Pacific pocket mouse Breeding and Reintroduction Update

Annual Partners Meeting
13 February 2023

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International Conservation Translocation Conference 2023

SAN DIEGO ZOO WILDLIFE ALLIANCE

Outline

Conservation breeding

- Current status
- 2023 reproduction
- Assisted Reproductive Technology
- Wild collection
- Preserving cell lines

Reintroductions

- Survival skill evaluation
- Site management
- Overwinter Survival
- Supplementation
- Success metrics

- Summary of research results
- Plans for 2024

Conservation Breeding

Captive Population Status

Age	Wild-caught founders		Captive-born		Total
	Male	Female	Male	Female	
YOY	-	-	-	-	
1	3	4	47	61	115
2	-	-	28	40	68
3	-	-	3	3	6
4	1	2	-	3	6
5	2	2	1	2	7
6	-	-	1	3	4
7	-	-	1	3	4
Total	6	8	81	115	210

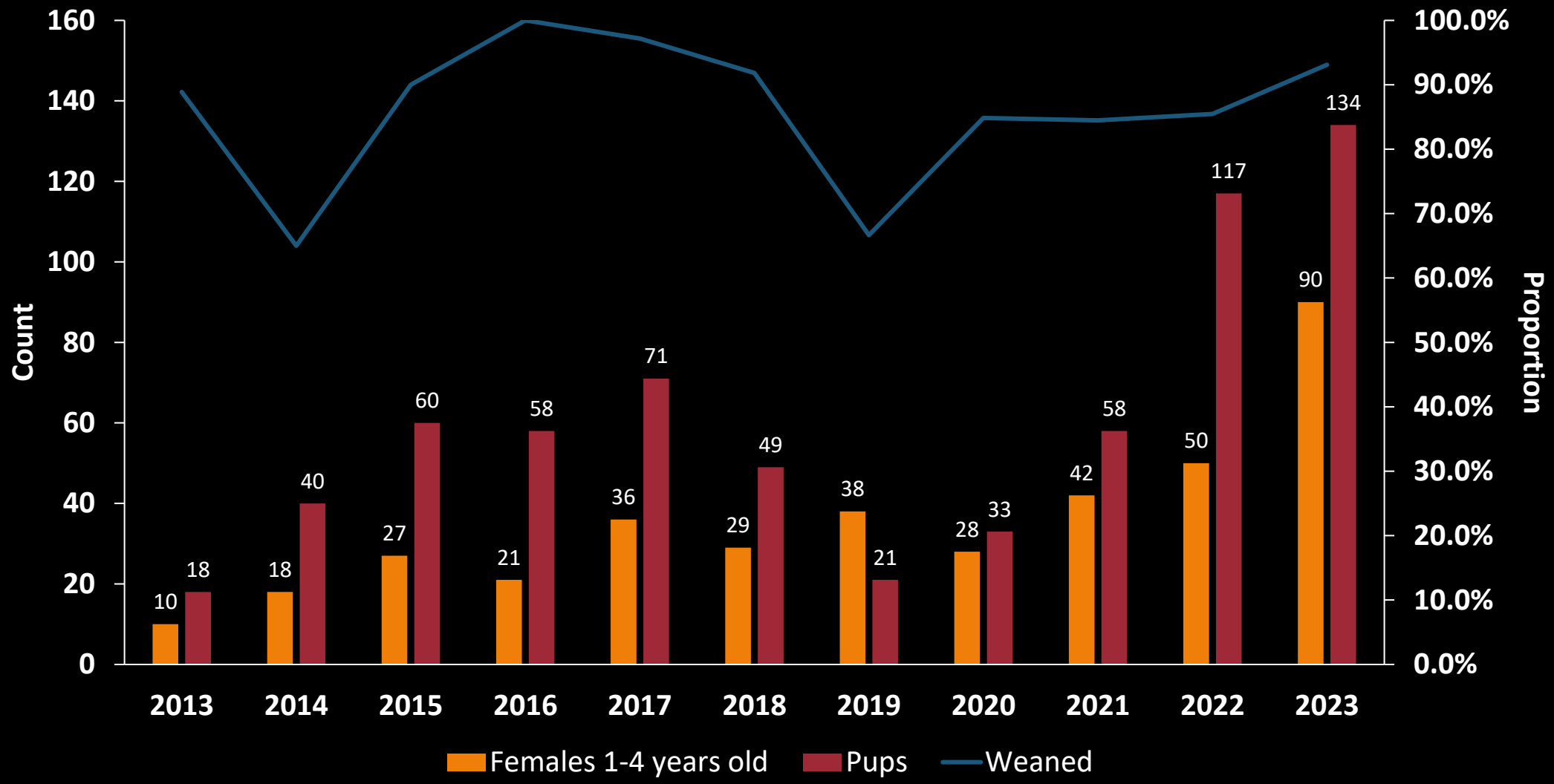
Reproduction 2023

Outcomes:

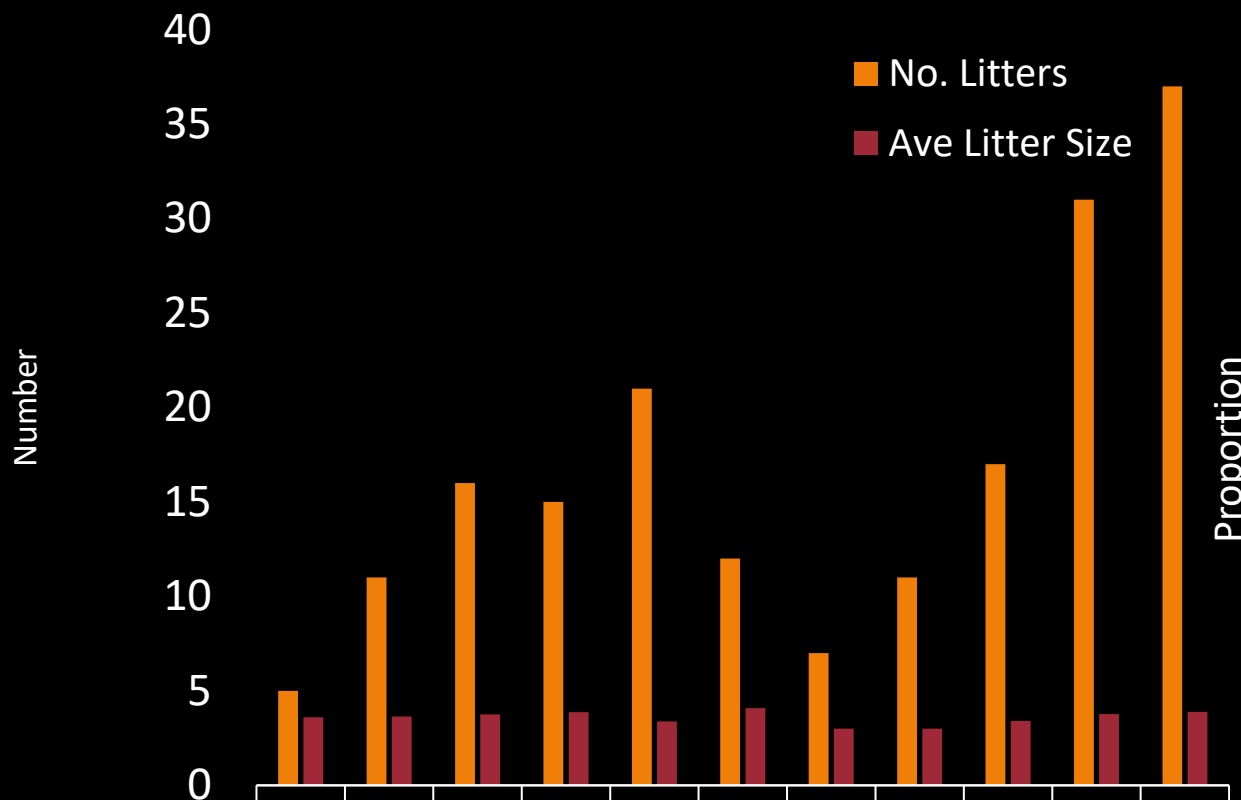
- 59 successful matings*
- 42 females confirmed pregnant*
- 31 litters produced*
- First litter born 12 March 2023
- Last litter born 28 September 2023*
  - February - September
- 134 pups weaned*

*Program records

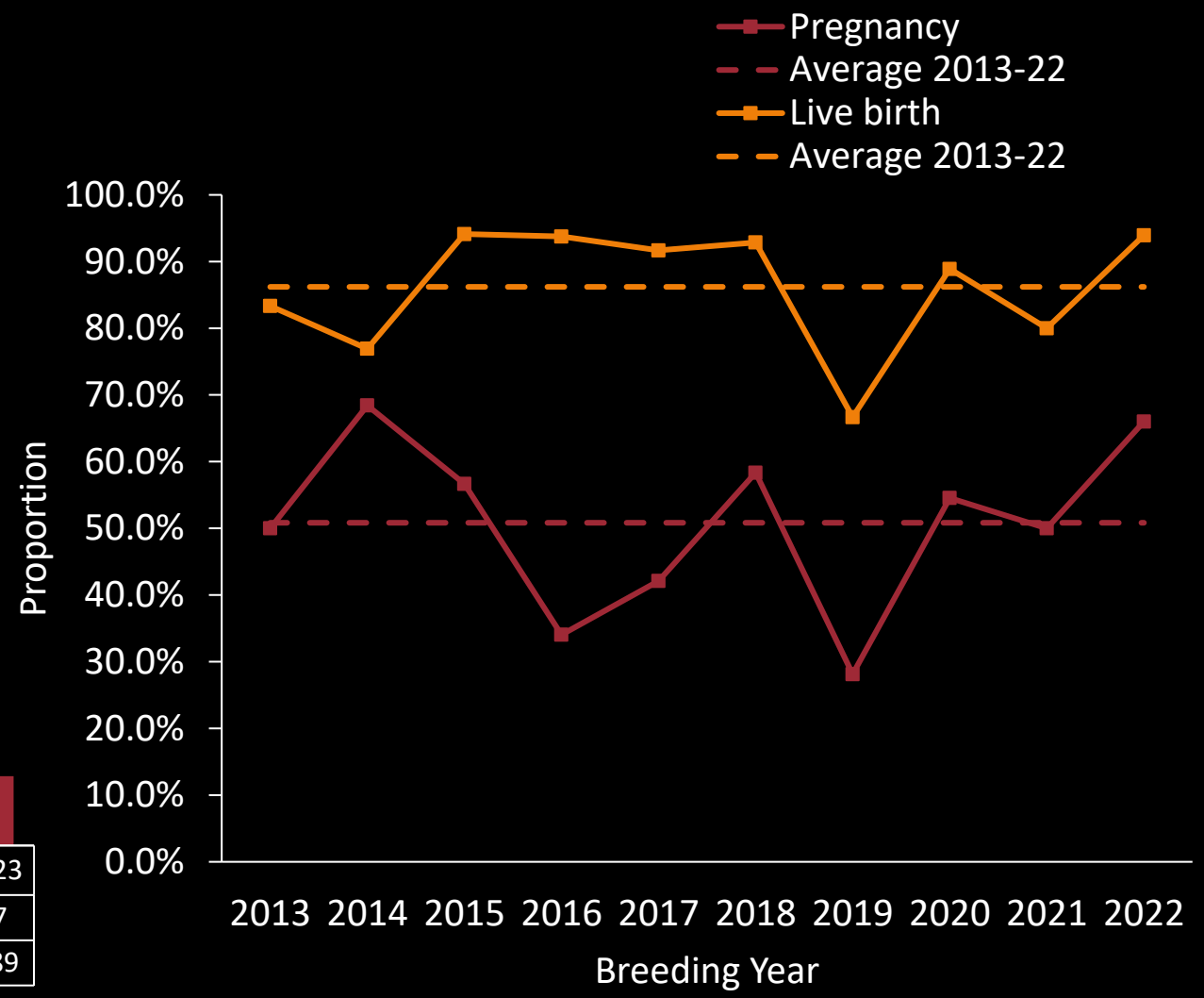
Reproductive Outcomes 2013-2023



Reproductive Outcomes 2013-2023



	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
No. Litters	5	11	16	15	21	12	7	11	17	31	37
Ave Litter Size	3.6	3.64	3.75	3.87	3.38	4.08	3	3	3.41	3.77	3.89



Wild Collection Recommendations

To have equal representation of all three populations in the captive population and maintain genetic diversity:

- Apr/May 2023: collect 4 PPM (2F:2M) from DP
- Apr/May 2024: collect two PPM (1F:1M) from both SM and SSM (N=4)
- Apr/May 2025: collect up to four PPM (equal sex ratio) from DP
- Oct-Dec 2025: re-evaluate collection strategy

*½ half of the animals collected will contribute to genetic diversity

GOALS

↑ DP representation

Replace the lineage lost from SM,

↑ number of SSM lineages

Collection

Dana Point Headlands

- Estimated abundance using CNLM track tube data
- 69 PPM during 5 weeks of monitoring
- Collection of 4 falls within criteria of 10% adults or 20% YOY
- August 16, 2023

	YOY	Adults
Female	7	0
Male	17	4
Unk	1	0

Collection

Santa Margarita

- Mitigation within Area 501
- Shana Dodd trapped 8 nights
- Collected 3
  - Second order relatives
  - Coccidia*

Collection Status 2012-2023

Year	Dana Point	Santa Margarita	South San Mateo
2012	10	10	2
2013	1	0	4
2014	0	4	0
2016	0	0	4
2019	0	0	4
2020	0	0	3
2023	4	3	0
Total	15	17	17

Total collected = 49

Total produced to date = 662

Captive Population Genetics

Preserving Cell Lines

Population	Number	Progress (cell lines)
Dana Point	5	Frozen
Santa Margarita	7	Frozen
South San Mateo	7	Frozen
Admixed	14	Frozen

Reintroductions & Field Work

Photo: Ken Bohn

Survival skill assessments

Fitness associated behaviors “Survival skills”

Predator defense:
Black-tailed prairie dog

Food finding:
Kea

Habitat selection and use:
mountain yellow-legged frogs

Behavioral Competency

	Shelter use	Foraging	Predator Response
			
Passed	98.1%	100%	Owl: 100% Snake 89.66%

Site Management

Habitat Management WT 2/3

Habitat surveys biannually

	Percent Cover (±SE)	
Habitat Feature	Ideal (Good)	Nov 2022
Bare Ground	21-60	61.28 (±1.50)
Cactus	N/A	0.14 (±0.07)
Forb	31-70	**2.21 (±0.2)**
Native Grass	16-40	**0.84 (±0.09)**
Non-native Grass	0-10 (11-20)**	1.97 (±0.35)
Shrub	0-20 (21-40)***	31.29 (±1.43)
Woody debris/leaf litter	1-20 (21-40)	2.26 (±0.18)

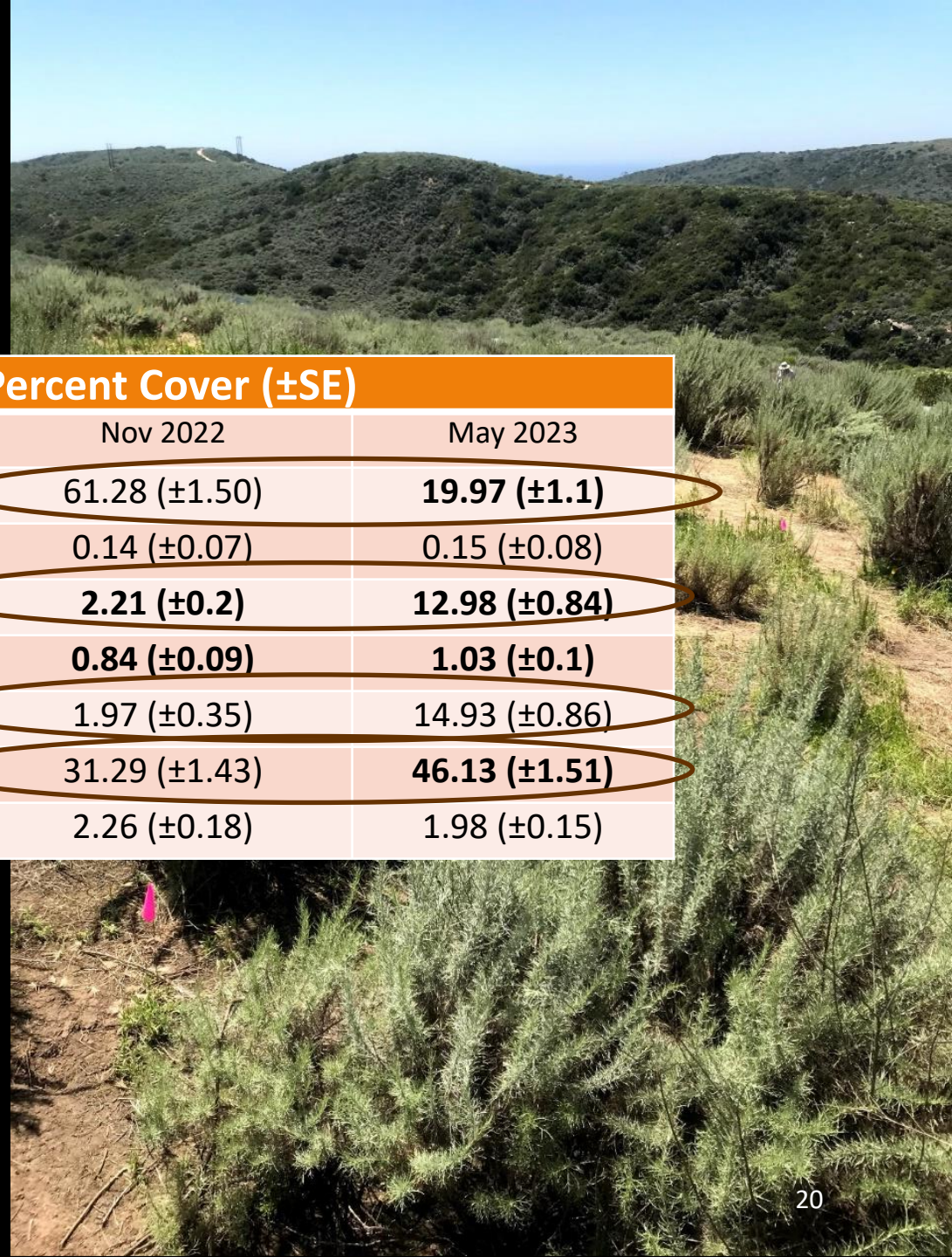
Feb: Spot treat non-native grass herbicide

March: Hand pulling non-native grass WT2

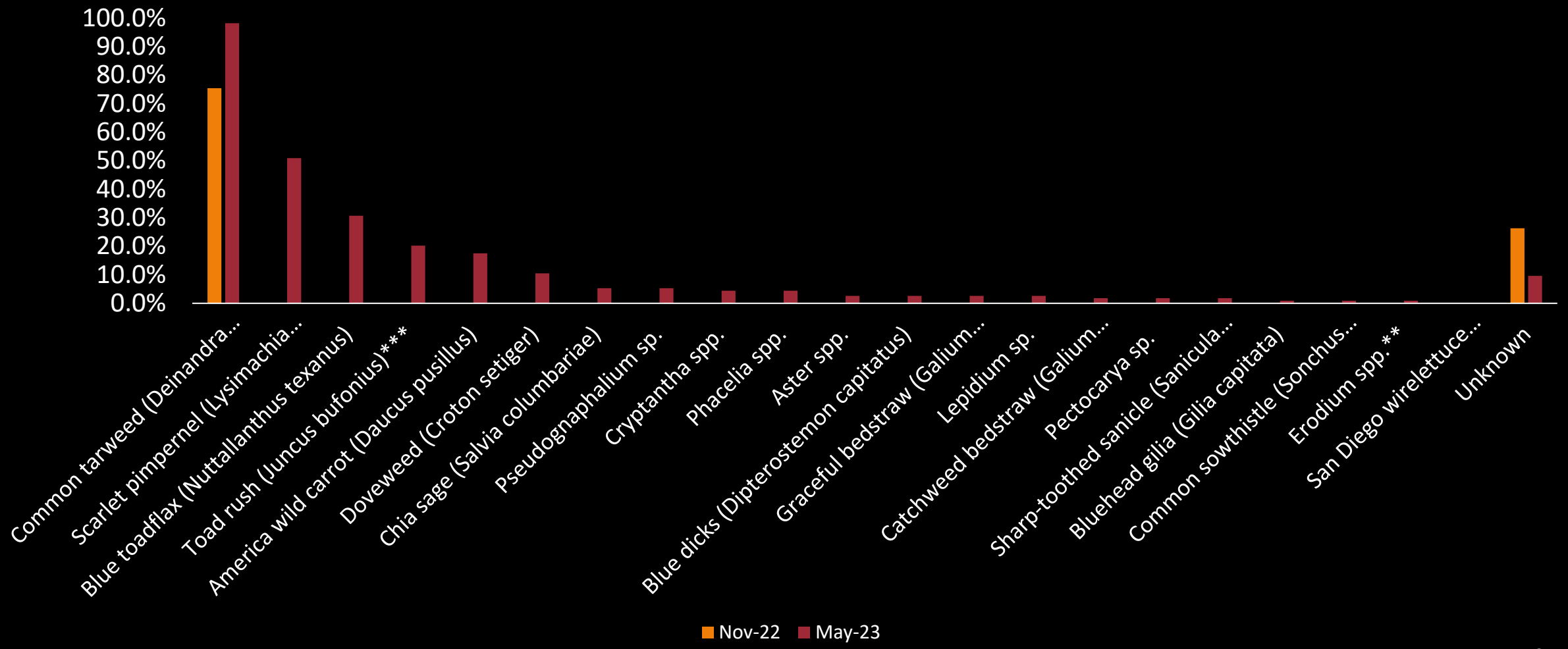
Habitat Management WT2/3

	Percent Cover (±SE)		
Habitat Feature	Ideal (Good)	Nov 2022	May 2023
Bare Ground	21-60	61.28 (±1.50)	**19.97 (±1.1)**
Cactus	N/A	0.14 (±0.07)	0.15 (±0.08)
Forb	31-70	**2.21 (±0.2)**	**12.98 (±0.84)**
Native Grass	16-40	**0.84 (±0.09)**	**1.03 (±0.1)**
Non-native Grass	0-10 (11-20)**	1.97 (±0.35)	14.93 (±0.86)
Shrub	0-20 (21-40)***	31.29 (±1.43)	**46.13 (±1.51)**
Woody debris/leaf litter	1-20 (21-40)	2.26 (±0.18)	1.98 (±0.15)

June and August: Hand pulling non-native grass and tarweed



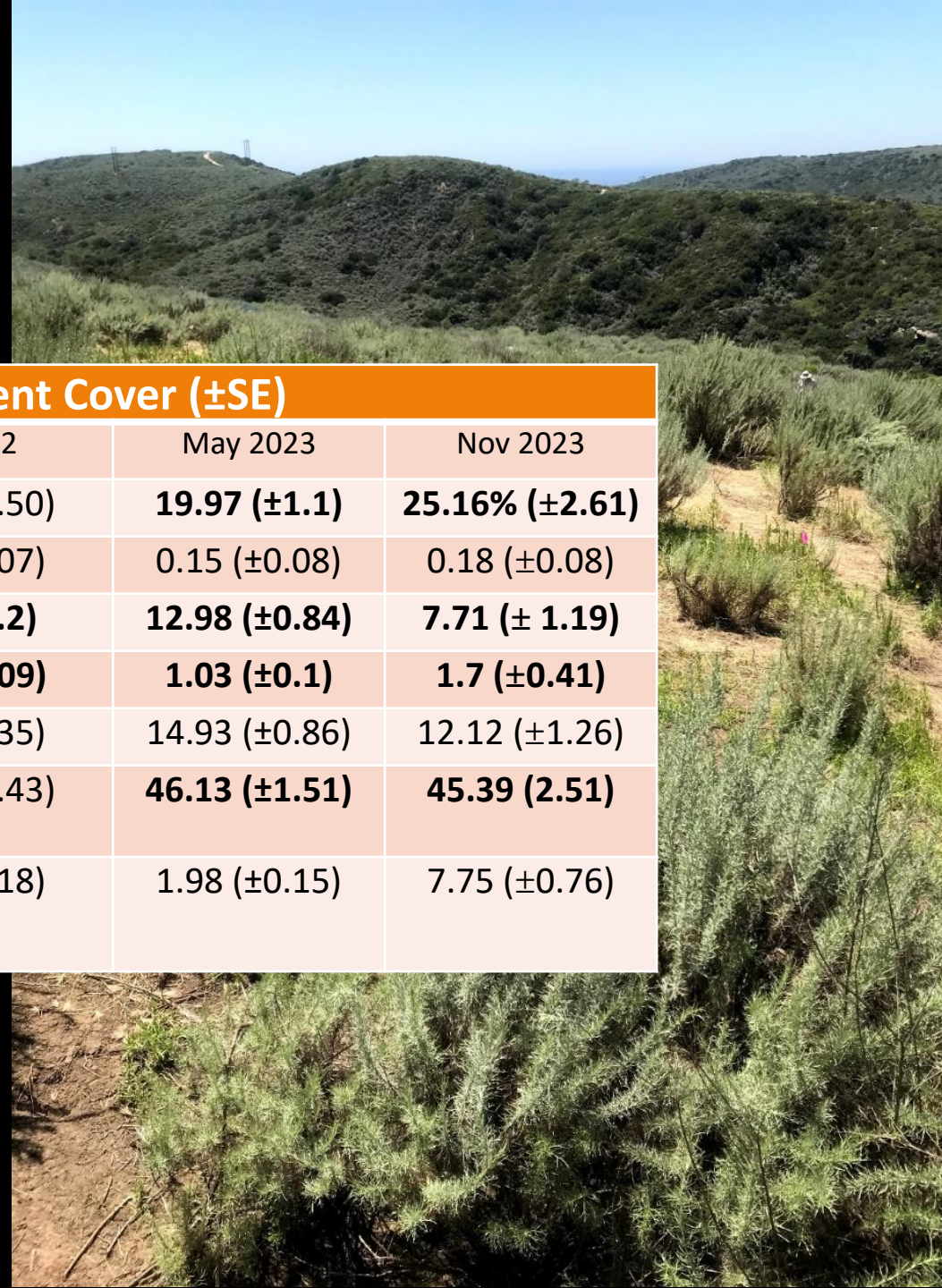
% Plots with Forbs and Rushes 3 Dominant sp
WT 2/3



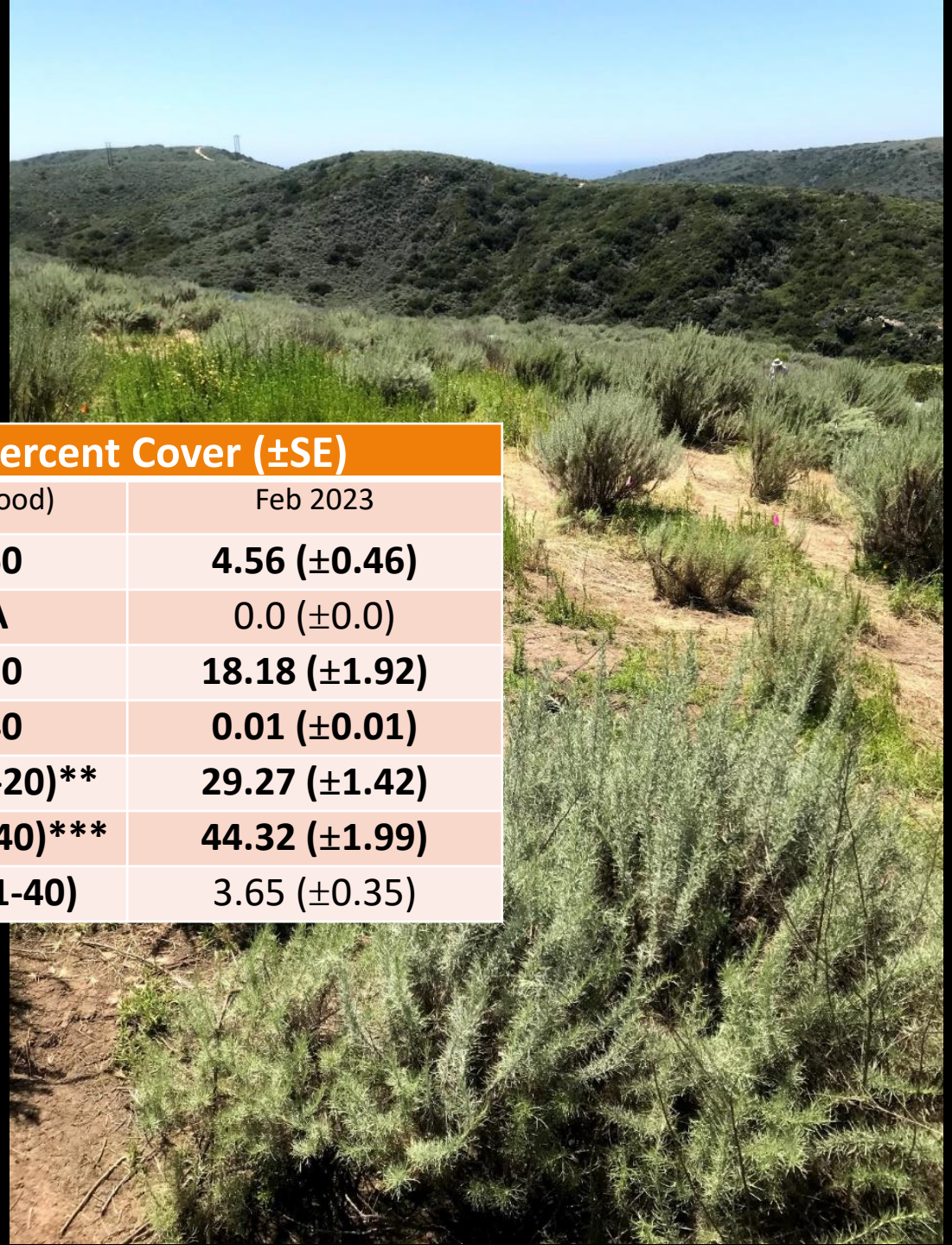
Habitat Management WT2/3

	Percent Cover (±SE)			
Habitat Feature	Ideal (Good)	Nov 2022	May 2023	Nov 2023
Bare Ground	21-60	61.28 (±1.50)	**19.97 (±1.1)**	**25.16% (±2.61)**
Cactus	N/A	0.14 (±0.07)	0.15 (±0.08)	0.18 (±0.08)
Forb	31-70	**2.21 (±0.2)**	**12.98 (±0.84)**	**7.71 (± 1.19)**
Native Grass	16-40	**0.84 (±0.09)**	**1.03 (±0.1)**	**1.7 (±0.41)**
Non-native Grass	0-10 (11-20)**	1.97 (±0.35)	14.93 (±0.86)	12.12 (±1.26)
Shrub	0-20 (21-40)***	31.29 (±1.43)	**46.13 (±1.51)**	**45.39 (2.51)**
Woody debris/leaf litter	1-20 (21-40)	2.26 (±0.18)	1.98 (±0.15)	7.75 (±0.76)

June and August: Hand pulling non-native grass and tarweed



Habitat Management WT 4



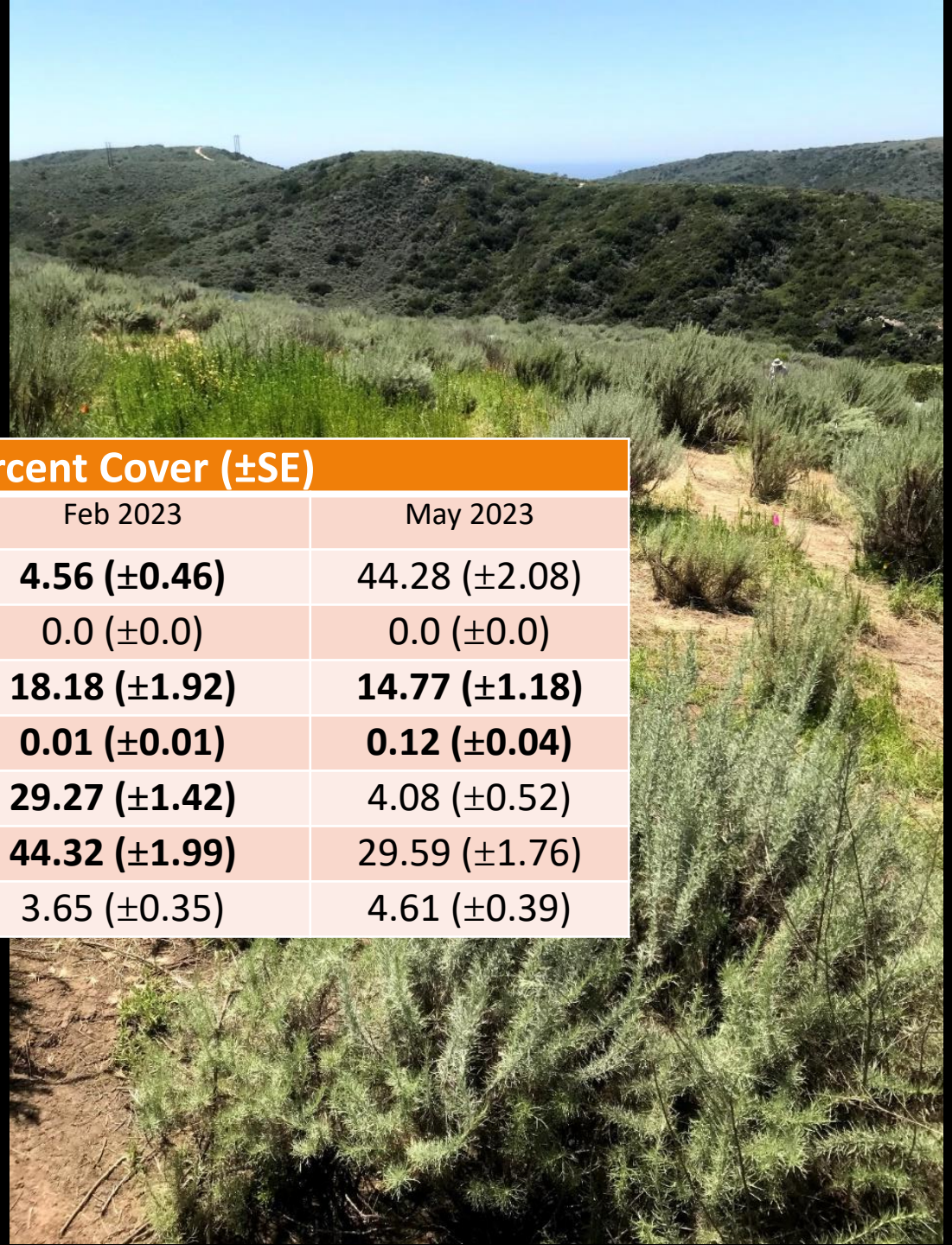
	Percent Cover (±SE)	
Habitat Feature	Ideal (Good)	Feb 2023
Bare Ground	**21-60**	**4.56 (±0.46)**
Cactus	**N/A**	0.0 (±0.0)
Forb	**31-70**	**18.18 (±1.92)**
Native Grass	**16-40**	**0.01 (±0.01)**
Non-native Grass	**0-10 (11-20)****	**29.27 (±1.42)**
Shrub	**0-20 (21-40)*****	**44.32 (±1.99)**
Woody debris/leaf litter	**1-20 (21-40)**	3.65 (±0.35)

Feb: herbicide

March and April: shrub thinning and hand removal of non-native grass

We planted 34 native shrubs in 16 plots to increase cover as part of experiment and added species diversity

Habitat Management WT 4



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Habitat Feature	Ideal (Good)	Feb 2023	May 2023
Bare Ground	**21-60**	**4.56 (±0.46)**	44.28 (±2.08)
Cactus	**N/A**	0.0 (±0.0)	0.0 (±0.0)
Forb	**31-70**	**18.18 (±1.92)**	**14.77 (±1.18)**
Native Grass	**16-40**	**0.01 (±0.01)**	**0.12 (±0.04)**
Non-native Grass	**0-10 (11-20)****	**29.27 (±1.42)**	4.08 (±0.52)
Shrub	**0-20 (21-40)*****	**44.32 (±1.99)**	29.59 (±1.76)
Woody debris/leaf litter	**1-20 (21-40)**	3.65 (±0.35)	4.61 (±0.39)

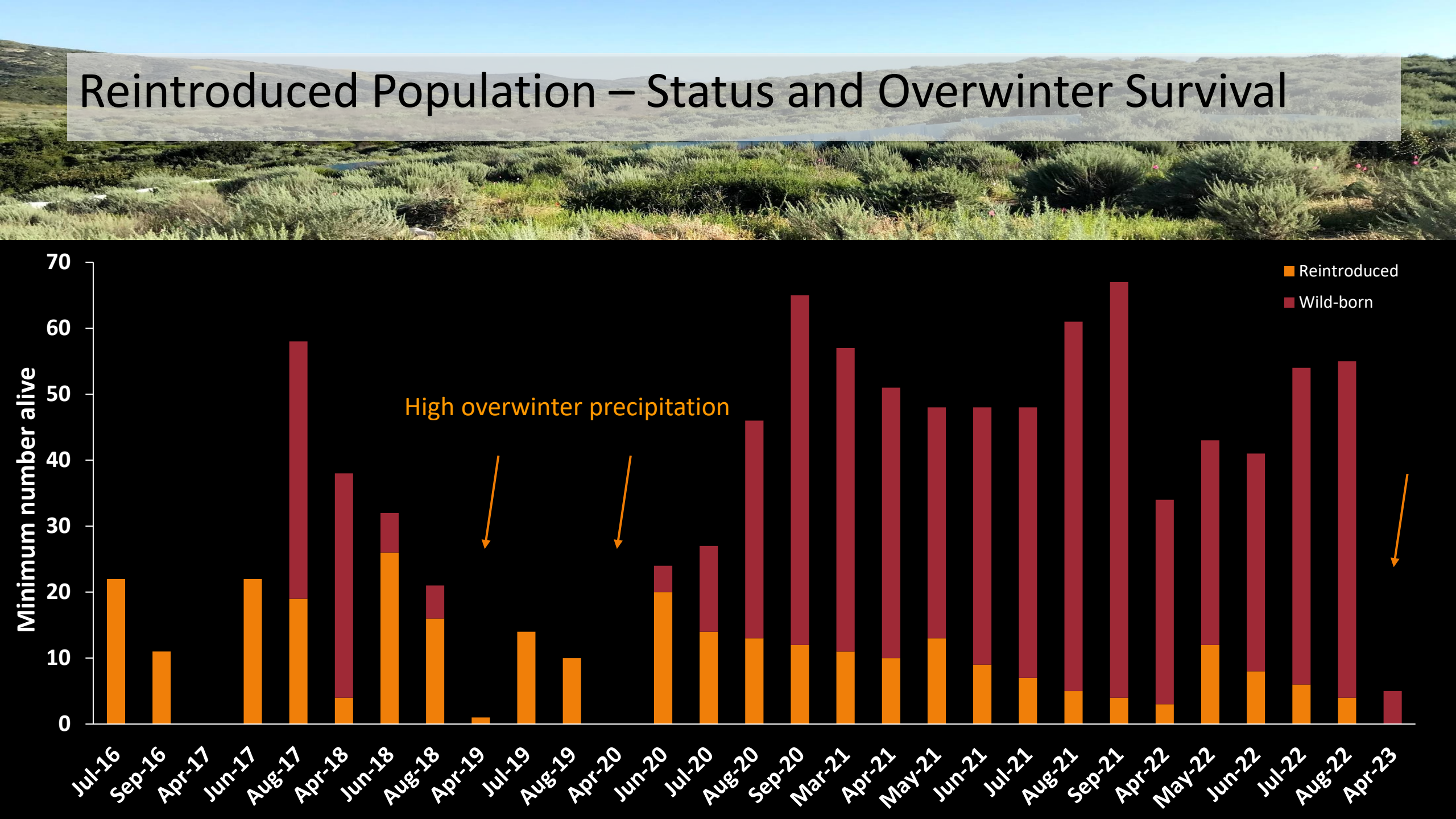
Native forb and grass seeds are added through supplementary feeding

Habitat Management WT 4

	Percent Cover (±SE)			
Habitat Feature	Ideal (Good)	Feb 2023	May 2023	Nov 2023*
Bare Ground	**21-60**	**4.56 (±0.46)**	**44.28 (±2.08)**	22.38 (±1.13)
Cactus	**N/A**	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
Forb	**31-70**	**18.18 (±1.92)**	**14.77 (±1.18)**	**20.03 (±1.37)**
Native Grass	**16-40**	**0.01 (±0.01)**	**0.12 (±0.04)**	**0.27 (±0.07)**
Non-native Grass	**0-10 (11-20)****	**29.27 (±1.42)**	4.08 (±0.52)	4.34 (±0.38)
Shrub	**0-20 (21-40)*****	**44.32 (±1.99)**	29.59 (±1.76)	35.65 (±1.67)
Woody debris/leaf litter	**1-20 (21-40)**	3.65 (±0.35)	4.61 (±0.39)	17.35 (±0.93)

Planned management: spot treatment non-native grass
Shrub thinning
Addition of native forb seeds

Reintroduced Population – Status and Overwinter Survival



Reintroduction (Supplementation)

- 50 PPM were released into unoccupied Water Tank 4(Laguna Coast Wilderness Park) on May 1, 2023 using standard methods*
- 20 PPM were released into WT 2/3 on June 27, 2023
- Management release
  - YOY
  - 24 hour holding period

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- Management release
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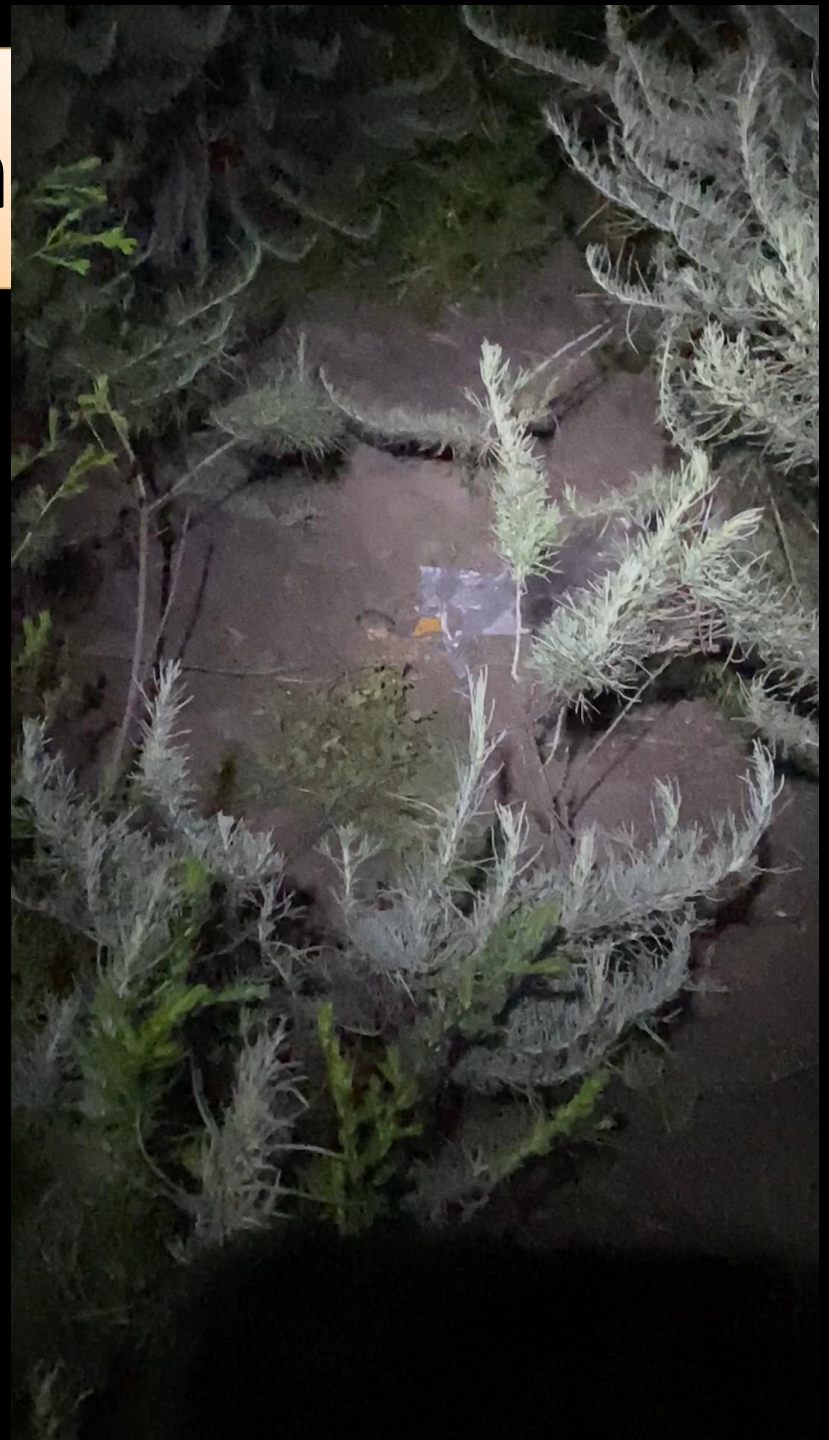
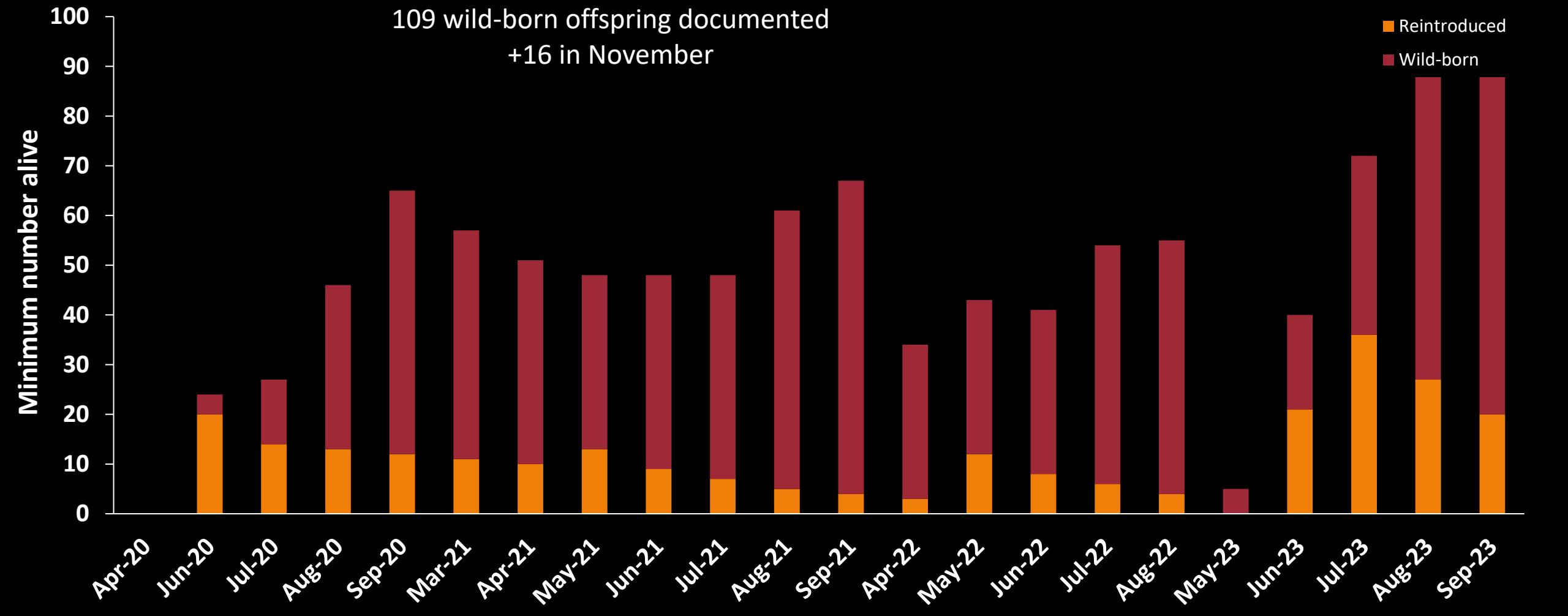
  - 24 hour holding period
- Behavioral Observation



Reintroduced Population Status

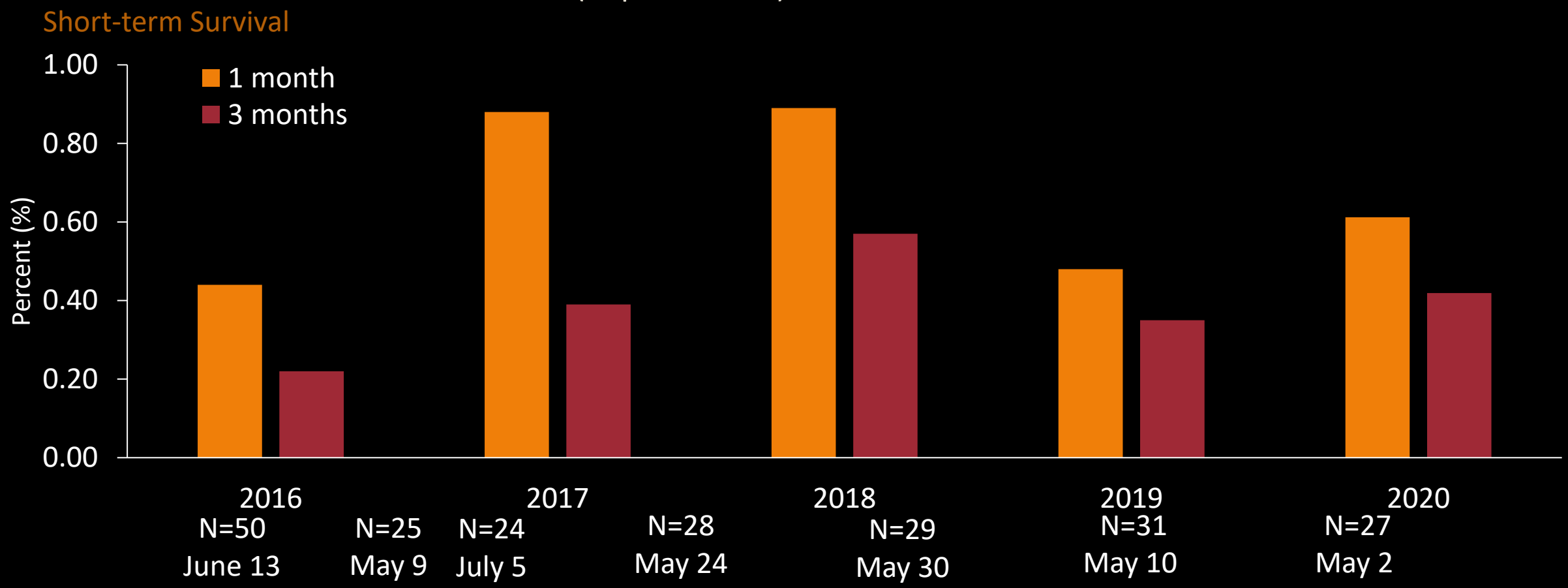


Identifying settlement and burrow ownership

Camera trap videos

Reintroduction Success Metrics

Founders Settle → **Survive** → Population Growth (Reproduction) → Population Regulates → Population Viability



Success criteria by Stage of Reintroduction

Stages	Term	Criteria for success	Time frame
Stage 1 – survival and growth of individuals in release cohort	Short-term	≥50% survival for release cohort members	1 month post-release
Stage 2 - evidence of reproduction	Short-term	≥50% surviving females are reproductive – evidence of cycling, pregnancy Wild-born offspring emerge on site	First active season (~0-5 months post-release)

Success criteria by Stage of Reintroduction

Stages	Term	Criteria for success	Time frame
Stage 3 population growth	Short to medium-term	The number of mice at the release site ≥ the number released	First active season (~0-5 months post-release)
Stage 3 population persistence	Medium -term	Overwinter survival ≥ % of mice that are expected in extant populations Consistently high number of individuals caught in each monitoring period (i.e., more than released) YOY observed in years when reproduction is observed in extant populations	>3 years post-release

Success criteria by Stage of Reintroduction

Stages	Term	Criteria for success	Time frame
Stage 3 – population regulation	Long-term	Population surveys document consistently medium to high density of PPM in April/June and September each year for 3-5 years with no supplemental mice* YOY observed in years when reproduction is observed in extant populations founders comprise a small proportion of captures Evidence of PPM dispersal and persistence outside of reintroduction perimeter fence	Minimum of 3-5 years post-initial release

Success criteria by Stage of Reintroduction

Stages	Term	Criteria for success	Time frame
Stage 4 – population viability	Long-term	Negligible probability of extinction (e.g. using PVA)	unknown

Summary of Research Results

2023 Research

- Genomics (Aryn Wilder/Erik Funk)
- Microbiome (Candace Williams/UCSD)
- Captive housing
  - Enrichment (cognitive challenge)
  - Seasonal temperature
- Reproduction
  - Multiple mating
- Survival skills and training
  - Antipredator (exposure vs training; dam experience)
  - Interspecific experience
- Reintroduction Methods
  - 7 vs 14 day acclimation
  - Full acclimation protocol vs management reintroduction
- Habitat suitability/Receiver site selection
  - burrow architecture
  - soil
- Stress – cortisol vs corticosterone
- Foraging preferences and Cache pilfering
- Torpor and anthropogenic noise
- Using cameras, quantify activity budget, and heterospecific interactions

Small rodent community

Dusky-footed woodrat

California pocket mouse

Dulzura kangaroo rat

Western harvest mouse

Pacific pocket mouse

Cactus mouse

California mouse

Deer mouse

8/16/2016 8:18 PM ID:18

Does experience with a heterospecific competitor affect the translocation success of the endangered Pacific pocket mouse?

Effect of experience with heterospecifics on PPM reintroduction success

2020-2021

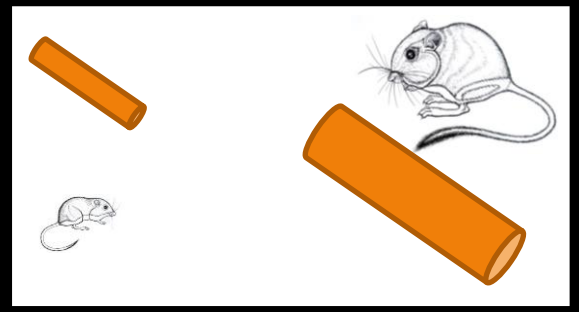
Collected 9 wild DKR

Pre-test 10 minutes

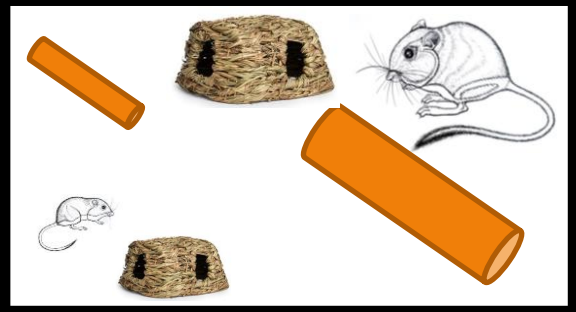
Familiarization Period ~2 hours

Post-test 10 minutes

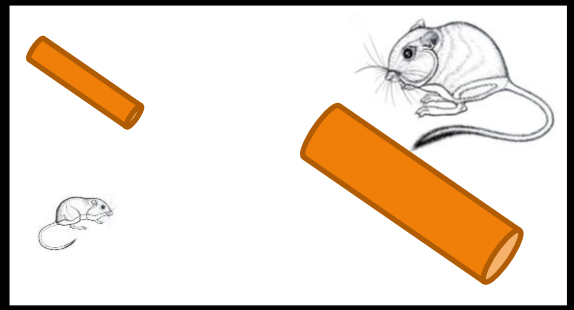
Experienced
n=26



Night 1



Night 2



Night 3

Control
n=18

No DKR experience

Effect of experience with heterospecifics on PPM reintroduction success

2022

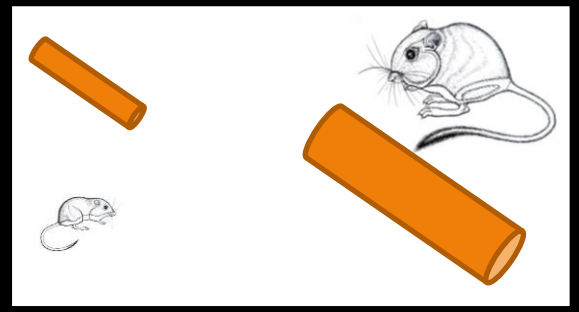
Collected 10 wild DKR

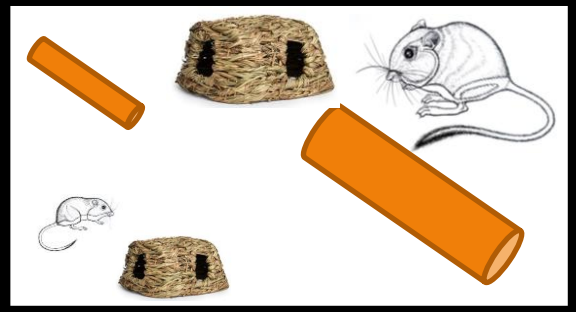
Pre-test 10 minutes

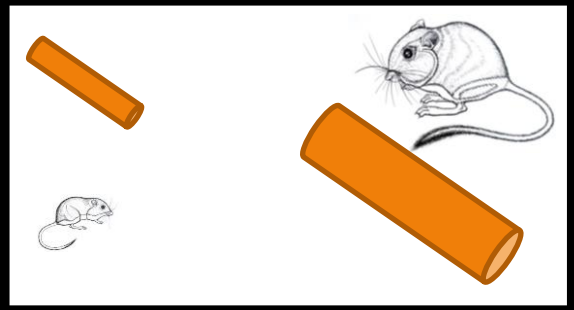
Familiarization Period ~2 hours

Post-test 10 minutes

Experienced
n=15





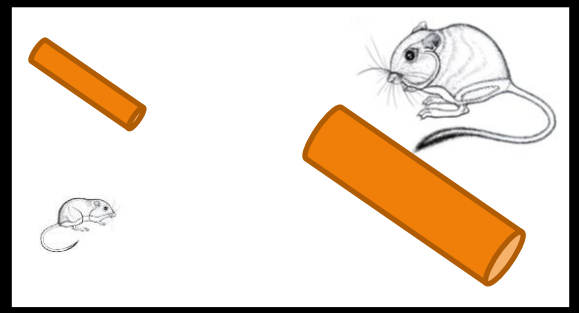


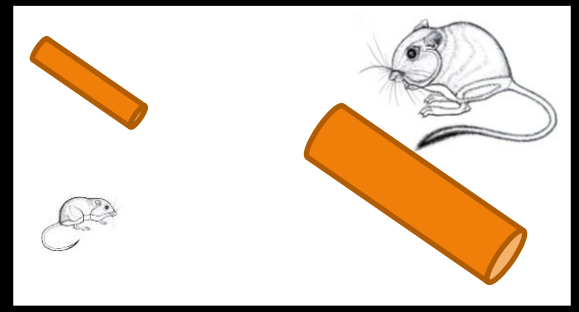
Night 1

Night 2

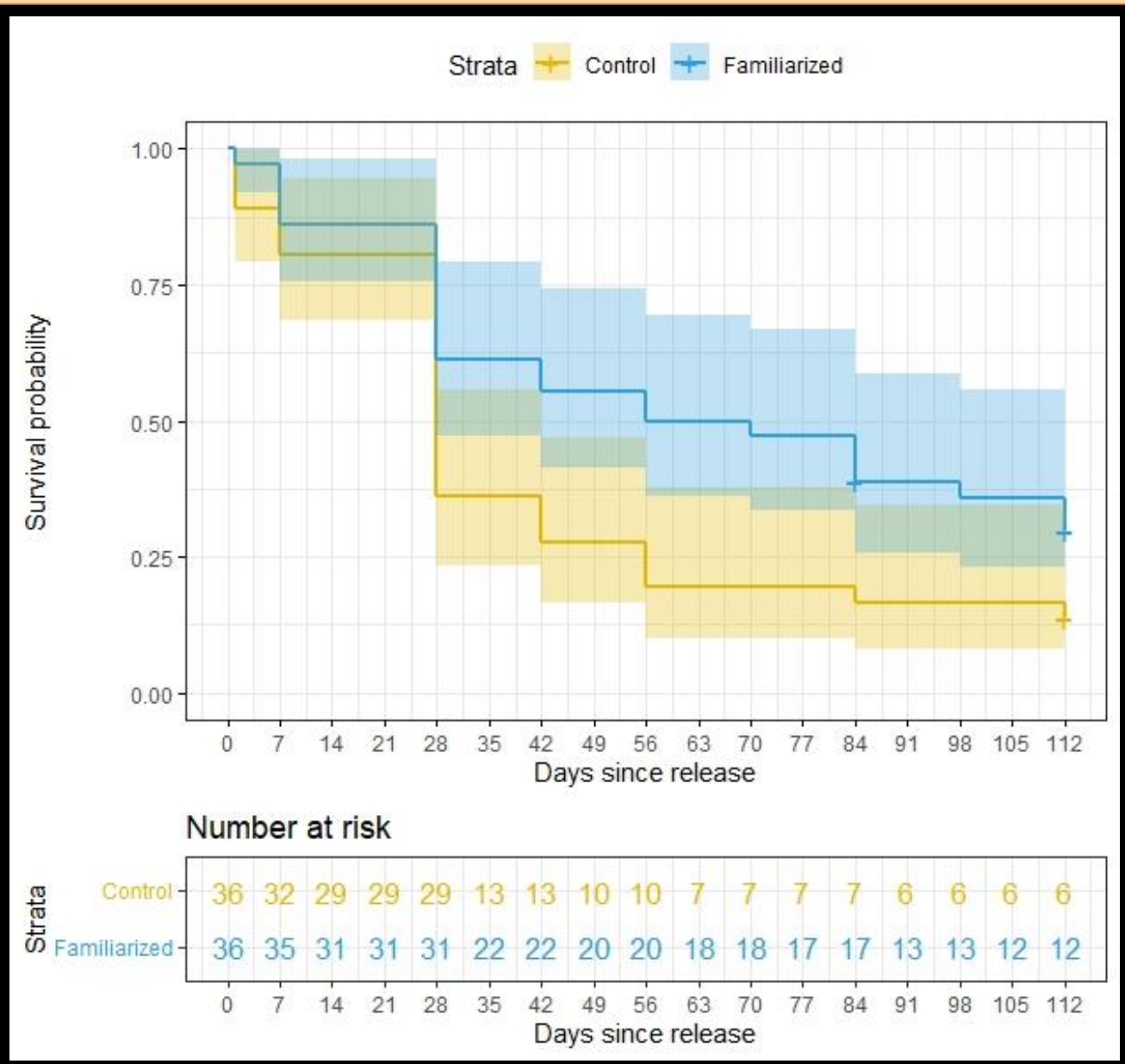
Night 3

Control
n=13



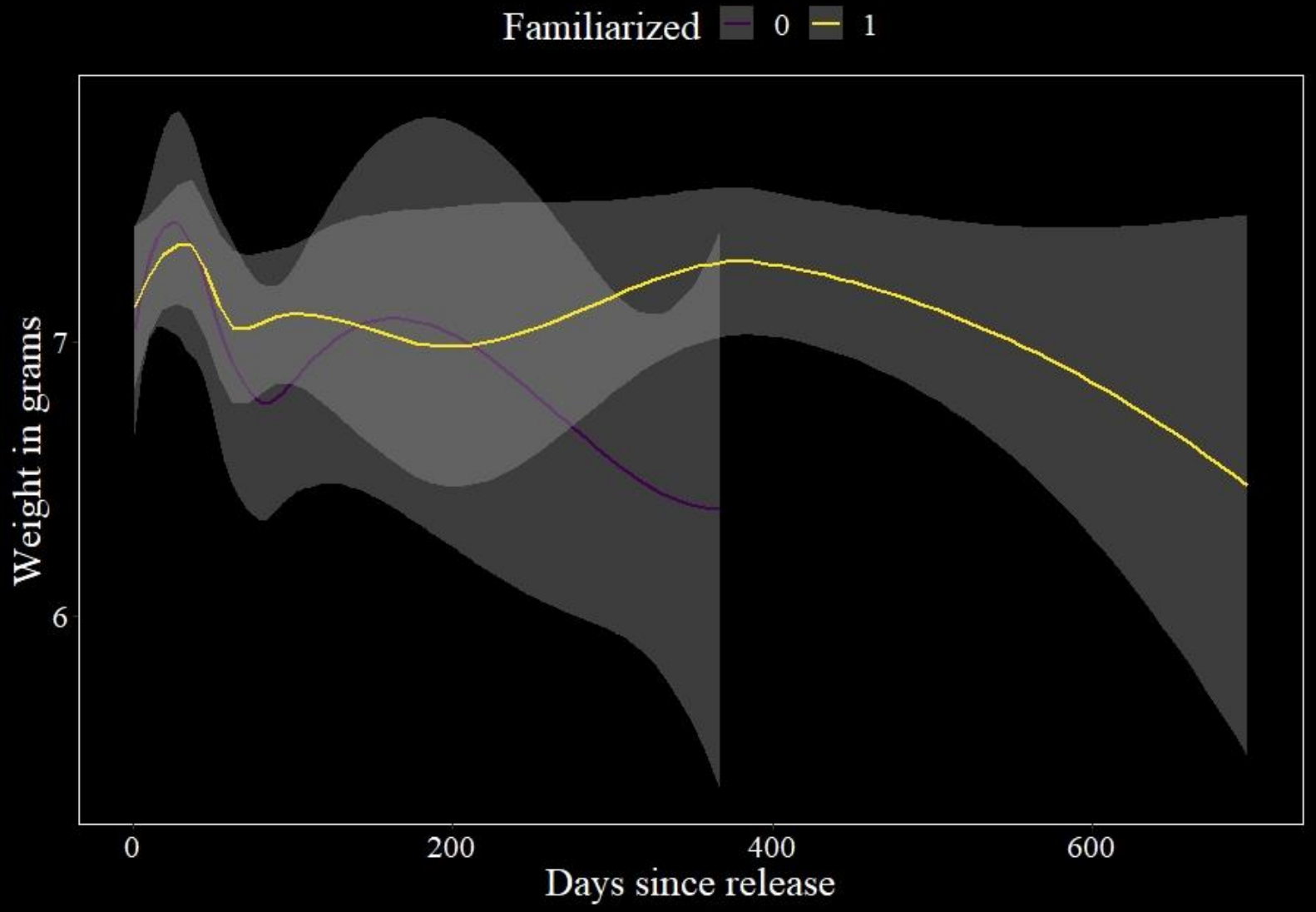


Evidence of effect of heterospecific training

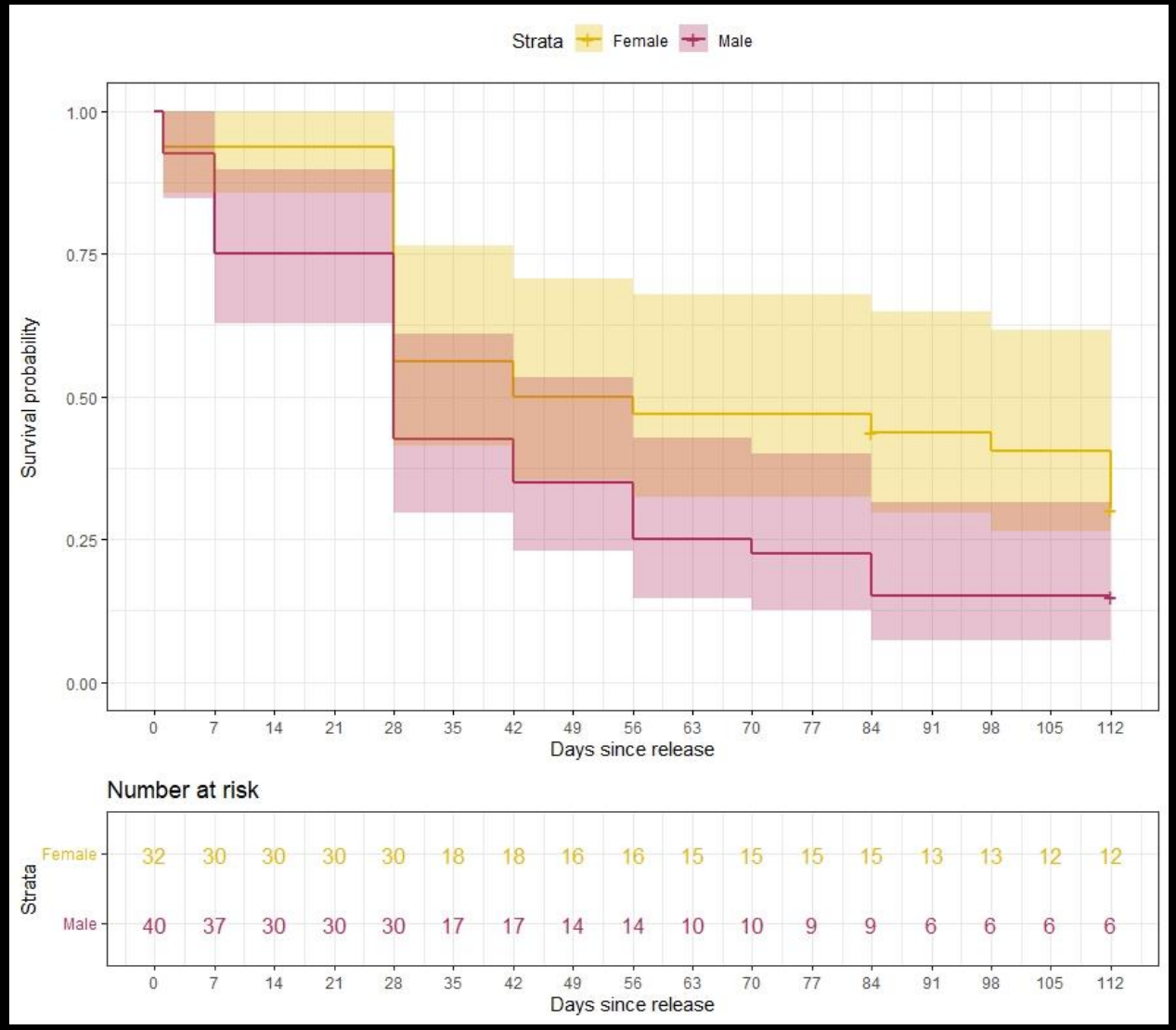


Evidence of effect of heterospecific training

Mechanism is still unclear



Sex differences



Fitness associated behaviors “Survival skills”

Predator defense:
Black-tailed prairie dog

Food finding:
Kea

Habitat selection and use:
mountain yellow-legged frogs

Adaptive Management

Experience with DKR improves fitness outcomes

- Incorporate experience with heterospecific competitors in our pre-release preparation
- Determine if other experience with other heterospecifics is beneficial
- Release equal numbers of both sexes where possible

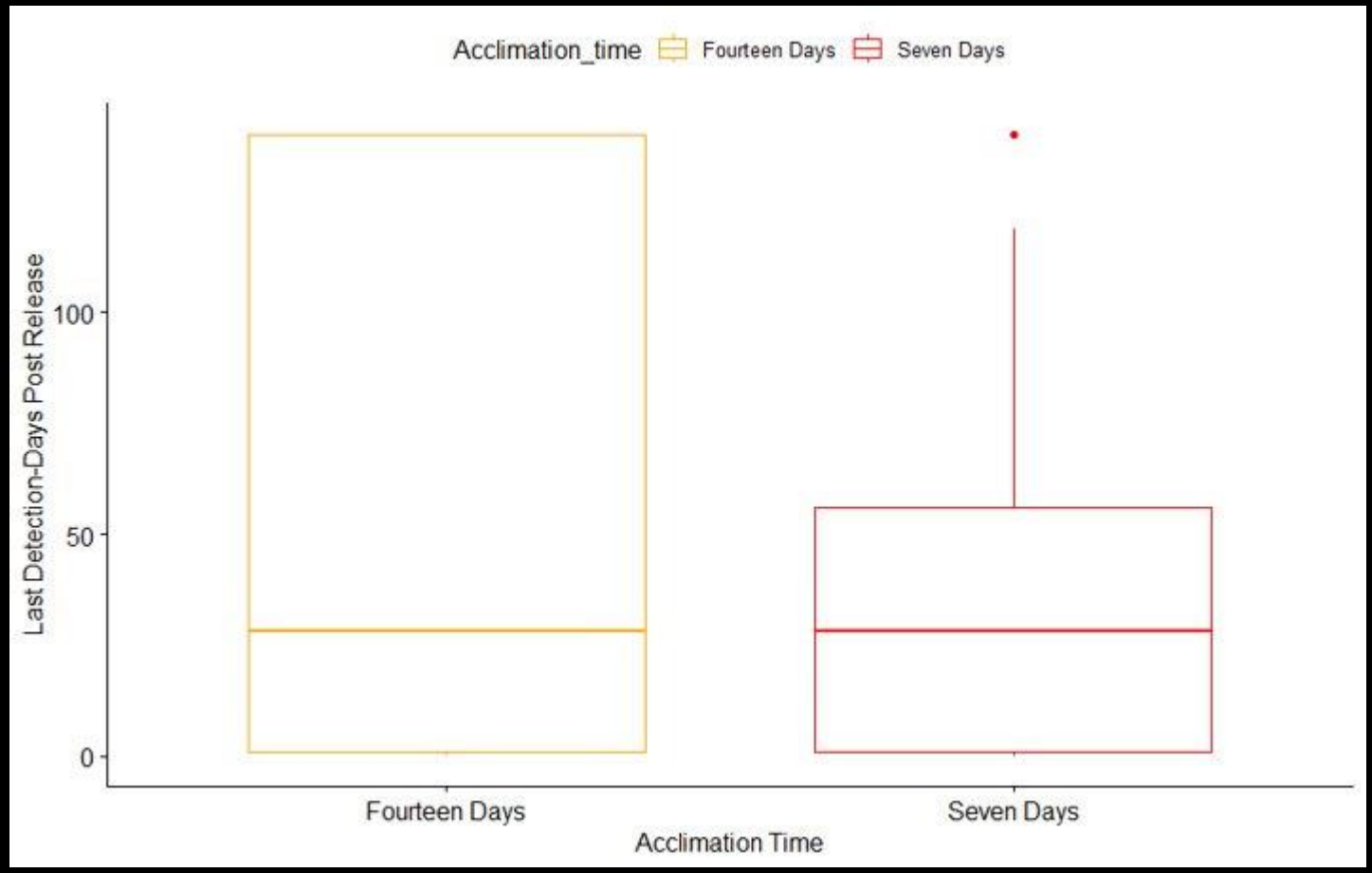
Reintroduction Methods

Optimal acclimation period may vary by source of founders

Captive vs wild caught

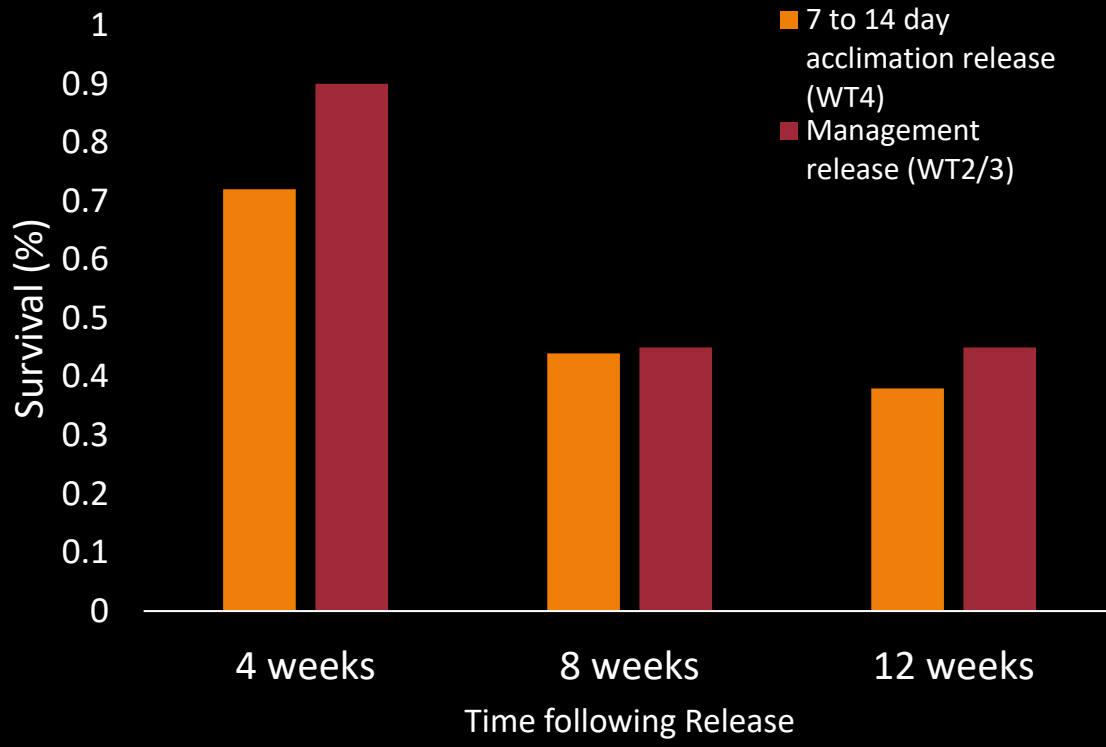
7 day acclimation (n=25)

14 day acclimation (n=25)



Full protocol
Behavioral competency including experience with heterospecifics
7 day acclimation
Released 1-3 year olds

Management release
Streamlined animal prep
24 hour acclimation
Released only YOY



Reintroduction Methods

Habitat Suitability - Soils

HOBO data logger(s) were deployed at extant PPM populations

Extant Sites

- South San Mateo
- Oscar
- Edson

Reintroduced population

- Laguna Coast Wilderness Park (Water Tank),

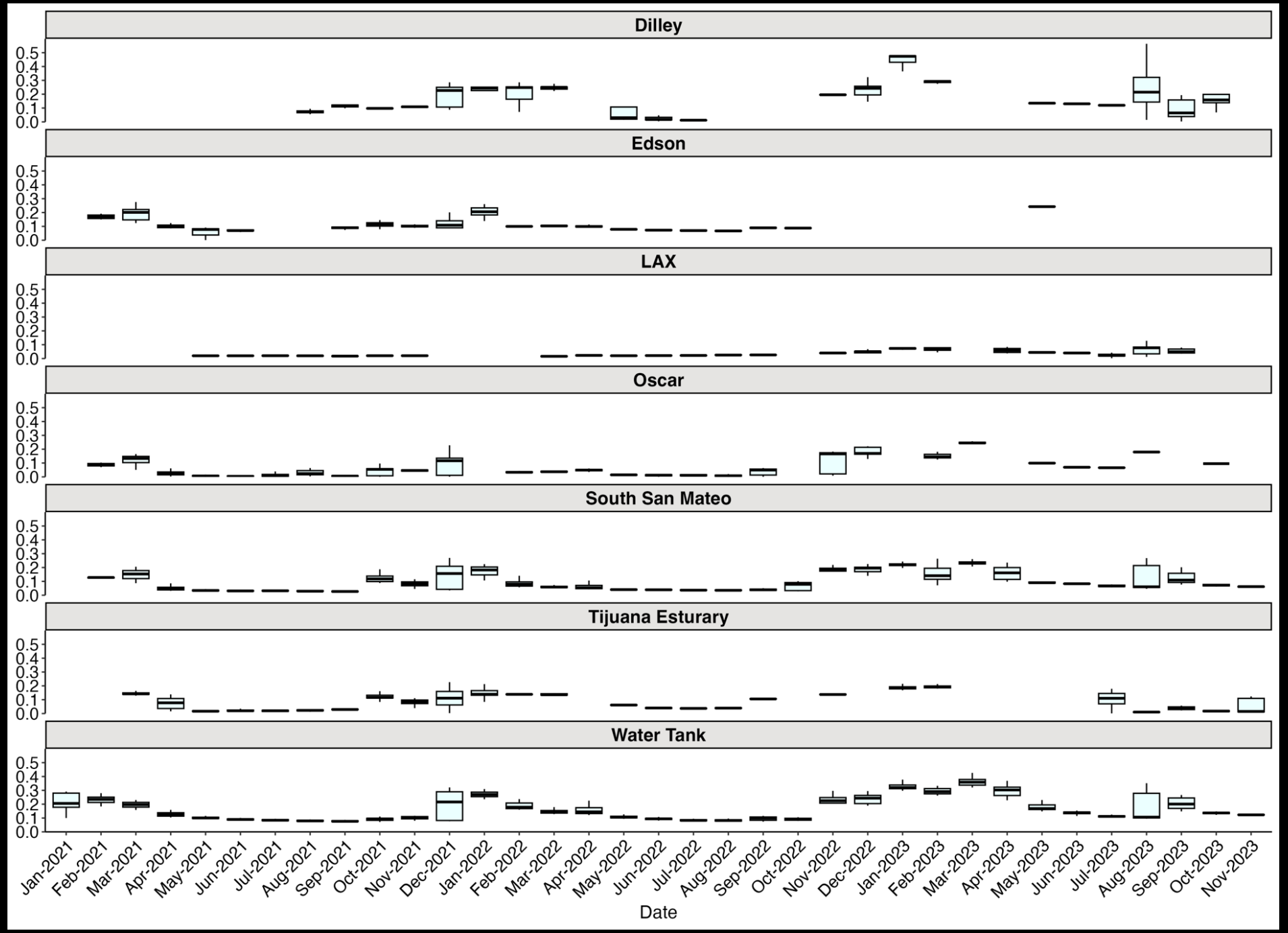
Potential receiver sites

- James Dilley Greenbelt Preserve *(Dilley)*,
- *Los Angeles Airport (*LAX*)*,
- Tijuana River National Estuarine Research Reserve *(Tijuana Estuary)*

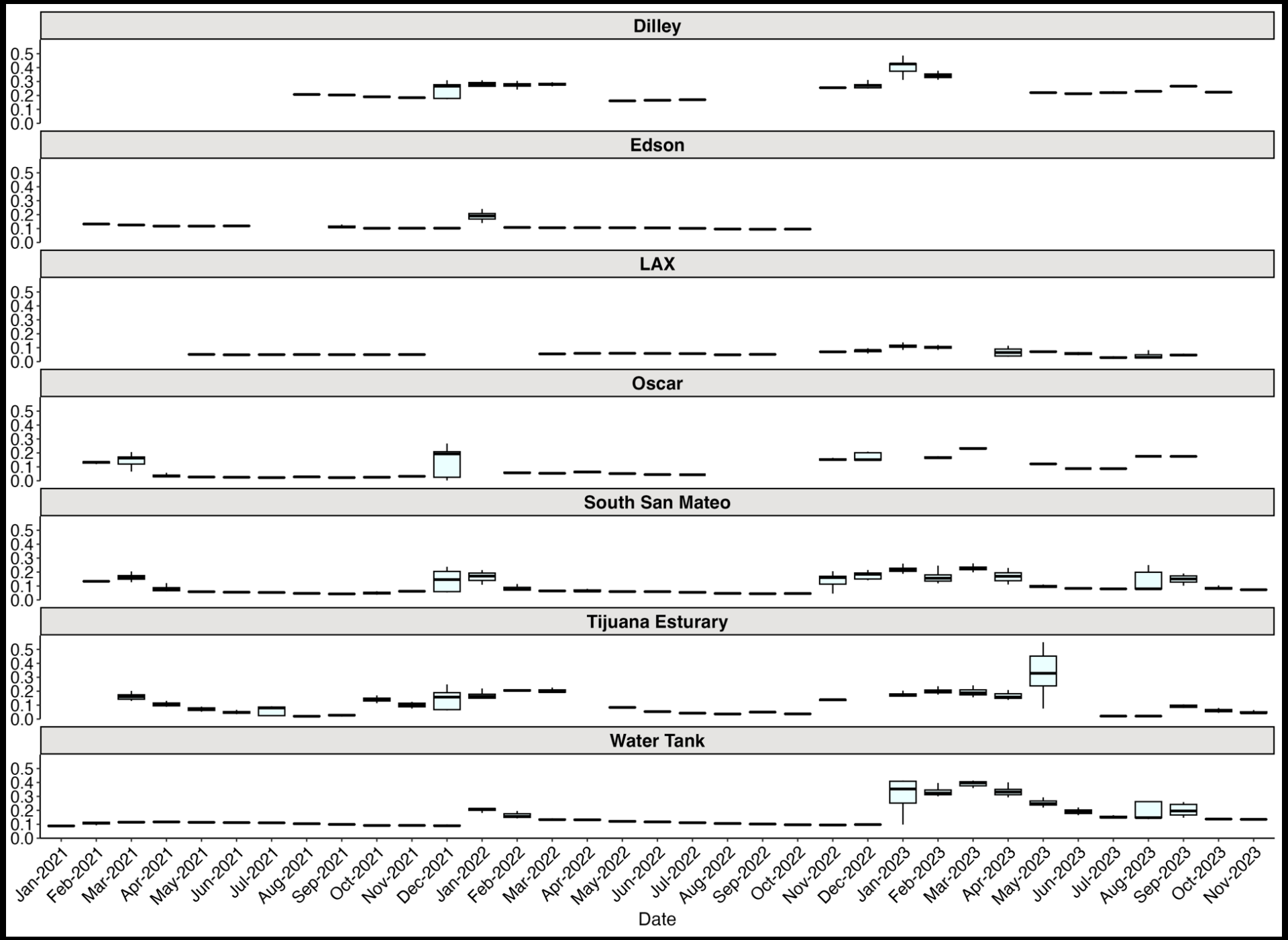
Data logger and 4-5 individual sensors attached directly to the unit with cables.

Downloaded data monthly, or when site access was granted.

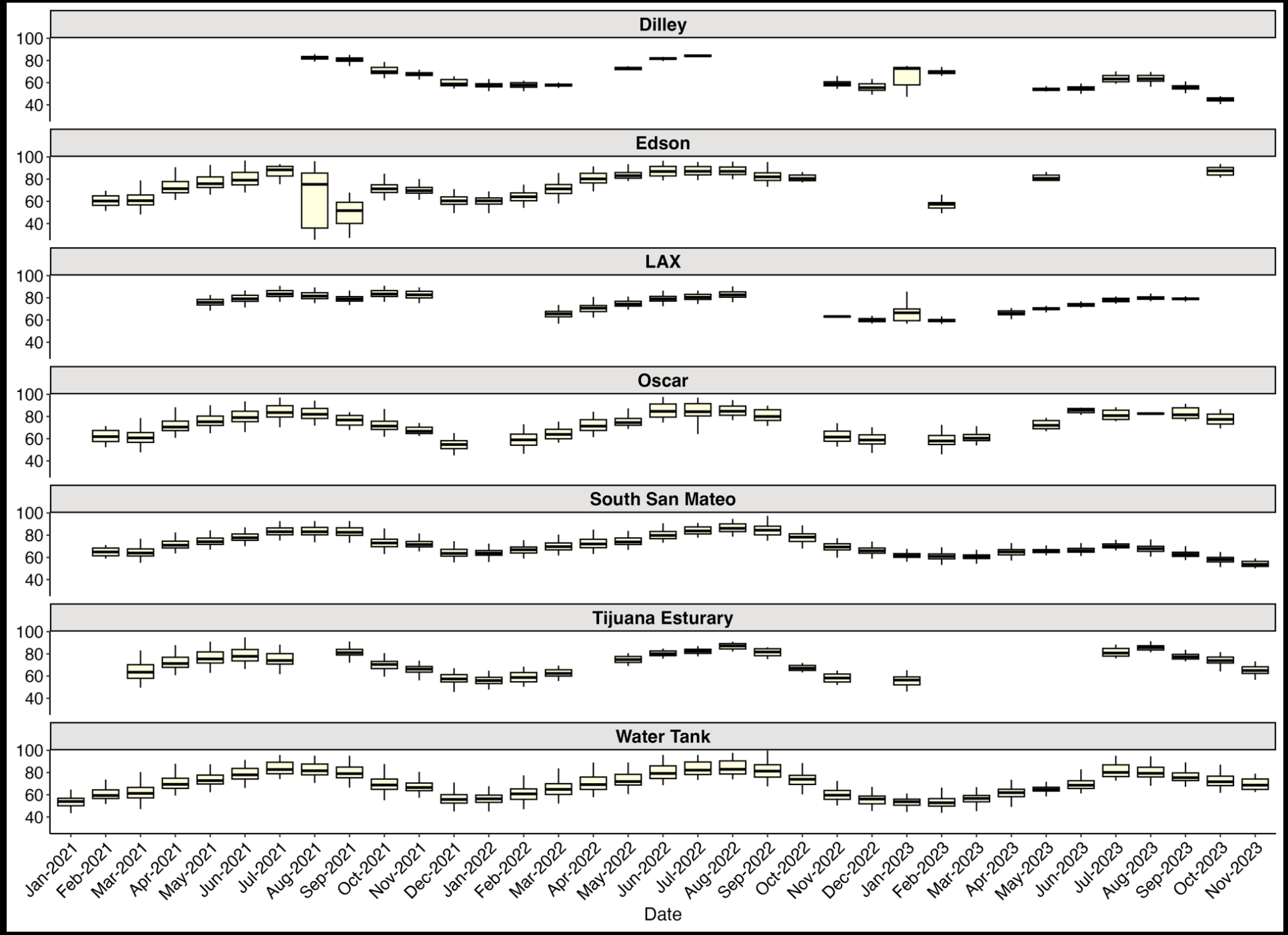
Moisture m3/m3
4 inches

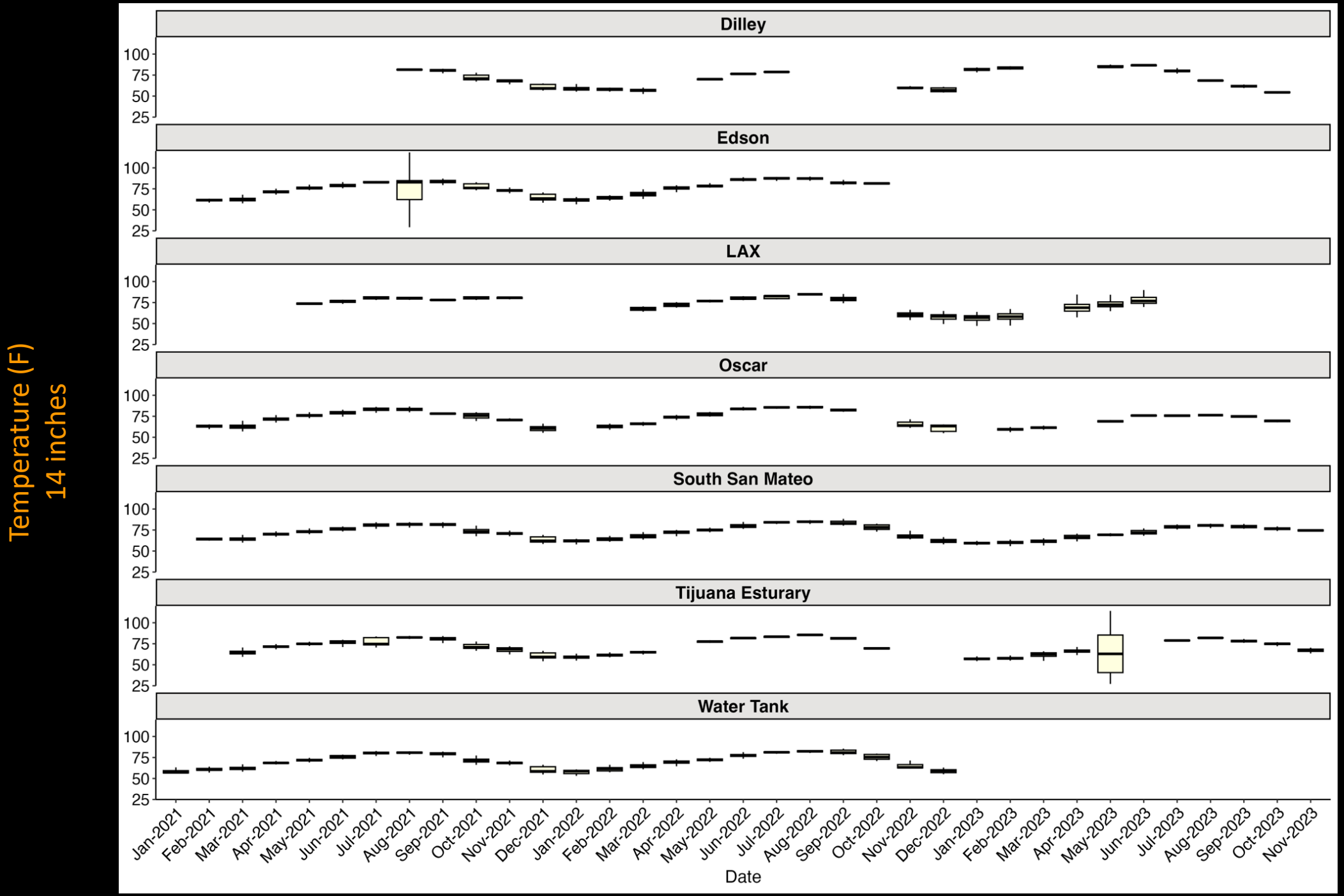


Moisture m3/m3
14 inches



Temperature (F)
4 inches




Temperature (F)
14 inches
Dilley
Edson
LAX
Oscar
South San Mateo
Tijuana Esturary
Water Tank
100
75
50
25
Jan-2021
Feb-2021
Mar-2021
Apr-2021
May-2021
Jun-2021
Jul-2021
Aug-2021
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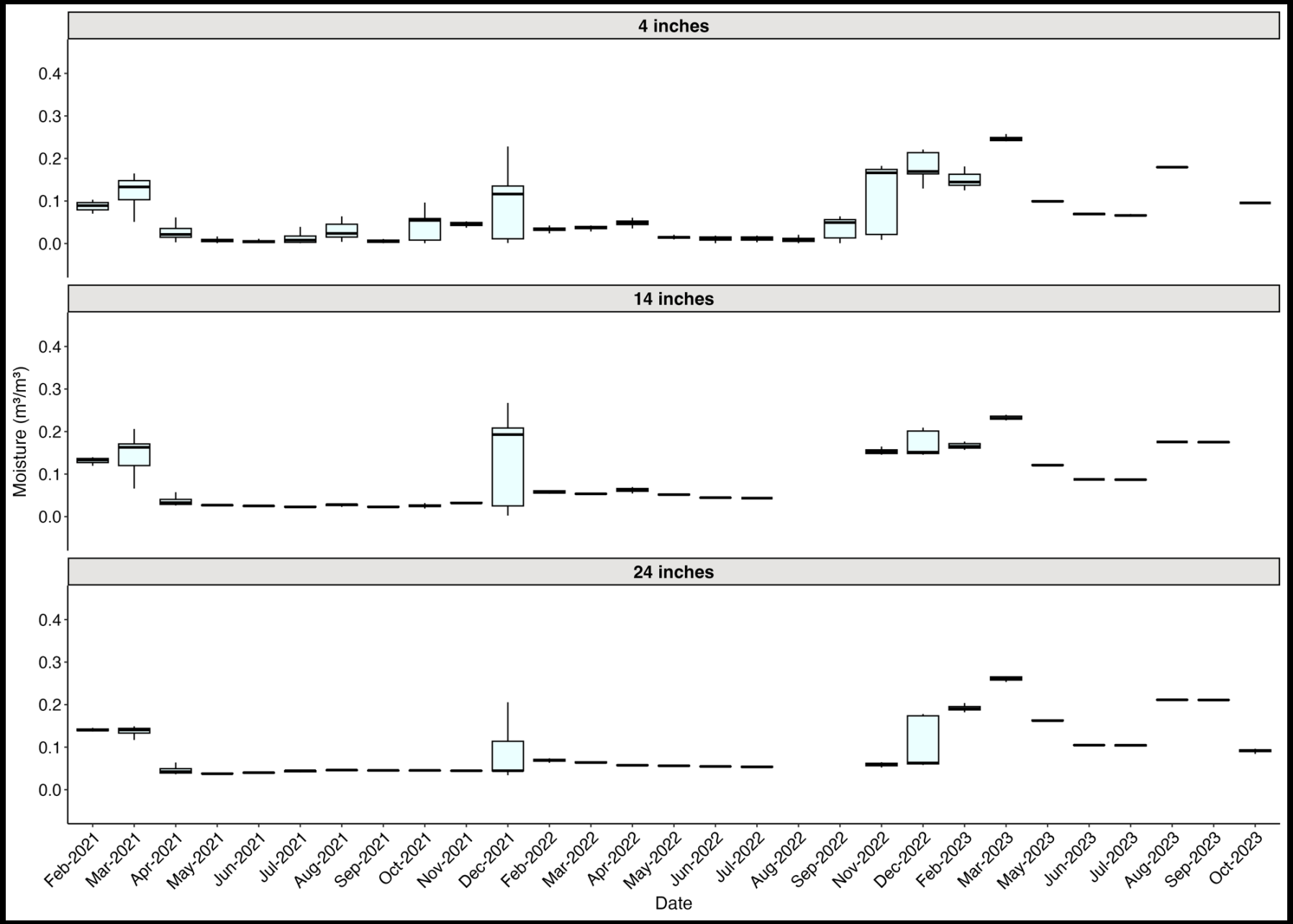
Sep-2023
Oct-2023
Nov-2023
Date

Santa Margarita – Oscar One

Moisture m3/m3



Current and Future Plans

- Cell line development
- Genomics (Aryn Wilder/Erik Funk)
- Microbiome (Candace Williams/UCSD)
- Captive housing
  - Enrichment (cognitive challenge)
  - Seasonal temperature
- Reproduction
  - Multiple mating
- Survival skills and training
  - Antipredator (exposure vs training; dam experience)
  - Interspecific experience
- Reintroduction Methods
  - 7 vs 14 day acclimation
  - Full acclimation protocol vs management reintroduction
- Habitat suitability/Receiver site selection
  - burrow architecture
  - soil

- Stress – cortisol vs corticosterone
- Foraging preferences and Cache pilfering
- Torpor and anthropogenic noise
- Using cameras, quantify activity budget, and heterospecific interactions

Other activities:

- Outreach and Education
  - Talks to local schools and development of educational module
- Conservation standards process

Breeding and Reintroduction(s) Plans 2023

Breeding

- Near capacity
- Until reintroduction, limit breeding to high priority females (founders, etc.)

Reintroduction Site 1 - Water Tank

- Habitat Management
- Trap April Overwinter Survival
- Decisions

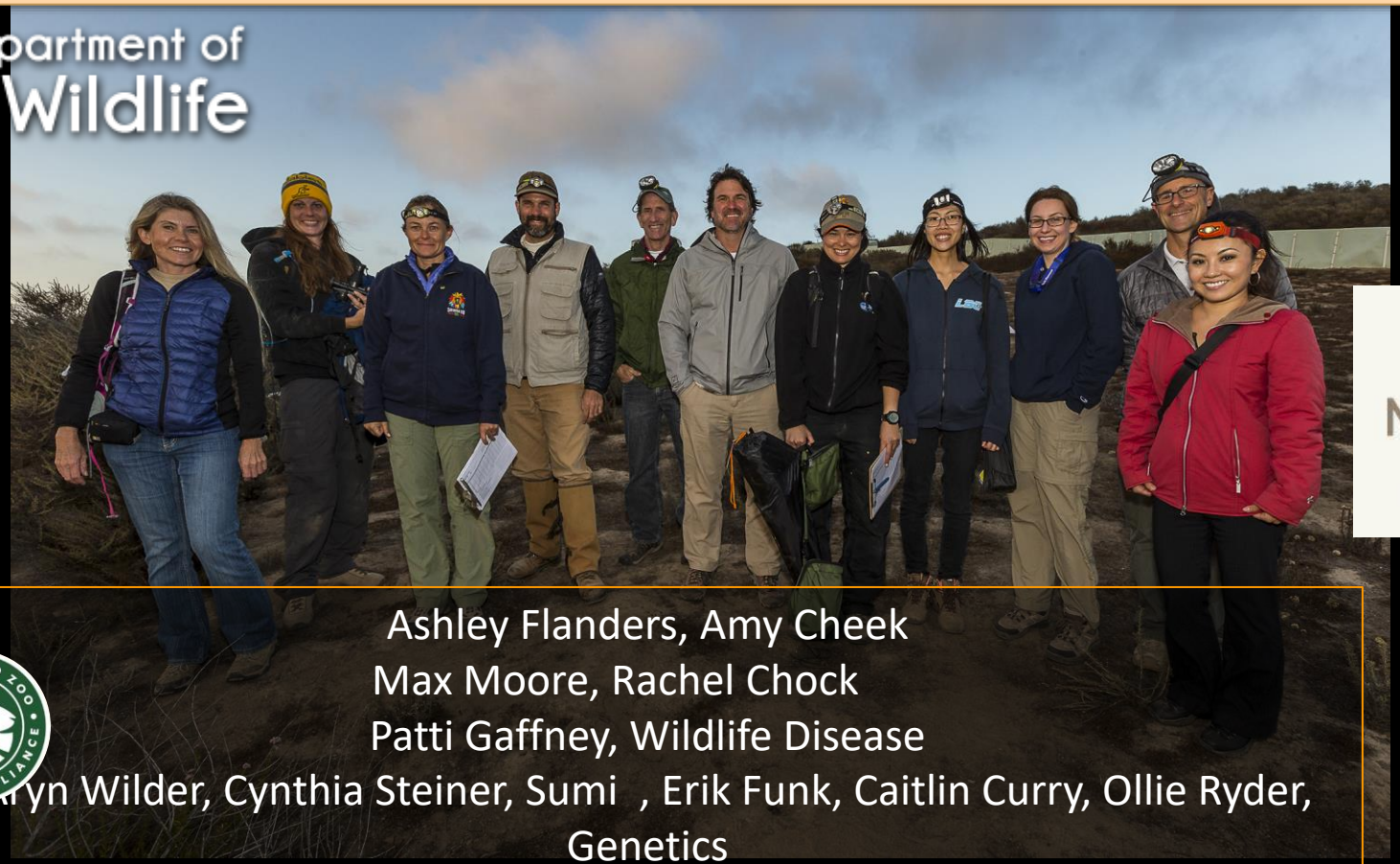
Reintroduction Site – Wire Mountain

- SWOT (Rachel)
- Decision to proceed?
- Research
  - Habitat cover suitability
  - Heterospecific experience - CHCA

Reintroduction Site - Dilley

- Proposal
- Habitat Assessment May/June
- Habitat Management

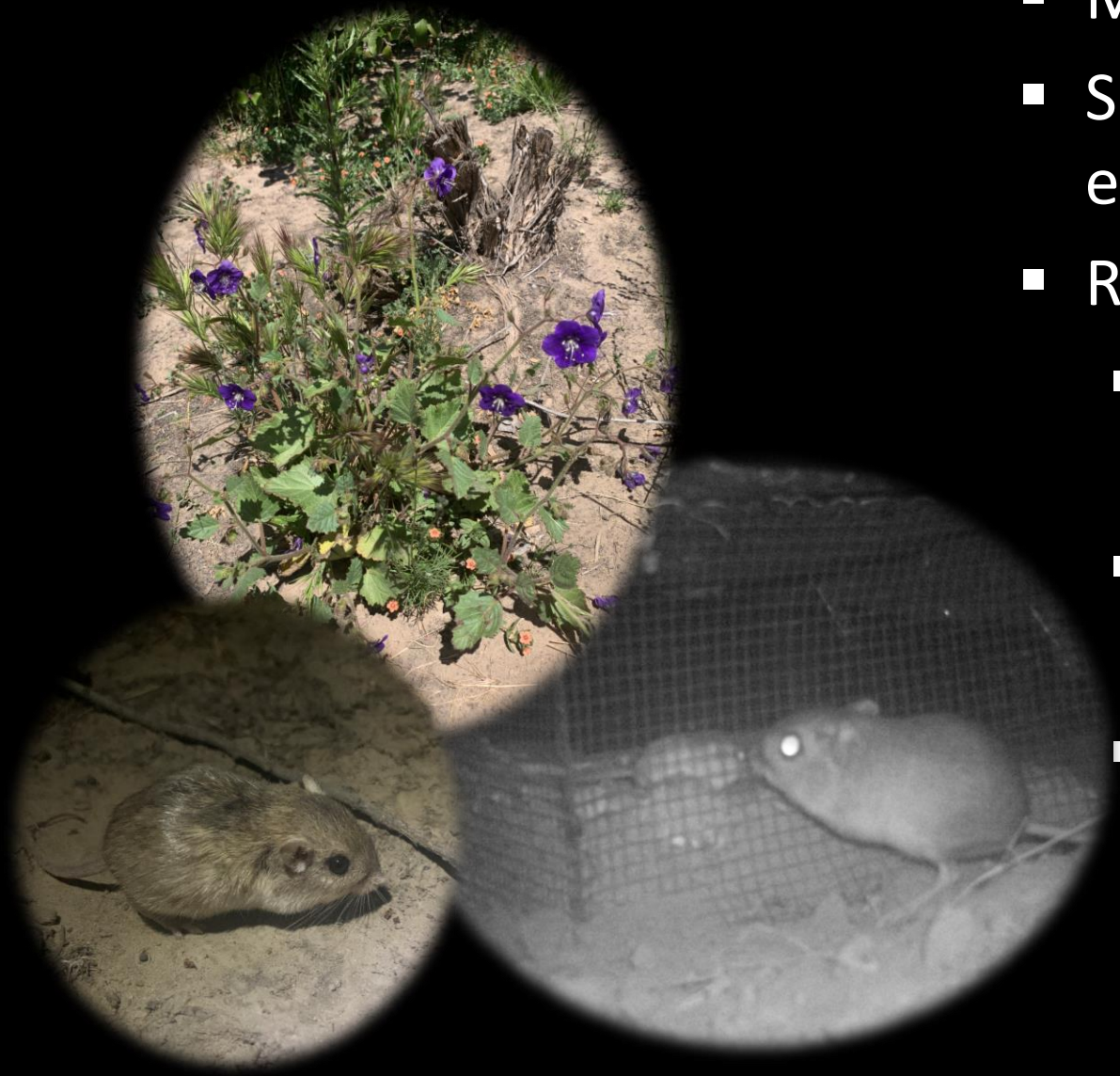
Team and Partners



Ashley Flanders, Amy Cheek
Max Moore, Rachel Chock
Patti Gaffney, Wildlife Disease
aryn Wilder, Cynthia Steiner, Sumi , Erik Funk, Caitlin Curry, Ollie Ryder, Genetics
Jennifer Damato-Anderson, Nutrition, Jim Haigwood, Collections, Park Vet Team, Countless dedicated volunteers

Henry W. & Ellen R Warne Family Endowment Fund Grant

2024 Plans



Reintroduction Population/Research

- Monitor release population
- Supplementations and expand into existing enclosures
- Research:
  - Habitat preference of reintroduced and dispersing PPM
  - Enhancing forb and native grass abundance
  - Using cameras, quantify activity budget, and heterospecific interactions

Research: Predictors of past Reproductive Success

ADAPTIVE MANAGEMENT TAKE HOME

Prioritize full scrotal males for breedings

Select results

Copulation ↑ Scrotal, not Partially Scrotal testes

(Binomial GLMM, N= B = 0.56±0.24, RI = 1.0)

Pregnancy ↑ Scrotal, not Partially Scrotal testes

(Binomial GLMM, B = 1.03±0.48, z = 2.15)

Litter size ↑ F weight during pregnancy

(Poisson GLMM, B = 0.14±0.04, RI = 1.0)

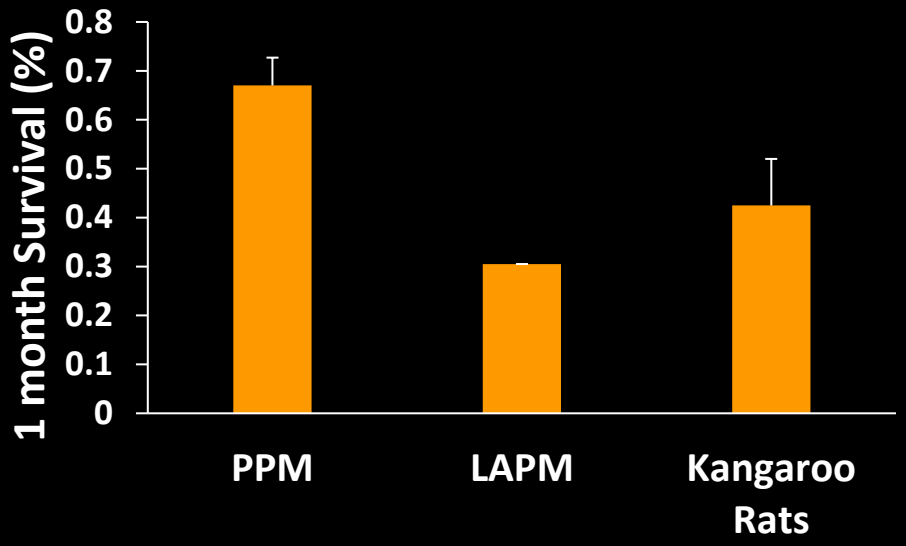
No predictors of weaning success

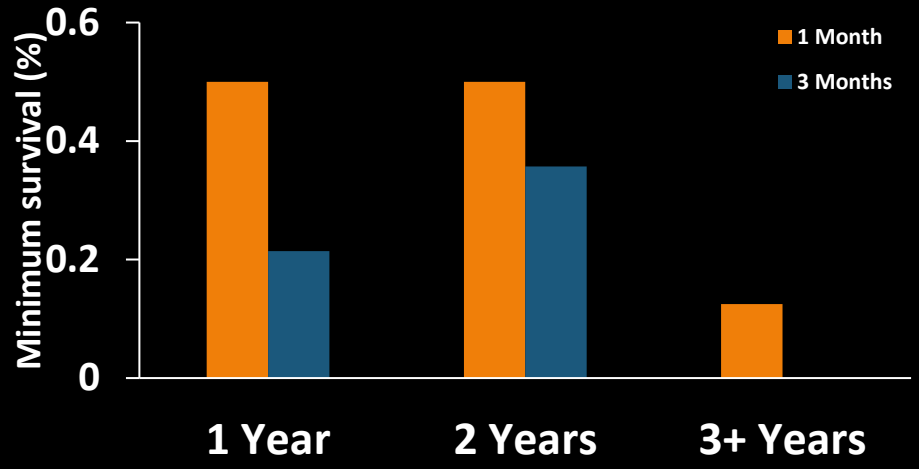
* Have not yet analyzed the effect of multiple mates

Reintroduction Success Metrics

Founders Settle → **Survive** → Population Growth (Reproduction) → Population Regulates → Population Viability

Short-term Survival





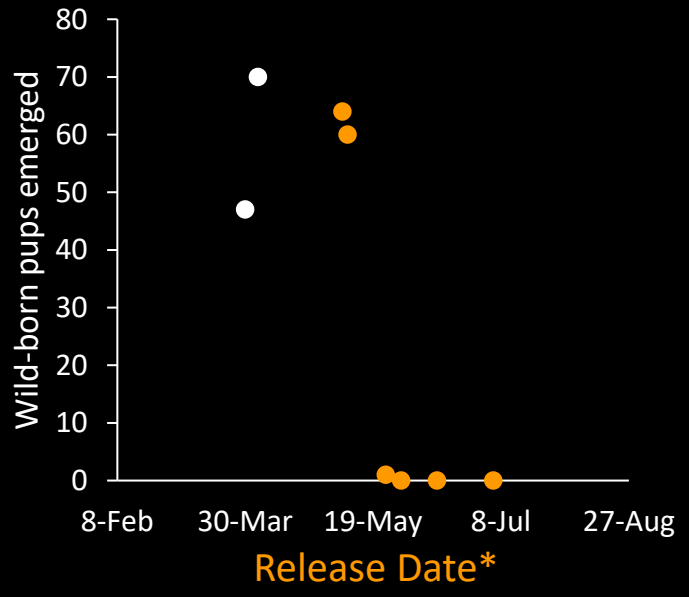
****with our protocols PPM have shown high early survival post release in comparison to efforts with other related species.**

Reintroduction Success Metrics

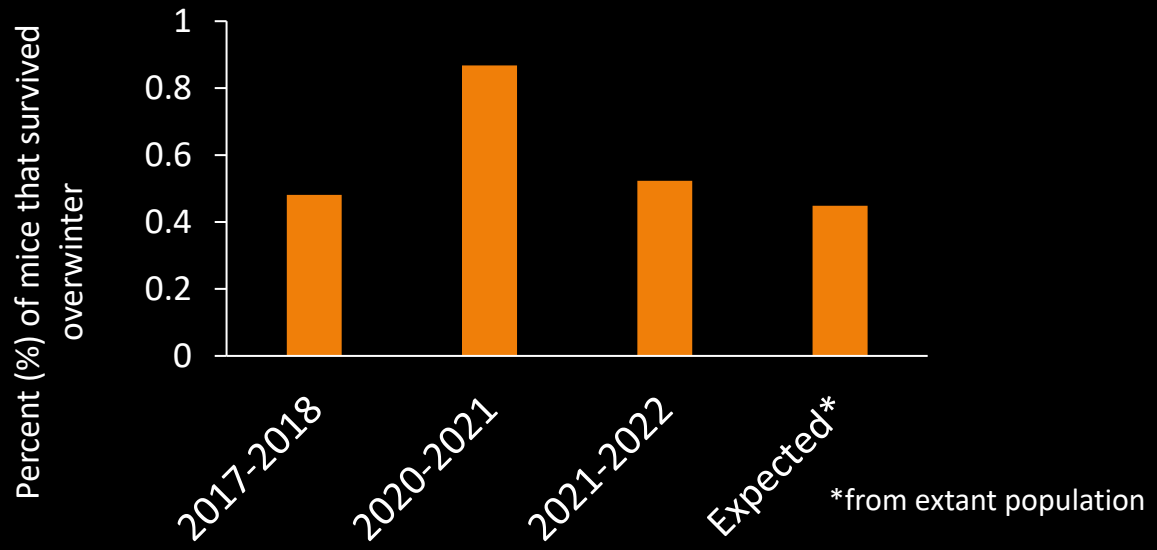
Founders Settle → **Survive** → Population Growth → Population Regulates → Population Viability

Reproduction in year of release, Over-winter survival, Reproduction and Spring/summer Survival

Reproduction



Overwinter Survival



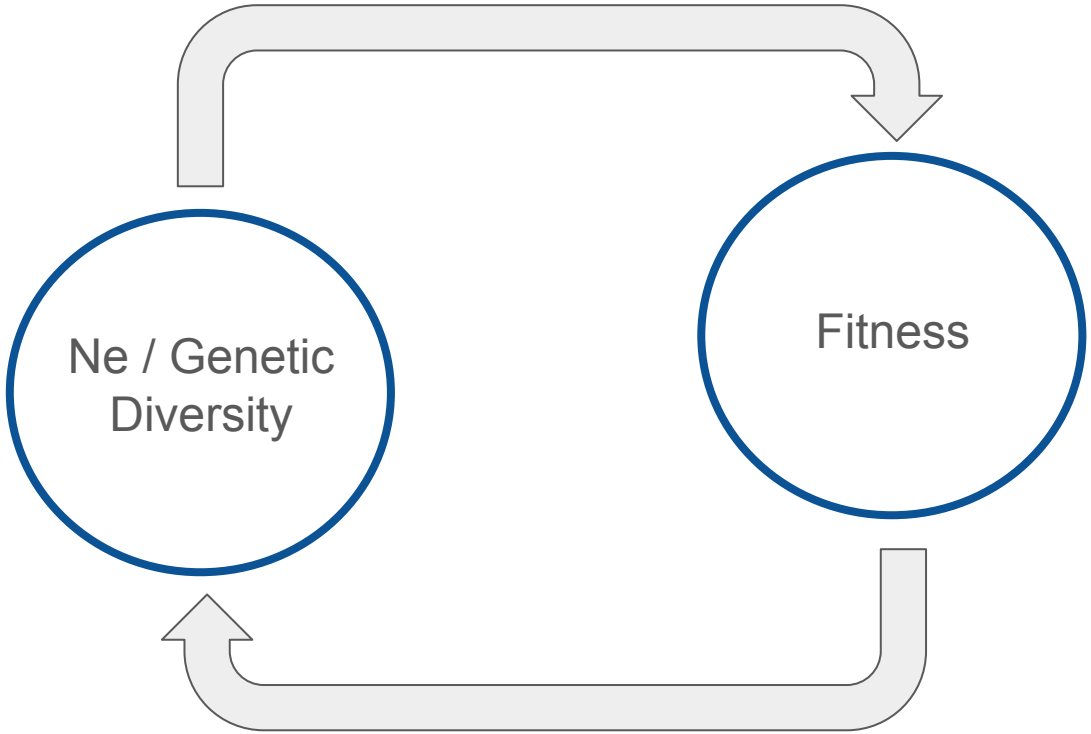
Pacific pocket mouse: Planned Research

PPM working group meeting
13 February 2024

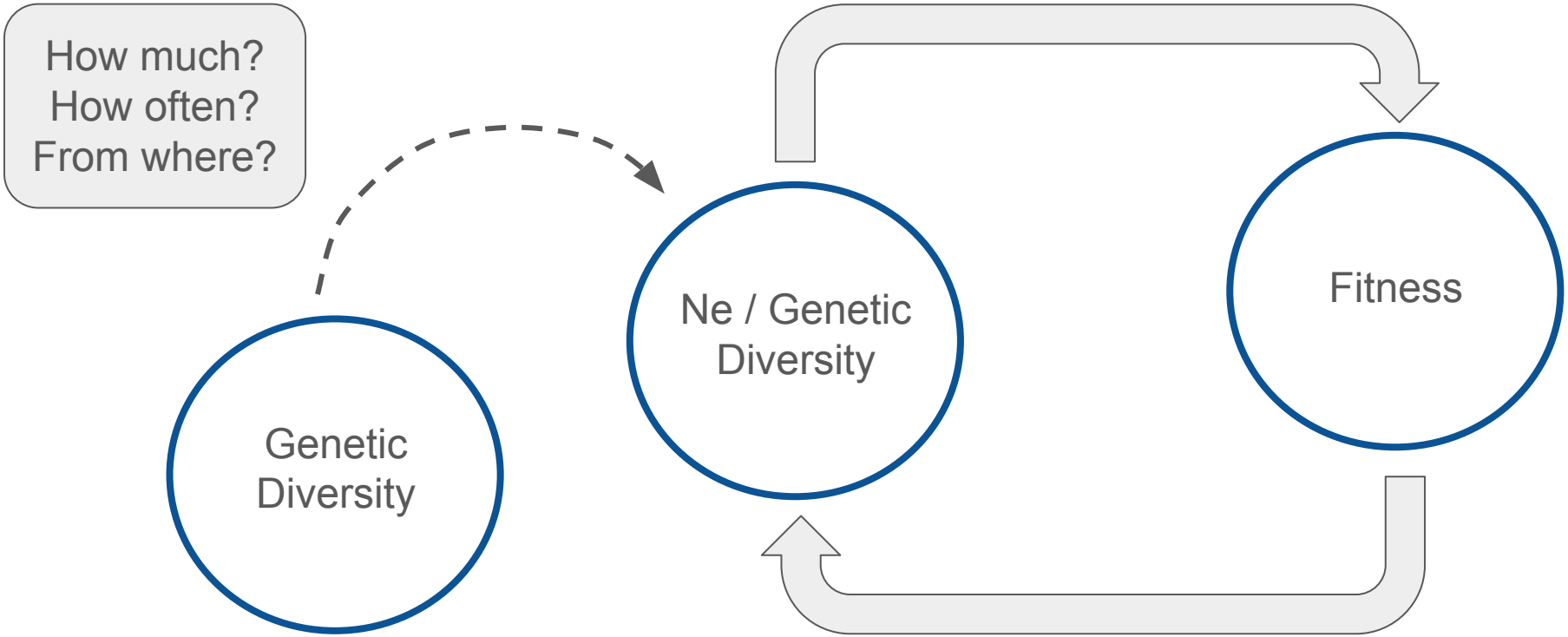
Erik Funk
Postdoctoral Researcher

San Diego Zoo Wildlife Alliance

What strategies maximize population persistence?



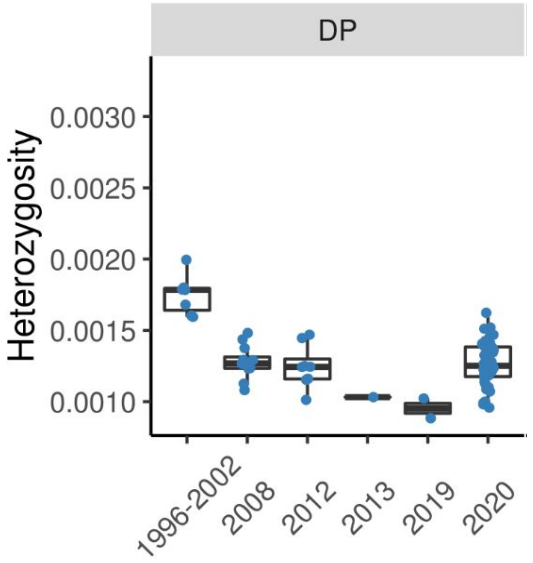
What strategies maximize population persistence?



Proposal in review: Genomic erosion and population viability of Dana Point Pacific pocket mice

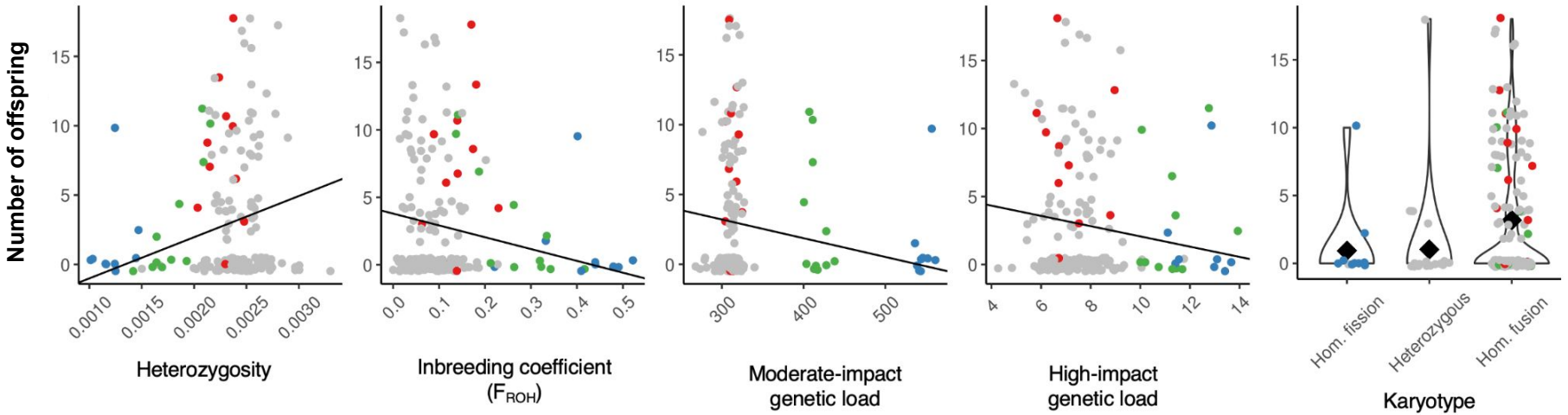
Objective 1. Quantify genetic changes at Dana Point over the long and short terms

- Sequence historical samples from ~1930s and new samples from 2023
- Quantify changes in genetic diversity, inbreeding, and load
- Model demographic changes



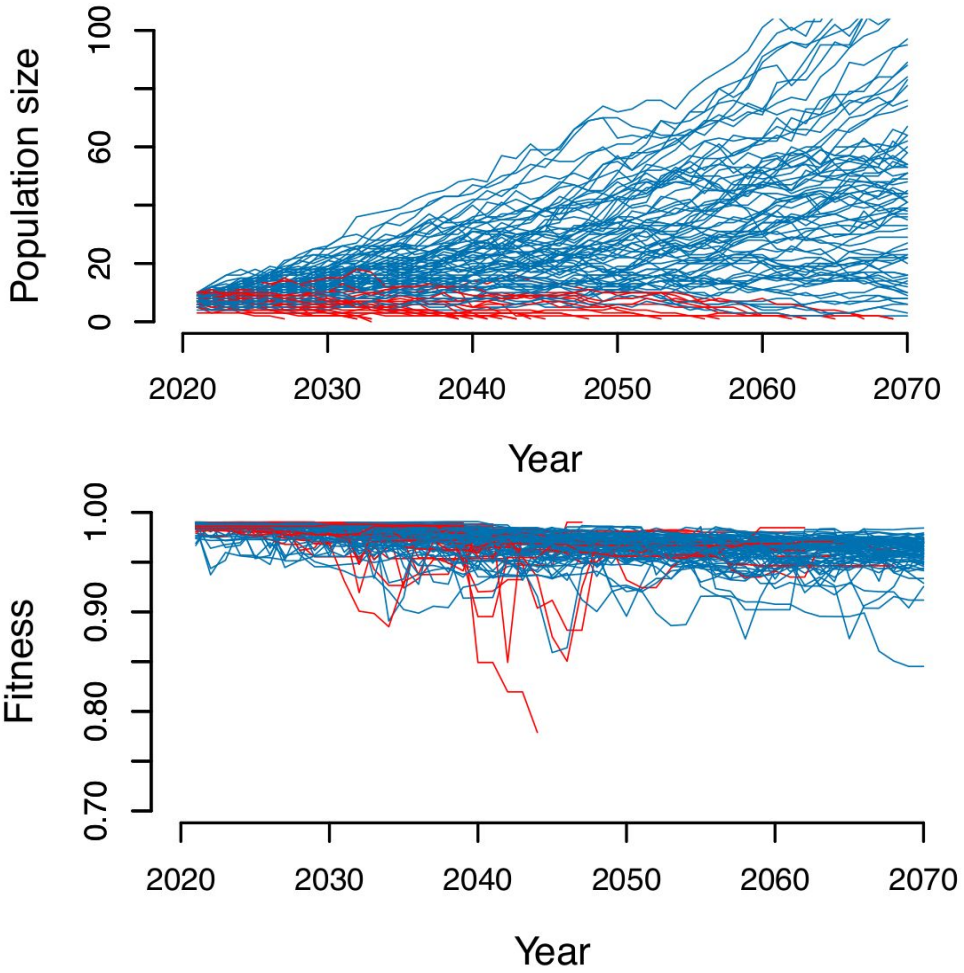
Objective 2. Evaluate the effects of translocation on long-term viability in Dana Point

Integrate empirically-informed estimates of fitness with forward-time simulations.



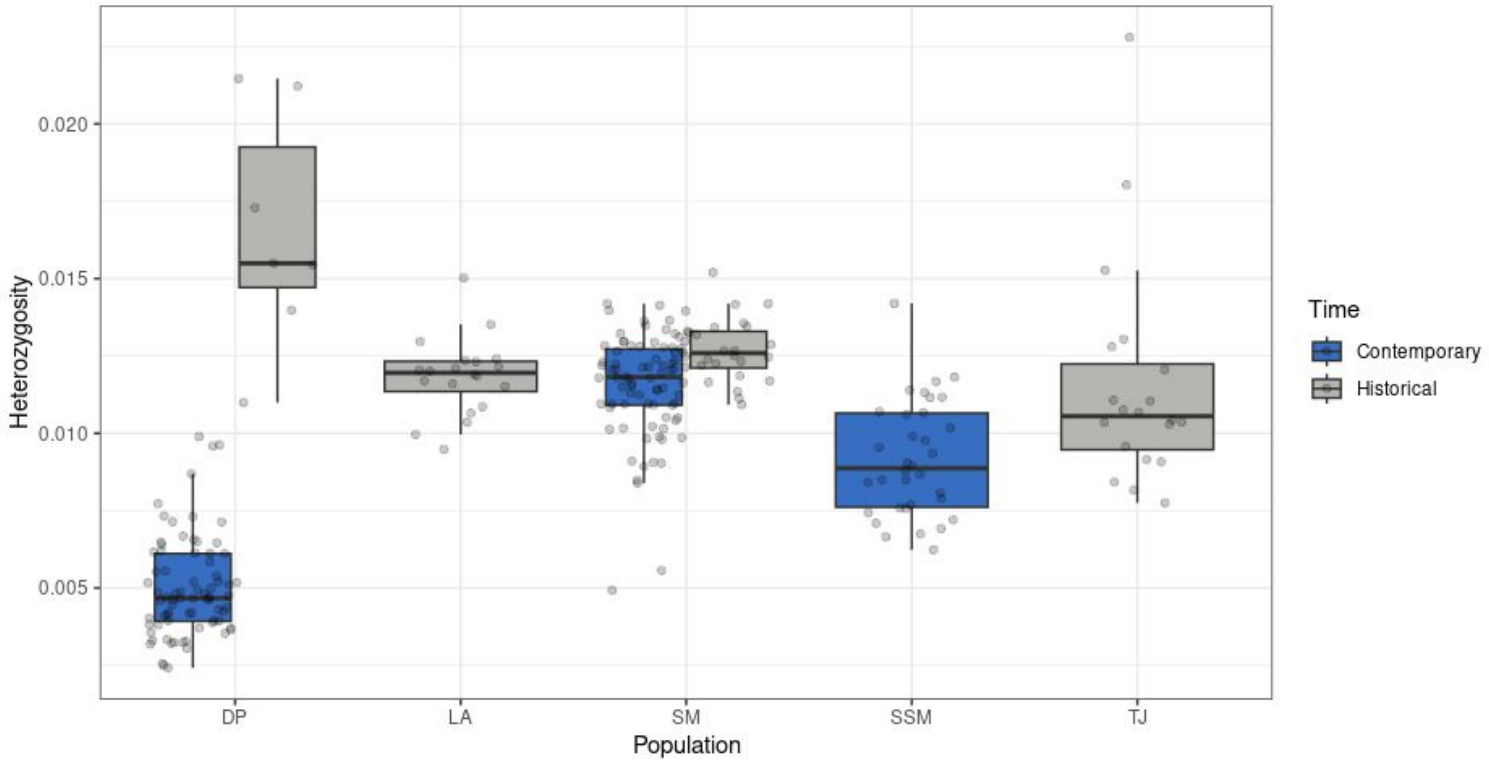
O2. Dana Point viability

- Examples from Vaquita (Robinson et al., right) and Rhino (Wilder et al.)
- Count proportion of iterations that move to extinction.
- Can test the effect of different translocation strategies.



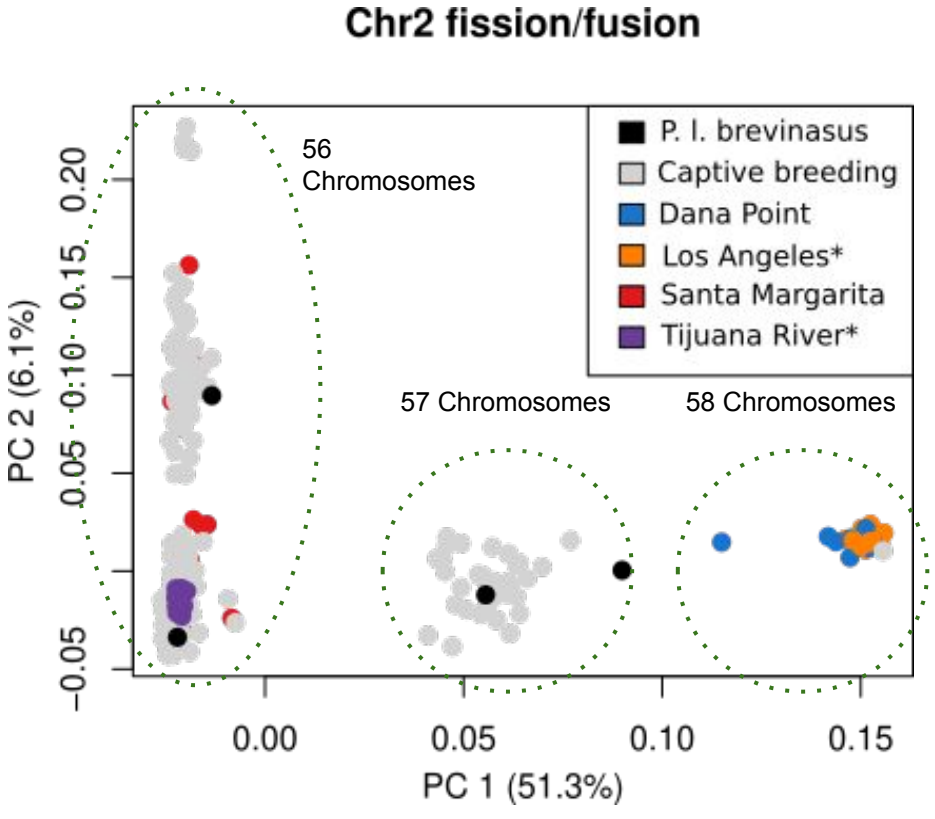
Robinson et al. 2022. Science.

Preliminary data - Historical heterozygosity



Preliminary data - Chr2 fission/fusion

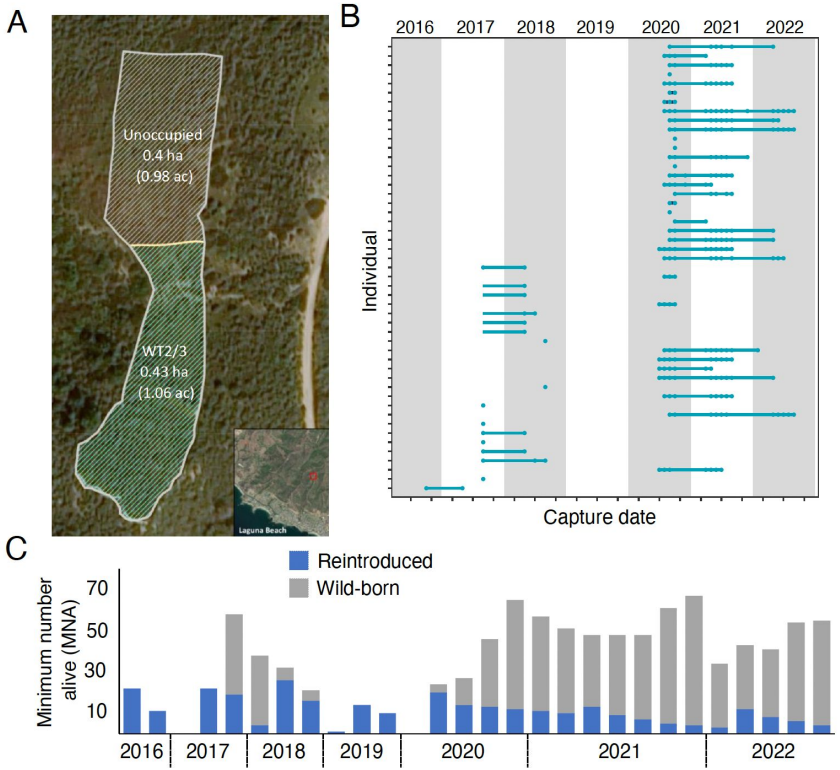
- Chromosome 2 fission may exist in other longimembris subspecies
- Can provide insight into the effects of chromosome number on fitness



Proposal in review: Genomic correlates of survival and reproduction in Laguna Coast Wilderness Park

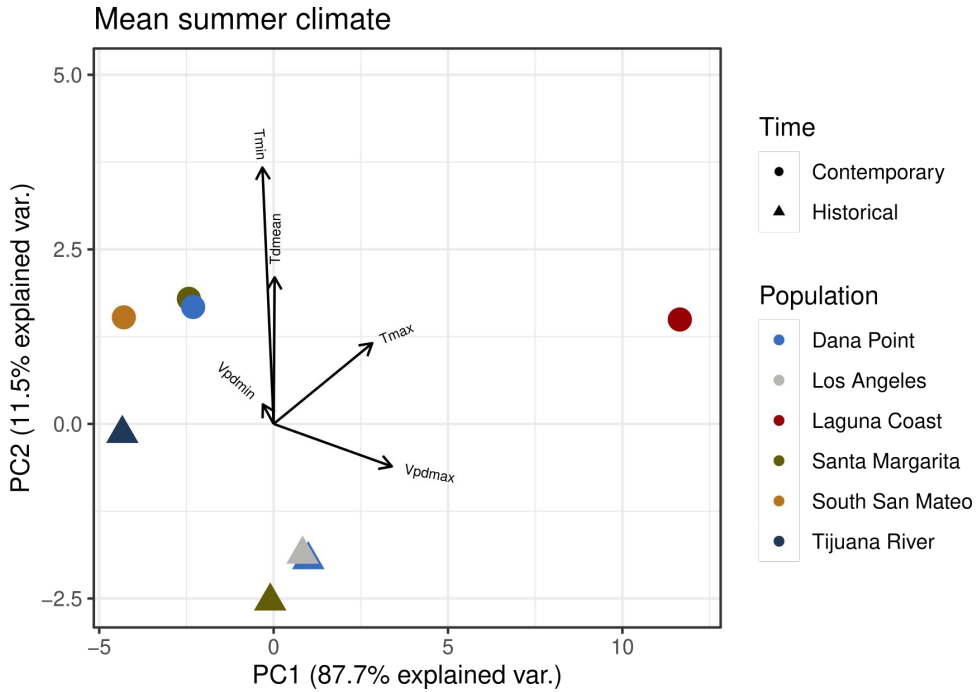
Objective 1: Estimate fitness coefficients using recapture data.

- Sequence the remaining released and wild born individuals
- Estimate minimum number of days alive
- Test for correlations with heterozygosity, inbreeding, chromosome number, and load using Kaplan-Meier survival curves

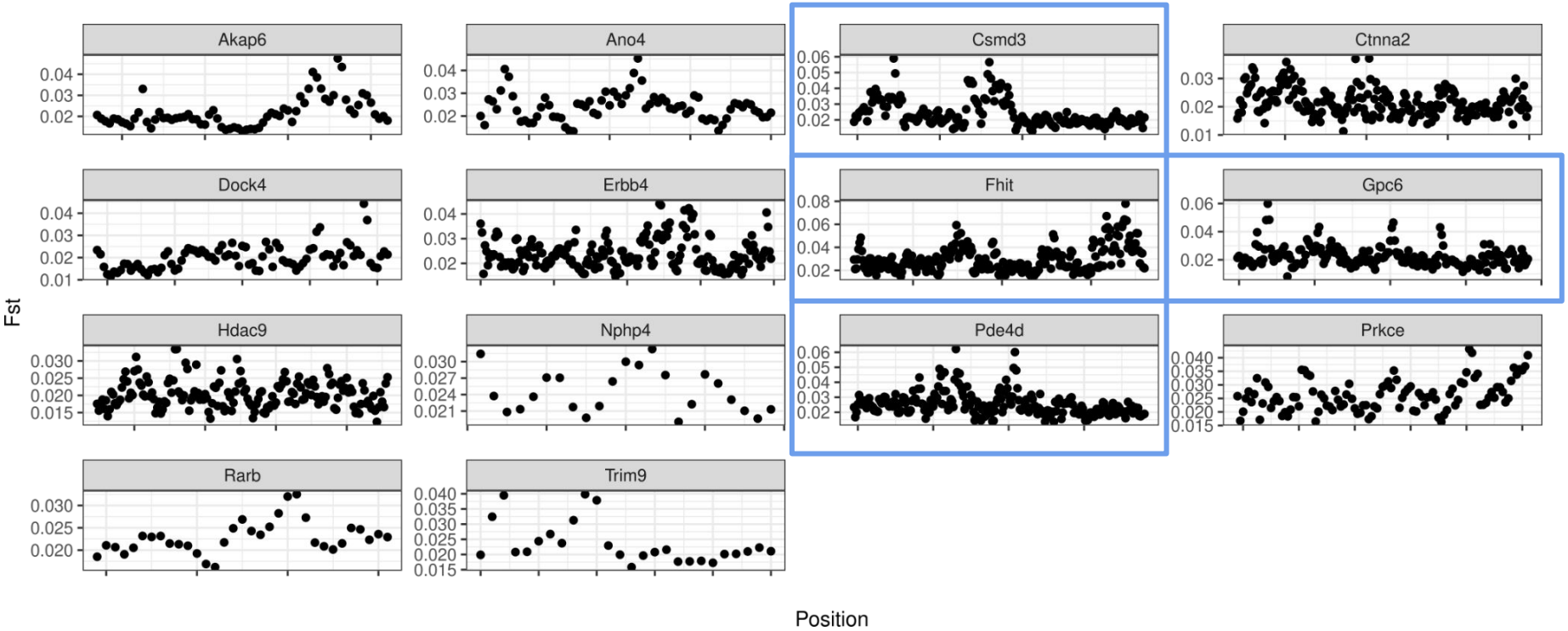


Objective 2: Track changes in allele frequency of climate associated variants.

Current work tests for correlations between genetic variation and climate

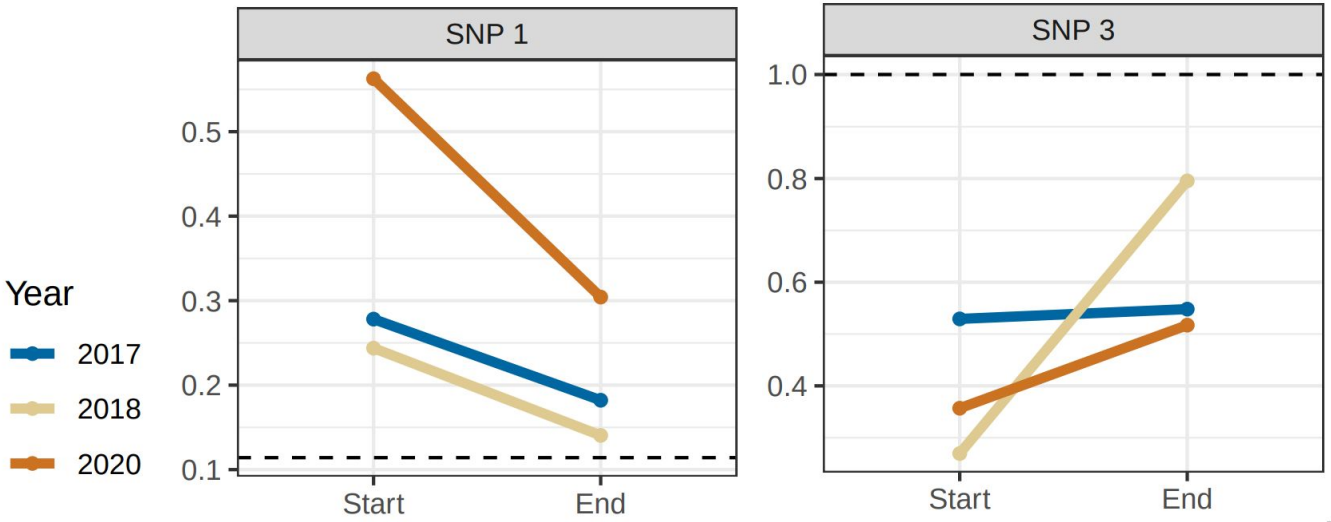


Objective 2: Track changes in allele frequency of climate associated variants.



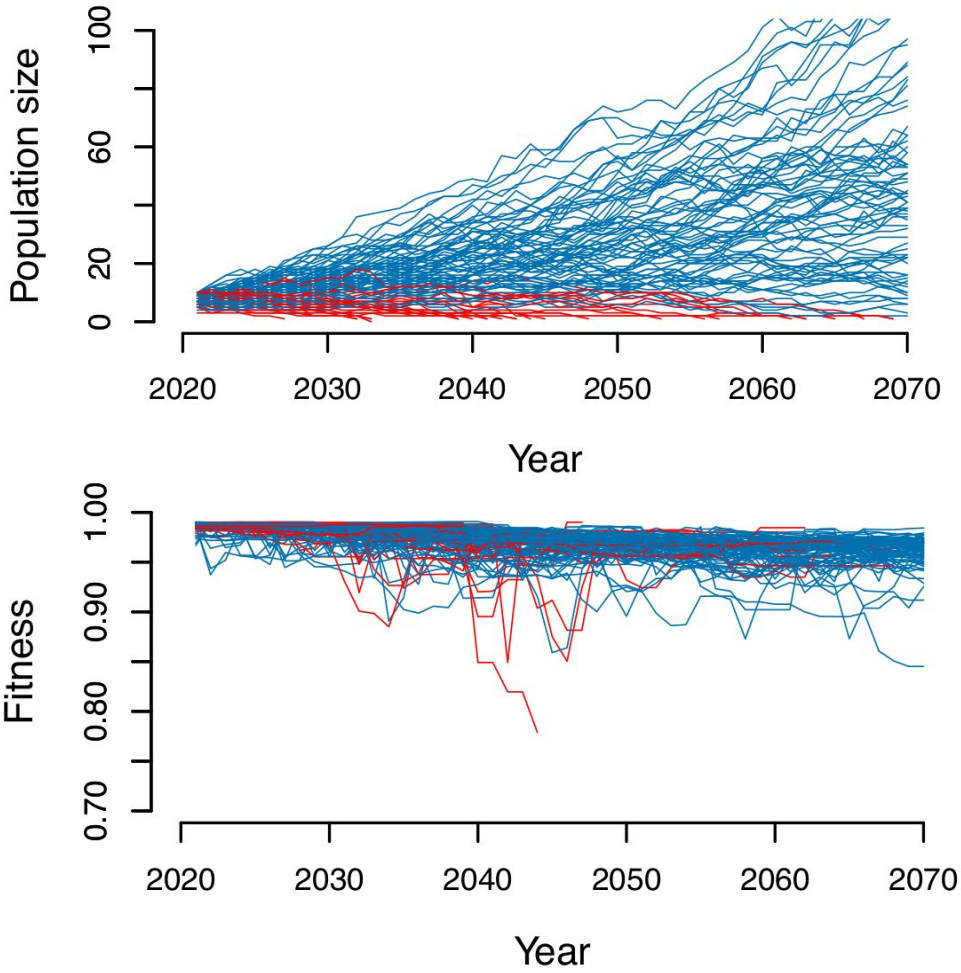
Objective 2: Track changes in allele frequency of climate associated variants.

- 14 genes identified through previous associations with climate.
- Measure allele frequency shifts and selection coefficients.



Objective 3: Simulate different levels of supplementation for population sustainability.

- Proportion of iterations that move to a population size of 0 indicate probability of extinction.
- Can test the effect of different reintroduction strategies.



Robinson et al. 2022. Science.

Planned Research

1. Quantify genomic erosion and assess rescue strategies in Dana Point
    a. Sequence the latest round of 25 mice captured during 2023 season
    b. Use historical and contemporary sequence data to quantify changes in heterozygostiy, load, and inbreeding over the past 100 years.
    c. Use simulation and empirical fitness estimates from captivity to evaluate the viability of Dana Point, and the effects of different translocation strategies.
2. Estimate fitness and population persistence in Laguna Coast Wilderness Park
    a. Sequence all individuals and estimate fitness by correlating genetic diversity with recapture survival data
    b. Track allele frequency changes at putative candidate climate loci
    c. Use simulation and empirical fitness estimates to evaluate the viability of Laguna Coast Wilderness Park, and the effects of different reintroduction strategies.

San Diego Zoo Wildlife Alliance

Erik Funk
Postdoctoral Researcher

PPM Working Group Feb. 13, 2024 Presentations

1 message

Miller, William B. <william_b_miller@fws.gov> Tue, Feb 20, 2024 at 8:44 AM
To: "Adsit-Morris, Devin T" <dadsit-morris@usgs.gov>, "james.asmus@usmc.mil" <james.asmus@usmc.mil>, "brad.barker@ocparks.com" <brad.barker@ocparks.com>, Abby Bratt <abby@proteus.co.nz>, "Brehme, Cheryl S" <cbrehme@usgs.gov>, "Burlaza, Melanie@Wildlife" <melanie.burlaza@wildlife.ca.gov>, Rachel Chock <RChock@sdzwa.org>, "Edgarian, Tristan K" <tedgarian@usgs.gov>, "Fisher, Robert N" <rfisher@usgs.gov>, "Brachylophusgau@gmail.com" <Brachylophusgau@gmail.com>, "Gray, Emily@Wildlife" <emily.gray@wildlife.ca.gov>, Alison Greggor <agreggor@sdzwa.org>, "darryl@proteus.co.nz" <darryl@proteus.co.nz>, "Major, Matt" <Matt.Major@ocparks.com>, "Tiffany M. Mcfarland" <tmmcfarland@ag.tamu.edu>, Korie Merrill <kmerrill@cnlm.org>, "Miller, William B." <william_b_miller@fws.gov>, Jennifer Naegele <Jennifer.naegele@ocparks.com>, lana meade <Lana.Nguyen@parks.ca.gov>, Barbara Norton <Barbara.Norton@ocparks.com>, Leonard Nunney <leonard.nunney@ucr.edu>, Zachary Principe <zprincipe@tnc.org>, Redetzke CIV Nathaniel R <nathaniel.redetzke@usmc.mil>, "Rice, Kyle@Wildlife" <kyle.rice@wildlife.ca.gov>, "Roberts, Carol" <carol_a_roberts@fws.gov>, "Dianne H. Robinson" <Dianne.Robinson@ag.tamu.edu>, Deborah Rogers <drogers@cnlm.org>, JEFF ROSALER <JROSALER@danapoint.org>, Oliver Ryder <ORyder@sdzwa.org>, Debra Shier <DShier@sdzwa.org>, "Sin, Hans@Wildlife" <Hans.Sin@wildlife.ca.gov>, "Snyder, Jonathan" <Jonathan_d_Snyder@fws.gov>, "andrea.souther@usmc.mil" <andrea.souther@usmc.mil>, Cynthia Steiner <CSteiner@sdzwa.org>, jim sulentich <jsulentich@occonservation.org>, Sullivan CIV Sherri <sherri.sullivan@usmc.mil>, "Terp, Jill" <Jill_Terp@fws.gov>, Thomas CIV Kristin H <kristin.thomas@usmc.mil>, scott tremor <stremor@sdnhm.org>, Sadie Trombley <strombley@sdzwa.org>, "Vandergast, Amy" <avandergast@usgs.gov>, "bvillanueva@danapoint.org" <BVillanueva@danapoint.org>, Aryn Wilder <awilder@sdzwa.org>, Candace Williams <cwilliams@sdzwa.org>, alisa zych <alisa.zych@usmc.mil>

All- I have uploaded the presentations from the recent PPM Working Group meeting to a fileshare site (link below) should you wish to view and download. Thanks for a productive meeting! - Will

https://fws-fileshare.box.com/s/swdck8wqxtzm9aybbwkvrslbqfewnrmo

William B. Miller, Biomonitor
U.S. Fish and Wildlife Service
Carlsbad Fish and Wildlife Office
2177 Salk Avenue, Suite 250
Carlsbad, California 92008
(760) 431-9440 Ext. 206
William_B_Miller@fws.gov

Pronouns: *He, Him, His*

PPM collections from Dana Point population

Deborah Rogers <drogers@cnlm.org> Mon, Jun 5, 2023 at 8:14 AM
To: "Miller, William B." <william_b_miller@fws.gov>
Cc: Korie Merrill <kmerrill@cnlm.org>, "Sin, Hans@Wildlife" <Hans.Sin@wildlife.ca.gov>, "Snyder, Jonathan" <Jonathan_d_Snyder@fws.gov>, "Emily@Wildlife" <emily.gray@wildlife.ca.gov>, "Karen@Wildlife" <Karen.Drewe@wildlife.ca.gov>, david mayer <david.mayer@wildlife.ca.gov>, Aryn Wilder <awilder@sdzwa.org>, Debra Shier <DShier@sdzwa.org>, "Brehme, Cheryl S" <cbrehme@usgs.gov>, "Fisher, Robert N" <rfisher@usgs.gov>, "Roberts, Carol" <carol_a_roberts@fws.gov>

Good morning, Will.

The email exchange has illuminated some areas to revise in the draft Genetic Management Plan (GMP) and also the need to prepare annual work plans for implementation. Just as a preserve management plan provides context, objectives, and strategies -- but requires annual work plans for details that may be condition-specifici and also to allow for adaptive management -- so too, does the implementation of the draft GMP. For example, a predicted April-May 'collection period' for mice requires putting together a specific and detailed plan for review and approval well in advance of this period as well as an agreement -- such as that which was executed in May 2019 between CNLM and San Diego zoo.

As described in our email of May 12th, there is continuing value in collecting more genetic information on the Dana Point PPM population, given that the current stats are based on restricted sampling over a relatively short period of time. We had suggested some trapping -- later in the summer, perhaps July -- to collect some demographic data and tissue samples for further genetic analysis. We also provided information on the likely status of the mice, indicating that PPM experts had advised it was probably already too late to be collecting PPM without causing unintentional harm given evidence of reproductive status at Camp Pendleton. We recommended that if take was to occur for crossing experiments this calendar year, it would be better to take young or post-lactation females a few months from then. I indicated that, as such, we still had some time to make the decision and to develop a more detailed plan.

For additional animals to be taken from Dana Point this year -- presumably in July or August (best time TBD with input from USGS scientists who are best positioned to advise based on status of mice at Camp Pendleton) -- reasonably, there would need to be a specific plan which provides more detail than in the draft GMP, including:
* number of trap nights proposed
* determination and flagging of route to be followed from trap to trap to minimize disturbance to habitat
* description of how abundance will be computed
* the maximum percentage of PPM for take that will have no impacts to the population
* criteria for taking mice for mice (sex, age)
* criteria for stopping trapping effort early (e.g., death or injury to target or nontarget species)
* commitment for regular information sharing on status of mice that are taken from Dana Point
* description of all experiments to be performed on DP mice
* commitment to taking tissue samples and processing these samples as quickly as possible to add to the genetic information of the population

If you can provide this information -- which is no doubt readily available but I don't see all of this in the draft GMP or in recent emails -- I can draft a 'Site Access for Research Agreement' . Please advise as to whether this agreement would be between CNLM and USFWS or the San Diego Zoo (it was the latter for the previous take (2017) of PPM for the captive breeding program).

Of course, this agreement would be for this season only and would not imply agreement either to future years' take of PPM or for introduction of PPM hybrids, if achieved, at some later date. Adaptive management will be critical to maintain -- with continued insight into the genetic status of the (still) wild population at Dana Point, and other knowledge or conditions that may be relevant. Detailed plans (as well as agreements) would be needed for all interventions that involve taking or adding mice.

Best,
Deborah

On Fri, Jun 2, 2023 at 1:58 PM Miller, William B. <william_b_miller@fws.gov> wrote:

Dear Deborah and Korie- Thought I would check in to see if you have reconsidered our request to collect PPM at Dana Point this year in light of Aryn Wilder's email of May 24? Thanks in advance for your prompt attention. - Will

William B. Miller, Biomonitor
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Carlsbad, California 92008
(760) 431-9440 Ext. 206
William_B_Miller@fws.gov

Pronouns: *He, Him, His*

From: Aryn Wilder <awilder@sdzwa.org>
Sent: Wednesday, May 24, 2023 8:49 AM
To: Deborah Rogers <drogers@cnlm.org>; Debra Shier <DShier@sdzwa.org>
Cc: Korie Merrill <kmerrill@cnlm.org>; Sin, Hans@Wildlife <Hans.Sin@wildlife.ca.gov>; Snyder, Jonathan <Jonathan_d_Snyder@fws.gov>; Emily@Wildlife <emily.gray@wildlife.ca.gov>; Karen@Wildlife <Karen.Drewe@wildlife.ca.gov>; david mayer <david.mayer@wildlife.ca.gov>; Brehme, Cheryl S <cbrehme@usgs.gov>; Fisher, Robert N <rfisher@usgs.gov>; Miller, William B. <william_b_miller@fws.gov>; Roberts, Carol <carol_a_roberts@fws.gov>
Subject: [EXTERNAL] Re: Query regarding PPM collections from Dana Point population

Dear Deborah,

Thank you for your detailed and thoughtful message. I agree that we are all working toward the same goal of conserving PPM, and open dialogue and sharing of ideas and information are essential to this goal. To this end, we wanted to weigh in to the conversation.

First, we want to reiterate what Will and Carol pointed out—that the Genetic Management Plan has undergone peer review by experts in genetics and heteromyid biology and evolutionary history. These experts agree that, while some details remain to resolved and the GMP needs polishing, the overall conclusions and conservation recommendations are sound and based on the best science and data available. And we have far more data than are available for nearly any other endangered species; whole genome sequences from hundreds of PPM, including ~70 PPM from Dana Point, collected over the past two decades, with matched survival and reproduction data for several hundred of them. Waiting for yet more data and a perfect understanding of every aspect of the system only delays conservation action that is needed given the loss of genetic diversity we've seen in the Dana Point population.

The genetic data show that the Dana Point population is small and has lost genetic diversity. Even when the samples collected from Dana Point in 2020 are considered, it is still clear that this population has lost genetic diversity. This trend indicates that the effective population size (Ne) is not large enough to retain genetic diversity. In addition, populations with small Ne tend to accumulate higher burdens of genetic load and suffer inbreeding depression. The genomic data suggest Dana Point PPM indeed carry more deleterious mutations, and the lower reproductive success of Dana Point founders compared to their admixed offspring in the conservation breeding program may be the result of inbreeding depression. We have no evidence that the Dana Point population has purged deleterious alleles, a process which is thought to occur only in populations with stably small Ne for many thousands of generations (e.g. possibly vaquitas and channel island foxes), which is different from the history of PPM. Species thought to have experienced purging are uncommon, and modeling suggests that this is because most populations that are small enough to purge deleterious alleles go extinct.

The recommendation to collect Dana Point PPM for the breeding program is not for the sole purpose of experimentation, but to provide a resource for safely boosting genetic diversity. We know that admixed PPM produce morphologically and behaviorally normal offspring. This observation over several generations in the breeding program is the best evidence that the populations are compatible, and that the chromosomal differences between populations have only a small impact, if any. Nonetheless, our proposed breeding strategy will enable the introduction of only PPM with the same chromosome number as the Dana Point PPM, and will provide more data on the impact of interbreeding. These direct observations of fitness of crosses bred within an ex-situ facility are the safest and best way to measure the impact of interbreeding. Although strongly negative impacts would likely have arisen when Dana Point founders were interbred in the breeding program starting in 2012, if with additional data we did find that interbreeding is harmful, we would not introduce outside PPM into Dana Point.

-

As has been pointed out, breeding admixed PPM that have matching Dana Point karyotypes would take at least two generations and several years, so the timeline for translocations would likely be years away. Given what looks like a population expansion in 2020, this year may be the best time to collect animals for the breeding program with minimal impact on the population. It makes no sense to wait for gene technology to help. Gene editing has not been done in a single endangered species so far, and the application of this technology in conservation is likely decades away. There are no other practical or financial considerations on the timing of collections beyond the scientific evidence that has been collected over time and presented to partners.

We absolutely agree that continuing other methods such as habitat management and reduction of stressors are critical for supporting population growth and persistence, but prioritizing maintaining isolation of a small population in decline will result in the loss of adaptive potential and possible extinction of the population. This is already the case for all other known PPM populations save the three remaining. Now is the time to act.

Sincerely,

Aryn and Debra

Aryn P. Wilder, PhD (she/her)

Scientist, Conservation Genetics

San Diego Zoo Wildlife Alliance

15600 San Pasqual Valley Rd

Escondido CA 92027

(760) 291-5453

awilder@sdzwa.org

sdzwa.org

From: Deborah Rogers <drogers@cnlm.org>
Date: Friday, May 12, 2023 at 6:11 PM
To: "Roberts, Carol" <carol_a_roberts@fws.gov>
Cc: Korie Merrill <kmerrill@cnlm.org>, "Sin, Hans@Wildlife" <Hans.Sin@wildlife.ca.gov>, "Snyder, Jonathan" <Jonathan_d_Snyder@fws.gov>, Debra Shier <DShier@sdzwa.org>, Aryn Wilder <awilder@sdzwa.org>, "Emily@Wildlife" <emily.gray@wildlife.ca.gov>, "Karen@Wildlife" <Karen.Drewe@wildlife.ca.gov>, david mayer <david.mayer@wildlife.ca.gov>, "Brehme, Cheryl S" <cbrehme@usgs.gov>, "Fisher, Robert N" <rfisher@usgs.gov>, "Miller, William B." <william_b_miller@fws.gov>
Subject: Re: Query regarding PPM collections from Dana Point population

Carol:

Thank you for your support of the Dana Point PPM population. While the draft genetic management plan has still not been reviewed to the extent it deserves and there is important work to be done on this that could impact recommended actions, I don't want to side-step your point about time sensitivity.

Yes, if the approach is to experiment with hybridization and backcrossing, this is an uncertain, risky endeavor. At some point, gene technology may well have advanced to the point of making such traditional breeding efforts unnecessary and allow more elegant and less impactful methods. Have other methods been considered that would be less impactful on PPM?

Another point that has some representation in the current draft, but has been recognized as needing more attention and analysis, is the Ne of the current population. It's still not clear (perhaps just to me) what is the risk of inbreeding depression given the genetic differentiation of this population and degree to which deleterious alleles may have been lost. Are you clear on this point? It's important to the sense of urgency of introducing (or not) more genetic diversity.

I am not aware of evidence of inbreeding depression. If I am missing this, I would very much appreciate a reminder about this.

As I know you're considering, temporal perspective is important. There is the timescale of divergence of this population, timeline for which additions to genetic diversity would be 'needed' (see above); and timeline for developing a traditional breeding approach to providing more genetic diversity, if needed. These are quite different. If my interpretation is correct that there is no crisis (regarding the first two points), then a season, year, or years for the latter would seem immaterial. Am I missing some other (e.g., practical? financial?) considerations here that have not been mentioned? I can only reflect on what I know and what is offered by others.

It's clear that a bad decision was averted by not allowing PPM from other populations to be introduced to the Dana Point population. It would seem prudent to better evaluate (and interpret, understand) genetic diversity within PPM populations before assuming that imminent (in evolutionary terms) intervention was needed. Both additional analyses (which has been recognized as a need for the current draft plan) and perhaps additional genetic samples (which we suggested) are important to the direction and the timeline of any genetic intervention. In the meantime, we continue to manage genetic diversity with professional management to support reproduction, removal or reduction of stressors, and maintenance of natural selection. Good population size and reproduction are critical to genetic management.

On a more practical level, PPM experts have commented that it's probably already too late to be collecting PPM without causing unintentional harm. We understand that adults are already reproductive (at least at Camp Pendleton) so it would not seem to be recommended to be taking females that may be in early stages of pregnancy (?) Rather, it has been recommended that if it is decided to 'take' animals this year, it would be better to take young animals or post-lactation females a few months from now. Make sense? If so -- and again, I only know the considerations that have been offered -- we still have some time to make this decision. Will seemed to imply, but perhaps I misunderstood, that trapping animals at Camp Pendleton or DP would be undertaken, but not both, this year.

Carol, I know that we are on the same page in wanting what is best for PPM -- both for the wild population at Dana Point and for the species. I also assume that this means that a genetic management plan should be tightly coupled with a recovery plan. With respect for your concern and shared interests, I am humbly offering my questions and perspective and look forward to your response.

Best,
Deborah

On Fri, May 12, 2023 at 4:55 PM Roberts, Carol <carol_a_roberts@fws.gov> wrote:

> Deborah,
>
> I understand your concerns, but I disagree with your characterization that these breeding experiments are not driven by a crisis situation. Will was very clear in his message to you as to why it is appropriate to proceed at this juncture:
>
> ...Importantly, there is consensus that the conservation research has been well done, the broad conclusions of the plan are sound and there is support for the recommendation of collecting additional animals from Dana Point to help investigate and pursue the strategy of backcrossing and reintroducing animals to increase genetic variation at Dana Point.
>
> Given the support for the GMP management recommendations and the time it will take to address all of the reviewer's comments, we are requesting permission to proceed this

Spring with the collection of animals from Dana Point as proposed in the GMP while we address the reviewers comments. Of course any collections would be regulated by the total number of animals captured during trapping to make sure that we are only removing a small proportion of the animals present. As has been demonstrated from our earlier collection efforts, our success breeding and preserving genetic variation of population founders is maximized by collecting animals as early in the season as practicable (e.g. during April and May)...

Given the amount of time it will take to make the appropriate back-crosses in support of reintroduction of individuals to Dana Point, and knowing that the numbers have been good there in the recent past, it is an opportune time to pursue collections during this year's trapping. Depending on the actual results, this may be moot. If the numbers continue to be good, collection of a few individuals for captive rearing, in the context of the consensus on the need for this measure, should be allowed to proceed. I expect that it will take on the scale of years to be ready to reintroduce individuals to the Preserve, and every year we wait is a year that could result in a biological challenge to the mice that they are not physiologically capable of responding to given their greatly reduced genetic diversity. Time **is** of the essence, and I believe the appropriate safeguards will be in place to protect the population at large. I ask that you and Korie reconsider your decision and allow for the possibility of collections this year should adequate animals be available.

Thank you,

-Carol (she, her, hers)

The Endangered Species Act is turning 50! https://www.fws.gov/esa50

Carol A. Roberts, Division Supervisor

Environmental Contaminants/Federal Projects/ESA Sec 7 & 10

Collateral Duty Safety Officer

Carlsbad Fish and Wildlife Office

2177 Salk Avenue, Suite 250, Carlsbad, CA 92008

760-431-9440, ext. 271 (note: I telework Thursday and Friday)

Mobile phone including spill emergencies: 760-607-9768

From: Deborah Rogers <drogers@cnlm.org>
Sent: Friday, May 12, 2023 10:34
To: Miller, William B. <william_b_miller@fws.gov>
Cc: Korie Merrill <kmerrill@cnlm.org>; Sin, Hans@Wildlife <Hans.Sin@wildlife.ca.gov>; Roberts, Carol <carol_a_roberts@fws.gov>; Snyder, Jonathan <Jonathan_d_Snyder@fws.gov>; Debra Shier <DShier@sdzwa.org>; Aryn Wilder <awilder@sdzwa.org>; Emily@Wildlife <emily.gray@wildlife.ca.gov>; Karen@Wildlife <Karen.Drewe@wildlife.ca.gov>; david mayer <david.mayer@wildlife.ca.gov>; Brehme, Cheryl S <cbrehme@usgs.gov>; Fisher, Robert N <rfisher@usgs.gov>
Subject: [EXTERNAL] (CNLM Ref: S033) Query regarding PPM collections from Dana Point population

Good morning, Will.

Korie and I have discussed your request to collect mice from the Dana Point Preserve that you provided to us two weeks ago. We understand this is a time-sensitive but also important decision which we have carefully considered in the context of the draft genetic plan, appropriate recovery actions, and the status of PPM on the Preserve. We agree that there has been much effort in drafting the plan and it represents a review of much experimentation and knowledge. We also agree, as you mentioned, that some additional analyses are needed towards getting at important information such as Ne, as well as further describing the patterns of genetic diversity in appropriate contexts. We believe that those revisions -- as well as incorporating information and questions from other reviewers -- are critical towards having a sound plan.

Any take of PPM from the Preserve is a serious decision and, given that there is a different awareness now of the DP population than even a few years ago, that taking mice for breeding experiments does not seem to be in response to a crisis situation, and that further analysis of genetic data (and more samples?) might strengthen the plan, we don't think that now is the time to take more mice from the Preserve.

Towards the point of getting more genetic information--both from additional analyses as well as, possibly, more samples -- if you think this would contribute significantly to our understanding of the genetic status of the DP PPM population, we would suggest some limited trapping later this summer to collect some more ear snips. If so, we suggest waiting until after our first session of track-tube monitoring has been completed (at the end of June). This would allow for trapping young of year (to confirm reproductive success) and provide current PPM occupied areas on the Preserve for more targeted trapping.

We still do intend to provide a better (than represented here) review of the draft genetic management plan -- it deserves that attention -- but are still currently very much occupied with other issues and activities that directly affect the current and future well-being of the Dana Point PPM.

Best,
Deborah

---------- Forwarded message ---------
From: **Miller, William B.** <william_b_miller@fws.gov>
Date: Mon, May 8, 2023, 09:58
Subject: Re: [EXTERNAL] (S033) Re: PPM Collections at Dana Point
To: Deborah Rogers <drogers@cnlm.org>
Cc: Korie Merrill <kmerrill@cnlm.org>, Sin, Hans@Wildlife <Hans.Sin@wildlife.ca.gov>, Roberts, Carol <carol_a_roberts@fws.gov>, Snyder, Jonathan <Jonathan_d_Snyder@fws.gov>, Debra Shier <DShier@sdzwa.org>, Aryn Wilder <awilder@sdzwa.org>, Gray, Emily@Wildlife <emily.gray@wildlife.ca.gov>, Drewe, Karen@Wildlife <Karen.Drewe@wildlife.ca.gov>, david mayer <david.mayer@wildlife.ca.gov>

Deborah- We have one more external reviewer that indicated she will try to get us comments by the end June. Anything you can do to provide us a response expeditiously is appreciated

so we can schedule to collect this month at MCBCP if not Dana Point. Thanks- Will

William B. Miller, Biomonitor

U.S. Fish and Wildlife Service

Carlsbad Fish and Wildlife Office

2177 Salk Avenue, Suite 250

Carlsbad, California 92008

(760) 431-9440 Ext. 206

William_B_Miller@fws.gov

(S033) PPM track tube monitoring session 1

Debra Shier <DShier@sdzwa.org> Tue, Jul 11, 2023 at 1:45 PM
To: Korie Merrill <kmerrill@cnlm.org>
Cc: "Miller, William B." <william_b_miller@fws.gov>

Wonderful! That makes this much easier. We'll be in touch soon.

Best,

Debra

Debra M. Shier, Ph.D. (she/her)

Brown Endowed Associate Director of Recovery Ecology

San Diego Zoo Wildlife Alliance

dshier@sdzwa.org

From: Korie Merrill <kmerrill@cnlm.org>
Sent: Tuesday, July 11, 2023 1:44 PM
To: Debra Shier <DShier@sdzwa.org>
Cc: Miller, William B. <william_b_miller@fws.gov>
Subject: Re: [EXTERNAL] (S033) PPM track tube monitoring session 1

Debra,

I think if you provide the requested details that should work. My assumption is that the email(s) from the request in late April-May has the background and justification for the proposal so if needed that would be incorporated in the SARA by Deborah.

On Tue, Jul 11, 2023 at 1:27 PM Debra Shier <DShier@sdzwa.org> wrote:

> Hi Will,

Thanks for looping me into the thread. I was out last week and am catching up on emails and everything else this week. But, I'm happy to get a proposal together.

Korie, Deborah indicated in her email that you need the following details:

* number of trap nights proposed

* determination and flagging of route to be followed from trap to trap to minimize disturbance to habitat

* description of how abundance will be computed

* the maximum percentage of PPM for take that will have no impacts to the population

* criteria for taking mice for mice (sex, age)

* criteria for stopping trapping effort early (e.g., death or injury to target or nontarget species)

* commitment for regular information sharing on status of mice that are taken from Dana Point

* description of all experiments to be performed on DP mice

* commitment to taking tissue samples and processing these samples as quickly as possible to add to the genetic information of the population

A question: do you need us to provide the background justification for the collection or can the proposal can simply include the requested details?

Please let me know and we'll get something to you as soon as we are able.

Best,

Debra

Debra M. Shier, Ph.D. (she/her)

Brown Endowed Associate Director of Recovery Ecology

San Diego Zoo Wildlife Alliance

dshier@sdzwa.org

From: Miller, William B. <william_b_miller@fws.gov>
Sent: Tuesday, July 11, 2023 12:57 PM
To: Korie Merrill <kmerrill@cnlm.org>

Cc: Debra Shier <DShier@sdzwa.org>
Subject: Re: [EXTERNAL] (S033) PPM track tube monitoring session 1

Hi Korie- Thank you for providing the track-tube detection data. We were waiting for the data to put together a trapping/collection proposal. I will coordinate with Debra and she or I will respond with a proposal ASAP. Thanks- Will

William B. Miller, Biomonitor

U.S. Fish and Wildlife Service

Carlsbad Fish and Wildlife Office

2177 Salk Avenue, Suite 250

Carlsbad, California 92008

(760) 431-9440 Ext. 206

William_B_Miller@fws.gov

Pronouns: *He, Him, His*

From: Korie Merrill <kmerrill@cnlm.org>
Sent: Monday, July 10, 2023 1:27 PM
To: Miller, William B. <william_b_miller@fws.gov>
Subject: [EXTERNAL] (S033) PPM track tube monitoring session 1

Hi Will,

Attached is our session 1 *preliminary* results for PPM (May-June) on the Preserve. The gis layer attached is for the subplot locations and whether or not PPM were detected at those points in session 1. These results are preliminary and subject to change with further quality control and review of the cards, it is not a permit report.

PS- CNLM is still waiting for the Zoo to send information to us so we can move forward with a Science and Research Agreement for PPM trapping and collection. Do you mind nudging Debra Shier to send us their proposal asap?

I'm not sure if she or you were waiting for our track tube data before sending that information to Deborah Rogers but that level of detail (e.g., gps location of traps) isn't required for us to start the Agreement.

Let me know if the layer package doesn't work and I can send you the shapefile.

--

Korie Merrill

Regional Preserve Manager - South Coast

Center for Natural Lands Management

Direct: 949.218.1145

Office: 760.731.7790 ext. 204

ANNUAL WORK PLAN FOR THE FISCAL YEAR 2024

(October 1, 2023 – September 30, 2024)

FOR THE

CNLM DANA POINT PRESERVE
PACIFIC POCKET MOUSE
ENHANCEMENT MANAGEMENT PLAN

CAN: N62473-20-2-0018

(CNLM Ref. No. G2036)

Provided to:

Marine Corps Base Camp Pendleton; Andrea Souther, andrea.souther@usmc.mil

NAVFAC Southwest; Roland Sosa, roland.a.sosa2.civ@us.navy.mil

United States Fish and Wildlife Service; William Miller, william_b_miller@fws.gov

California Department of Fish and Wildlife; Emily Gray, emily.gray@wildlife.ca.gov

Prepared by:

Korie Merrill; Regional Preserve Manager, kmerrill@cnlm.org

Center for Natural Lands Management

27258 Via Industria, Suite B

Temecula, CA 92590

Phone: 760.731.7790

27 September 2023

I. Introduction

The Center for Natural Lands Management's (CNLM) Dana Point Preserve (Preserve) is located in the City of Dana Point, Orange County, California, and was established in December 2005. Pacific pocket mouse (*Perognathus longimembris pacificus;* PPM) focused enhanced management on the Preserve following guidelines under the Enhanced Management Plan (EMP; CNLM 2020). These activities will be conducted using funds provided by the U.S. Marine Corps through the Readiness and Environmental Protection Integration (REPI) program through a Cooperative Agreement (Agreement; N62473-20-2-0018). Enhanced management will be focused on the more suitable areas of the Preserve—i.e., areas with suitable geomorphology (e.g., slopes less than 15%) rather than expend resources in areas not likely to sustain PPM such as steep cliff faces of coastal bluff scrub. Within the Preserve, this focal area is approximately 8 ha (20 ac) of mature coastal sage scrub vegetation with slopes less than 15% and sandy soils. This work plan describes the EMP management activities planned for the Fiscal Year (FY) 2024 (October 1, 2023 – September 30, 2024).

The primary management objectives for the fourth year (Year 4, FY 2024) of the Agreement will be to continue the management goals and objectives listed in the EMP. These objectives, which will drive enhanced management on the Preserve are:

- Write an annual work plan for FY 2024
- Continue the implementation of the five-year vegetation management plan
- Monitor for and analyze PPM response to management activities
- Collaborate with other PPM experts and land managers
- Enhance protection of PPM from external threats such as trespass and uncontrolled public access
- Integrate the EMP and Habitat Management Plan (HMP)
- Report these activities in an annual summary report

Enhanced management for PPM on the Preserve will be based on relevant scientific principles and paradigms, and current science-based information from CNLM, U.S. Fish and Wildlife service (USFWS), U.S. Geological Survey (USGS) and other experts in the field (e.g. San Diego Zoo Wildlife Alliance). Enhanced management of PPM on the Preserve will use monitoring data conducted under the HMP in combination with this EMP such as: increased use of wildlife cameras, remote sensing, and specific PPM monitoring point vegetation monitoring to be used for habitat modeling.

II. Protection

CNLM will increase their presence on the Preserve by increasing CNLM Ranger patrols. Ranger presence will enhance protection of PPM from external threats such as trespass and educate the visiting public about the importance of protecting PPM. To aid in protection against threats, 50h of additional patrols by CNLM rangers will be conducted over the year. These additional patrols can provide an increase in staff presence to help prevent or mitigate trespass as visitation to the Preserve increases annually. Additional patrols will also be provided during sensitive times such as the PPM breeding season.

To further protect PPM, CNLM will collaborate with other PPM experts and land managers via virtual workshops and meetings to discuss and assess threats to PPM on the Preserve such as invasive species and/or public use of the trail.

Monitoring

CNLM will continue to develop appropriate long-term monitoring and management protocols through assessments of the current vegetative/open space conditions on the Preserve. Vegetation data will be collected in 2024 at each track tube point, during the biologically relevant time (typically April- May), following the USGS rapid assessment protocol (Brehme et al. 2016). Remote sensing data, specifically aerial imagery, color-infrared (CIR) and normalized difference vegetation index (NDVI) was captured in early spring 2023, this data will be used in conjunction with rapid assessment data to determine habitat characteristics for each grid.

PPM will be monitored using track tubes over two sessions: Session 1 in the spring (typically May) and Session 2 in the summer (typically August). Two track tubes will be placed in each grid at the subplot level, similar in design to 2023, 2022 and 2020 monitoring efforts. Vegetation and PPM data collected will be analyzed in collaboration with USGS.

Additional monitoring activities such as camera trapping for PPM and predator species use will be conducted throughout the year. The cameras will be checked monthly at which time the data downloaded, reviewed, and entered in the Preserve's wildlife database.

Vegetation Manipulation

Vegetation manipulation will be conducted following CNLM's established protocols (CNLM 2022) and is anticipated to occur between November 2023 and January 2024, when PPM above ground activity is low. Based on the vegetation management plan and on the ground observations, vegetation manipulation activities for FY 2024 will concentrate in the north corner of the Preserve adjacent to Dana Strand Road and in the southeast near the Scenic Gate (Figure 1), with additional maintenance (limited raking and debris removal) in areas manipulated within the last three years. Vegetation manipulation activities will be conducted in designated polygons by qualified restoration contractors. The planned activities will not be significantly dissimilar from those conducted in 2017-2023 and will be overseen by CNLM staff to ensure appropriate sensitivity to focal species.

It is anticipated this year CNLM will work in approximately three acres of the Preserve, removing dead and downed plant material and raking to create open sandy patches. Due to the location of the target area, time per acre (and therefore cost) will be similar to 2023 -- plant material may need to be hauled off site with a truck and trailer rather than a green waste bin due to issues with the City's encroachment permit conditions.

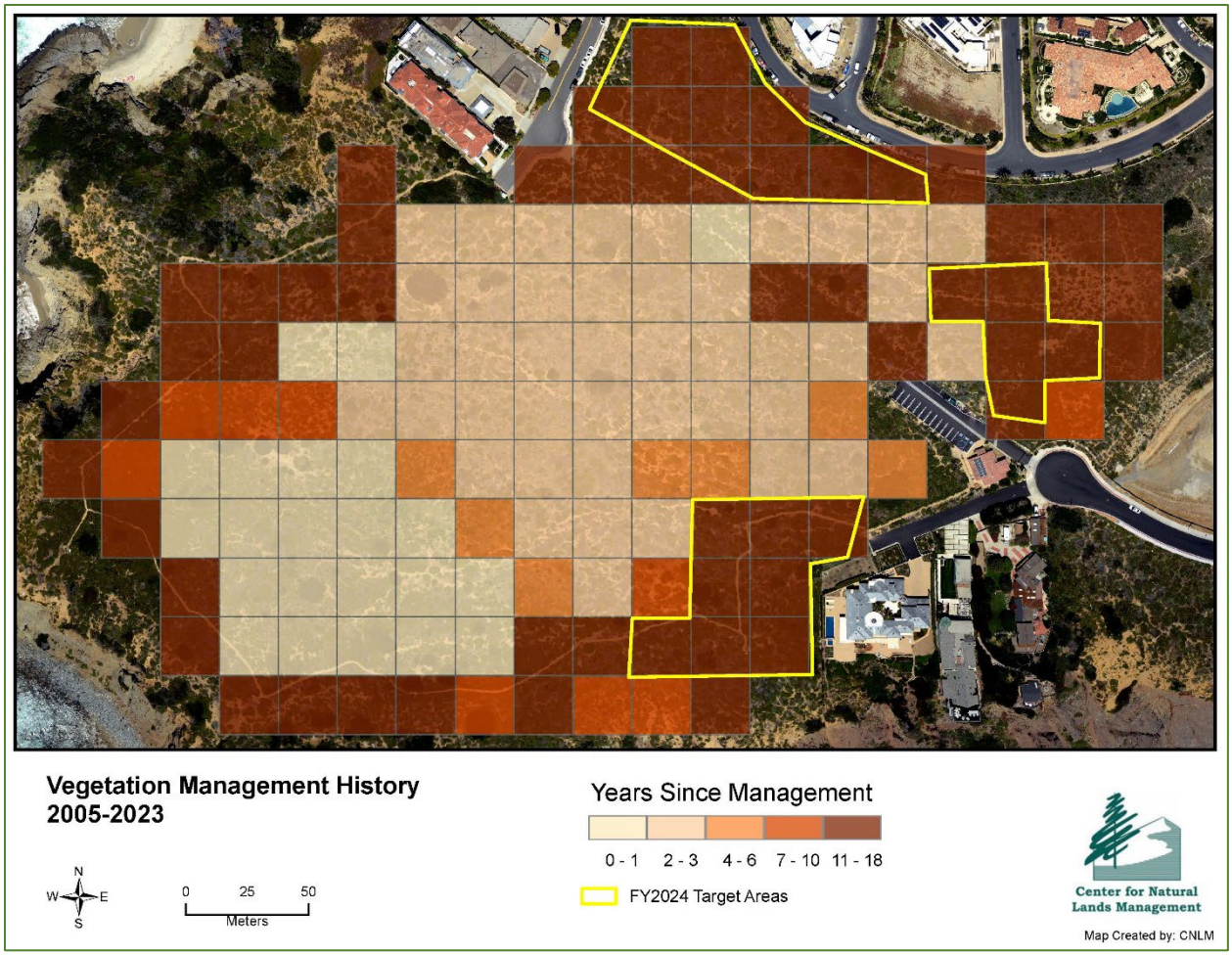

Figure 1. FY 2024 map of priority grids for vegetation manipulation activities. The darker the shade, the higher the priority based on years since extensive vegetation management occurred using 2005 - 2023 data.

III. Reporting

The EMP has been written to offer robust, long-term guidance to optimize the use of regulatory relief funds provided by U.S. Marine Corps for enhanced management. Routine monitoring and management objectives described are intended to help ensure PPM health and persistence in perpetuity. An annual work plan will be prepared by CNLM as a separate section of the general preserve annual work plan and will provide a description of general activities for the upcoming fiscal year (October 1 – September 30). These work plans will be provided to CDFW, USFWS, and U.S. Marine Corps for the purpose of soliciting input. An annual budget for the upcoming fiscal year will be prepared by CNLM and will be based on funding from REPI Funds. An annual summary report will be prepared by CNLM and will be completed near the beginning of the new calendar year, with a target date no later than 1 February, for the previous fiscal year. The EMP annual report will be included as an attachment to CNLM's Dana Point Preserve report provided to CDFW, USFWS and U.S. Marine Corps. Annual financial reports will be prepared by CNLM and provided to USFWS and the U.S. Marine Corps with a target date no later than 1 February for the previous fiscal year.

IV. Work Schedule

Table 1. Anticipated work schedule for FY 2024.

FY 2024 Task	Anticipated Completion Date
Increase on site presence and patrols	Ongoing
Implement threat reduction strategies	Ongoing
Enhance our knowledge of PPM activity on the Preserve	Ongoing
Collaborate with other PPM experts to update targets for preferred habitat conditions	Ongoing
Monitor wildlife use on the Preserve	Ongoing
Analyze previous years monitoring data	1 December 2023
Conduct enhanced vegetation manipulation	1 January 2024
Provide an annual summary report	1 February 2024
Provide an annual fiscal report	1 February 2024
End of I&C Funds Phase	1 May 2024
Conduct rapid assessment vegetation surveys	1 June 2024
Conduct enhanced PPM monitoring	30 September 2024
Provide a work plan	30 September 2024

V. Budget

The expected budget for enhancement management activities on the Preserve FY 2024 totals $23,682.30 (Table 2). It is anticipated that the Initial and Capital (I&C) funds provided in 2021 will be exhausted at the end of the three-year period in April 2024, thus May-October 2024 activities will be funded through the perpetual endowment.

Table 2. Anticipated budget for FY 2024.

PPE Enhanced Management

Task	Cost ($)
85100 · Biotic Surveys	
85130 · Non-labor costs	800.00
Total 85100 · Biotic Surveys	**800.00**
85150 · Field Equipment	
85173 · CNLM Vehicle Mileage	200.00
Total 85150 · Field Equipment	**200.00**
85220 · Habitat Maintenance	
85250 · Contractor labor	6,000.00
85260 · Non-labor costs	400.00
Total 85220 · Habitat Maintenance	**6,400.00**
85345 · Labor - Staff	
85346 · Labor - Preserve Manager	3,339.60
85348 · Labor - Preserve Ranger	11,442.70
Total 85345 · Labor - Staff	**14,782.30**
85399 · Operations	
85430 · Non-labor costs	1,500.00
Total 85399 · Operations	**1,500.00**
Total Expense	**23,682.30**

VI. References

Brehme, C.S., D. Clark, M.A. Burlaza, and R.N. Fisher. 2016. Pacific Pocket Mouse Habitat Protocol. U.S. Geological Protocol. 38p.

[CNLM] Center for Natural Lands Management. 2020. Dana Point Preserve (S033) Pacific Pocket Mouse Enhanced Management Plan. 2020-2030. 31 July 2020.

[CNLM] Center for Natural Lands Management. 2022. Dana Point Preserve Pacific Pocket Mouse 5-year Enhanced Vegetation Management Plan. 03 January 2022.

03/24/24 – 03/30/24

RANGER REPORT

08:30– 17:30

03/24/24 SO33-S032 Sunday

- Patrol S032, SO33
- Set gate timers. 19:06
- Trail Hours are now posted with daily sunset times.

03/26/24 SO32, SO33 Tuesday

08:30 to 17:30

- SO33, S032 patrol.
- S033 Maintenance.

03/27/24 SO32- SO33 Wednesday

08:30 to 12:30

- Rain Day
- Patrol SO32,SO33.
- Gate timer reset 19:07

03/28/24 SO33 – Thursday

08:30– 17:30

- Patrol S033. SO32

03/30/23 SO33 Saturday

08:00 – 13:00

- PTO

Vegetation trimming was completed in the preserve no additional trimming should not take place until CAGN nesting is done.

Weather slowed the trimming on Preserve boundary adjacent to Selva drive.

No further trimming will take place until nesting season is over I observed at least one nesting pair along preserve Selva boundary fence line.

However I observed what looks like evidence of herbicide spraying along the Selva boundary dead and dying vegetation was not real apparent until it rained dying vegetation can be seen as a brown line from the employee gate to the end of the Preserve boundary on Selva.

Selva fence boundary to preserve

01/28/24 – 02/03/24

RANGER REPORT

08:00– 17:00

01/28/2024 SO33 Sunday

SO32 Trail patrol

- Continued trail trimming SO33.

Set gate timers. 17:18

01/30/24 SO30 -Tuesday

08:00 to 17:00

- S032 patrol
- S033 continued trail trimming SO33.

01/31/24 SO33 - Wednesday

09:00 to 16:30

- Trail trimming continues SO33.

02/01/24 SO33 – Thursday

- SO32 patrol

08:00– 17:00

Trail trimming completed SO33.

- Began selva fence clean up.

02/03/23 SO33 Saturday

08:00 – 13:00

- Short day


My Altitude
33° 27′ 40″ N -117° 42′ 55″ W
39.66m 130.13ft
Feb 8, 2024 at 13:51
55°F, Mostly Cloudy
Wind Speed: 22.99 mph Bearing: 270°
SO33 trimming complete


My Altitude
33° 27′ 41″ N -117° 42′ 54″ W
40.49m 132.84ft
Feb 8, 2024 at 13:49
55°F, Mostly Cloudy
Wind Speed: 22.99 mph Bearing: 270°
SO33 trimming complete

SITE ACCESS FOR RESEARCH AGREEMENT ("Agreement")

Pacific pocket mouse trapping and conservation breeding program

Pertaining to the CNLM Dana Point Preserve (S033)

Effective as of August 4, 2023, the Center for Natural Lands Management ("CNLM") hereby grants to Zoological Society of San Diego d/b/a San Diego Zoo Wildlife Alliance ("SDZWA") access to enter certain property owned and managed by CNLM for the purpose of locating, trapping, and removing a small number of Pacific pocket mice (*Perognathus longimembris pacificus*) for inclusion in a captive breeding project.

RECITALS

A. CNLM is a nonprofit organization dedicated to the protection and management of sensitive and significant natural habitats and rare and listed species through perpetual, science-based stewardship.

B. CNLM is the owner and habitat manager of certain real properly known as the Dana Point Preserve ("Preserve"), County of Orange, California (Exhibit A). This iconic preserve retains the distinction of having been the only recently confirmed occupied Pacific pocket mouse habitat in 1994, and the preserve whose imminent threat of development was driving force in the federal listing of the species on February 3, 1994 (USFWS 1994). CNLM has the legal authority to enter into this Site Access for Research Agreement (Agreement) as holder of fee title of the Preserve since December 2005.

C. SDZWA proposes to conduct Research, as further described in Exhibit A attached hereto, on Preserve, and CNLM intends to permit access for such Research pursuant to the terms of this Agreement.

D. CNLM, in providing conditional access to SDZWA for the stated purposes, is not committing to: i) additional take of PPM from the Preserve in the future; or ii) allowing any captive-bred or translocated PPM to be placed on the Preserve.

NOW THEREFORE, in consideration of the promises and mutual covenants contained herein, CNLM and SDZWA hereby agree as follows:

TERMS AND CONDITIONS

1. Purpose. The purpose of this Agreement is to define the terms and conditions under which SDZWA will be granted site access to the Preserve for conducting the Research.

2. Term. This Agreement shall be in effect through September 30, 2023.

3. Fee. CNLM is imposing no financial requirements on SDZWA.

4. Take of PPM. A maximum of four (4) individuals of Pacific pocket mouse may be removed from the Preserve for the Research. They may be composed of any number of either sex and of any age. Tissue samples (ear snips) may be taken from every PPM captured.

5. Notice. Prior to any entry onto the Preserve, SDZWA shall give CNLM reasonable notice, at a minimum of seven (7) days, of its intention to enter the Preserve. Unless otherwise provided herein, such notice shall be to the Preserve Manager. Alternatively, Preserve Manager and SDZWA may agree in advance to a Preserve access schedule.

6. Access. Access onto the Preserve shall be limited to individuals, equipment and materials, the presence of which on the Preserve is necessary in order to conduct the Research. This Agreement does not grant SDZWA the right to undertake any activity on the Preserve which will in any way interfere with activities being conducted on the Preserve by CNLM or at its direction. Special access conditions, if any, are detailed in Exhibit B.

7. Acknowledgements and Research Reports/Publications. SDZWA agrees to formally acknowledge CNLM contributions to Research, including the use of Preserve, intellectual contributions by CNLM employees, and other CNLM assistance, in any reports or publications that arise from Research. SDZWA further agrees to provide CNLM in a timely fashion a copy of SDZWA's publications published as a result of the Research, including any thesis or dissertation derived from said Research.

SDZWA agrees to provide quarterly reports to CNLM of all activities and results in the conservation breeding program that include mice collected from the Preserve.

8. Special Provisions and Conditions. Special provisions and conditions, if any, imposed upon and agreed to by SDZWA, are described in Exhibit C, attached.

9. Non-Interference. SDZWA shall use its best efforts to avoid interfering in any way with the activities being conducted on the Preserve by CNLM. SDZWA shall immediately halt any and all activities on the Preserve at the request of CNLM if CNLM determines that any activity is (a) being conducted in violation of this Agreement, (b) inconsistent with or not covered by this Agreement, or (c) likely to negatively impact the native species, habitat, or environmental values of the Preserve as determined by CNLM.

10. Conduct of Activities. SDZWA will conduct Research in compliance with all Federal, state, and local laws, ordinances, and orders including, without limitation, any permit, notice, or approval requirements.

11. Habitat Protection and Damage. SDZWA agrees that (a) it is responsible for any and all damage or destruction it causes to the Preserve, Preserve species and habitat, and/or CNLM property (including fences, gates, signage, roads, and trails); (b) no trash or discarded material will be left on Preserve at any time by SDZWA; (c) no exotic or non-local native species will be introduced into the Preserve; and (d) all equipment, stakes, temporary fences, enclosures, and similar materials will be completely removed from the Preserve at the conclusion of each research period/season and the Research.

12. Responsibility and Accountability.

a. SDZWA shall have full and sole responsibility for the safety of any of its agents, and contractors, and for the operation, safety, security, and proper handling of all equipment and materials brought on or about the Preserve. SDZWA shall hold harmless, indemnify, and defend CNLM and its directors, officers, employees, agents, and invitees (collectively "Indemnified Parties") from and against all liabilities, penalties, costs, losses, damages, expenses, causes of action, claims, demands, or judgment, including without limitation, reasonable attorneys' fees (collectively "Claims"), arising from or in any way connected with this Agreement including, without limitation, Claims for injury to or the death of any person, or physical damages to any property, resulting from any act, omission, condition, or other matter related to or occurring on or about the Preserve, but only in proportion to and to the extent that such Claims arise from the negligent or intentional acts or omissions of SDZWA, its officers, agents, or employees.

b. SDZWA agrees that all individuals participating in Research on the Preserve will be under its direct supervision and are subject to the terms and conditions of this Agreement.

c. SDZWA and Research Assistants shall maintain a copy of this Agreement and other supporting authorizations and permits on its person at all times while on the Preserve and shall present same to CNLM employees or agents, the property landowners (if different), and other authorities (including law enforcement and resource agency officials), when requested.

d. SDZWA will ensure that all individuals participating in Research on the Preserve will execute a release of liability form, a sample of which is attached hereto as Exhibit D; and SDZWA assumes all liability and responsibility for failure of any such individual to execute such form.

13. "INTENTIONALLY LEFT BLANK"

14. Dispute Resolution. In the event a dispute shall arise between the parties to this Agreement that cannot be resolved by negotiating in good faith or through

mediation, it is agreed that the dispute shall then be referred to an arbitration service selected by agreement of the parties. The arbitrator's decision shall be final and legally binding and judgment may be entered thereon. Each party shall be responsible for its share of the arbitration fees in accordance with the applicable rules of arbitration. In the event a party fails to proceed with arbitration, unsuccessfully challenges the arbitrator's award, or fails to comply with the arbitrator's award, the other party is entitled to costs of suit, including a reasonable attorney's fee for having to compel arbitration or defend or enforce the award.

15. Notices. All notices to be given under this Agreement shall be in writing and addressed to the recipient as set forth below:

SDZWA:	Ron Swaisgood Brown Endowed Director of Recovery Ecology San Diego Zoo Institute for Conservation Research 15600 San Pasqual Valley Road Escondido, CA 92027 Telephone: (760) 291-5427 Email: RSwaisgood@sandiegozoo.org
And a copy to:	Wendy Bulger General Counsel San Diego Zoo Wildlife Alliance 2550 5th Ave., Suite 520 San Diego, CA 92103 Telephone: (619) 685-3264
CNLM:	Dr. Deborah L. Rogers Co-Executive Director & Director of Conservation Science and Stewardship Center for Natural Lands Management 27258 Via Industria, Suite B Temecula, CA 92590 Telephone: (760) 731-7790 Ext. 103 Facsimile: (760) 731-7791 Email: drogers@cnlm.org

All notices shall be deemed effectively given: (a) when delivered, if delivered personally or by couriered mail service (such as, for example only, Federal Express or DHL); (b) five (5) days after such notice has been deposited in the United States mail postage prepaid, if mailed certified or registered U.S. mail, return receipt requested; or (c) when received by the party for which notice is intended if given in any other manner.

In an emergency, contact all other parties immediately.

16. General Provisions.

a. Applicable Law. The validity of this Agreement and of its terms or provisions, as well as the rights and duties of the parties hereunder, shall be governed and interpreted in accordance with the laws of the State of California.

b. Nonwaiver. No provision of this Agreement shall be waived, by conduct or otherwise, except in writing signed by both parties. No assent or waiver whether expressed or implied, of any breach of any one or more of the covenants, conditions, or provisions set forth in this Agreement shall be deemed to be a waiver of any subsequent breach of the same or any other covenant, condition, or provision hereof.

c. Severability. If any provision of this Agreement shall be adjudged invalid by any court, the remaining provisions of this Agreement shall remain valid and enforced to the full extent permitted by law.

d. Authority. The individuals signing this Agreement represent that they are authorized to execute this document, that their execution of this document shall be binding on the parties that they represent, and that the other party hereto may rely upon such representation.

e. Integrated Agreement. This Agreement, including all Exhibits attached hereto, contains the entire understanding and agreement between the parties, sets forth all the rights and duties of the parties with respect to the subject matter hereof, and replaces and supersedes all previous agreements or understandings, whether written or oral, relating thereto. No modification of this Agreement or any of its terms shall be effective unless in writing and signed by the duly authorized representatives of the parties.

f. Cancellation. Either party reserves the right to terminate this Agreement, in whole or in part, with thirty (30) days prior written notice if it determines it to be in the best interest of either party.

SIGNATURES ON FOLLOWING PAGE.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the day and year first above written.

Zoological Society of San Diego
d/b/a San Diego Zoo Wildlife Alliance

By: DocuSigned by: Shawn Dixon 741EB4932ED04AF...

Shawn Dixon
Chief Operating Officer

Date: 8/10/2023

References

Center for Natural Lands Management

By: Deborah L. Rogers

Deborah L. Rogers
Co-Executive Director & Director of
Conservation Science and Stewardship

Date: August 11, 2023

EXHIBIT A

Research Description

Provided by Dr. Debra M. Shier
Brown Endowed Associate Director of Recovery Ecology
San Diego Zoo Wildlife Alliance
Email: dshier@sdzwa.org

The Pacific Pocket Mice (PPM) collected from the Dana Point Preserve (DP) will be used in the conservation breeding program to increase the genetic diversity of the captive population and maintain representation of DP alleles in an ex situ population. We will conduct both intrapopulation matings and interpopulation matings (between DP and South San Mateo or Santa Margarita) to better evaluate the fitness of admixed and non-admixed animals and increase our power to detect fitness impacts of outcrossing. Finally, as described in the Genetic Management Plan, we wish to further explore in captivity the ability to perform backcrosses to maintain the karyotype characteristic of DP in admixed PPM.

Because the goal of collection is to increase genetic diversity of the captive population, we propose to set trapping lines throughout habitat to maximize the distance between mice that are collected. This then provides an opportunity for the collection of tissue for the evaluation of the status of the DP population. We will take a genetic sample using the ear snip method from every PPM captured. The PPM genetic management plan recommends collection of tissue from an estimated 1-3 mice per 50 m x 50 m area. Because the suitable PPM habitat in the DP headlands includes approximately 25 50 x 50m grids, collection of tissue from no more than 75 individuals would be used for a full evaluation of the status of the genetics of the DP population. Lower sample sizes are still valuable for genetic monitoring. We will conduct microsatellite analyses on tissue samples from the mice captured at the DP headlands by the end of 2023. A full evaluation of the current status of the DP population using SNP-based analysis will be conducted as soon as possible.

To evaluate abundance of PPM currently present at the Dana Point (DP) headlands, we used the 2023 track tube data from CNLM and followed protocols established in 2013 for the use of track tube data in support of collections at South San Mateo. Because the relationship between track tube detections and number of PPM is not well understood, in 2013 we used PPM home range to approximate how many track tubes a single PPM might be likely to visit on average over one week (i.e., the time interval of the track tube data). Based on a home range greater than 12 meters but less than 25 meters in diameter, we assumed a PPM would be likely to visit track tubes within 12.5 meters of one another but not 25 meters apart. Accordingly, we assumed a single PPM could visit

up to 4 track tubes during a week, suggesting a maximum population density of around 16 PPM per 1 hectare sampling grid at Camp Pendleton.

At DP headlands, trap tubes are placed in two 12 m x 12 m quadrants of each 24 x 24 m grid cell resulting in lower track tube densities than are used at Camp Pendleton and staggered trap spacing of 16.9 meters between traps within the same grid cell and 24 m among grid cells. Because combining the two quadrants of data for each grid cell at DP is of a similar scale to combining the 4-track tube data at Camp Pendleton, we assumed the combined grid cell level data at Dana Point approximates the size of a PPM's home range. Based on this assumption, we estimate that up to 69 individual PPM (average= 48.6) have been detected during the five weeks of monitoring at Dana Point year (Table 1).

Table 1. Number of Grid Cells within which PPM were detected each week from May 31 – Jun 27, 2023.

Survey Date	May 31	June 6	June 13	June 20	June 27
Number of PPM detections	28	52	69	56	38

As detailed in the Draft Genetic Management Plan, we would like to collect 4 mice (2 females and 2 males) to bring into the captive colony which, based on the approximated size of the DP population, is consistent with the criteria that we have been using that collections will not exceed 10% of adults or 20% of young of the year to minimize the impact to the source population.

Preferably we would like to collect 4 young of the year, but if fewer than 4 young of the year are captured, in addition to captured young of the year, we propose to collect no more than 3 adults to reach our goal.

EXHIBIT B

Access Special Conditions

1. Access for Research is provided for a maximum of five (5) trap-nights.

2. SDZWA will only enter and exit the Preserve through the access gates. The Preserve Manager will provide access if entrance occurs after gates are locked (sunset daily).

3. SDZWA will not allow access onto the Preserve for anyone who is not associated with the research project or who has not signed a Release Form (Exhibit D).

4. When working after public-access hours (i.e., sunset to 7:00 a.m. daily), if the SDZWA observes any members of the general public onsite and if CNLM staff are not present at that time, the SDZWA will:

    i. If safe, approach and notify the individual(s) that the Preserve is closed and he/she/they must leave;
    ii. If not deemed safe or in the event of non-compliance, notify law enforcement ('911'); and
    iii. Notify the Preserve Manager of any trespassers observed or encountered on-site during business hours or by email.

5. SDZWA will secure the site before, during, and after work is completed. No gates shall be left open, ajar, or unlocked.

Preserve Manager Contact Information:

Korie Merrill
CNLM Regional Preserve Manager, South Coast Region
Email: kmerrill@cnlm.org
Phone: (760) 731-7790 Ext. 204
Direct: (949) 218-1145

EXHIBIT C

Special Provisions and Conditions

1. Field activities shall be conducted in such a manner as to not injure or kill federally threatened coastal California gnatcatcher (*Polioptila californica californica*). The Preserve Manager will brief SDZWA on any necessary precautions and these will be observed at all times while on the Preserve.
2. Field activities will also be conducted in such a manner to avoid impacts on other sensitive resources such as rare plants, the general location and sensitivity of which to be provided by Preserve Manager, as appropriate.
3. Trapping route (to trap locations) will be determined, flagged, and faithfully followed to minimize disturbance to habitat and avoid any active rodent burrows. Trapping route and trap locations must be approved by the Preserve Manager.
4. Trapping will be immediately stopped if there is a trap injury or death of any PPM or any other injury or death, any trap injury or death of any nontarget animal, or at the discretion of the Preserve Manager. Additionally, if trap success is low enough to suggest that the size of the Dana Point PPM population has been significantly overestimated (e.g., failure to capture at least 20 individuals), SDZWA will confer with CNLM and likely postpone collections to another time.
5. No pets (including dogs on-leash) are allowed within the Preserve.
6. Guidance for Pacific pocket mouse trapping: Trapping strategy for capturing individuals for the captive breeding program shall be strongly informed by minimizing stress on Preserve pocket mice and other listed or sensitive species, minimizing likelihood of repeated captures of same individuals, and reducing impact on the breeding activity of the resident wild population.
7. Limits for removal of Pacific pocket mice from Preserve: No pregnant or lactating individuals are to be removed from the Preserve. A maximum of four (4) individuals of Pacific pocket mouse may be removed from the Preserve for the Research. They may be composed of any number of either sex and of any age. Tissue samples (ear snips) may be taken from all (unique) mice captured. Only one sample per mouse is allowed. The Preserve Manager must approve: 1) the selection of mice for removal and 2) the means by which the selections will be made (e.g., length of time in traps or other types of captivity while decisions are made).

EXHIBIT D

RELEASE FORM

ACCESS FOR RESEARCH ASSISTANCE ON (S033) DANA POINT PRESERVE ("Preserve")

Dear _____
(PRINT COMPLETE NAME OF INDIVIDUAL SEEKING LIMITED SITE ACCESS)

You have requested permission from the Center for Natural Lands Management (CNLM) to enter upon the Preserve for the purpose of conducting research on the Pacific pocket mouse as more fully described in the August 2023 Site Access for Research Agreement ("Agreement') between CNLM and Zoological Society of San Diego d/b/a San Diego Zoo Wildlife Alliance ("SDZWA").

In consideration of CNLM, its officers, directors, employees, and agents (collectively, "Released Parties") agreement to permit your participation in the foregoing activity on the Preserve at various times between (date of Agreement execution) and September 30, 2023, you agree to each of the following conditions:

1. You acknowledge that you are aware and have been informed that the Preserve may present risk to your safety. You agree to assume all risks involved in your visit to the Preserve. You agree to take all necessary precautions while on or about the Preserve to avoid injury to yourself or others. You also acknowledge that you are physically able to undertake the activities contemplated on the Preserve. You agree to follow the rules for the Preserve provided to you by CNLM, or by SDZWA as may be required by the Agreement. You agree that although they may not choose to be present during your visit to the Preserve, neither CNLM nor any other Released Party shall have any obligation to you.

2. You hereby release each and all of the Released Parties from any and all liability, claims, causes of action, damage, loss, casualty, expense, or similar items, in law or in equity, whether known or unknown, that you may suffer or incur as a result of, or relating to, your visit(s) to the Preserve.

3. You hereby agree to indemnify, defend and hold each and all of the Released Parties, harmless in all respects from any and all claims causes of action, judgments costs, expenses, attorney's fees, and liabilities (collectively "Claims"), whatsoever, arising in any way in connection with your visit(s) to the Property, but only in proportion to and to the extent that such Claims arise from the negligent or intentional acts or omissions of SDZWA, its officers, agents, or employees.

4. You agree to accept any and all financial and legal responsibility for any fire-fighting activities that are a direct result of your activities while on the Preserve.

Please indicate your agreement to the above by signing this letter.

Sincerely yours,

CENTER FOR NATURAL LANDS MANAGEMENT

The above is hereby agreed to and confirmed this _____ day of _____, 2023.

PRINT NAME SIGNATURE:

_____ _____

Address: _____

Telephone: _____