State of California
Natural Resources Agency
Department of Fish and Wildlife
Wildlife Branch

Captive Breeding, Anti-Predator Behavior and Reintroduction of the Pacific Pocket Mouse (*Perognathus longimembris pacificus*)

2012-2014

By

Dr. Debra Shier

Nongame Wildlife Program, 2014-03

Final Report

To

State of California
Department of Fish and Wildlife
South Coast Region
3883 Ruffin Road
San Diego, CA 92123

Captive Breeding, Anti-Predator Behavior and Reintroduction of the Pacific Pocket Mouse (*Perognathus longimembris pacificus*)

For the period
June 15, 2012 – June 14, 2014

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ABSTRACT

The primary goal of this project was to use captive breeding techniques to increase numbers of the endangered pacific pocket mouse (*Perognathus longimembris pacificus;* PPM), learn about the species behavioral ecology as it related to their reproduction and survival skills, genetics and stress, prepare naïve animals for reintroduction to the wild, and reintroduce them to sites within their historic range from which they have previously been extirpated. We established a captive breeding facility and collected 30 founders from the wild. To ensure that interbreeding animals from the 3 extant populations was an appropriate strategy for recovery, we conducted mate choice tests to determine if females showed mate preferences for males from their own population. The results of this study indicated that females showed no preferences; therefore, we began breeding in May of 2013. At the time of writing of this report, we have produced 41 pups in captivity with 73.1% (30) surviving to weaning. In 2014, we successfully bred captive born animals and produced F2 generation mice. Initial antipredator experiments with wild-caught founders revealed that in captivity wild-caught PPM exhibit vigilance behavior and utilize crypsis as an antipredator strategy. In the presence of owls, wild-caught PPM use visual cues to detect a looming stimulus, and while in the presence of snakes, these same mice use multimodal cues (visual, motion and scent) for predator detection. Foraging trials show that PPM preferred to forage under cover and thus minimize potential interactions during seed collection. Initial results from interspecific competition trials indicate that PPM may use territoriality to coexist with putative competitors. During Phase I of this project, we genotyped all founders and offspring produced and used this information to determine relatedness and to develop breeding priorities each year. We examined stress by developing methods for non-invasive fecal glucocorticoid analysis. PPM showed no consistent diurnal cortisol activity over a 48 hour period. Initial results indicate that wild-caught PPM showed a decrease in cortisol after they acclimated to captivity.

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[1] Shier, D.M. 2014. Captive Breeding, Anti-Predator Behavior and Reintroduction of the Pacific Pocket Mouse (*Perognathus longimembris pacificus*), 2012-2014. California Department of Fish and Wildlife, Wildlife Management, Nongame Wildlife Unit Report, 2014-03. Sacramento, CA 70 pp + Appendices.

INTRODUCTION

The pacific pocket mouse (*Perognathus longimembris pacificus;* PPM) is endangered and only 3 extant populations are known to exist. The largest remaining population, the Santa Margarita population, is located in the Oscar One and Edson training areas on Marine Corps Base Camp Pendleton. PPM populations expand and contract in response to rainfall patterns. Southern California experienced low levels of rainfall for several years in a row (70% of normal mean precipitation 2005-2006, 52% of normal mean precipitation 2006-2007, and 39.5% of normal mean precipitation 2007-2008 with 2013 being the driest on record for decades. In 2006 we initiated a translocation program for PPM with the goal of developing translocation methods for the species. However, population surveys for PPM conducted on the Santa Margarita population between 2006 and 2008 indicated that this population had contracted significantly (Shier 2008, 2009). Between 2006 and 2008, PPM numbers in the Santa Margarita population were too low to conduct a translocation. Thus, in 2008, we submitted a proposal to captively breed and reintroduce PPM.

A conservation breeding program was initiated by the San Diego Zoo Institute for Conservation Research in 2012.

Objectives

The primary goal of this project is to use non-invasive captive breeding techniques to increase numbers of PPM, prepare naïve animals for reintroduction to the wild, and reintroduce them to sites within their historic range from which they have previously been extirpated. The long term goal is to establish several additional wild populations across the historic range of the species. In the process we are learning about the species behavioral ecology, physiology and genetics. In particular, we have designed experiments to examine the species antipredator behavior, mating behavior, foraging behavior, interspecific interactions, stress and genetics.

PERSONNEL

The following people conducted research on PPM associated with this project: Dr. Debra Shier designed and setup the captive facility, and conducted and supervised field and captive research. Maryke Swartz assisted with the establishment of the facility and conducted captive and field research. Amaranta Kozuch and Andrew Heath conducted captive research and daily husbandry. Rachel Chock and Thea Wang conducted field research.

CAPTURING FOUNDERS

Our goal was to bring 30 founders into captivity to begin the PPM breeding program. These individuals were to come from the 3 remaining extant populations: 1). Dana Point (DP); 2). Santa Margarita (SM); and 3) South San Mateo (SSM). The established target number was N = 10 from each population. For captive breeding to be successful, it is important to have both experienced breeders (adults) and young animals (young of the year; YOY) that will survive in captivity long enough to produce multiple litters. Thus, of the 10 animals from each population, we planned to bring in 2 adult males: 2 adult females: 3 (YOY) males: 3 YOY females constituting no more than 10% of adults or 20% of juveniles in each population to minimize impacts to the wild populations. To reach our goal of 4 adults and 6 YOY, the required abundance estimate in each population was a minimum of 70 PPM. Individuals were to be captured in a dispersed manner throughout the populations with the goal of maximizing genetic diversity of the founders.

Methods

General trapping protocol

There were 2-4 people in the field team. We opened no more than 200 traps each night. On all sites we placed flags in high quality PPM habitat (sandy areas with open vegetation) and placed 2 traps at each flag. Flags were spaced >10m apart to maximize the probability of collecting unrelated animals. We used 9-inch Sherman traps with shortened doors to prevent tail severance. We baited traps with white millet that was cooked for 1 minute in a microwave to prevent germination. Traps were set just before sunset (18:30-20:00) and checked at midnight and dawn unless otherwise noted. If we found a trap with ants inside or within 6 inches of it, we closed the trap for the night. We took a GPS location at every trap in which we captured a PPM.

Processing

All animals captured were weighed, aged (adults = tawny brown pelage, weight ≥ 6.0g; YOY = pelage grey or partially grey with molt line and/or weight < 6.0g), sexed and assessed for reproductive condition. If a female was obviously pregnant, she was released. All animals were inspected for physical condition (e.g. pelage condition and ectoparasites) and for previous marks by United States Geological Survey (USGS) biologists. All animals previously marked were released. If the animal captured was selected as a founder for the captive facility, it was transferred to a holding cage (19 X 30 X 20 cm) for transport that contained 5 cm of clean sand, millet and a 6 inch section of 1inch PVC tubing.

Dana Point

In May of 2012, the Dana Point population was estimated to be >75 individuals (W. Miller, FWS pers comm.). Thus, we were permitted by United States Fish and Wildlife Service (FWS) and California Department of Fish and Wildlife (DFW) to capture 10 individuals from the DP population. We acquired trapping data from May to determine current locations of PPM. On

June 19, 2012, we set out 105 flags in open sandy patches and placed 2 9-inch Sherman traps with shortened doors at each flag (Figure 1). We avoided areas of sensitivity for coastal California gnatcatchers (*Polioptila californica*) and prebaited all traps. On June 20, 2012 we opened and set traps between 18:30 and 20:00 hours. We checked traps at midnight and again at 02:00 and closed all traps at the 02:00 trap check.

Santa Margarita

We trapped PPM from the Santa Margarita population from June 26 to July 3, 2012. In the spring/early summer of 2012, the Santa Margarita population was surveyed by USGS with a combination of trapping and track tube methodology. The population was estimated to be >75 PPM with a larger portion of individuals found within the Edson range than expected. Thus, we were permitted to capture 10 individuals. Because the Santa Margarita population is spread over a large geographic area and our goal was to trap a genetically diverse set of founders, we chose 10 locations spread throughout the population's range (Figure 2) with the plan of trapping 1 animal from each.

South San Mateo

The third extant population, South San Mateo (SSM), is located in the northern part of Marine Corps Base Camp Pendleton (Figure 3.). As of July 5, 2012, USGS had trapped 7 adult PPM in SSM (6 from their trap grid 1452 and 1 from 1518). By July 25th USGS had documented PPM presence at 6 additional track tube grids. To date, there is insufficient data to assess the relationship between track tube detections and number of PPM. Thus, in consultation with USFWS, we decided to error on the conservative side and count a single PPM at each USGS track tube grid (Figure 4) that contained < 10 PPM detections and 2 PPM at each USGS track tube grid that contained ≥ 10 PPM detections. Using this approach for the track tube data along with the numbers of individual PPM captured by USGS, 15 individual PPM were documented by USGS. To document additional individuals, we trapped the SSM population from July 17 to August 16, 2012. We surveyed SSM with assistance from Will Miller from FWS. We identified 15 additional areas off of the USGS grids to target for trapping (Figure 5). We trapped each of the 15 sites plus 5 USGS track tube grids for 3-5 consecutive nights for a total of 3290 trap nights.

Figure 1. United States Geological Survey Topographic map of the Dana Point population. The location of the population is shown with a red box.

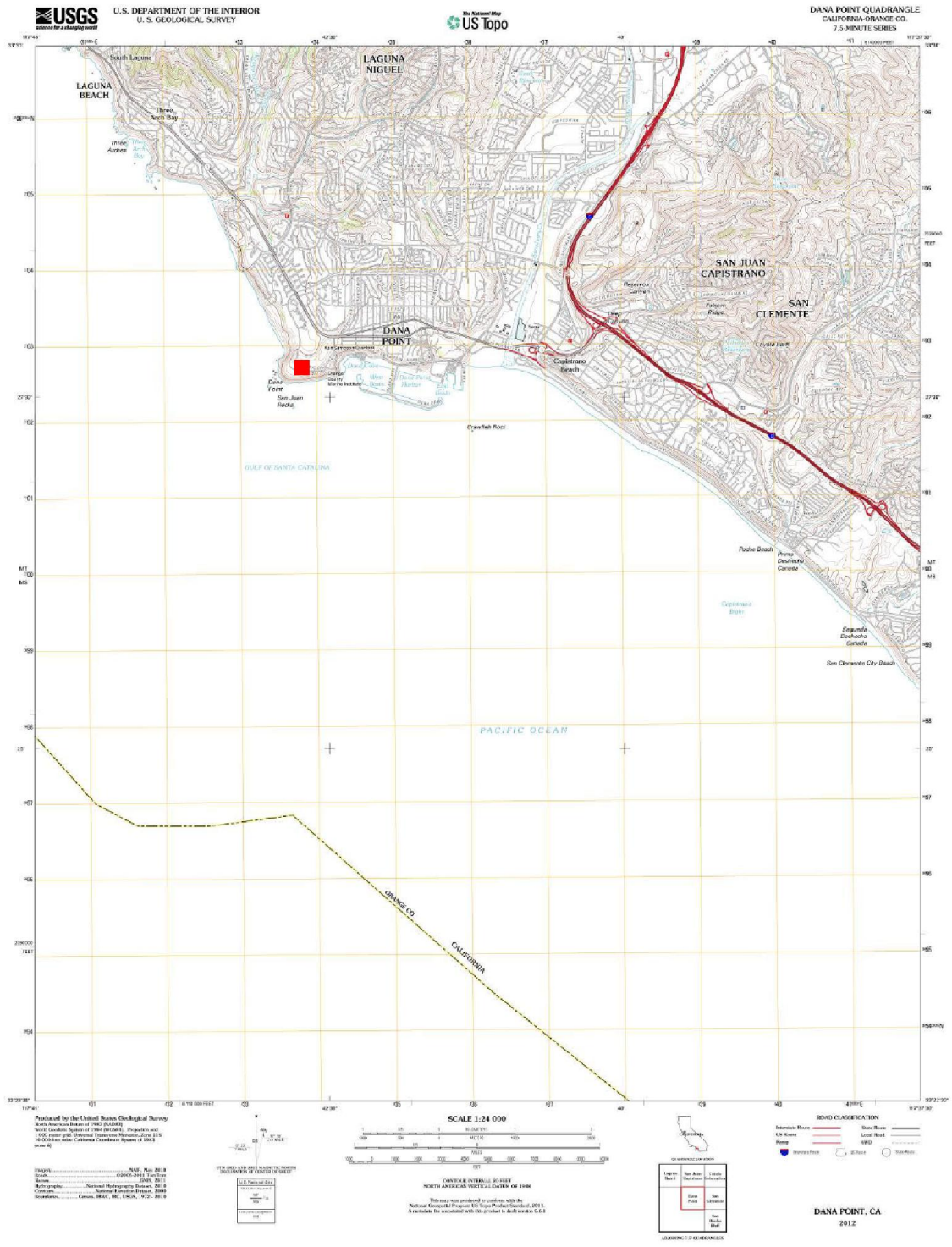


Figure 2. United States Geological Survey Topographic map of the sites trapped within the Santa Margarita population of PPM on Marine Corps Base Camp Pendleton. Red boxes show sites trapped.

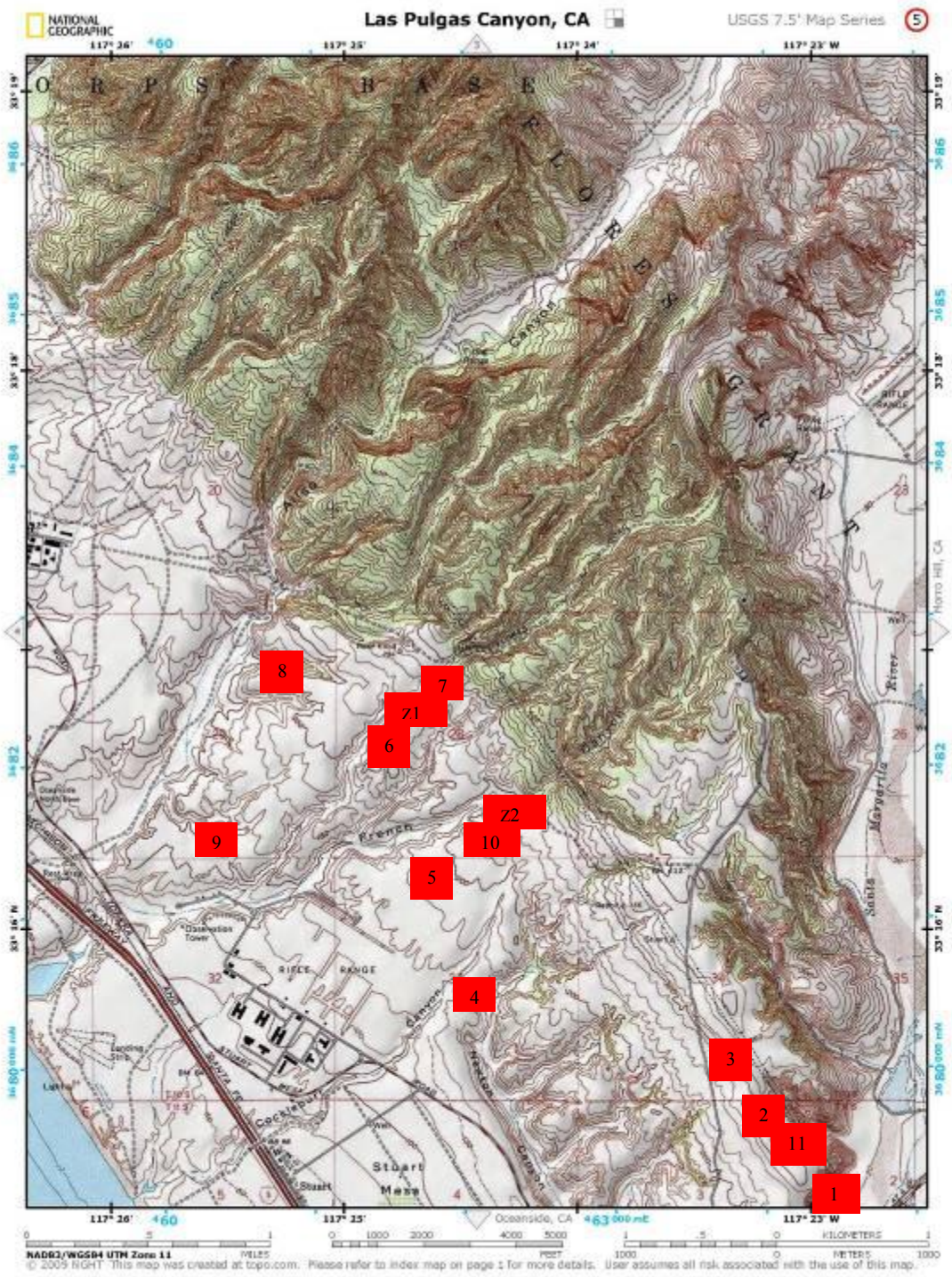


Figure 3. United States Geological Survey Topographic map showing the location of the South San Mateo population (marked with the red square).

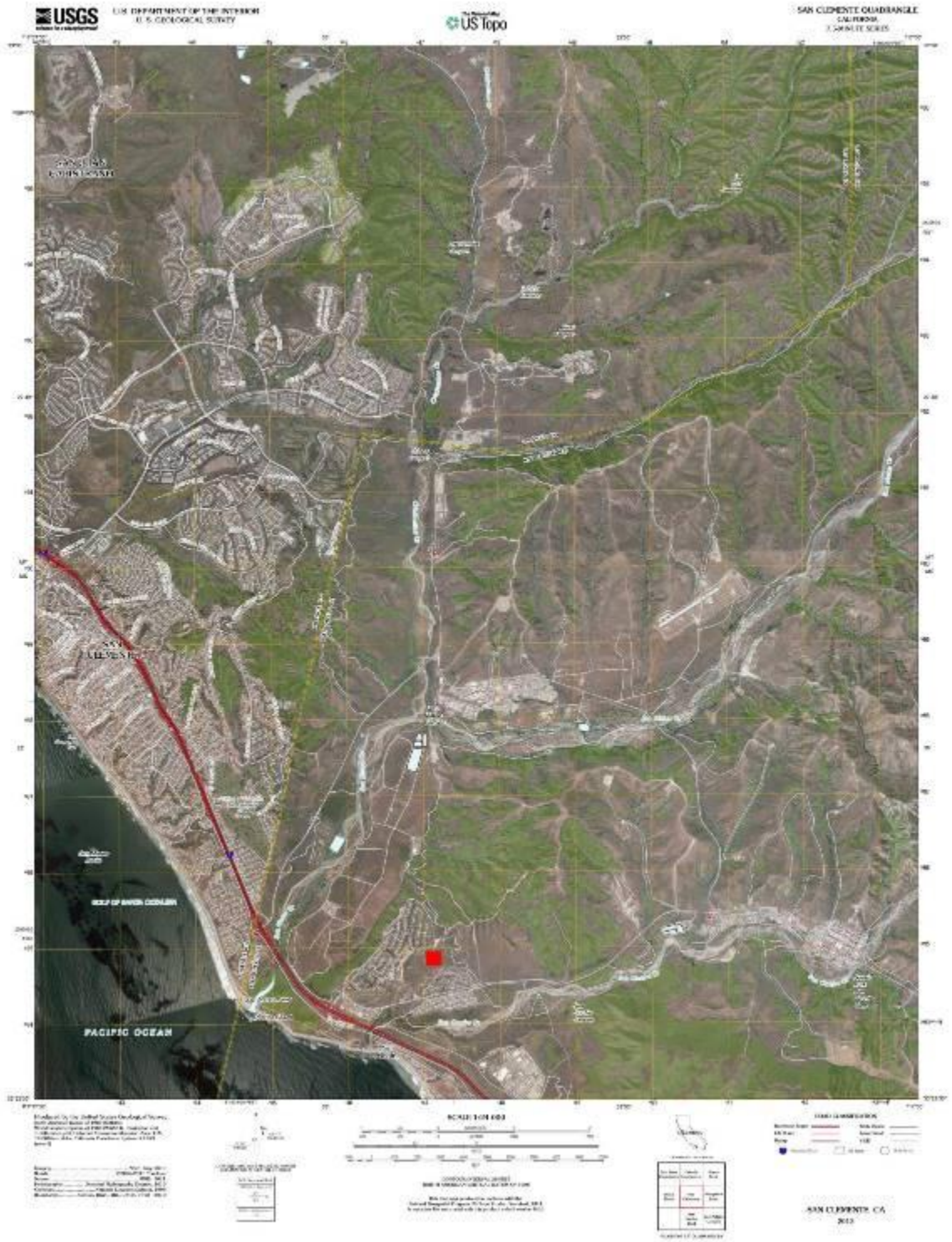


Figure 4. United States Geological Survey (USGS) PPM grids within the San Mateo South population on Marine Corps Base Camp Pendleton showing locations with PPM detections used to determine PPM density for possible removal. Numbers in red boxes indicate number of track tubes with PPM prints in July of 2012 (Note: Site 1518 did not have PPM prints documented in July, but USGS had evidence of a PPM at this site earlier in the year.

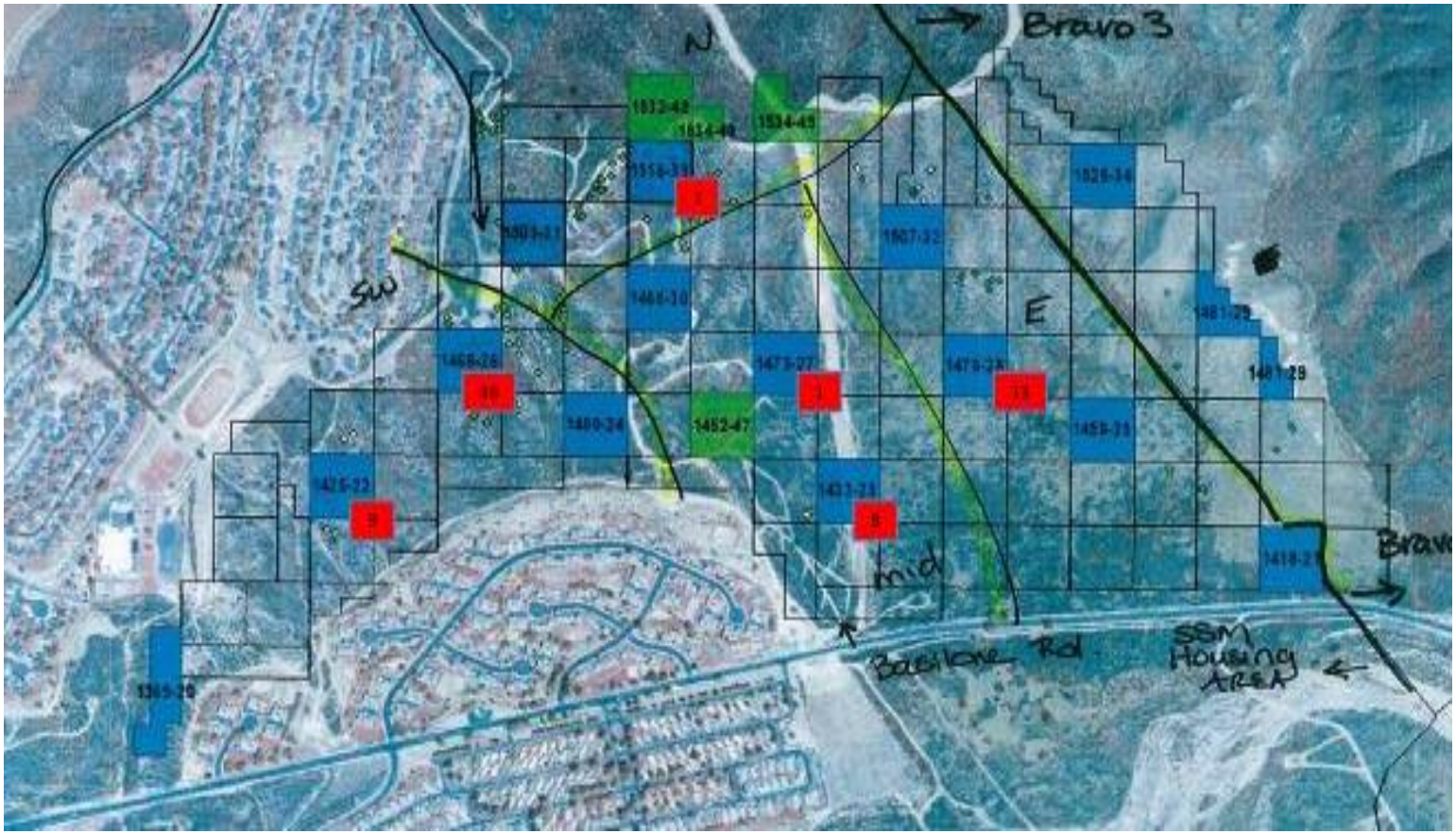
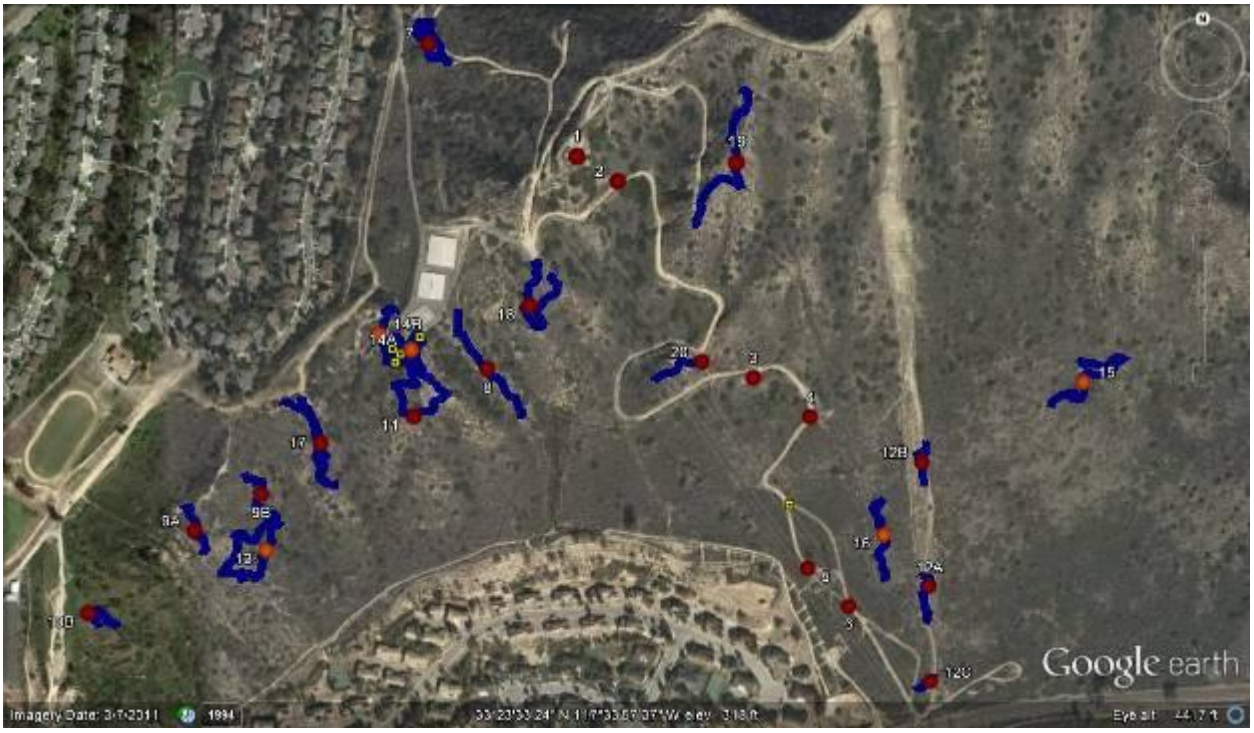


Figure 5. Satellite map showing 2012 trapping sites within the SSM population on Marine Corps Base Camp Pendleton.



Results

Dana Point

In 2012, we captured 27 unique individuals (Table 1). Of those, we selected 10 PPM as founders for the breeding program. They were selected based on geographical dispersion and age/sex of the individuals. Of the 10 PPM brought in from Dana Point that night we identified the animals as belonging to the following age/sex categories: two adult males, two adult females, three juvenile females and three juvenile males spread throughout the entire site including north of the old road (Figure 6). No injury or mortality of PPM occurred as a result of the trapping.

While there were no injuries to PPM from our trapping efforts we did observe 8 individuals (3 F: 5M) that had bald spots on their heads and/or hips. These were the result of the use of sharpie pens (red and blue) during the May trapping effort to assess the population numbers. We brought only 1 of these individuals into captivity (Male 4). No ectoparasites were observed on any of the PPM captured.

During the quarantine entrance exam on the morning of June 22, 2012, we determined that Female 9 was misidentified as a male. Thus, our sex ratio of founders was skewed 6F:4M. We consulted with DFG, FWS and CNLM and decided to return a female to Dana Point and collect 1 additional male. We selected to return Female 6 to Dana Point that night since we had collected another animal in an adjacent trap. That night, we returned to Dana Point, set 18 traps (3 per 6 flags) where we had successfully captured male PPM the previous night and released Female 6 at her point of capture.

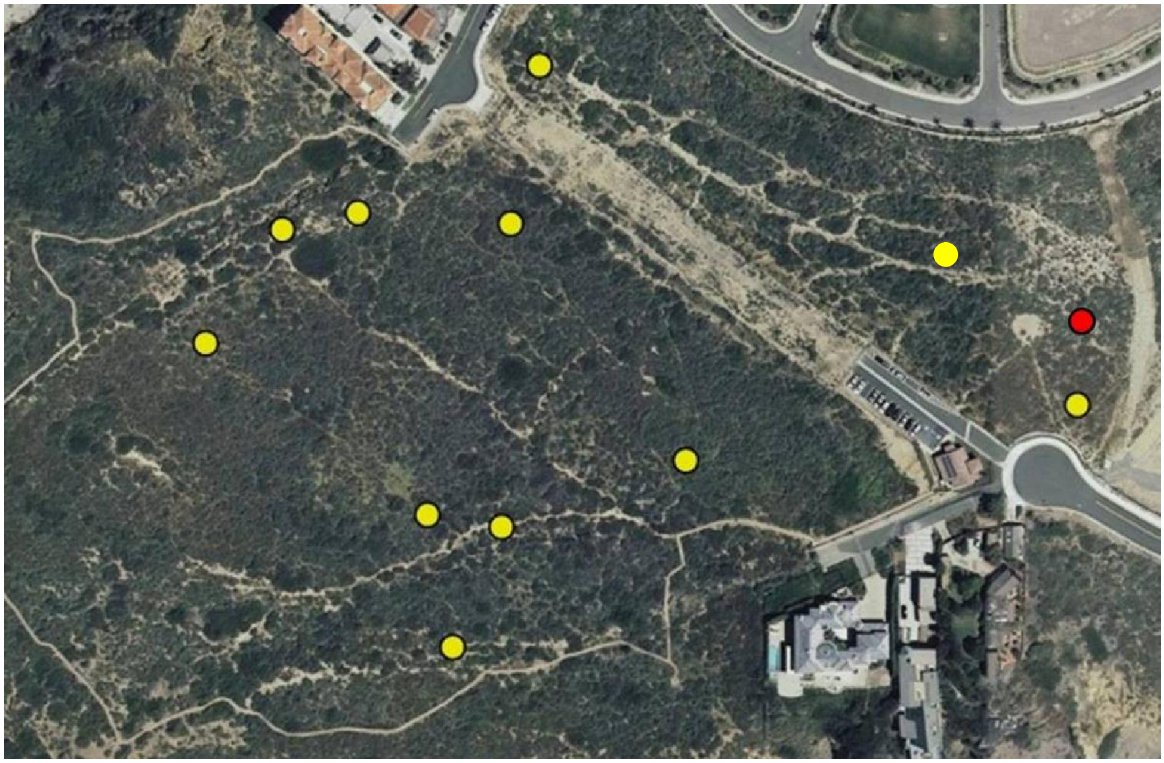
Unfortunately, only after juveniles from 2012 matured and started to become reproductive, did we realized that a second juvenile was misidentified. In addition to female 9, we misidentified a juvenile male as a juvenile female. The sex ratio of PPM collected from Dana Point was 4 females and 6 males. Thus, in 2013, we collected 1 additional female from Dana Point to reach our goal of 5 female founders.

On April 2, 2013 we set out 50 flags along the old road bed and north of the old road and placed 2 traps per flag. We opened traps on April 3, 2013 and captured 8 adult PPM (4F:4M). All PPM males were scrotal and 3 of the 4 females had visible nipples but none were in estrus or pregnant. We brought into captivity 1 female (Female 24; Table 1, Figure 6).

Table 1. Capture Data for PPM Captive Breeding Founders from Dana Point

Date							Reproductive Condition			Released/Removed	Captive ID	Notes
	Flag No.	X	Y	Sex	Age	Weight (g)	Estrous Swelling	Nipples	Scrotal			
6/21/2012	4	433756	3702812	male	YOY	7			Scrotal	removed	7	
6/21/2012	12	433840	3702778	male	adult				Scrotal	released		bald spot on head and hind leg (dorsal side)
6/21/2012	25	433933	3702739	female	adult		1	post lactation		released		bald spot on head and hind leg (dorsal side)
6/21/2012	30	433932	3702723	male	adult				Scrotal	released		
6/21/2012	34	433944	3702692	male	adult	7.2			Scrotal	removed	1	
6/21/2012	37	433911	3702698	male	adult				Scrotal	released		bald spot on head
6/21/2012	41	433754.72	3702758.95	male	adult				Scrotal	released		bald spot on head and hind leg (dorsal side)
6/21/2012	42	433750.67	3702763.37	female	YOY					released		bump on left rear leg
6/21/2012	43	433745.46	3702756.8	female	adult	6.9		visible		removed	2	
6/21/2012	47	433691.86	3702760.94	male	adult				Scrotal	released		
6/21/2012	49	433665.23	3702755.19	female	YOY	6.3				removed	3	
6/21/2012	50	433670.06	3702771.68	female	adult					released		bald spot on head; inflammed
6/21/2012	52	433654	3702760	female	adult		pregnant	visible		released		bald spot on head and hind leg (dorsal side)
6/21/2012	56	433621	3702733	female	YOY			visible and red		released		lactating
6/21/2012	59	433637.96	3702715.65	male	adult	6.3				removed	4	bald spot on head; inflammed
6/21/2012	71	433760.92	3702692.75	female	adult		bloody discharge	visible and red		released		
6/21/2012	78	433782.54	3702645.61	male	adult				Scrotal	released		
6/21/2012	79	433761.71	3702653.57	female	adult			visible and red		released		
6/21/2012	80	433757	3702656	female	YOY			post lactation		released		
6/21/2012	82	433741.48	3702650.83	female	YOY	5.7				removed	5	
6/21/2012	83	433715.48	3702655.2	female	YOY	5.8				released	9	classified as male in field but misidentified
6/21/2012	84	433709.09	3702662.61	female	YOY	6.1		visible		removed	6	removed from field for 24 hours but returned to capture location
6/21/2012	86	433698.02	3702673.54	male	YOY				Scrotal	removed		
6/21/2012	95	433724	3702609	male	adult	6.8			Scrotal	removed	8	
6/21/2012	97	433749	3702621	female	adult		possibly pregnant	visible		released		
6/21/2012	103	433806.49	3702673.54	male	adult	6.3			Scrotal	removed	10	
6/22/2012	37	433911	3702698	male	adult				Nonscrotal	released		bald spot on head and hind leg (dorsal side)
6/22/2012	47	433691.86	3702760.94	male	adult	7.5			Scrotal	removed	11	recapture?
4/3/2013		433939.38	3702717.49	female	adult	7.0	2	visible	n/a	removed	24	

Figure 6. Map of Dana Point Headlands Capture Locations. Yellow dots indicate collection location of 10 animals in 2012, red dot indicates collection location of single adult female in 2013.



Santa Margarita

We captured a total of 18 PPM over 6 nights of trapping in 2012 and brought 10 into captivity. Table 2 provides details for each animal captured. We brought 10 animals into captivity from the SM population. No ectoparasites were observed on any of the PPM captured.

Table 2. PPM Captures at Santa Margarita population Marine Corps Base Camp Pendleton

Date								Reproductive Condition			Released/Removed	Captive ID	Notes
	Site No.	Flag No.	X	Y	Sex	Age	Weight (g)	Estrous Swelling	Nipples	Scrotal			
6/27/2012	2	12	463879	3680105	male	yoy	5			Scrotal	removed	12	
6/28/2012	1	16	464026	3679532	female	yoy	5.9	1			removed	13	
6/29/2012	3	25	4633835	3680217	female	yoy		1			released		yellow crust on genitals and base of tail
7/2/2012	3	19	463778	3680183	female	adult	7.4	1	visible		removed	14	
7/2/2012	7	1	4622152	3682221	male	adult	5.8			nonscrotal	removed	16	
7/2/2012	9	9	460630	3681423	male	yoy	5.8			nonscrotal	removed	15	
7/2/2012	6	3	462019	3681990	male	yoy	5			nonscrotal	removed	17	
7/2/2012	5	2	462424	3681490	female	adult		1	post-lactation		released		
7/2/2012	5	10	462314	3681442	male	yoy				nonscrotal	released		
7/2/2012	5	13	462295	3681435	male	adult					released		
7/2/2012	5	18	462273	3681429	female	yoy	5.8	1			removed	18	
7/3/2012	4	5	462150	3681075	female	yoy	4.6	1			removed	20	
7/3/2012	8	11	461392	3682390	female	adult	7	1	post-lactation		removed	19	
7/3/2012	8	13	461383	3682402	male	yoy				nonscrotal	released		
7/3/2012	8	19	461361	3682463	male	adult	6.8			nonscrotal	released		
7/3/2012	8	21	461366	3682482	male	yoy				nonscrotal	released		
7/4/2012	10	3	462596	3681348	female	yoy	5.5	1			released		
7/4/2012	10	16	462629	3681351	male	adult	6.3			nonscrotal	removed	21	
4/10/2014	Z18	D1	461556	3682623	female	adult	6.2	2	visible		removed	52	
4/11/2014	1	G1	464049	3679472	male	adult	7.8			Scrotal	removed	51	
4/20/2014	2	B1	463916	3679682	male	adult	7.2			Scrotal	removed	58	
4/21/2014	1	E1	464049	3679493	male	adult	7.5			Scrotal	released		
6/3/2014	11	E4	463996	3679530	female	adult	6.5	2	visible		removed	71	

South San Mateo

In 2012, we captured a total of 5 unique PPM over 16 nights (3290 trap nights) (Table 3). One of these individuals was previously marked by USGS. Thus, taking together the 15 PPM documented by USGS and the additional 4 individuals that we captured, 19 individual PPM were documented in 2012. We were permitted to bring into captivity 2 adult PPM in 2012. (1M: 1F). No ectoparasites were observed.

In order to reach our goal of 10 founders from each extant population, in spring of 2013, we began documentation of PPM numbers. We conducted track tube surveys at 13 sites in SSM between March 21 and July 12 2013 (Figure 7). Each track tube site had 10 track tubes spaced 20m apart in a primarily linear fashion. Track tube sites were checked twice a week for the first 3 weeks and once each week for 4 additional weeks. To date, there is insufficient data to assess the relationship between track tube detections and number of PPM. Thus, we used PPM home range to estimate the number of track tubes a single PPM may visit regularly. We assumed that track tubes separated by 20m would be visited by a single PPM, but that a single PPM could visit as a cluster of 4 track tubes if they were spaced approximately 6.5m apart (USGS protocol).

Using this protocol, we documented 22 unique PPM on our sites. Taken together with data accumulated by end of April 2013 by the USGS which indicated 40 unique individuals, we were permitted to take 6 additional founders. We set traps on May 2, 2013 and captured 3 PPM (2M: 1F; Male 25, Female 26 and Male 27) 1 from each of our Sites 2, 6 and 7. On May 11, 2013, we attempted to collect additional PPM. We trapped USGS grids 1425, 1468, 1452, and 1433. We only trapped 2 female PPM. Both females were previously marked by USGS and were released. We trapped SSM to collect additional founders on June 13, 2013 and again on June 26-27, 2013. On the night of June 13, we captured 2 PPM on USGS grids both were previously marked. We released both animals. On June 26, 2013, we trapped no PPM and on June 27, we trapped 1 unmarked adult female PPM on USGS grid 1433 and took it into captivity (Female 30). This brings our current total SSM founders to 6. Scott Tremor from the San Diego Natural History Museum conducted additional trapping efforts in SSM during July 2013 and failed to capture any PPM. Thus, we decided to end our efforts at collection in SSM for 2013.

During the 2014 partners meeting, we decided to forego further PPM captures from SSM given the data from our efforts and those of USGS during 2012 and 2013. We, instead, made the decision to pull the remaining 4 founders from the SM population which has been shown to contain the largest amount of genetic variation in the subspecies.

Final Collection from Santa Margarita

On March 30, 2014, USGS placed track tubes in within the Edson range in the SM population. We checked tubes on April 5, 2014 and documented PPM at 4 separate tube locations. Taken together with USGS detections also listed in the table 39 PPM were detected in SM. Thus, we were permitted to take 3 PPM from SM with a need to document 1 additional PPM before taking the fourth and final founder into captivity. On April 9, 2014, we set 4 trapping grids of 25 traps each (2 in Edson: Z18, Z29; 2 in Oscar: 1, 3; see Figure 2) and trapped for 3 nights. We captured 1 adult female and 1 adult male PPM and transferred them to the Safari Park for quarantine (Table 2). We pulled traps from our Edson Z18 grid and set Grid 2 in Oscar. We opened traps again April 20 and trapped for 2 nights. We captured 2 adult male PPM and brought 1 of the

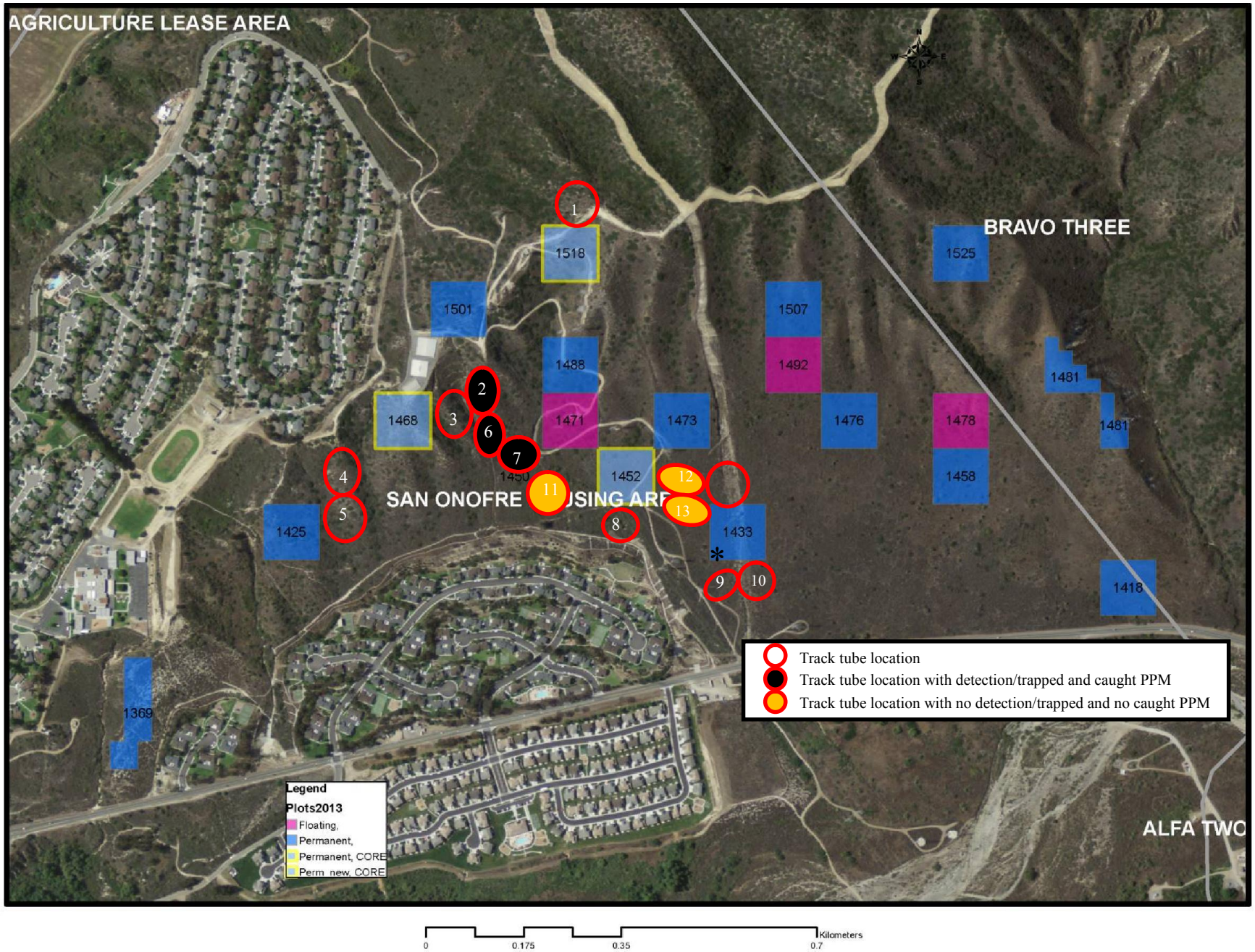
males into captivity for quarantine (Table 2). The second male brought our total PPM documented in SM for 2014 to 40 and thus we were permitted to capture a 4th and final PPM. We trapped for 3 nights beginning May 12, 2014 and captured no PPM. Because we suspected that the local heat wave and full moon conditions may have reduced our PPM capture probability, we waited for a couple of weeks before attempting capture of the final female. On June 1, 2014, we prebaited 2 existing grids (1 and 2), pulled traps at Grid 3 on which we had no previous PPM captures in 2014 and set up Grid 11 in Oscar which was located just north of USGS grid 221 (Figure 2). We opened traps on June 3, 2014 and captured 2 female PPM at the midnight trap check. One female was released at the point of capture and 1 female was taken into captivity. We pulled all traps and flags immediately after capture. The final female was transported to the Safari Park for quarantine.

Table 3. PPM Captures at South San Mateo Marine Corps Base Camp Pendleton

Date	Location								Reproductive Condition			Captive ID
	Site	Flag No.	X	Y	Sex	Age	Capture History	Weight (g)	Estrous Swelling	Nipples	Scrotal	
7/18/2012	2012-5	23	447563	3694791	Male	Adult	New*				Nonscrotal	
7/19/2012	2012-5	25	447560	3694787	Male	Adult	Recapture				Nonscrotal	
7/20/2012	2012-5	24	447560	3694787	Male	Adult	Recapture				Nonscrotal	
8/7/2012	2012-14	23	447104	3694973	Male	Adult	New				Nonscrotal	
8/7/2012	2012-14	19	447114	3694967	Female	Adult	New	6.4	1	Not visible		22
8/8/2012	2012-14	25	447104	3694978	Male	Adult	Recapture				Nonscrotal	
8/9/2012	2012-14	25	447104	3694978	Male	Adult	Recapture				Nonscrotal	
8/9/2012	2012-14	1	447138	3694986	Female	Adult	New		1	Visible		
8/9/2012	2012-14	25	447104	3694978	Male	Adult	Recapture				Nonscrotal	
8/15/2012	2012-14	24	447560	3694962	Male	Adult	Recapture				Nonscrotal	
8/16/2012	2012-14	27	447108	3694959	Female	Adult	New		1	Not Visible		
8/16/2012	2012-14	27	447108	3694959	Female	Adult	Recapture					
8/16/2012	2012-14	18	447122	3694950	Female	Adult	Recapture					
8/16/2012	2012-14	24	447560	3694962	Male	Adult	Recapture				Nonscrotal	
8/16/2012	2012-14	2	447133	3694980	Female	Adult	Recapture		1			
8/17/2012	2012-14	1	447138	3694986	Female	Adult	Recapture		1			
8/17/2012	2012-14	24	447560	3694962	Male	Adult	Recapture	6.9			Partially	23
8/17/2012	2012-14	27	447108	3694959	Female	Adult	Recapture					
5/2/2012	2013-6	F	447262	3694991	Female	Adult	New	6.5	1	Visible	n/a	26
5/2/2013	2013-2	L	447280	3694917	Male	Adult	New	7.9	n/a	n/a	partially	25
5/2/2013	2013-7	G	447323	3694888	Male	Adult	New	8.0	n/a	n/a	Scrotal	27
6/27/2013	USGS-1433		447644	3694708	Female	Adult	New	6.9	1	Visible	n/a	30

* Animal was previously marked by USGS

Figure 7. Track tube site locations within the South San Mateo population of Marine Corps Base Camp Pendleton.



Quarantine

All PPM were transferred to quarantine at the Harter Veterinary Medical Center (HVMC) at the Safari Park on the night of capture for a 14 or 30-day quarantine. We used an all in all out protocol such that the quarantine period began on the day that the last animal from each population was brought into the HVMC. All animals were inspected by a veterinarian upon arrival and weighed. Weights were then taken weekly.

HEALTH AND DISEASE

Very little is known about disease in PPM. In Phase 1, we began to gather information to develop a disease risk assessment and mitigation plan for PPM by conducting health assessments. Upon capture of founders we documented the physical condition of captured PPM, our veterinarians conducted health assessments upon entry into quarantine and we are in the process of developing a preliminary risk assessment plan based on the information we have gathered on known viruses and bacterial pathogens found in the species and any that we have seen in captivity, the species assemblage present at the source and potential release sites, and the PPM captive diet.

Methods

For each founder, we assessed physical condition via inspection of the animals in the wild. We assessed the following: weight, pelage condition, external body condition and ectoparasite load. Founders were combed systematically and any ectoparasites (e.g., fleas, ticks, lice) were collected into ethanol to be counted. A fecal sample was taken from each wild-caught founder and a fecal o/p test (o = ova, p = parasite) was run to examine individuals for endoparasites. The o = ova and the p = parasite. The PPM fecal samples were soaked in saline and placed into a zinc sulfate solution for a flotation technique to increase the yield of any ova in the sample.

Results and Discussion

Several Dana Point animals collected in 2012 had raw bare spots on their bodies (see Table 1 above). We released all but one of these animals rather than using them as captive founders. The male (#4) that had a raw bare spot healed and the fur on his head regrew over the subsequent weeks. All founders were judged as healthy and only two (Female 26 from SSM; Male 58 from SM) had ectoparasites (ticks) upon capture. The ticks were found on her ear pinna and were removed. All fecal assays for endoparasites were negative. A detailed health summary for the period of quarantine is attached as Appendix A.

Over the course of fall 2012, we saw a nasal crust develop in several of the PPM. We took a sample of the crust from a few individuals and sent it to a lab for cytological evaluation. The results rule out blood as the source of the crust, however, the samples are too small to definitively determine composition. Veterinary staff assumed it is composed of porphyrins based on the color, location (nares), species (rodents) and the fact that it seems to be a self-limiting problem. We see a low level of morbidity but no serious disease or damage from this chronic intermittent crusty material. Of interest, captive born and wild caught PPM all have it. A possible cause is a nonspecific stressor in their environment, however, it will be difficult to determine the exact cause given the size of the samples. There is no evidence that it is toxic, contagious, or infectious agent or a nutritional problem. We will continue to monitor individuals for the presence of the crust.

PPM CAPTIVE PROPAGATION AND RESEARCH

General Methods

Housing

The mice were housed in the renovated PPM facility. The facility contained 3 animal rooms, a room for behavioral experiments, a bathroom, and a separate space for food prep, sample storage and computer work. A central air conditioning/heating unit was installed to maintain facility temperature between 68 and 75˚F. Each animal room contained skylights to maintain natural lighting conditions. Target humidity (50 - 60%) was achieved using tanks of water and/or humidifiers. Shelving was installed in each room to increase the capacity of the facility. Each room contained 3 wooden shelves along the perimeter of the room and in the 2 larger rooms, 2 additional shelves were built in the center of the room to function as tables or accommodate expansion as numbers of PPM increase. In total, the facility was designed to hold as many as 275 PPM.

Social Cages

At the end of the quarantine period, animals were moved to social cages in the PPM facility. Social cages are clear, acrylic boxes divided into 2-6 compartments (each compartment measuring 30 x 12 x 30 cm) which share a long side. Mice were separated by a clear, acrylic barrier such that 2 to 6 mice were housed individually in a single social cage unit. The number of units in a box was determined by the length of the wall in each room. To allow olfactory and some tactile contact between neighboring animals, the clear barriers have 1 cm wide slots every 3 cm along the bottom third of the divider and 5 mm holes in the top third. In addition, each unit has 5 mm holes in the top 1/3 in the exterior walls of the short end so that animals in adjacent units will be able to smell each other. These cages have been shown to facilitate socialization and thus maintain estrous cycling in other heteromyid rodents (Yoerg 1999, Yoerg and Shier 2000). Males had only female neighbors and vice versa. Each mouse was provided an artificial nest chamber. In the social cages, a 12 cm piece of 2.5 cm white PVC pipe joined to a T-section formed an entrance/exit “burrow” to the nest jar.

Data Collection

Weights

All animals were weighed weekly for the first 4-6 weeks after being brought into captivity to assess their physical condition. Thereafter, females were weighed weekly and males were weighed every 2 weeks.

Estrus

Reproductive condition was assessed for all females every 1-3 days during non-estrous periods. To assess estrous condition, females were scored according to the scale in Table 4.

Table 4. Scoring System for Estrous Condition in Heteromyids after Villablanca, unpublished

Genitals		
	1	Clitoris large, vulva not swollen
	2	Vulva slightly risen, diameter greater than clitoris
	3	Vulva noticeable risen, longer than wide
	4	Vulva large, top flat, edges round
	5	Vulva taut, top flat, edges straight
Discharge		
	1	None
	2	Dry white crust
	3	Stiated cast
	4	Plug (mucous, black, post-copulatory noted)
	5	Fresh or dried blood

Weight as an indicator of physical condition in captivity

Methods

See general methods.

Results and Discussion

Data indicate that PPM from each population increased in weight once brought into captivity (Table 5). Weight peaks for males and females of each population occurred in July or August of 2012 and stabilized over subsequent months. By the end of December 2012 weights for Dana Point and SM females were the same. Data for males show the same result. The 2012 data from SSM represent only 1 male and 1 female. The weight for the SSM female was the same as for females from the other 2 populations by the end of December. While, the weight of the male from SSM was higher than the average for the other 2 populations, his weight was within the range of variation for males of the other 2 populations. These data indicate that PPM from each of the populations consumed the captive diet and increased in weight. This weight increase stabilized for animals in each population during the fall. Weight can be used as an indicator of how well wild animals are adjusting to a captive environment. The weight data indicate that mice from each population adjusted well to the captive environment.

In 2013, adult weights were fairly stable throughout the year. Weight minimums occurred in February for both for male and female founders and peaked in September (Figure 8). Weight data are comparable to those from captivity in 2012 and from PPM in the wild and provide one indicator that captive PPM remain in good condition.

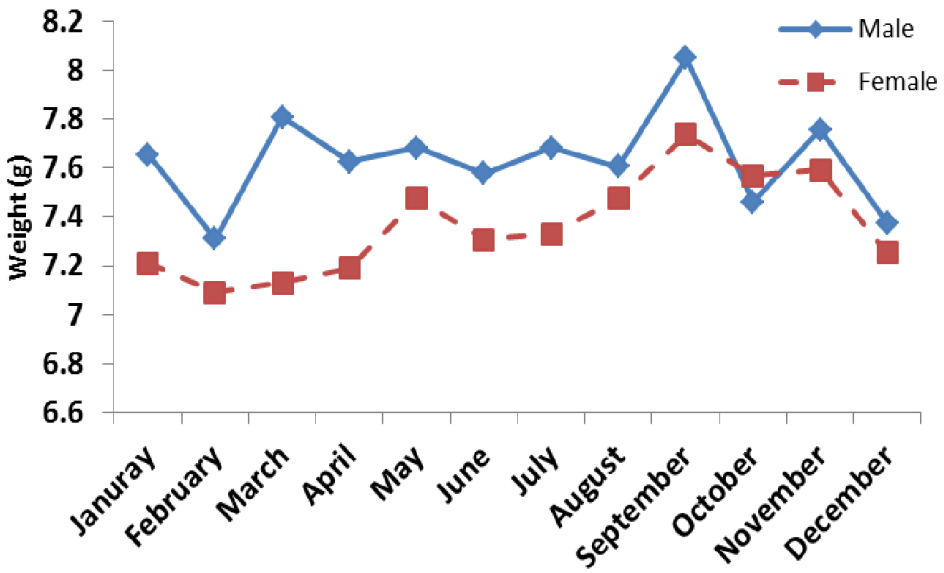
Table 5. Weights of PPM founders by population upon capture and at the end of each month through the end of 2012.

		Weight (g)*						
		Capture	July	August	September	October	November	December
Dana Point								
	Male	6.9	7.8	8.3	8.0	8.0	8.0	7.7
	Female	6.2	7.5	8.2	8.1	7.9	7.9	7.6
Santa Margarita								
	Male	5.6	7.4	7.1	6.5	6.7	6.8	7.0
	Female	6.1	7.6	7.1	6.6	7.0	6.9	7.5
South San Mateo**								
	Male	6.9		7.2	6.8	6.5	7.4	8.0
	Female	6.4		8.9	7.5	6.5	8.2	7.4

* Data represent weights at the end of each month

** Data from this population represents weights from only 1 male and 1 female

Figure 8. Average Monthly Weight of Male and Female PPM 2013



Estrus Cycling

Methods

Reproductive condition was assessed for all females every 1-3 days during non-estrous periods. See general methods for a description of the estrous scale.

Results and Discussion

Of the 10 females that were taken from the wild, only 1 (Female 5) exhibited estrus cycling during the period from July 20, 2012 to January 21, 2013. Female 5 was in peak estrus on July 25, 2012 while in quarantine. This was her only estrous cycle in captivity in 2012. All female PPM founders (10 out of 10) brought into captivity in 2012 came into estrus at least once during 2013. Two of the three adult females brought into captivity in 2013 came into estrus during the active period. However, none of the captive born females came into a full estrus (minimum of a 3 on a 5 point scale and perforate) during the season in which they were born.

Females cycled between January 22nd and September 17, 2013 and averaged 4.53 cycles during that period (range = 1-19). Cycle length averaged 15.31 days.

Mate Choice Tests

There are three extant populations remaining and we chose to begin the PPM captive propagation program with 10 founders from each population. Two approaches to the captive population management were possible: 1. manage the populations as separate Evolutionary Significant Unit (ESU's); or 2. manage a single captive population by interbreeding founders from all three extant populations. Given the genetic data and the historic range of the species, we elected the latter approach. The genetic data suggests that the extant populations were likely once contiguous. Thus, although there is some evidence that the three PPM populations have become genetically differentiated (Swei et al. 2003; S. Thomas, unpublished data), it is assumed that this is the result of recent isolation due to human activities rather than evidence of adaptive evolution.

We conducted mate choice tests to provide further evidence to confirm our decision to manage a single intermixed captive population. If animals from the 3 extant populations readily interbreed, this will provide support for cross breeding. If, however, females showed strong preferences for males from their own population compared to males from one of the other populations, this would indicate that we would need to manage all 3 populations separately.

Methods

Females (n=9) showing signs of estrous condition (i.e. minimum estrous swelling of 3-5 on a 5-point scale and perforate) were paired with one male from the female's population (SAME) and one male from another population (OTHER). To remove the effects of familiarity, at the beginning of the breeding season, we moved animals home cage locations to ensure that each female had both a (SAME) and (OTHER) male neighbor. The order of presentation of SAME vs. OTHER males was counterbalanced to eliminate order effects.

Introduction of pairs occurred in our standard introduction arena (30 x 50 x 100cm) and were 10 minutes in duration. The floor was covered with 5 cm of sand. One-half cup of sand was taken from the female and male's enclosures and spread across the top of the "new" sand to minimize stress for individuals and reinforce familiarity. The top 1 cm of sand was removed and replaced with new sand between mate pairings. A burrow consisting of a 12 cm piece of 2.5 cm PVC pipe joined to a T-section was located at each end of the aquarium. All introductions were staged at least 1 hour after dusk and recorded via video camera for later transcription. Red light bulbs illuminated the aquarium from above.

If any aggressive behavior (e.g. biting or another other harmful contact) was observed, the animals were separated and the trial ended. If behaviors that precede mating were displayed (i.e. female on her side with a leg in the air, or male mounts female), the animals were separated to avoid mating. At the end of the trial, we returned both animals to their home cages. All trials were videotaped and transcribed using Jwatcher software. The following ethogram was used to quantify behavior (Table 6).

Table 6.

Behavior		Description
Affliative	Nose to Nose	2 animals oriented with noses together within 1 cm of each other
	Sandbathing	rubbing side or ventrum against the sand
	Circling	2 animals move rapidly in a tight circle allowing oral genital contact
	Follow/Hesitate	animal 1 repeatedly approaches animal 2 and animal 2 pauses, shortening the distance b/w them
	Lay on Side	female lays on side with leg in air
Aggressive	Chase/Flee	rapid movement toward/away from other animal
	Jump/Avoid	animal jumps upwards and back while facing the other animal
	Lunge Threat	animal makes a sudden movement towards the other
	Fight	sparring, biting, or locked attack

Results and Discussion

Female PPM showed no preference for males from their own population when directly compared with males from a different population. They spent the same amount of time exhibiting affiliative behaviors (sandbathing, hesitating following an approach, nose to nose contact, circling, and laying on their side: independent t-test: $t = -0.519$; $p = 0.611$) and aggressive behavior (fight, jump avoid, lunge threat, chase: independent t-test: $t = -1.223$; $p = 0.239$). These results provide further support for the decision to manage the captive population as a

single interbred population. Thus, after consultation with Will Miller, FWS, we proceeded with our first matings in May of 2013.

Mate Pairings, Copulations, Pregnancies, and Litters

We paired females with males during peak estrus (defined above). Our genetics team examined the genotypes of the founders and provided a list of individuals not to breed. We initially selected a male that came from a different population from the female in estrus. In addition, we used several other characteristics to select males; for example: 1. Familiarity to female, 2. Degree of affiliation in previous encounters, 3. Degree of testes distention, 4. Size relative to female, 5. Mating experience. In some cases, females mated with the first male selected. However, in other cases, females had to be paired several times before allowing a male to mount. In several cases, females never mated during an estrous cycle despite being paired with as many as 4 or 5 males.

In 2013, there were 14 copulations that resulted in 5 confirmed pregnancies. In the first half of 2014, there were 13 copulations that resulted in 7 pregnancies.

Successful males exhibit high frequency of sandbathing, approach the female, but are not aggressive. Precopulatory behaviors include: scent deposition (sandbathing and digging) by both male and female, approach/hesitation, and circling. Females lie on their side and lift their leg and copulation begins. Matings consist of multiple intromissions and ultimately the male ejaculates and begins self-grooming. Female behavior changes immediately following ejaculation to that of aggression in the form of chasing and light sparring upon contact. Not all females that mate become pregnant, even if a copulatory plug is present following intromission. Because in the wild females likely mate with multiple males and sperm competition may be at play, we decided to begin to determine if females would mate multiply in our captive facility and if so determine if mating with multiple males increases the percentage of females that become pregnant and which male(s) sire(s) the offspring. To date we have mated 2 females with multiple males. We have learned that some females are willing to mate multiply.

In an effort to increase captive reproductive success, in 2014, we modified our mate pairing protocol to provide females with an opportunity to select a mate. Prior to a pairing, we placed a female into a central compartment in the introduction arena. We then placed a scrotal male into a compartment on either side of her with a clear perforated barrier between the female and each male. We then allowed the female to explore the cage for 15 minutes. If the female spent significantly more time next to one male compared to the other, she “selected” that male and was subsequently paired with him. To continue to improve captive reproductive success, we will begin to explore if hormone treatment is safe and effective in rodents and could be implemented with PPM.

To date, successful copulations produced 41 PPM pups. These copulations have involved 5 female and 5 male founders. In addition, in 2014, 5 female and 4 male from our F1 generation have mated and produced F2 offspring. 6 of the 7 litters (85.7%) in 2014 have been produced by F1 animals. Table 7 shows the breeding contribution by population.

Table 7. Breeding Contribution by Population.

Date of	Female	Population	Male(s)	Population Origin	No. of pups
5/18/2013	13	Santa Margarita	25	South San Mateo	2 (1 dead)
6/11/2013	22	South San	21	Santa Margarita	4
6/16/2013	18	Santa Margarita	1	Dana Point	2
7/12/2013	19	Santa Margarita	23	South San Mateo	4
8/8/2013	14	Santa Margarita	4	Dana Point	6 (1 dead)
3/8/2014	29	Captivity	40	Captivity	4 (1dead)
3/20/14	32	Captivity	44	Captivity	5 (5 dead)
4/3/14	39	Captivity	41	Captivity	4 (3 dead)
4/13/14	34	Captivity	46	Captivity	4
4/30/14	33	Captivity	44	Captivity	4
5/15/14	13	Santa Margarita	25	South San Mateo	2
5/20/14	29	Captivity	40	Captivity	Currently pregnant

Litters

Forty-one pups resulted from the 12 successful pregnancies. Pup survival rate was 73.1%. Litter size ranged from 2-6 and averaged 3.72. Eleven of 41 pups died prior to or at weaning. Nine of these pups were born to inexperienced captive born mothers. Pup loss is common among first time mothers, thus we expect the percentage of pups lost to decrease with maternal experience. A detailed explanation of pup loss is found under the incidental death section below. The current sex ratio of pups produced in captivity is 9 females: 15 males and 17 of unknown sex.

ANTIPREDATOR EXPERIMENTS

Numerous studies have shown that once a program has established sufficient breeding, the challenge is to successfully release captive-born offspring to the wild. Perhaps most problematic is the fact that captive environments often fail to provide the experiences necessary to ensure survival upon release of captive born young into native habitat (Beck 1995; Beck et al. 1994). Numerous studies have shown that captive-born animals have a higher mortality rate than wild-caught animals after release in the wild (Beck et al. 1994; Ginsberg 1994; Griffith et al. 1989; Miller et al. 1994a). The survival skills of wild-caught individuals may also erode while in captivity (Yoerg and Shier 1997). In several species, the increased mortality has been linked to ineffective antipredator behavior (Biggins et al. 1999; Fischer and Lindenmayer 2000; Frantzen et al. 2001; Wallace 1994; Yoerg and Shier 2000).

Perhaps surprisingly, predator recognition in many animals depends upon experience (reviewed by Griffin et al. 2000). Anti-predator behavior often must be functional when a predator is first encountered, but animals can improve their responses with experience (Shriner 1995). A substantial empirical literature demonstrates that animals that initially show no recognition of fear can be conditioned to respond to live and model predators (Griffin et al. 2000). The type (habituation, social learning or facilitation etc.) and specificity (how specific to the target predators is the enhanced response?) of learning in nature will elucidate the factors that affect the development of survival skills, and therefore play important roles in the development of training protocols.

To date, predator training research has been conducted across several taxa [(fish; Brown and Leland 2003), (birds; Maloney and McLean 1995; McLean et al. 1999), (mammals; Griffin and Evans 2003; Griffin et al. 2001; McLean et al. 2000; Miller 1990; Miller et al. 1994b; Mineka and Cook 1988)] and recent research indicates that these training programs can be effective in terms of long term post-release survival (Shier and Owings 2006, 2007). In rodents, predator training research has shown that the social environment may play a critical role in predator training protocols. For prairie dogs, pairing alarm vocalizations with predator exposure can enhance training (Shier and Owings 2006) and juveniles trained in the presence of experienced adult demonstrators were more wary with predators than control juveniles (Shier and Owings 2007). Perhaps most interesting and pertinent to this research was a study conducted on Heermann's kangaroo rat. Yoerg and Shier showed that despite being solitary, young kangaroo rats learn antipredator behavior from their mothers (Yoerg and Shier 1997). Young kangaroo rat pups shadow their mothers in the presence of a snake, but not in tests without the snake present (Yoerg and Shier 1997).

Nothing is known about the antipredator behavior of pocket mice in the wild. We are in the process of conducting a series of experiments to determine how pocket mice avoid predators, if their antipredator behavior varies by predator type, and what kind of experiences, if any, are necessary for juveniles to develop effective antipredator skills.

To begin, we set out to learn about PPM antipredator behavior in the presence of owls.

Antipredator behavior of wild-caught PPM in the presence of Owls

Methods

Subjects were all 22 captive PPM. Each individual mouse was tested in 3 treatments: 1. Owl (mounted owl swooped down over testing area on pulley system); 2. PVC (control treatment in which a PVC pipe approximating the size of the Owl model is swooped down over the testing arena); and 3 Control (sham control in which the mouse is placed in the arena, but nothing is swooped down over the arena). For the owl model we used a taxidermically mounted Barn Owl (*Tyto alba*) with its wings out and talons extended during all tests. All tests occurred in a testing arena. The clear acrylic testing arena (48 x 24 x 12 in) rested on the floor of the room and was filled with 5 cm of sand. The test arena was divided into four equal-sized quadrants delineated on the side of the cage for the observers (Quadrant 4 nearest the Owl; Quadrant 1 was furthest away.). A small spring of Buckwheat (*Eriogonum fasciculatum)* was placed in the center of each quadrant to provide cover. A pulley system was mounted to the walls and ceiling of the testing room such that the owl or PVC pipe control could "sit" high in the room against a wall and a curtain drawn in order that the test mouse could not view the stimulus when placed into the arena. The room was illuminated from the ceiling with a single 100watt red light bulb. A video camera was mounted on the ceiling to record all tests for later transcription in JWatcher. The behaviors documented are listed in Table 8.

Table 8. Ethogram of Behaviors in Anti-predator Experiment

Behavior	Description
Digging	movement of sand either with fore or hind feet
Sand Bathing	rubbing side or ventrum against the sand
Grooming	scratching with fore or hind feet and or licking their fur
Running	rapid movement
Walking	slowly moving; not running
Foraging	collection of seeds into cheek pouches and/or caches and/or eating
Freeze/Still	does not move, frozen in one spot
Scanning/ Looking	head up (eye shine visible) moving head around with all four feet on ground
Rearing up/ Standing	stretching up on rear feet and looking around
Climbing	crawling up buckwheat vegetation within enclosure
Out of Sight	cannot see animal or distinguish the behavior

Procedure

Prior to a test, the owl or PVC pipe was pulled up into ready position above the arena and the curtain closed. A PPM was then carried from its home cage to the testing room in its nest jar and placed in the center of the testing arena. An observer stood quietly against the wall to manipulate the curtain and pulley system. Animals were given 5 minutes to acclimate to the testing arena. The test was 7.5 minutes divided into three 2.5 minute periods (pre-stimulus control during which the stimulus was behind a curtain; sit, during which the curtain was removed to reveal the stationary PVC or owl and swoop, during which the stimulus (PVC or owl) was released to swoop down over the top of the focal subject). At the end of the test, the PPM was removed from the test arena in its nest jar and returned to its home cage. Before another mouse was tested, the sand in the arena was thoroughly sifted and mixed and any feces or urine was removed. A cup of clean sand and 1 teaspoon (per quadrant) of seeds were sprinkled on top before each test. No animal was tested in different treatments on one night.

Control tests were identical to the Owl or PVC tests except that no stimulus was present.

The treatment order was counterbalanced to avoid order effects. Tests were conducted between dusk and 11 pm. Females were anestrous during tests.

Data Analysis

Three composite variables were used for data analysis. 1. vigilance = freeze/still, scanning/looking, rearing up/standing; 2. escape = running/climbing; 3. maintenance = digging, sandbathing, grooming and foraging.

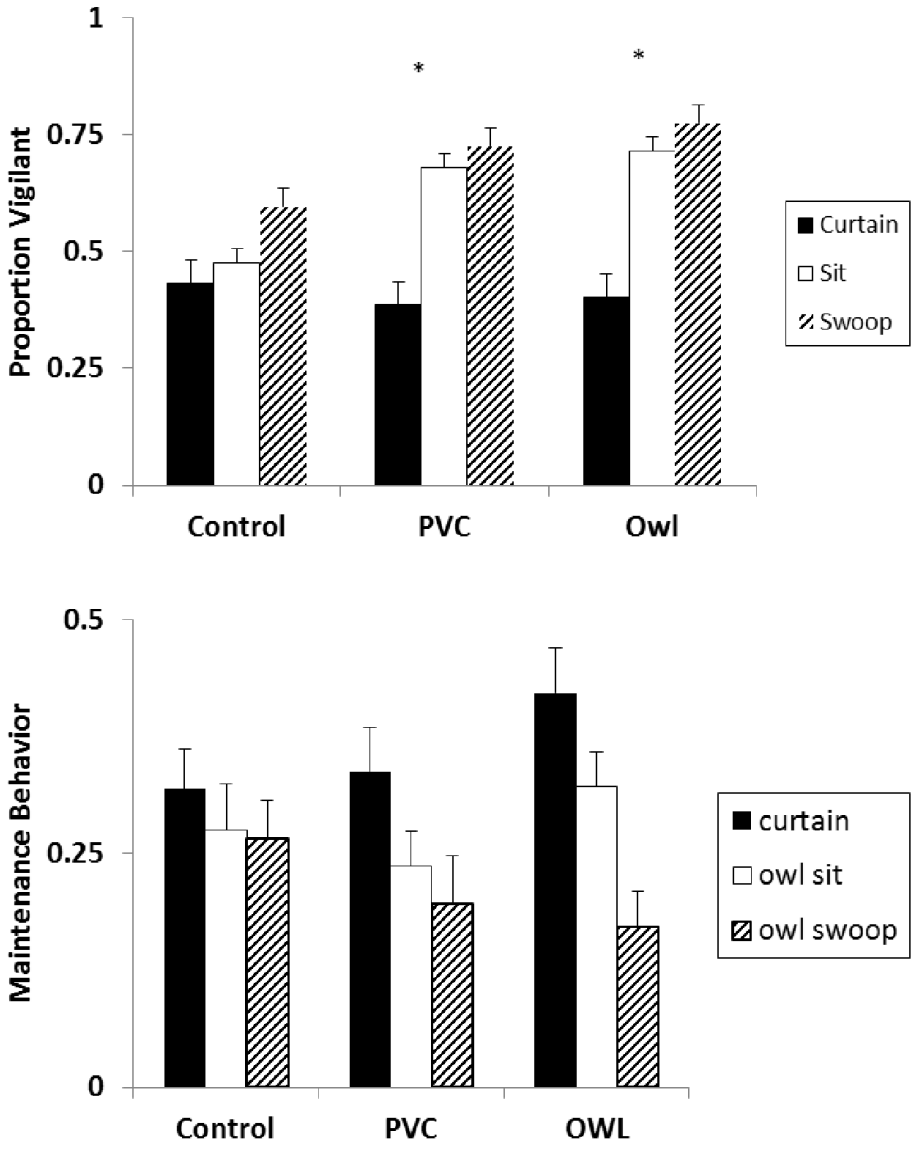
For each variable, I computed the change in behavior from pre-stimulus to stimulus swoop periods (swoop minus pre-stimulus) and used General Linear Modeling (GLM) to assess treatment effects. I then conducted post-hoc tests to determine the effect of visual and motion cues on each treatment separately. I conducted all data analyses in SPSS version 20.

Results and Discussion

Not surprisingly, wild-caught PPM behaved strongly to the presence of an overhead stimulus. Wild-caught PPM exhibited significantly different behavior across treatments (vigilance: $F = 7.544$, $p = 0.007$; escape: $F = 4.007$, $p = 0.019$; maintenance = $F = 4.114$, $p = 0.020$; Figure 9). From pre-stimulus to the swoop period, PPM exposed to the two looming stimuli (PVC and owl) increased vigilance ($F = 10.997$, $p = 0.001$) and decreased maintenance behaviors ($F = 32.878$, $p < 0.001$). There was no change in escape behaviors for PPM in PVC and Owl treatments from pre-stimuli to swoop periods ($F = 1.357$, $p = 0.248$). These results indicate that PPM exhibit vigilance and escape behaviors in the presence of a looming stimulus. Looming stimuli have been shown to elicit overhead fright responses in several taxa (Giles 1984; Maier et al. 2004; Shier and Owings 2007).

Overall, there was no difference in the vigilance or escape behavior of wild-caught PPM between the PVC stimulus and the Owl stimulus trials (vigilance: $F = 1.262$, $p = 0.266$; escape: $F = 2.868$; $p = 0.96$), suggesting that visual cues are not important for antipredator behavior in PPM. To determine if the data supported this hypothesis, I examined the periods in which the PVC and owl stimuli were stationary (sit) and swooping (swoop) separately. There were no significant differences in the vigilance behavior of PPM due to the treatment [vigilance (sit period: $F = 0.192$, $p = 0.663$; swoop period: $F = 0.735$, $p = 0.395$); escape (sit period: $F = 0.835$, $p = 0.365$; swoop period: $F = 0.202$, $p = 0.655$)]. These data provide further evidence that PPM are attending to the motion of the stimulus rather than visual cues in the presence of an aerial threat. Given the nocturnal activity of PPM, visual cues may be less important than motion for predation detection. These results provide the first demonstration of antipredator behavior in wild-caught PPM and can be used as a baseline to which to compare captive born PPM behavior in order to determine if predator training is required and to establish levels for assessing behavioral competency. As these tests are conducted on captive-born animals and they become adults, we will be able to determine if captive-born PPM exhibit similar antipredator behavior as that of wild-caught adults.

Figure 9. Behavior of wild-caught PPM across period and treatment



Antipredator behavior of wild-caught PPM in the presence of Snakes

Methods

Subjects were all 22 captive PPM. Each individual mouse was tested in 3 treatments: 1) Snake (presentation of king snake, *Lampropeltis getula californiae,* into the stimulus compartment of the arena; 2) Stick (presentation of stick approximately the same size and shape of the snake when elongated into the stimulus compartment of the arena; and 3) Empty (empty compartment control). The stick condition served to as a control used examine the effect of a visual stimulus similar approximating the size and shape of the snake. The same king snake was used during all snake tests. All tests occurred in the same testing arena as described previously except that the pulley system was not used during snake trials. Instead, a clear perforated barrier was placed between the stimulus compartment and the mouse compartments which allowed for visual and olfactory cues between the snake and focal mouse. In addition, we placed an artificial burrow (6inch long PVC tube) into each quadrant for cover.

Procedure

Prior to a test, we sifted the sand in the arena and sprinkled 1 cup of clean sand on top of the existing sand. We placed the snake or stick into the stimulus compartment and slid a black opaque cardboard between the stimulus compartment and the mouse quadrants. In the snake trials, we allowed the snake to acclimate to the arena for 10 minutes prior to introducing the mouse. A PPM was then carried from its home cage to the testing room in its nest jar and placed in the center of the testing arena for a 5 minute acclimation period. The test was 10 minutes in duration. After the first 5 minutes (pre-visual stimulus), we removed the black cardboard to expose the stimulus compartment (visual stimulus). At the end of the test, the PPM was removed from the test arena in its nest jar and returned to its home cage. Before another mouse was tested, the sand in the arena was thoroughly sifted and mixed and any feces or urine was removed. A cup of clean sand and 1 teaspoon of seeds (per quadrant) was sprinkled on top before each test.

Control tests were identical to the Snake or Stick tests except that no stimulus was present.

Each focal subject was tested in a single treatment each night and all 3 tests were completed over the course of 3 nights. Treatment order was counterbalanced to avoid order effects. Tests were conducted between dusk and 11 pm. Females were anestrous during tests.

Data Analysis

Three composite variables were used for data analysis. 1. vigilance = freeze/still, scanning/looking, rearing up/standing; 2. shelter = in burrow, under cover; 3. maintenance = digging, sandbathing, grooming and foraging.

I initially examined the main effects of treatment on total time (s) allocated to: vigilance, shelter and maintenance behaviors.

For each composite variable, I then computed the change in behavior from pre to post-stimulus periods and used General Linear Modeling (GLM) to assess treatment effects. I then conducted post-hoc tests to determine the effect of visual and scent cues on each treatment separately. I conducted all data analyses in SPSS version 20 and STATA12.

Results and Discussion

Wild-caught PPM allocated a different amount of time to vigilance behavior across the three treatments (vigilance: $F = 4.796$, $p = 0.012$). However, there was no main effect of treatment on shelter seeking or maintenance behavior. Stimulus treatment was a reliable predictor of the change in behavior when exposure to different stimuli across testing periods. Overall, wild-caught PPM increase vigilance ($t = 2.01$, $p = 0.05$; Figure 10a) and shelter seeking behavior ($t = 2.29$, $p = 0.026$; Figure 10b) from pre-visual stimulus period to the visual stimulus period, indicating heightened vigilance response as the test proceeded. However, the increase in vigilance and shelter seeking behavior from pre-visual stimulus to visual stimulus periods was greater during stimulus trials (snake and stick) compared to control trials. By contrast, wild-caught PPM show a decrease in maintenance behaviors (foraging, grooming, etc. described above) only during snake tests (snake: $t = 3.188$, $p = 0.005$; stick: $t = -0.103$, $p = 0.991$; control: $t = -1.651$, $p = 0.114$; Figure 10c). Taken together, these data indicate that PPM respond to snake predators by reducing maintenance behaviors, and increasing vigilance and shelter seeking behavior (e.g. freezing). These changes of behavior from pre-visual stimulus to the visual stimulus period are due to the visual cues of the stimuli presented. The stick approximated the size and shape of the snake and thus it is not surprising that PPM seek shelter in response to the presence of the stick. However, it is important to note that wild-caught PPM allocated significantly more time to vigilance and less to maintenance behavior in the presence of the snake compared to the stick. Thus, these data indicate that PPM use some combination of visual and/or motion cues specific to the snake for detection and behavioral modification in the presence of a snake predator.

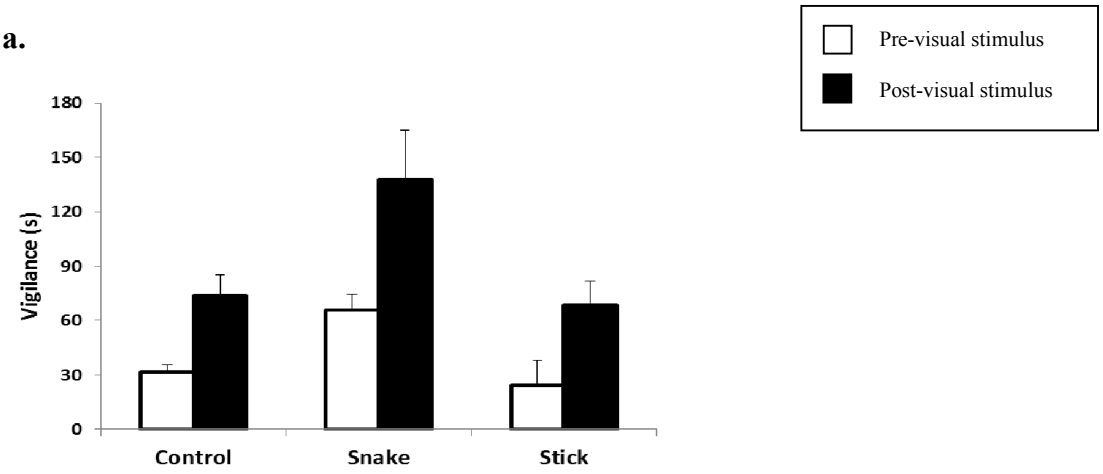
Differences across treatments seen during pre-visual stimulus periods indicating that PPM use also use scent cues to detect snake predators ($F = 3.584$; $p = 0.034$). During the pre-visual stimulus testing period, there was an opaque barrier placed between the stimulus compartment and the mouse compartment of the testing arena. Thus, any differences in mouse behavior during this period, was due to scent cues. Therefore wild-caught PPM use multimodal cues to detect snake predators and exhibit anti-predator behavior.

From these experiments we are beginning to develop and understanding of wild PPM survival skills. This information will be used as a competency goal for captive born PPM. The next step is to examine the antipredator skills of captive born PPM. During Phase I, we conduct antipredator tests (n=16) with both Owls and Snakes at two time points: weaning and the spring after they were born (~ 7 months of age). These tests will continue as we wean additional pups and they become adults. During Phase II, once we have 20-25 pups that have been tested both at weaning and as adults, we will analyze these data. Results from these experiments will: 1.

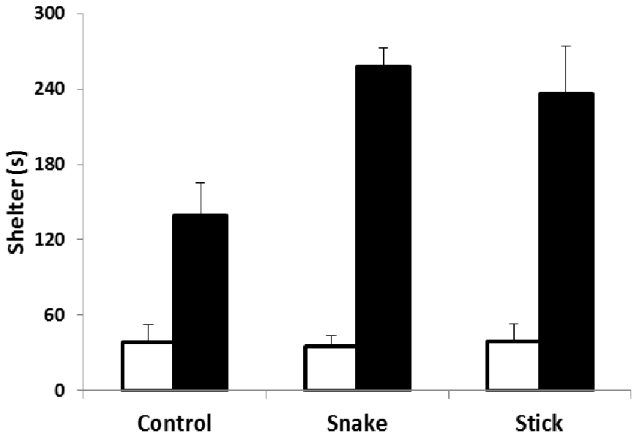
indicate if captive born young exhibit similar antipredator skills to wild-caught adults, 2. elucidate the role of development in that process and 3. allow us to determine if captive born PPM require antipredator training to development effective antipredator behavior prior to release.

Figure 10. Behavior of wild-caught PPM across period (pre-visual to visual) and treatment (control, stick and snake)

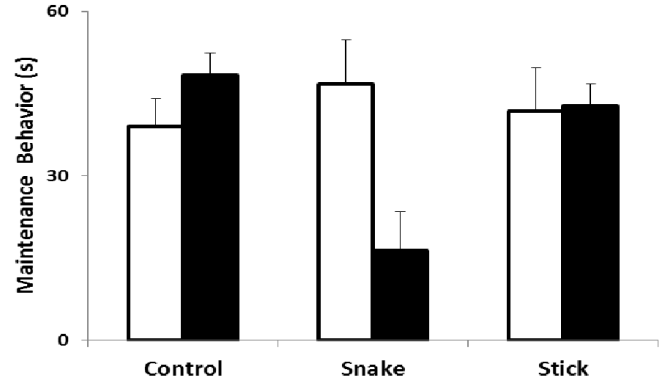
a.



b.



c.



INTERSPECIFIC COMPETITION EXPERIMENTS

Studies of interspecific competition in intact ecosystems offer valuable insights into how species achieve stable coexistence (e.g. Ziv and Kotler 2003). Competitive relationships provide an avenue for understanding interactions with heterospecifics, and while theory predicts that population persistence is more likely when competition is low (MacArthur & Wilson 1967), competitive relationships are rarely considered when planning species reintroductions. Success rates of captive breeding and reintroduction programs have increased slowly since early reintroduction programs, with greater attention now being paid to habitat type, food availability, dispersal, and predation risk (Fischer and Lindenmayer 2000; Seddon et al. 2007). Predation has been a major cause of reintroduction failure; although species normally coexist with predators, temporarily excluding predators from release sites can improve the odds of establishing stable populations (Moseby et al. 2012). Just as temporarily excluding predators can improve the success of reintroduction programs, so might the exclusion or targeted density reduction of a key competitor.

To understand direct interactions, we staged dyadic encounters between PPM and three sympatric species. There is a long precedent of experimentally determining dominance relationships within a community of small mammals through the use of staged dyadic encounters in an arena (reviewed in Harper & Batzli 1997). These paired encounters can be used to understand dominance if trials are conducted in a neutral arena, or site-specific dominance (i.e. territoriality) by conducting trials within and subsequently outside of an individual's territory (Wolff et al. 1983; Stokes et al. 2012).

To date, dominance relationships in the small rodent community in the coastal region of Southern California have been examined only by (Meserve 1972; Meserve 1976b, c). In these studies, PPM were tested in a neutral laboratory setting against each of 8 other rodent species (*Neotoma fuscipes, N. lepida, Dipodomys agilis, Peromyscus californicus, P. eremicus, P. maniculatus, Perognathus fallax, Rethrodontomys megalotis*). Meserve found an almost linear relationship between species size and the outcome of staged encounters. His results indicate that PPM, the smallest species in the community, and *Reithrodontomys megalotis* (REME), the closest in body size to PPM, are behaviorally subordinate to all other species but mutually intolerant of one another (exhibiting equal aggression or avoidance).

While Meserve's research provides evidence that PPM are subordinate to or avoid all other species in a neutral captive setting, its application is limited here. There is evidence that some animals behave differently in captivity than in the wild (Meserve 1972; Wolff et al. 1983); Wolff et al. 1983) and wild PPM have been observed to chase larger competitors from their territory (D. Shier, pers. obs.). This may be due, in part, to defense of resources in the wild. Wolff and colleagues (1983) paired two species of *Peromyscus* in the field to determine whether coexistence was mediated by interspecific territoriality. They found an individual was more often dominant in its own home range but subordinate in another animal's home range, regardless of species. They determined that dominance was *site-specific and not species-specific*.

To investigate indirect interactions we began to study cache pilfering in the community. Caching (storing) seeds that are collected while foraging is a tactic employed by many species of heteromyids (Leaver and Daly 2001; Murray et al. 2006; Price et al. 2000). While PPM utilize both scatter hoards (shallowly buried seeds near a burrow) and larder hoards (a stockpile inside of the burrow) (R. Chock, pers. obs. from both field and laboratory), it is likely that larder hoards are essential to survive their estivation period during winter when resources are scarce (Kenagy 1973). Caches are susceptible to cache pilferage, or the removal of food items by an individual other than the cacher. Studies in small mammal communities have found that pilfering of both natural caches (Clarke and Kramer 1994; Daly et al. 1992; Leaver and Daly 2001) and artificial caches (Pyare and Longland 2000; Stapanian and Smith 1978; Vander Wall 2000) occurs at a rate of 2-30% per day (reviewed in Vander Wall & Jenkins 2003), suggesting that the entire cache could be depleted within one to several weeks.

Cache pilfering is a form of competition that could have severe negative consequences for PPM if they are pilfered from, or could have a positive impact on their survival if they are able to pilfer from other caching species in the community. In a study of heteromyids of varying sizes, it was suggested that smaller species pilfer from the caches of larger, more efficient foragers. This dynamic, indirect competition can lead to a stable coexistence (Leaver and Daly 2001). A reverse relationship, where the caches of small estivating species are pilfered by larger opportunistic species, could lead to competitive exclusion.

Trapping

We trapped in the Oscar One training area of the Santa Margarita PPM population on Marine Corps Base Camp Pendleton from May 15, 2013 to August 23, 2013 (Table 9/Figure 11). There were 2-3 people in the field team and we opened no more than 200 traps each night. On all sites we placed flags in high quality PPM habitat (sandy areas with open vegetation) and placed 2 traps at each flag. We used 9-inch Sherman traps with shortened doors to prevent tail severance. We baited traps with white millet that was cooked in a microwave for 1 minute to prevent germination. Traps were set just before sunset (18:30-20:00) and checked at 22:00 and 02:00 each night to allow time to make behavioral observations and release animals before dawn. If we found a trap with ants inside or within 6 inches of it, we closed that trap for the night. We took a GPS location at the trap of the initial capture of a PPM. We captured a total of 40 individual PPM over 73 nights of trapping.

Figure 11. Satellite map showing trapping sites in Oscar One of the Santa Margarita population on Marine Corps Base Camp Pendleton.

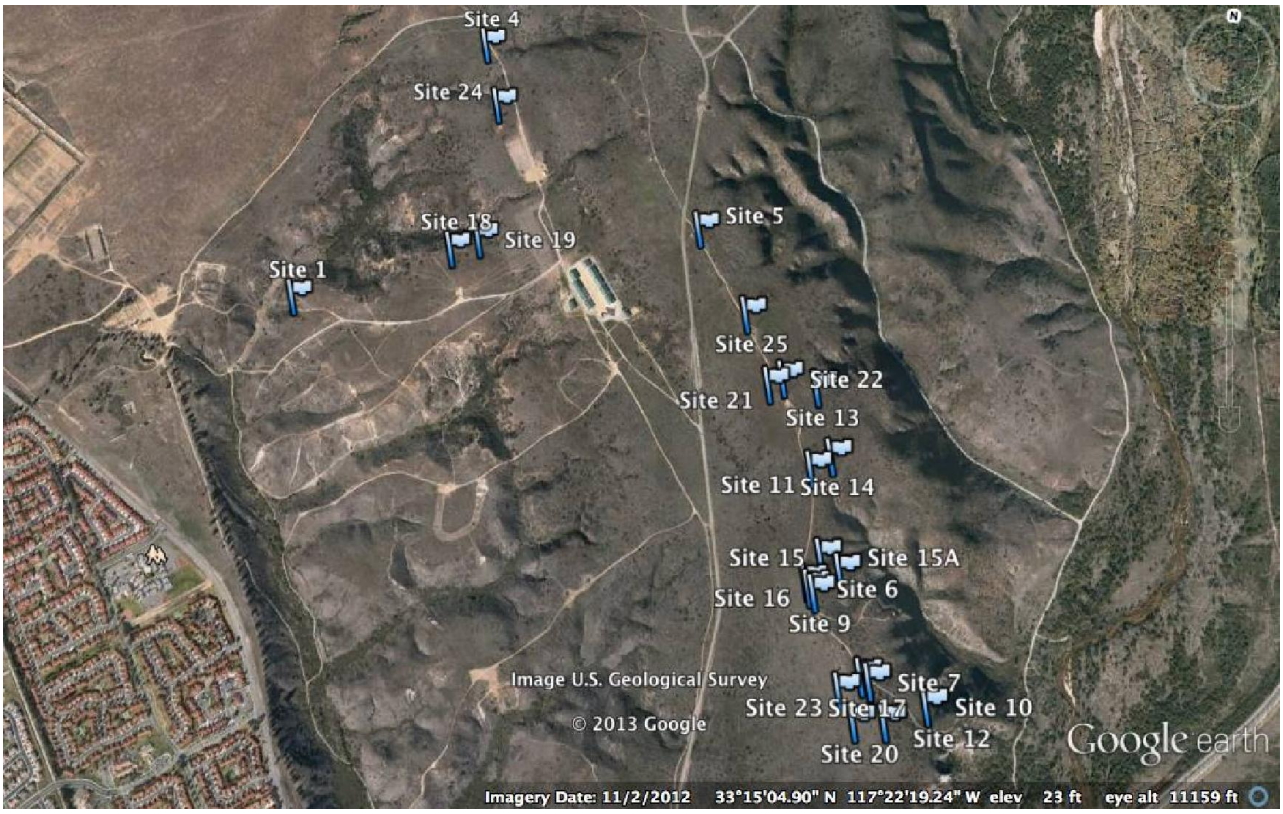


Table 9 Unique PPM captures in Oscar One in the Santa Margarita population on Marine Corps Base Camp Pendleton.

Date	Site	Flag	Weight	Sex	ReproCond	GPS Position	History	VIE	Notes
May 15, 2013	7	3	5.5	F	NNV	N33 15.168 W117 23.091	New	999	VIE: right red, red, red
May 16, 2013	6	15	5	F	JV	N33 15.318 W117 23.234	New	777	VIE: right yellow, yellow, yellow
May 16, 2013	7	5	5	M	JV	N33 15.149 W117 23.087	New	977	VIE: right red, yellow, yellow. White on flanks
May 19, 2013	7	10	5.4	F	VN	N33 15.146 W117 23.110	New	779	VIE: right yellow, yellow, red
May 20, 2013	7	13	6	M	NS	N33 15.168 W117 23.090	New	979	VIE: right red, yellow, red. Yellow mark small
May 20, 2013	7	7	6.6	M	S	N33 15.143 W117 23.096	New	69-	sharpie belly; weight cannot be right
May 20, 2013	7	14	6.3	F	VN	N33 15.173 W117 23.098	New		green/orange
May 21, 2013	7	6	7.1	F	VN	N33 15.146 W117 23.094	New	666	VIE: right blue, blue, blue
May 22, 2013	9	6	4.6	M	JV	N33 15.287 W117 23.179	New	966	VIE: red, blue, blue
May 22, 2013	7	5	5.7	M	JV	N33 15.151 W117 23.087	New		sharpie belly
May 22, 2013	7	3	6	M	JV	N33 15.168 W117 23.090	New		sharpie belly; missing tip of tail
May 27, 2013	6	11	7	F	VN	N33 15.337 W117 23.262	New		pink
May 27, 2013	9	5	4.3	M	JV	N33 15.284 W117 23.188	New		sharpie
May 28, 2013	12	5		F	VN	N33 15.096 W117 23.052	New		green
May 28, 2013	6	2	6.9	M	S	N33 15.303 W117 23.237	New		orange sharpie
May 28, 2013	9	5	7.4	F	VN	N33 15.284 W117 23.188	New		purple
May 29, 2013	4	7	5.9	M	JV	N33 16.152 W117 23.975	New		orange/purple sharpie; baby coat
May 29, 2013	7	10	5.4	M	JV	N33 15.150 W117 23.108	New		pink/orange; lots of anal mites
May 30, 2013	12	3	5.7	M	PS	N33 15.108 W117 23.056	New		green/purple
May 30, 2013	7	7	7.2	M	PS	N33 15.143 W117 23.094	New		pink/green
May 30, 2013	7	14	6	M	NS	N33 15.173 W117 23.100	New		pink/purple
June 4, 2013	12	9	7	M	PS	N33 15.089 W117 23.051	New	42	black/orange
June 4, 2013	6	9	5.1	M	JV	N33 15.318 W117 23.262	New	0-1,55-	Left:pink,-,blue; Right:pink,pink,-(marked by USGS)
June 4, 2013	7	7	4.6	M	JV	N33 15.143 W117 23.095	New		black/pink
June 5, 2013	12	12	4.8	F	JV	N33 15.094 W117 23.032	New		black/green
June 5, 2013	6	12	7.2	M	S	N33 15.339 W117 23.258	New		black/purple
June 6, 2013	12	13	4.4	F	JV	N33 15.096 W117 23.026	New		blue
June 6, 2013	6	2	6.1	F	VN	N33 15.305 W117 23.255	New		green/blue
June 6, 2013	9	1	5.5	M	JV	N33 15.292 W117 23.203	New		pink/blue
June 10, 2013	6	15	5.8	F	VN	N33 15.318 W117 23.235	New		blue/orange
June 13, 2013	12	5	5.1	F	JV	N33 15.102 W117 23.056	New		blue/purple; female? Hard to tell
June 14, 2013	12	4	6.5	F	VN	N33 15.103 W117 23.059	New		blue/black; dark coat
June 27, 2013	6	28	4.4	F	JV	N33 15.271 W117 23.209	New		blue/blue
July 7, 2013	12	11	6.1	F	VN	N33 15.089 W117 23.036	New		pink/pink molting adult to adult coat
July 7, 2013	7	25	6	F	VN	N33 15.115 W117 23.060	New		purple/purple (full cheeks) scraped nose
July 8, 2013	7	7	6.3	M	NS	N33 15.144 W117 23.095	New		green/green
July 8, 2013	12	10	3.7	M	JV	N33 15.093 W117 23.044	New		orange/orange; full cheeks
July 17, 2013	18	21	6.9	M	S	N33 15.806 W117 23.923	New		pink/black stubby tail
August 5, 2013	20	7	6.6	F	NNV	N33 15.109 W117 23.116	New		blue/green
August 9, 2013	23	5	6.6	M	NS	N33 15.158 W117 23.172	New		purple/orange

Methods for Paired Interactions

To test dominance and territoriality in the field, we staged 5-minute dyadic encounters between PPM and three putative competitors: *Chaetodipus californicus,* (CHCA); *Peromyscus maniculatus* (PEMA); *Reithrodontomys megalotus* (REME). We conducted 10 PPM/PEMA trials, 4 PPM/REME trials, and 2 PPM/CHCA trials. Trials were between adults of the same sex and no individual was tested more than once. All animals were tagged for identification. Unique ID, species, sex, weight, and trap location was recorded. Adult males and anestrous but sexually mature females were used in dyadic encounters. For each pairing one species was randomly assigned to be the resident, and the encounter was staged by the trap station where the resident animal was captured.

We used a clear Plexiglas open-bottomed, rectangular interaction arena (24" x 24" x 24") in the field. Animals were on natural substrate and able to see their surroundings. A removable cardboard partition split the arena into two sides, and a PPM and an individual of one of the competitor species was placed on separate sides to acclimate (indicated by commencement of foraging) before the trial began. At the start of the trial, the barrier was removed and the two individuals observed for 5 minutes. In the event of a locked battle, animals would be separated to prevent injury and the trial will be terminated. *No injuries were sustained or locked battles occurred in our 16 trials with PPM.* Trials were recorded with an infrared camera and transcribed into JWatcher, an event logging software used to code behaviors and interactions for analysis.

Frequencies of agonistic behaviors (Table 10) were calculated for each species pair. Mann-Whitney U tests were used to look for differences in number of dominant and submissive behaviors exhibited between species. We examined territoriality for each species pairing by comparing a species' behavior as a resident to when it was an intruder. Persistence was measured by comparing number of approaches in each species pair.

Table 10. Ethogram of behavior for interspecific interactions.

Behavior	Definition
Approach/Leave	Animal moves towards/away from other animal
Displace	One animal approaches and the other leaves
Fight	Sparring, biting, or locked battle
Jump/Avoid	Animal jumps upwards and back while facing other animal
Chase/Flee	Rapidly moving towards/away from other animal
Sandbathe	Rubbing side or ventrum against sand

Cache Pilfering

We shook 5g of hulled millet with 0.5g non-toxic fluorescent pigment (ECO Pigment series, DayGlo Color Corp, Cleveland, OH; approved by FWS for use with heteromyids) that passes through an animals' digestive tract and is visible in feces (Stapp et al. 1994; Longland & Clements 1995). A minimum of a 1-week inter-test interval was observed for each individual used in both staged encounter and cache pilfering trials.

To provision an individual with seeds we opened traps in its home range. Once the individual was trapped, we checked and emptied its cheek pouches of any previously collected seeds. We placed the mouse inside a clear Plexiglas open-bottomed, rectangular arena (24" x 24" x 24") with a tray of 5g of fluorescent seeds. The animal typically filled its cheek pouches and dug under the edge of the arena to return to its burrow. It was able to return to the seed tray by reentering under the edge of the arena, and could make as many trips back and forth as necessary. The arena detained the animal long enough for it to identify the seed tray as a food source, and in 8 out of 9 trials with PPM in the field, the individuals made multiple trips from the seed tray to

their burrow until the 5g of seeds was entirely depleted. In one trial the PPM pit cached 2.5g of seeds inside the arena, and after an hour was released from the arena and did not return to the seed tray.

After provisioning an animal with dyed seeds we trapped a 16-trap grid (4x4 traps, 4m spacing) for 5 consecutive nights and collected fecal samples from all trapped individuals of each species. Traps were wiped clean prior to opening each night and feces were collected from the trap. Feces were present in all traps at the first trap check. We examined all feces under a black light for traces of fluorescent pigment, which indicated cache pilfering.

Results and Discussion

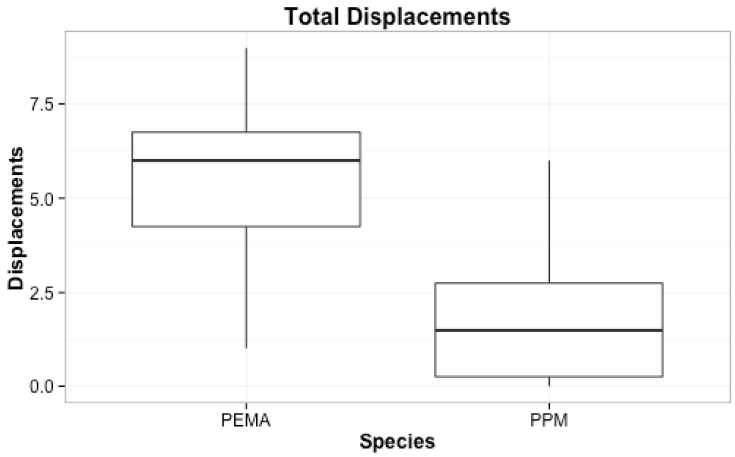
Paired Interactions

Our initial pairings with PPM and PEMA provide preliminary evidence for successful territoriality as a mechanism for PPM and PEMA coexistence. Overall displacement (approach by an individual causing the other to move away) of PPM by PEMA is more frequent than vice versa (n=10; p=0.002, Mann-Whitney U Test, Figure 12A). This supports previous findings that PEMA are behaviorally dominant to PPM in a neutral setting (Meserve 1976c). When PEMA residents are paired with PPM intruders, PEMA again displace PPM more often than they are displaced by PPM (n=5; p=0.008, Figure 12B). However, when PPM are residents there is no difference in displacements by PEMA or PPM (n=5; p=0.3, Figure 12C), suggesting that dominance may be site-specific. Additionally, only two attacks (lunging/jumping at opponent) occurred in the 10 trials, and both were initiated by PPM residents, resulting in the displacement of their PEMA opponent. PPM residents approached the opponent more often than PPM intruders did (p=0.02), which suggests that persistence may be more important than dominance in maintaining territorial ownership (Stamps & Krishnan 1995). PPM also spent more time foraging as residents than intruders (16% and 5% of the time, respectively, Figure 13). This indicates a lower missed-opportunity foraging cost in the presence of a competitor when PPM are in their own territory. While our sample size was too small for conclusive results, they suggest that territoriality may be an important mechanism for PPM coexistence with larger heterospecifics and requires further investigation.

Additionally, a few preliminary trials were conducted between PPM and CHCA and PPM and REME. Two trials between PPM and CHCA, both with PPM as the resident, included no instances of PPM displacing CHCA, though one CHCA displaced the PPM it was paired with 3 times in the 5 minutes. No aggressive interactions or attacks were observed. When PPM was paired with REME in four trials, resident PPM (n=3) displaced REME once, though multiple approaches by PPM were observed. REME displaced the resident PPM twice, with fewer total approaches. When PPM was the intruder (n=1) REME displaced PPM three times, PPM displaced REME once, and again more approaches by PPM were observed. There were no aggressive interactions or attacks by either species in the PPM vs. REME trials. While there are too few pairings of PPM with each of these species to truly begin to understand the relationships between these species pairs, our preliminary observations suggest there is little overt aggression between any of the species pairs, and further study will help us understand whether PPM interact with each species differently.

Figure 12. Displacements between PEMA and PPM

A.



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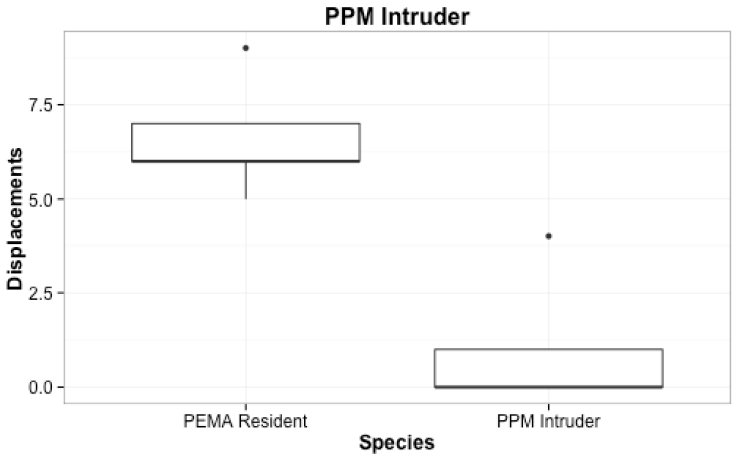


C.

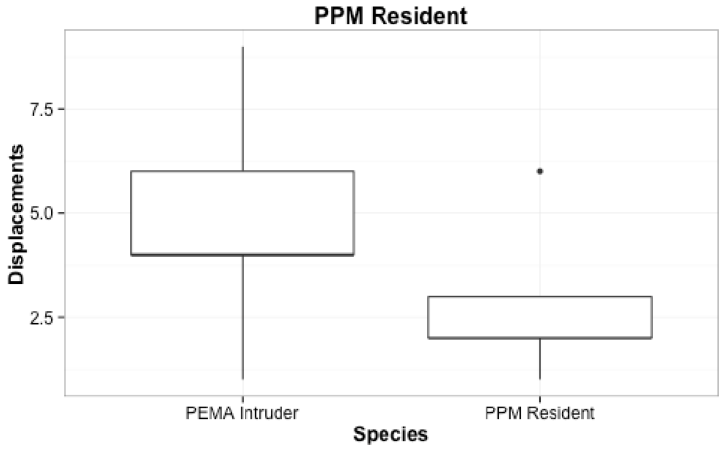
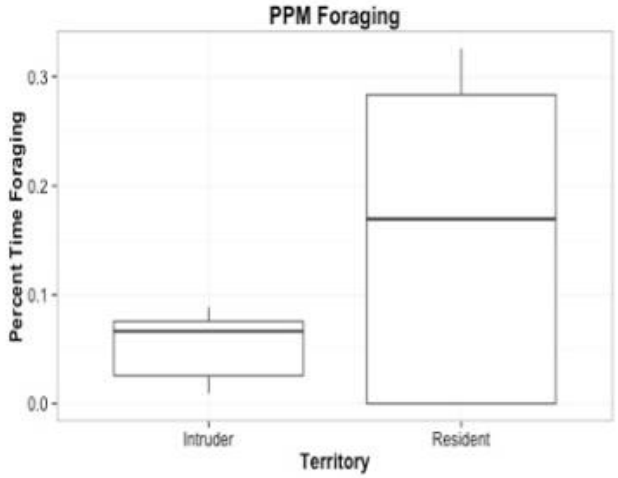


Figure 13. PPM Foraging comparison when resident vs intruder



Cache Pilfering

In 9 trials we found evidence of 2 PEMAs pilfering from PPM caches, while 15 PEMAs and 1 CHCA who were trapped in the area showed no sign of pilfering. While PEMA are capable of pilfering from PPM surface pit caches, initial results suggest that it is infrequent. Number of CHCA and REME were too low in the vicinity of the PPM captures to determine whether they pilfer seeds from PPM. As stated above, PPM spent more time on average foraging as residents than intruders (16% and 5% of the time, respectively). While this result is not significant ($p=0.6$, Mann-Whitney U Test) with the small sample size of $N=5$, it may indicate a lower missed-opportunity foraging cost in the presence of a competitor when PPM are in their own territory. We propose to further investigate the role of cache pilfering in the community and test the reciprocal relationship; whether PPM pilfer from other species. Studying cache pilfering will provide important information on which species have a negative indirect impact on PPM by pilfering their seeds, and which species positively influence PPM's seed acquisition when PPM is the pilferer.

FORAGING EXPERIMENTS

The foraging behavior of PPM has been little studied. Meserve (1976) found PPM showed diet specialization on grass and forb seeds. Relatively little utilization of shrubs was documented with the exception of one occasionally important shrub, California Buckwheat (*Erigonium fasciculatum*; Meserve 1976a; Meserve 1976b). However, the study was limited in several ways: 1. the results are based on preferences of only 2 to 5 individuals; 2. the study was conducted in the dry season when most flowers and fruits of perennials shrubs were not available; and 3. PPM did not complete excretion of lab provided foods resulting in discarded early samples. Because preferred food items are likely to be eaten first, the current data on diet preferences need to be interpreted with caution.

We are in the process of conducting a series of experiments on foraging in PPM. We examined foraging decisions under predation pressure in Phase I of the project and plan to examine diet preferences and foraging efficiency in the next phase of the project. We will examine whether these behaviors vary across founder population. Our goal with these experiments is to develop an understanding of the species foraging behavior, cues and/or food types that are required for captive born young to develop effective foraging skills prior to release.

Foraging under Predation Pressure

Predators are important to a prey population in more than one respect. Clearly they exert a direct effect by catching and subsequently killing the prey, but predators may also indirectly affect prey by changing their activity pattern (Banks et al. 1999; Eilam et al. 1999; Hendrie et al. 1998), foraging behavior, growth rate (Gotthard 2000), and reproduction (Desy et al. 1990; Herman and Valone 2000; Lima and Dill 1990). In order to balance food quality/quantity and predation risk, several prey species indeed show the ability to modify their use of a feeding patch when subjected to changes in predation pressure (Brown 1988; Brown et al. 1988; Holmes 1984; Kotler and Brown 2004). Such modifications could be seen as: 1. changes in the amount of food intake due to less time spent and/or reduced foraging effort at a given "risky" patch; and/or 2. tendency to forage in covered feeding patches even though food may be less abundant or of poorer quality than in open patches and/or 3) changes in harvest rates and apprehension or vigilance (Brown et al. 1988; Kotler 1992).

We will use Giving Up Density (GUD) as a measure of foraging under predation pressure. The GUD estimates the quitting harvest rate (Brown 1988). The assumption with the GUD is that if there was no perceived threat, the animal would forage until just before the density of food is so low that it would take as much energy to search as it would gain from the food. If there is a perceived threat, then the animal will forage in the patch less and there will be more food left over. If the foraging patches follow a gradient from most impacted to least impacted the threat should be reflected in the GUD of the patches.

Methods

We tested 20 wild-caught adult PPM in each of 3 treatments: Snake, Owl and Control (taxidermic mount of a mouse). The mouse control is used to simulate a nonpredatory heterospecific and thus control for the effect of predator pressure during the predator trials. All trials were conducted in the introduction arena located in Room A of the captive breeding facility. The arena was divided into 3 quadrants and all sand in the arena sifted prior to the onset of the experiment and between each trial. Quadrant 1 was the stimulus presentation (SP) compartment for the Snake and Control tests and was left empty during Owl tests. A barrier was placed between quadrants 1 and 2. We sprinkled ½ cup sand scented with the focal subject's own scent on top of quadrants 2 and 3. Trials were 15 minutes in duration. We placed a buckwheat "bush" (cluster of sprigs of native buckwheat) along the wall in quadrants 2 and 3. We then placed a tray with 1.00g of millet seed each under the buckwheat and in the open in each quadrant (on alternating sides in the two compartments). We counterbalanced the order of treatments to prevent order effects. For the Snake treatment, we transferred our kingsnake into the SP quadrant of the testing arena. For the Owl treatment, we placed our mounted Barn Owl on the cable looming in a stationary position located over the (SP) quadrant of the testing arena. In the control treatment, the mouse control stimulus was placed 4 inches from the barrier in the SP quadrant. We then removed a focal subject from their home cage in their PVC tunnel and placed them into the testing arena in quadrant 3 with their PVC tunnel. The test began when the observer started the video camera. After the 15 minute trial, we returned the focal individual to its home cage. We then removed each seed tray and placed the seeds into separate bags. All trials were conducted at least 30 minutes after dark. At the end of the experiment, all bags of seeds were counted and weighed.

Results and Discussion

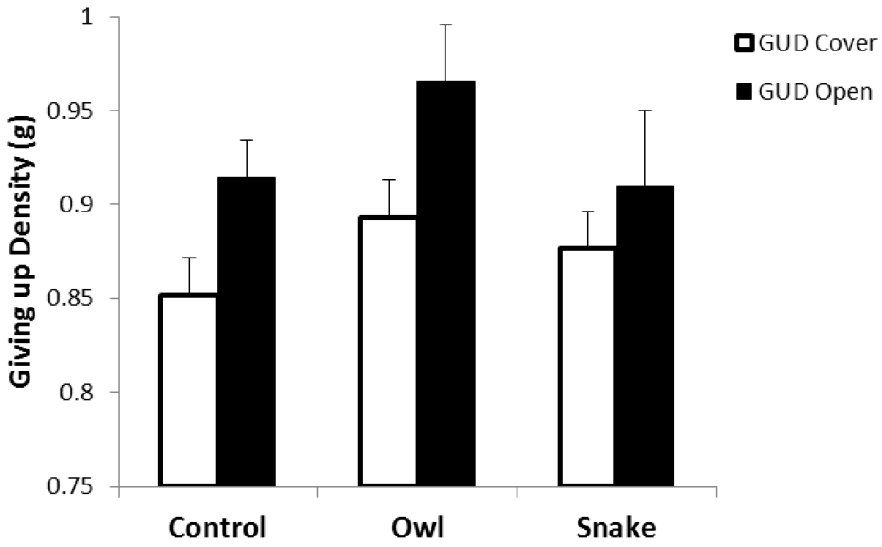
Results from this experiment indicate that wild-caught PPM forage more under cover than in the open ($t = -2.562$, $p = 0.013$). PPM leave significantly more seeds in microhabitat patches in the open compared to under cover of a bush (Figure 14). Males foraged more than females, taking more seeds from trays overall ($F = 5.627$, $p = 0.021$). There were no main effects of predator treatment ($F = 0.983$, $p = 0.381$), indicating that the GUD of foraging patches was unaffected by the predator stimulus present. Thus, PPM appear to have a single strategy of foraging under the protection of cover regardless of predator presence.

A second line of evidence indicates PPM foraging preferences. The first trays that PPM chose to visit were the farthest distance from both heterospecific stimuli (predator and non-predator) and were significantly more likely than expected by chance to be under cover ($X^2 = 17.15$, $p < 0.001$). These data provide support for the notion that behaviorally, PPM avoid interaction with heterospecifics and minimize risk by foraging under cover.

Taken together, wild PPM from the 3 populations foraged at similar rates. However, there are significant effects of population origin for males ($F = 4.733$, $P = 0.016$). Males founders from Dana Point foraged significantly more than male founders from the other 2 populations. These

are the first data to elucidate PPM foraging strategy. Future studies will examine diet preferences of wild-caught PPM.

Figure 14. PPM Giving Up Density in the Open vs. Under Cover



GENETICS

In this conservation breeding project, we had the rare opportunity to assess genetic relatedness of founders at the outset. This information can be used to direct breeding efforts. In particular, it has allowed us to determine which mate pairings would results in maximum genetic diversity of the captive population in order maintain allelic diversity.

Previous efforts by the ICR Genetics division from 2008-2012 focused on establishing a microsatellite library to characterize the genetic diversity in wild populations of PPM. Earlier analyses have shown that the extant populations in the wild represent three distinct genetic clusters (Dana Point, SSM, and SM).

The goal for genetic analysis was to provide information on the genetic diversity of the captive population relative to the wild populations, and to use this information to make breeding recommendations.

Methods

Sampling

We collected genetic samples from all 26 PPM founders and from 17 of the 18 offspring produced in captivity. To do this, we used standard ear snip protocol (Alexander and Riddle 2005; Loew et al. 2005; Metcalf et al. 2001; Waser et al. 2006). Ear snips can be as small as a pencil point and provide ample genetic data for analysis of parentage, genetic relationships and dispersal (Waser et al. 2006). We obtained ear snips by sterilizing scissors with 70% ethanol, holding the scissors on a tangent from the edge of the pinna, and snipping a sliver (~0.5mm) off the edge of the pinna. Tissue samples were then transferred to and stored in a vial with 95% ethanol. Scissors were sterilized between animals.

Due to their small size, entire ear snips were used for DNA extraction, resulting in sufficient yields for genetic analysis. The DNAs were genotyped at 19 PPM-specific microsatellite markers which were previously developed by the Genetics Division. These data were used in several analyses, summarized below.

DNA extraction, polymerase chain reaction, and microsatellites

DNA was extracted from ear snips using the QIAamp DNA Mini Kit (Qiagen Inc.) following the manufacturer's protocol. The extractions resulted in sufficient yields for genetic analysis. DNA samples were stored at -20 °C post-extraction. Along with DNA profiling for individual identification, we banked tissues and extracted DNA from all mortalities.

In 2013, an attempt was made to multiplex the polymerase chain reaction (PCR) in order to conserve resources. The 19 PPM-specific microsatellite markers, which were previously developed by the ICR Genetics Division, were pooled into seven different multiplexes and successfully amplified using the QIAGEN Multiplex PCR Kit (Qiagen Inc.). PCR amplicons

were separated using capillary electrophoresis on an ABI 3130 genetic analyzer (Applied Biosystems). Alleles were scored relative to an internal size standard (500 ROX) using GeneMapper 3.0 (Applied Biosystems). These data were used in several analyses, summarized below.

Basic summary statistics

The computer program Cervus 3.0.3 (Kalinowski et al. 2007) was used to calculate the number of alleles per locus and observed heterozygosity (summarized in Table 11). The sample sizes were too small to evaluate statistically significant deviations from Hardy-Weinberg equilibrium or genotypic disequilibrium (though previous analyses of data from wild PPM samples showed no evidence for either). High levels of heterozygosity were observed in the captive born population, representing higher genetic variation than any of the founder populations.

Table 11. Summary statistics for microsatellite data among the founder populations and captive born PPM.

Population	# Individuals	Alleles/locus	Observed Heterozygosity
Santa Margarita	10	6.12	0.63
South San Mateo	5	2.47	0.40
Dana Point	11	2.05	0.34
Captive born	17	5.68	0.74

Population genetics

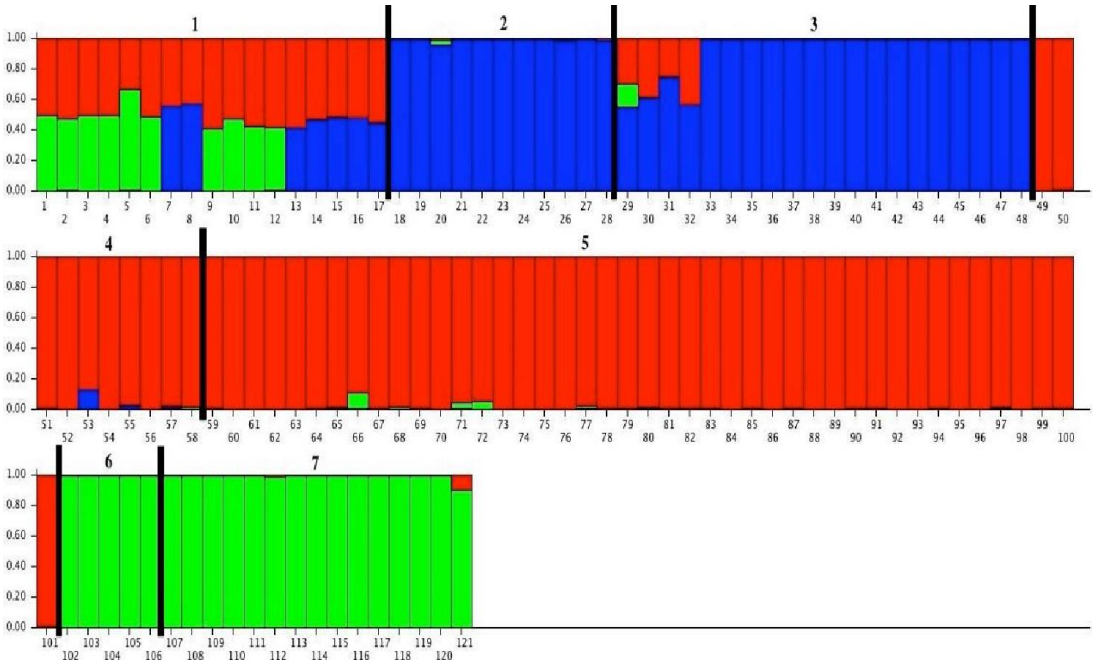
The computer program Genepop 4.2 (Rousset 2008) was used to perform basic population genetic analyses. For all founder populations, the genetic diversity captured among the founders is comparable to what had been surveyed in previous years with larger sample sizes (diversity estimates are summarized in Table 12). The captive born population has high levels of genetic diversity within individuals and among individuals within samples compared to the wild and founder populations from Dana Point, SM, and SSM.

Table 12. Diversity estimates (heterozygosity) within individuals ('1-Qintra'), and among individuals within samples ('1-Qinter') averaged over all loci/population.

Sample	*N*	1-Qintra	1-Qinter
Dana Point (wild)	20	0.363	0.388
Dana Point (founders)	11	0.345	0.311
Santa Margarita (wild)	43	0.677	0.756
Santa Margarita (founders)	10	0.626	0.747
South San Mateo (wild)	15	0.495	0.497
South San Mateo (founders)	5	0.400	0.438
Captive born	17	0.737	0.686

To evaluate hierarchical population genetic structure, a Bayesian clustering analysis was performed using the computer program Structure 2.3.2 (Pritchard et al. 2000). This analysis is used to objectively determine the number of genetic clusters that exist among a set of individuals using their multilocus genotype data, without any preconceived notions regarding possible population structure. Previous analyses have shown the extant populations represent three distinct genetic clusters. Including the founders in the analysis shows that the founders group predictably with the genetic cluster from which they were sampled (Figure 15). The F_1 offspring of the captive population also assigned predictably to populations of its founder parents. Individuals 1-6 came from two separate litters but had one parent from SSM and another from SM. Another litter of individuals, 7 and 8, are offspring of PPM founders from Dana Point and SM. Individuals 9-12 came from a litter from founders from SSM and SM, and individuals 13-17 came from a litter from founders from Dana Point and SM.

Figure 15. Structure bar plot showing the proportion of each individual's genetic information (y-axis) that assigns to each of three genetic clusters (represented by the three colors). 1=Captive born, 2=Dana Point Founders, 3=Dana Point Wild, 4=Santa Margarita Founders, 5=Santa Margarita Wild, 6=South San Mateo Founders, 7=South San Mateo Wild



Relatedness

To aid in the captive breeding efforts, relatedness analyses were performed to determine if any pairs of individuals should not be mated together. The computer program Coancestry 1.0 (Wang 2011) was used to estimate relatedness using three different estimators. Simulated genotypes across four relationship categories (parent-offspring, full sibling, half sibling, and unrelated) were generated using the allele frequencies among wild individuals (including wild founders) for each population. Relatedness estimates were bootstrapped and 95% confidence intervals were calculated. These relatedness confidence intervals were then compared to empirical relatedness values, calculated in the computer program SPAGeDi (Hardy and Vekemans 2002), for each possible pairing within each founder population, to identify pairs that should not be mated.

The following conclusions were made for breeding:

- For the SM population there was no pair of individuals that exhibited relatively high relatedness. They can, for the most part, be considered equally related.
- For the Dana Point population there were two pairs that exhibited relatively high relatedness: 4x5 and 5x8. This population has lower allelic variation, which results in less power when trying to determine who might be closely related. However, dyad 4x5 appears to have a relatedness value at the level of full-sibling to parent-offspring, and

dyad 5x8 appears to have a relatedness value at the level of half-sibling to parent-offspring. Recommendations have been made to avoid mating these pairs.

- For the SSM population, there were two pairs that exhibited relatively high relatedness: 22x23 and 26x27. Both dyads have a relatedness value that falls within the 95% confidence interval for all four relatedness categories estimated. Recommendations were made to avoid mating these pairs.

STUDBOOK PEDIGREE

The studbook pedigree indicates the current captive-born portion of the population is descended from 10 founders with 15 potential founders still remaining (Figure 16/Table 13). The gene diversity of the captive-born portion of the population is 92.38%, which is equivalent to that found in approximately 6 or 7 unrelated animals (FGE = 6.56).

Figure 16. Graph illustrating the distribution of founder representations in the captive-born portion of the PPM population.

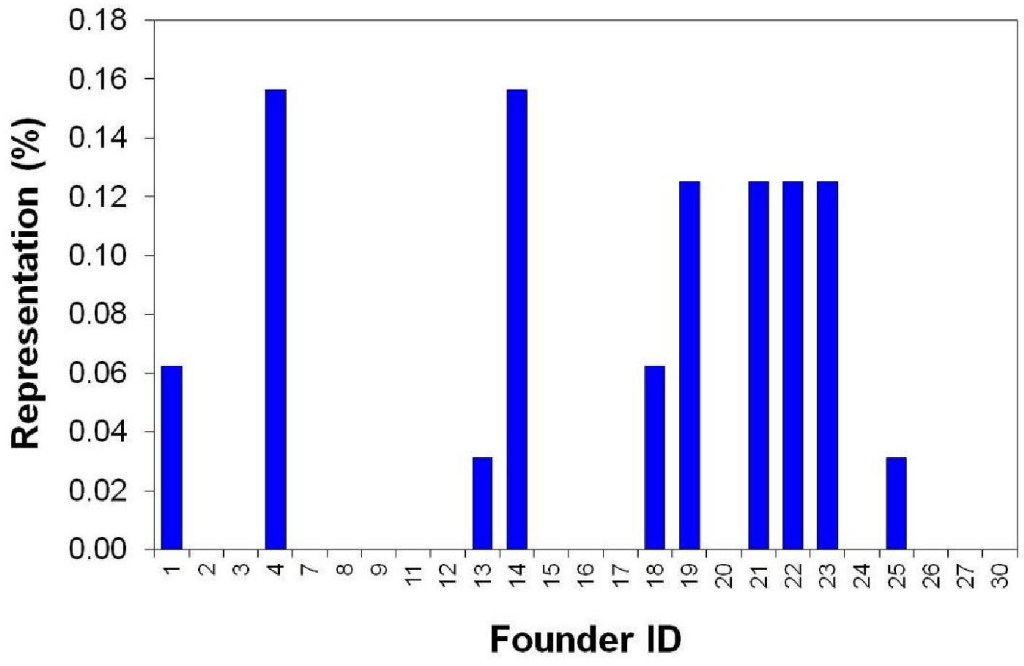


Table 13. Captive Population Genetics Summary from Studbook – definitions of terms are included in Appendix B

	2014	Current Potential
Number of Founders	10	15 additional
Founder Genome Equivalents (FGE)	6.56	25.00
Gene Diversity Retained (%)	92.38	98.00
Population Mean Kinship	0.0762	-----
Mean Inbreeding (F)	0.0000	-----
% Known Pedigree	100	-----
% Certain Pedigree	100	-----

These data combined the with Studbook Pedigree information were used to decide on breeding priorities for 2014 (see Appendix C for breeding recommendations for 2014).

ENDOCRINOLOGY

Much stress physiology research, particularly in non-human animals, has focused on refining species-specific standards for measuring the activation of the hypothalmic-pituitary-adrenal cortex (HPA) axis via glucocorticoid (GC) production (Moberg 2000). Traditionally, adrenal cortex activity has been measured through analysis of GC concentrations in plasma, but blood sampling has drawbacks (Harper and Austad, 2000; Keay et al., 2006; Lane, 2006), including the increase in GCs that the sampling procedure itself may cause (Cook et al., 2000).

An alternative approach is to analyze GCs and their metabolites in feces (Touma et al. 2004). In this case, the collection method is non-invasive and hence less likely to affect GC production, and the timing of sample retrieval is more flexible than other methods (which is useful when direct access to animals is limited or not possible). In addition, fecal GC (FGC) levels represent an aggregate of GCs and their metabolites over a period of time (Keay et al., 2006).

Therefore, FGC levels can be useful in understanding how persistent stressors can affect animals and their welfare. This use extends to assessing stress in animals that are part of conservation programs as these typically involve some combination of stressors, such as captivity, marking, monitoring, transport and handling, in addition to environmental and social disturbance. Mitigating stress responses to these procedures may ultimately be important for the success of conservation efforts (Dickens et al. 2010; Teixeira et al. 2007). As a result, conservation researchers have begun to investigate the effect on FGC levels of common conservation practices (e.g., trapping, Harper and Austad, 2001; transport, Millspaugh et al., 2007; radio transmitters, Pereira et al., 2009; Wells et al., 2003; captivity, Rothschild et al., 2008).

Many wildlife conservation projects in recent years involve the translocation of free-ranging animals. However, translocation as a practice has been historically associated with a high animal mortality rate (Griffith et al., 1989). As typical translocations involve at least all of the stressors aforementioned, a few studies have examined FGC levels in response to translocation events (Dickens et al., 2009a, 2009b; Franceschini, 2008; Pinter-Wollman, 2009; Viljoen et al., 2008). While FGCs and their metabolites have been examined in a growing number of species of conservation concern, the biological relevance of the technique has been validated in only a small percentage of the species (Touma and Palme 2005). Because of the high variability in GC metabolism and excretion within and across species, validation steps are crucial to reliably assess adrenocortical activity for a given species (Hunt et al., 2004).

Methods

Fecal Sample collection

We began by collecting fecal samples from all individuals at multiple time points to store for future analysis. We collected fecal samples from all 22 captive PPM that were captured from Dana Point, SM and SSM in 2012 at: 1) capture in the wild, 2) 6-8 weeks in captivity, 3) 3 months in captivity and 4 months in captivity, 4) 6 months in captivity and 5) 1 year in captivity.

In 2013, we analyzed the samples from 1 and 2 above. The samples from 3-5 will be analyzed during summer of 2014. Here I compare the fecal corticosterone of females and male founders from the 3 extant populations.

Assessment of diurnal activity

A first step in developing a methodology to assess whether fecal corticoids will be useful to examine stress in PPM is to examine the diurnal variation in fecal corticoids. In fall of 2013, we collected fecal samples from 10 wild-caught PPM (5 males; 5 females) over a 48 hour period--every 2 hours during the night and every 4 hours during the day. The day before collection began, we sifted the cages of all PPM to remove any fecal pellets. We collected fecal pellets from each subject's cage and placed them into an individually labeled vial. Vials were immediately placed into a standard freezer and transferred to a -80 freezer at the end of the collection period.

Antibody characterization by high performance liquid chromatography (HPLC)

Preliminary analyses by radioimmunoassay (RIA) indicated that PPM fecal extracts contain analytes that are reactive with cortisol and corticosterone antibodies (unpubl. data). This is consistent with previous findings in Merriam's kangaroo rat (*Dipodomys merriami*), in which significant concentrations of both GCs were measured in plasma (Preston, 2001). In an effort to characterize and validate an antibody for the measurement of GC concentrations in PPM feces, we separated pooled fecal extracts by reversed phase HPLC followed by RIA of each fraction using three different antibodies: monoclonal mouse anti-cortisol (071210107; MP Biomedicals, Costa Mesa, CA), polyclonal rabbit anti-corticosterone (07120113; MP Biomedicals), and polyclonal rabbit anti-corticosterone (CMJ06; supplied by Coralie Munro; University of California, Davis). We lyophilized all fecal pellets from each subject. We then transferred the freeze-dried pellets to 12 x 75 mm borosilicate tubes and extracted them by shaking in 1 ml of 80% methanol for 30 minutes. The extracts from each individual were combined into a clean tube, dried down under vacuum, reconstituted in water, and then concentrated on C18 cartridges (WAT051910 Sep-Pak, Waters, Milford, MA) that had been conditioned with 10ml each of 100% methanol and then water. The samples were then washed with 10ml of water, eluted from the cartridges with 5 ml of 100% methanol, dried down under vacuum, and reconstituted in 0.2 ml of 100% methanol.

Following solid-phase extraction, we injected 20 µl of each fecal extract pool into a Beckman System Gold 3-piece unit (Programmable Solvent Modules 125/406 and Diode Array Detector Module 186, Beckman Coulter) and separated on a Nova Pak C18 column (WAT086344, 3.9 x 150mm, Waters) as previously described (Harris et al., 2012). Samples were separated along an acetonitrile gradient beginning with 2:98 (acetonitrile:water, v/v) and increasing to 75:25 over 75 minutes at a flow rate of 1 ml per min. Fractions were collected at 1 minute intervals, dried down, and reconstituted in phosphate buffered saline (PBS, pH 7.0). We prepared each of the 75 fractions in duplicate for measurement by RIA (as described in Fecal glucocorticoid assays below) with each of the antibodies. We compared the elution times of immunoreactive fractions to the elution times of several commercially available (Steraloids, Newport, RI) steroids (corticosterone, cortisol, testosterone, estrone sulfate, and progesterone) and steroid metabolites

(5β-androstane-3α-ol-11-17-dione and 5α-pregnane-3β,11β,21-triol-20-one) similarly prepared and detected by absorbance at 205 nm.

Fecal glucocorticoid assays

Fecal pellets were lyophilized and weighed to the nearest 0.001 g and transferred to 12 x 75 mm borosilicate tubes for extraction. To extract fecal GCs we shook lyophilized pellets in 1 ml of 80% methanol for 30 minutes. Following extraction, we transferred 0.9 ml of the extract to a clean tube, dried it down under vacuum, and reconstituted it in 0.5 ml of assay buffer. Cortisol standards were serially diluted in PBS from 0.16 – 20 ng/ml and prepared in duplicate for each assay, along with quality control standards of approximately 0.8 and 8.0 ng/ml. In order to reduce nonspecific binding, 400µl of 0.4% bovine serum albumin (Fraction V; Fisher Scientific, Pittsburgh, PA) in PBS was added to each tube. Finally, tritiated cortisol (1,2,6,7-^{3}H; Perkin Elmer, Waltham, MA; 10,000 cpm per 0.1ml) and cortisol antibody (1:3000 in 0.1 ml of PBS) were added, bringing the final assay volume to 0.70 ml. Each assay was incubated overnight at 4^{o}C, and bound-free separation was performed by adding 250ml of 5% charcoal/0.5% Dextran in PBS, incubating at 4^{o}C for 30 minutes, and centrifugating at 2000 x g for 15 minutes. The supernatant was combined with 3.5 ml of scintillation cocktail and counted on a Beckman LSC6500 scintillation counter. The concentrations of cortisol standards were plotted against the log-logit transformation of the % ^{3}H-CORT bound. Fecal glucocorticoid concentrations were expressed as ng/g fecal dry weight.

Results and Discussion

Transition to captivity

Overall there appeared to be a decrease in fecal corticoids after animals had time to acclimate to captivity. However, there was no significant difference in fecal corticoids collected during capture from the wild and those taken 6-8 weeks after founders were transferred to the PPM facility (ANOVA: $F = 1.941$, $p = 0.184$; Figure 17). There were no differences in fecal cortisol levels across population either at trapping ($F = 1.036$; $p = 0.337$) or 6-8 weeks in captivity ($F = 0.748$, $p = 0.402$) and while males show an almost significant reduction in fecal corticoids from capture to 6-8 week post captivity (paired t-test: $t = 2.142$; $p = 0.069$), females show no differences.

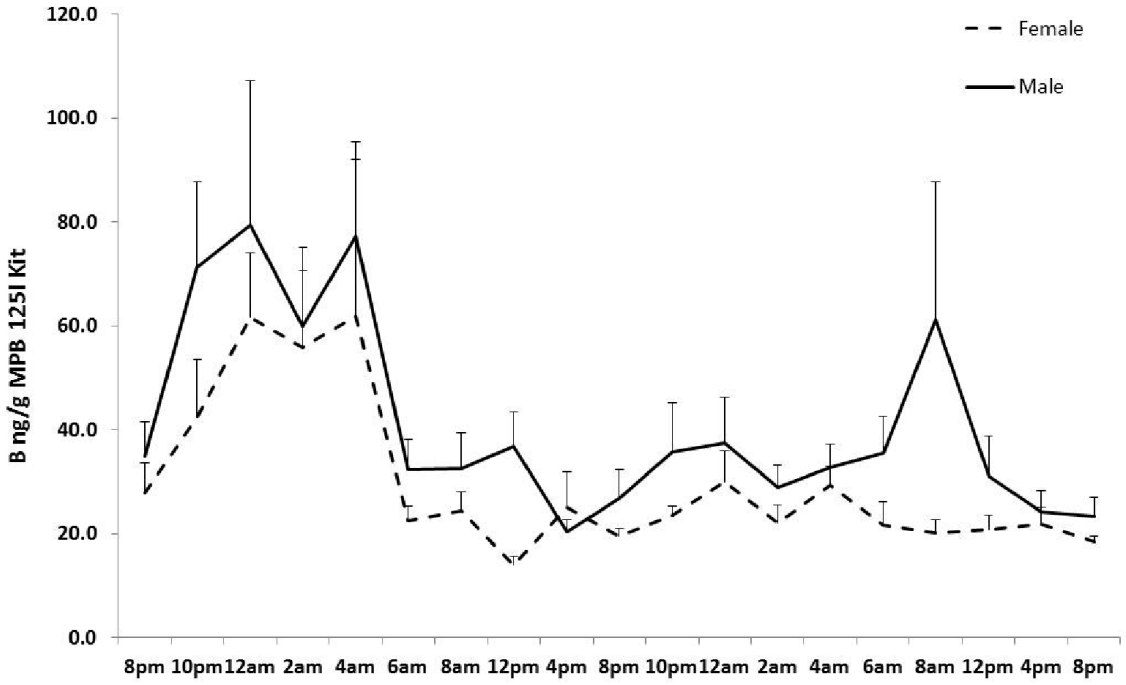
These results are extremely preliminary as the use of fecal corticoids to measure stress in PPM must first be validated. However, preliminary results are promising and indicate that PPM may have transitioned into captivity smoothly.

Diurnal activity

There is no clear pattern of diurnal activity of cortisol for male or female PPM (Figure 17). While it appears that there may have been a spike in cortisol during the first several timepoints, individual variation was high for males (as indicated by the standard errors shown). A validation of this method using a pharmacological challenge will enable us to interpret the data more clearly. We plan to conduct the validation test during Phase 2 of this project. This validation will allow us to develop a complete stress profile for PPM. Among other things, this will enable

us to determine the amount of time that it takes for cortisol to enter the feces and thus identify potential environmental stressors, including various experiences in captivity.

Figure 17. Diurnal cortisol profile for male and female PPM over a 48 hour period.



CONCLUSIONS

During Phase I we initiated captive breeding of the endangered Pacific Pocket Mouse. We set up a captive facility on exhibit at the San Diego Zoo Safari Park, conducted surveys and assessed the status of the 3 extant populations to ensure that our removal of PPM from the wild did not exceed 10% of adults or 20% of juveniles, and captured and transferred 30 founders to captivity.

Within the captive breeding facility, the health of all of the collected founders has been assessed and each individual has been genotyped. Mate choice tests have been performed with females that have come into estrus to verify the compatibility of males and females from different source populations. Behavioral tests to determine wild PPM survival skills and to establish behavioral competency measures for captive born and reared animals have been initiated. To date, anti-predator tests have been conducted with wild-caught founders and indicate that PPM utilizes its cryptic coloration and size paired with vigilance and avoidance as antipredator strategies. A foraging experiment was conducted to examine wild PPM foraging strategies. This experiment showed that PPM prefer to forage under cover similar to other pocket mice and at a distance from heterospecifics. Interspecific interactions have been assessed in the wild and caching trials have been conducted. Initial results suggest that dominance between PPM and its putative interspecific competitors may be site specific. Methods are being developed to use fecal corticoids to understand stress in this species. We have conducted an experiment to examine the diurnal cycle of glucocorticoids in PPM fecal samples which will facilitate interpretation of future experiments that examine the effects of stress. In addition, we have collected samples at several time points to assess PPM stress during transition to captivity. Current data indicate that stress levels at capture are similar to those after being in captivity for approximately two months. Because PPM like other heteromyids are desert adapted and gut processing time is extended, the amount of cortisol in fecal pellets at capture reflect the stress levels several hours prior to the capture event. Thus, the stress level at capture is a reliable baseline measure of wild PPM stress. This suggests that daily captive life is no more stressful for PPM than live in the wild. However, validation of this protocol with a known stressor is required before these results can be confirmed.

In June of 2013, breeding of animals was initiated and the successful pairing of individuals has proven that the breeding protocol is effective. To date, 27 pairings have resulted in 12 litters, producing 41 pups. Pup survival to date is 73.1%, thus our current captive population size is 58. We have established a studbook to track genetic relatedness of individuals in the captive population and provide breeding recommendations for potential pairs.

Our genetic management plan established a goal of growing the captive population to 200 before removal of 50 animals for reintroduction to the wild. Because we have not yet met that goal, we were unable to reintroduce PPM during Phase 1 of this project. In Phase 2, we will explore ways to improve reproductive success of captive animals. While 68.7% have cycled at least once since January of 2014, only 20 % of females have cycled in the last month. In addition, several females are not going through a full estrous cycle that includes perforation. While this is

common in a captive facility, increasing the number of females that go through a full estrous cycle and/or the frequency of cycling will increase the number of breeding opportunities.

We are just beginning to understand behavioral competency of wild-caught PPM. Thus, in Phase 2, we will continue to conduct experiments to examine survival skills in this species.

INCIDENTAL DEATHS

We had 13 PPM mortalities associated with this research during Phase 1.

Death of adult founders:

1. Male 10, a founder from Dana Point, was brought into captivity as an adult. He was found on December 21, 2012 with its toes curled and its foot in an unusual position but otherwise he was active. The animal was transferred to our Harter Veterinary hospital. They x-rayed it and found that his leg was broken. The veterinarian was concerned that if left alone the bone would eventually push through the skin. We decided to put a splint on the leg and watch to see if he would tolerate it. After successfully splinting the leg, the male was kept in the HVMC for observation. He was eating well and moving around the cage. However, on December 25th he was having labored breathing and was treated. On December 28, 2012 his splint had to be adjusted again and he died several minutes after being caught for restraint. The necropsy report indicates that no clear cause of death was found.

2. Female 5, a founder from Dana Point, was brought into captivity as an adult. She died on the night of January 10, 2014. She was in torpor and found dead in her cage. She was an adult female at capture. She had a history of weight loss from the previous week and poor food consumption thought to be due to torpor. Gross necropsy did not reveal a cause of death. No fat stores were seen. Seeds were present in the cheek pouches. Histopathology is pending. Necropsy revealed that bacterial endocarditis was the cause of death in this mouse.

Death of pups produced in captivity:

1. Pup # 28 was born significantly smaller than its littermate and it was eaten by its mother (Female 13) in the first days after birth.

2. Pup #45 was the runt of a large litter of 6 and did not survive weaning.

3. Pup # 50 was born significantly smaller than its littermates and it was found dead outside of the nest in the first days after birth.

4. Pups #53-57 were all from the same litter. Their mother, captive born female #32 did not lactate.

5. Pups #60-62 were all from the same litter. Their mother, captive born female #39. One pup was significantly larger than the rest.

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APPENDIX A - HEALTH STATUS OF FOUNDERS DURING QUARANTINE

All PPM were quarantined for up to 30 days at HVMC. All PPM were inspected upon entry into quarantine and a bodyweight taken. Weekly bodyweights were obtained. A single fecal o/p exam was done and results were negative. A visual exam was done during the quarantine period by a veterinarian. All PPM appeared to be in good health. Significant individual history during the quarantine period is recorded below.

PPM #1 Male #612245: none

PPM #2 Female#612246: none

PPM #3 Female#612247: none

PPM #4 Female#612248: Upon entry into quarantine this PPM had a focal area of erythema and inflammation on the top of the head. This is presumed to be from a previously applied Sharpie marker (used during field work for ID purposes). This was monitored and within 7 days was resolved.

PPM #5 Female#612249:none Note**:** ***Died*** 10 Jan 2014 (bacterial endo-carditis is cause of death)

PPM #6 Female#612250: noneNote: only here 1 day then returned

PPM #7 Male #612251: none

PPM #8 Male #612252: none

PPM #9 Female#612253: none

PPM #10 Male #612254: noneNote**:** ***Died*** 28 Dec 2012 (left tibia fracture).

PPM #11 Male #612257: none

PPM #12 Male #612262: Note: Tip of tail was accidently avulsed off during capture. Tip is clean and dry. No intervention needed.

PPM #13 Female#612264: none

PPM #14 Female#612267: Note: wound on proximal tail (dry, scab) presumed trauma. This resolved with time but residual quiet swelling present.

PPM #15 Male #612271: noneNote: Later developed bilateral Keratitis which was medically treated and resolved. Also had interscapular hair loss which improved with time (no treatment).

PPM #16 Male #612272:none

PPM #17 Male #612273: none

PPM #18 Female#612274: none

PPM #19 Female#612275: none

PPM #20 Female#612276: none

PPM #21 Male #612279: none

PPM #22 Female#612318: Notes: small kink in tail (old problem)

PPM #23 Male #612328: none

PPM #24 Female#613106: none

PPM #25 Male #613181: none

PPM #26 Female#613182: Two Ixodes ticks (larvae) removed from right pinna.

PPM #27 Male #613183: none

PPM #30 Female#613261: none

PPM#51 Male #614129: none

PPM#52 Female#614130: none

PPM#58 Male #614136: One tick (*Dermacentor occidentalis* larvae) removed from right pinna.

PPM#71 Female #614290: none

APPENDIX B -- DEFINITIONS OF GENETIC TERMS

Current Gene Diversity (GD) -- The proportional gene diversity (as a proportion of the source population) is the probability that two alleles from the same locus sampled at random from the population will not be identical by descent. Gene diversity is calculated from allele frequencies, and is the heterozygosity expected in progeny produced by random mating, and if the population were in Hardy-Weinberg equilibrium.

Founder – An individual obtained from a source population (often the wild) that has no known relationship to any individuals in the derived population (except for its own descendants).

Founder Genome Equivalents (FGE) – The number wild-caught individuals (founders) that would produce the same amount of gene diversity as does the population under study. The gene diversity of a population is 1 - 1 / (2 * FGE).

Founder Representation – Proportion of the genes in the living, descendant population that are derived from that founder. I.e., proportional Founder Contribution.

Inbreeding Coefficient (F) – Probability that the two alleles at a genetic locus are identical by descent from an ancestor common to both parents. The mean inbreeding coefficient of a population will be the proportional decrease in observed heterozygosity relative to the expected heterozygosity of the founder population.

Mean Kinship (MK) – The mean kinship coefficient between an animal and all animals (including itself) in the living, captive-born population. The mean kinship of a population is equal to the proportional loss of gene diversity of the descendant (captive-born) population relative to the founders and is also the mean inbreeding coefficient of progeny produced by random mating. Mean kinship is also the reciprocal of two times the founder genome equivalents: MK = 1 / (2 * FGE). MK = 1 - GD.

Percent Pedigree Certain – Percent of an animal's genome that is traceable to known Founders through specific ancestors (MULTS not included). Percent certain represents a higher degree of knowledge than percent known, and is therefore always less than or equal to percent known.

Percent Pedigree Known – Percent of an animal's genome that is traceable to known Founders, through identification of either specific ancestors or all possible ancestors (MULTs included).

APPENDIX C -- BREEDING RECOMMENDATIONS FOR 2014

A ranked list of potential mates is provided for each female, with the most desirable mates at the top of the list. Any animals listed at the same rank can be considered interchangeable (of equal priority for breeding). Try to breed each female with the best potential mate possible, while also trying to maximize the number of breeding males.

Females 2, 3, 9, 20, 24, 26, 30 (unrepresented wild-caught)

1. Males 7, 8, 11, 12, 15, 16, 17 (unrepresented wild-caught)
2. Male 25
3. Male 1
4. Male 36
5. Males 21, 23
6. Males 31, 37, 38, 40
7. Male 4
8. Males 41, 42, 44, 46

Female 13

1. Male 25
2. Male 1
3. Male 36
4. Male 21, 23
5. Male 31, 37, 38, 40
6. Male 4
7. Males 41, 42, 44, 46
8. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 29

1. Male 36
2. Male 1
3. Males 31, 37, 38, 40
4. Males 21, 23
5. Males 41, 42, 44, 46
6. Male 4
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 22

1. Male 21
2. Male 37, 38, 40
3. Male 4
4. Males 41, 42, 44, 46
5. Male 1
6. Male 36
7. Male 25
8. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Females 32, 33, 34

1. Male 37, 38, 40
2. Males 41, 42, 44, 46
3. Male 4, 23
4. Male 36
5. Male 1
6. Male 25
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 18

1. Male 1
2. Male 25
3. Males 21, 23
4. Males 31, 37, 38, 40
5. Male 4
6. Males 41, 42, 44, 46
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Male 35

1. Male 25
2. Males 31, 37, 38, 40
3. Males 21, 23
4. Males 41, 42, 44, 46
5. Male 4
6. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 19

1. Male 23

2. Male 21
3. Male 31
4. Male 4
5. Males 41, 42, 44, 46
6. Male 25
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 39

1. Male 31
2. Males 41, 42, 44, 46
3. Male 4, 21
4. Male 36
5. Male 1
6. Male 25
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 14

1. Male 4
2. Males 21, 23
3. Males 31, 37, 38, 40
4. Male 1
5. Male 36
6. Male 25
7. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)

Female 43

1. Males 31, 37, 38, 40
2. Males 21, 23
3. Male 36
4. Male 1
5. Male 25
6. Males 7, 8, 11, 12, 15, 16, 17, 27 (unrepresented wild-caught)