The Use of Canine Scent Detection, Tracking Tubes, Dynamic Occupancy Modeling and Genetic Analysis of Scat to Better Understand and Inform Recovery of the Pacific Pocket Mouse

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PPM

- Heteromyid (6g)
- External cheek pouches
- Subspecies of Perognathus longimembris
- Critically Endangered
- 4 extant populations (3 on Camp Pendleton)

Habitat

- West facing gentle slopes w/in 4 km of ocean
- Sandy Substrates
- Open CSS with forbs and grasses

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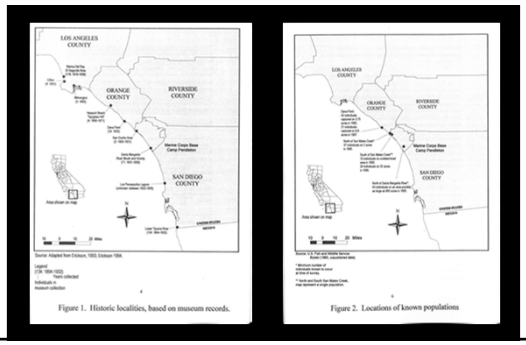
PPM Distribution Historic & Current



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Behavior

- Nonsocial, nocturnal
- Dust Bath, Cache seeds
- Torpor in Summer to Winter (related to seed availability?)
- Temporal Activity Variable by sex/age
- Forage primarily on seeds (forbs, grasses, shrubs)
- Reproduction- 1-2 litters (gestate 2 weeks, wean 30 days)
- Home Range estimates (.017 ha- 0.25 ha)

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Program History

2007- Monitoring Workshop with Scientific Panel
Set Program Goals
2008- Live-Trap North & South San Mateo
2009- Sample Methodology Study
2010- PPM Discovery Effort
2011- Creation of Monitoring Plan
2012- Monitor Year 1
2013- Monitor Year 2 + Argentine Ant Sampling
2014- Monitor Year 3 + Genetic Barcode Assay Development

Acknowledgements

MCB Camp Pendleton
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- Scientific Panel

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- Conservation Canines (UW)

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- SDZoo:

Debra Shier, Amaranta Kozuch

- Many Others!!!

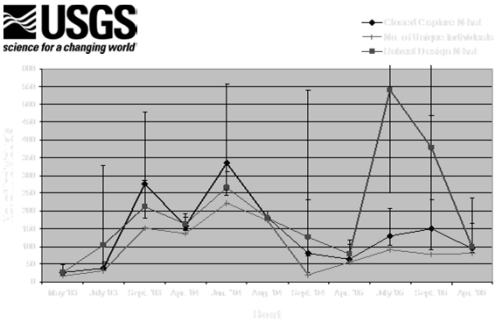
Goals of Monitoring

- Monitor status of PPM
  - Long term trends/ annual trends
  - Response to natural environmental & human induced perturbations
  - Scientifically robust & cost effective
- Increase knowledge of PPM
  - Natural history (habitat & natural variation)
  - Response to biotic & abiotic variables
- Feedback loop (adaptive management & monitoring)
  - Test meaningful/relevant *apriori* hypotheses
  - Response to management actions (i.e. fire, training, vegetation thinning, other)
  - Targeted studies/ programs

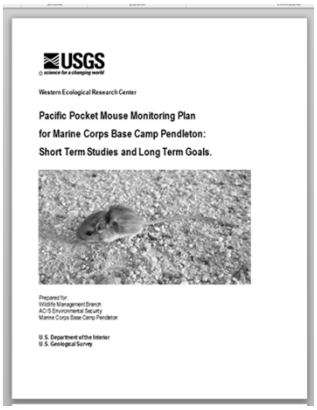
USFWS Oscar One Research Some Lessons Learned

Extremely challenging and time intensive (at best) to get accurate estimates of annual abundance on which to make informed decisions.

- Abundance highly variable: seasonal and annual
- Difficult to distinguish YOY from adults (recruitment?)
- Capture probabilities highly variable: time, space, individuals
- Little known about spatial distribution or variation in distribution over time



How much is enough? 600 traps x 2 Grids x 4 Biologists/Grid x 10 Nights x 4 Times /yr = Large Confidence Intervals (FWS 2007)



General Consensus for LT Monitoring

- Spatial Monitoring Scheme- Annual Occupancy Index
  - Smaller N & S San Mateo populations may require more intense sampling
  - Information Gap- Current Size and distribution
- Program will be closely tied to management
  - Collect habitat, biological, environmental data multiple scales- assess drivers of distribution & inform mgmt
  - Will incorporate BACI studies to inform and assess mgmt actions
  - Info on distribution will help inform Base on better mgmt decisions for training
- Adaptive
- Some effort to 'discovery' (not confined to MCBCP)

Program Specifics

Sampling:

- Permanent vs. Random Plots (75/25)
- Passive Sampling preferred
  - Information Gap- Require preliminary studies to assess & validate

Measures:

- Proportion Area Occupied
- Relative Index of abundance/activity
  - Information Gap- depends upon sampling technique, validation
- Some measure of reproductive success
  - High vs. low recruitment year (feasibility- identification issue)

Program Specifics

Environmental Covariates (large & small spatial scales)

- Presence of sandy patches? (Y/N with estimated proportion)
- Vegetative cover (growth forms: herbs/forbs, grasses, shrubs, trees, open ground, leaf litter)
- Dominant plant species
- Slope
- Recent human disturbance
- Other rodent species
- Soil descriptors

Other:

- Ant species
- Historic land use
- Soil depth, permeability, hardness, other

Size of Sample Plot- TBD

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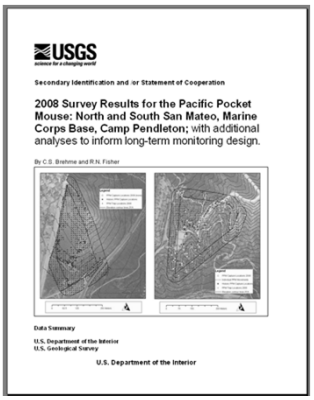

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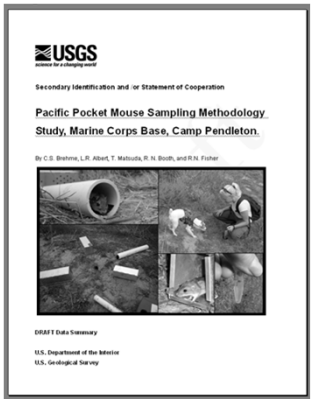

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Short term Goals 2009

What is the optimal sampling method for annual monitoring of PPM?

Evaluation Criteria:

- accuracy
- detection probability
- impact to species
- impact to habitat
- cost

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2009 Study Goals

Objectives:

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 PPM behavioral responses using infrared video cameras.
3. Develop species identification assay for rodent scat to distinguish PPM from other rodent species.

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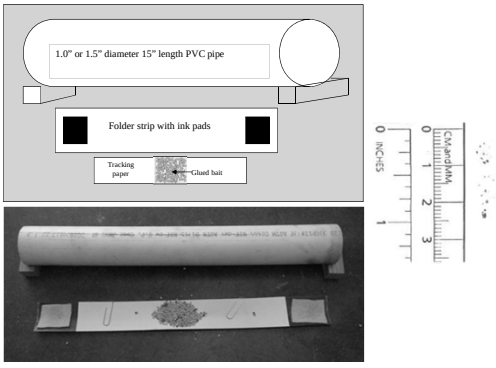
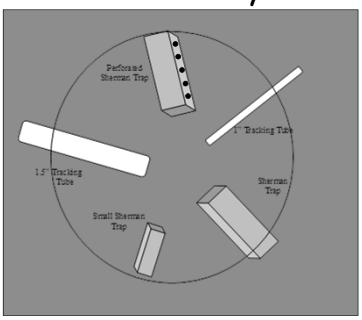


Trap & Tracking Tube Arrays



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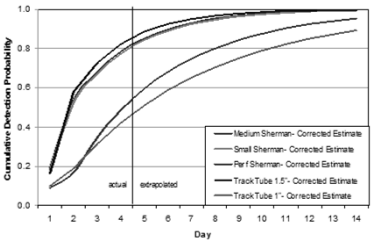
Probability of Detection Comparison



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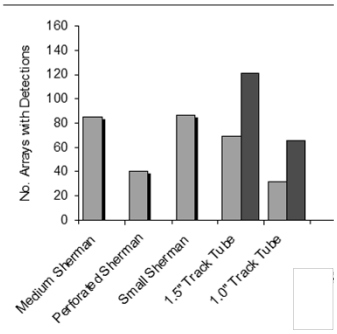
Weeks 3-7



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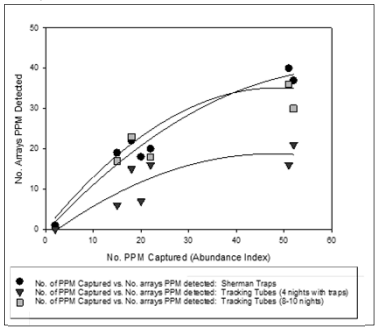
Association between PPM Captures and Detections

No. arrays with tracks as index of abundance



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- **PPM active all night**
- **Ranged 1 – 260 minutes in video before detection**
- **Averaged 7-12 entries into Sherman traps before capture**
- **Approached and entered Sherman traps & 1.5" track tube most often.**

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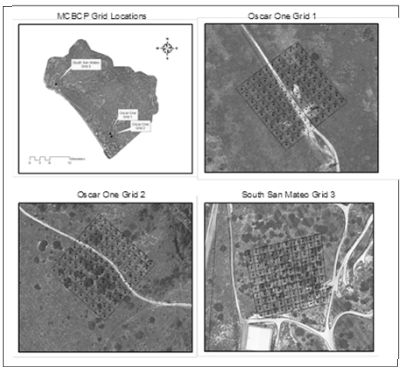
Canine Survey Grids

100m x 100m



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Protocol

1. Search each 10m x 10m cell (limit 2 hits /~5 minutes)

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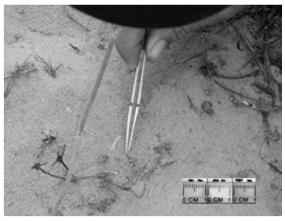
Protocol

2. Search for scat
   - Collect in vials
   - Collect and sift sand



Protocol

1. Set up tracking tubes (1 per cell)
2. Run for 7 days
3. If "Hits" not verified by tubes, scat sent to lab
   (PCR Assay for species identification- University of Washington)

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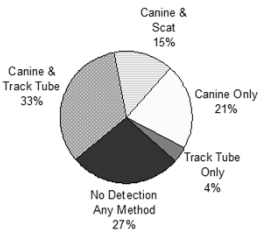
Results

3 grids X
100 cells each



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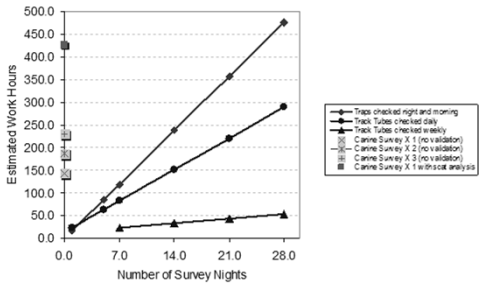
Relative Cost/ Effort



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Live- Trapping

Advantages

- Standard proven method
- Allows for calculations for abundance, demographic and health data (age, sex, condition).
- The small and medium live-traps had the high detection rates in this study.
- High probability of detection (3 days for a 50m2 grid =0.93),
- 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.

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Live- Trapping

Disadvantages

- Abundance estimates highly variable due to low capture probabilities for individual PPM, variable activity within seasons, identification of YOY.
- 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.
- Midnight and morning trap checks, along with rebating traps in the afternoon require at least 3 trips to each location per trap night = extensive disturbance to habitat.
- Trapping and handling PPM likely causes stress to the animal.

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Track Tubes: Advantages

- PPM tracks can be easily distinguished from other rodent species.
- 1.5" Tracking tube detection probability was very close to live-traps.
- Tracking tubes can be checked on a weekly basis. A month of tracking tube surveys = approximately 6 days of live-trapping.
- Surveying for a prolonged amount of time:
  - reduces detectability issues from variable PPM activity periods
  - increases the probability of detecting PPM to almost 1.0.
- The lower cost and effort increase the number of sample plots.
- Checking the tubes periodically (i.e. weekly) reduces disturbance to PPM habitat.
- The tubes likely do not cause the animal any stress.

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Track Tubes

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.

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Canine Scent Detection

Advantages

- How long is 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.
- 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.
- Passive method: The dogs likely do not cause the animals any stress.

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Canine Scent Detection

Disadvantages

- How long is PPM scent detectable by canines? Possible Disadvantage to long term annual monitoring.
- Training continuously challenging due to our inability to reliably locate PPM sized scat or PPM sized burrows.
- Unknown if detections are truly PPM unless verified by another method. Increases costs
- Reliability and repeatability of detections can vary within and among survey days. Difficult to account for in statistical models.

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Conclusions

- Tracking tubes most suitable sampling method for long-term occupancy monitoring.
  - Occupancy positively associated with abundance.
- Live-trapping most suitable for assessing if reproduction is occurring (medium & small traps).
- Canines most suitable for discovery
- Scat DNA Assay suitable method for discovery, verification
- We now have 4 detection methods for PPM!!!

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2010 Study Goals

1. Better establish PPM population boundaries for long-term monitoring
2. Survey suitable PPM habitat across Base for discovery.
   a. Canine scent surveys
   b. Verification by tracking tubes, live-trapping & scat DNA assay

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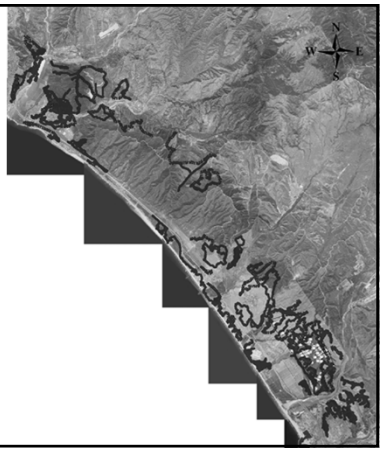

537.7 km surveyed

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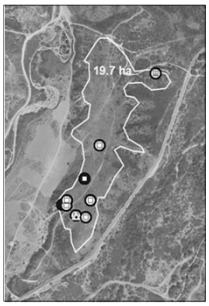
Proposed Initial Monitoring Boundaries

NSM



Papa One

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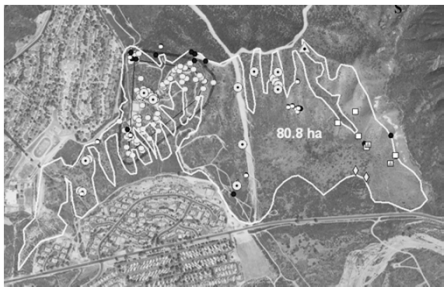
Proposed Initial Monitoring Boundaries



SSM

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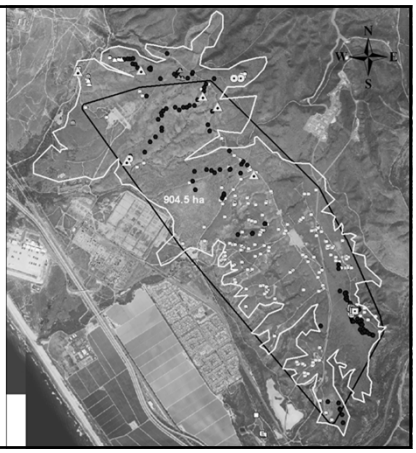
Proposed Initial Monitoring Boundaries

Oscar One & Two/ Edson

Note: Edson clay soils



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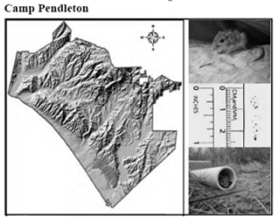
Western Ecological Research Center

Pacific Pocket Mouse Monitoring Protocol for MCB Camp Pendleton



Prepared for:

Wildlife Management Branch
AC/S Environmental Security
Marine Corps Base Camp Pendleton

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Sample Plot Size v. Occupancy

Occupancy Monitoring:

Recommended PAO 0.2- 0.8 (MacKenzie et al. 2006)

- Power to model covariates
- Power to detect trends

How to control:

- Size of Sample Area
- Size of Sample Plot

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Sample Plot Size v. Occupancy

Mice patchily distributed over 10 ha

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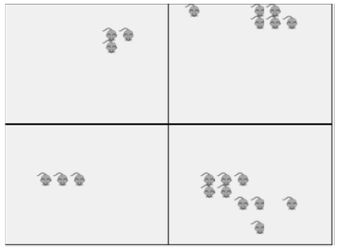
Sample Plot Size v. Occupancy

4 plots
2.5 ha each

PAO= 1.0
Area Occupied= 10 ha



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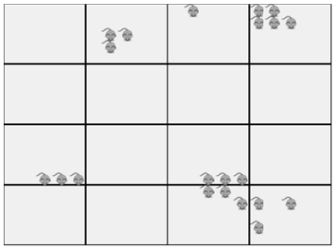
Sample Plot Size v. Occupancy

16 plots
0.4 ha each

PAO= 0.44
Area Occupied= 4.4 ha



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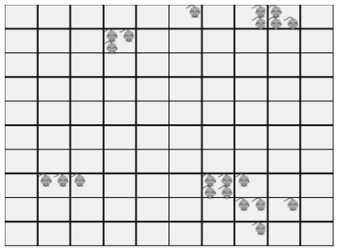
Sample Plot Size v. Occupancy

100 plots
0.1 ha each

PAO= 0.11
Area Occupied= 0.11 ha



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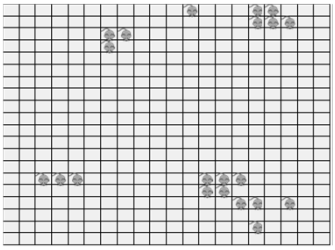
Sample Plot Size v. Occupancy

400 plots
0.025 ha each

PAO= 0.05
Area Occupied= 0.5 ha



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Sample Plot Size v. Occupancy

Probability plot occupied very high
Any random plot- expect to be occupied
Large survey area for each plot
Few total plots to get precise estimate

TOO BIG

too small

Probability plot occupied very low
Survey 20 random plots- expect 1 to be occupied
Low and imprecise PAO estimate

Both:
Imprecise PAO estimates
Little power to detect trends
Little info on spatial patterns over time
Little info on correlations with habitat covariates

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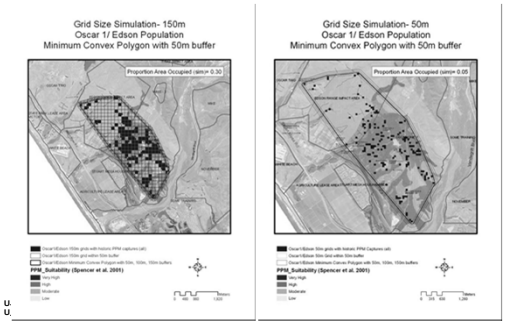
Sample Plot Size Simulations MCBCP



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Sample Plot Size Simulations MCBCP

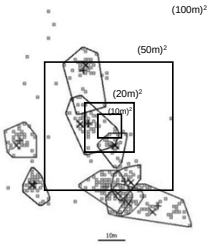
(Grid size ranges: 50-150m² (Oscar 1/ Edson) and 25-100m² (N&S San Mateo))
(sample area buffer width ranges from 0 to 150m)

	Defined Sample Area*	Area (ha)	Grid Size 150m # grids*	Grid Size 150m PAO*	Grid Size 100m # grids*	Grid Size 100m PAO*	Grid Size 50m # grids*	Grid Size 50m PAO*
Oscar 1/ Edson	MCP (0m)	729.1	323	0.29	734	0.16	2916	0.05
	50m	791.9	347	0.30	799	0.16	3170	0.05
	100m	854.5	363	0.27	852	0.15	3413	0.05
	150m	915.7	406	0.25	921	0.13	3650	0.04
	No of Grids Occupied*		95/MCP1,103		121/MCP1,124		166/MCP1,161	
North San Mateo	MCP (0m)	5.6	5.0	0.80	22.0	0.45	90.0	0.27
	50m	9.7	8.0	0.89	41.0	0.41	159.0	0.18
	100m	14.4	16.0	0.63	59.0	0.31	238.0	0.12
	150m	19.9	21.0	0.48	85.0	0.21	325.0	0.09
	No of Grids Occupied*		4/MCP1,8		10/MCP1,17		24/MCP1, 29	
South San Mateo	MCP (0m)	6.3	9.0	0.78	34.0	0.32	141.0	0.13
	50m	16.3	20.0	0.80	67.0	0.24	298.0	0.09
	100m	23.2	24.0	0.50	103.0	0.16	380.0	0.05
	150m	31.1	33.0	0.35	135.0	0.12	441.0	0.04
	No of Grids Occupied*		7/MCP1,12		11/MCP1,16		19/MCP1, 24	

Sample Plot size Other Considerations

Plot size:

- home range movements
  - Mean diameter 170m2 (13x13m) Shiel
  - Median 10-14m in 3-10 days- FWS
- detectability
- sample unit density



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Monitoring Program Highlights

- Habitat-based monitoring program for PPM occupancy.
- Multi-scaled Sample Plots (50 -3200).
- Stratified and optimized sampling of PPM populations (13 ha, 105 ha, and 885 ha).
- Continuous monitoring of all plots during active season (April-July) with tracking tubes*.

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Monitoring Program Highlights

- Incorporation of more intensive core sample plots + live trapping (phenology, reproduction, abundance)
- Allocation of effort over time- Permanent (80%) vs. new sample plots (20%).
- Discovery Effort
- Adaptive Protocol.

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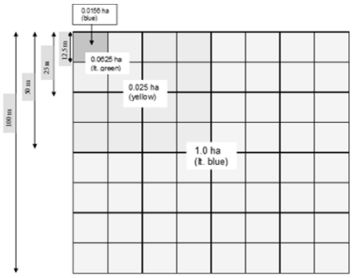

24 S Marg
18 SSM
9 NSM

50 Grids across 3 populations

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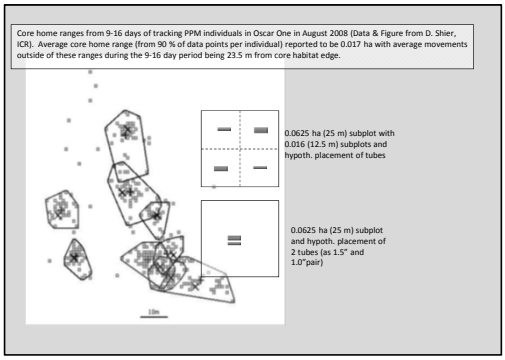

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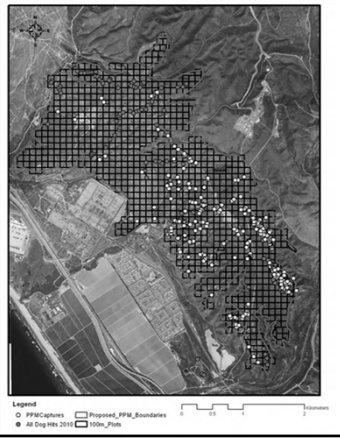
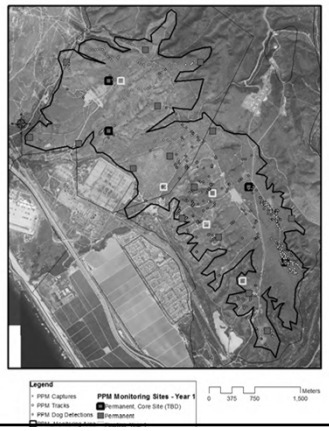
Estim. average 1 tube = 1 mouse

Santa Margarita
Oscar/Edson
885 ha

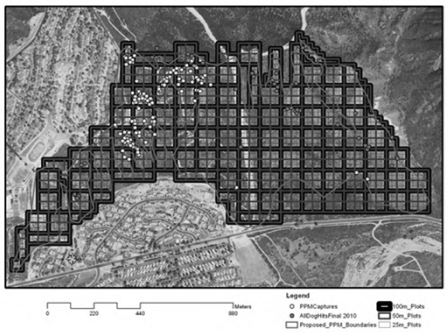


USGS

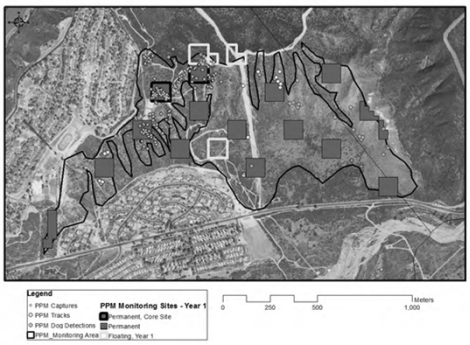


USGS

SSM
105 ha



USGS



USGS

NSM
13 ha

USGS

Habitat and other Covariates

- **Other Rodents** (CHSP, PESP, Reme)
- **Slope, Aspect**
- **Dominant Soil Types**
- **% Sandy Patches**
- **Vegetation Cover** (OG, LL, Herb/Forb, Grasses non-native v. native, shrub, tree)
- **PPM Food Plants** (Ca. buckwheat, White sage, Deerweed, CA croton, Cryptantha, Ca aster)
- **Roads/Trails** (Dirt, gravel, paved)
- **Disturbance** (Fire, training, recreational)

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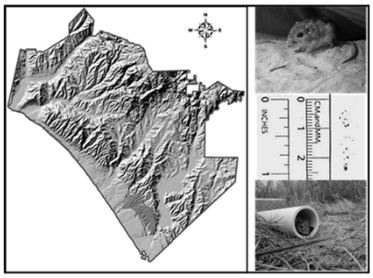
PPM Monitoring Results and Findings 2012-2014



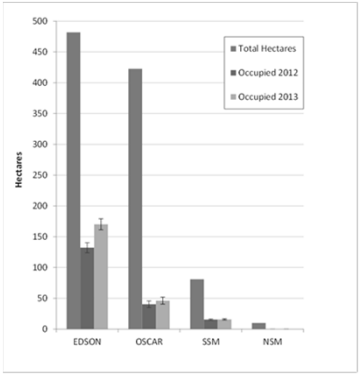
U.S. Department of the Interior
U.S. Geological Survey

Occupancy Estimates 2012-14

>70% of PPM occupied habitat on MCBCP is in Edson Impact Area

NSM-no PPM detections

(.016 ha scale)



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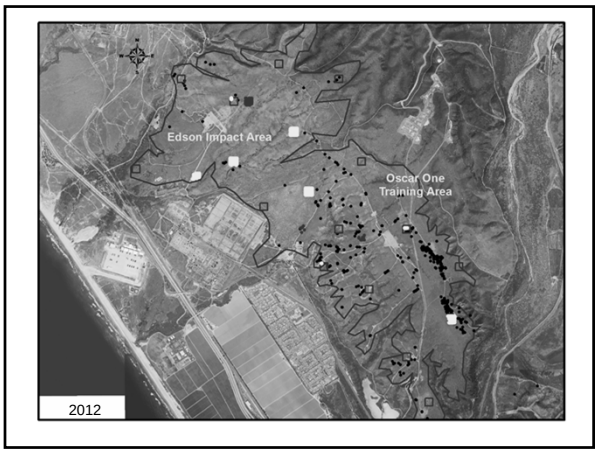
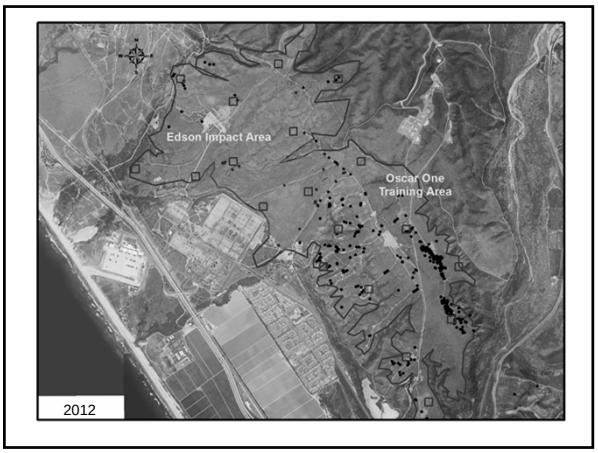
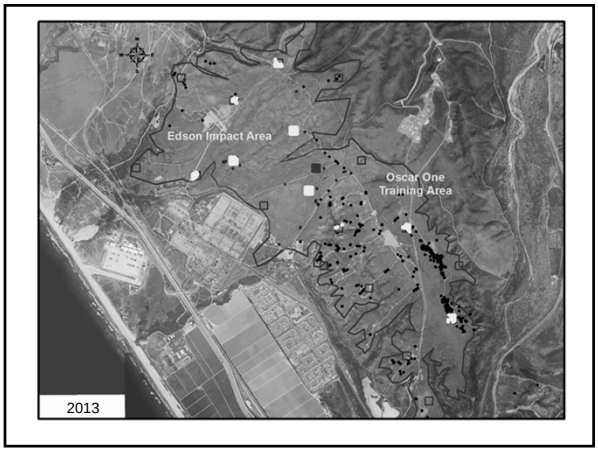
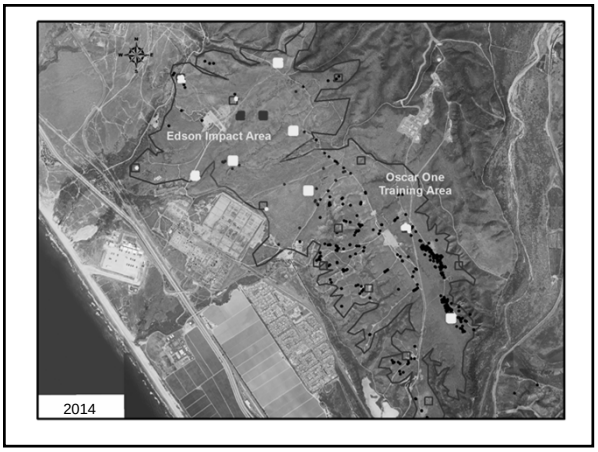
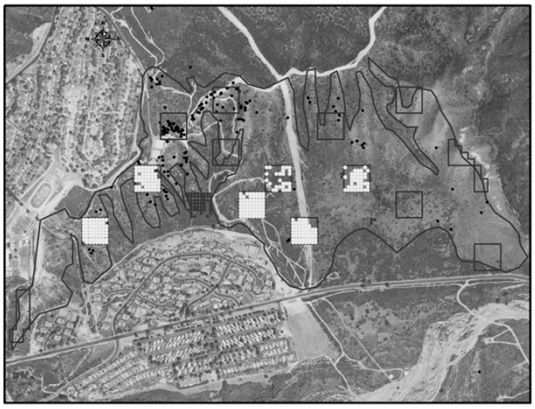

Edson Impact Area
Oscar One
Training Area
2012

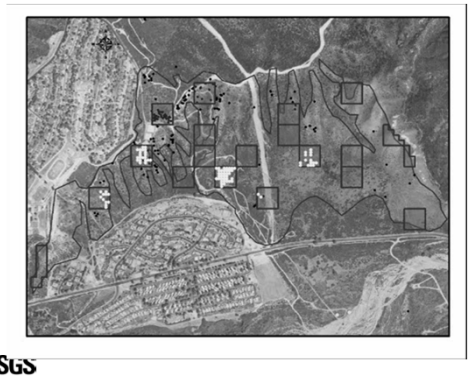

Edson Impact Area
Oscar One
Training Area
2012


Edson Impact Area
Oscar One
Training Area
2013


Edson Impact Area
Oscar One
Training Area
2014



Within Season Dynamics



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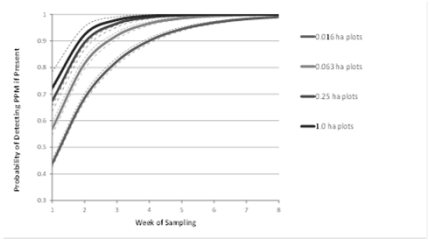
Highlights- Modeling Results (ρ)

- The cumulative probability of detecting PPM using the tracking tubes over the 2 month monitoring period was estimated to be near perfect at all spatial scales



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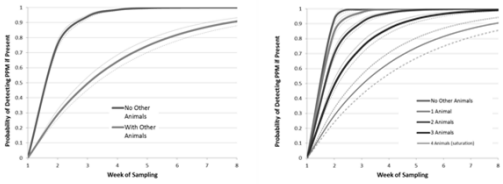
Cumulative Detection Probability

0.016 ha (1 tube) **0.063 ha (4 tubes)**



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Occupancy Modeling Results (ψ)

Spatial Scale (ha)	Spatial Scale (m)	Top model (ρ)	Top model (ψ)
.016	12.5 x 12.5	All Animals (-)*	Site * Forbs * Non-native Grass (NNG)
.063	25 x 25	All Animals (-)	Forbs + NNG$^{\wedge 2}$ + Foot Training Index
.250	50 x 50	All Animals (-)	Forbs + NNG + Foot Training Index
1.00	100 x 100	All Animals (-)	Forbs

* Dana Point Data included at this spatial scale, covariate for ρ in model

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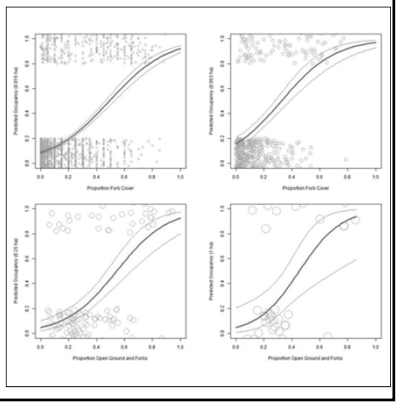
Relationship to PPM
Occupancy (ψ)

Forb
Cover



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Correlations: Forb Cover

Pearsons R (vary between 0-1)

(-) Woody Debris (R= 0.50)
(-) Shrub (R= 0.59)
(-) Years Since Last Fire (R= 0.55)
(+) Fire Frequency (R= 0.52)

.0625 Scale

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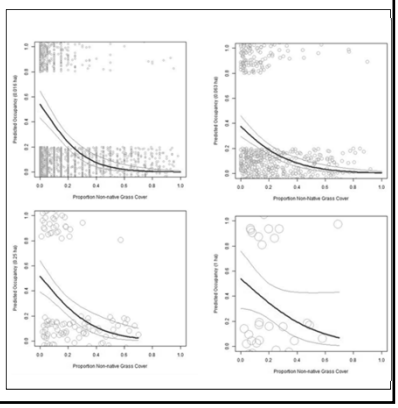
Relationship to PPM Occupancy (ψ)

Non-native Grass Cover



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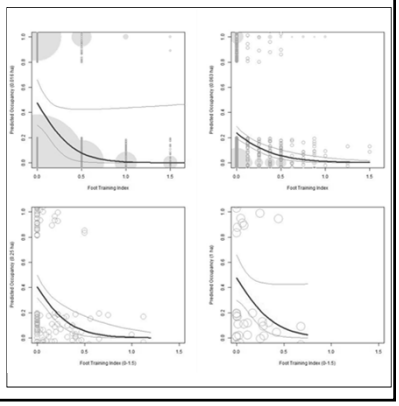
Relationship to PPM Occupancy (ψ)

Foot Training Index

Index (0-4):
Light foot traffic= 0.5
Heavy foot traffic= 1.5
Bivuac= 1
Trash= 0.5
Dogs= 0.5



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Site Specific Modeling Results 2013

Site	Top model (ρ)	Top model (ψ)	Top forb (subshrub) species predictors
Edson	All Animals (-)*	Forbs * Non-native Grass (NNG)	Erodium
Oscar	All Animals (-)	Forbs + REME	Croton + Erodium
SSM	All Animals (-)	Forbs	Croton + Mustard
Dana Point	All Rodents (-)	Herbs (Forbs+Grasses) + Disturbance	Croton

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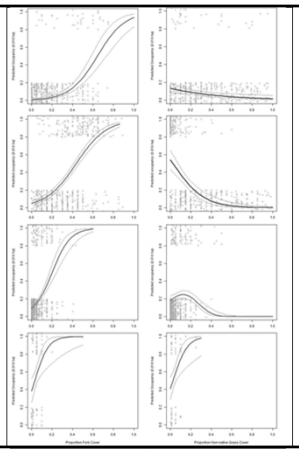
Relationship to PPM (ψ)

Site Specific

Forb Cover & Non-native Grass Cover



Oscar One

Edson

SSM

Dana Point

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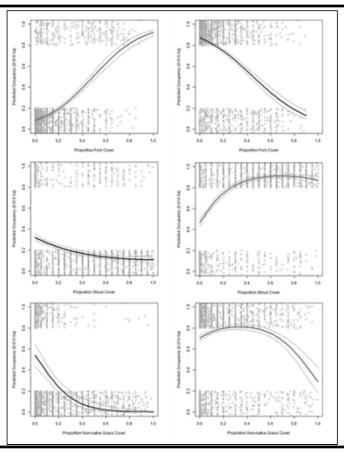
PPM vs. REME



Forbs

Shrubs

Non-native Grass

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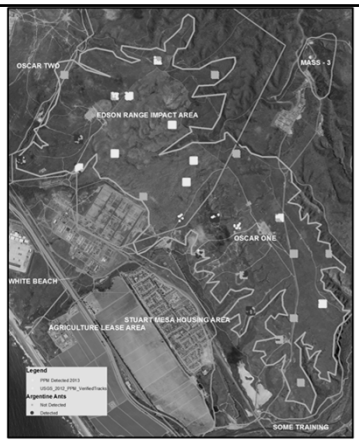
Santa Margarita
(Oscar & Edson)

Argentine Ant Detections 2013



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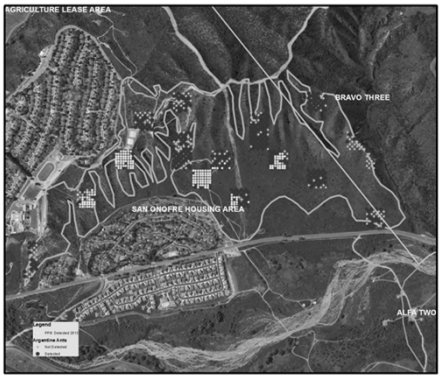
South San Mateo Argentine Ant Detections 2013



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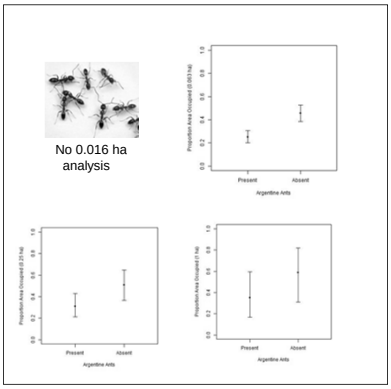
Argentine Ants in 2013 PPM Models



No 0.016 ha analysis

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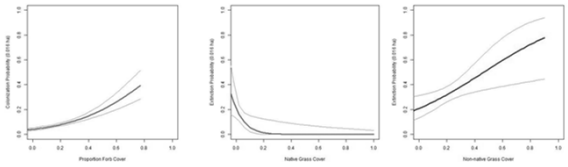
Multi- Year Modeling Results (γ,ε)

Spatial Scale (ha)	Top model (ρ)	Top model (ψ)	Top model (γ)	Top model (ε)
.016	All Rodents	Forbs^2 + NNG^2 Foot Training Index	Forbs	Native Grass(+) + Non-native Grass (-)



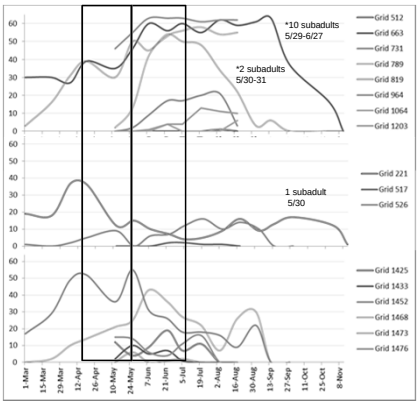
USGS Permanent plots only

Temporal Activity

Edson

Oscar One

SSM



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Live Trapping

	Oscar	Edson	SSM
§April 7-10	Adults, males scrotal, Pregnant female	Adults, males scrotal	Adults, Perforate female
§May 12-4	Adult (only 1 cap)	Adults, males scrotal	Adults, males scrotal
§June 3-5	Adults, Subadults, males scrotal	Adults, Subadults, males scrotal, Lactating female	Subadults
§July 8-10	Adults, Subadults	Adults, Subadults, males scrotal, Pregnant female	Adults, Subadults, males scrotal
§Aug 19-21	Adult scrotal male(only 1 cap)	Adults, Subadults, males not scrotal, Lactating female, Pregnant female YOY	Adults, Juv/Subadults, males not scrotal
total unique individuals captured	13 ppm	27 ppm	10 ppm

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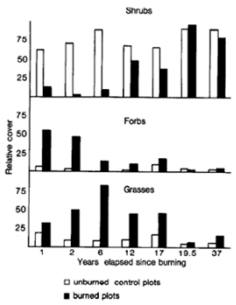
Conclusions

- PPM require adequate cover of forbs.
- Association of PPM with sandy habitat likely indirect
  - **Sandy**
  - **Frequent fire**
- *Paradigm shift*

*The **Pacific Pocket Mouse** is a Federally Endangered animal in California. It lives in sandy coastal soils….*



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Conclusions, Cont.

- Non-native grasses are positively associated with PPM only in sites with very low forb cover.
- Non-native grass cover <30% are negatively associated with PPM over all sites.
- Heavy foot traffic is a negative predictor of PPM at all spatial scales.
- Bulldozing associated with local extirpation of upper SSM
- Argentine Ants potentially have negative impact on PPM with minimal overlap in occurrence within the Santa Margarita population(s).
- PPM Recruitment success spatially and temporally variable

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Recommendations

- **Incorporate modeling results into PPM habitat management goals** (favoring high forb cover, intermediate bunch grass and open ground, low cover of shrubs and woody debris/leaf litter)
- Manage for forb growth
  - Prescribed burning
  - Hand thinning of shrubs, woody debris, leaf litter
  - Herbicides (non-native grasses)
  - Controlled grazing
  - Seeding forbs

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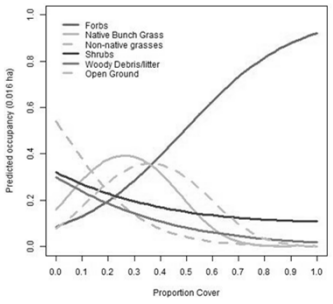

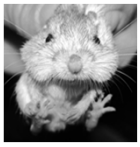

Cover Type	Goal	Range
Forbs	60%	> 25%
Open Ground	25%	20-50%
Native Grass	15%	10-40%
Non-native Grasses	0%	<20%
Shrubs	5%	<25%
Woody Debris	0%	<20%

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Habitat Restoration Goals

Recommendations, continued

- **Minimize "footprint" of foot traffic from military training and biology** (particularly in sand dominated areas and steeper slopes; i.e. remove core plots in hilly sandy areas, clearly mark walking trails in all core plots)
- **Xerify habitat to reduce Argentine ant population density and probability of further colonization within PPM habitat.** (Reduce leaf litter, shrub cover, water seepage)
- **Further research and analysis of PPM diet in relation to resource availability and reproductive success to better inform habitat and population management.**
- **Management Plan**

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Research Questions:

1. How are forbs related to PPM phenology: Activity and Reproductive Success
   - Total cover of forbs
   - Specific forb species
   - Forb phenology
   - PPM diet vs. seed availability (Yvonne & Kris)
2. Do home ranges vary across population sites? Are movements related to seed availability?

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Research Questions:

4. Are Argentine Ants negatively impacting PPM populations- habitat/ recruitment?
   - NSM & SSM (Part) 2010
   - SSM, Santa Margarita 2013*
   - Dana Point- needed

4. Further data analysis*
   - Forb cover- phenology
   - Species interaction modeling
   - Juveniles/recruitment by track size
   - Abundance vs. Track Index (over time)
   - Development of occupancy-abundance models

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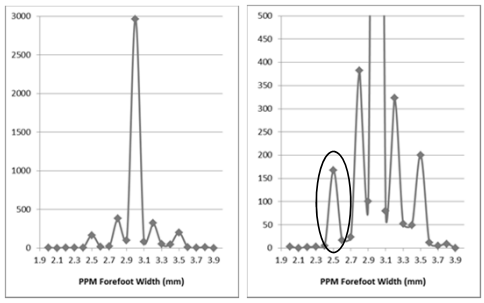
New Developments :
PPM Track size to document Recruitment



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PPM Scat Assay- Barcoding

- We are using standard universal primers that amplify a ~500bp plant chloroplast gene that is commonly used to barcode plant species.
  - Can distinguish among genera and in many cases can produce species-level identifications.
- We amplify the gene region from the stool samples and collect sequence data using an illumina MiSEQ next generation sequencer
  - collects 1000s to 100,000s of sequences from each sample
  - should have coverage for most of the plant species that are present and amplified in a stool sample.

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PPM Scat Assay

- The sequences are then compared against a reference library-- ours is comprised of a mixture of field collected reference samples from plants in the habitat and others pulled from BOLD-- the online plant species barcoding database. Sequences with a high percentage match are assigned that species/genus ID.
- We have preliminary data back on a handful of samples that was encouraging.
  - Single scat sensitivity (1/2/4)
  - Plant species identified correspond to those in habitat (wild) and provided diet (Zoo)
  - Optimization of sample preparation
  - 2nd extraction done on 2 samples (PPM & PEMA) for arthropods. No arthropods in PPM scat sample; Arthropods present in PEMA sample

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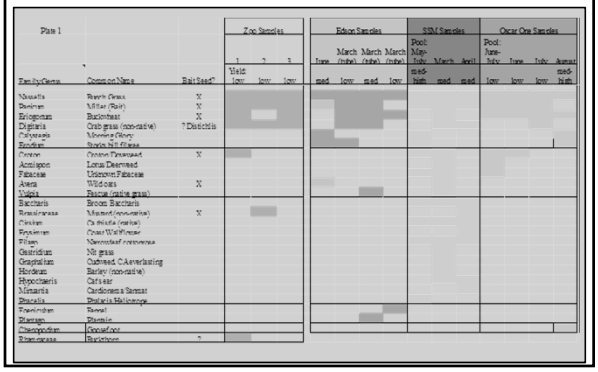
Some Initial Results



Next Plate- In Process

- Currently running a second larger batch of samples with replication to look at repeatability, and refining the "bioinformatics" end (library referencing, data matching algorithms and verifying calls).
- 6 individual PPM samples (3 zoo, 3 wild)
  - 1 vs. 2 vs. 4 scat; wild: fresh vs. in trap
  - 2 PCR's per sample- repeatability
- 45 Individual PPM samples from Oscar, SSM, Edson & Dana Point representing spring, summer/fall

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PPM Recovery- Near Future

- SD Zoo (&FWS)- Captive Breeding Program
  - Planned introductions of 1-2 new populations
  - Behavioral Studies- Caching, seed preference in captivity & species interactions
- PPM Management Plan for MCBCP
- Dietary and Resource Use Study to further inform habitat restoration
- Continued Monitoring & Modeling*
- Argentine Ant Control- possible

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Acknowledgements

U.S. Department of the Interior
U.S. Geological Survey