Pacific Pocket Mouse Sampling Methodology Study, Marine Corps Base, Camp Pendleton.

By C.S. Brehme, L.R. Albert, T. Matsuda, R. N. Booth, and R.N. Fisher

Data Summary
Prepared for:

AC/S Environmental Security, Marine Corps Base Camp Pendleton

U.S. DEPARTMENT OF THE INTERIOR
U.S. GEOLOGICAL SURVEY
WESTERN ECOLOGICAL RESEARCH CENTER

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Suggested citation:
Brehme, C.S, L.R. Albert, T. Matsuda, R. N. Booth, and R.N. Fisher. 2010. Pacific Pocket Mouse Sampling Methodology Study, Marine Corps Base, Camp Pendleton. Prepared for AC/S Environmental Security, Marine Corps Base, Camp Pendleton. 47p.

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By C.S. Brehme, L.R. Albert, T. Matsuda, R. N. Booth, and R.N. Fisher.

Abstract of 2008 Study

In preparation for developing a comprehensive long term monitoring plan for the Pacific pocket mouse (PPM), a scientific panel agreed that several preliminary 'pilot' studies were warranted to generate information needed to draft a quality program. This report describes the second short term study identified as a priority, which was to determine if an alternate, accurate, sampling tool or method can be found that will increase probability of detection and increase cost effectiveness, as well as decrease negative impacts to the species and its habitat.

From May 5 to July 13, 2009, we conducted surveys for PPM within Oscar One training area and South San Mateo. Our main goals were: 1) to evaluate methods for detecting PPM (Sherman live-traps (small, medium standard, medium with perforations), tracking tubes of different diameters (1.5" vs. 1.0"), and canine scent detection (Conservation Canines, University of Washington), 2) record behavioral responses of the mice to the different traps and tracking tubes using infrared video cameras, and 3) develop a species identification assay for rodent scat to distinguish PPM from other rodent species.

In order to directly compare Sherman live-traps and tracking tubes, arrays of tubes and traps were subjectively placed at suspected PPM burrows and open sandy patches across the study area and run for 4 nights at each location. After the third week, we also left tracking tubes out for additional nights where PPM were detected in traps, due to the unavailability of PPM for detection after being captured in a Sherman trap. We set a total of 468 arrays over the study period. Probabilities of detection were highest for the small and medium Sherman live-traps and the 1.5" tracking tube. Cumulative detection probability regression plots predict a > 90% chance of detection after 6 to 9 nights (uncorrected and corrected estimates). Estimates using the perforated traps and 1 inch tube were substantially lower with a 90% probability of detection after 11 to >14 nights. These probabilities are at a very small spatial scale of a single array location to be used for

comparison. Detection probabilities when setting larger grids of traps or tubes would be substantially greater.

A single Jack Russell terrier, Casey, successfully completed the PPM scent training by mid-June. We could not survey the same array locations due to canine behavioral changes associated with human scent, so we set up three independent 100 m^2 survey grids with 100-10 m^2 survey cells (2 in Oscar One, 1 in South San Mateo). Casey surveyed all survey cells one time, and half of the cells a second time due to time constraints. Of these, he had positive behavioral changes "hits" at 193 cells. Forty-six percent were verified by tracking tubes, 20% by scat DNA testing, and 34% were unverified. We were unable to determine whether unverified hit locations were occupied by PPM. As a separate validation, we secretly placed PPM scat (7-10) in 24 cells during the course of the study. Of these Casey detected 54% on the first survey, 84% after being redirected to search specific location, and did not detect 17%.

In summary, all three sampling methods evaluated were successful in detecting the Pacific Pocket Mouse (PPM). Each method has advantages and disadvantages that are reviewed in this report. The use of any method should be chosen and evaluated based upon the objectives of the study.

For long term monitoring of PPM, the optimal method will have high probability of detecting PPM, low impact to PPM, and be reasonable in cost (Brehme and Fisher, in collaboration with scientific panel. 2009). The use of 1.5" tracking tubes best meet these criteria of the sampling methods evaluated in this study. We also recommend the use of 1" tube in combination with the 1.5" tube even though detection probabilities were quite low using this method. When other more dominant species are highly abundant in comparison to PPM, the inclusion of the 1" tube excludes most other small mammal species maximizing the potential for PPM detection.

The positive correlation between PPM counts and the numbers of arrays where PPM were detected show that number of arrays could be used as a relative index of density. This relationship is not linear, however, and will always be limited by the number and density of tracking tubes. The sensitivity lies at the lower end of the range which may be the more critical range for assessing declines and/or needs for management actions.

Live-trapping is necessary in order to determine if PPM are reproducing in a given year. It is unknown if successful reproduction is mostly governed by general climate and rainfall conditions or local environmental factors. In order to get a better understanding of population dynamics across their habitat, we recommend establishing a live-trapping grid within each of the

populations (Santa Margarita (Oscar One and Edson Training Areas), North San Mateo, and South San Mateo)) to be run for a limited time each year.

We recommend canine scent surveys as the most suitable method for discovery. This is by allowing them to search and wander over large spatial areas (up to $2km^2$ per day) and follow scents (rather than being confined to a cell or grid). In this way, rather than use for detecting individual mice, dogs would be used to detect PPM populations or subpopulations. Positive detections could then be used for targeted verification studies using tracking tubes, live-trapping, and/or scat DNA testing. In 2008 and 2009, we also learned that PPM exist well outside of their known boundaries at South San Mateo. Use of the dogs is also recommended to better establish PPM population study boundaries for the long-term monitoring program.

Unfortunately, we were unable to confirm the presence of PPM at North San Mateo for a second year using the canine scent surveys with targeted verification by scat DNA assay and tracking tubes.

The video recordings of PPM behavior in relation to the traps and tubes were very helpful in understanding why capture probabilities of individual mice are fairly low. We observed many instances (35%) in which mice were active but not detected by traps or tracking tubes within a given night. In many of these instances, the mice showed no interest in the traps or tubes. In the videos that did show high interest by a PPM individual, it was typically a long period of time before they were detected (average of 83 minutes). Often, PPM were in the Sherman traps many times before being captured. We do not know if this is due to the animal avoiding the treadle (staying in front or jumping to back- both behaviors were observed) or due to the treadle not being sensitive enough. All traps were set as sensitive as possible without automatically closing to a sensitivity of at least 5g. In most instances, the animal was eventually captured in the same trap on the same night. Other behaviors observed like digging, burrow tending, burrow sharing, and mating all add to our life history understanding for the species.

Finally, the scat DNA assay for species identification is a very useful tool to confirm presence of PPM from scat. Scat can be collected at suspected burrows in combination with any of the other detection techniques to help establish presence or absence at a study site.

Introduction

The primary mission for Marine Corps Base Camp Pendleton (MCBCP) is "to operate an amphibious training Base that promotes the combat readiness of operating forces by providing facilities, services, and support responsive to the needs of Marines, Sailors, and their families" (MCB Camp Pendleton Strategic Plan 2002). In addition, the base has committed to fulfill stewardship and regulatory requirements for the natural resources on base. This includes monitoring and management for the endangered Pacific pocket mouse (*Perognathus longimembris pacificus*, PPM) as described in the MCBCP Integrated Natural Resources Management Plan (October 2001). The U.S. Geological Survey was contracted to develop a scientifically valid, effective, and cost-effective monitoring program for the PPM on MCBCP in 2007. The monitoring program should document trends in the status of PPM on base and identify results criteria for management action.

In 2007, the USGS, representatives from MCBCP, and a scientific review panel reviewed literature on PPM ecology and previous monitoring efforts in order to construct a long term monitoring plan for PPM on MCBCP. A two-day workshop was held on September 6th and 7th in which many independent and agency scientists, consultants, and land managers gave additional input. The major consensus along with workshop materials and panel comments are presented in the document “Pacific Pocket Mouse Monitoring Plan for Marine Corps Base Camp Pendleton: Short Term Studies and Long Term Goals” (2009). In general, the panel agreed upon a ‘proportion area occupied’ (PAO) spatial approach to monitoring the largest population within the Oscar One and Edson training areas. This approach may be used for the smaller San Mateo populations; however, it is suspected that if population sizes are very small, these areas may require a more intensive annual census. In addition, a portion of annual effort should go toward discovery of ‘new’ populations of PPM within suitable habitat less than 5km from the coast.

In preparation for developing a comprehensive long term monitoring plan for PPM, the panel agreed that several preliminary 'pilot’ studies were warranted to generate information needed to draft a quality program. In 2008, we conducted the first short term study, which was to immediately and comprehensively assess the status of North and South San Mateo PPM populations (Brehme and Fisher 2009).

For the second ‘pilot study’, the USGS and scientific review panel agreed the current sampling methodology is time intensive, destructive to habitat, and suffers from a low probability

of capture. The use of this method as a primary sampling tool limits the amount of area that can be surveyed effectively in any given year, thus reducing the robustness of any spatial long-term trend indices. Because of this, USGS and the panel determined that a study is warranted to determine if an alternate accurate sampling tool or method can be found that will increase probability of detection and cost effectiveness, as well as decrease negative impacts to the species and its habitat. This report describes the second short term study conducted in 2009 to assess the effectiveness of alternate sampling methodologies for PPM.

Pacific Pocket Mouse

The Pacific pocket mouse (PPM) is one of 19 subspecies of the little pocket mouse (*Perognathus longimembris*) in the heteromyid rodent family. This subspecies was historically rare and patchily distributed along coastal southern California. They were thought to be extinct until rediscovered in 1993 on Dana Point. PPM were federally listed as endangered on September 29, 1994 and were subsequently found in three locations within MCB, Camp Pendleton in 1995 (North San Mateo, South San Mateo, and North Santa Margarita within the Oscar One Training Area). These four locations comprise the only currently known extant populations of this subspecies.

PPM have been historically found on southern California marine terraces and alluvial plains within 4 km of the coast. They are typically associated with open patches of sandy soils within coastal sage scrub communities, although vegetation characteristics, such as shrub and grass cover, vary considerably. Their diet is comprised primarily on small seeds of grasses (*Bromus*, other) and herbs/forbs (*Croton californicus, Heterotheca grandiflora, Pluchea sericea, Hordeum murinum, Centaura melitensis, Corethrogyne filaginafolia, Gnapthalium sp., Calacadenia sp*., other) and shrubs (*Eriogumun fasciculatum*, Meserve 1976b, Germano 1997). PPM have a long lifespan for a rodent of up to 8 years in captivity and 3 to 4 years in the wild (French et al.1967, Hayden and Lindberg 1976).

PPM are nonsocial, nocturnal, and physiologically adapted to warm and dry climates. They go into variable amounts of facultative torpor during the winter and in response to low resource conditions. Above ground activity is thought to coincide with seed availability, which may extend from March through September, but is most dependable in late April, May and June (Meserve 1976a, Shier 2009). The demography of foraging mice has been shown to change within the active season. From early to late season, the dominant demographic group will change from adult males

to adult females to young of the year. This is consistent with reproductive activity (Miller and Pavelka 2008, Shier 2009).

The onset of breeding is typically in early spring. Females gestate young for about 23 days and wean young after 30 days. There may be no reproduction in low resource years. However, in high resource years, adult females may have up to two litters with their female offspring mating and reproducing in a single season (Miller and Pavelka 2008). Because of this, PPM abundance can be highly variable within and among years.

PPM exhibit typical behaviors of heteromyid rodents including sand bathing to keep pelage clean and healthy, collection of seeds in external cheek pouches, and caching of seeds below ground and within burrow systems for sustenance throughout the year.

PPM movements of up to 181m in a single night have been documented, with average movement distances reported of 10m to 30m between successive captures (Dodd et al. 1998, 1999; Miller and Pavelka 2008). The average home range size from 9 individuals was estimated to be 0.017 ha for this species (Shier 2009), which is smaller than that reported for desert *Perognathus longimembris* subspecies (home ranges of 0.12 to 0.56 ha in Joshua Tree (Chew and Butterworth 1964) and 0.3-3.1 ha in Nevada (Maza et al. 1973).

There are many potential threats and stressors to current PPM populations. These include habitat loss (development, soil compaction and associated vegetative loss from heavy use), habitat alteration (overgrowth of non-native grasses or native shrubs), fragmentation (roads, development), increased predation risks (domestic cats, dogs, Argentine ants), and increased competition for seed resources (Argentine ants)(USFWS 1997, Brehme and Fisher 2009).

Objectives

Our main objectives were to:

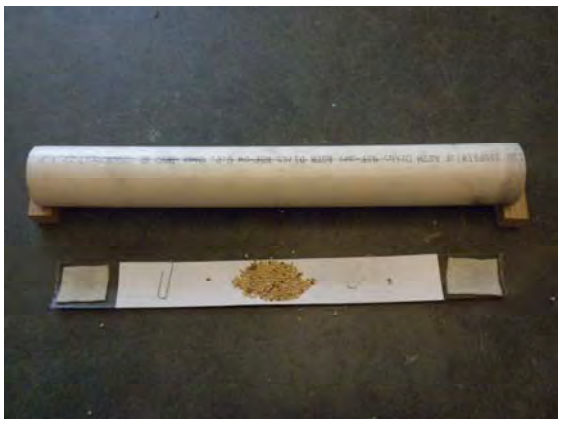
1. Evaluate methods for detecting PPM.
    a. Sherman traps of different sizes, with and without perforations
    b. Tracking tubes of different diameters
    c. Canine scent detection
2. Record behavioral responses of the mice to the different traps and tracking tubes using infrared video cameras.
3. Develop species identification DNA assay for rodent scat to distinguish PPM from other rodent species.

Materials and Methods

Tracking tubes:

Tracking tubes are used to identify small animals by their tracks (Glennon et. al 2002, Loggins et. al 2010, Matsuda et al. 2010). Our tracking tubes were a modified version of Loggins et al. 2010, currently being used by the Florida Fish and Wildlife Commission to monitor the endangered Choctawhatchee Beach Mouse (*Peromyscus polionotus allophrys*) and the endangered Perdido Key Beach Mouse (*Peromyscus polionotus trissyllepsis*), and by the Fish and Wildlife Service, Daphne Field Office to monitor the endangered Alabama beach mouse (*Peromyscus polionotus ammobates*). The tracking tubes used by these agencies are open only at one end. Our tracking tubes are slightly modified in that they allow free access and exit at both ends, allowing mice to escape from any potential predators or other aggressive mice. Two sizes of tracking tubes were used for the study (Figure 1).

- 1.0” diameter X 15” length PVC pipe
- 1.5” diameter X 15” length PVC pipe



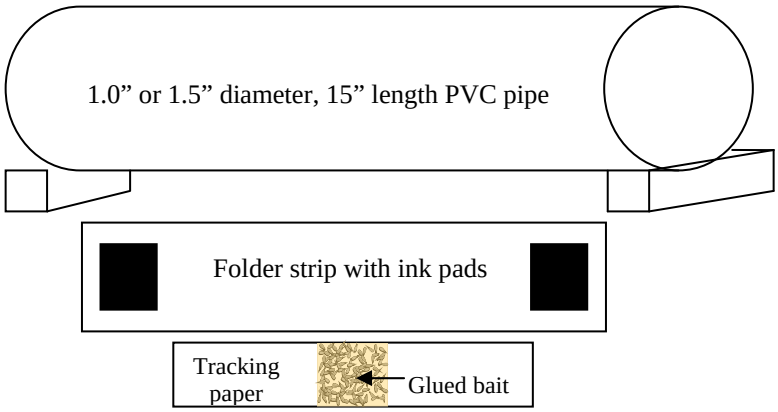


Figure 1. Photograph and Diagram of Small Mammal Tracking Tube

Tracking tubes were made out of standard 1.0” and 1.5” diameter PVC pipe cut to a length of 15”. They were fitted with wood blocks routed and glued to fit the contour of the edges of the tube to prevent rolling and debris build up in the tube. This retrofit was added 6/1/09. Tubes

contained inserts with ink pads, tracking paper, and bait. Inserts were made of 15” length strips of filing folders in the corresponding 1” or 1.5” width wrapped with contact paper. This was necessary to prevent ink from being absorbed into paper. 1.5” long felt pads were then glued to each end to serve as ink pads. Ink pads were saturated with ink solution and a tracking paper (replaceable heavy cardstock, Wausau Paper). was placed in between the ink pads and secured using a standard paper clip. The ink solution was composed of 2 ½ parts of food grade mineral oil (STE Oil Company, San Marcos, TX) to 1 part carbon lampblack (Fisher Scientific, Catalog # C198-500) as described by Mabee 1998, Glennon et al. 2002; Nams and Gillis 2003, and Loggins et. al 2010). Securing the tracking paper to the insert is necessary to ensure the paper did not come in contact with the felt pad and absorb ink from the pad. A small amount of starch glue was then applied to the center of the tracking paper with a tablespoon of bait placed on top. We applied a small thin layer of glue (<100uL) to attach the first layer of bait to the tracking surface. The starch glue formula (Pritt) is made from 90% renewable resources and is non-toxic, solvent-free, and acid-free – environmentally-friendly, yet with a strong bonding strength. This allowed the tube to remain an effective attractant, even if other mice or ants remove the loose seeds on top. This was important to evaluate for possible use in a long term monitoring program. Currently, beach mouse programs check tubes monthly. This allows them to cover of a large spatial area over a long period of time. In PPM habitat, there are many other species of mice and ants which can predate the seed bait. If the tubes do not need to be checked daily for presence of seed, we could potentially have substantial increases in sample size and the spatial area covered. The tube was then gently placed on the ground.

Live-Traps

We tested three different types of Sherman live-traps (H.B. Sherman, Inc., Tallahassee , FL);

- 3x3.5x9" Large Folding Aluminum Sherman live animal trap with Galvanized Treadles and Doors; w/ shortened doors modification (LFATDG SD).

- 3x3.5x9" Perforated Large Folding Aluminum Sherman live animal trap with Galvanized Treadles and Doors; w/ shortened doors modification (PLFATDG SD).

- 2 x 2.5 x 6.5" Small Folding Aluminum with Galvanized Treadles and Doors w/ modified shortened doors (SFATDG SD)

Trapping & Tracking tube arrays:

Between May 1 and July 10, 2009, we placed 50-75 detection arrays over seven sampling periods of four nights each (468 locations). In order to maximize the potential for detection, we limited our study to areas with records of PPM captures within the Oscar One Training Area and South San Mateo (SSM), such as the eastward powerline road off of MACS Road. North San Mateo (NSM) was not targeted for this study as no PPM were captured at this location in 2008 (Brehme et al. 2010). Each week, we trapped different sets of plots and surveyed for four nights. (Note: FWS recommends a minimum of five nights of trapping for presence/absence surveys. The purpose of these surveys was not to establish absence- see Introduction/ Objectives sections).

Detection arrays within these areas were subjectively placed near possible PPM burrows and open sandy patches. Arrays were placed at a minimum distance of 10m apart from one another and locations were recorded using a handheld GPS. Detection arrays consisted of the following traps and tubes placed within a 1m diameter circle (Figure 2).

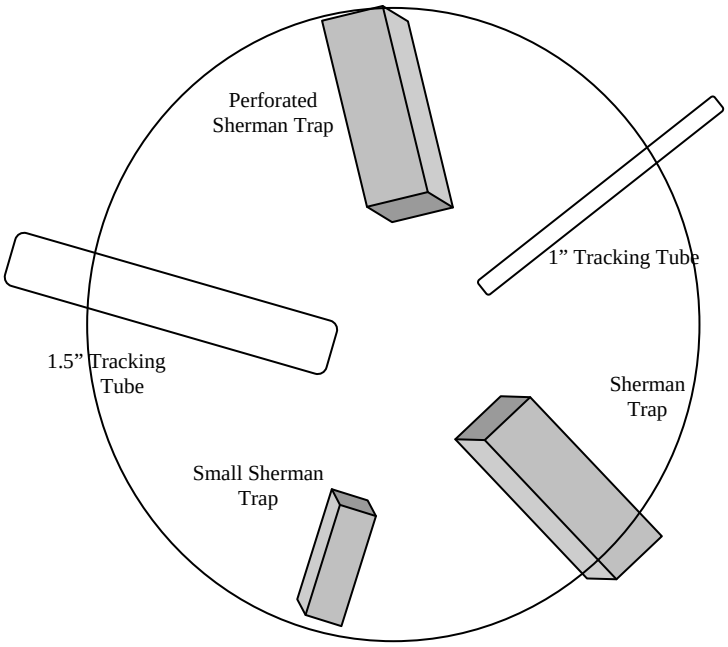


Figure 2. Diagram of Trap and Tracking Tube Array

Traps and track tubes were set on the first day and baited with birdseed composed primarily of millet. They were checked for 4 consecutive nights approximately 11:00PM to 2:30AM and again in the mornings from 5:30AM to 10:00AM. Sherman traps were closed each morning and re-baited and opened each afternoon after 3:00PM. Captured PPM were measured (hind foot, body length, tail length, ear length) and weighed. Subadults were identified by the presence of partial grey pelage on the body or legs. Sexual reproductive status was noted. All animals were temporarily batch marked by clipping a small amount of fur from the hip area to document recaptures. The clips were unique to time and day of trapping (first night/morning = upper/lower right hip clip, second night/morning = upper/lower left hip clip, third night/morning = upper/lower back clip, fourth night/morning = upper/lower back of neck/head clip). Tracks were also taken of some captured individuals for use as track vouchers.

Tracks from tracking tubes were measured (hindfoot and forefoot width and length) and identified to species or genus. Note: Successful identification of PPM tracks was determined to be feasible by pilot studies conducted by the USGS (2008) and Peter Meserve (1976a). PPM tracks are definable by both size (hindfoot and forefoot width and length) and toe pattern.

On the third week of the study, if PPM were captured in Sherman traps but no tracks were left in the tracking tubes, we left the tracking tubes out for four more nights. Similarly, if PPM tracks were detected at an array, with no captures in Sherman traps, we set out the Sherman traps for four more nights. On the 4th through 7th week of the study, we set out tracking tubes only for four subsequent nights due to sampling and cost constraints and because tracking tubes were still being validated as an new detection method. The schedule for trapping is presented in Table 1.

Table 1. Schedule for Trapping and Tracking Tube Arrays

	Primary Sampling			Continued sampling*		
Week	Dates	Location	# arrays	Dates	Total # arrays	Sizes
1	5/4-5/9	Oscar 1- crucible	71	na	0	na
2	5/11-5/15	Oscar 1- Powerline	72	na	0	na
3	5/18-5/22	Oscar 1- Powerline	75	5/25-5/28	25 tubes/ 6 traps	1.5" only
4	5/25-5/29	Oscar 1- Powerline	65	5/29-6/3	16 tube pairs (both size)	Both sizes
	6/1/2005	**No new trapping- Retrofit of tubes with wood end pieces**				
5	6/8-6/12	SSM	60	6/16-6/19	10 tube pairs (both size)	Both sizes
6	6/15-6/19	SSM	75	6/22-6/26	16 tube pairs (both size)	Both sizes
7	6/22-6/26	Oscar 1- Powerline	50	6/29-7/3	all	Both sizes

* Left track tubes out at arrays where there were captures in traps but no tracks after first week

Personnel

FWS Permit TE-045994-8

Cheryl Brehme, Laura Albert, Denise Clark - Independent

Dana McLaughlin- Supervised

Analyses

Relative detection probabilities were estimated using the single-year single season logistic model in program PRESENCE as described by MacKenzie et al. 2006. For input data, we only used data at array sites where we captured or detected PPM by one or more methods. Therefore, we knew the proportion area occupied (psi) was 1.0 for these sites. In order to directly compare relative detection probabilities, psi was fixed at 1.0 for all analysis. We condensed night and morning detection data, so that there was one data point for each night of the survey (4 in total). This was due to poor model fits when using both night and morning data.

After an animal is captured in a trap, it cannot be detected in another trap type or tracking tube. Because of this, both an uncorrected and a corrected analysis was done for all traps and tubes. For the uncorrected analysis, if an animal was captured in one of the traps during a trap occasion, the occasion was treated as a "no detection" for the other traps and tubes (i.e. for Program Presence a "0" vs. "1" detection). For the corrected analysis, if an animal was captured in one of the traps during a trap occasion, the occasion was treated as "no survey" for the other traps and tubes. In other words, the PPM was not available for detection in the other traps and tubes (i.e. for Program Presence a "-", vs. 1 or 0). It is likely that the true detection probability lies somewhere between these estimates, as there would be an undetermined amount of time the animal was available to be detected in another trap or tracking tube prior to being capture in one of the Sherman traps.

In modeling relative detection probability, we compared models where ρ was constant (.), varied by each individual trapping session (t). Because small mammals may be more likely to enter a trap after a period of acclimation, we tested models where ρ differed between the first one or two sessions and all subsequent sessions (Day 1_other, Day1_2_other). Cumulative probabilities of detection over number of trap occasions were calculated in the same manner as cumulative capture

probabilities (Equation 1). We used detection probabilities from the latter nights to extrapolate cumulative detection for prolonged surveys. For instance, if the best model was (Day1_other) with an estimated p of 0.2 the first night and 0.4 for subsequent nights, we used p=0.4 to extrapolate cumulative detection for a hypothetical 14 nights. Since only sample arrays where PPM were detected by any method were used in these analyses, these estimates are expected to be biased slightly higher than true values, but are valid for comparative purposes.

Equation 1:

$$\text{Cumulative } \rho = 1 - (1-\rho_1)*(1-\rho_2)*\ldots*(1-\rho_n)$$

where ρ = capture probability

n= trap session

PPM Video Surveillance Cameras

A total of 8 camera set-ups (provided by USGS) were used simultaneously throughout the study in addition to capture data to determine PPM trap preference. The cameras were set up along with trap arrays on the first day at active PPM burrows. Each camera set up consisted of a weatherproof Sony Super Night Vision Camera (model M020-S53-002) with built in IR illuminators (IR-2HAD) and a 6.0mm fixed focus lens. Each camera was mounted on a 57.5” tall Manfrotto tripod (model 190XPROB) that was fitted with bird deterrent spikes. The cameras were connected to a SVAT Mini Portable Digital Video Recorder (model Clear Vu Pro CVP800) using AV out wiring. During set up an AV input wire connected the digital recorders to a hand held video viewer (Archos modelAV400 20 GB Video Player/Recorder) to ensure that the placement of the camera angle was facing the trap entrances and optimally captured the 1 meter diameter trap set up (Figure 3). The cameras and digital recorders were plugged into a 200W inverter contained in a weather proof case below the tripods. The inverters were switched off during the morning trap checks and turned back on during the afternoon baiting of traps to conserve battery life. The inverters were powered by rechargeable Super Start car or marine deep cycle boat batteries. Wires connecting the devices were encased in plastic wire protecting split loom coil to prevent wire damage from chewing by rodents or other animals (Figure 3).

Figure 3. Surveillance Camera; a) Set-up and b) View.

The digital recorders were set to continuously record in high quality mode between the hours of 10pm and 5am. The digital recorders contained two AA batteries in them at all times to prevent recording settings from being reset. The motion detection setting was ineffective due to the small size of the PPM. A 16GB memory card was placed in each recorder, swapped each morning with a blank card and the data was uploaded onto a computer for later viewing. The memory cards were labeled with the corresponding camera number.

AVS4YOU video software was used to view the footage. This was done in a stepwise manner. First, we viewed video from arrays that had positive animal detections from the trap and/or tracking tube data. The remaining video was considered negative footage and viewed at 16X real time. This allowed us to view all video footage obtained between the set hours of 10pm and 5am. Upon watching the video we counted the number of times an individual;

1) Approached a trap type: Approached a trap entrance without entering the trap.
2) Entered a trap type: Completely entered the trap from the cameras aerial view.
3) Was detected: Captured in the trap or left tracks in the tubes.

We also recorded the start and end PPM activity time stamp and the number of PPM in each session. Notes were kept of all animal interaction and behavior.

Fecal (Scat) Species Identification Assay

The overall goal was to identify Pacific Pocket Mouse (PPM) scat samples that were collected in the field, and to discriminate them from other potential non-target species. This led to 2 main goals in the lab (Center for Conservation Biology- CCB, University of Washington): 1) design primers and develop a PCR assay that discriminates PPM from other species, and 2) validate a reliable DNA extraction method for these extremely small scat samples

Initial Research: USGS provided scat and ear tissue samples of 5 male and 5 female PPM, and sympatric non-target small mammal species captured in South San Mateo and Oscar One training areas; *Chaetodipus californicus, Reithrodontomys megalotis* (REME), *Peromyscus maniculatus* (PEMA), *P. eremicus* (PEER). *P. californicus* (PECA), and *Mus musculus* (MUMU). First CCB searched the NCBI online database for mouse DNA sequences from similar mitochondrial regions, and then imported those sequences from PPM as well as all other non-targets species into the software program, CLC DNA Workbench. This program then aligned those sequences and forward and reverse primers were designed from conserved regions of DNA. From this sequence alignment and primer design, CCB was able to predict the size of DNA band that would be amplified, and if any restriction enzymes could cut the band for specific species (which it did indicate for a few species, including PPM).

PCR Test: CCB then extracted the DNA from tissue samples sent by USGS using Qiagen DNeasy Tissue kit. Then CCB performed PCR reactions using the designed primers and our standard Species ID PCR recipe and thermocycler settings (Wasser et al. 2004), and then performed a restriction digest with enzyme Hinf1 on a portion of the PCR product. The samples were then loaded onto a 3100 Genetic Analyzer (Applied Biosystems Inc.), and the band sizes were viewed and analyzed using the software Genotyper (ABI).

These results showed all mouse species produced a band of 348bp, but when digested with the Hinf1 enzyme, PPM would be cut into a 274bp band, CHCA into a 230bp band, some PEMA into a 154bp band, and all other species didn't get cut at all, so remained a 348bp band (PEER, REME, PECA, and MUMU). This confirmed our predicted results from the software program, and was successful in discriminating PPM.

DNA Extraction test: To ensure DNA could be extracted from the extremely small scat samples, with the most efficient method possible, CCB tried 3 different extraction methods: Qiagen Stool Kit, Qiagen DNeasy Tissue kit (modified), and AquaStool kit. Scat samples obtained from a

pet mouse (MUMU) were used for the initial extraction test, as numerous pellets were available from the same individual to rule out any sample variation. After extracting the DNA, the same PCR assay and Hinf1 digest was performed as with the tissue samples, (including a few tissue samples for positive controls), and run on the 3100 Genetic Analyzer. The results showed that both of Qiagen's kits worked equally well (100%) whereas the AquaStool kit only worked on 50% of the samples. The modified Qiagen DNeasy Tissue kit was chosen since it is the most efficient (less labor-intensive than the Qiagen Stool kit). CCB then extracted all of the PPM and non-target scat samples provided by USGS, and performed the PCR, Hinf1 digest and 3100 run as before. These results matched the tissue samples' results, and completed the validations for this Species ID assay.

Canine Scat Detection

The USGS provided scat collected from captured PPM individuals to the Center for Conservation Biology (CCB, University of Washington). With this scat, CCB initially trained 2 detection dogs (Jack Russell Terriers "Casey" and "Timmy") 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".

After the initial training, two handlers (Julie Ubigau, Michelle Manza) travelled to San Diego on June 8 to train the dogs in actual PPM habitat on MCBCP. The Powerline Road area of Oscar One was chosen for the high density of PPM in the area. USGS personnel walked the area and flagged what they perceived as freshly dug PPM burrows (fresh mounds of sand, PPM sized

scat, entrance hole of ~2.5 cm typically triangular in shape). The dogs were trained on PPM burrows using same reinforcements as above. Training on MCBCP was also occasionally supplemented with placed PPM scat. Dogs were regularly taken to coastal sage scrub areas outside of MCBCP (i.e. San Elijo ecological reserve in northern San Diego County) to ensure they would not 'hit' on non-target small mammal species scat. Behavior changes or 'hits' were typically defined as the dog showing an increased interest in a small area followed by sitting down. After sitting down, the dog was instructed to "show me", and would typically point to an area on the ground with its nose. Training on MCBCP lasted from June 8 to June 17. One dog, "Casey" successfully completed the training. Audubon cottontail and California Quail proved to be too much of a distraction for Timmy to overcome.

Canine Survey Grids

We marked off two 100m X 100m grids in the Oscar One training area and one 100m X 100m grid in South San Mateo Housing Area (see Figure 4). 100m grids were then subdivided into one hundred 10m X 10m search 'cells'. This design was based upon consultation with Heath Smith (Conservation Canines) and Mark Pavelka (USFWS). Each cell was searched by Casey for PPM scat for an average of 5 minutes. Each day, Casey was able to work for an average of 5 to 6 hours and complete one-half of a grid. A USGS biologist assisted as orienteer and for data collection. For repeatability, one half of each grid (50 cells per grid) was surveyed a second time (see Table 2 for Schedule).

Canine surveys were conducted as follows; Casey was led on leash by his handler (Julie Ubigau, Conservation Canines) and instructed to search each cell. The search was limited to approximately five minutes or a maximum of two hits per cell, then the dog was led to the next cell. After a dog 'hit', the handler would state the confidence level in both the dog response as well as the handlers' confidence in the dog response. Handlers' confidence is based upon their assessment of the dog's behavior. This may be useful to separate out when the dog is responding but not detecting PPM scent (i.e. seeking approval from the handler) versus getting a genuine PPM scent detection. Observers then searched for PPM scat, collected any found using tweezers, and placed into a small vial. If scat was not found on top, we collected sand from the area pinpointed by the terrier.

Sand was then sifted through two increasingly fine soil sieves (nos. 12 and 25) to remove any PPM size scat for further analysis and verification. Using scat collected from captured

individuals, we determined that PPM scat can be separated using the no. 25 sieve. This mixture was then carefully hand sorted to separate any PPM looking scat from other small detritus. The scat was placed in test tubes with a confidence level of low or high based upon shape and color.

A two- step least cost approach was used to verify dog 'hits'. After the canine surveys were complete, a tracking tube was placed within each cell of each grid and run for 7 days. If there was a canine 'hit' in a cell that was not verified by tracks, then the best looking scat sample (if scat recovered) from that cell was sent to the lab for species identification.

Table 2. Schedule for Canine Scent Surveys

Grid	Portion of Grid	Dates of Initial Canine Survey	Date(s) of Repeat Canine Survey	Dates of Tracking Tube Survey
1	East half	6/18	7/1, 7/3	6/22- 6/29
	West half	6/22	NA	6/29- 7/6
2	East half	6/22	NA	6/22- 6/29
	West half	6/24	6/29	6/29- 7/6
3	South half	6/26	7/2	7/6- 7/13
	North half	6/30	7/2	7/6- 7/13

Additional Survey Areas

On July 5, we took Casey to survey North San Mateo and around pitfall array # 12 in South San Mateo. A Pacific pocket mouse was captured during a herpetofauna pitfall trap survey on June 23 at this array, which was 400 m southeast of the known population boundaries at this site. Nearby herpetofauna array # 13 was also searched. At North San Mateo, the search was concentrated along the trail that goes around the site.

Accuracy in Canine Detection of Placed PPM Scat

In order to get a better understanding of the sensitivity of Casey to placed scat, USGS personnel placed PPM scat in some of the cells the day before a survey was scheduled. Scat was placed in 2 different arrangements to simulate concentrated odor vs. spread out odor; 1) 7-8 scat placed together and 2) a grouping of 3-4 scat placed in 3 distinct locations within 10cm of each other (i.e. points of a triangle). The number of scats for these trials was chosen based upon the availability of PPM scat from captured PPM from the trap/tracking tube array efforts.
Trials were conducted during grid surveys for a total of 12 trials per placement type. The handler was unaware of which cells contained the placed scat. When conducting the trial, if Casey searched

the cell without detecting the placed scat, the USGS orienteer would point out the general area of placed scat. The handler would then take Casey a second time, directing him to search around the directed location. Thus, there were three categories of response; 1) detection upon first search, 2) detection upon 2nd directed search, and 3) No detections after 2 searches. We report the percentages of each response based upon type of placed scat.

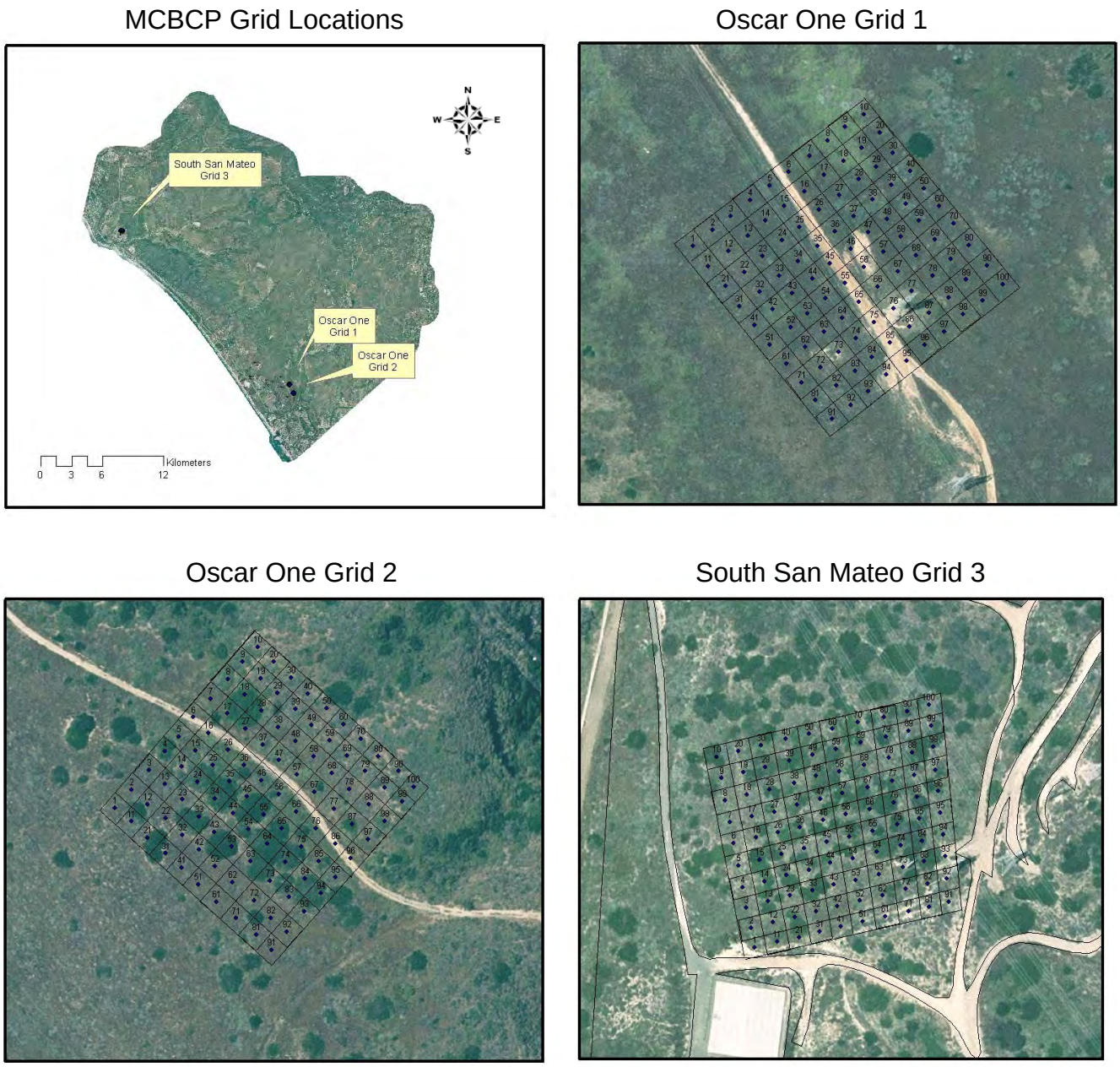


Figure 4. Locations of 100m^2 Canine Scent Survey Grids in MCB, Camp Pendleton

Results

Live-Trap and Tracking Tube Arrays

At 468 subjectively placed arrays, we detected PPM at a total of 171 arrays or 37% (Table 3). Of all positive arrays, medium and small standard Sherman traps had the highest detection rates within the 4 day period (63%), followed by 1.5” tracking tubes (45%), medium perforated traps (32%) and 1.0” tubes (20%). Because PPM were unavailable to be detected by other methods after being captured and were more likely to go back to the same trap after first detection, starting in the 3rd week we left the tracking tubes out at locations with a Sherman trap capture but no tube detection for four to six more days. This resulted in an increase in detection rates to 70% for 1.5” tubes and 40% for 1.0” tubes.

In the Sherman traps, we captured four individuals at a single array (0.6%), three individuals at 6 arrays (3.3%), two individuals at 24 arrays (13.3%), and one individual at the remaining 110 arrays (61.1%). We documented movement between a minimum of 20 arrays (12% of total arrays, distance 10 to 15m), where the first capture at the array was a recaptured individual.

Table 3. Number of PPM Detections among Traps and Tracking Tubes

			Number of Arrays with Detections after 4 nights (after 8-10 nights)				
			Sherman Traps			Tracking Tubes	
Week	Location	# arrays	Medium Standard	Medium Perforated	Small Standard	1" Diameter	1.5" Diameter
1	Oscar 1- crucible*	71	0	1	1	0	0
2	Oscar 1- Powerline	72	10	7	7	3	7
3	Oscar 1- Powerline	75	28 (32)	9	23	3	15 (33)
4	Oscar 1- Powerline	65	26	15	24	9 (19)	20 (28)
5	SSM	60	11	5	16	8 (16)	13 (21)
6	SSM	75	9	5	11	3 (14)	5 (14)
7	Oscar 1- Powerline	50	11	6	13	9 (13)	16 (17)
Total PPM Detections			95	48	95	35 (68)	76 (120)
Relative Detection Rate			63%	32%	63%	20% (40%)	45% (70%)
Total PPM Detections: 3rd-7th week			85	40	87	32 (66)	69 (114)
Relative Detection Rate			57%	27%	58%	21% (44%)	46% (76%)

* Working out methods with cameras and tracking tubes

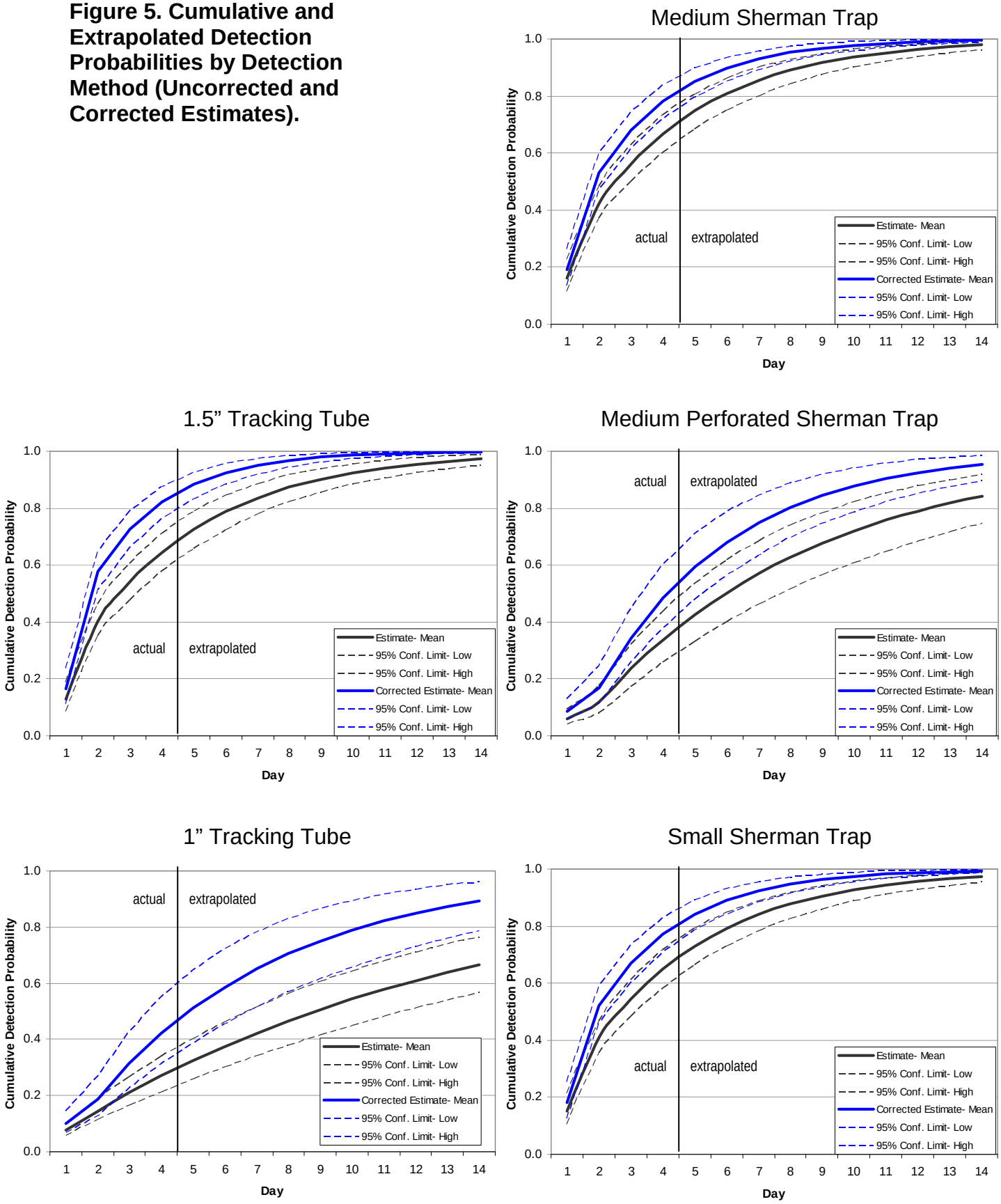
Of the simple occupancy models tested, the best model for each trap and tracking tube type is presented in Table 4. The fit of all models (as measure by the deviance statistic *c-hat;* where *c-hat* = 1 is a perfect fit) increased after the data correction based upon non-availability of PPM to be detected after being capture in a single Sherman trap (highlighted in yellow, see Methods:Analysis section for correction details). This correction did not take into account the time available before capture or the possibility of more than one individual being present, so the actual model and detection probabilities are likely somewhere between the corrected an uncorrected versions. Almost all models also showed an increased probability of detection for each trap and tube type after the first or second night of the survey ("Day1_other", "Day12_other").

Relative detection probabilities estimated from each model are presented in Table 4 and Figure 5 by survey day and detection method. To simulate longer term sampling, cumulative detection probabilities from model parameter estimates are also extrapolated out to 14 days (Figure 6). Data was limited to all arrays with a PPM detection and the occupancy parameter (psi) was fixed to "1.0" for all model evaluations (i.e. all sites occupied). Because of this, the estimates for ρ are likely biased slightly high. Medium Sherman traps, small Sherman traps, and 1.5" tracking tubes all predict a PPM detection probability close to 1.0 after 14 survey days (corrected and uncorrected models).

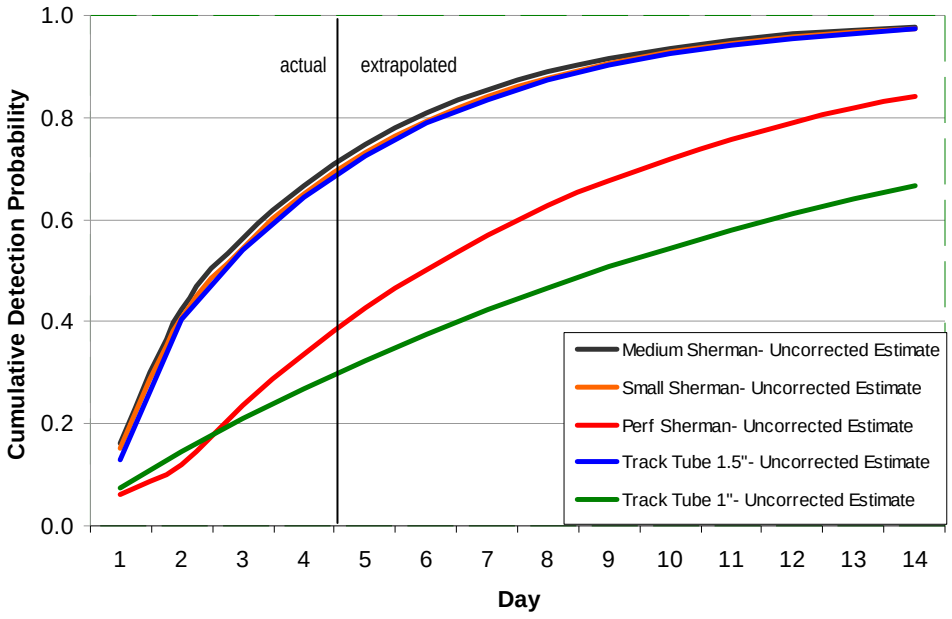
Table 4. Detection Probabilities among Traps and Tracking Tubes (Uncorrected and Corrected Estimates).

Detection Method	Best Model for (p)	Detection Probability (p) by day p- day 1	p- day 2	p- day 3	p- day 4	Model Fit c-hat
Medium Sherman	Day1_other	.163 (.028)	.241 (.019)	.241 (.019)	.241 (.019)	2.8
Medium Sherman corr.	Day1_other	.193 (.033)	.317 (.024)	.317 (.024)	.317 (.024)	1.4
Perforated Sherman	Day12_other	.061 (.013)	.061 (.013)	.134 (.018)	.134 (.018)	3.2
Perforated Sherman corr	Day12_other	.087 (.018)	.087 (.018)	.213 (.028)	.213 (.028)	1.6
Small Sherman	Day1_other	.151 (.027)	.231 (.019)	.231 (.019)	.231 (.019)	2.6
Small Sherman corr.	Day1_other	.181 (.032)	.309 (.024)	.309 (.024)	.309 (.024)	1.7
All Sherman	Day1_other	.319 (.036)	.491 (.023)	.491 (.023)	.491 (.023)	3.3
Tube 1 inch	p constant	.075 (.010)	.075 (.010)	.075 (.010)	.075 (.010)	42.1
Tube 1 inch corr.	Day12_other	.099 (.020)	.099 (.020)	.155 (.027)	.155 (.027)	1.6
Tube 1.5 inch	Day1_other	.129 (.026)	.228 (.019)	.228 (.019)	.228 (.019)	8.9
Tube 1.5 inch corr.	Day1_other	.165 (.032)	.351 (.027)	.351 (.027)	.351 (.027)	3.6
All tubes	Day1_other	.140 (.027)	.252 (.019)	.252 (.019)	.252 (.019)	9.4
All tubes corr.	Day1_other	.180 (.033)	.380 (.027)	.380 (.027)	.380 (.027)	3.6

Figure 5. Cumulative and Extrapolated Detection Probabilities by Detection Method (Uncorrected and Corrected Estimates).



A. Models not corrected for unavailability of PPM for detection after capture in trap.



B. Models corrected for unavailability of PPM for detection after capture in trap.

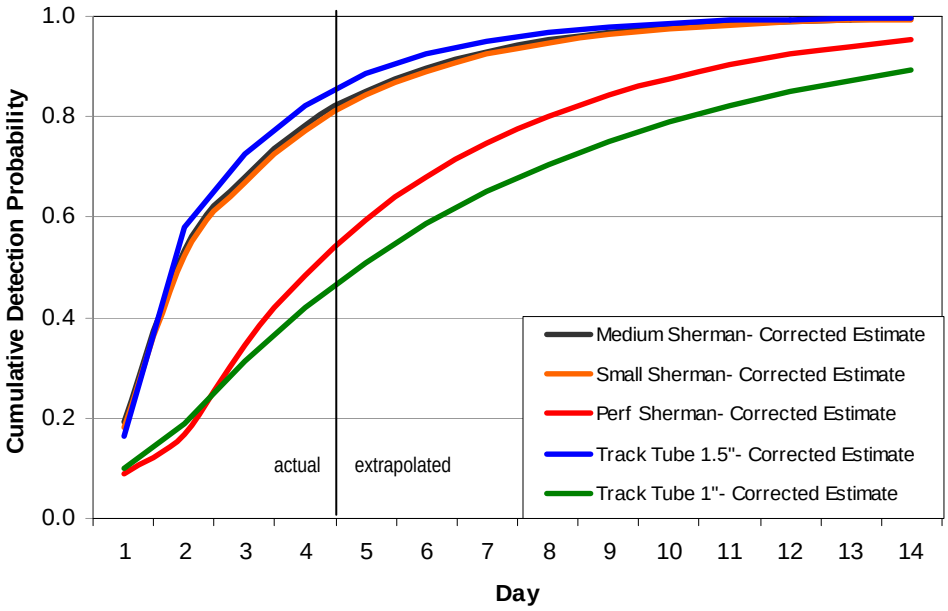


Figure 6. Cumulative and Extrapolated Detection Probabilities by Detection Method (Uncorrected and Corrected)

The relationship between PPM counts and number of arrays where PPM was detected was explored to determine if the latter could be used as a relative density index (Figure 7). Results show significant positive correlations for both detection techniques. The totals for arrays deployed each week were used as data points. The best fit regressions were all quadratic showing that the slope of the relationship decreased as PPM densities increased.

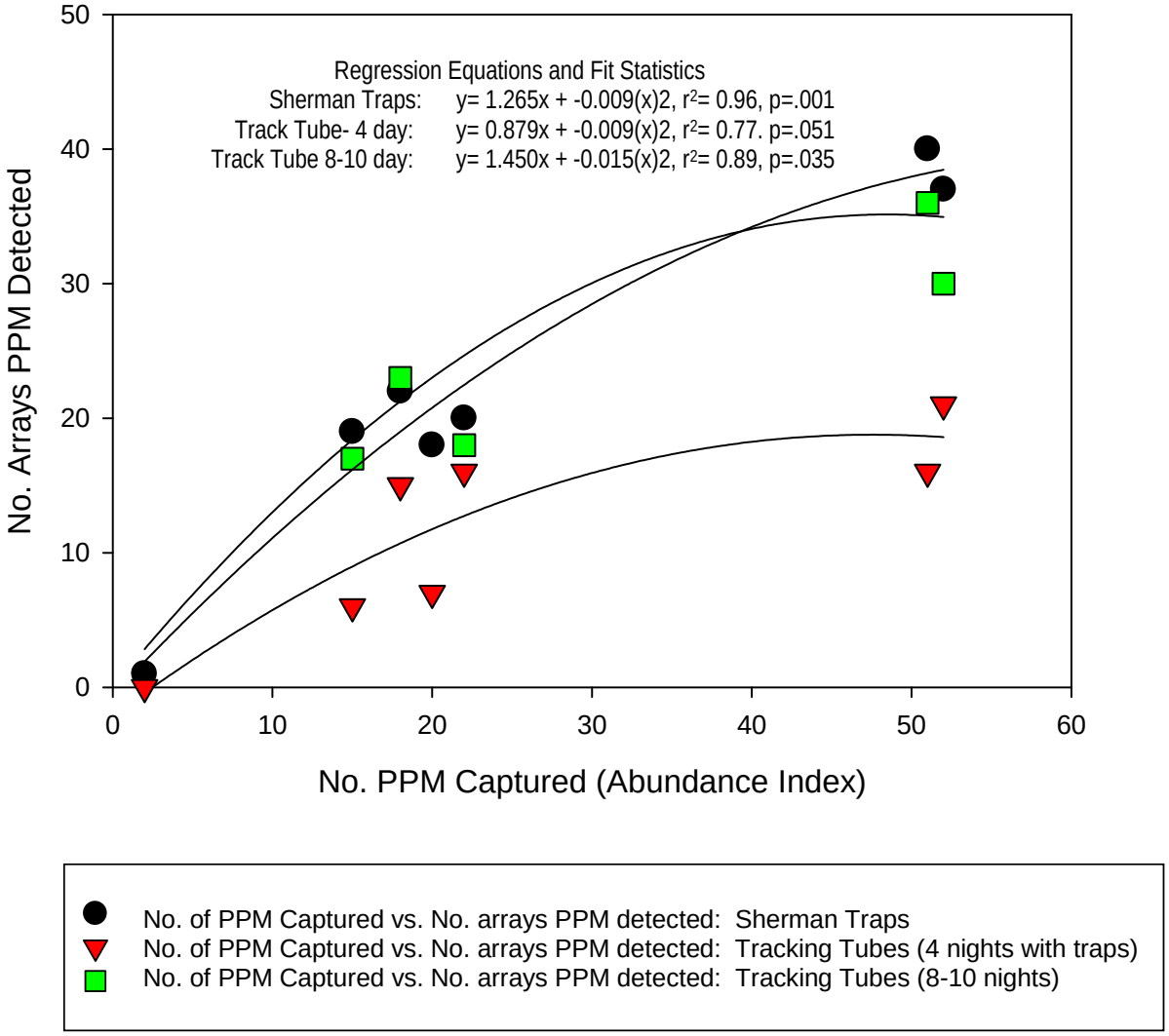


Figure 7. Relationship between Individual PPM Counts and Number of arrays PPM
PPM Video Surveillance Materials & Methods

Video Behavioral Observations

A total of 108 videos were viewed, of those 20 were positive for PPM footage. It was possible to identify the PPM in the videos due to their small size and jumping mode of movement in comparison to other mice species. It was not possible to identify the PPM to an individual. There were 50.75 hours of PPM footage from the 10pm to 5am time frame. PPM were active throughout the time period, but found to be the most active between the hours of 11:00pm and 3:00am. (Figure 8). In 7 (35%) of the videos, PPM were observed but not detected by any method. When detected, PPM spent an average of 83 minutes (range 1-261) in the cameras view before being captured in a Sherman trap or detected on tracking paper. (Figure 9).

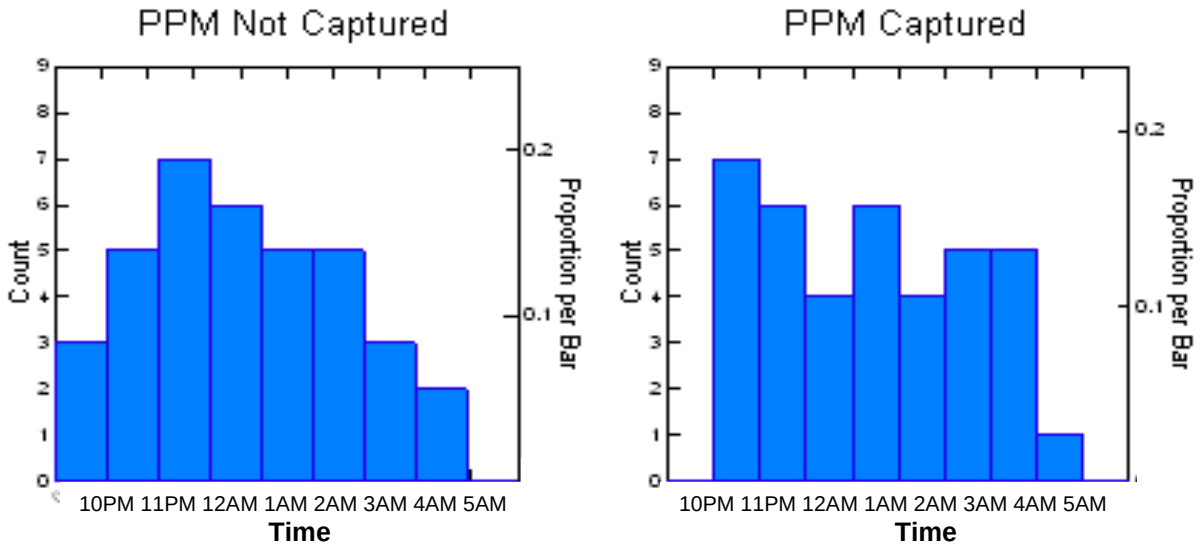


Figure 8: Times of PPM Activity Across All Videos

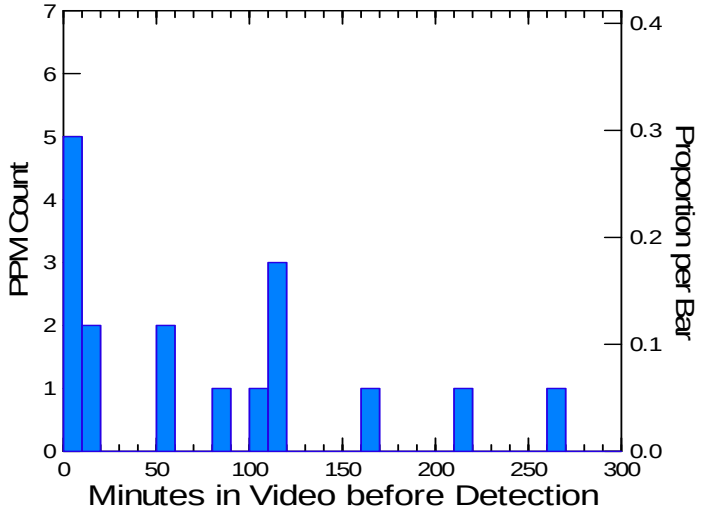


Figure 9: PPM Activity Minutes in Video before detection

PPM approached the medium and perforated Sherman traps most frequently at a median of 1.5 and 3 times a night, respectively, and approached the 1” tracking tube the least amount of times a night (Figure 10). The perforate Sherman trap had the most total approaches and entrances with a median of 1.5, ranging from 1 to 29. While the 1” tracking tube was approached and entered the least number of times. The medium Sherman, small Sherman and 1.5” tracking tube were approached and entered a similar amount of times and had the most PPM detections (Figures 10 and 11). Trap entries without capture raised the question of where in the trap the PPM is located. The mouse may stay in the front of the trap and not trigger the treadle or it may jump to the rear of the trap completely avoiding the treadle and not activating the trap door. We observed both behaviors while out at night. These occurrences count as entries and not captures.

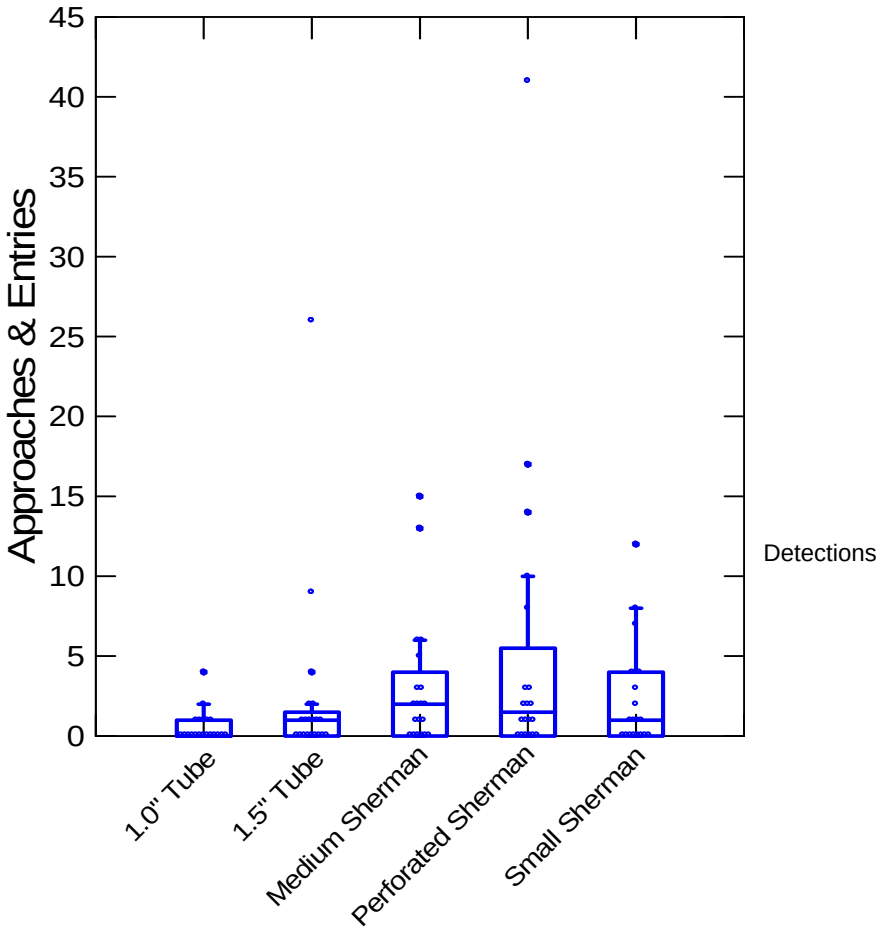


Figure 10 : Box Plots with Median Numbers of PPM Approaches & Entries per Video Night by Detection Method

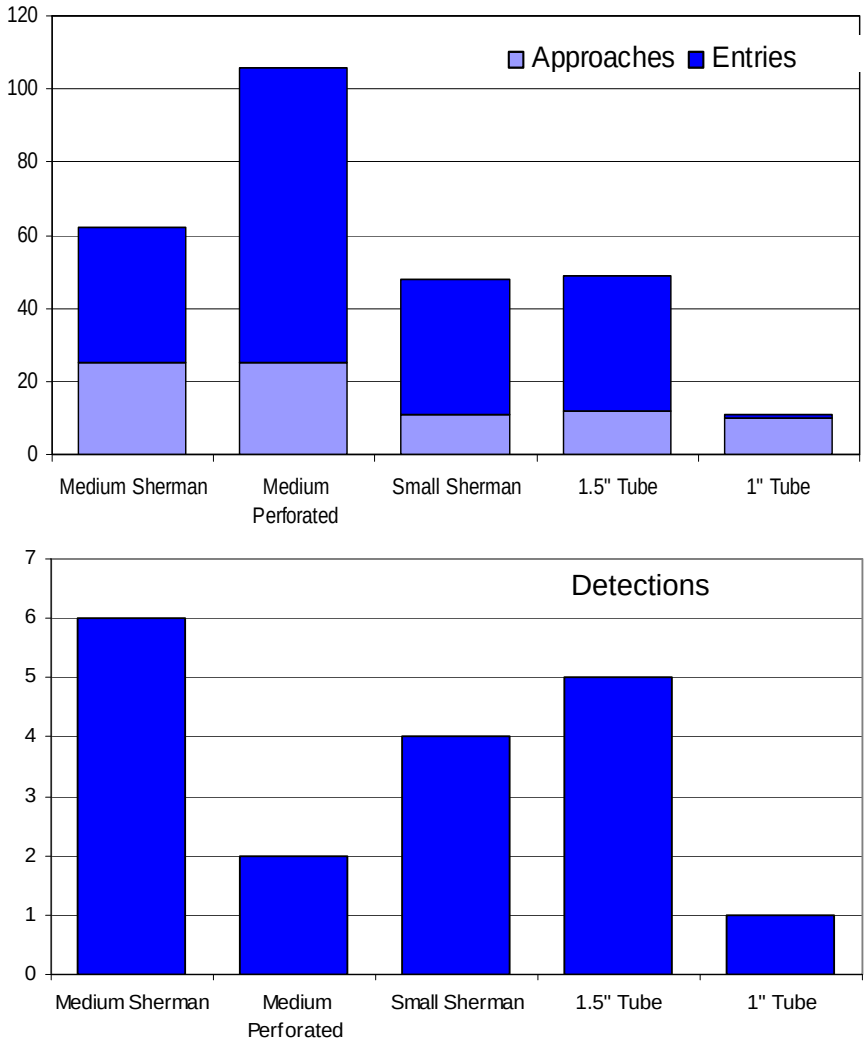


Figure 11: Total Numbers of Approaches , Entrances, and Detections by Detection Method.

Notable behavior observations:

Several notable PPM behaviors were observed while viewing the footage. Constant digging was the most obvious behavior. Arrays that were placed around a suspected PPM burrow recorded hours of "burrow tending" by the PPM. One night of video shows an individual PPM "burrow tending" from 10pm to 4:20am. The mouse digs furiously and enters and exits the burrow for hours. The digging around the burrow entrance is an almost constant effort. In one video, an individual PPM was recorded to have entered a single perforated Sherman trap 29 times without being captured. The cause of the trap malfunction was due to the animals' burrow tending. The

placement of the trap happened to be in the direction of the animals digging. The animal spent hours digging around its burrow and filling the trap with dirt, preventing the trap from functioning correctly. This behavior was an outlier for the number of approaches and entrances to the perforated Sherman trap (Figure 12).

Figure 12: Trap No Longer Effective Due to PPM digging behavior.

Most of the data collected show individual PPM entering the same trap type over and over again. Once it entered a specific trap or tube, it would tend to go back to that trap/tube repeatedly during its activity. It was observed several times that an animal would enter a trap, then enter a burrow and return to the trap again. This behavior appeared to be seed caching with the mouse using the trap as a safe food source.

Several of the videos contained footage of multiple mice active in the 1 m^2 view of the camera. If a non-target mouse species was in the same array as the PPM, both mice would run away, showing avoidance of the other species. We observed multiple PPM foraging in the same area around the trap array (Figure 13). The reaction of multiple PPM in the small area ranged from avoidance, attempted mating to aggressive behaviors. Aggressive behaviors included chasing, charging and jumping at the other PPM. These interactions ended with both mice running away and out of the camera view.

Figure 13: Two PPM Foraging Around Single Array.

One video captured a PPM sharing a burrow, possibly for mating. The first PPM is observed tending to its burrow entrance. The mouse enters the burrow and stays for periods of time and then exits and continues digging at the burrow opening. At one point, while the PPM is inside of the burrow for several minutes, another PPM enters the occupied burrow. Ten minutes pass without any activity. One PPM exits the burrow and seems to consider re-entering the hole when the other PPM exits and chases it away.

A mating attempt was recorded on May 13, 2009 at 11:20pm. The mating attempt began with the mice circling each other and the male attempting to mount the female. The mice move across the 1 m^2 view of the camera. The interaction ends when the mice run off in different directions.

PPM Scat DNA Assay:

The species identification assay was successful in discriminating PPM from other mice with a unique amplified base pair length of 274 after enzyme cleavage. The other sympatric heteromyid mice, *C. californicus* and *C. fallax*, also had distinctive base pair lengths of 230 and 190, respectively. *Peromyscus* species and *Mus musculus* have base pair lengths of 348 after. Thus, they can be distinguished from the heteromyids but not from one another. *R. megalotis* is completely cut and therefore cannot be distinguished from a sample with no amplified DNA.

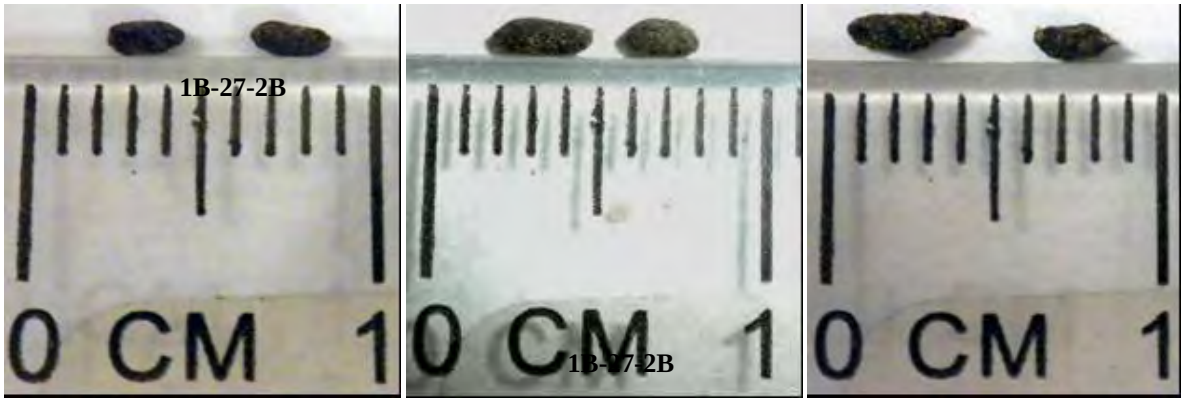
It was hypothesized that exposure to sand may remove DNA from the PPM scat as silica is commonly used for DNA extraction. In our tests, all 6 scat samples that were sifted (either same day or following week) resulted successful DNA amplification and species identification. Confidence in the samples appeared to be a better predictor of success as the proportion of samples that tested positive for PPM decreased with decreasing confidence. Confidence is based upon size, shape and color of scat and is currently a subjective index. High confidence PPM scats are typically 2 to 3 mm in length, fairly smooth, dark, oval or teardrop in shape, and pinched at one end. Photos of high, medium and low confidence scats are provided. Any scat bigger than ~3.5 mm would be "no confidence" and not be considered for testing.

Table 5. Results of scat DNA assays based upon time before sifting and confidence in appearance (to be PPM vs. other species).

Species:	Scientific name	Common name	DNA bp size after enzyme cut
PPM	*Perognathus longimembris*	Pacific Pocket Mouse	274
CHCA	*Chaetodipus californicus*	California Pocket Mouse	230
PEMA	*Peromyscus maniculatus*	Deer mouse	348, some males 154
REME	*Reithrodontomys megalotis*	Western harvest mouse	none (completely cut)
MUMU	*Mus musculus*	House mouse	348
PECA	*Peromyscus californicus*	California mouse	348
CHFA	*Chaetodipus cfallax*	San Diego pocket mouse	160
ND	*NA*	NA	No DNA amplified

Confidence	Sorted	Sample ID	Date collected	Date Sorted	photo	DNA bp size	Result	
high	fresh	1B-27-2A	7/1/2009		LRA632	**274**	PPM	
high	fresh	3A-61-2	6/26/2009		LRA630		ND	
high	fresh	1B-67-2A	7/1/2009		LRA626	**274**	PPM	
high	same day	1B-27-2B	7/1/2009	7/1/2009	LRA625	**274**	PPM	
high	same day	1B-67-2B	7/1/2009	7/1/2009	LRA617	**274**	PPM	
high	same day	1B-47-2	7/1/2009	7/1/2009	LRA628	**274**	PPM	
high	one week	1A-96-3	6/18/2009	6/30/2009	LRA621	**274**	PPM	
high	one week	1A-84-1	6/23/2009	6/27/2009	LRA623	**274**	PPM	2nd PCR
high	one week	1A-90-2	6/18/2009	6/25/2009	LRA622	**274**	PPM	
medium	fresh	3A-45-1	6/26/2009		LRA631	**274**	PPM	2nd PCR
medium	fresh	3A-41-2	6/26/2009		LRA627	**274**	PPM	
medium	fresh	2B-43-1	6/29/2009		LRA620		ND	
low	same day	1B-69-2	7/1/2009	7/1/2009	LRA619		ND	
low	same day	2B-64-2	6/29/2009	6/29/2009	LRA634		ND	
low	same day	2B-2-1	6/29/2009	6/29/2009	LRA633		ND	

“High Confidence Scat that tested positive for Pacific Pocket Mouse”



“Low Confidence Scat that did not test positive for PPM (Result indicated).

N.D

P. maniculatus

C. californicus

Figure 14. Examples of High vs. Low Confidence Scat.

We also tested three scat samples collected during the canine survey at North San Mateo (see p. 35). None tested positive for PPM. Two of the samples were *P. maniculatus* and the other ND (No DNA amplified- possible *R. megalotis*).

Canine Detection Surveys:

Canine scent detection had a higher rate of PPM detections in comparison to the tracking tubes. Over all three grids (269 searched cells), Casey gave positive PPM behavioral responses ('hits') in 193 out of 269 cells on the first and/or second visit. Of these, we verified 66% of these 'hits' with PPM tracks in the track tubes (46%) or PPM scat DNA assays (20%). For verifications, all 269 cells were surveyed using tracking tubes, but scat was only assayed in cells that had positive canine detections not verified by tracking tubes. It is unknown whether unverified canine detections were occupied by PPM and not verified, or unoccupied by PPM. PPM were detected by tracking tubes and not canines in 11 cells (4%). Cumulative results are presented in Table 6 and Figure 9. Results for individual grids are presented in Tables 7-9 and Figures 16-18.

Table 6. Numbers of Verified and Unverified Canine PPM Detections over all Search Cells and Grids

		PPM Survey Results (# Search Cells)				Lab Test Results of Unverified		
	No. Cells	Canine Detection	Track Tube (+)	Scat DNA Assay (+)	No verification	Other Species	No test result	No scat found
Canine Detections (+)	193	193	92	41	59	6	37	16
Canine Nondetections (-)	76	0	11	na	na	na	na	na

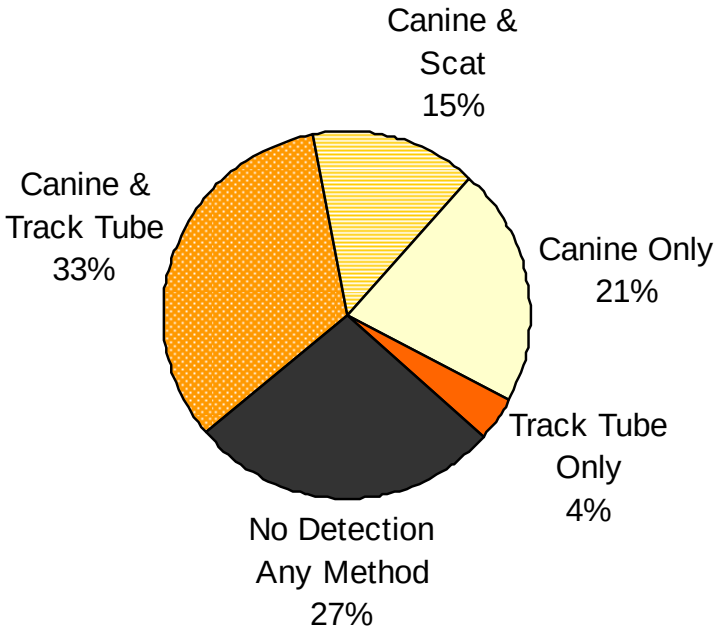


Figure 15. Percent Verified and Unverified Canine PPM Detections over all Search Cells and Grids

Table 7. Numbers of Verified and Unverified Canine PPM Detections for Grid 1, Oscar One Training Area

		PPM Survey Results (# Search Cells)				Lab Test Results of Unverified		
	No. Cells	Canine Detection	Track Tube (+)	Scat DNA Assay (+)	No verification	Other Species	No test result	No scat found
Canine Detections (+)	100	100	59	82	18	1	11	6
Canine Nondetections (-)	0	0	0	NA	NA	NA	NA	NA

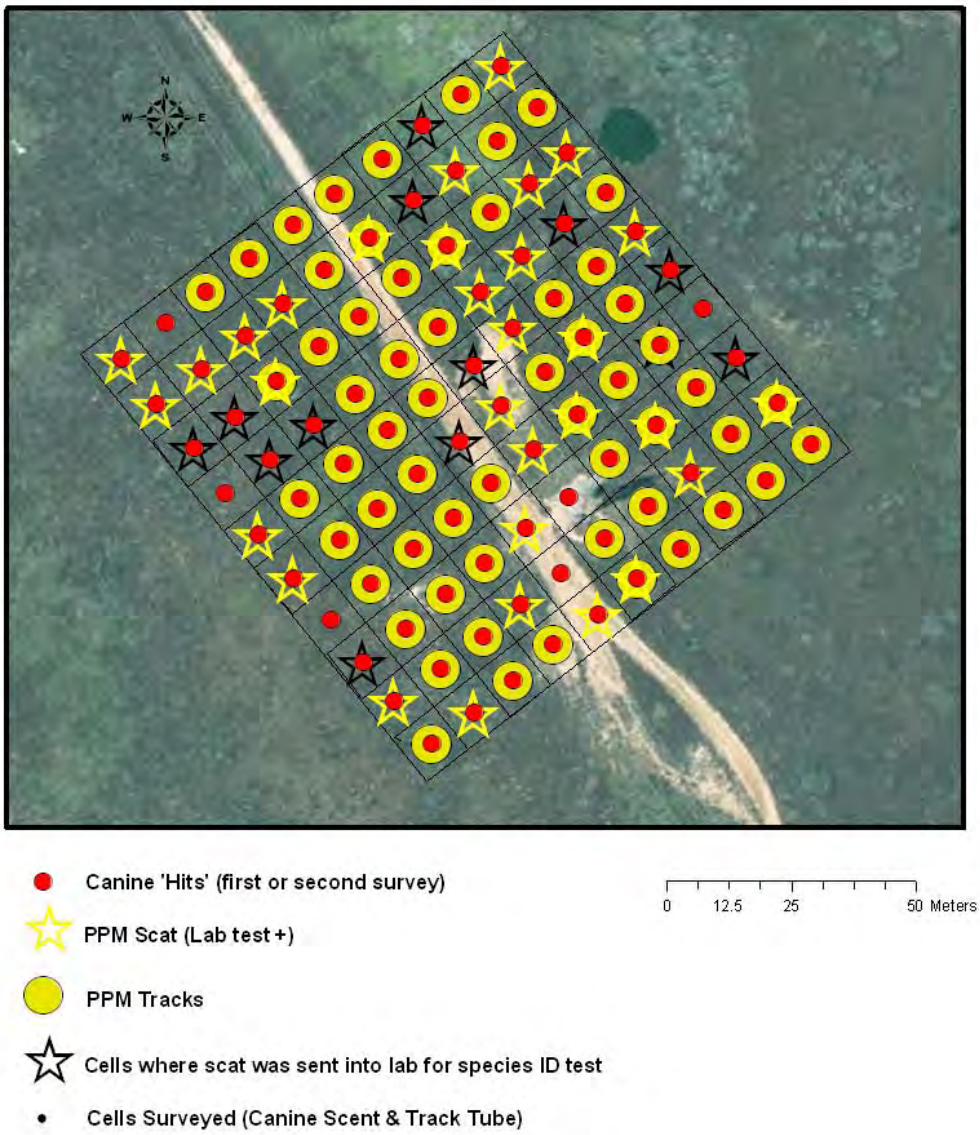


Figure 16. Results of Canine Scent, Tracking tube, and PPM Scat Surveys in Grid 1, Oscar One Training Area.

Table 8. Numbers of Verified and Unverified Canine PPM Detections for Grid 2, Oscar One Training Area

		PPM Survey Results (# Search Cells)				Lab Test Results of Unverified		
	No. Cells	Canine Detection	Track Tube (+)	Scat DNA Assay (+)	No verification	Other Species	No test result	No scat found
Canine Detections (+)	40	40	1	9	30	4	20	6
Canine Nondetections (-)	57	0	0	NA	NA	NA	NA	NA

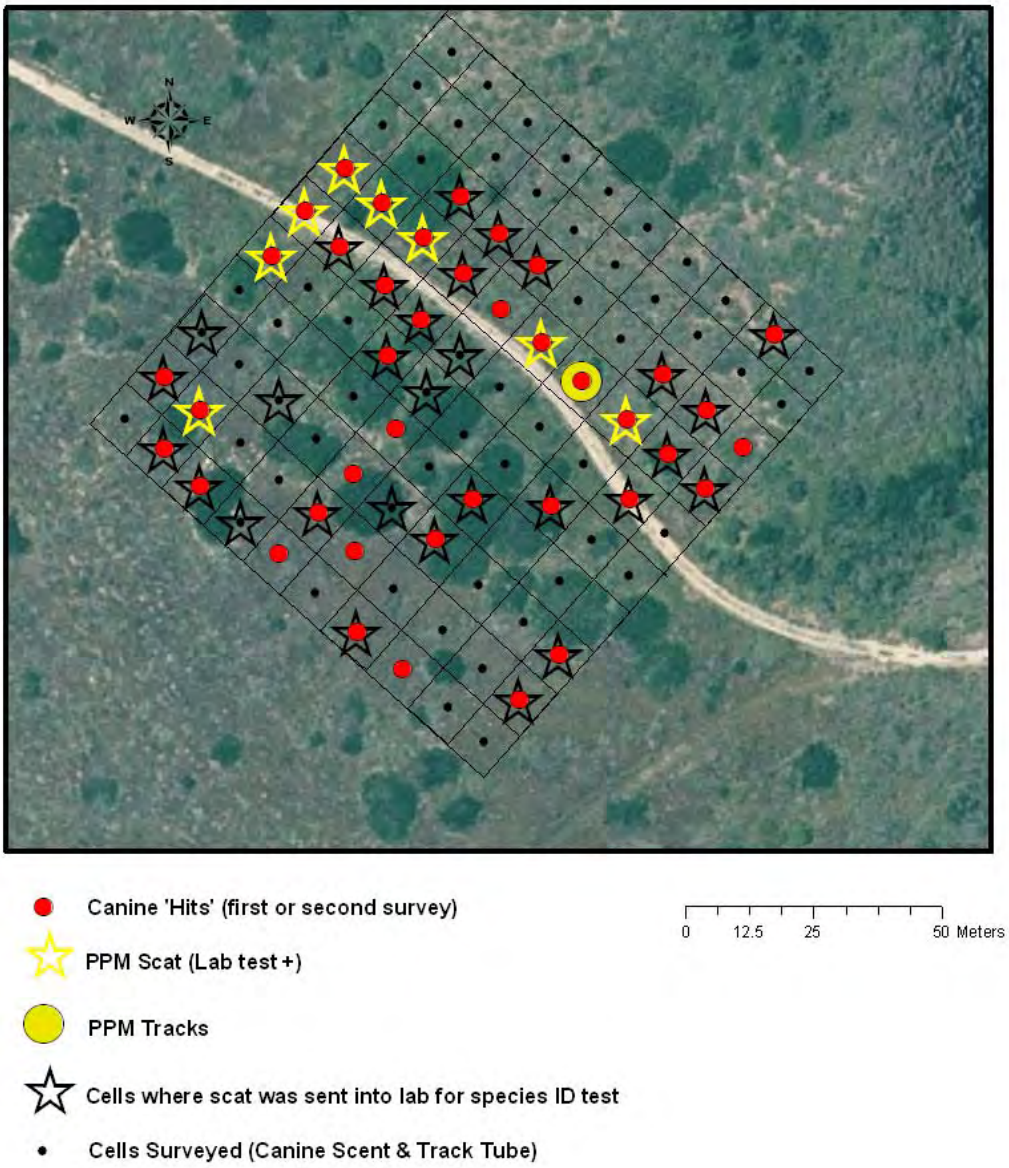


Figure 17. Results of Canine Scent, Tracking tube, and PPM Scat Surveys in Grid 2, Oscar One Training Area.

Table 9. Numbers of Verified and Unverified Canine PPM Detections for Grid 2, Oscar One Training Area

		PPM Survey Results (# Search Cells)				Lab Test Results of Unverified		
	No. Cells	Canine Detection	Track Tube (+)	Scat DNA Assay (+)	No verification	Other Species	No test result	No scat found
Canine Detections (+)	53	53	32	42	11	1	6	4
Canine Nondetections (-)	19	0	11	NA	NA	NA	NA	NA

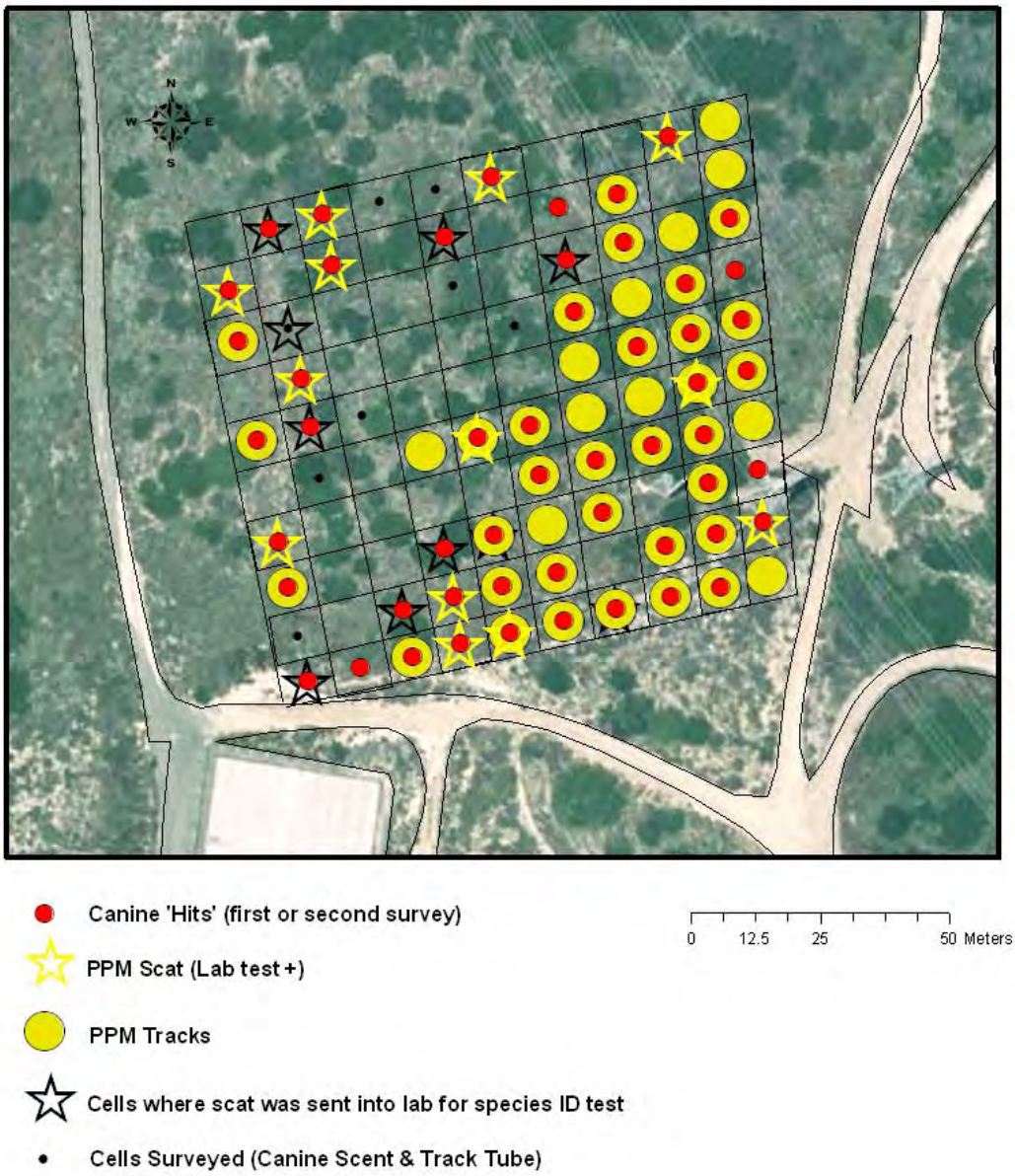


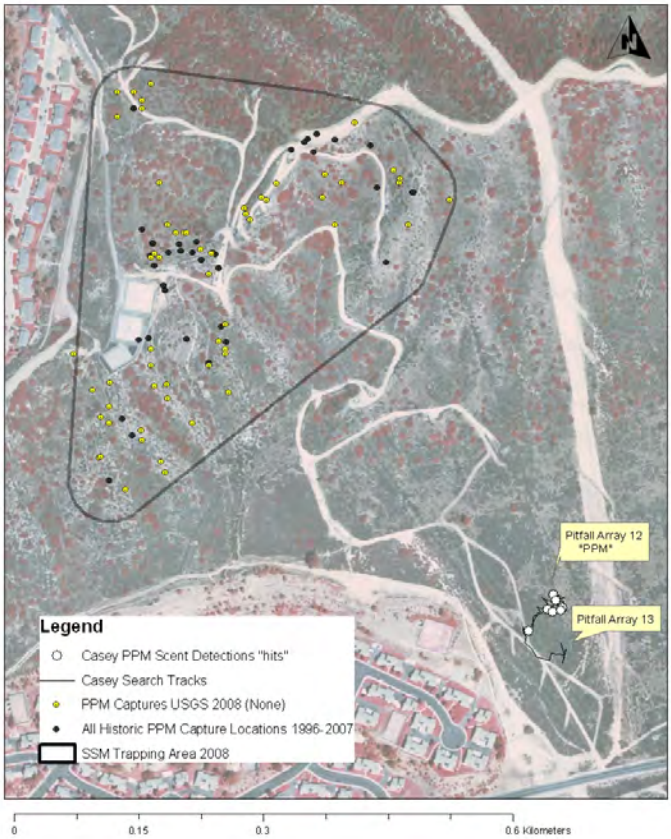
Figure 18. Results of Canine Scent, Tracking tube, and PPM Scat Surveys in Grid 3, South San Mateo Housing Area.

Additional Survey Areas

A PPM was captured in herpetofauna pitfall array #12 (other USGS study) on June 17, 2009. On July 7, Casey got seven positive detections at this location and no detections at the nearby array # 13, where no PPM were captured. Tracking tubes also confirmed two of the detection locations at Array 12. Other species detected were *Chaetodipus* sp, *Peromyscus* sp, and *R. megalotis*.

During the survey of North San Mateo, Casey also had a total of seven detections. There was no confirmation of the PPM detections at North San Mateo with tracking tubes or the scat DNA assay. Tracking tubes recorded *Chaetodipus* sp. and *Peromyscus* sp. Three scat samples were tested using the scat DNA assay. None tested positive for PPM. Two of the samples were *P. maniculatus* and the other ND (No DNA amplified- possible *R. megalotis*).

South San Mateo



North San Mateo

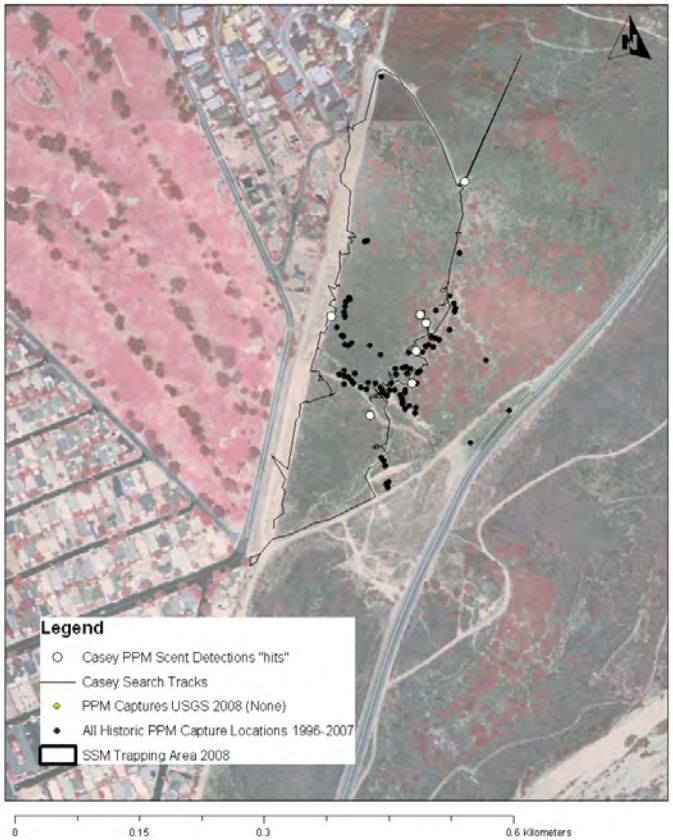


Figure 19. Canine Search Areas and Scent Detections in North San Mateo and South San Mateo Pitfall Array #12.

Casey detected placed PPM scat in a search cell 54% of the time during the first survey attempt. The detection rate was higher when scat was concentrated in one spot rather than in multiple locations within a 10 cm diameter (Table 10). After being directed to search the specific location a second time, the detection rate increased to 83%. Of the search cells where scat was placed but not detected, three out of four had two other "hits", potentially from naturally deposited PPM scat. Two out of the four were confirmed to be occupied by PPM from tracks.

Table 10. Accuracy in Canine Detection of Placed Scats

3-4 PPM scat placed in multiple locations (within 10cm of each other)

			Canine Detections					
Grid	Cell	Date	1st go through	2nd go through with direction	No detection after 2 go throughs	Confidence Level (1-5)	Number other hits	Notes
1	99	6/18		X		5	1	
2	47	6/22			X	na	2	
1	5	6/23			X	na	2	
1	91	6/23		X		4	0	
2	93	6/24		X		3	1	animal in bushes
2	95	6/24		X		nr	0	
3	11	6/26	X			3+	0	
3	93	6/26			X	na	0	distraction/ pet dog
2	93	6/29	X			3	0	
3	11	7/2	X			3+	0	
3	93	7/2	X			3	1	
1	99	7/3	X			4	1	
		Total	5	4	3			
		Percentage	41.7%	33.3%	25.0%			

7-8 PPM scat placed in single location

			Canine Detections					
Grid	Cell	Date	1st go through	2nd go through with direction	No detection after 2 go throughs	Confidence Level (1-5)	Number other hits	Notes
1	86	6/18			X	na	2	
1	89	6/18	X			2	1	
2	7	6/22	X			3+	1	
2	86	6/22		X		nr	0	tired, rabbit nearby
1	72	6/23		X		4	1	
2	11	6/24		X		3	0	very distracted
2	42	6/24	X			nr	0	
3	42	6/26	X			3+	1	
3	71	6/26	X			3	0	
1	86	7/1	X			4	1	
3	42	7/2	X			3+	1	
3	71	7/2	X			4	1	
		Total	8	3	1			
		Percentage	66.7%	25.0%	8.3%			

Relative Survey Effort Comparison between Live-Traps, Tracking Tubes, and Canine Scent Surveys

In order to make decisions regarding sampling, cost and effort evaluations may be warranted. Estimates of relative effort in hours of surveying for PPM using the different detection methods are presented in Figure 14, Table 11. Estimates for tracking tubes include checking the tubes daily vs. weekly. Estimates for canine surveys include initial training. Advantages should be weighed based upon study objectives (see Discussion)

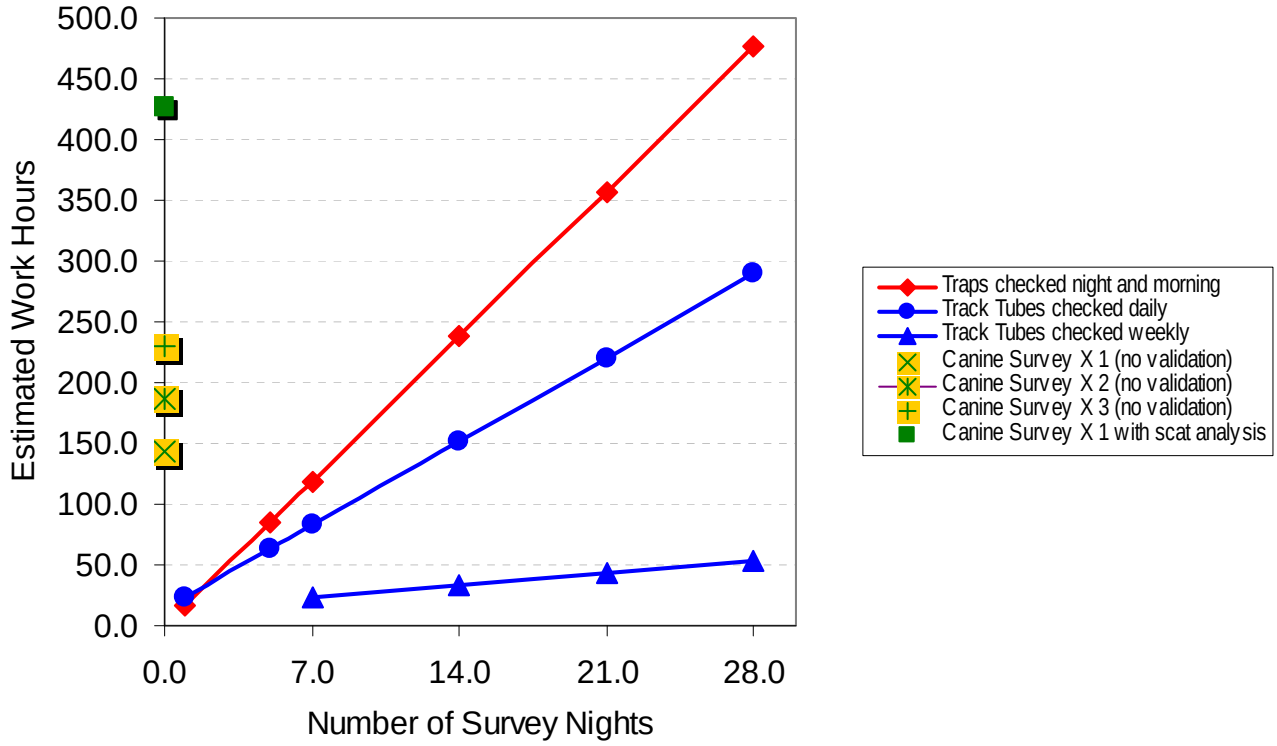


Figure 20. Estimated Relative Hours for Surveying PPM with Live-Traps, Tracking Tubes, and Canines.

Table 11. Estimated Relative Hours for Surveying PPM with Live-Traps, Tracking Tubes, and Canines.

	Estimated Relative Hours for Survey Portions								Estimated Relative Hours for Continuous Surveys								
	Setting& baiting (1st day only)	Each Trap-night				Last Trap Morning			Checked every day					Tubes checked once/week			
		Check midnight	Check morning	Reset dusk	Sub-total	Check midnight	Check morning	Sub-total	1 Night	7 Nights	14 Nights	21 Nights	28 Nights	7 Nights	14 Nights	21 Nights	28 Nights
Live-traps	4.2	4.2	4.2	4.2	12.5	4.2	4.2	8.3	12.5	87.5	175.0	262.5	350.0	-	-	-	-
Travel (0.5 hr. each way, 0.5 hr between grids)	1.5	1.5	1.5	1.5	4.5	1.5	1.5	3.0	4.5	31.5	63.0	94.5	126.0	-	-	-	-
Total Time: Live-traps	5.7	5.7	5.7	5.7	17.0	5.7	5.7	11.3	17.0	119.0	238.0	357.0	476.0	-	-	-	-
Tracking Tubes	12.5	-	4.2	-	4.2	-	4.2	4.2	16.7	41.7	70.8	100.0	129.2	16.7	20.8	25.0	29.2
Measure Tracks	-	-	4.2	-	4.2	-	4.2	4.2	4.2	29.2	58.3	87.5	116.7	4.2	8.3	12.5	16.7
Travel (0.5 hr. each way, 0.5 hr between grids)	1.5	-	1.5	-	1.5	-	1.5	1.5	3.0	12.0	22.5	33.0	43.5	3.0	4.5	6.0	7.5
Total Time: Track tubes	14.0	-	9.8	-	9.8	-	9.8	9.8	23.8	82.8	151.7	220.5	289.3	23.8	33.7	43.5	53.3

						Scat Collection & Analysis			Estimated Relative Hours (No Scat Collection or Analysis)					Estimated Relative Hours with Scat Collection and Analysis			
	Mark Plots	Training (10 days)	Survey		Sub-total	Collect/ Process Scat	Lab Test Scat	Sub-total	1 survey	2 surveys	3 surveys	4 surveys		1 survey	2 surveys		
Canine Scent Surveys	8.3	80.0	41.7	-	130.0	54.2	250.0	304.2	130.0	171.7	213.3	255.0	-	434.2	780.0	-	-
Travel (0.5 hr. each way, 0.5 hr between grids)	1.5	10.0	1.5	-	13.0	-	-	-	13.0	14.5	16.0	17.5	-	13.0	14.5	-	-
Total Time: Live-traps	9.8	90.0	43.2	-	143.0	12.5	250.0	304.2	143.0	186.2	229.3	272.5	-	447.2	794.5	-	-

Note: Times are relative estimates for comparison only and not to be used for budget estimates. Actual time will vary substantially depending upon many factors including survey location(s), numbers of grids, distance between grids and traps, and numbers of animals detected/captured.

Time assumptions (traps): Set time = 1 min each, Check/ process animal/rebait time= 1.5 min. each

Time assumptions (tubes): Set time = 2 min each tube; Check/ replace paper/ re-ink/ bait time = 1.5 min. each tube; Measure and input tracks = 2 min each tube.

Time assumptions (Canines): Mark Plots = 2 min. each; Scent Survey = 5 min. each plot X 2 people; Collect Scat = 3 min. each, Process/ sort scat = 10 min. each; Lab test = 60 min. each

Time assumptions (Canines with Scat Analysis): Collect Scat = 3 min. each, Process/ sort scat = 10 min. each plot; Lab test = 60 min. each sample (based on cost).

Discussion

All three sampling methods evaluated were successful in detecting the Pacific Pocket Mouse (PPM). Each method has advantages and disadvantages. The use of any method should be chosen and evaluated based upon the objectives of the study. We review these for each method.

Live-Trapping

Advantages:

- Use of Sherman live-traps is a standard proven method for detecting PPM and other rodents.
- Trapping allows for calculations of abundance by marking individuals, as well as collection of demographic and health data (age, sex, condition).
- The small and medium live-traps had the highest detection rates in this study and would thus be the optimum choice for long term monitoring if based on this criteria alone.
- Use of live-trapping as a method of species detection is very good with high probability of detection when species are present over a period of 3 days for a $50m^2$ grid (0.93, Brehme and Fisher 2009).
- Estimates of survivorship, immigration and emigration can also be calculated for large and repeated trapping efforts (Miller and Pavelka 2008).
- Very young juveniles and subadults can still be distinguished from adults. This is important in understanding species trends as it has been shown that PPM do not successfully reproduce in some years (Shier 2008).

Disadvantages:

- Low capture probabilities for individual PPM, even large efforts have resulted in abundance estimates with very wide confidence intervals, reducing their information value.
- Because PPM have highly variable above ground activity levels and adults and young of the year cannot be reliably distinguished from one another, abundance estimates are highly variable within the seasonal period of activity, as well as among years.
- Very high cost and effort limit the number of sample plots that can be run on an annual basis and over a wide spatial area. Maximizing number of sample plots important for precise occupancy estimates.

- Midnight and morning trap checks, along with rebating traps in the afternoon require at least 3 trips to each location per trap night. This results in extensive disturbance to the habitat even after only three trap-nights, particularly on sandy slopes (see Brehme et al. 2009). Crushing of PPM burrows may also occur.

- Trapping and handling PPM likely causes stress to the animal. Some individuals may become hypothermic even under temperate weather conditions. Stress may be reduced after multiple recaptures.

Tracking Tubes

Advantages:

- PPM tracks can be easily distinguished from other rodent species (Matsuda et al. 2010).

- 1.5" Tracking tube detection probability was very close to that of small and medium Sherman live-traps.

- Tracking tubes can be checked on a weekly basis. A month of tracking tube surveys is equivalent in cost and effort to approximately 6 days of live-trapping.

- The lower cost and effort increase the number of sample plots that can be run on an annual basis and over a wide spatial area. Maximizing number of sample plots important for precise occupancy estimates.

- Checking the tubes periodically (i.e. weekly) reduces disturbance to PPM habitat.

- Surveying for a prolonged amount of time reduces detectability issues from variable PPM activity periods due to rain, moonlight, short term torpor, or other unknown factors.

- Surveying for a prolonged amount of time increases the probability of detecting PPM if present to almost 1.00.

- The tubes likely do not cause the animal any stress.

Disadvantages:

- Tracking does not allow for collection of demographic or health information (age, sex, abundance, condition).

- Tracking tubes, wood stabilizers, inserts, track paper, and ink solution must be built, cut, and prepared by hand (i.e. not easily ordered from catalog).

- Tracking tubes are more time consuming to set up in the field and the ink solution is messy to work with.

Canine Scent Detection

Advantages:

- Do not know how long PPM scent detectable by canines. Possible that dogs can smell scat from previous year. Advantage to discovery of unknown populations or other PPM occupied habitat.

- Canines have extremely sensitive sense of smell, allowing them to locate PPM scat on surface of the ground while PPM are underground in burrows. The dogs likely do not cause the animals any stress.

- Canines can cover large areas in a relatively short amount of time. This decreases costs of using dogs to survey large amounts of habitat in comparison to other methods.

Disadvantages:

- Do not know how long PPM scent detectable by canines. Possible that dogs can smell scat from previous year. Disadvantage to long term annual monitoring.

- Training dogs and rewarding them in the field is continuously challenging due to our inability to reliably locate PPM sized scat or PPM sized burrows after they communicate a possible detection.

- Unknown if detections are truly PPM unless verified by another method (traps, track tubes, or scat DNA test). This increases costs of using dogs to survey standard plots and grids in comparison to other methods.

- Reliability and repeatability of detections can vary based upon many environmental factors (wind, temperature), dog (observer) variability, effectiveness of communication between dog and handler, and other unknown factors. These factors can vary greatly within and among survey days. Difficult to account for in statistical models.

For long term monitoring of PPM, the optimal method will have high probability of detecting PPM, low impact to PPM, and be reasonable in cost (Brehme and Fisher, in collaboration with scientific panel. 2009). The use of 1.5” tracking tubes best meet these criteria of the sampling methods evaluated in this study. We also recommend the use of 1” tube in combination with the

1.5” tube even though detection probabilities were quite low using this method. This size tube is effective in excluding most other small mammal species (except for *R. megalotis*). When other more dominant species are highly abundant in comparison to PPM, the inclusion of the 1” tube maximizes the potential for PPM detection.

Although the primary monitoring index is recommended to be proportion area occupied, it is preferred to have an index of relative abundance/ density or activity (Brehme and Fisher, in collaboration with scientific panel. 2009). The positive correlation between PPM counts and the numbers of arrays where PPM were detected show that number of arrays could be used as a relative index of density. This relationship is not linear, however, and will always be limited by the number and density of tracking tubes. For instance, if a there is a $100m^2$ grid (1 ha) with 100 tracking tubes. The maximum number of detections is 100, regardless of whether the density is 100 PPM/ha or 1000 PP/ha. The sensitivity lies at the lower end of the range which may be the more critical range for assessing declines and/or needs for management actions.

Live-trapping is necessary in order to determine if PPM are reproducing in a given year. It is unknown if successful reproduction is mostly governed by general climate and rainfall or local environmental factors. In order to get a better understanding of population dynamics across their habitat, it would be beneficial to establish a live-trapping grid within each of the populations (Santa Margarita (Oscar One and Edson Training Areas), North San Mateo, and South San Mateo) to be run for a limited time each year. Because of the sandy substrate and ‘hilly’ landscape at South San Mateo, habitat destruction is a concern for any such grid.

We do not recommend use of canine scent surveys for the disadvantages stated earlier in the discussion. However, we do recommend this as the most suitable method for discovery of PPM populations or other PPM occupied habitat. The surveys across the $100m^2$ grid with $10m^2$ cells were relatively time consuming and appeared awkward for the dogs and handlers. The collection of scat is also very time consuming for a single grid. Although Casey did accomplish the grid surveys with 66% of his detections validated, we feel the dogs are best used as they are in large animal studies. This is by allowing them to search and wander over large spatial areas (up to $2km^2$ per day) and follow scents (rather than being confined to a cell or grid). These large areas are extremely difficult to trap effectively. In this way, rather than use for detecting individual mice, dogs would be used to detect PPM populations or subpopulations. Positive detections could then be used for targeted verification studies using tracking tubes, live-trapping, and/or scat DNA testing.

Since it was proposed that some monitoring funds be used for discovery of unknown PPM populations, we propose this to be the most suitable methodology for this purpose. In 2008 and 2009, we also learned that PPM exist well outside of their known boundaries at South San Mateo. Use of the dogs is also recommended to better establish PPM population study boundaries for the long-term monitoring program.

Unfortunately, we were unable to confirm the presence of PPM at North San Mateo for a second year using the canine scent surveys with targeted verification by scat DNA assay and tracking tubes.

The video recordings of PPM behavior in relation to the traps and tubes were very helpful in understanding why capture probabilities of individual mice are fairly low. We observed many instances (35%) in which mice were active but not detected by traps or tracking tubes within a given night. In many of these instances, the mice showed no interest in the traps or tubes. In the videos that did show high interest by a PPM individual, it was typically a long period of time before they were detected (1-261 minutes). Often, PPM were in the Sherman traps many times before being captured. We do not know if this is due to the animal avoiding the treadle (staying in front or jumping to back- both behaviors were observed) or due to the treadle not being sensitive enough. All traps were set as sensitive as possible without automatically closing to a sensitivity of at least 5g. In most instances, the animal was eventually captured in the same trap on the same night. Other behaviors observed like digging, burrow tending, burrow sharing, and mating all add to our life history knowledge for the species.

Finally, the scat DNA assay for species identification is a very useful tool to confirm presence of PPM from scat. Scat can be collected at suspected burrows in combination with any of the other detection techniques to help establish presence or absence at a study site. Interestingly, there were only two tracking tubes found with PPM scat in comparison to many scat (>10) typically found in the traps. We hypothesize that this is due to decreased time spent in the tubes, as well as lower stress for the animal.

In summary, we now have several additional tools for detecting the Pacific pocket mouse!

Considerations and Recommendations for Monitoring: Summary from 2009 Studies:

These recommendations are in addition to those from Brehme et al. 2009 and 2010a.

1. Survey suitable PPM habitat outside of the currently known PPM population boundaries to better understand PPM distribution, potentially discover unknown populations, and establish initial boundaries for long term monitoring. Use canine scent surveys followed by targeted verification by scat DNA assay, tracking tubes, and/or live-trapping.
2. Use tracking tubes as the primary sampling method for long term occupancy monitoring.
3. Use limited live-trapping within each population to understand annual reproductive success across populations.

Acknowledgements

We thank Stacie Hathaway, Denise Clark, and Dana McLaughlin (SDNHM) for their tremendous and high quality work effort and Roland Sosa, MCBCP, for coordination and support. The Conservation Canine Group (University of Washington) were extremely hard working and a pleasure to work with (Julie Ubigau, Michelle Manza, Heath Smith, Casey and even Timmy (he tried!). We thank members of the PPM Scientific Panel; Ken Burnham (USGS), Peter Meserve (NIU), Mark Pavelka (FWS), Wayne Spencer (CBI) William Miller (FWS), and Doug Deutschman (SDSU) for providing input into study design. U.S. Fish and Wildlife Service, California Department of Fish and Game, and San Diego Gas and Electric provided permits for animal handling and land use. The work was funded by the Assistant Chief of Staff, Environmental Security, Natural Resource Management Division, Marine Corps Base, California. MIPR M0068109MPFE049.

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