San Francisco Bay Area, badgers were negatively associated with suburban land use and road lengths (Lay 2008). In 1986, the American badger was listed as a California Department of Fish and Wildlife (DFW) Species of Special Concern due to a substantial reduction of their distribution and abundance.

Badgers were extensively hunted for their pelts in 1930's and 1970's, and are still reportedly being trapped in high numbers (Williams 1986, Quinn 2008). Currently, a California DFW Trapping License is required for any for-profit trapping or hunting of badgers with no limits to the number of individuals. Depredation and predator control that is not for-profit does not require a permit or reporting. This species has long been considered a pest species for agriculture. It is hypothesized that there are many more badgers killed for depredation and it is unknown how much this has contributed to their decline (Williams 1986, Quinn 2008). To date there is little known about the ecology of the badger in coastal southern California.

Primary stressors to the American badger in southern California include:

1. Road mortality
2. Habitat loss
3. Habitat fragmentation: Lack of open habitat and/or corridors for movement and dispersal.
4. Hunting and trapping: Predator control/ sport shooting/ fur trapping
5. Consumption of pesticides through small mammal prey

Methods

Canine Scent Surveys

The Center for Conservation Biology (CCB, University of Washington) obtained American badger scat from the Washington Zoo. With this scat, CCB initially trained one detection dog "Pips" at their training facility in Eatonville, Washington, following the methods outlined in Wasser et al 2004.

> "Dogs selected for the program were initially introduced to target species odor (scat) utilizing a scent box. The scent box is a 2 m × 30 cm × 30 cm hinged rectangle with five compartments open to the outside by a 5-cm hole. Scat is placed in one of the five compartments. The search is initiated by the verbal command "find it". The dog is guided to investigate each compartment of the scent box and encouraged to smell at the hole openings. Initially, the "find it" command is verbalized between each hole. Upon sniffing the hole containing the sample, the dog is immediately rewarded with a well-timed toss of a tennis ball

> across its visual field followed by verbal praise and ~90 s of play. The dog quickly learns to associate sample detection with the reinforcement of the reward. This maintains a strong motivation level for these high play drive dogs to locate the source of target odors throughout the day. Samples are next hidden at multiple indoor locations, varying height, and degrees of detection difficulty. After 1–2 days, the scent box is again briefly used to teach the dog to sit at the sample prior to receiving the reward. This keeps the dog focused on the scat until the handler can confirm its presence. Scat samples are then gradually hidden over a progressively larger, defined area in the field. Samples are set out in the training area at least several hours prior to any given training session. This allows the scat scent to percolate into the environment and any human scent trail to dissipate. Dogs are introduced to scat from many different individuals of each target species".

Once in San Diego, the canine scent detection team surveyed targeted sites from March 31 to May 9, 2014. The schedule was typically 3 days on with one day off as recommended by Conservation Canines. USGS biologists (Cheryl Brehme and Stacie Hathaway) assisted as orienteers and for data collection. On several days, we would survey more than one site in a day. All routes and detection locations were recorded using a GPS unit attached to the dog. GPS coordinates were taken and pin flags were placed at locations where the dog indicated a scent detection (behavior change, "hit"). After a dog "hit", the handler would state the confidence level in the dog's response as well as the handler's confidence in the dog's response. All scat was collected with gloved hands, placed into a plastic bag, and stored frozen until DNA testing. The orienteer also recorded information on the condition of the scat (color, freshness, and contents), vegetation type, dominant soil type, and took photos of the scat and representative habitat.

Badger Sign Surveys

In addition to canine surveys for scat, we also surveyed the landscape for potential badger sign (burrows, digs, and tracks). The surveyor would walk the site while scanning for mounds and burrows. Burrows were measured and confirmed as badger if they were the correct size and shape (approximately 8-12 inches wide and 6-10 inches in height) and contained characteristic horizontal claw marks within the burrow (approximately one inch spacing between claws). Freshness was determined by evidence of loose soil at the entrance indicative of recent digging. Other evidence included body 'drags' and/or tracks observed at the burrow entrance. Older burrows were identified as such if they had new or substantial growth of grasses or forbs at the entrance, there was no evidence of recent digging, or contained evidence of recent squirrel use.

Scat DNA Testing

The goal of scat DNA testing was to identify if scat samples collected in the field were from the American badger. CCB developed a badger specific identification assay that amplifies two American badger specific DNA markers and tested all samples.

The surfaces of all samples were swabbed in duplicate to remove mucosal cells for DNA extraction. DNA on the swabs was extracted using a modified version of Qiagen's DNeasy Tissue DNA extraction kit. These DNA extracts were then PCR amplified three times on the duplicate extracts using two previously developed and validated badger-specific mitochondrial DNA markers, BGR1 and BGR3. Fragments were separated by size using capillary electrophoresis on an ABI 3730 and then visualized and scored using SoftGenetics' GeneMarker software. Negative controls were used throughout each step of the process, and positive controls of known badger DNA and various non-target species were amplified along with experimental samples. Because of the specificity of the assay, all positive results can be interpreted as DNA from the American badger. Negative results should be interpreted as either being from another species or from the scat of an American badger where the DNA was too degraded to amplify in the PCR's.

To test for the potential of another animal's urine or contamination of DNA on the exterior surface of the scats, a subset of samples was swabbed on both the interior and exterior of the scats. In addition to amplification with the badger-specific markers, both the interior and exterior swabs of these samples were PCR amplified and digested using primers that amplify mammalian mitochondrial DNA and differentiate numerous species based on a restriction digest enzyme that cuts the amplified DNA into species-specific fragment sizes (Foran et al 1997). A small subset of these samples were also PCR amplified using BGR1, BGR3, COI and ATP6 markers from the mitochondrial genome, and the amplified DNA was sequenced using an ABI 3730 and analyzed using MEGA software.

Hair Snags

When fresh badger burrows were identified at sites where repeat visits were possible, hair snags were placed within the burrow entrances. Hair snags were constructed according to protocol provided by American badger researcher, Richard Klafki (British Columbia), who travelled to San Diego and shared his expertise and methods with the USGS from May 1 to May 6, 2014.

Snags were made from 30 cm (12”) of 2-cm (3/4”) wide metal strapping formed into a ‘D’ (Figure 1). Two 3-inch nails were inserted through holes drilled at the base of the ‘D’ and were used to secure the snag inside the burrow. Three rivets were placed at each edge and middle to secure the strapping in its shape. Two squares (approximately 3-4 cm by 2 cm) of pinned-knaplock (used to anchor carpet in doorways) were riveted to the curved edge of the metal strapping. The teeth of the knaplock were slightly bent down to better force hair into the snag and prevent injury to an animal.

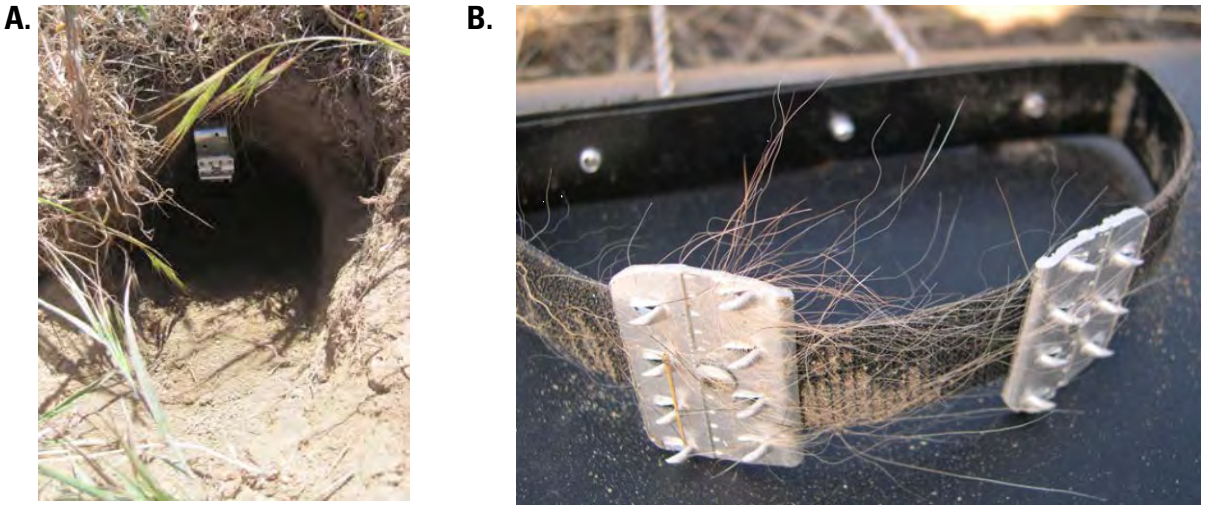

Figure 1. (A) Hair snag in burrow in Volcan Mountain, (B) Close-up of snag with hair provided by Richard Klafki.

Infrared Cameras

Infrared cameras were set near and facing areas with fresh burrows in attempts to document badger activity. Reconyx PC800 HyperFire Professional Semi-Covert IR were set to medium sensitivity for motion detection and automatic time-lapse photo captures every 1 minute.

Outreach Efforts

Outreach efforts to the public and other wildlife professionals are commonly used in order to gain information on badger localities and their spatial and temporal use of habitat (Ministry of Environment Ecosystems 2007). We created and distributed a poster for public and professional outreach for this information in July 2014 (Figure 2). The poster was modified from a version provided to us by Richard Klafki. We distributed the poster to wildlife professionals, land managers, and others, as well as posted to the Western Ecological Research Center and San Diego Monitoring and Management Program websites. Many people kindly forwarded this to others to further the outreach

efforts as well as posted in information kiosks (Figure 2). We also established a "San Diego Badger Hotline" (phone and email) to collect any sighting information.

Figure 2. Badger information outreach poster with hotline information (adapted from version provided by Richard Klafki). Example of poster in kiosk at Barnett Ranch Preserve.