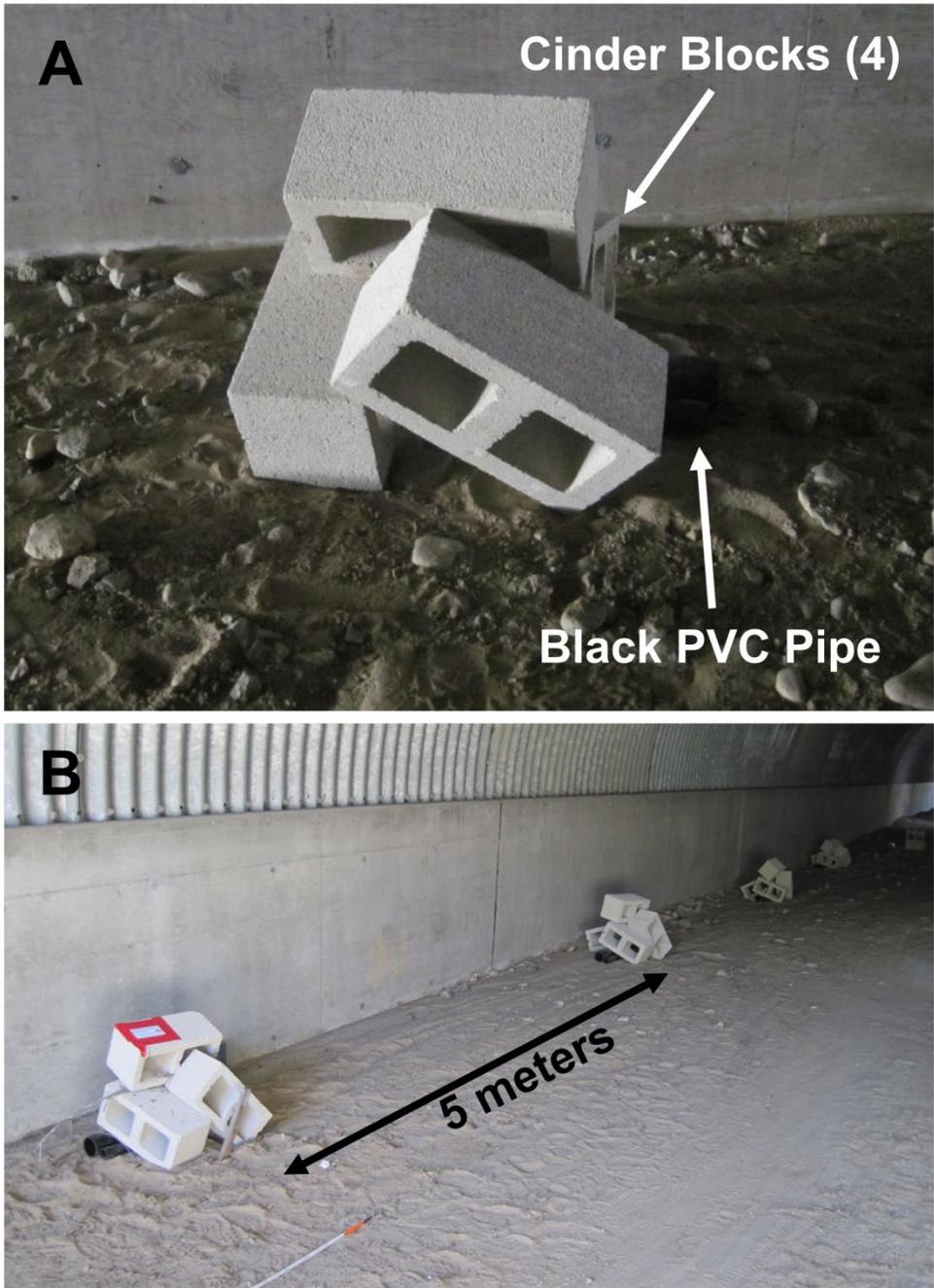


Figure 4: Treatments applied to randomly selected underpasses. Each treatment structure consisted of four cinder blocks, one 0.5-meter length of 1-inch black PVC pipe, and one 0.5-meter length of 1-inch black PVC pipe (A). Structures were placed at 5 meters intervals along one wall for the entire length of the treated underpass (B). Thus, the number of structures placed in the control underpasses varied based on the length of the underpass (Table 1).

Table 2: Summary of data collection effort and numbers of photos by camera.

| Site | Camera Placement | Treatment | | 2012 | | | | 2013 | | | |
|--------------------------------|------------------|-----------|------|------------|----------|-------------------------|--------------------|------------|----------|-------------------------|--------------------|
| | | Site | Side | Start Date | End Date | #Photos Motion Infrared | #Photos Time-Lapse | Start Date | End Date | #Photos Motion Infrared | #Photos Time-Lapse |
| CCN | interior | Yes | Yes | 6/8 | 10/3 | 1,180 | 35,628 | 3/20 | 9/25 | 1,961 | 54,442 |
| CCN | interior | Yes | No | 6/8 | 10/3 | 2,007 | 39,646 | 3/20 | 9/25 | 2,101 | 54,445 |
| CCN | exterior | Yes | No | 6/27 | 10/3 | 52,895 | 25,891 | 3/20 | 9/21 | 91,193 | 49,753 |
| CCS | interior | No | No | 6/8 | 10/3 | 23,171 | 33,639 | 3/20 | 9/25 | 44,430 | 54,446 |
| CCS | interior | No | No | 6/8 | 10/3 | 14,438 | 37,658 | 3/20 | 9/25 | 25,659 | 54,446 |
| CCS | exterior | No | No | 6/8 | 6/22 | 27,831 | 843 | NA | NA | 0 | 0 |
| HFT | interior | Yes | Yes | 6/19 | 10/3 | 2,367 | 30,454 | 3/19 | 9/25 | 5,849 | 54,693 |
| HFT | interior | Yes | No | 6/19 | 10/3 | 4,889 | 30,456 | 3/19 | 9/25 | 5,928 | 50,370 |
| HFT | exterior | Yes | No | 7/3 | 10/3 | 25,093 | 25,009 | 3/19 | 9/25 | 100,602 | 48,724 |
| HFT | ledge | Yes | Yes | 7/3 | 9/26 | 2,855 | 24,354 | NA | NA | 0 | 0 |
| SPP | interior | No | No | 5/15 | 10/3 | 5,702 | 37,661 | 3/19 | 9/25 | 5,462 | 54,693 |
| SPP | interior | No | No | 5/16 | 10/3 | 1,278 | 37,654 | 3/19 | 9/25 | 3,599 | 54,690 |
| SPP | exterior | No | No | 5/16 | 10/3 | 6,268 | 35,337 | 3/19 | 9/25 | 46,023 | 54,701 |
| SVR | interior | Yes | Yes | 6/14 | 10/3 | 2,580 | 29,474 | 3/20 | 9/25 | 4,186 | 54,441 |
| SVR | interior | Yes | No | 6/14 | 10/3 | 2,511 | 27,692 | 3/20 | 9/25 | 4,338 | 54,443 |
| SVR | exterior | Yes | No | 7/3 | 10/3 | 61,856 | 18,543 | 3/20 | 9/25 | 184,168 | 49,912 |
| VCM | interior | Yes | Yes | 5/25 | 10/3 | 2,739 | 41,709 | 3/19 | 9/25 | 3,879 | 54,679 |
| VCM | interior | Yes | No | 5/25 | 10/3 | 7,092 | 37,691 | 3/19 | 9/25 | 7,757 | 54,685 |
| VCM | ledge | Yes | Yes | 5/25 | 10/3 | 6,265 | 37,692 | 3/19 | 9/25 | 7,573 | 54,681 |
| VCM | exterior | Yes | No | 8/15 | 10/3 | 1,017 | 14,056 | 3/19 | 9/25 | 15,079 | 52,676 |
| VCN | interior | No | No | 5/31 | 10/3 | 4,554 | 31,924 | 3/19 | 9/25 | 4,564 | 54,683 |
| VCN | interior | No | No | 5/31 | 10/3 | 5,335 | 35,938 | 3/19 | 9/25 | 4,720 | 54,681 |
| VCN | ledge | No | No | 5/31 | 10/3 | 7,767 | 31,913 | 3/19 | 9/25 | 2,362 | 54,683 |
| VCS | interior | No | No | 5/24 | 10/3 | 18,392 | 46,094 | 3/19 | 9/25 | 18,373 | 54,689 |
| VCS | interior | No | No | 5/24 | 10/3 | 9,391 | 48,111 | 3/19 | 9/25 | 11,233 | 54,683 |
| VCS | ledge | No | No | 5/24 | 10/3 | 5,333 | 47,922 | 3/19 | 9/25 | 8,094 | 54,687 |
| VCS | exterior | No | No | 8/15 | 10/3 | 32,471 | 9,260 | 3/19 | 9/25 | 29,381 | 48,386 |
| Totals by Year and Camera Type | | | | | | 337,277 | 852,249 | | | 638,514 | 1,337,412 |
| Totals by Year | | | | | | 1,189,526 | | | | 1,975,926 | |
| Grand Total | | | | | | 3,165,452 | | | | | |

Image Processing

We used a cross-platform Java program to process the first subset of approximately 200,000 motion-trigger images (Tracey et al. 2014). Using the Python TensorFlow API (<https://www.tensorflow.org/>), we constructed an image data pipeline for efficient storage and loading of the image data from disk, functions for preprocessing the image data, and a convolutional neural network model for supervised image classification (ResNet50 architecture as described in He et al. 2015). While considerable time was spent in the development of a machine learning algorithm to classify the remaining photos, this was not fully developed and functional in time for the due date for this report (See Appendix 4 for details). While considerable time was spent in the development of a machine learning algorithm to classify the remaining photos, this was not fully developed and functional in time for the due date for this report. It appeared that the algorithm also learned the backgrounds of images where target species were most likely to occur, which in turn resulted in a large number of images without any animals (i.e., background only) being classified as target species (J. Tracey, pers. comm.). Therefore, in May and June of 2022, we reviewed the approximately 3 million remaining images, pre-classified by the machine-learning algorithm, ‘by hand’. Images were first reduced to 244 x 244 pixels and examined in batches of 25,000 per folder. Reviewers opened each folder in Windows with the view set to extra large icons. Reviewers then scrolled through photos in sets of 3 (width) and moved photos to folders corresponding to a focal species group (mouse, rat, lizard, snake, squirrel, rabbit, roadrunner, skunk, raccoon, fox, bobcat, coyote, and deer) (Table 3). Photos of other animals outside the focal groups, such as birds and humans, and photos with no identifiable animal were not classified during this process. There were 602,546 images identified by the machine learning algorithm as “None” and 209,953 images identified as “Human”. For these, we reviewed two representative batches of 25,000 images each. After careful review, fewer than 10 images per batch belonged to one of our focal groups (0.04%). Therefore, we did not review the remaining folders in these two categories due to time constraints and because the additional small amount of data would not likely change the results of the analyses.

Table 3: Species groups used for data analysis

| Species Group | Species Included |
|-------------------|--|
| Mouse | North American Deer Mouse (<i>Peromyscus maniculatus</i>), Deer Mouse (<i>Peromyscus</i> spp.), Pocket Mouse (<i>Chaetodipus</i> spp.), California Vole (<i>Microtus californicus</i>), Brush Mouse (<i>Peromyscus boylii</i>), California Mouse (<i>Peromyscus californicus</i>), Unknown Mouse Species |
| Rat | Bryant’s Woodrat (<i>Neotoma bryanti</i>), Big-eared Woodrat (<i>Neotoma macrotis</i>), Woodrat (<i>Neotoma</i> spp.), Kangaroo Rat (<i>Dipodomys</i> spp.), Black Rat (<i>Rattus rattus</i>), Unknown Rat Species |
| Lizard | Western Fence Lizard (<i>Sceloporus occidentalis</i>), Orange Throated Whiptail (<i>Aspidoscelis hyperythrus</i>), Western Whiptail (<i>Aspidoscelis tigris</i>), Granite Spiny Lizard (<i>Sceloporus orcutti</i>), Sceloporus Species (<i>Sceloporus</i> spp.), Side Blotched Lizard (<i>Uta stansburiana</i>), Unknown Lizard Species |
| Snake | Southern Pacific Rattlesnake (<i>Crotalus oreganus</i>), Red Diamond Rattlesnake (<i>Crotalus ruber</i>), California kingsnake (<i>Lampropeltis californiae</i>), Unknown Snake Species |
| Squirrel | Western Gray Squirrel (<i>Sciurus griseus</i>), California Ground Squirrel (<i>Spermophilus beecheyi</i>), Unknown Squirrel Species |
| Rabbit | Desert Cottontail Rabbit (<i>Sylvilagus audubonni</i>), Black-tailed Jackrabbit (<i>Lepus californicus</i>), Brush Rabbit (<i>Sylvilagus bachmani</i>), <i>Sylvilagus</i> not identified to species (<i>Sylvilagus</i> spp.), Unknown Rabbit Species |
| Roadrunner | Greater Roadrunner (<i>Geococcyx californianus</i>) |
| Skunk | Striped Skunk (<i>Mephitis mephitis</i>), Spotted Skunk (<i>Spilogale putorius</i>) |
| Raccoon | Raccoon (<i>Procyon lotor</i>) |
| Fox | Gray Fox (<i>Urocyon cinereoargenteus</i>) |
| Bobcat | Bobcat (<i>Lynx rufus</i>) |
| Coyote | Coyote (<i>Canis latrans</i>) |
| Deer | Mule Deer (<i>Odocoileus hemionus</i>) |

Data Analysis

At each camera, we condensed unique species use events within 10-minute intervals; images of the same species occurring <10 minutes apart were not considered independent. Therefore, if there were 6 images of a coyote within a 10-minute interval, it would count as a single unique use event. We defined 28, 7-day periods within each year and calculated the activity or ‘abundance of use’ events during each 7-day period for each species by summing species unique use events over each sampling period. These then are relative measures of the proportion and frequency of space-use by each species (Gilbert et al. 2020). We accounted for any differences in sample effort by creating an effort covariate. For passive infrared trigger camera data, effort covariate was the sum of the number of days each camera was active during each 7-day period (i.e., “day effort”). For time lapse camera data, where

cameras were set to take a photo only every 5 minutes, we conservatively multiplied day effort by the ratio of total trigger to time lapse photos containing target species and groups (1:25). This ratio was calculated from data where the passive infrared trigger and 5-min time-lapse photos were both active in the same cameras during the duration of the study. Therefore, we assumed for the model that time lapse photos had 1/25th the sampling effort applied and accounted for this in the detection portion of the model.

To model activity (abundance of use events) of target species groups relative to camera placement and treatment, we used closed- and open-population N-mixture models, respectively. To compare the abundance of use events between exterior, interior, and ledge placements, we used closed population N-mixture models (Royle 2004). We compared exterior and interior abundance of use events for all species groups and additionally ledge camera placements for 3 groups (lizard, mouse, and rat), as we did not observe larger species using ledge habitat. We modeled abundance of use as a function of placement separately for 2012 and 2013 but used 2012 as our primary analysis for effect of placement due to confounding of treatment effects for interior placement in 2013. For each species, observed counts $Y_{i,j}$ were estimated:

$$Y_{i,j} \sim \text{binomial}(p_{i,j}, N_i) \text{ (Equation 1)}$$

where p is the detection probability at camera i on visit (7-day period) j and N is the latent abundance of use events at camera i . Latent abundance of use events is defined as the unobserved absolute abundance of use events derived from the model, based on observations and estimated detection probability. We estimated detection probability as a function of camera sampling effort (Equation 2).

$$\text{Logit}(p_{i,j}) = \alpha_0 + \alpha_{\text{effort}} * \text{effort}_{i,j} \text{ (Equation 2)}$$

We estimated site-specific latent abundance N_i from a Poisson distribution, with rate parameter λ_i modeled as a function of site and placement (Equations 3 and 4), with a log-link.

$$N_i \sim \text{Poisson}(\lambda_i) \text{ (Equation 3)}$$

$$\log(\lambda_i) = \beta_0 + \beta_{1i}[\text{site}_i] + \beta_2 * \text{placement}_i \text{ (Equation 4)}$$

where site and treatment were treated as factors. Here site_i refers to the primary road associated with each underpass, with a total of 5 levels. The term placement_i was a 2 or 3-level factor, corresponding to exterior, interior, and ledge (in lizard, mouse, and rat models) placement.

To evaluate the effect of underpass structure treatment on animal use, we used an open-population N-mixture model (Dail and Madsen 2011), extended to estimate intrinsic rate of abundance of use (population growth) increase from one year to the next (Hostetler and Chandler 2015). For small animal groups (lizard, mouse, rat, snake, squirrel, rabbit), the site was the side of the underpass with/without treatment (Treatment Side, n=16). For the remaining medium and large animal groups, the site was the entire underpass with/without treatment (Treatment, n=8). If species groups increased their use of underpasses after the addition of structural treatments, we would expect to see increased rates of use for treated underpasses in comparison to controls (untreated underpasses) from 2012 to 2013. Here, observed counts were indexed $Y_{i,j,t}$, representing counts of animals at site i , during 7-week periods j , in year t . Again, counts were generated from a binomial process with parameters detection probability and $N_{i,t}$. We estimated detection probability using the same structure as equation 2. Unlike the closed-population model, latent abundance $N_{i,t}$ was indexed on primary period, and estimated based on initial abundance $N_{i,t-1}$ and the rate of growth γ (Equations 5 – 8).

$$N_{i,t} \sim \text{Poisson}(\exp(\gamma_i) * N_{i,1}) \text{ (Equation 5)}$$

$$N_{i,1} \sim \text{Poisson}(\lambda_{i,1}) \text{ (Equation 6)}$$

$$\log(\lambda_{i,1}) = \beta_0 + \beta_1[\text{site}_i] \text{ (Equation 7)}$$

$$\log(\gamma_i) = G_0 + G_i * \text{treatment}_i \text{ (Equation 8)}$$

To understand the effect of treatment on growth rate of use, we averaged site-specific growth rate ($\exp(\gamma_i)$) across all treatment and non-treatment sites. We then estimated the percent difference between growth rates in treatment and non-treatment sites, to determine if significant differences between growth rate existed between treatment conditions. Although we sampled for a longer time period in 2013, any differences due to increased sampling or seasons among years were accounted for by the BACI design by incorporating any concomitant changes in growth rates of the controls (untreated underpasses) from 2012 to 2013.

Data were prepared for analysis using CamTrap-R (Niedballa et al. 2016). We fit all models in R v. 4.1.2 (R core team) using package Unmarked (Fiske and Chandler 2011). All models were run using default specifications, using a Poisson mixture, and setting the index of integration to 100. We describe significant effects using 90% confidence intervals, unless otherwise specified.

Results

We analyzed photos for the presence of 13 focal groups: lizard, mouse, rat, snake, squirrel, rabbit, roadrunner, skunk, raccoon, fox, bobcat, coyote, and deer. Representative photos of groups are presented in Appendix 2. From 2012 – 2013 we documented 13,922 unique use events across all species groups (Table 4). Detection probabilities for a single use event ranged from 0.01 to 0.23 for species groups (Appendix 3).

Table 4. Number of unique use events in treatment and non-treatment sites in 2012 and 2013 (reduced to maximum of 1 occurrence event every 10 min.).

| | | Non-Treatment | | Treatment | | Total |
|---|------------|---------------|------|-----------|------|-------|
| | | 2012 | 2013 | 2012 | 2013 | |
| | no. sites | 12 | 12 | 4 | 4 | 16 |
| Small Animal Groups (site= each side of each underpass) | LIZARD | 76 | 285 | 111 | 258 | 730 |
| | MOUSE | 786 | 498 | 747 | 1474 | 3505 |
| | RAT | 72 | 228 | 65 | 466 | 831 |
| | SNAKE | 8 | 14 | 2 | 13 | 37 |
| | SQUIRREL | 158 | 162 | 221 | 335 | 876 |
| | RABBIT | 810 | 1089 | 31 | 29 | 1959 |
| | no. sites | 4 | 4 | 4 | 4 | 8 |
| Medium/Large Animal Groups (site= each underpass) | ROADRUNNER | 138 | 81 | 13 | 27 | 256 |
| | SKUNK | 61 | 206 | 40 | 58 | 365 |
| | RACCOON | 202 | 84 | 90 | 65 | 441 |
| | FOX | 100 | 158 | 0 | 19 | 277 |
| | BOBCAT | 28 | 107 | 145 | 273 | 553 |
| | COYOTE | 939 | 1448 | 114 | 531 | 3032 |
| DEER | 344 | 314 | 160 | 242 | 1060 | |
| | | 3722 | 4674 | 1739 | 3790 | 13922 |

Relative Activity Outside vs. Inside Underpass

In 2012, prior to placement of any underpass treatments, model estimates for the relative activity of most animal groups were not significantly different within vs. outside the underpasses (Figure 5, Table 5). However, mice and bobcats were significantly more active within the underpasses than in exterior habitat. In comparison to exterior habitat, average bobcat activity was 7.2 times greater within the underpasses, while mice activity averaged 4.8 times greater within the underpasses and 10.7 times greater on the ledges. Additionally, mice (especially *Peromyscus* spp.) were often documented looking and jumping off the ledges and jumping around on the floors of the underpasses (Figure 6).

Lizards and rats were also documented on the ledges, but less so than the floor of the underpasses. In contrast, the activity of rabbits and roadrunners was significantly lower within the underpass than the exterior habitat. The activity of rabbits was 4.7 times lower within the underpasses, while roadrunner activity was 16.5 times lower than outside the underpasses. Although not significant, average activity of rats, snakes, squirrels and foxes was also lower within the underpass than exterior habitat (Figure 5, Table 5).

In 2013, the direction of the ratio of relative activity inside vs. outside of the underpasses (treatment and non-treatment combined) remained the same across most animal species, with the exception of rats and foxes (negative to positive) and bobcats and coyotes (positive to negative; Table 5).

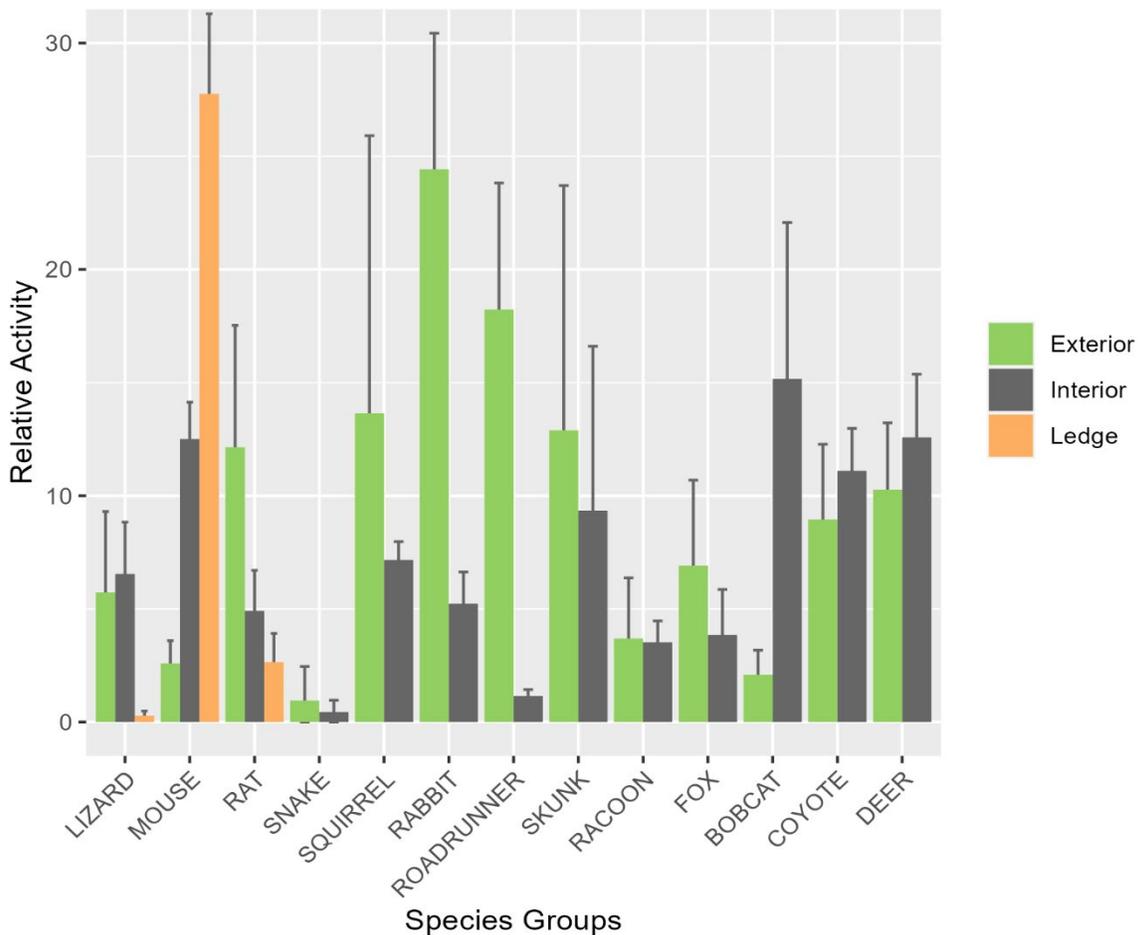


Figure 5. Relative activity of species groups in exterior, interior, and ledge locations. Error bars indicate the mean +/- 1SE. Ledge cameras were only included in the lizard, mouse, and rat models. Results are presented for 2012, prior to any effect of treatment

Table 5. Model estimates of animal activity outside (exterior) vs. within (interior) underpasses. For lizards, mice, and rats, relative activity on ledges within underpasses are included. Means and standard errors in 2013 are across both control and treatment sites.

| Group | Year | Exterior | | Interior | | Ledge | | Ratios | | | |
|------------|------|----------|------|----------|-----|-------|-----|-----------------------|-----|--------------------|-----|
| | | Mean | SE | Mean | SE | Mean | SE | Interior/ Exterior | SE | Ledge/ Exterior | SE |
| LIZARD | 2012 | 5.8 | 3.6 | 6.7 | 2.4 | 0.3 | 0.2 | 1.1 | 1.7 | 0.0 | 2.5 |
| | 2013 | 12.5 | 4.8 | 16.1 | 3.2 | 1.3 | 0.5 | 1.3 | 1.4 | 0.1 | 1.6 |
| MOUSE | 2012 | 2.5 | 1.0 | 12.3 | 1.7 | 25.5 | 3.7 | 4.9 | 1.5 | 10.1 | 1.5 |
| | 2013 | 8.3 | 2.8 | 17.8 | 2.4 | 9.4 | 2.0 | 2.1 | 1.4 | 1.1 | 1.4 |
| RAT | 2012 | 9.6 | 4.5 | 4.4 | 1.7 | 2.1 | 1.1 | 0.5 | 1.5 | 0.2 | 1.7 |
| | 2013 | 7.7 | 2.5 | 27.0 | 3.4 | 4.6 | 1.3 | 3.5 | 1.3 | 0.6 | 1.5 |
| SNAKE | 2012 | 0.9 | 0.9 | 0.4 | 0.4 | NA | NA | 0.5 | 4.1 | NA | NA |
| | 2013 | 1.0 | 0.9 | 1.0 | 0.7 | NA | NA | 1.0 | 2.3 | NA | NA |
| SQUIRREL | 2012 | 11.8 | 10.7 | 7.2 | 0.9 | NA | NA | 0.6 | 2.5 | NA | NA |
| | 2013 | 3.9 | 2.0 | 7.4 | 1.1 | NA | NA | 1.9 | 1.7 | NA | NA |
| RABBIT | 2012 | 21.8 | 5.5 | 5.1 | 1.4 | NA | NA | 0.2 | 1.2 | NA | NA |
| | 2013 | 39.8 | 7.9 | 8.6 | 2.0 | NA | NA | 0.2 | 1.2 | NA | NA |
| ROADRUNNER | 2012 | 5.6 | 5.6 | 0.9 | 0.3 | NA | NA | 0.2 | 3.2 | NA | NA |
| | 2013 | 14.3 | 14.3 | 0.7 | 0.3 | NA | NA | 0.1 | 3.1 | NA | NA |
| SKUNK | 2012 | 12.9 | 10.8 | 9.3 | 7.3 | NA | NA | 0.7 | 1.5 | NA | NA |
| | 2013 | 20.7 | 11.3 | 14.0 | 6.2 | NA | NA | 0.7 | 1.4 | NA | NA |
| RACOON | 2012 | 4.0 | 2.9 | 3.5 | 0.9 | NA | NA | 0.9 | 2.0 | NA | NA |
| | 2013 | 6.3 | 3.6 | 4.5 | 1.8 | NA | NA | 0.7 | 1.6 | NA | NA |
| FOX | 2012 | 6.9 | 3.8 | 3.8 | 2.0 | NA | NA | 0.6 | 1.4 | NA | NA |
| | 2013 | 12.7 | 3.5 | 18.4 | 2.9 | NA | NA | 1.4 | 1.3 | NA | NA |
| BOBCAT | 2012 | 2.1 | 1.1 | 15.2 | 6.9 | NA | NA | 7.3 | 1.6 | NA | NA |
| | 2013 | 7.5 | 2.3 | 4.5 | 1.2 | NA | NA | 0.6 | 1.3 | NA | NA |
| COYOTE | 2012 | 8.5 | 3.2 | 10.9 | 1.9 | NA | NA | 1.3 | 1.4 | NA | NA |
| | 2013 | 25.3 | 5.8 | 13.9 | 2.1 | NA | NA | 0.5 | 1.2 | NA | NA |
| DEER | 2012 | 10.3 | 2.9 | 12.6 | 2.8 | NA | NA | 1.2 | 1.3 | NA | NA |
| | 2013 | 7.0 | 2.2 | 11.9 | 2.7 | NA | NA | 1.7 | 1.3 | NA | NA |

A)



B)



Figure 6. A) Deer mice (*Peromyscus* spp.) A) peering over edge of ledge and B) jumping off of ledge.

Effect of Structure within Underpasses

Following placement of the concrete blocks and PVC pipes (treatments) along the interior of underpasses, activity significantly increased for 5 species groups (mice, rats, rabbits, fox, and coyotes: Figures 7-8, Tables 6-7). For the species that responded positively to treatment, growth rate of use averaged 2.3 times greater than growth rates documented by non-treatment cameras, with the largest differences being foxes (3.6 times greater) and mice (3.2 times greater). Although not significant at the 95% confidence level, snake activity on average increased by a factor of 0.5, and snakes were also observed moving within the concrete structures (Figure 9). No substantial or significant effects were documented for squirrels, racoons, or deer.

Conversely, treatment was significantly negatively associated with activity by 2 species groups (skunks and bobcats). The activity of bobcats, and skunks decreased by an average factor of 0.5 in treatment areas (i.e., decreased by half), with the strongest negative effect observed for bobcats (0.4 times greater, i.e., decreased by a factor of 0.6).

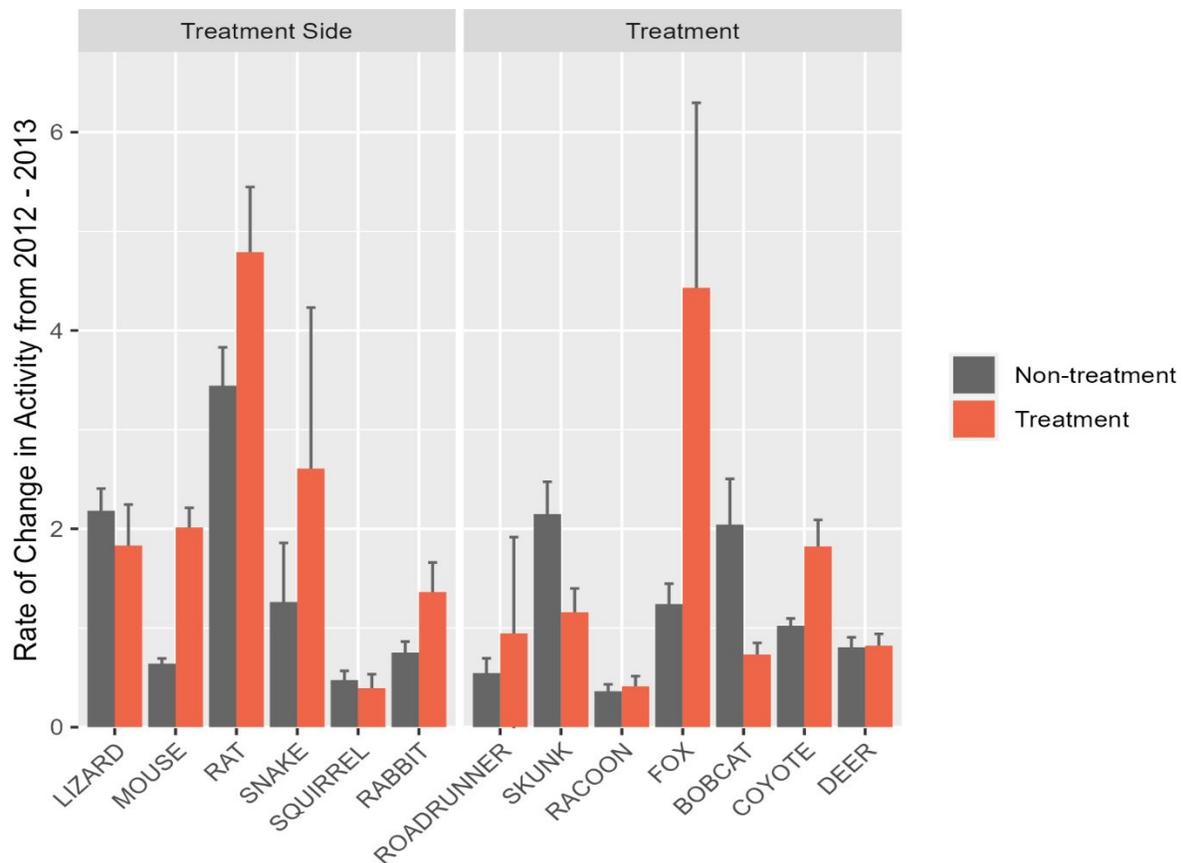


Figure 7. Comparison of change in activity from 2012 – 2013 in treatment and non-treatment sites. Treatment was analyzed at 2 spatial scales depending upon species sizes. For small animal groups, the site was the side of the underpass with/without treatment (Treatment Side, n=16). For medium and large animal groups, the site was the entire underpass with/without treatment (Treatment, n=8). Error bars indicate the mean +/- 1 SE.

Table 6. Model predictions for initial abundance (2012) and abundance following treatment in 2013. Abundance estimates represent the average use, across all underpasses, for treatment and non-treatment cameras. Estimates for 2012 are derived from the model estimate directly. Estimates for 2013 are derived from the predicted abundance in 2012 multiplied by the mean and SE of the growth parameter (γ). Treatment was analyzed at 2 spatial scales depending upon species sizes. For small animal groups, the site was the side of the underpass with treatment (n=16). For medium and large animal groups, the site was the entire underpass with treatment (n=8).

| | Group | Year | Interior-Non treatment Control | | Interior- Treatment | | Ratio- Treatment/ Control | |
|--|------------|------|--------------------------------------|------|------------------------|------|---------------------------------|------|
| | | | Mean | SE | Mean | SE | Mean | SE |
| Small Animal Groups (site=each side of each underpass) | LIZARD | 2012 | 9.5 | 1.7 | 4.9 | 1.1 | 0.52 | 0.83 |
| | | 2013 | 20.7 | 2.2 | 9.7 | 1.7 | 0.47 | 0.52 |
| | MOUSE | 2012 | 21.5 | 1.7 | 20.2 | 2.1 | 0.94 | 1.95 |
| | | 2013 | 14.5 | 1.2 | 33.0 | 3.2 | 2.28 | 0.77 |
| | RAT | 2012 | 6.8 | 1.2 | 7.9 | 1.7 | 1.16 | 0.23 |
| | | 2013 | 23.8 | 2.7 | 35.9 | 4.8 | 1.51 | 1.25 |
| | SNAKE | 2012 | 1.3 | 0.7 | 1.4 | 0.9 | 1.08 | NA |
| | | 2013 | 1.6 | 0.8 | 2.7 | 1.5 | 1.69 | NA |
| | SQUIRREL | 2012 | 48.5 | 22.4 | 29.7 | 14.5 | 0.61 | 0.48 |
| | | 2013 | 23.6 | 4.7 | 13.2 | 3.6 | 0.56 | 1.38 |
| | RABBIT | 2012 | 8.2 | 2.1 | 14.7 | 3.6 | 1.79 | 0.08 |
| | | 2013 | 6.1 | 0.9 | 15.4 | 3.0 | 2.52 | 0.06 |
| Medium/ Large Animal Groups (site=each underpass) | ROADRUNNER | 2012 | 2.4 | 0.5 | 1.3 | 0.4 | 0.54 | 0.02 |
| | | 2013 | 1.3 | 0.4 | 1.2 | 1.3 | 0.92 | NA |
| | SKUNK | 2012 | 20.5 | 5.2 | 9.3 | 2.7 | 0.45 | 0.83 |
| | | 2013 | 44.1 | 6.7 | 10.7 | 2.2 | 0.24 | 0.48 |
| | RACOON | 2012 | 13.8 | 2.5 | 7.8 | 1.8 | 0.57 | 0.73 |
| | | 2013 | 5.0 | 1.0 | 3.3 | 0.8 | 0.66 | 0.49 |
| | FOX | 2012 | 1.5 | 0.5 | 1.7 | 0.5 | 1.13 | 0.42 |
| | | 2013 | 1.8 | 0.5 | 6.9 | 3.3 | 3.83 | 0.46 |
| | BOBCAT | 2012 | 3.6 | 1.2 | 10.8 | 2.5 | 3.00 | 5.02 |
| | | 2013 | 7.3 | 1.6 | 7.9 | 1.3 | 1.08 | 0.24 |
| | COYOTE | 2012 | 24.5 | 2.8 | 13.5 | 2.0 | 0.55 | 0.51 |
| | | 2013 | 25.0 | 1.8 | 24.6 | 3.6 | 0.98 | 0.15 |
| DEER | 2012 | 17.1 | 3.2 | 15.0 | 3.3 | 0.88 | 0.44 | |
| | 2013 | 13.8 | 1.7 | 12.3 | 1.8 | 0.89 | 0.66 | |

Table 7. Change in activity (growth rate of use events) from 2012 – 2013 in treatment vs. non-treatment underpasses. Rate ratios represent the ratio of growth rate values between treatment and non-treatment sites. Treatment was analyzed at 2 spatial scales depending upon species sizes. For small animal groups, the site was the side of the underpass with/without treatment (Treatment Side, n=16). For medium and large animal groups, the site was the entire underpass with/without treatment (Treatment, n=8). Black bolded values indicate significant positive responses to treatment, where 95% CIs for the effect of treatment were positive. Red bolded values indicate significant negative values, where the 95% CIs for the effect of treatment was negative.

| | | Change in Activity 2012 - 2013 (Growth Rates) | | | | | |
|--|------------|--|------|------------------|------|---|------|
| | | Non-treatment | | Treatment | | Log Ratio (Treatment/Non-treatment) | |
| | | Mean | SE | Mean | SE | Mean | SE |
| Small Animal Groups (treatment = each side of underpass) | LIZARD | 2.17 | 0.23 | 1.84 | 0.41 | -0.16 | 0.24 |
| | MOUSE | 0.68 | 0.05 | 2.13 | 0.21 | 1.15 | 0.13 |
| | RAT | 3.52 | 0.39 | 5.14 | 0.73 | 0.38 | 0.17 |
| | SNAKE | 1.26 | 0.60 | 2.61 | 1.62 | 0.73 | 0.70 |
| | SQUIRREL | 0.49 | 0.10 | 0.41 | 0.15 | -0.18 | 0.38 |
| | RABBIT | 0.74 | 0.11 | 1.36 | 0.30 | 0.61 | 0.25 |
| Medium/Large Animal Groups (treatment = each underpass) | ROADRUNNER | 0.55 | 0.15 | 0.94 | 0.97 | 0.55 | 1.06 |
| | SKUNK | 2.15 | 0.33 | 1.16 | 0.24 | -0.62 | 0.23 |
| | RACOON | 0.36 | 0.07 | 0.42 | 0.10 | 0.15 | 0.31 |
| | FOX | 1.25 | 0.31 | 3.99 | 1.90 | 1.16 | 0.54 |
| | BOBCAT | 2.04 | 0.46 | 0.73 | 0.12 | -1.03 | 0.27 |
| | COYOTE | 1.02 | 0.07 | 1.82 | 0.27 | 0.58 | 0.16 |
| | DEER | 0.81 | 0.10 | 0.82 | 0.12 | 0.02 | 0.19 |

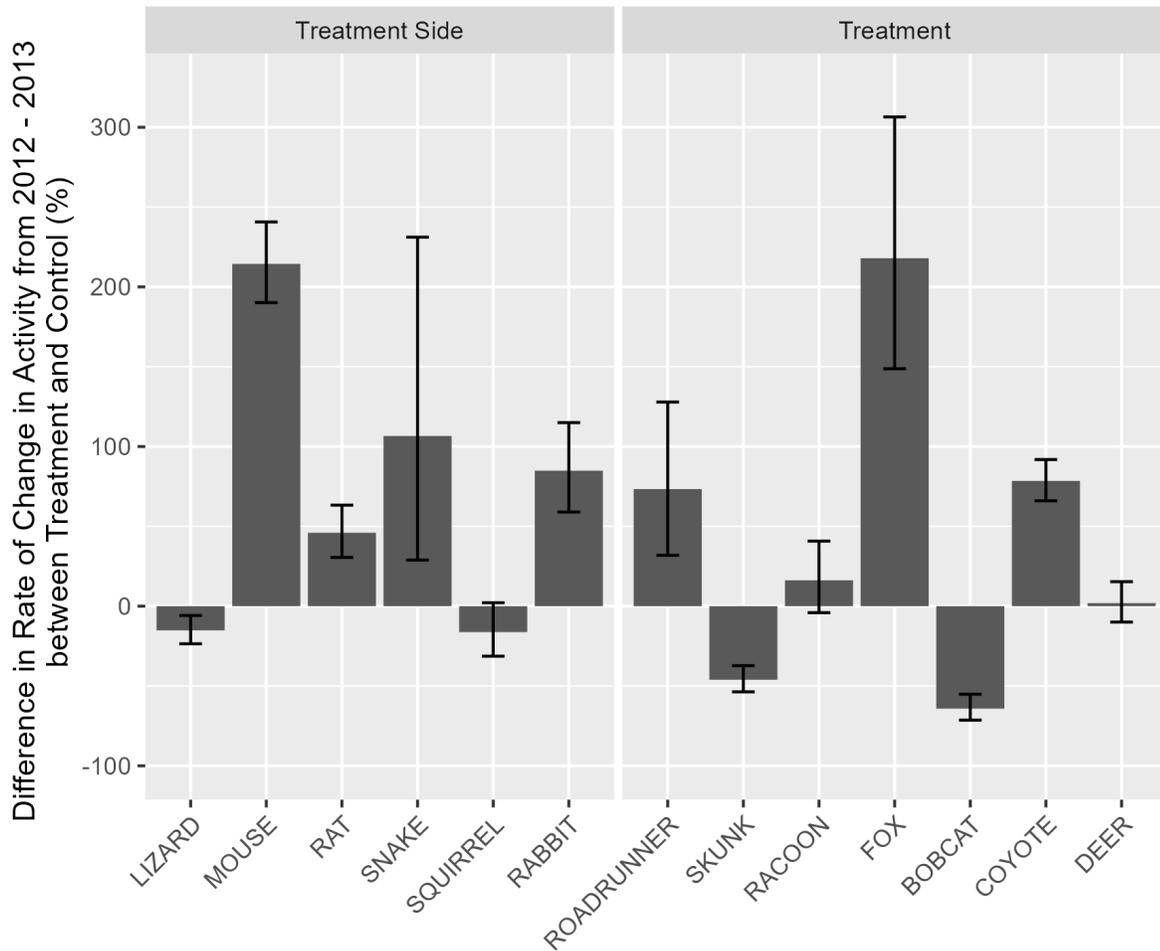


Figure 8. The percent difference in change in activity rates between treatment and non-treatment sites. Positive values indicate increased use of underpasses with structure added while negative values indicate decreased use. Treatment was analyzed at 2 spatial scales depending upon species sizes. For small animal groups, the site was the side of the underpass with/without treatment (Treatment Side, n=16). For medium and large animal groups, the site was the entire underpass with/without treatment (Treatment, n=8). Error bars indicate the mean +/- 1 SE.

A)

B)



Figure 9. A) California kingsnake (*Lampropeltis californiae*) moving along treatment side of Underpass and B) unknown snake species interacting with treatment.

Discussion

To our knowledge, this is the first study to assess the effects of internal structures/cover (in this case concrete block piles) on the use of large underpasses by a community of small and large animals. A recent review of 270 empirical papers on the effectiveness of wildlife crossing structures by Denneboom et al. (2021) concluded that there are many studies on the use of underpasses by large mammals, but small mammals and reptiles are not well represented. Additionally, only a small proportion of underpass use studies incorporate or compare animal activity within vs. outside of the underpass (Denneboom et al. 2021). We found only one unpublished study on the effect of internal cover treatments. A Masters' thesis by Connolly-Newman (2008) studied the effects of internal cover (woody debris) on small mammals in a series of 10 underpasses and reported positive, but insignificant responses (primary capture North American deer mice, *Peromyscus maniculatus*). Other than this, we could not find any such studies from this review and others (Ascensão et al. 2015, Smith et al. 2015, Denneboom et al. 2021) or from using applicable search terms on google scholar (underpass, passage, or crossing & rocks, logs, downed wood, or cover). Finally, because we used a BACI design, any differences in activity of species or groups of species related to changes in abundance over time should not have affected our results, as we expect broad temporal abundance patterns to be similar across our upland coastal sage scrub sites (Rodriguez et al. 1997, van der Ree et al. 2015a).

Underpass Permeability

Prior to any addition of structure within the underpasses, our results indicated that species groups (lizards, snakes, squirrels (primarily ground squirrels), skunks, fox, coyote, and deer) showed no significant differences in activity within and outside the underpasses suggesting that there was no strong avoidance or preference of these passages. Mice and bobcats exhibited significantly higher activity within the underpasses, likely indicating that underpasses were regularly used for foraging or home range movements. Habitat generalists and many carnivores have been reported to use large passages regularly (e.g., Mata et al. 2005, Mata et al. 2009, Grilo et al. 2008). We captured many photos of mice that appeared to show them hunting large invertebrates (particularly beetles) within the underpasses, suggesting they were using the passages as part of their habitat.

In contrast, rats (primarily native woodrats), rabbits, fox and roadrunners likely avoided use of large open underpasses, based on their significantly lower activity inside the underpasses. Rats and rabbits are primary prey species of the suite of medium and large carnivores in our study area (e.g., Koehler and

Hornocker 1991). Gray foxes (*Urocyon cinereoargenteus*) are largely associated with brushy habitats and avoid open areas and areas of high coyote predation risk (Fedriani et al. 2000). In the Santa Monica Mountains, 8 of 12 recorded fox deaths were due to coyote predation (Fedriani et al. 2000). The combination of predator avoidance and lack of cover may explain avoidance of the underpass interior for these species. Roadrunners (*Geococcyx californianus*) are terrestrial diurnal omnivores that prey upon many large invertebrates (80% of diet), lizards, and small mammals and actively defend their territories (Maxon 2005). Very high activity outside versus within the open underpasses indicates that these passages are not part of their home ranges. Although we did not survey invertebrates, we did not observe many on the underpass floor during camera checks or observe them on the daytime photos (in contrast to noticeable beetle activity on night photos). Therefore, the lack of prey may explain their avoidance of the underpasses.

Response to Treatment (Internal Structure/Cover)

The addition of structure/cover treatments resulted in a variety of responses from the wildlife community potentially associated with predator-prey interactions and alteration of interference competition between predators. Structure/cover treatments were associated with significant increases in use by mice, rats, rabbits, fox, and coyotes and substantial, but not significant, increase in activity of snakes and roadrunners. Mice, rats, and rabbits are all primary prey species for larger carnivores, and we presume they felt safer entering these underpasses with cover, while the positive responses of snakes, fox, and coyotes may have been due to increased presence and scent of prey species. The analysis for small animals was specific to the side of the underpass with the cover treatment, so these species groups were responding directly to the placed cover. In reviewing photos, mice, rats, and snakes were observed moving within the openings of the concrete cinderblock piles, while rabbits were observed next to the piles. Although not significant, roadrunner activity substantially increased after treatment. We postulate this may have been due to increased use of the underpass by large diurnal invertebrates, although invertebrate responses to cover treatment need to be verified.

Skunk and bobcat activity were significantly lower after the addition of structure/cover treatments. We don't know why skunks were less active after treatment, but as coyote prey (Shedden et al. 2020), it may be associated with increased coyote activity (but see Prange and Gehrt 2007). Similarly, coyotes and bobcats compete over the same prey resources, and there are many studies that report spatial avoidance between these species (see review by Dyck et al. 2022). Coyotes are behaviorally dominant over bobcats; negative responses in abundance and the use of habitat by bobcats

have been documented in response to increased coyote activity elsewhere (e.g., Fedriani et al. 2000, Wilson et al. 2010, Dyck et al. 2022).

There were no large or significant effects of the addition of structure/cover treatments on activity of lizards, squirrels, raccoon, or deer. The most common lizard species in our study were Western fence lizards (*Sceloporus occidentalis*, habitat generalists) and California whiptails (*Aspidoscelis californicus*, open habitat specialists; Stebbins and McGinnis 2012). Cinderblock piles within the underpass do not offer opportunities for thermoregulation; however, we would expect lizards to have a positive response if invertebrate activity increased due to the treatments. Fence lizards have also been positively associated with cover (Grover 1996). Further research may shed light on these results. Deer may be very wary of cover objects that predators may hide behind; however, the cinderblock piles were not large enough to potentially conceal any large carnivore predators, such as coyote or mountain lion. Also, deer often travel in groups as a general anti-predator strategy (Lingle 2001).

Ledges

Ledge cameras recorded very high activity of mice on ledges, compared to activity recorded by ground level interior and exterior cameras. None of the ledges in our study had ramps leading up to them, as they were not constructed to facilitate animal movement. Therefore, animals in our study had to jump or climb the walls of the underpass to reach a ledge. Ledges were used occasionally by rats and lizards in our study, but in 2012 prior to treatments, mice were 5 times more active on ledges than the interior floor and 10 times more active on ledges than exterior habitat. Many pictures of mice using the ledges appeared to show use of the ledges as a vantage point to prey upon invertebrate species below. There were many pictures of them peering over the edge and a few showing them jumping from the ledge. We believe they were hunting beetles and other large invertebrates moving along the floor of the underpass, as beetles could be seen on nighttime time-lapse photos as many small black dots that changed positions throughout the evenings. However, ground level activity of mice increased greatly after addition of structure and the use of ledges was reduced. This suggests that, in addition to using them as a hunting perch, mice also used ledges as a safe haven from predators, a function the structures could also have when present. The use of ledges as safe havens might also explain why mice were more active in untreated open underpasses than the rat guild was. Mice, predominantly *Peromyscus* spp., could presumably escape a predator by jumping and climbing onto a ledge, whereas Bryant's woodrats (*Neotoma bryanti*), the most abundant species representing the rat group, are likely less

efficient in scaling a vertical 4-foot concrete wall due to their size and weight. Additionally, woodrats primary foods (stems and leaves) are not present in the underpass, while the mice, which are more omnivorous, might find arthropod prey in the underpass (e.g., Meserve 1976). These results support incorporation or addition of ledges to enhance use and connectivity for small mammals, as well as potentially herpetofauna and medium sized mammals depending upon the presence of a ramp and size of ledge (e.g., Clevenger and Huijser 2011). Ledges specifically made for animal movement with on-ramps and off-ramps can facilitate movement for a wide variety of animal species (e.g., Dolan 2005, Villalva et al. 2013, Smith et al. 2015).

Limitations

We interpreted lower activity within the underpass to be avoidance and higher activity to be preference. However, the underpasses may act as a funnel for movement, resulting in increased probability of detection within the underpass compared to exterior habitat. For our analysis and due to relatively low sample sizes (numbers of cameras), we only incorporated relative effort as a covariate for the detection probability. Due to the number of cameras available, we also sampled the exterior with fewer cameras than were used within the underpasses. Therefore, exterior activity may be underestimated. We plan to construct additional Bayesian models that may better accommodate camera placement for the detection parameter, as well as incorporate random variables of site and year to better estimate the relative activity of species groups within and adjacent to the underpasses (exterior, interior without treatment, and interior with treatment, ledge).

We chose a threshold of 10 minutes to represent individual movement events, which is reasonable for an animal moving through an underpass or the adjacent habitat (Burton et al. 2015). Each 10-minute window could include multiple animals and/or single animals with detection sites in their home ranges (i.e., a mixture of abundance and behavior; Burton et al. 2015). In the case of the latter, death, emigration, or immigration of a single animal could have a large influence on the results. Although the underpasses had all been constructed at least 10 years prior to our 2-year study, some species may take longer to respond to the underpass treatments (e.g., Clevenger and Waltho 2003, Seidler et al. 2018). Therefore, continued study of the use of these underpasses could be valuable. Finally, additional studies across more sites and habitats would help us to better understand how taxa respond to large underpasses and internal structure.

Conclusions and Future Research

Our study showed that many small to medium sized prey species (i.e., rats, rabbits) appear to avoid the use of large open underpasses and that addition of internal structure/cover treatments may increase underpass use by these taxa. Internal structures such as the cinderblock rock piles in our study as well as the boulders and downed logs recommended in many guidance documents appear to provide an inexpensive way to increase underpass use by a wide variety of species. Our results indicate that responses may be associated with trophic interactions, such as bottom-up and top-down effects (Hanley and La Pierre 2015). Internal structural cover can enhance habitat value by shielding moisture which in turn can support more diverse microbial communities that are often the base of food chains (Warren-Rhodes et al. 2013). Internal cover also offers protection for invertebrates and small animal species. Positive responses of small animals and their predators in our study (mice, rats, rabbits, snakes, roadrunners, fox, coyote) may provide supportive evidence for bottom-up effects as a result of increased resources. However, increased use by large predators, such as coyote, may have resulted in top-down effects from predation and competition pressure such as decreased use of underpasses by medium sized prey species (i.e., skunks) and intra-guild competitors, such as bobcats.

As of this writing in 2022, the structures/cover are still in the underpasses used in this study. It would be interesting and potentially valuable to document longer term responses to the treatment, after 10 years of acclimation. Surveys specific to large invertebrates in the open areas and under the cover structures could help address hypotheses regarding responses of invertebrates and animals that prey upon these species.

Although we can acquire hundreds of thousands, or millions, of images at a relatively low cost, processing the images requires a tremendous investment of human resources. Our machine learning algorithm used in the photo analysis for this report was not completely accurate. However, we are continuing development of this algorithm to improve our ability to process large numbers of photos accurately and efficiently in the future.

Continued analyses of these data are warranted using Bayesian models to better incorporate random variables for the abundance and growth rate parameters of the N-mixture models, as well as to incorporate camera placement as a covariate in the detection probability parameter. Additional analyses that incorporate the responses of wildlife groups to human presence and activity are also planned.

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Appendix 1: Underpasses and Cameras

Valley Center Road

A. Valley Center North (VCN)



B. Valley Center Middle (VCM)



C. Valley Center South (VCS)



| Underpass | Site ID | Group | #Cameras | Camera Positions |
|----------------------|---------|-----------|----------|---------------------------------|
| Valley Center North | VCN | Control | 3 | 2 interior, 1 ledge |
| Valley Center Middle | VCM | Treatment | 4 | 2 interior, 1 exterior, 1 ledge |
| Valley Center South | VCS | Control | 4 | 2 interior, 1 exterior, 1 ledge |

Cameras:



VCN01



VCN02



VCN03



VCM01



VCM02



VCM03



VCS01



VCS02



VCS03

Scripps Poway Parkway (SPP)



| Underpass | Site ID | Group | #Cameras | Camera Positions |
|-----------------------|---------|---------|----------|------------------------|
| Scripps Poway Parkway | SPP | Control | 3 | 2 interior, 1 exterior |

Cameras:



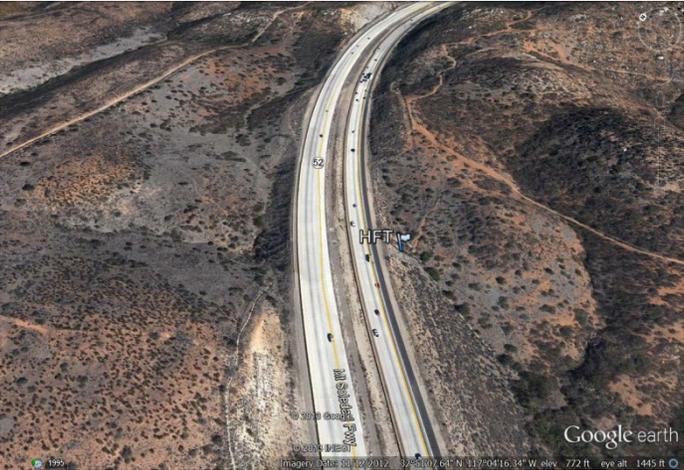
SPP01

SPP02



SPP03

Highway 52 (HFT)



| Underpass | Site ID | Group | #Cameras | Camera Positions |
|------------|---------|-----------|----------|---------------------------------|
| Highway 52 | HFT | Treatment | 4 | 2 interior, 1 exterior, 1 ledge |

Cameras:



HFT01



HFT02



HFT03



HFT04

Carmel Country Road (CCN, CCS)



| Underpass | Site ID | Group | #Cameras | Camera Positions |
|---------------------------|---------|-----------|----------|------------------------|
| Carmel Country Road North | CCN | Treatment | 3 | 2 interior, 1 exterior |
| Carmel Country Road South | CCS | Control | 3 | 2 interior, 1 exterior |

Note: Both underpasses have a 3-lane road above it.

Cameras:



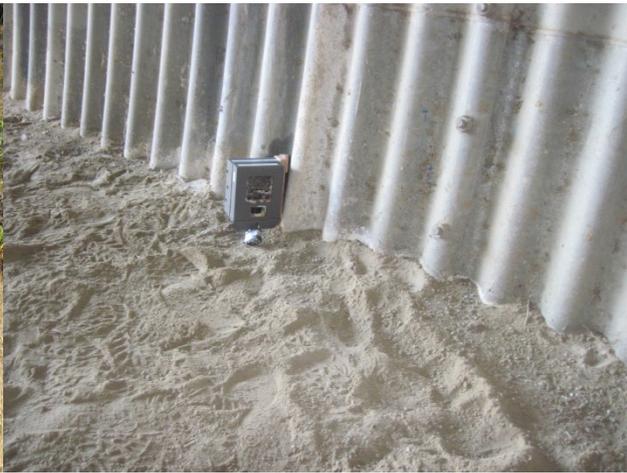
CCN01



CCN02



CCN03



CCS01



CCS02



CCS03

Note: In the photo of CCN03, we initially placed the camera at a greater height and angled it toward the ground to reduce the number of motion detection images due to plants moving in the background. However, this was ineffective, and the camera was moved to ground level.

Sorrento Valley Road (SVR)



| Underpass | Site ID | Group | #Cameras | Camera Positions |
|----------------------|---------|-----------|----------|------------------------|
| Sorrento Valley Road | SVR | Treatment | 3 | 2 interior, 1 exterior |

Cameras:



SVR01



SVR02



SVR03

Appendix 2: Representative Animal Photos

Lizards



Aspidoscelis hyperythrus (Orange-throated Whiptail)



Sceloporus occidentalis (Western Fence Lizard)



Sceloporus orcutti (Granite Spiny Lizard)

Mice



Peromyscus californicus (California Mouse)



P. maniculatus (North American Deer Mouse)



Microtus californicus (California Vole)



Chaetodipus fallax (San Diego pocket mouse)

Rats



Neotoma spp. (Woodrat)



Dipodomys simulans (Dulzura kangaroo rat) -top left
Neotoma spp.- (Woodrat) bottom right



Neotoma spp. (Woodrat) appearing to prey on beetles (circled)



Snakes

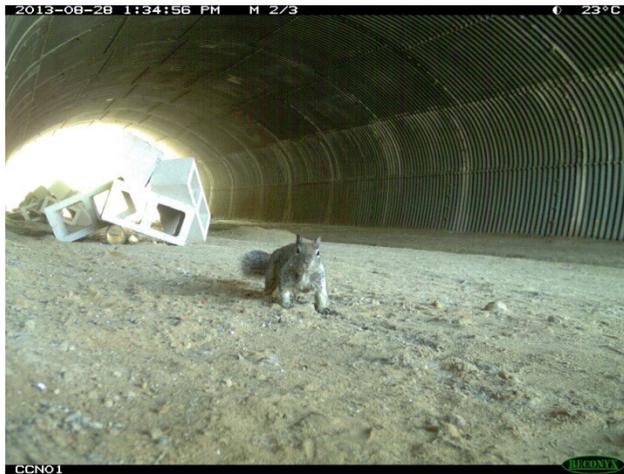


Crotalus oreganus helleri
(Southern Pacific rattlesnake)



Thamnophis hammondi
(Two-striped Gartersnake)

Squirrel



Spermophilus beecheyi (California Ground Squirrel)

Rabbits



Sylvilagus spp. (Cottontail species)

Roadrunner



Geococcyx californianus (Greater Roadrunner)

Skunk



Spilogale putorius (Spotted Skunk)



Mephitis mephitis (Striped Skunk)

Raccoon



Procyon lotor (Raccoon)

Fox



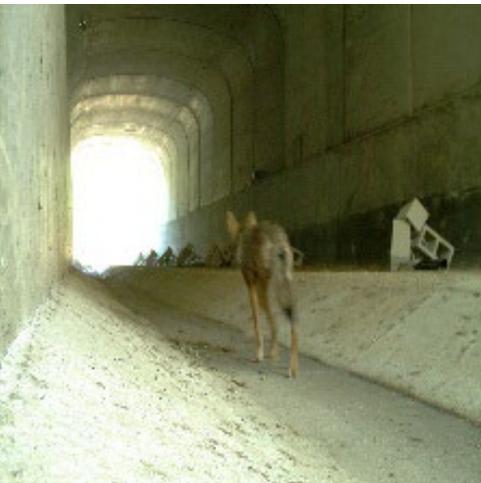
Urocyon cinereoargenteus (Gray fox)

Bobcat



Lynx rufus (Bobcat)

Coyote



Canis latrans (Coyote)

Deer



Odocoileus hemionus fuliginatus (Southern mule deer)

Appendix 3: Detection Probabilities of Species Groups

Table 1. Individual detection probability. Mean estimates for individual detection, holding camera sampling effort constant at 7 days.

| Detection: Open-Population Model | | |
|----------------------------------|------|------|
| | Mean | SE |
| BOBCAT | 0.09 | 0.01 |
| COYOTE | 0.17 | 0.01 |
| DEER | 0.08 | 0.01 |
| FOX | 0.06 | 0.01 |
| LIZARD | 0.06 | 0.01 |
| MOUSE | 0.21 | 0.01 |
| RABBIT | 0.05 | 0.01 |
| RACCOON | 0.08 | 0.01 |
| RAT | 0.05 | 0.00 |
| ROADRUNNER | 0.23 | 0.02 |
| SKUNK | 0.02 | 0.00 |
| SNAKE | 0.03 | 0.01 |
| SQUIRREL | 0.01 | 0.00 |

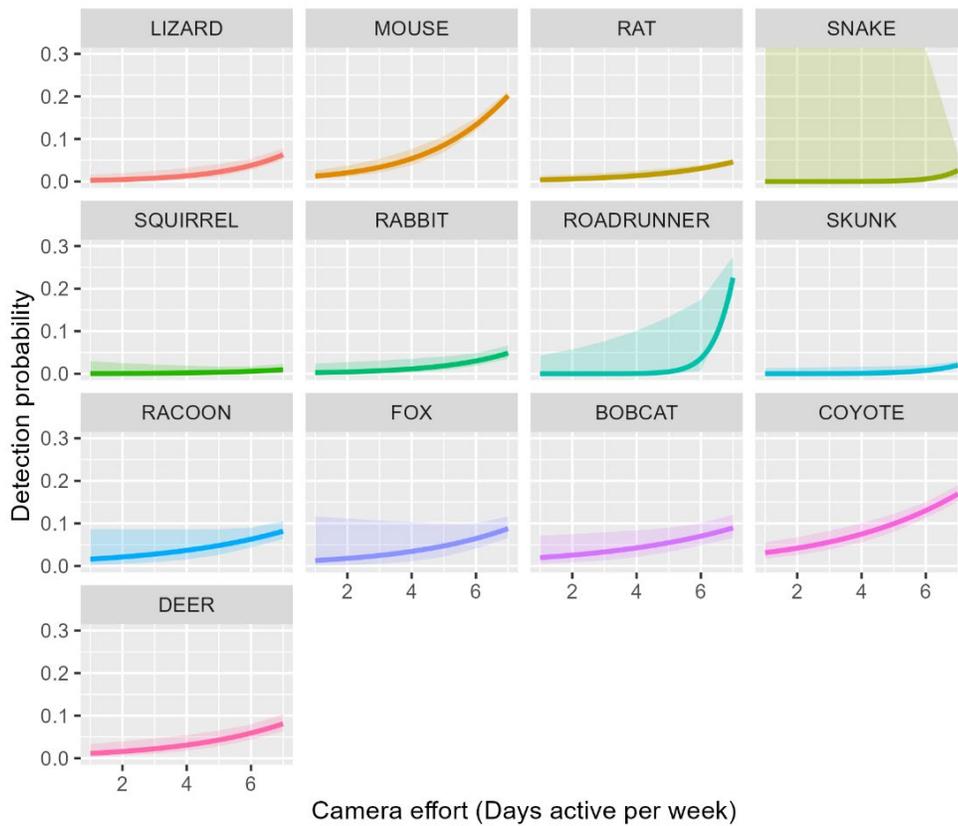


Figure 1. The effect of number of days on detection probability across underpasses for a single week. Lines represent the mean response. Intervals represent 1 SE above and below the mean respectively.

Appendix 4: Machine Learning Results

Machine learning model

Initial model results based on our training dataset of ~200,000 images suggested high sensitivity (mean = 0.983) and specificity (0.975) for 6 species groups tested. Based on these results analysis expanded to include all 13 species groups and the entire dataset of remaining images (~2.8 million photos). Of these, the initial photo validation step dropped 38,993 photos due to images not meeting preprocessing requirements. These photos were reviewed manually following the machine learning model.

Once applied across the entire dataset, the machine learning model had extremely low specificity and most images identifications were false positives. Of the 2,642,042 images included in the machine learning model, 1,385,370 were predicted to be target species. After human classification, only 31,575 of the machine learning model images were identified as target species, or 2.2% of the amount predicted by the model. The model had the lowest rate of false positive identifications for squirrels (86.4%) and the highest for bobcats (99.3%), lizards (99.2%), and skunks (99.0%). Although we did not corroborate every image classified as “NONE” by the model, after reviewing 30,000 images, only 81 contained target species, or a ~0.27% false-positive rate.

Model sensitivity was higher and 75% of images containing target species were correctly identified, when compared to accurate human classification (23,638/31,575; Table 1). When the dataset of images was reduced to only those considered unique observations (>10 minutes apart), the model compared less favorably (70%, 5237/7493; Table 2). The model was most accurate for roadrunners (94%), bobcats (83%), deer (81%), mice (81%), and squirrel (82%, Table 1). Conversely, the model poorly identified racoons (33%), snakes (34%), and foxes (42%). All other species had 64 – 73% accuracy, well below previously reported levels based on training data.

For the entire dataset of species images, the most common species group that the model assigned incorrectly was bobcats identified as other species groups including coyotes (14%), foxes (29%), mice (10%), rabbit (14%), racoon (36%), and skunk (9%). A further 3 species groups were most frequently misidentified as birds including lizards (17%), snakes (30%), and squirrels (6%).

Discussion

The machine learning model under development had relatively modest sensitivity and low specificity at the time of this report. Most false positive identifications were images with no data, taken by motion capture cameras. The inability of the model to handle images from motion capture cameras, particularly those facing outside with high amounts of vegetation in view, limited the application of the current version of this model to classify images from those cameras. Without manually reviewing the images classified by the model, counts of animals would have been overestimated by between 5.9 – 142.9x, depending on the species. Further, ~25% of images that contained species would have been incorrectly grouped by the model, with bias varying between species groups (Tables 1 and 2).

It is unclear what caused the model to not properly discern between species groups, when training datasets had shown high sensitivity and specificity (TPF Q1 2022 quarterly report). Previous training datasets had high levels of accuracy and precision, and the full model failed to meet these performance metrics. Most incorrect identifications were improperly identified empty or ‘None’ images. The model may have trained on the background present in species group images and assigned empty photos with similar backgrounds to species groups with similar background (J. Tracey pers. comm.). Further development of the model to discern empty images more effectively will likely help the accuracy of model predictions. In addition, training the model on a broader set of images, with different properties (lightings, terrains, and placements of animals within images), may also improve model performance.

Table 1: Model accuracy for all human-identified images. Values represent the percent of correctly identified (human-identified) images that were classified in each machine learning category. Cells labeled green represent the percentage of images classified as the same species by both the machine learning model and human identification. Orange cells represent the species group that the machine learning most frequently misidentified, relative to the actual (human determined) identity.

| | | HUMAN - IDENTIFIED | | | | | | | | | | | | |
|--------------------|------------|--------------------|--------|------|-----|--------|-------|--------|---------|-----|------------|-------|-------|----------|
| | | BOBCAT | COYOTE | DEER | FOX | LIZARD | MOUSE | RABBIT | RACCOON | RAT | ROADRUNNER | SKUNK | SNAKE | SQUIRREL |
| MODEL - IDENTIFIED | BIRD | 0% | 1% | 1% | 1% | 17% | 0% | 11% | 0% | 0% | 3% | 0% | 30% | 6% |
| | BOBCAT | 83% | 14% | 5% | 29% | 0% | 10% | 14% | 36% | 0% | 0% | 9% | 0% | 1% |
| | COYOTE | 12% | 66% | 9% | 5% | 3% | 0% | 3% | 11% | 1% | 2% | 2% | 0% | 5% |
| | DEER | 3% | 11% | 81% | 6% | 0% | 3% | 3% | 0% | 0% | 0% | 4% | 0% | 0% |
| | FOX | 0% | 1% | 0% | 42% | 1% | 0% | 1% | 1% | 0% | 0% | 2% | 0% | 0% |
| | LIZARD | 0% | 1% | 1% | 4% | 69% | 0% | 1% | 0% | 0% | 0% | 0% | 21% | 4% |
| | MOUSE | 0% | 0% | 0% | 0% | 0% | 81% | 0% | 0% | 19% | 0% | 0% | 1% | 0% |
| | RABBIT | 0% | 4% | 2% | 6% | 2% | 0% | 64% | 7% | 5% | 2% | 4% | 10% | 1% |
| | RACCOON | 1% | 1% | 0% | 1% | 0% | 0% | 0% | 33% | 1% | 0% | 6% | 0% | 0% |
| | RAT | 0% | 0% | 0% | 0% | 0% | 2% | 3% | 1% | 70% | 0% | 0% | 1% | 0% |
| | ROADRUNNER | 0% | 1% | 0% | 0% | 1% | 0% | 1% | 0% | 0% | 94% | 0% | 0% | 1% |
| | SKUNK | 0% | 1% | 0% | 4% | 0% | 5% | 0% | 11% | 3% | 0% | 73% | 0% | 0% |
| | SNAKE | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 34% | 0% |
| | SQUIRREL | 0% | 0% | 0% | 3% | 7% | 0% | 0% | 0% | 0% | 0% | 0% | 3% | 82% |

Table 2: Model accuracy for 10-minute unique images used in models. Values represent the percent of correctly identified (human-identified) images that were classified in each machine learning category. Cells labeled green represent the percentage of images classified as the same species by both the machine learning model and human identification. Orange cells represent the species group that the machine learning most frequently misidentified, relative to the actual (human determined) identity.

| | | HUMAN - IDENTIFIED | | | | | | | | | | | | |
|--------------------|------------|--------------------|--------|------|-----|--------|-------|--------|---------|-----|------------|-------|-------|----------|
| | | BOBC | COYOTE | DEER | FOX | LIZARD | MOUSE | RABBIT | RACCOON | RAT | ROADRUNNER | SKUNK | SNAKE | SQUIRREL |
| MODEL - IDENTIFIED | BIRD | 1% | 1% | 1% | 0% | 0% | 0% | 10% | 0% | 0% | 6% | 0% | 0% | 0% |
| | BOBC | 0% | 0% | 4% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | COYOTE | 0% | 0% | 0% | 7% | 3% | 0% | 4% | 19% | 2% | 0% | 2% | 0% | 8% |
| | DEER | 4% | 10% | 0% | 8% | 0% | 3% | 8% | 1% | 0% | 0% | 5% | 0% | 0% |
| | FOX | 0% | 1% | 0% | 0% | 1% | 0% | 0% | 1% | 0% | 0% | 2% | 0% | 0% |
| | LIZARD | 0% | 1% | 1% | 8% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 5% |
| | MOUSE | 0% | 0% | 0% | 0% | 0% | 0% | 1% | 0% | 0% | 0% | 0% | 4% | 0% |
| | RABBIT | 0% | 5% | 3% | 7% | 3% | 0% | 0% | 6% | 8% | 6% | 5% | 15% | 3% |
| | RACCOON | 1% | 1% | 0% | 1% | 0% | 0% | 0% | 0% | 2% | 0% | 6% | 0% | 0% |
| | RAT | 0% | 0% | 0% | 0% | 0% | 2% | 1% | 0% | 0% | 0% | 0% | 4% | 0% |
| | ROADRUNNER | 0% | 1% | 0% | 1% | 1% | 0% | 1% | 0% | 0% | 0% | 0% | 0% | 2% |
| | SKUNK | 0% | 0% | 0% | 3% | 0% | 6% | 2% | 11% | 4% | 0% | 0% | 0% | 0% |
| | SNAKE | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | SQUIRREL | 0% | 1% | 1% | 4% | 6% | 0% | 0% | 0% | 0% | 0% | 0% | 4% | 0% |



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