

**Determining and Measuring Environmental Influences
on the Distribution and Abundance of
Casey's June Beetle (*Dinacoma caseyi*)**

FINAL REPORT

2015–2022



U.S. Fish and Wildlife Service
Palm Springs and Southern Nevada Fish and Wildlife Office
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IN MEMORIAM

This report is dedicated to the late Dr. Christopher James Gregory (1970–2018), without whom this study would not have occurred. The study was envisioned and created by Chris, whose efforts began in 2014, continued until his passing, and included securing funding and collaborations, study design, gathering and inspiring volunteers, and tireless efforts in the field to innovate methods, complete surveys, and ensure rigorous data collection. His creative initiative, hard work, and dedicated commitment to Casey’s June beetle conservation has contributed invaluable and significant progress toward understanding the foundational ecology of an otherwise largely unknown species.



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Title Page Photo: A male Casey's June beetle. Photo Credit: Jon Avery (retired), U.S. Fish and Wildlife Service.

In Memoriam Photo: Christopher Gregory in Palm Canyon Wash. Photo Credit: Noelle Ronan, U.S. Fish and Wildlife Service.

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PERMITS

In accordance with section 10(a)(1)(A) of the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*), fieldwork was conducted under U.S. Fish and Wildlife Service recovery permits. Activities conducted in 2016–2018 were authorized under Recovery Permit TE-837760 (Kendall H. Osborne), in 2019 under Carlsbad Fish and Wildlife Office (CFWO) Recovery Subpermit FWSCFWO-37, in 2020 under CFWO Recovery Subpermit FWSCFWO-38, in 2021 under CFWO Recovery Subpermit FWSCFWO-40, and in 2022 under CFWO Recovery Subpermit ES-034097. A U.S. Fish and Wildlife Service Letter of Authorization

(LOA-3154) for the possession and use of Casey's June beetle genetic samples was provided to Dr. Daniel Rubinoff, University of Hawaii at Mānoa. Access to study locations in portions of Palm Canyon Wash and Tahquitz Creek were authorized under the Riverside County Flood Control and Water Conservation District's No-Fee Access Permits which were issued annually for the duration of the study. Access to study locations in Smoke Tree Ranch was authorized by written communication via email from Tracy Conrad, Chief Operating Officer, with Chris Gregory and Noelle Ronan, U.S. Fish and Wildlife Service. Access to study locations in the Tahquitz Creek Golf Resort in 2021 and 2022 was authorized under written approval by the City of Palm Springs via email communication with Noelle Ronan, U.S. Fish and Wildlife Service.

ABSTRACT

Casey's June beetle (*Dinacoma caseyi*) is a narrow endemic species found only in Palm Springs, Riverside County, California. It was listed as a federally endangered species on September 22, 2011, in accordance with the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*). Most of the species' historical desert alluvial fan habitat has been lost to development. Once-contiguous habitat has been fragmented into a patchwork of small habitat areas within a mostly developed landscape. Its distribution is now restricted to a greatly reduced area of suitable habitat. Commensurate with losses of habitat and interruptions of ecosystem processes, the threats facing the species continue to increase, making the need for protection and management vital.

Little information is available on Casey's June beetle. This is the only study to date to collect basic ecological data and evaluate abundance and distribution with habitat and environmental conditions. We established 28 long-term survey plots throughout the species' range in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek to estimate male Casey's June beetle abundance, distribution, and flight distances as a function of light attraction using mark-recapture and assessed vegetation, habitat, and environmental conditions associated with emergence and breeding. Associated derivative studies evaluated genetic structure within the Casey's June beetle population. We also conducted observational field studies and pilot laboratory observations of adults and immature beetles to evaluate fundamental aspects of biology and life history.

From April 2016 to June 2022, we captured and marked adult male Casey's June beetle using black light traps at survey plots as the primary method to record capture and recapture counts, evaluate flight movements among traps, emergence and activity patterns associated with environmental variables, body size and relative body condition, and collect specimens for genetic analysis. We conducted vegetation mapping, and sampled vegetation at survey plots. We deployed wireless temperature data loggers (Kestrel Drop D1®) underground at survey plots to examine the influence of soil temperature variation on Casey's June beetle emergence. From 2015–2017, we mapped Casey's June beetle emergence holes to describe relative activity patterns. In 2019 and 2022, we conducted small-scale soil excavations to locate larvae and pupae for description and determine burrowing depths. Larvae were collected for observational laboratory rearing to document life history information and identify species. We documented adult male Casey's June beetle longevity under mild outdoor captive conditions and evaluated the durability and retention of the marking method applied to adult males. Our study was not focused on female Casey's June beetle; however, we collected a small sample of gravid adult females for a pilot laboratory study of their biology and ecology.

We found wide inter-annual fluctuation in relative abundance, and variation in scale and covariates influencing Casey's June beetle population dynamics. Based on data from 2018, there was an average daily emergence of 9,554.4 (95 percent CI=7,790.1–11,191.7) male Casey's June beetles during the April–May breeding season. The average recapture probability was 0.096 (95 percent CI=0.082–0.109) and there was a consistent low rate of recaptures (4.7 percent). The effective capture radius around traps was 253.6 feet [ft; 77.3 meters (m)]. We documented a

maximum flight distance of 2,543 ft (775 m) within a single evening, which are the first flight distances recorded for Casey's June beetle. Casey's June beetles were associated with both native and nonnative vegetation with no specific plant species association. We confirmed the previously unknown taxonomy of Casey's June beetle as a unique species. Although differentiation within the Casey's June beetle population was hypothesized, our results documented no population substructure. Data collected on Casey's June beetle emergence holes, breeding season length, and descriptions of larvae, pupae, and adults based on soil surveys and field and laboratory observational studies are the first for this species.

This study provides an array of descriptive statistics, analyses estimating male abundance, and information on population dynamics, ecology, and habitat associations which improves our understanding of habitat management and conservation measures beneficial for the population. The relative abundance estimates, and inter-annual fluctuations represent an initial modeling of the capture results from data during a 6-year time span. Given that sampling artifacts in trend estimation decrease in time series with more than 10 time-points, and that statistical power increases in time series with more than 15 time-points, our complete 7-year dataset is still in progress. Additional surveys are needed to develop the baseline and long-term time series needed to understand population trends.

Keywords: Casey's June beetle, *Dinacoma*, abundance, mark-recapture, habitat assessment, Coachella Valley, Southern California, insect conservation, endangered species

TABLE OF CONTENTS

IN MEMORIAM	ii
ACKNOWLEDGEMENTS	v
Permits	v
ABSTRACT	vii
List of Figures	xii
List of Tables	xv
INTRODUCTION	1
Species Overview	1
Purpose and Objectives	5
METHODS	7
Study Area and Sampling Design	7
Abundance and Distribution.....	8
<i>Black Light Trapping</i>	8
<i>Rangewide and Sentinel Surveys</i>	13
<i>Mark-Recapture</i>	14
Vegetation Communities.....	15
Underground Soil Temperature.....	16
Emergence Holes.....	17
Body Size, Body Condition, and Emergence Hole Size	18
<i>Comparisons with Dinacoma marginata</i>	18
Field and Laboratory Observations	19
<i>Male Observations</i>	19
<i>Female Observations, Collection, and Laboratory Studies</i>	19
<i>Larvae and Pupae</i>	22
Genetics.....	23
Data Analysis	26
<i>Abundance and Distribution</i>	26
<i>Ecology, Vegetation Communities, and Genetics</i>	28
<i>Assumptions and Caveats</i>	30
RESULTS	32
Sampling Design	32
Abundance and Distribution.....	35
<i>Abundance Estimation, Capture Patterns, and Environmental Associations</i>	35

<i>Survey and Capture Summary – Descriptive Statistics and Results</i>	46
Vegetation Communities	57
Underground Soil Temperature.....	65
Emergence Holes.....	70
Body Size, Body Condition, and Emergence Hole Size	78
<i>Body Size</i>	78
<i>Emergence Hole Size</i>	80
<i>Comparisons with Dinacoma marginata</i>	81
<i>Relative Body Condition</i>	81
Field and Laboratory Observations	83
<i>Male Observations</i>	83
<i>Female Observations, Collection and Laboratory Studies</i>	84
<i>Larvae and Pupae</i>	90
<i>Incidental Observations of Predation</i>	98
Genetics	99
DISCUSSION	103
Abundance and Distribution.....	103
<i>Capture and Recapture Patterns</i>	104
Vegetation Communities	104
Hydrology.....	105
Biology and Ecology	106
<i>Male Observations</i>	108
<i>Body Size and Emergence Holes</i>	108
<i>Soil Excavation Surveys and Larvae</i>	109
<i>Genetics and Connectivity</i>	109
<i>Incidental Observations</i>	110
Conservation.....	111
<i>Seasonal Restriction for Species Protection</i>	111
<i>Effective Survey Area</i>	112
<i>Prospects for Population Augmentation</i>	112
<i>Habitat Management and Conservation Measures</i>	113
<i>Best Practices for Herbicide Use in Casey’s June Beetle Habitat</i>	114
Recommendations and Future Research	116
REFERENCES	118
APPENDIX A – Biologist, Staff, and Volunteer Participants.....	127

APPENDIX B – Casey’s June Beetle Long-Term Survey Plots	135
APPENDIX C – Casey’s June Beetle Marking Scheme	138
APPENDIX D – Vegetation Communities.....	141
APPENDIX E – Genetics Publications and Report.....	209
APPENDIX F – Population Analysis	240
APPENDIX G – Survey and Capture Summaries 2016–2022	293
APPENDIX H – Underground Soil Temperature 2017 and 2019.....	302
APPENDIX I – Emergence Hole Map Series.....	306
APPENDIX J – Buried Salvaged Vegetation in Palm Canyon Wash	361

LIST OF FIGURES

Figure 1. Current and historical range of Casey's June beetle in Coachella Valley, Riverside County, California.	3
Figure 2. The pronounced sexual dimorphism of Casey's June beetle between the smaller, white-colored males and the larger, brown, wingless females.	4
Figure 3. Casey's June beetle Rangewide and Sentinel survey plots distributed systematically throughout the species' range in Palm Canyon Wash ($n=12$), Smoke Tree Ranch ($n=4$), and Tahquitz Creek ($n=12$) over the duration of the study from 2016–2022.	9
Figure 4. Casey's June beetle study area in Palm Springs, California.	10
Figure 5. Black light trap used to capture male Casey's June beetles during surveys at long-term survey plots.	11
Figure 6. A male Casey's June beetle from the study prior to release after being marked with a unique identifier.	15
Figure 7. Temperature data loggers (Kestrel Drop D1®) attached to an aluminum stake prior to underground installation to record soil temperature variation at a long-term survey plot. ...	16
Figure 8. Variation in body wear and size of male Casey's June beetles.	20
Figure 9. Male and female Casey's June beetles emerging to breed and the resulting emergence holes created from burrowing through soil.	21
Figure 10. Soil excavation surveys for Casey's June beetle larvae and pupae in 2019 and 2022.	24
Figure 11. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and percent moonphase (turquoise line).	36
Figure 12. Annual and across-year temporal variation in peak captures of Casey's June beetles.	38
Figure 13. Probability of detecting (i.e., capturing) an individual male Casey's June beetle, given that it has emerged and is present in the trapping area.	39
Figure 14. Within-year estimated corrected abundance at each trap site, after accounting for imperfect detection of individual male Casey's June beetles within a given evening.	40
Figure 15. Corrected site abundance estimates of male Casey's June beetles on each of five trapping evenings during 2021.	41
Figure 16. Estimated mean nightly abundance of male Casey's June beetles between April 26th and May 24th, 2018.	43
Figure 17. Predicted site abundance of Casey's June beetles as sites are located further upstream from the Cathedral City gauge station, Palm Springs, California, 2016-2018.	44

Figure 18. Predicted increase in average abundance of male Casey's June beetles as a function of plant species richness at a site, regardless of whether the species was native or naturalized, 2016 and 2019.	44
Figure 19. Median Casey's June beetles captured by trap (x-axis) during Rangewide surveys from 2016–2020 in Palm Canyon Wash (blue), Smoke Tree Ranch (orange), and Tahquitz Creek (gray).	49
Figure 20. Median Casey's June beetles captured by trap during Rangewide surveys from 2021–2022 in Tahquitz Creek and Palm Canyon Wash.	50
Figure 21. Median (top), maximum (middle), and CPUE (catch per unit effort; bottom) of Casey's June beetles captured during Sentinel surveys from 2016–2020.	51
Figure 22. Total Casey's June beetle captures by month during Sentinel surveys for all years combined, 2016– 2020.	52
Figure 23. Casey's June beetle total catch per total survey effort (CPUE; hour) during Rangewide and Sentinel surveys with annual rainfall by year.	53
Figure 24. Vegetation communities and land cover types mapped within Casey's June beetle critical habitat in the study area.	59
Figure 25. Mean and standard deviation (SD) of underground soil temperature (degrees Celsius) recorded at three depths at long-term survey plots ($n=8$ traps combined) in Palm Canyon Wash in 2017.	66
Figure 26. Mean and standard deviation (SD) of underground soil temperature (degrees Celsius) recorded at three depths at long-term survey plots ($n=4$ traps combined) in Palm Canyon Wash in 2019.	66
Figure 27. Daily underground soil temperature (degrees Celsius) recorded hourly in March 2017 (March 7 at 16:00 to March 31 at 23:00) for three depths at survey trap 6.	69
Figure 28. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash in 2015.	71
Figure 29. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash in 2016.	72
Figure 30. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash and the upper reach of Tahquitz Creek in 2017.	73
Figure 31. The mean and standard deviation (error bars) of Casey's June beetle adjusted emergence hole counts by year at long-term survey plots ($n=12$ traps) for 2015–2017 data combined.	74
Figure 32. The mean and standard deviation (error bars) of adjusted emergence hole counts by long term survey plot ($n=12$ traps) for 2015–2017 data combined.	75
Figure 33. Casey's June beetle body measurements from April to June in Palm Canyon Wash in 2017.	79

Figure 34. Casey's June beetle body weight from May to June in Palm Canyon Wash in 2017.	80
Figure 35. Frequency of relative body condition categories of Casey's June beetles in Palm Canyon Wash and Smoke Tree Ranch by month in 2016.	82
Figure 36. Frequency of relative body condition categories of Casey's June beetles in Palm Canyon Wash and Tahquitz Creek by month in 2017.	83
Figure 37. No obvious negative effects on individual Casey's June beetles from marking were observed.	85
Figure 38. Male Casey's June beetles incidentally observed in grass vegetation (A) and in shrubs (B) in April and May 2020, during surveys in Palm Canyon Wash.	86
Figure 39. Casey's June beetle eggs and larvae from laboratory study in 2016 and 2020, respectively.	89
Figure 40. Casey's June beetle larvae propagated from an egg laid by a female Casey's June beetle collected in 2021 and maintained under captive rearing conditions.	91
Figure 41. Beetle larvae found during soil excavation surveys in 2019 and 2022, Palm Canyon Wash.	92
Figure 42. Casey's June beetle pupa and larval exuvium found during a soil excavation survey at the Sentinel plot in March 2022, in Palm Canyon Wash.	95
Figure 43. Morphological description of Casey's June beetle larvae.	97
Figure 44. Morphological description of male Casey's June beetle pupa collected from Palm Canyon Wash, March 2022.	101

LIST OF TABLES

Table 1. Climate and environmental variables recorded during Casey's June beetle surveys.	12
Table 2. Adult male Casey's June beetle (<i>D. caseyi</i>), <i>Dinacoma marginata</i> , and <i>Dinacoma sanfelipe</i> collected for genetic analysis.	25
Table 3. Casey's June beetle survey areas by survey type, location, years, number of traps, and purpose.	33
Table 4. The number and timing of Casey's June beetle Rangewide and Sentinel surveys by year, 2016–2022.....	46
Table 5. Casey's June beetle first and last emergence dates based on timing of first and last surveys during Sentinel surveys in 2016–2020.	47
Table 6. Casey's June beetle total captures and catch per unit effort (CPUE; survey hours) during Rangewide and Sentinel surveys with rainfall by year.	53
Table 7. Average recapture rate (percent of total captures) during Rangewide surveys by capture frequency category and location, 2016–2022 combined.	55
Table 8. Casey's June beetle total captures and recaptures, and median and maximum recapture distances, calculated as the minimum straight-line distance from the release location to the recapture trap, 2016–2022.	56
Table 9. Vegetation communities, land cover types, and acres within long-term survey plots sampled in 2016 using the relevé method.	60
Table 10. Percent vascular plant cover by stratum, change in percent cover between 2016 and 2019, and median number of Casey's June beetles captured at traps during Rangewide and Sentinel surveys in 2016 and 2019.....	63
Table 11. Monthly mean underground soil temperature (degrees Celsius) recorded at 15 cm, 65 cm, and 115 cm depths at long-term survey plots, combined, in Palm Canyon Wash in 2017 and 2019.	67
Table 12. Casey's June beetle emergence hole survey area and total emergence hole counts from 2015–2017.....	74
Table 13. Casey's June beetle emergence hole counts, captures, vegetation, and percent compacted soil in long-term survey plots (trap), 2015–2017.....	76
Table 14. Percent of Casey's June beetle emergence holes observed in dominant vegetation communities, 2015–2017.	78
Table 15. Casey's June beetle body measurements from Palm Canyon Wash and Tahquitz Creek in 2017.	79
Table 16. Casey's June beetle emergence hole measurements sampled from Palm Canyon Wash and the upper reach of Tahquitz Creek.....	81

Table 17. Relative body condition of Casey's June beetles in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek, 2016–2017, summarized as the frequency of each category. ..	82
Table 18. Results of gravid female Casey's June beetle and beetle larvae study under small-scale captive rearing laboratory conditions, 2016–2022.	88
Table 19. Soil excavation locations surveyed for beetle larvae in 2019 and 2022 in Palm Canyon Wash.	93
Table 20. Incidental predation observations of Casey's June beetle during surveys from 2016–2021.	102

INTRODUCTION

Casey's June beetle (*Dinacoma caseyi*) is a narrow endemic scarab beetle known only from the alluvial fans, bajadas, and river wash areas at the base of the San Jacinto Mountains in the vicinity of Palm Springs, California. In accordance with the Endangered Species Act of 1973 (Act), as amended (16 U.S.C. 1531 *et seq.*), on September 22, 2011, the U.S. Fish and Wildlife Service (Service) listed Casey's June beetle as a federally endangered species due to loss and modification of populations and habitat [76 FR 58954 (Service 2011)]. Approximately 594 acres (ac) [240 hectares (ha)] of critical habitat was designated in the same Federal listing rule. The California Endangered Species Act does not provide protection for insect species; therefore, Casey's June beetle has no state listing status.

Casey's June beetle is currently represented by a single, small population that has exhibited a significant decline in its available habitat and a substantially reduced distribution. About 97 percent of their historical range has been lost due to development (Service 2011). No empirical information is available to determine the finite rate of population change for Casey's June beetle, outside of the closely related rate of loss of available habitat over the last several decades. Population size has likely been declining, concomitant with the loss of habitat to development.

Loss of occupied habitat has continued since the early 1990s. In the final Federal listing rule (Service 2011), the Service determined that 28 percent (287 ac [116 ha]) of the 1,018 ac (412 ha) of contiguous suitable habitat for Casey's June beetle identified as extant (based on 1991 aerial photographs) had been lost to development. From 2003 to 2005, the loss of occupied Casey's June beetle habitat occurred at a rate of 3.8 percent per year. At the time of Federal listing, the Service estimated the amount of undeveloped habitat occupied by the species was approximately 605 ac (245 ha) (including all non-contiguous habitat containing any soil types used by Casey's June beetle) within the City of Palm Springs (Service 2011). The species' current distribution is estimated at 1,989 ac (805 ha; Service 2025), which is based on information provided by this study and suitable habitat modeling that included soils, vegetation, and hydrology in occupied habitat.

Most of the occupied area remaining in the City of Palm Springs is threatened by habitat destruction, degradation, and fragmentation due to urban and recreational development and flood control maintenance and damage reduction activities. Other likely threats include ground and vegetation disturbance, the attraction effect of artificial lighting and swimming pools, hydrology changes within habitat areas, and their small and declining (presumed by habitat loss) population size. In addition to having a restricted range and small population size, the species also has limited dispersal capabilities (Hovore 2003). These conditions most likely increase the degree of threat due to chance events, such as floods or drought, which are beyond the natural variability of the ecosystem (Lande 1993). The risk of local extinction is widely noted to increase as the fraction of occupied habitat patches, occupied patch area, and density of occupied patches decrease (Forman and Godron 1986; Hanski 1991; Hanski *et al.* 1995; Hokit and Branch 2003).

SPECIES OVERVIEW

Casey's June beetle was first collected in 1916 in the Palm Springs area and later described by Blaisdell based on male specimens (Blaisdell 1930; Service 2011). The genus *Dinacoma* and

approximately 106 other genera constitute the New World members of the subfamily Melolonthinae (May beetles, June beetles, and chafers) of the scarab beetle family (Scarabaeidae) (Evans and Smith 2009). Casey's June beetle is part of a genus of beetles with naturally restricted ranges and is adapted to specialized habitat and soil types. The exact historical distribution of Casey's June beetle is unknown due to the generality and paucity of location descriptions from early collection records (Service 2006; Service 2011). However, museum specimen records indicate the historical range can be described from the City of Palm Springs south to the community of Indian Wells, prior to the development of the Coachella Valley. Currently, the only remaining population of Casey's June beetle occurs in and adjacent to Palm Canyon Wash and Tahquitz Creek in Palm Springs (Figure 1), an area of less than 1,900 ac (769 ha) of suitable habitat (Service 2025). The species only occurs on the desert floor; its known elevation ranges from 335 ft (102 m) to 580 ft (177 m). Its habitat is a dynamic desert wash ecosystem including alluvial soils, seasonal hydrology from the surrounding mountainous watershed, and native and nonnative desert wash and desert scrub vegetation. Therefore, important habitat characteristics include climate, hydrology, soil type, and vegetation (Service 2025).

Casey's June beetle is a medium-sized June beetle between 0.56 to 0.70 inches (1.4 to 1.8 centimeters [cm]) long (Evans and Hogue 2006). Male Casey's June beetles have a dusty brown or whitish coloring, and brown and cream longitudinal stripes on the elytra (wing covers and back) (Blaisdell 1930; Bruyey 2006). Most of the body has a covering of whitish scales, supplemented on much of the head, thorax, and ventral surfaces with fine, white hairs, giving the males a chalky appearance (Osborne 2015). Their reddish-brown antennae are clubbed, as is common to scarab beetles. The clubbed ends consist of a series of leaf-like plates that can be held together or fanned out to detect scents. A pronounced sexual dimorphism is evident between the smaller, white-colored males and the larger, brown, wingless females (Figure 2). Females are flightless and are characterized by an enlarged abdomen, reduced legs and antennae, and metathoracic wing reduction (reduction in flight wing size in adults).

The majority of the Casey's June beetle life cycle is spent underground where egg laying, larval, and pupal development occurs until the adult ecloses (the process of moulting or emerging into adult), burrows to the soil surface to breed aboveground during their flight season, typically from early April to June. Adult males emerge and begin flying around dusk, with peak emergence and flight happening up to two hours after dusk. The males fly swiftly and low over the ground, searching for females. Adult females emerge and emit pheromones to attract males. The flightless females immediately burrow underground after mating. Current evidence supports the hypothesis that Casey's June beetle larvae do not require particular host plant species for feeding (Service 2011). Like other species of June beetle, the food source for Casey's June beetle larvae appears to be plant rootlets and organic detritus (Hovore 2003; Osborne 2020, unpubl. data; Osborne 2022a, unpubl. data). Males are generally distributed across the species range. Distribution of the flightless females is unknown, but we assume that dispersal and genetic mixing is attributed to males.

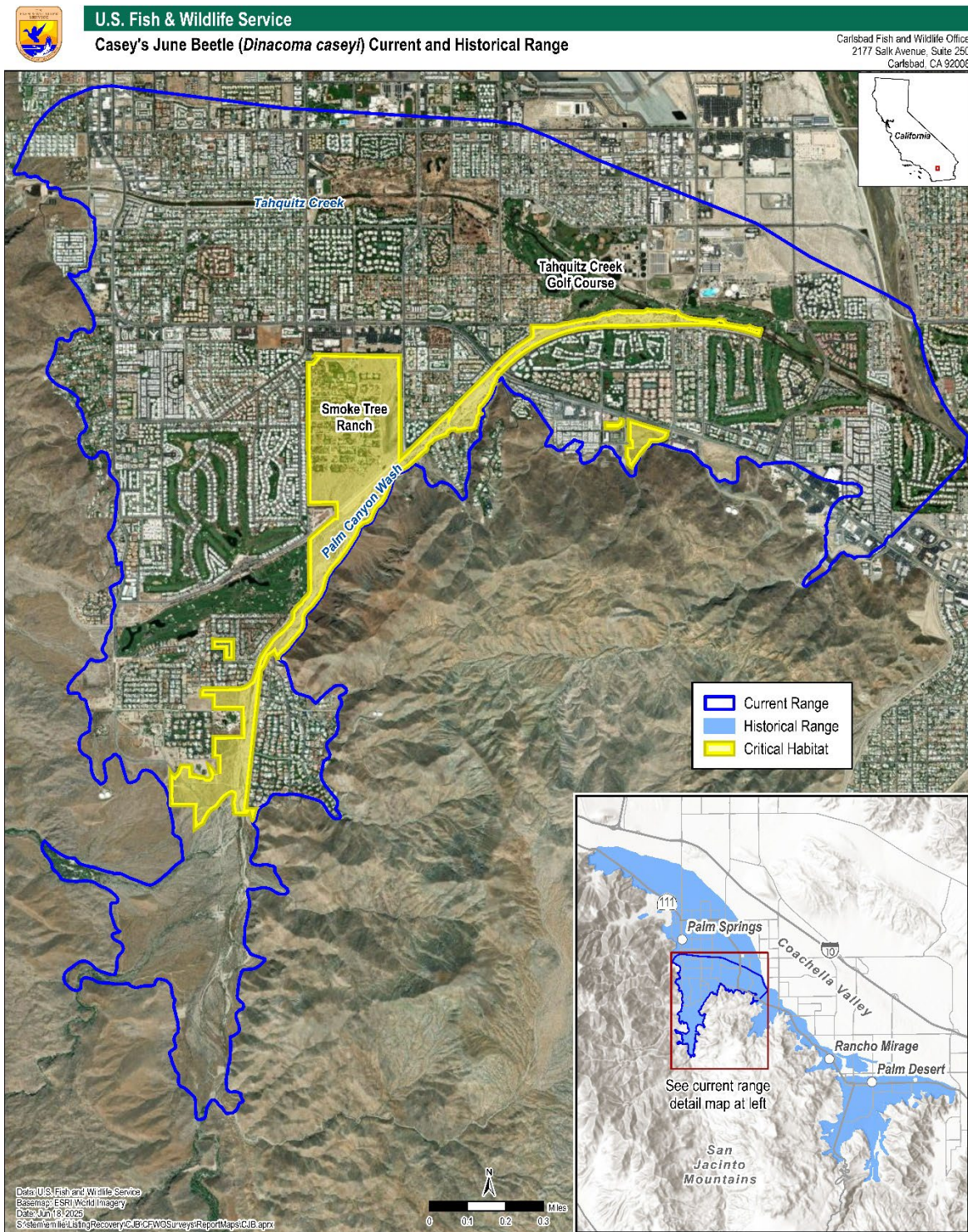


Figure 1. Current and historical range of Casey's June beetle in Coachella Valley, Riverside County, California.

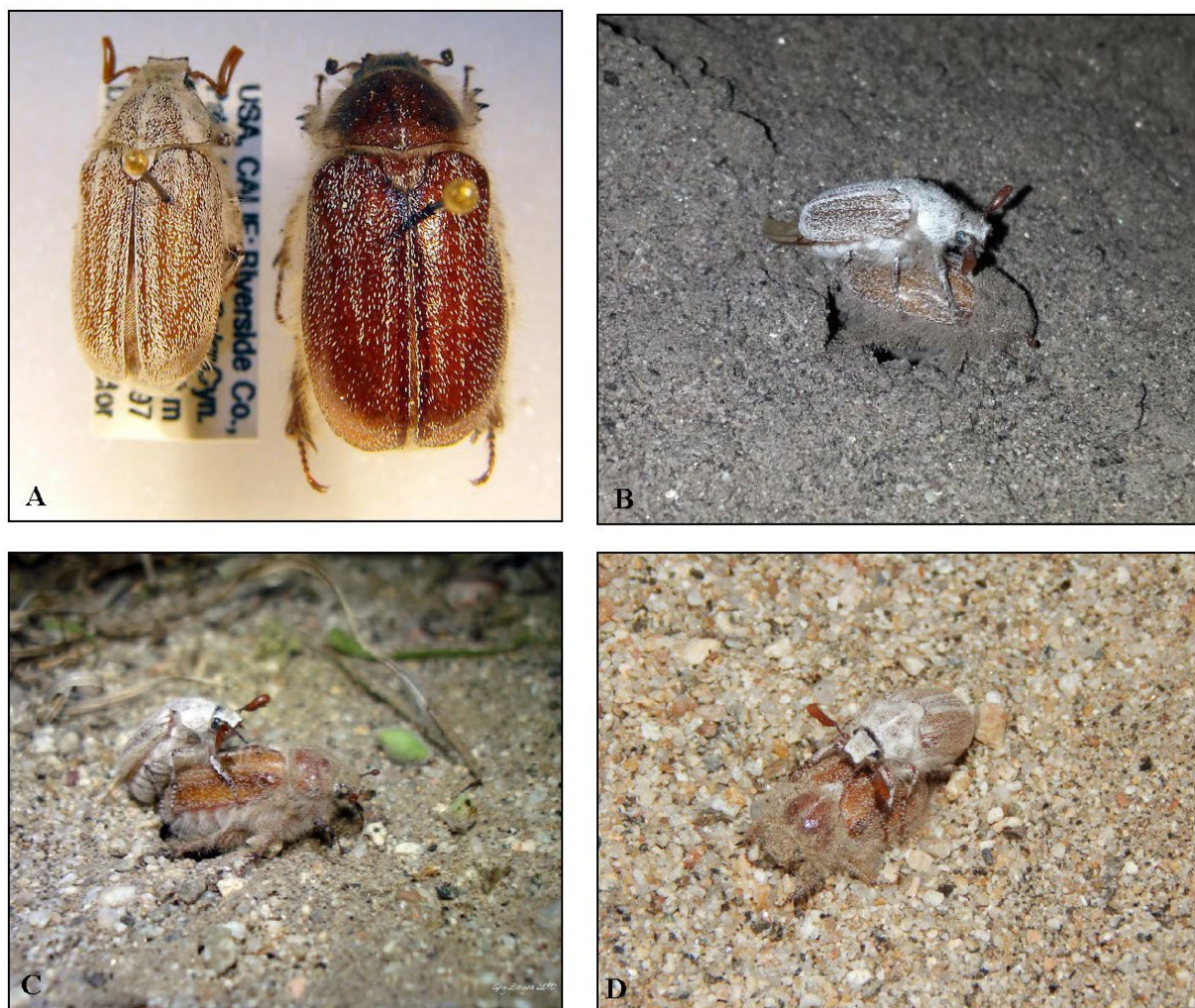


Figure 2. The pronounced sexual dimorphism of Casey's June beetle between the smaller, white-colored males and the larger, brown, wingless females. (A). Pinned specimens, San Diego Natural History Museum reference collection, illustrating the size and color difference between the male (left) and female (right). Specimens collected in the Palm Springs vicinity, April 15, 1986. (B, C, D). Mating during the breeding season.²

Casey's June beetle is associated with broad, gently sloping alluvial fans (bajadas) at the base of the Santa Rosa and San Jacinto Mountains in the Coachella Valley (Service 2009). Historically, these alluvial fans were sandy, dry washes with meandering channels, ephemeral flows and dry upland areas associated with soil deposition resulting from rainfall and erosion (Lancaster *et al.* 2012). Casey's June beetle adapted within this hydrological regime that presumably provided such benefits as water inputs to support vegetation; sediment and detritus deposition, an important food resource; maintaining moist soil conditions for larvae; and aiding dispersal of

² Photos: D.A. La Rue (A), Christopher Gregory, U.S. Fish and Wildlife Service (B), Guy Bruyey (C), Philip Clevinger, Wood Environment and Infrastructure Solutions, Inc. (D).

individuals, including the flightless females. Such natural hydrological regimes likely maintains the habitat functions (Poff *et al.* 1997) that Casey's June beetle depends upon. However, most of the historically occurring alluvial fan habitat has been developed and the dry washes constrained within flood control structures that concentrate rather than disperse flows across a broader alluvial floodplain (Alluvial Fan Task Force 2010). Palm Canyon Wash and Tahquitz Creek are largely channelized with structures that contain flows (District 1982), resulting in more common higher magnitude events, greater flow velocities, and greater sediment scour with downstream deposition.

Casey's June beetle is commonly associated with the Carsitas gravelly sand soil series (CdC), described as gravelly sand on 0 to 9 percent slopes (USDA 2016) but is also associated with Riverwash (RA) and Carsitas cobbly sand (ChC) soils. Their burrowing habits suggest that Casey's June beetle requires substrates not composed predominately of larger rocks, compacted soils, heavy silts or clays, or soils that otherwise are too difficult for burrowing. Casey's June beetle is typically associated with the natural community type of Sonoran (Coloradan) desert scrub vegetation (Sonora mixed woody and succulent scrub) located on the alluvial fans and bajadas. Sonoran desert scrub is characterized as scattered assemblages of broad-leaved microphyll shrubs with an open canopy (Mayer and Laudenslayer 1988). This microphyll scrub occurs in areas where soil water availability is somewhat higher than surrounding terraces occupied by creosote bush scrub (*Larrea tridentata*). Vegetation in Palm Canyon Wash primarily consists of desert willow (*Chilopsis linearis*), smoke tree (*Psoralea arguta*), cheesebush scrub (*Ambrosia salsola*), and tamarisk (*Tamarix* spp.). Adjacent areas consist of a variety of commercial, residential, and recreational developments or ruderal plants.

PURPOSE AND OBJECTIVES

Little information is available on Casey's June beetle life history, and no published scientific studies of Casey's June beetle life history, population size, distribution, and dynamics, or individual movement exists (Service 2013). Along with expanding the Casey's June beetle distribution and restoring and protecting suitable habitat, the Service (2013) recommended designing and implementing a rangewide survey and monitoring scheme as a recovery action for the species. To achieve this recovery action, we conducted a rangewide study from 2015 through 2022 to evaluate Casey's June beetle population and habitat to support data-driven management decisions and aid species conservation. We established rangewide long-term survey plots (hereafter, survey plot or trap) to estimate male Casey's June beetle abundance, distribution, and flight distances as a function of light attraction; and assessed habitat and environmental conditions at long-term survey plots throughout Casey's June beetle habitat. Associated derivative studies evaluated genetic structure within the Casey's June beetle population, and pursued pilot laboratory observations of adults and immature beetles to determine fundamental aspects of biology, life history, and immature morphology of Casey's June beetle.

This is the only study to date to systematically collect basic ecological data and evaluate abundance and distribution with habitat and environmental conditions for Casey's June beetle. Our primary goal was to inform our understanding of the species' ecology to improve

conservation outcomes and habitat and population management actions. By conducting a rangewide ecological study, our intent was to:

1. Estimate relative abundance and distribution of male Casey's June beetle using black light trap surveys.
2. Collect ecological and environmental data associated with Casey's June beetle emergence and breeding.
3. Characterize and delineate vegetation communities in Casey's June beetle habitat.
4. Evaluate male Casey's June beetle flight distances as a function of black light attraction using mark-recapture.
5. Evaluate correlations of Casey's June beetle presence and relative abundance with climatic and environmental conditions.

This study was a joint effort led by the Service's Palm Springs Fish and Wildlife Office, in partnership with Osborne Biological Consulting, Dudek, The Living Desert Zoo and Gardens, the University of Hawaii, Heron Ecological, LLC, Riverside County Flood Control and Water Conservation District (District), and volunteers.

METHODS

STUDY AREA AND SAMPLING DESIGN

From March 2015 to June 2022, we studied Casey's June beetle ecology throughout much of its current range in Palm Springs, Riverside County, California. The study area included Palm Canyon Wash, outside of Agua Caliente Band of Cahuilla Indian lands; the terrace floodplain adjacent to Palm Canyon Wash in Smoke Tree Ranch; and portions of Tahquitz Creek, a tributary of Palm Canyon Wash (Figure 3). Palm Canyon Wash, channelized with a concrete-lined levee since the mid-1950s, consists of a constrained, dry desert wash fluvial system supporting native and non-native vegetation (Figure 4). Smoke Tree Ranch is a gated community with dispersed, low impact residential development in the floodplain terrace adjacent to, and fluvially separated by a levee from Palm Canyon Wash, which has been occupied by Casey's June beetle for decades (Service 2013). A portion of Smoke Tree Ranch includes a conservation area for Casey's June beetle with suitable soils and undisturbed desert scrub vegetation (Service 2012; Figure 4). Tahquitz Creek is a tributary to Palm Canyon Wash, portions of which have been modified since 1991 for flood control and recreation. The upper reach of Tahquitz Creek has been modified by a concrete-sided flood control channel surrounded by urban and residential development; the middle reach is not channelized and flows through Prescott Preserve, an abandoned golf course acquired for conservation and restoration in 2022 (previously the Mesquite Golf and Country Club); and the lower reach flows through the operative Tahquitz Creek Golf Resort, which has resulted in a highly modified channel (Figure 4).

During the study, a notably large flood event occurred in Coachella Valley that affected the study area and presented an opportunity to study Casey's June beetle after this episodic hydrologic event. On February 14, 2019, a 200-year storm event resulted in peak flows of approximately 6,000 cubic feet per second (ft^3/s ; 170 cubic meters per second [m^3/s]) and deposited approximately 250,000 cubic yards [yd^3 ; 191,139 cubic meters (m^3)] of sediment in areas of Palm Canyon Wash, which was the highest recorded peak flow since 1980 (Service 2023). The District received emergency U.S. Army Corps of Engineers (Corps) authorization to conduct sediment removal and levee repair in Palm Canyon Wash to maintain the levee's flood control capacity. The District removed a total of 270,000 yd^3 of sediment from a 42-ac (17-ha) area extending from approximately 200 linear ft (61 m) upstream of South Araby Drive (in the middle portion of the wash) and ending 700 linear ft (213 m) downstream of Gene Autry Trail (the lower portion of the wash). Sediment removal occurred between July 29, 2019, and February 28, 2020. The District conducted levee repair in the mid-reach of Palm Canyon Wash to repair damage from extensive flood-related scour. Levee repair occurred along an approximately 3,327 linear ft (1,014 m) reach in Palm Canyon Wash along the levee adjacent to Smoke Tree Ranch, which impacted 12.82 ac of Casey's June beetle habitat. The levee repair occurred from September 1 to December 16, 2020.

We established long-term survey plots where male distribution and abundance, habitat, emergence hole, and underground soil temperature surveys were conducted, individual males were collected for genetic analysis and a small sample of individual females were collected for laboratory study of their biology and ecology. Environmental variables (Table 1) and male

Casey's June beetle size measurements were recorded at plots during distribution and abundance surveys (see *Rangewide and Sentinel Surveys* below). We conducted soil surveys for larvae near plots with higher adult male abundance based on survey results and opportunistically where flood control maintenance activities were occurring.

We placed long-term survey plots systematically to achieve a representative sample distributed throughout the wash and terrace floodplain habitats (Figure 3). In Palm Canyon Wash, Smoke Tree Ranch Casey's June beetle conservation area (hereafter, Smoke Tree Ranch), and the upper reach of Tahquitz Creek, survey plots were approximately 1,640 ft (500 m) apart. In the golf course habitat of the lower reach of Tahquitz Creek, survey plots were 820 ft (250 m) apart. We did not have permission to survey the middle reach of Tahquitz Creek or Agua Caliente Band of Cahuilla Indian lands in Palm Canyon Wash. To accomplish simultaneous surveys at plots throughout the species' range, we used a team of biologists from the Service, Dudek, Osborne Biological Consulting, Ecological Conservation and Management, Wood Environment and Infrastructure Solutions, Inc. (Wood), The Living Desert Zoo and Gardens, and volunteers from partner agencies and the local community (Appendix A; Table A1).

Female Casey's June beetle life history varies from the males, including their detection probability and survey methodology. Our study focused on male Casey's June beetle. Because study of females was outside our scope, information on female Casey's June beetle was mostly limited to incidental observations (see *Female Observations, Collection and Laboratory Studies* below).

ABUNDANCE AND DISTRIBUTION

Black Light Trapping

Insect collecting traps using a black (ultraviolet) light is an effective, commonly used method to capture light-attracted, night-flying insects of a variety of taxa, including Coleoptera (Triplehorn and Johnson 2005; White *et al.* 2016; Zemel and Houghton 2017; Owens and Lewis 2018). We captured adult male Casey's June beetle using black light traps at long-term survey plots as the primary method to collect capture and recapture counts to evaluate distribution and estimate abundance, Casey's June beetle movements among traps, emergence and activity patterns associated with environmental variables, body size measurements, relative body condition, and specimens for genetic analysis. The Service's Casey's June beetle presence/absence survey guidelines (Service 2016), which are typically used to assess Casey's June beetle presence on proposed development sites, was the framework for the survey methodology, modified to provide broader information on distribution, and emergence and activity patterns. We modified surveys to include a wider range of temperature and wind conditions, and seasonal timing. Other survey specifications were modified to achieve study goals including the frequency of surveys, spacing of black light traps, survey length (number of hours per survey, longer survey season), and marking individual Casey's June beetles.

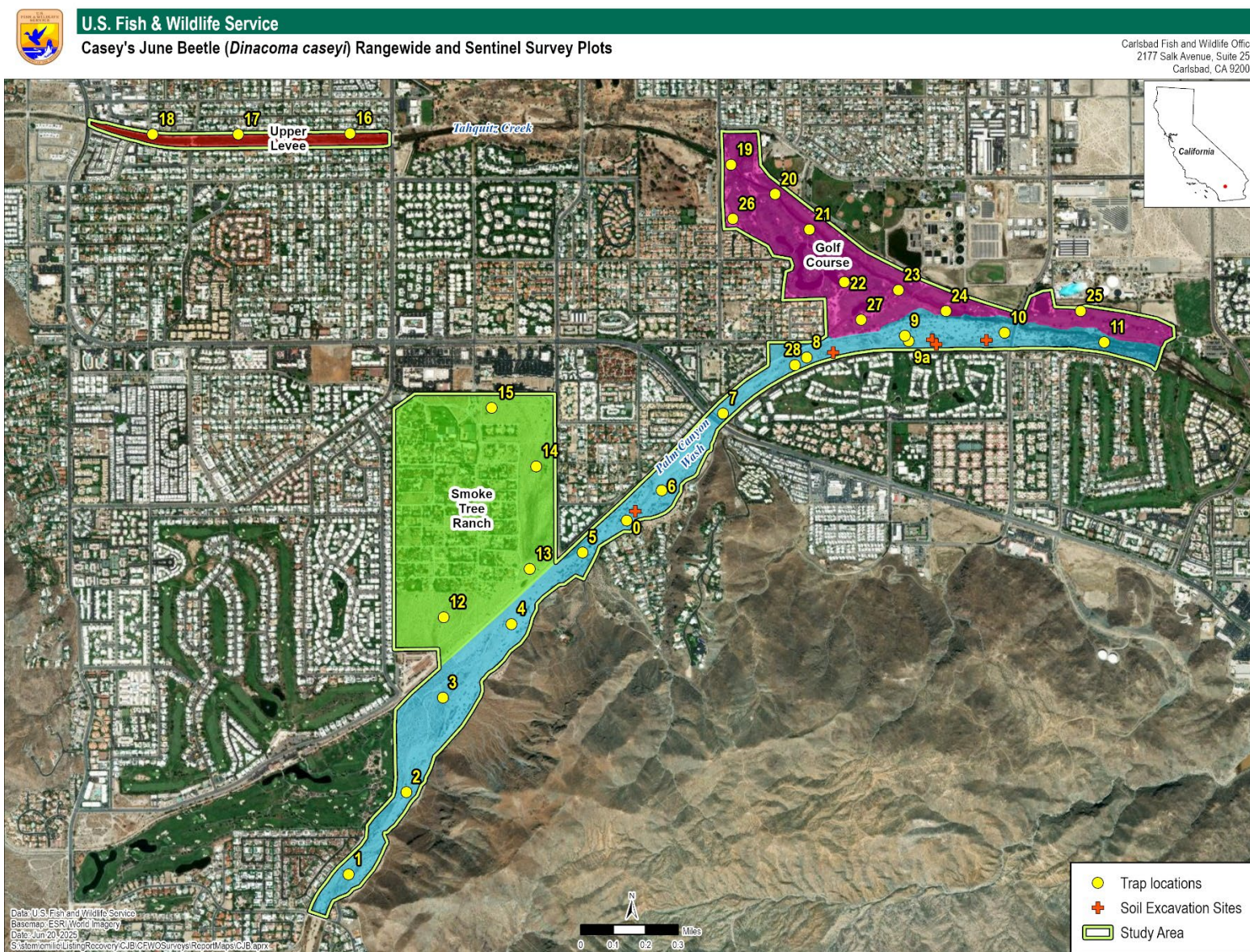


Figure 3. Casey's June beetle Rangewide and Sentinel survey plots distributed systematically throughout the species' range in Palm Canyon Wash ($n=12$), Smoke Tree Ranch ($n=4$), and Tahquitz Creek ($n=12$) over the duration of the study from 2016–2022.



Figure 4. Casey's June beetle study area in Palm Springs, California. **(A).** The dry desert wash habitat in Palm Canyon Wash where the species' current distribution is centered. **(B).** The cheesebush shrubland in the Smoke Tree Ranch Casey's June beetle conservation area. **(C).** The non-turf patches occupied by Casey's June beetle in the lower reach of Tahquitz Creek within the operative Tahquitz Creek Golf Resort. **(D).** The upper reach of Tahquitz Creek where the species occupies the ornamental and desert tree and shrub habitat on top of the levee, along the paved sidewalk.³

³ Photos: Noelle Ronan, U.S. Fish and Wildlife Service (A, C, D), Dudek (B).



Figure 5. Black light trap used to capture male Casey's June beetles during surveys at long-term survey plots. **(A).** Black light trap at the Sentinel survey site, Palm Canyon Wash. **(B).** Male Casey's June beetles on the sheet around the trap bucket. The attraction effect of the black light causes beetles to be sluggish and remain on the sheet for capture by hand.⁴

⁴ Photos: Noelle Ronan, U.S. Fish and Wildlife Service (A), Philip Clevinger, Wood Environment and Infrastructure Solutions, Inc. (B).

We used black light trap specifications previously used by Osborne (2008, 2012, 2015) and consistent with the Service’s survey guidelines (Service 2016) which included one 15-watt black light tube suspended over a 5-gallon (gal) bucket topped with an aluminum funnel (8–10 inches [20–25 cm] tall, 2 inches [5 cm] wide at its lower opening) that leads to the inside of the bucket. The bucket apparatus is placed on top of a 6.5 ft (2.0 m) square white sheet to help observe Casey’s June beetles that miss the bucket and land on the ground (Figure 5). The attraction effect (positive phototaxis) of the black light causes beetles to be ‘stunned’ and sluggish so they remain on the sheet for capture by hand. Black lights were powered using a 12-volt deep cycle lead acid golf cart battery. We assumed a 328-ft (100-m) radius survey plot size, based on the current understanding of beetle attraction to black lights (Service 2016). We temporarily held captured beetles in gallon and quart size plastic bags for counting and marking (see *Mark-Recapture* below). Except for specimens collected for genetic analysis (see *Genetics* below), Casey’s June beetles were released after recording data and marking. Female Casey’s June beetles are flightless and not attracted to black lights, therefore we did not capture females. We recorded incidental observations of females, but no females were included in capture survey or abundance analyses.

We recorded a suite of climate and environmental variables either once at the survey start (sunset time, moon phase) or hourly during capture surveys (Table 1). Sunset times and moon phase illumination were determined from online sources (Moongiant 2022; U.S. Navy 2022). We recorded temperature 3.2 ft (1.0 m) above ground, wind speed, and relative humidity using a Kestrel® 3000 weather meter. Temperature at ground level and 4.0 inches (10.0 cm) below ground was recorded using a food-grade temperature thermometer.

We provided standardized training to surveyors on trap deployment, data collection, and marking technique. Only individuals trained, qualified, and permitted under Service recovery permits (see *Permits*, page v.) handled and marked Casey’s June beetles. The study’s lead biologists and lead entomologist conducted field oversight to assist volunteers during black light surveys.

Table 1. Climate and environmental variables recorded during Casey’s June beetle surveys.

Variable	Data Type	Units
Sunset time	Continuous	24-hour clock
Moon phase	Continuous	Percent illumination
Hourly time when variables are recorded	Continuous	24-hour clock
Temperature 1 meter above ground	Continuous	Degrees Celsius
Temperature at ground level	Continuous	Degrees Celsius
Temperature 10 centimeters below ground	Continuous	Degrees Celsius
Wind speed	Continuous	Kilometers per hour
Wind direction	Categorical	Cardinal and ordinal directions
Relative humidity	Continuous	Percent

Rangewide and Sentinel Surveys

We conducted two types of capture (black light trap) surveys to evaluate the spatial and temporal variability of Casey's June beetle abundance during the breeding (flight) season. To sample on a rangewide macro-spatial scale, all plots were surveyed simultaneously on one evening every 2 weeks during the typical April-through-May flight season (hereafter, Rangewide surveys). To observe temporal variation on a micro-scale, one plot was surveyed frequently, every 1 or 3 days, depending on year, until the end of the flight season (hereafter, Sentinel surveys).

We established 28 Rangewide survey plots distributed systematically throughout the species' range in Palm Canyon Wash ($n=12$), Smoke Tree Ranch ($n=4$), and Tahquitz Creek ($n=12$) over the duration of the study (Figure 3; Appendix B, Table B1). Rangewide surveys began 1 hour before sunset and ended 3 or 4 hours after sunset. Black light traps were checked hourly to record environmental data and capture totals, mark individuals (see *Mark-Recapture* below), and release captured beetles (except those collected for genetic analysis). We did not record beetle size measurements or relative body condition during Rangewide surveys. Not all traps were surveyed each year. Due to logistics and funding, from 2019 to 2022, we surveyed a subset of the Rangewide plots that represented low, medium, and high Casey's June beetle relative abundance, based on survey data. We established additional survey plots in previously un-surveyed areas based on new information during the study. Casey's June beetle was discovered in Tahquitz Creek in 2017 as a result of the study. We established Rangewide survey plots in Tahquitz Creek in 2019 and additional plots covering downstream areas in the Tahquitz Creek golf course in 2021 (Figure 3; Appendix B, Table B1).

We established one Sentinel survey plot (trap 0) at the beginning of the study (2016) in Palm Canyon Wash near South Araby Drive (Figure 3; Appendix B, Table B1) where entomologists and Service biologists had previously observed high numbers of Casey's June beetle relative to other locations within the species' range. To evaluate the date of first emergence at the beginning of the flight season, surveying at the Sentinel plot began in late March most years. According to current understanding, the start of the flight season was considered to be early April (Service 2011). To evaluate the length of the flight season, we conducted Sentinel surveys until Casey's June beetle captures ceased. Based on capture data during the first study year, we used the metric of zero Casey's June beetle captures for three consecutive survey evenings after May 31, the presumed end of the flight season according to published information (Service 2011), to indicate the probable end of the flight season. Sentinel surveys began a half hour before sunset and ran until 1 hour after sunset. The trap was checked every half hour to record environmental variables, capture totals, and mark and release beetles. From 2017–2020, we surveyed at additional traps (supplemental traps) later in the flight season, using the Sentinel survey protocol, to supplement documentation for the length of the flight season.

We noted incidental observations of Casey's June beetle predation during Sentinel and Rangewide surveys. We confirmed a predation event of a Casey's June beetle when we observed a beetle being pursued or eaten within close range, where the white color of the male beetle was clearly evident. Some of the predation events were observed through binoculars and some observations were photographed. For bird species, predation observations were made from

around sunset until dark. We defined a predation event as an observation of one or more individuals of a predator species exhibiting hunting and feeding behavior of Casey's June beetle on a single evening.

Mark-Recapture

To assess the effectiveness of black light attraction, rate of recaptures, document flight movements among traps, and estimate population abundance, we marked adult male Casey's June beetles using a fine or ultra-fine point color Sharpie® according to a predetermined marking scheme unique to each survey plot, which included color, side of the body where a number is applied (number placed on the right or left elytra), and whether a dot is applied to the pronotum (Figure 6; Appendix C, Table C1). Individual Casey's June beetles were marked using sequential numbering, unless noted otherwise. We evaluated several commonly used, non-toxic insect marking materials that would remain intact during the survey interval and not adversely affect beetles (e.g., marking does not affect behavior and is assumed invisible to predators such that marked insects are no more or less likely to be removed from the population due to predation; Butler, *et al.* 2012; Tikkamaki and Komonen 2011; Walker and Wineriter 1981). Prior to the first Rangewide survey in 2016, we field tested paint pens, nail polish, Liquid Paper® (white out), and color markers of different point sizes (Sharpie®) for their effectiveness, ease of application, and ease of distinguishing colors. We selected fine and ultra-fine point Sharie® markers of six different colors because it was quick to apply, required minimal pressure on the beetle's body, was easiest to use on the small elytra area, and remained intact. We selected the colors most visible and discernable to the surveyor at night viewed under a headlamp.

We evaluated the effectiveness of black light attraction as the distance a Casey's June beetle traveled from the point of release around the capture trap to the location of the recapture trap. Marked Casey's June beetles were released at three distances from the capture trap: 130 ft (40 m), 328 ft (100 m), and 820 ft (250 m). Beetles were released in randomly chosen cardinal directions with approximately equal proportions of beetles per distance from the capture trap hourly during surveys. If marked Casey's June beetles were recaptured, the unique identifier and the time of recapture was recorded. By recording the unique identifier of recaptured beetles at traps, we documented flight movements among traps.

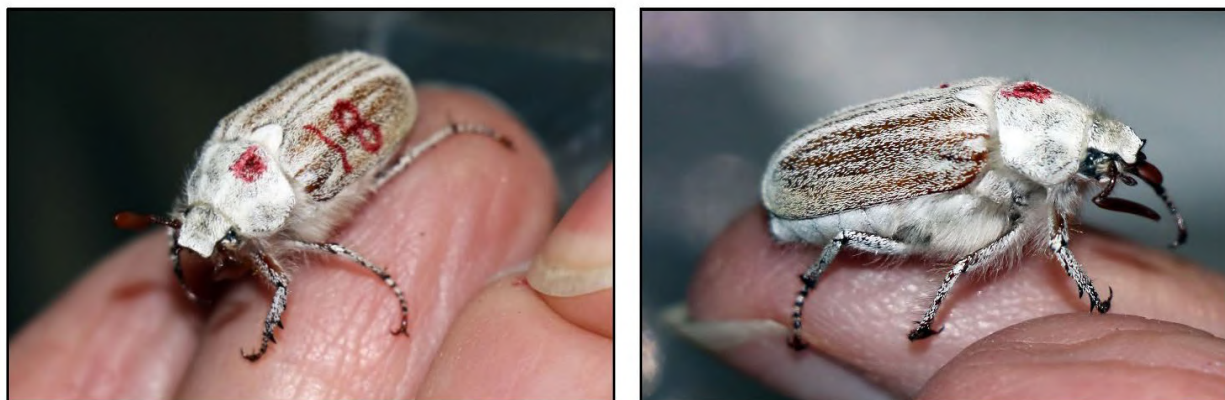


Figure 6. A male Casey's June beetle from the study prior to release after being marked with a unique identifier. Top front (left) and lateral (right) views are shown.⁵

VEGETATION COMMUNITIES

To examine associations of Casey's June beetle ecology and demographics with vegetation characteristics in 2015, 2016, and 2019, Dudek botanists compiled a plant inventory, and conducted vegetation mapping and sampling in Palm Canyon Wash and Smoke Tree Ranch (Dudek 2016; Strittmater and Amoaku 2019). Plant inventory, mapping, and sampling methods are described in Strittmater and Amoaku (2019; Appendix D) and summarized here.

In 2015, Dudek conducted a plant inventory and vegetation mapping within the 594-ac (240-ha) Casey's June beetle critical habitat area (Dudek 2016; Strittmater and Amoaku 2019; see Appendix D). Latin and common names followed the California Native Plant Society's Inventory of Rare and Endangered Plants (plant species with a California Rare Plant Rank; CNPS 2019), the Jepson Interchange List of Currently Accepted Names of Native and Naturalized Plants of California (Latin names for plant species without a California Rare Plant Rank; Jepson Flora Project 2019), and the U.S. Department of Agriculture's Natural Resources Conservation Service PLANTS Database (common names for plant species without a California Rare Plant Rank; USDA 2018).

Dudek mapped vegetation communities and land uses in the field. Vegetation community classifications followed the Manual of California, Second Edition (Sawyer *et al.* 2009), with modifications made to accommodate the lack of conformity of the observed communities using Oberbauer, Kelly, and Buegge (2008) and Holland (1986). Natural communities were mapped to the association level. If non-native vegetation covered approximately 20–50 percent absolute cover, vegetation communities were mapped as the disturbed form.

In 2016 and 2019, Dudek conducted vegetation sampling at long-term survey plots [328-ft (100-m) radius area] to evaluate changes in plant species composition and structure. Vegetation was sampled using the most recent California Native Plant Society relevé protocol, which is a semi-

⁵ Photos: Joanna Gilkeson, U.S. Fish and Wildlife Service.

quantitative, visual estimation of plant cover rather than a count of plant species along a transect line or other precise measurement technique (CDFW and CNPS 2016). The relevé method provides a standardized visual assessment of vegetation communities to classify and map large areas in a limited amount of time, minimizes damage to vegetation, and limits establishment of trails during sampling visits.

UNDERGROUND SOIL TEMPERATURE

From 2017–2019, we sought to examine the influence of underground soil temperature variation on Casey’s June beetle emergence and flight season activity. To evaluate spatial and temporal variation, we deployed wireless temperature data loggers (Kestrel Drop D1®) at the center of 12 long-term survey plots in Palm Canyon Wash year-round at three depths: 6 inches (15 cm), 26 inches (65 cm), and 45 inches (115 cm). To avoid vegetation and soil microbial crust disturbance, data loggers were not deployed at Smoke Tree Ranch. Data loggers were attached to a 3.5-ft (1-m) perforated aluminum stake (Figure 7) to maintain consistent underground logger placement among survey plots. We programmed data loggers to record temperature hourly for the life of the batteries, re-deployed loggers as logistically feasible, and periodically downloaded data wirelessly. To retrieve data loggers, a metal detector was used to locate the buried loggers when surface markers were no longer present. For seasonal comparisons, we defined spring as March 1 to May 31, summer as June 1 to August 31, fall as September 1 to November 30, and winter as December 1 to February 28.



Figure 7. Temperature data loggers (Kestrel Drop D1®) attached to an aluminum stake prior to underground installation to record soil temperature variation at a long-term survey plot.⁶

⁶ Photo: Noelle Ronan, U.S. Fish and Wildlife Service.

EMERGENCE HOLES

From 2015–2017, we mapped Casey’s June beetle emergence holes throughout all or a sample of Palm Canyon Wash and the upper reach of Tahquitz Creek, depending on year, to describe Casey’s June beetle presence and relative activity and evaluate possible correlates with environmental conditions. In 2015, we surveyed the entirety of the Palm Canyon Wash study area and, with access permission from the Agua Caliente Band of Cahuilla Indians, a portion of tribal land in Palm Canyon Wash upstream of Bogert Trail. In 2016, we surveyed a sample of the Palm Canyon Wash study area that included the 100-meter radius area at long-term survey plots. In 2017, in addition to surveying the Palm Canyon Wash sample area we also surveyed the 328-ft (100-m) radius area at long-term survey plots in the upper reach of Tahquitz Creek. To avoid vegetation and soil microbial crust disturbance, emergence holes in Smoke Tree Ranch were not surveyed.

Visible emergence holes are determined by the soil substrate and history of disturbance (Ronan *et al.* 2015–2017, pers. obs.), therefore we only surveyed areas of compacted soils where emergence holes were visible. In Palm Canyon Wash, much (but not all) of the compacted soil was a thick layer of highly cemented (super-compacted) fine sediments deposited from upstream flows during a 2014 flood event that occurred after an extensive fire within the Palm Canyon drainage basin. Dudek qualitatively estimated the percent of compacted soils within each long-term survey plot during vegetation community surveys in 2016 (Strittmater and Amoaku 2019).

We recorded Casey’s June beetle emergence hole counts along meandering transects up to approximately 90 ft (30 m) wide [45 ft (14 m) on either side of a transect] in June and July, after the flight season and before emergence hole degradation. In 2015, we surveyed emergence holes during 10 days in June–July, 7 days in July 2016, and 5 days in July 2017. Because other beetles and insects have a similar subterranean life cycle and emerge in the spring and summer, not all emergence holes can be attributed to Casey’s June beetle (Service 2025). We determined the physical characteristics of Casey’s June beetle emergence holes based on multiple observations of emerging male and female Casey’s June beetle in the field (see also *Body Size*, *Body Condition*, and *Emergence Hole Size* below), and comparative observations in the field of smaller and larger emergence holes that could be attributed to other beetles such as *Cyclocephala* spp., and later in the season, Apache cicadas [*Diceroprocta apache*] that emerge in May to late June. To minimize counting emergence holes made by other insect species, we only recorded emergence holes that exhibited clear attributes to those observed as Casey’s June beetle.

We collected global positioning system (GPS) locations for all recorded emergence holes. Depending on availability, Trimble GeoXT 6000 and Trimble Geo 7X GPS units were used to record emergence hole locations with submeter accuracy or handheld Garmin GPS units with 10-ft (3-m) accuracy. When emergence holes occurred in clusters, we counted the total number of holes in an approximately 10-ft (3-m) area and recorded the GPS location at the center of the cluster. Transect spacing and spatial awareness was maintained using the GPS unit or Avenza Maps (Avenza Systems Inc.) track function.

For consistency and to control for surveyor bias and variance, the same Service biologists (Christopher Gregory and Noelle Ronan) conducted all emergence hole surveys. Prior to the first data collection survey, we tested the feasibility of the meandering transect method in the field and conducted a calibration (i.e., quality control) survey to assess consistency and resolve discrepancies in assigning emergence holes to Casey's June beetle between the surveyors.

BODY SIZE, BODY CONDITION, AND EMERGENCE HOLE SIZE

For a subset of Sentinel surveys in 2016 and 2017, we collected size [length and width in millimeters (mm)] and weight [grams (g)] measurements of captured male Casey's June beetles. For beetle size, the greatest body width across the opposing elytra (humeral angle or "shoulder") and body length [clypeus (tip of head) to the pygidium (tip of abdomen)] was measured using a graduated scale with 1.0 mm demarcations. Beetles were weighed in the field to the nearest 0.01 g using a battery-operated digital scale (Jennings JSR 50).

In 2016 and 2017, we recorded relative body condition of a subset of captured male Casey's June beetles at the Sentinel survey plot and a small sample at plots in Smoke Tree Ranch and upper Tahquitz Creek. To assess the relative age composition of male beetles alive during the flight season, we developed a body condition index based on the relative wear of white scales and hairs on the elytra and body (Figure 8) as a proxy to days since emergence. In 2016, a categorical scale of 1 (newly emerged, clean white) to 3 (very worn, with no or little white remaining) was used to represent no or little wear, moderate wear, and heavy wear as a relative body condition index to compare the degree of wear among sampled beetles. In 2017, we used an expanded categorical scale of 1 (newly emerged, clean white) to 5 (very worn, with no or little white remaining).

In 2016 and 2017, we collected a sample of Casey's June beetle emergence hole measurements from Palm Canyon Wash in the Sentinel survey plot area and the upper reach of Tahquitz Creek near trap 16. Emergence holes (length and width) were measured using a graduated scale with 1.0 mm demarcations during the early (April) and peak (May) flight season (Figure 9).

Comparisons with *Dinacoma marginata*

We compared Casey's June beetle body size and emergence hole size with those of the closely related *Dinacoma marginata*. On May 29, 2016, Osborne collected a sample of *D. marginata* emergence hole dimensions and adult body size measurements while collecting specimens for study at Bautista Canyon, Riverside County (see *Genetics* below). Osborne observed many undamaged emergence holes on a hard, compacted ("cemented") substrate of dried mudflow. *D. marginata* was present in large numbers to the exclusion of other scarab species. These emergence holes appeared as vertical shafts in the same manner as those of Casey's June beetle. Osborne measured all holes in a small area (several square meters) of the "cemented" substrate using a scale with 1.0 mm demarcations, measured to the nearest 0.5 mm. With an interest in documenting the relation of emergence hole size to the size of emerging *D. marginata*, Osborne measured, using the same scale to the nearest 0.5 mm, the greatest width (on a line across the opposing elytral humeral angles) of adult male *D. marginata* specimens from that collection effort.

FIELD AND LABORATORY OBSERVATIONS

Male Observations

We conducted a small-scale observational study to (1) document adult male Casey's June beetle longevity under mild outdoor captive conditions; and (2) evaluate the durability and retention of the Sharpie® marker we applied to adult males for mark-recapture surveys, as well as assess any potential negative effects on the beetles from the marker. On April 20, 2016, Gregory, Osborne, and Anderson (Service entomologist) collected 11 male Casey's June beetles near the Sentinel trap site at the South Araby Drive crossing with Palm Canyon Wash. Five beetles were marked with a black Sharpie® marker and six were left unmarked. Osborne housed all 11 beetles in a large, screen-topped terrarium with 5 inches (13 cm) of sand, in a shaded outside location. Over the duration of the experiment, Osborne kept the sand only slightly moist at the bottom, while dry within the upper few inches, and recorded general behaviors, the number of beetles still alive, and the condition of the marks (i.e., general fading and readability) approximately once a week until no beetles remained alive.

Female Observations, Collection, and Laboratory Studies

Casey's June beetle female life history is not well understood or studied. Our study did not focus on female Casey's June beetle; however, we gathered incidental information during Rangewide and Sentinel surveys. Females were encountered at random, except when surveyors opportunistically searched for females aboveground by observing and following males attracted to calling (releasing pheromone) females (i.e., the males leading the observer to discovery of a female). Descriptive information and photographic documentation was collected when females were observed.

In 2016, 2017, 2019, 2020, and 2021, we collected a small sample of gravid adult female Casey's June beetle from Palm Canyon Wash for a pilot laboratory study of their biology and ecology and to explore the feasibility of captive propagation. After observing mating in the field, Osborne collected gravid females for general observation, dissection, or captive rearing of the laid eggs. Females collected from the field were placed in containers filled with native soil from Palm Canyon Wash and kept in a temperature-controlled room, maintained at approximately 70 degrees Fahrenheit [°F; 21 degrees Celsius (°C)] with a naturally occurring light cycle.

On April 4, 2016, one mated female was collected and placed into a 24-ounce [oz; 710 milliliter (ml)] glass jar with moist sand the following night. The container of sand was sorted on April 20, 2016, to evaluate presence of eggs or larvae. One gravid female collected on April 6, 2016, was dissected for microscopic examination. On April 8, 2016, one mated female (collected the night before) was placed into a wood-framed, clear plexiglass, vertical "ant-farm" viewing stadium filled to a depth of 8.2 ft (2.25 m) with damp sand collected from Palm Canyon Wash, to observe the rate of descent by burrowing of the female into the soil. The viewing stadium inside dimensions measured 94.75 inches (height) by 17 inches (width) by 0.5 inches (the width separating two transparent sheets of plexiglass) (240.6 by 43.2 by 1.3 cm). Female progress was observed for 3 days.

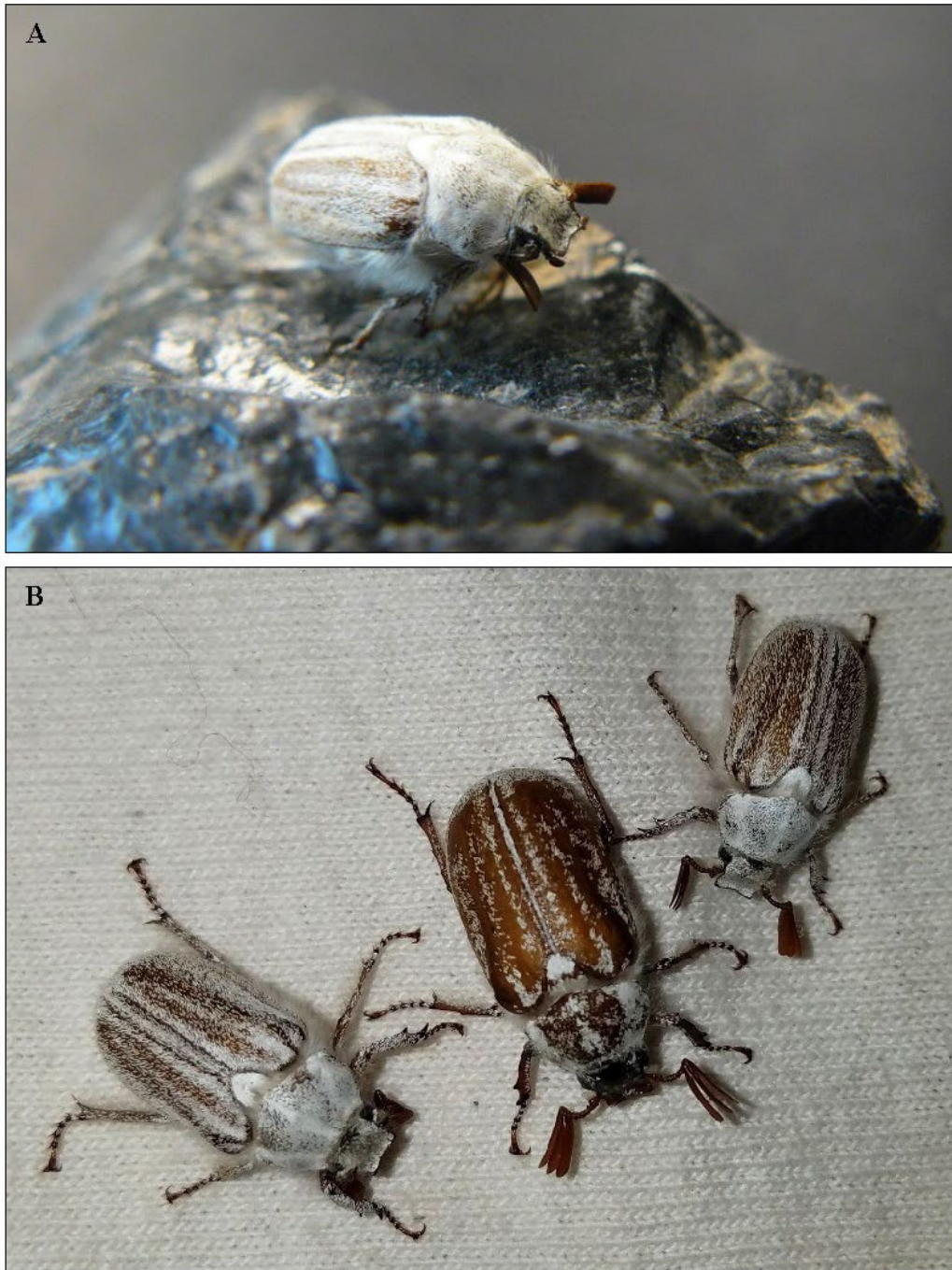


Figure 8. Variation in body wear and size of male Casey's June beetles. Males show wear during the breeding season as the scales and hairs rub off. **(A).** Newly emerged male showing fresh, white hairs and scales on the body. **(B).** Males on the left and right show moderate wear, and the male in the center is heavily worn. Size variation is also evident, with the center beetle noticeably larger and the beetle on the right is smaller. Smaller size and clypeal shape (concave) differentiates the *D. caseyi* in the center from *Polyphylla cavifrons*.⁷

⁷ Photos: Jon Avery (retired), U.S. Fish and Wildlife Service (A), Noelle Ronan, U.S. Fish and Wildlife Service (B).



Figure 9. Male and female Casey's June beetles emerging to breed (A, B) and the resulting emergence holes created from burrowing through soil (C, D, E).⁸

⁸ Photos: Christopher Gregory, U.S. Fish and Wildlife Service (A), Philip Clevinger, Wood Environment and Infrastructure Solutions, Inc. (B), Alison Williams-Anderson (retired), U.S. Fish and Wildlife Service (C, D), Noelle Ronan, U.S. Fish and Wildlife Service (E).

In 2017, Osborne placed one gravid female into a 4-ft (1.2-m) polyvinyl chloride (PVC) Standard Dimensional Ratio 35 sewer drainpipe with a live, rooted (potted within) *Chilopsis*, and placed another gravid female into a plastic 5-gal bucket with a potted *Chilopsis* (embedded into Palm Canyon Wash) to obtain eggs and potential larvae.

In 2019, gravid females were placed in 24 fluid oz (710 ml) glass containers; in 2020, females were placed in a 5-gal (23-liter [L]) plastic bucket; and in 2021, females were placed into the 4-ft PVC pipe (described above) with alternating layered alluvial sand and rotten wood/compost materials.

In 2019, Osborne reared larvae collected from the field (see *Larvae and Pupae* below) in 24 fluid oz (710 ml) glass containers filled with different possible food items to understand feeding behavior, including carrots, potatoes, willow (*Salix* spp.) roots, fresh desert willow (*Chilopsis linearis*) roots, and organic rotten wood. In 2020 and 2021, Osborne used substrate composed of clean, relatively uniform sand collected from Palm Canyon Wash, interspersed with one or two layers of compost and rotten wood, to house female Casey's June beetles collected from the field for egg laying and subsequent larvae development. The sand from Palm Canyon Wash was filtered through screens twice. The compost consisted of decomposed wood (various species except Eucalyptus [*Eucalyptus* spp.] due to the potential toxicity released from decomposing wood), twigs, and leaves. The rotten wood tree species were pine and oak collected from Palm Canyon Wash; these tree species would have been washed downstream from headwater locations. Once a month, Osborne added 16 oz (473 ml) of water to the 5-gal bucket and the PVC drainpipe (added every 1 ft [0.3 m] through drilled access holes) to keep the soil slightly damp in the mid and lower sections. Approximately 4 months after one gravid female was placed into the 5-gal rearing bucket (2020) and 8 months after one female was placed into the PVC drainpipe (2021), Osborne investigated the contents by sifting the substrate through a ¼-inch (6 mm) screen, followed by sifting through a 1/16-inch (4 mm) screen to search for larvae. Larvae found during sifting were placed in 5-gal buckets and periodically checked to record larval growth. Captive larvae were later placed in separate 60-dram (222-ml) vials with moist sand and organic debris to continue rearing until pupation. If Casey's June beetle larvae were reared to adult, they were retained as museum specimens and not released.

Larvae and Pupae

Casey's June beetle larvae and pupae have not been previously observed or described. To locate larvae and pupae for description and determine the range of burrowing depths, we conducted soil excavation surveys in 2019 and 2022. In November and December 2019, Osborne, Ronan, and biologists from the District conducted soil excavation surveys for Casey's June beetle larvae in conjunction with Corps and Service permitted emergency sediment removal activities conducted by the District in Palm Canyon Wash. The sediment removal was conducted from July 2019 to February 2020. Sediment removal was necessary to maintain flood control capacity within Palm Canyon Wash following the February 14, 2019, 200-year storm event that resulted in peak flows of approximately 6,000 ft³/s and deposited approximately 250,000 yd³ of sediment in Palm Canyon Wash.

Based on previous survey data, we non-randomly selected an area of high Casey's June beetle relative abundance where a large quantity of sediment was being removed for flood control maintenance, an approach that facilitated surveying at greater depths for larvae otherwise not logistically feasible without heavy equipment. We excavated soil by systematically digging at 4-inch (10 cm) layers followed by soil sifting through a ¼-inch screen suspended from an approximately 5-ft (1.5-m) wooden A-frame tripod (Figure 10). Soil excavation started from the bank walls created by the heavy equipment used to remove sediment. We recorded depth larvae were found, developmental stage, weight (g) using a battery-operated digital scale, and photographed specimens. Larvae were collected for observational laboratory rearing to document life history information (e.g., food preferences, growth rate) and identify species. Beetles were not released after removal from the wild.

On March 27, 2022, Osborne and Ronan conducted a soil excavation survey to search for larvae and pupae in the upper soil layers. We hypothesized that Casey's June beetle larvae and pupae would move to the upper soil layers prior to the breeding season in preparation to eclose (moult and emerge) in April. We delineated a small soil excavation area near native vegetation within the Sentinel survey plot area in Palm Canyon Wash where Casey's June beetles were consistently relatively abundant (Figure 10). The survey was focused on the upper portion of soil therefore depth was determined by where larvae or pupae were first found and the same systematic soil excavation method previously described was used. We recorded depth larvae or pupae were found and their developmental stage, photographed specimens, and collected larvae and pupae for species identification by anatomical comparison under a dissecting microscope. Specimen measurements were made in the laboratory. No beetles were released after removal from the wild.

We did not systematically excavate soil around living trees or shrubs (e.g., desert willow, smoke tree) to search for larvae feeding on plant roots. All soil surveys were conducted in the hydrologically active portion of the channel and not the upland floodplain terrace habitat.

GENETICS

Rubinoff *et al.* (2020) and Rubinoff and San Jose (2021) used genomic sampling of genome-wide nuclear deoxyribonucleic acid (DNA) and 1480 base pairs of mitochondrial DNA (mtDNA) data from *cytochrome oxidase subunit I* (COI) to examine the previously undescribed genetic population structure and diversity of Casey's June beetle. Sample collection, nuclear and mtDNA extraction protocols and sequencing, library preparation, and sequencing read (an inferred sequence of base pairs corresponding to all or part of a single DNA fragment) methods are described in Rubinoff *et al.* (2020; Appendix E) and briefly summarized here.



Figure 10. Soil excavation surveys for Casey's June beetle larvae and pupae in 2019 and 2022. Soil was systematically dug at 4-inch (10-cm) layers to document depths that larvae or pupae were found (A, B, 2019). Soil was sifted through a screen suspended from a wooden A-frame stand (C, 2019; D, 2022).⁹

In 2016, 2019, and 2020, we collected 31 adult male Casey's June beetle specimens by hand at black light traps in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek, which represented most of the extent of the species' range (Table 2). Genetic samples were collected during Rangewide and Sentinel surveys, and black light surveys conducted specifically for genetic sample collection in Palm Canyon Wash and Tahquitz Creek. In 2016, we collected 20

⁹ Photos: Noelle Ronan, U.S. Fish and Wildlife Service.

Casey's June beetle from the middle and lower reaches of Palm Canyon Wash and Smoke Tree Ranch. In addition, Osborne collected putative *Dinacoma marginata* at black light traps from the San Felipe Valley in San Diego County (42 adult males), and Bautista Canyon near Hemet in Riverside County (89 males and 2 females). Fourteen males from each of the two sites provided a reference genome for *Dinacoma* (Table 2). The additional males were used for morphological examination, with those from San Felipe Valley ultimately becoming type material for a new species description (*Dinacoma sanfelipe*; Gillett *et al.* 2020; Appendix E). In 2019 and 2020, we collected 11 additional Casey's June beetle samples from the upper reach of Palm Canyon Wash and previously unknown occupied locations in Tahquitz Creek to obtain a more representative sample of the range extent. The *Dinacoma* specimens were preserved in 70 percent ethyl alcohol.

A combination of Sanger sequencing and double-digest restriction-site associated DNA sequencing (ddRAD) was used; separate DNA extractions were prepared for Sanger sequencing of the mtDNA and ddRAD sequencing. To identify an outgroup for Sanger sequencing, we used the most closely related Scarab from the melolonthine subfamily using COI sequence data from the Barcoding of Life Database, which was *Amblonoxia fieldi* (Dusty June beetle; $n=2$ individuals), a species that occurs broadly throughout the southwestern United States. Raw Illumina sequencing reads were processed using the STACKS v. 2.1 pipeline.

Table 2. Adult male Casey's June beetle (*D. caseyi*), *Dinacoma marginata*, and *Dinacoma sanfelipe* collected for genetic analysis. *D. marginata* was the reference genome for *Dinacoma*.

Date Collected	Species	Locality ¹	Trap ²	Number Collected
4-Apr-2016	<i>D. caseyi</i>	Palm Canyon Wash – middle reach	0	8
29-Apr-2016	<i>D. caseyi</i>	Smoke Tree Ranch – upland terrace	14	2
29-Apr-2016	<i>D. caseyi</i>	Smoke Tree Ranch – upland terrace	15	2
1-May-2016	<i>D. caseyi</i>	Palm Canyon Wash – lower reach	10/11	8
27-May-2016	<i>D. marginata</i>	Bautista Canyon, Hemet	-	16
3-Jun-2016	<i>D. sanfelipe</i>	San Felipe Valley, San Diego Co.	-	12
1-Jun-2019	<i>D. caseyi</i>	Tahquitz Creek – upper reach	16	6
5-May-2020	<i>D. caseyi</i>	Palm Canyon Wash – upper reach	3	2
6-May-2020	<i>D. caseyi</i>	Palm Canyon Wash – upper reach	1	2
6-May-2020	<i>D. caseyi</i>	Tahquitz Creek – middle reach	19	1

¹ *D. caseyi* were collected from Palm Springs, Riverside County, *D. marginata* were collected from Bautista Canyon, Hemet, Riverside County, and *D. sanfelipe* were collected from San Felipe Valley, San Diego County, California. Bautista Canyon is located southeast of Hemet, California at an elevation of 2,223 ft (678 m). San Felipe Valley is in eastern San Diego County, east of the town of Julian, California at an elevation of 2,574 ft (785 m).

² Long-term survey traps where *D. caseyi* were collected in Palm Springs. Trap 10/11 refers to specimens collected at the approximate midpoint between these two traps. For trap 19, the individual was collected approx. 410 ft (125 m) west of trap 19. Trap number does not apply to *D. marginata* and *D. sanfelipe*.

DATA ANALYSIS

Abundance and Distribution

We sampled Casey's June beetle using repeated counts (i.e., repeated measures) at multiple locations with a spatiotemporal distribution and marked individuals with a unique identifier. Ronan explored the feasibility of analyzing the Casey's June beetle mark-recapture data to estimate population abundance and survival using program MARK, software that provides a suite of sampling methods and models based on data from marked individuals (capture-recapture methods) (White and Burnham 1999). Preliminary analyses were run for open populations using POPAN, with hourly sampling occasions for the Rangewide and Sentinel surveys. Individual encounter histories were structured with one group, and analyses using release distance (1, 40, 100, 250 m) as a covariate were attempted. Possible additional covariates that may influence encounter probability were considered including date, time, air and soil temperature, wind speed, humidity, and moon illumination. However, Ronan found the sampling occasions, Casey's June beetle life history and open population structure (e.g., continuous immigration, emigration, birth, and death), and low recapture rate did not fit the maximum likelihood models, resulting in lack of convergence for most models.

We subsequently funded Heron Ecological, LLC (Seth Harju, biometrician) to estimate Casey's June beetle emergence and relative abundance patterns with environmental covariates using models that accommodate low detection probability, and Casey's June beetle life cycle and open population structure. Although individual Casey's June beetles were marked, for abundance estimation, the data was treated as if the counts were of unmarked individuals. Using 2016–2020 data, and 2021 data from Tahquitz Creek, Harju (2021 and 2022) evaluated the temporally rich, but spatially restricted, Sentinel survey dataset for Casey's June beetle capture (emergence) patterns and the spatially rich, but temporally restricted, Rangewide survey dataset for abundance patterns.

Along with the Casey's June beetle capture survey and vegetation sampling data, the Service provided Harju with streamflow data for Palm Canyon Wash and Tahquitz Creek. Local streamflow discharge (cubic feet per second) data for Palm Springs was obtained at three gauging stations (Palm Canyon Wash headwaters: site 10258500, Palm Cyn C NR Palm Springs; Palm Canyon Wash downstream: site 10259050, Palm Cyn Wash NR Cathedral City; Tahquitz Creek headwaters: site 10258000, Tahquitz C NR Palm Springs) for 2015–2020 from the United States Geological Survey (USGS) National Water Information System (USGS 2020).

Data analyses are described in Harju (2021 and 2022; Appendix F) and summarized here. To examine capture patterns for the Sentinel survey data from 2016–2020, Harju addressed four questions:

1. When is the period (time) that population closure can be assumed?
2. Are weather or moon phase factors associated with whether Casey's June beetles are captured?

3. Are weather or moon phase factors associated with how many Casey's June beetles are captured?
4. What is the inter-annual temporal pattern in captures?

Harju calculated the maximum number of days between initial captures and subsequent recaptures of marked beetles and used a Poisson trend line to assess the period within which population closure can be assumed (question 1); used logistic regression constructed with a generalized additive mixed model (GAMM) for a daily trend in capture rates and an autocorrelation term to adjust for correlation of captures on the previous night (question 2); used GAMMs that incorporated environmental predictor variables of percent moon phase, humidity, wind speed, and temperature (question 2); used a quasi-Poisson GAMM with predictor variables of year, air temperature, humidity, maximum wind speed, and percent moon phase for the total numbers of captured beetles (question 3); and using the final sub-model structure, Harju calculated the predicted expected numbers of captured beetles as a function of Julian day to compare inter-annual variation in peak abundance (question 4).

To examine the Rangewide surveys for patterns in abundance, Harju addressed three questions, separately for each year:

1. What drives the probability of capturing an individual beetle, given that it emerged and is present in the trapping area?
2. What drives initial corrected abundance of captures on the first survey night of the year?
3. What is the true (corrected) abundance of male beetles over the course of sampling sessions within each year?

Harju used the R package 'unmarked' which analyzes data from surveys of unmarked animals involving spatial sampling using hierarchical models¹⁰ that estimate occupancy, abundance, and density of animals that cannot be detected with certainty (i.e., imperfect detection) (Fiske and Chandler 2019, Fiske and Chandler 2011). The probability of detection was calculated using N-mixture abundance modeling of unmarked individuals using the 'pcountOpen' function (R package 'unmarked' [R v3.6.3]), whereby repeated surveys are conducted within a period of assumed population closure (Harju 2021). Once the most meaningful detection probability was determined, Harju tested abundance on the first trapping night, differences by general habitat type (i.e., wash or upland), and variation among traps. The best model within each year was used to estimate corrected abundance of male beetles with activity areas overlapping traps for each survey night within each year.

¹⁰ Hierarchical models are multi-level models that sort lower levels of data under a hierarchy of successively higher-level units. Data is grouped at one or more levels, and the influence of the clusters on the data points considered in analysis. The inferences which are made about one quantity affect the inference about another such that the data points are not independent.

Harju analyzed new survey data collected from Tahquitz Creek in 2021 and re-analyzed the 2016–2020 Rangewide dataset to adjust for the influence of the February 14, 2019, flood event and update the layer for the expected range of Casey’s June beetles (Harju 2022). Using the same methods, a corrected abundance estimate of Casey’s June beetles in Tahquitz Creek from the 2021 surveys was calculated. To examine influences from the flood event, Harju re-analyzed the estimated corrected abundance of beetles from Rangewide data in 2018 and included the preceding January–March streamflow (discharge as cubic feet per second) data. Site-level vegetation differences from 2016 and 2019 in relation to corrected abundance were also evaluated (Harju 2022).

Harju addressed the following new questions, using the existing datasets (2016–2020) and the new 2021 capture data:

1. What were the site-level corrected abundances of male Casey’s June beetles on each trapping night in 2021?
2. What is an effective distance within which to delineate population geographic closure?
3. What was the detection probability-corrected estimated average nightly rangewide abundance of Casey’s June beetles in 2018?
4. Was average corrected abundance related to preceding January–March streamflow?
5. Were 2016 and 2019 average corrected abundances related to the site-level vegetation community?

Harju (2022) describes additional methods for the new analyses, including the following: distance detection functions in package ‘Distance’ in Program R (v4.0.5) to evaluate effective distance to delineate population closure (question 2); a discretized hexagonal geographic closure grid overlapping all Rangewide traps and the expected Casey’s June beetle range to calculate an estimate of average Daily Emerged Males (DEM) (question 3); a linear regression model with model fits compared using Akaike’s Information Criterion corrected for small sample sizes (AICc) to evaluate abundance in relation to streamflow and/or distance from the downstream Cathedral City gauge station (question 4); and linear regression and AICc for selection of models with vegetation and habitat variables including native/naturalized species richness and percent cover, and wash or upland location (question 5).

Ecology, Vegetation Communities, and Genetics

We calculated descriptive statistics using DATAtab (DATAtab Team 2024) and R 4.2.2 (R Core Team 2022). Basic statistical analyses were conducted as analysis of variance (ANOVA) and regression, with the significance threshold set at $p=0.05$. Correlation analyses were conducted using the Spearman correlation test for nonparametric data. The Mann-Whitney U-test for differences between groups was used for nonparametric data. Mapping and spatial analyses were conducted in ArcGIS® Pro 2.9.9 or higher (ESRI 2021). Rainfall data was downloaded from the

National Oceanic and Atmospheric Administration's integrated database, Global Historical Climatology Network daily (GHCNd) (NOAA 2020).

We present descriptive statistics and some basic relative indices based on captures to describe the occurrence and distribution of Casey's June beetle in the study area. We report both the mean and median of total counts of Casey's June beetle captures and recaptures. The mean is included for consistency with some of the raw data summary presented by Harju (2021 and 2022) in the abundance *Results*, but for discussions outside of the corrected abundance estimation (Harju 2021, 2022), the median is a more useful descriptive summary of captures. Though commonly used, the mean is an inaccurate representation of central tendency when data are skewed or contain outliers; it is sensitive to extreme observations and data that does not fit a normal distribution, as are the Rangewide and Sentinel survey captures. The median is a better measure of central tendency for data with a non-normal distribution because it falls in the middle of the distribution regardless of spread (i.e., variation) and distribution shape (Gotelli and Ellison 2004). We report the mean and standard deviation (SD) and the median and range as measures of variation and spread in the observed data, throughout.

We report uncorrected (raw) total counts of Casey's June beetle captures and recaptures only for simple, relative comparisons among survey plots and years. Raw total counts are not representative of abundance because they are confounded by capture probability, survey effort, and environmental covariates. Relative abundance estimation, including detection probability, was conducted by Harju (2021 and 2022; Appendix F).

To account for the variation in survey effort among traps and years, we present an approximation of catch per unit effort (CPUE; number of captures per survey hour, from surveys using standardized black light trap equipment) as an additional relative index of captures. The CPUE can be used to document spatiotemporal change and provide an index of abundance when assumptions are met, and other analyses conducted (Hubert and Fabrizio 2007). We present it as a companion descriptive statistic to further describe the capture structure and as a potential useful relative index of trends over time with future long-term monitoring. We caution against interpreting it as a true CPUE due to departures from at least some of the assumptions underlying the methods, lack of data transformation to correct for non-normal distribution, or tests for temporal correlation between survey plots (Hubert and Fabrizio 2007).

We calculated an approximation or index of Casey's June beetle density at black light traps as the number of Casey's June beetle captured per area surveyed using the effective survey area at black light traps estimated by Harju (77.3-m radius [2022]).

To evaluate male Casey's June beetle flight distances and movements among traps from mark-recapture data collected during Rangewide and Sentinel surveys, the distance individual Casey's June beetle traveled was calculated as the straight-line distance from the release location to the recapture trap location. We excluded data if surveyors did not record sufficient release location information to indicate whether the release occurred upstream or downstream of the capture trap. Flight distances of males recaptured at black light traps are a function of light attraction and capture probability and are not an estimate of dispersal.

Dudek digitized polygons from vegetation communities and land uses mapped in the field in 2015 using ArcGIS to create a geographical information system (GIS) coverage. Once in ArcGIS, the acreages of each vegetation community and land cover type was calculated (Strittmater and Amoaku 2019; Appendix D). Dudek also created a georeferenced map series (Dudek 2016; see Appendix D).

To determine the effective survey area of Casey's June beetle emergence holes and correct for incomplete detectability of emergence holes outside of compacted soils, we multiplied emergence hole counts by percent compacted soils within the survey area to calculate an adjusted emergence hole count. Dudek qualitatively estimated the percent compacted soils during vegetation surveys in 2016. Based on field observations, we assumed that percent compacted soil did not differ significantly during the emergence hole survey period from 2015–2017. Descriptive statistics and analyses were conducted using the adjusted emergence hole counts.

Analyses of Casey's June beetle population genetics structure and substructure for the mitochondrial and ddRAD datasets are described in Rubinoff *et al.* (2020, p. 2191–2192; Appendix E).

Assumptions and Caveats

This study's scope was focused on male Casey's June beetle ecology and demographics therefore, interpretation of results are applicable to the male portion of the population only. The data is not intended to be extrapolated to females of the population, which pose a different set of survey requirements and conditions according to their specialized behavior and biology. We present limited information for females based only on incidental observations or very small samples associated with pilot investigations. While these observations provide interesting natural history notes and may generate hypotheses for future exploration, they should not be interpreted as statistically significant.

Uncorrected (raw) total counts of Casey's June beetles should not be interpreted as a relative abundance or used to extrapolate a population estimate, as summarized in the Data Analysis section above and described in Harju (2021 and 2022). Maximum recapture distances among black light traps is assumed to represent a minimum flight distance capability but is not an estimate of dispersal.

During study design for capture surveys at black light traps, we assumed an attraction distance to 15-watt black lights to be 328-ft (100-m) radius, based on the current understanding of beetle attraction to black lights at the time (Service 2016). The surveys were designed, in part, to test this assumption.

To accomplish Rangewide surveys that included simultaneous surveys at multiple traps on the same night, we relied on many volunteers and personnel. If the number of volunteers and personnel was not sufficient to cover all traps on a given survey night, traps with the lowest Casey's June beetle captures were not surveyed, which maximized collecting data on more beetles while accommodating logistic-related constraints.

Based on the life history of similar June beetles, during study design and mark-recapture protocol development, we assumed that individual Casey's June beetles did not survive between subsequent Rangewide surveys (every 2 weeks).

Emergence Holes

Individual Casey's June beetles may create multiple holes as they emerge and burrow each day. A single hole may represent one Casey's June beetle emerging and re-entering (burrowing back down) the same hole, a behavior we observed in the field. Furthermore, we did not distinguish between male and female emergence holes. Therefore, emergence hole counts represent a relative activity index rather than absolute Casey's June beetle abundance or activity.

Although our survey methods minimized surveyor bias and incorrect counts, we acknowledge the possibility that some emergence holes included in the count may not be from Casey's June beetle but rather other beetle or insect species. However, we assume that our survey methods minimized error such that any inaccuracy is insignificant. Our interpretation of emergence hole counts and their ecological relevance is presented throughout as a relative activity index and not equivalent to abundance.

Larvae and Pupae

Our field soil survey results for Casey's June beetle larvae and pupae provided examples for further morphological evaluation but were inconclusive with respect to determination of Casey's June beetle larval food resources. Although field observations of emergence holes indicate an association with large trees and shrubs such as *Chilopsis*, it remains unclear the extent to which, if any, Casey's June beetle larvae may utilize these living plant roots, the organic drift captured by these plants, or both. Our laboratory studies of larvae demonstrated detritivory through extended larval development. Although the laboratory studies did not include repeated feeding trials with varied dietary choices, choices among varied types of decaying organic materials, varied rearing conditions, or multiple individuals from differing portions of the Casey's June beetle range, the results are nevertheless the best and *only* available science. Until more extensive larval investigations show otherwise, our results of larval detritivory may be tentatively assumed for the Casey's June beetle population.

RESULTS

SAMPLING DESIGN

To accommodate logistics and accomplish various goals based on new information over the duration of the study, not all traps were surveyed each year, and some trap sites were added during the study (Table 3). The number of surveys varied by year and trap depending on weather, logistics, and available personnel (Table 3). We conducted Rangewide surveys focused on Palm Canyon Wash from 2016–2020 and Rangewide surveys focused on the Tahquitz Creek golf course in 2021–2022. From 2016–2018, 15 traps in Palm Canyon Wash ($n=11$) and Smoke Tree Ranch ($n=4$) were surveyed. In 2019, to accommodate logistics, a subset of the Rangewide survey traps ($n=8$) were surveyed and three trap sites were added in the newly discovered, occupied upper Tahquitz Creek levee for a total of 11 traps. The sample for the subset of the Rangewide traps was spatially representative (i.e., upstream to downstream locations) and encompassed the range of captures (i.e., low, moderate, and high numbers of captures) we had documented. In 2020, Rangewide surveys in the Palm Canyon Wash focal area included 8 traps, which were the 6 subset traps in Palm Canyon Wash and 2 traps in the upper Tahquitz Creek levee area. In 2020, we were not allowed access to Smoke Tree Ranch due to COVID-19 restrictions.

In 2021 and 2022, we focused Rangewide surveys on the Tahquitz Creek golf course where previous survey information was sparse. In 2014, there were positive detections of Casey's June beetle at some black light traps placed along the northern edge of the golf course along a dirt pathway between recreational fields and the grass fairways (AMEC 2014). However, survey information throughout the entire golf course was absent. Directing survey effort to the Tahquitz Creek golf course was an important focus shift. Understanding relative abundance and connectivity of Casey's June beetle in the Tahquitz Creek drainage in relation to Palm Canyon Wash was critical for assessing the rangewide population and opportunities for future conservation. Because there was no baseline information for the entirety of the golf course area, we continued to survey some traps in Palm Canyon Wash and the upper Tahquitz Creek levee to serve as reference sites. We opted to include some trap locations in the sediment removal area to gather additional years of abundance data following the 2019 flood event. We omitted one trap location (trap 25) in the golf course from surveys in 2022 due to equipment theft in 2021 and safety concerns in the field from an adjacent homeless encampment.

We focused Sentinel surveys on the primary trap 0 for all years. We surveyed supplemental sites from 2017–2020 near the end of the breeding season to better document the presumed last Casey's June beetle flight (Table 3). In some years, Casey's June beetles were no longer captured at trap 0 near the end of the season but they were captured for a longer time period at one or more of the supplemental sites.

Table 3. Casey's June beetle survey areas by survey type, location, years, number of traps, and purpose.

Survey Type	All Years	Location	Years	Number of Traps	Purpose
Rangewide – Palm Canyon Wash	2016–2020	Palm Canyon Wash	2016–2018	11	Primary survey and population area
Rangewide – Palm Canyon Wash	2016–2020	Palm Canyon Wash	2019–2020	6	Primary survey and population area
Rangewide – Palm Canyon Wash	2016–2020	Smoke Tree Ranch	2016–2018	4	Primary survey and population area
Rangewide – Palm Canyon Wash	2016–2020	Smoke Tree Ranch	2019	2	Primary survey and population area
Rangewide – Palm Canyon Wash	2016–2020	Smoke Tree Ranch	2020	0	No access due to COVID-19 restriction
Rangewide – Palm Canyon Wash	2016–2020	Upper Tahquitz Creek - levee	2019	3	Addition of new discovery area
Rangewide – Palm Canyon Wash	2016–2020	Upper Tahquitz Creek - levee	2020	2	Addition of new discovery area
Rangewide –Tahquitz Creek	2021–2022	Tahquitz Creek Golf Course	2021	9	Golf course primary focus area
Rangewide –Tahquitz Creek	2021–2022	Tahquitz Creek Golf Course	2022	8	Golf course primary focus area
Rangewide –Tahquitz Creek	2021–2022	Upper Tahquitz Creek - levee	2021–2022	1	Reference site
Rangewide –Tahquitz Creek	2021–2022	Palm Canyon Wash	2021–2022	3	Reference sites. Sediment removal area.
Sentinel	2016–2020	Trap 0	2016–2020	1	Primary Sentinel survey site

Survey Type	All Years	Location	Years	Number of Traps	Purpose
Sentinel	2016–2020	Supplemental Traps	2017	4	Assess end of flight season. Assess new upper Tahquitz Creek sites.
Sentinel	2016–2020	Supplemental Traps	2018	5	Assess end of flight season. Assess new upper Tahquitz Creek sites.
Sentinel	2016–2020	Supplemental Traps	2019	3	Assess end of flight season
Sentinel	2016–2020	Supplemental Traps	2020	1	Assess end of flight season

ABUNDANCE AND DISTRIBUTION

Abundance Estimation, Capture Patterns, and Environmental Associations

Results from Harju's analyses (2021 and 2022) of the 2016–2021 Casey's June beetle survey, capture, and habitat datasets are the first to estimate Casey's June beetle abundance and emergence patterns and evaluate habitat and environmental influences on aspects of its ecology. Results of abundance and associated analyses are described in Harju 2021 and 2022 (see Appendix F) and summarized here.

The earliest Sentinel survey was on March 20 in 2018, and the latest on July 26, 2019. The earliest Rangewide survey was on April 2, 2020, and the latest on May 30, 2019. The longer survey period for the Sentinel site was designed to document a wider breadth of the Casey's June beetle breeding season, while the Rangewide survey period was consistent with the Service's presence/absence survey protocol. Summary statistics and plots of mean and raw capture data from the Rangewide and Sentinel surveys, along with environmental covariates revealed interesting patterns. For the Sentinel surveys, mean captures were between 7.0 and 9.0 Casey's June beetles per trap night from 2016–2019, but the mean captures per trap night (mean=18.3) was markedly higher in 2020. Rangewide survey traps had comparatively low captures per trap per night in 2016 (mean=7.4) but markedly higher captures per trap per night from 2017 through 2020 (mean=14.9–24.0).

Comparison of Sentinel survey captures between Julian day from 2017–2020 with environmental factors differed in the strength of the association as indicated by coinciding peaks in the data. There was a sharp increase in the number of captured beetles between Julian day 100–140, depending on year, which coincided with the period of new and waxing lunar phases. Peak captures occurred at approximately 50 percent waxing full moon (Figure 11; Figure 2 in Harju 2021). Raw plots of captures with air temperature at sunset, maximum wind speed, and percent humidity, did not show the same consistent association with peak beetle capture each year (Figures A1, A2, A3 in Harju 2021).

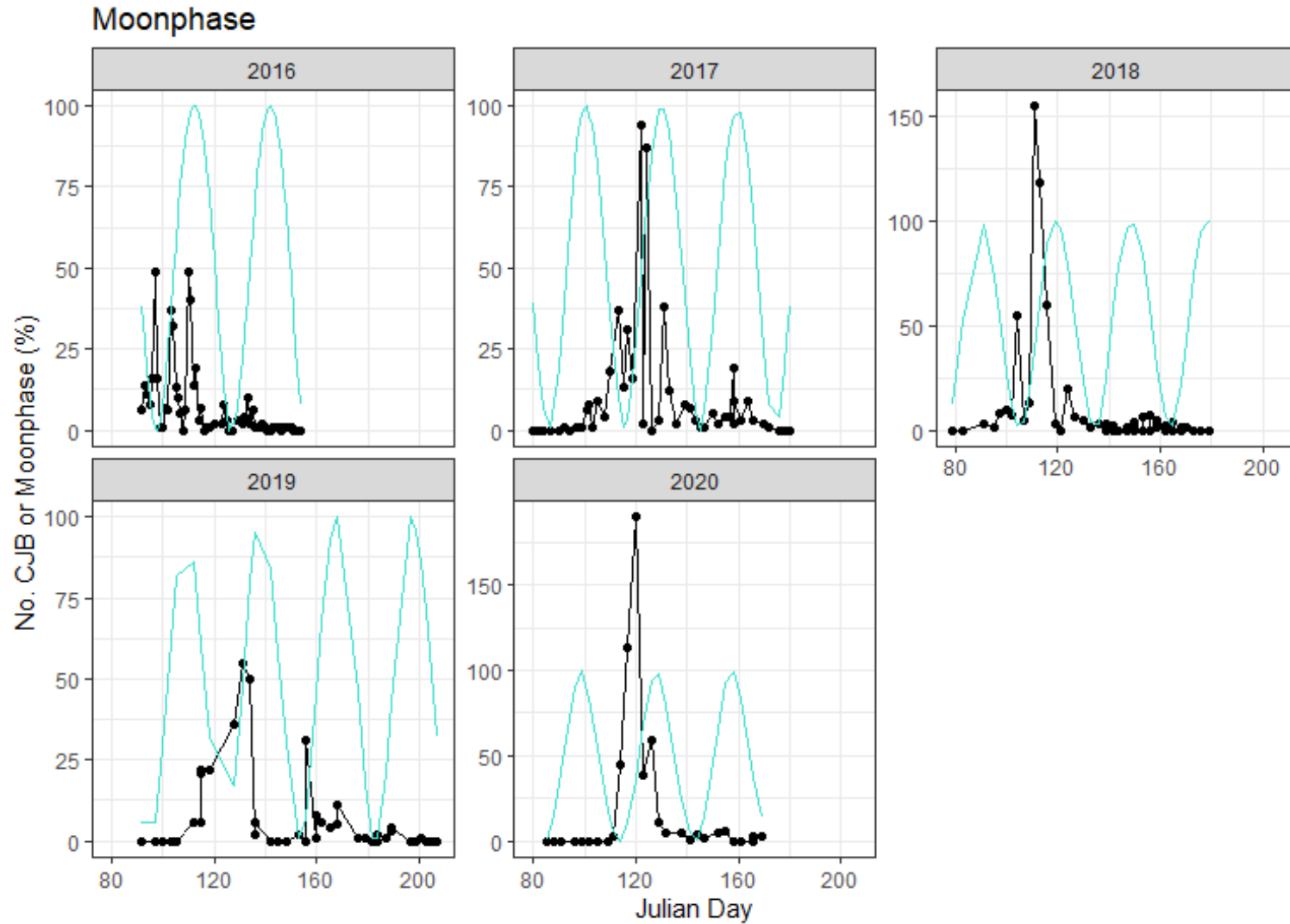


Figure 11. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and percent moonphase (turquoise line).¹¹

¹¹ Taken directly from Harju 2021 (Figure 2, Page 8).

Capture Patterns (Sentinel Survey)

From 2016–2020, there were a total of 5,471 uniquely marked Casey’s June beetle captures, with only 4.7 percent of those being recaptured. Most beetles were recaptured on the same night as originally marked (91.5 percent) and only 19 (7.3 percent) were recaptured the next day. Only three (1.2 percent) were recaptured three days after original capture and marking and no beetles were recaptured more than three days after initial marking (see also *Male Observations* below). Therefore, temporal population closure, and time at which trap surveys can be considered independent, may be assumed to occur after 3 days.

Results from analyses of captures with time over the breeding season, weather, and moon phase factors provided both new information for some variables and support for other previously assumed environmental relationships which had been factored into the Service’s presence/absence protocol survey design. There was a nonlinear pattern of higher capture rates in the middle of the season compared to the beginning and end, and a nonlinear capture probability which increased with air temperatures above 59 °F (15 °C), becoming constant with air temperatures above 90 °F (32 °C). Information previously unknown or uncertain, was that percent lunar illumination (as opposed to the timing within the lunar cycle) and humidity were unrelated to captures. However, as mentioned above, while the percent lunar illumination does not correlate with Casey’s June beetle captures, the highest captures are associated with the new and waxing portion of lunar phases. The final model also showed that maximum wind speed at sunset may have been positively related to capturing any beetles. That is, Casey’s June beetles were less likely to be captured as maximum wind speed increased (Harju 2021), information that provides data-driven support for field observations and previous assumptions of effects on captures.

This pattern generally held when evaluating the association of these same environmental variables with relative abundance of Casey’s June beetle captures (as opposed to probability of capture). Relative abundance of Casey’s June beetles peaked in late April and early May and was positively associated with warmer air temperature. Relative abundance at the Sentinel survey plot increased from 2016 through 2020, by a factor of 1.17 (95 percent confidence interval [CI]=1.00–1.39) every year. Humidity, maximum wind speed, and percent lunar illumination at sunset were not related to relative abundance.

Temperature influences capture rates because ectothermic insects have increased metabolic activity with increasing temperatures (Triplehorn and Johnson 2005). General “agility” increases across a range of temperatures from a low, where torpor is induced, through increasing activity across the increasing range of normally experienced temperatures, to a point where extreme high temperatures cause activity to cease (i.e., beyond metabolic tolerances). For example, in general, hot humid nights are best for finding insects at lights (Osborne, pers. obs.). During Rangewide surveys, the capture rate at black light traps was highest one hour after sunset (Appendix G, Figure G1), likely influenced by the increasingly warmer temperatures.

Inter-annual patterns of relative abundance of Casey’s June beetle captures from the five years of Sentinel survey data showed strong non-linear patterns with capture peaks occurring in late April

or early May, which aligned with the 50 percent full waxing moon phase during the same time period. There was some variation in the steepness of the linear declines among years [Figure 12 (Figure 5 in Harju 2021)].

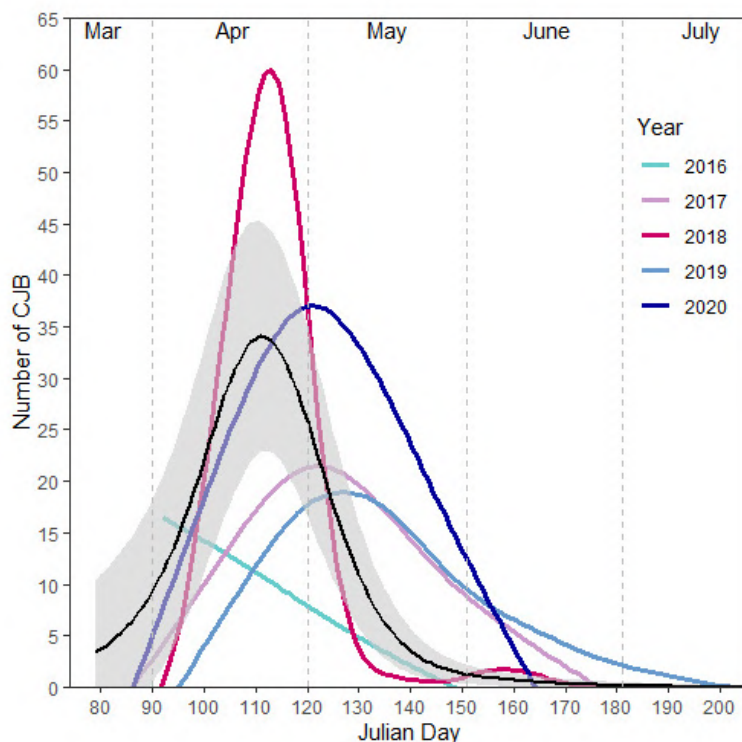


Figure 12. Annual and across-year temporal variation in peak captures of Casey's June beetles. Solid black line is across-year model-adjusted relative abundance, with gray 95% C.I. band.¹²

Abundance Patterns (Rangewide Survey)

Based on the 2016–2020 Rangewide data, considering the average observed wind speed or belowground temperatures, the probability of capturing an emerged individual Casey's June beetle varied among years but was always low (e.g., between 0.02 and 0.14; Figure 13 [Figure 6 in Harju 2021]). Capture probability was negatively correlated with belowground temperature, maximum wind speed, or both, depending on the year, so that individual beetles were less likely to be captured as belowground temperature or maximum wind speed increased. Site-specific features and the general habitat type where survey traps were located (wash or upland) explained the corrected (adjusted for detection probability) capture abundance at the beginning of a survey season. Capture abundance was best explained by site-specific variation among the trap locations in 2016, 2017, and 2019, however, general habitat type (wash or upland) was a contributing explanatory factor that performed better than the null model (no variation among sites). General habitat type was the best explanatory factor for 2018.

¹² Taken directly from Harju 2021 (Figure 5, page 11).

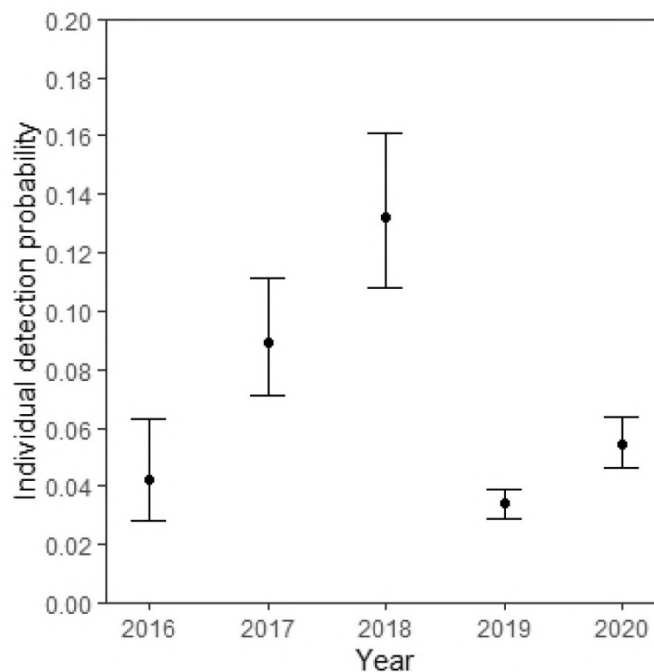


Figure 13. Probability of detecting (i.e., capturing) an individual male Casey's June beetle, given that it has emerged and is present in the trapping area. Error bars are 95% confidence intervals.¹³

The within-year corrected abundance of Casey's June beetles at survey traps over the duration of a survey season varied spatially and temporally (Figure 14; Figure 7 in Harju 2021). For traps in the Palm Canyon Wash Rangeland surveys from 2016–2020, some showed constant abundance while others had peaks and more variation. In 2016–2018, those traps with constant abundance also tended to have lower beetle numbers. This pattern was associated with Smoke Tree Ranch (traps 12–14) and some upstream locations (e.g., trap 1, 3). Data from 2019 showed more variation, lower abundance at more trap sites, and more variation over the survey season. Temporal resolution of the Rangeland data, which was collected every two weeks, was too low to demonstrate the spring peak evident in the more frequently collected Sentinel survey data (every 1–3 days). Abundance was not estimated for 2020 due to model convergence errors.

Extending this analysis to derive corrected abundance estimates for the Rangeland surveys in Tahquitz Creek in 2021 (Harju 2022), there is a similar pattern of abundance peaks associated with overall higher estimated abundance at some traps while other traps showed gradual, consistent abundance increases through the survey season (Figure 15; Figure 1 in Harju 2022). Estimated corrected abundance was lower and more constant within the golf course portion of Tahquitz Creek while the survey trap locations with higher estimated abundance and greater peak fluctuations were at the upper reach of Tahquitz Creek on the levee (trap 16) and the trap most contiguous with the less disturbed Palm Canyon Wash habitat (trap 24).

¹³ Taken directly from Harju 2021 (Figure 6, page 12).

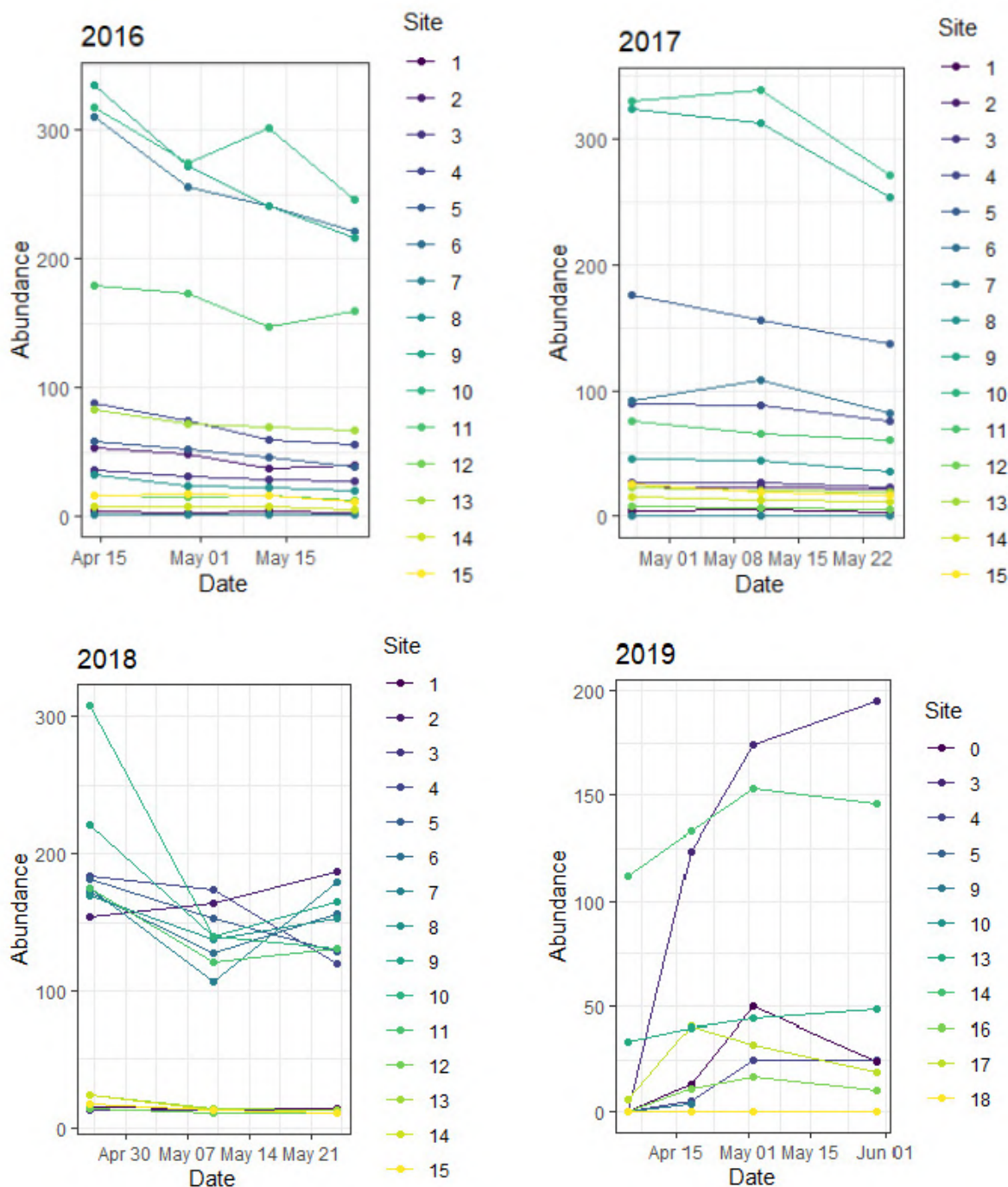


Figure 14. Within-year estimated corrected abundance at each trap site, after accounting for imperfect detection of individual male Casey's June beetles within a given evening. Abundance is the estimated number of male beetles with activity areas overlapping each trap site on a given night. Estimated abundance in 2020 is not provided because of convergence errors for the estimates. Note variable y-axes.¹⁴

¹⁴ Taken directly from Harju 2021 (Figure 7, page 14).

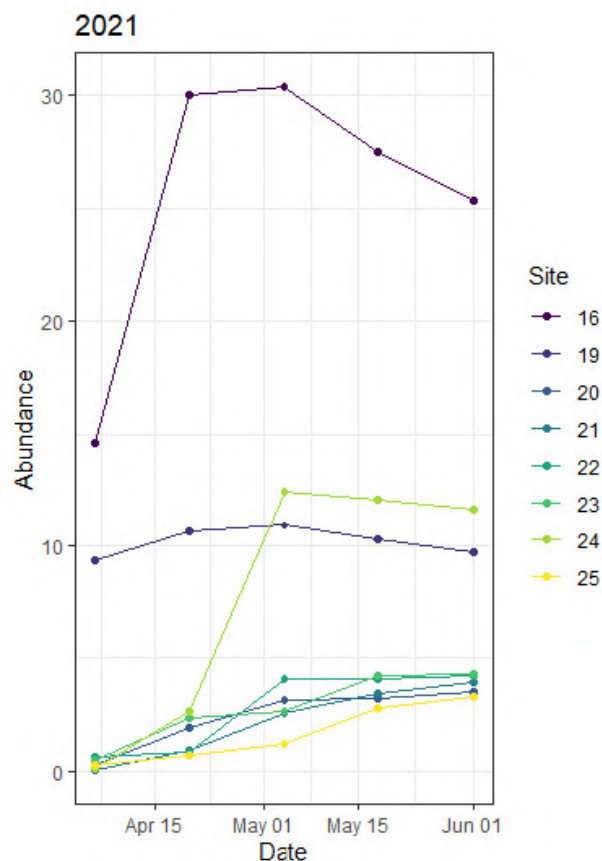


Figure 15. Corrected site abundance estimates of male Casey's June beetles on each of five trapping evenings during 2021.¹⁵

From analysis of the Casey's June beetle release-to-recapture distances using a distance decay function, there was an average recapture probability of 0.096 (95 percent CI=0.082–0.109) and an effective capture radius around any given point (i.e., trap) of 253.6 ft (77.3 m) (Harju 2022). This recapture probability updates and replaces the similarly low estimate previously calculated by Harju (2021). It is logically consistent with the low rate of recaptures (4.7 percent, Harju 2021) evident in the Sentinel survey data. The effective capture distance is the distance that delineates population closure. A Casey's June beetle emerging <77.3 m from a trap was more likely to be caught in that trap than not and any beetle emerging >77.3 m from a trap was less likely to be caught in that trap than caught. This estimate provides statistical support for a more accurate effective survey area around the 15-watt standardized black light traps typically used to survey for Casey's June beetle. It demonstrates a lower effective survey area (74 ft [23 m] less) than previously assumed (328 ft [100 m] radius) and upon which the Service's presence/absence Casey's June beetle protocol survey is based.

Harju estimated a probability-corrected average nightly rangewide abundance of male Casey's June beetles in 2018, deriving a single-day emergence estimate. There was an estimated average

¹⁵ Taken directly from Harju 2022 (Figure 1, page 6).

of 9,554.4 (95 percent CI=9,554.4–11,191.74) new male Casey's June beetles emerged rangewide per night between April 26 and May 24, 2018 (Figure 16, see Figures 4–6 in Harju 2022).

Average corrected abundance of male Casey's June beetles was influenced by the preceding January–March streamflow, which supports our supposition of the ecological significance of the dry wash hydrology on the beetle's abundance and distribution. Average daily streamflow volume (cubic feet per second) and distance upstream from the Cathedral City stream gauge, which is near the confluence of Palm Canyon Wash and the Whitewater River, explained the variation in average site abundance of Casey's June beetles. In other words, estimated abundance of Casey's June beetles declined the further upstream a site is (Harju 2022). With zero stream flow, the estimated average site abundance at the downstream Cathedral City gauge station was 200.9 (95 percent CI=115.7–286.2) male Casey's June beetles. Average abundance declined by 32.3 (7.2–57.4, $p=0.019$) beetles for every one kilometer (km) further upstream from the Cathedral City gauge station (Figure 17, Figure 8 in Harju 2022).

Analyses of vegetation community composition between 2016 and 2019, showed that plant species richness was generally lower in 2019 than at the same sites in 2016, a likely outcome from the scour and sediment deposition that occurred with the February 2019, flood event in Palm Canyon Wash. Harju modeled Casey's June beetle estimated abundance with native and non-native plant species, species richness, and percent cover. After reducing model selection to the most parsimonious model that considered only plant species richness, the average abundance of male Casey's June beetles increased by 3.6 (1.5–5.7) beetles for every additional plant species at the site, regardless of whether it was a native or non-native plant ($p=0.002$; Figure 18, Figure 11 in Harju 2022). This result provides statistical support for field observations of emergence hole counts and areas of relative high Casey's June beetle numbers where both native and non-native plant species occurred, and some locations were co-dominant (e.g., trap 11).

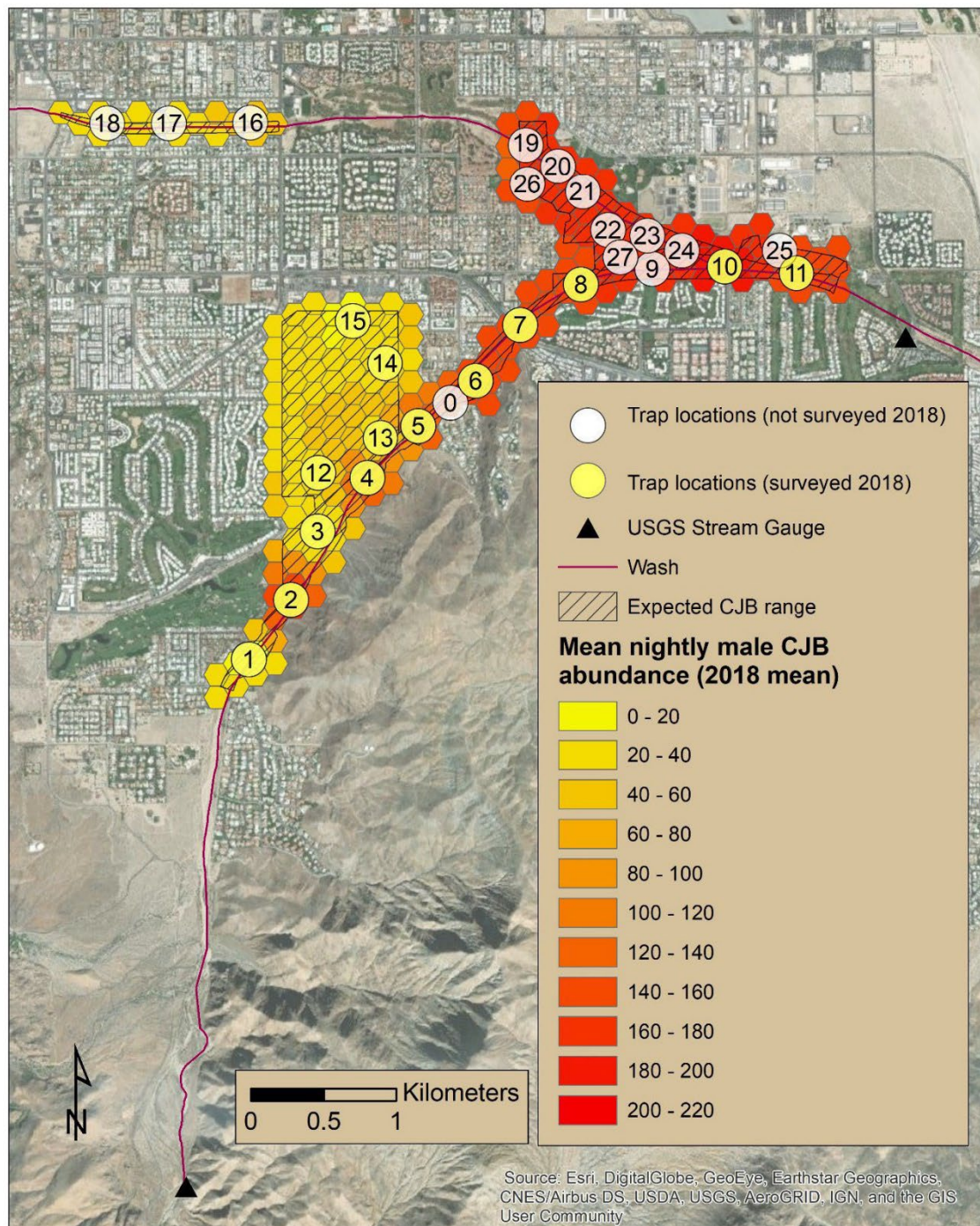


Figure 16. Estimated mean nightly abundance of male Casey's June beetles between April 26th and May 24th, 2018.¹⁶

¹⁶ Taken directly from Harju 2022 (Figure 4, Page 9).

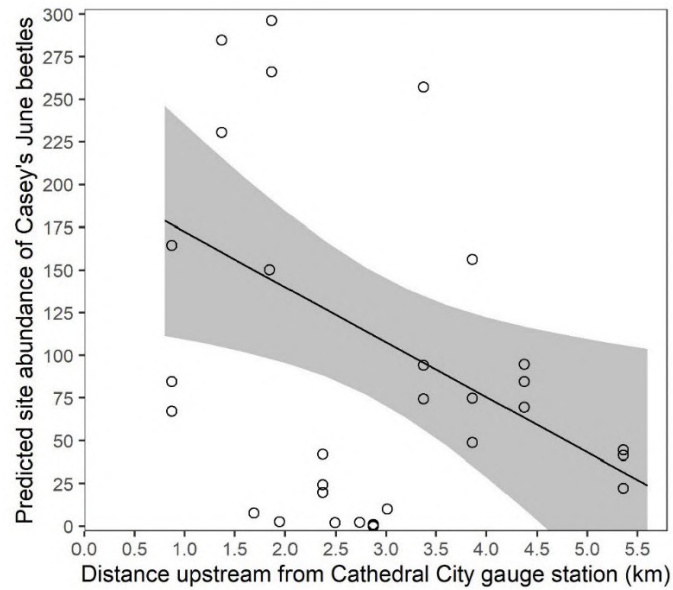


Figure 17. Predicted site abundance of Casey's June beetles as sites are located further upstream from the Cathedral City gauge station, Palm Springs, California, 2016-2018.¹⁷

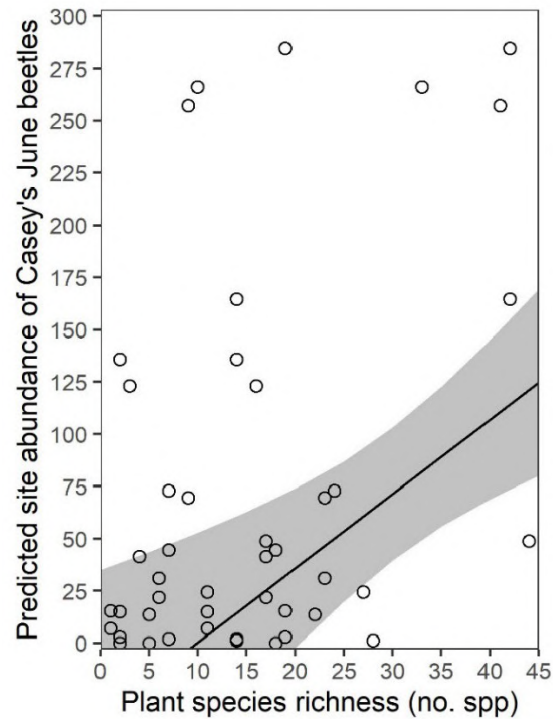


Figure 18. Predicted increase in average abundance of male Casey's June beetles as a function of plant species richness at a site, regardless of whether the species was native or naturalized, 2016 and 2019.¹⁸

¹⁷ Taken directly from Harju 2022 (Figure 8, page 14).

¹⁸ Taken directly from Harju 2022 (Figure 11, page 17).

Results Overview – Abundance Estimation, Capture Patterns, and Environmental Associations

There was notable variation in abundance across trapping events among sites for all data from 2016–2021. The Tahquitz Creek golf course sites represented the majority of the 2021 dataset. The total abundance estimated in 2021 was among the lower abundance sites compared with the Palm Canyon Wash sites from the 2016–2020 data. None of the traps in 2021 had an estimated abundance over approximately 30 male beetles per evening, a marked difference driven by contrasting habitat disturbance regimes for the Palm Canyon Wash sites and the Tahquitz Creek golf course sites.

Across all years, distance from release-to-recapture of Casey’s June beetles served as a proxy for emergence-to-capture distance. The distance decay model estimated an average recapture probability of 0.096 (95 percent CI=0.082–0.109) and an effective capture radius of 77.3 m, suggesting that a beetle emerging <77.3 m from a trap was more likely to be caught than not, and a beetle emerging >77.3 m from a trap was less likely to be caught than to be caught. Casey’s June beetles have a continuous distribution within occupied habitat, but with high spatial variability in density, and other variables affecting population dynamics, which presents statistical challenges in population estimation. Transferring continuous variables of beetle dynamics into discrete counterparts can facilitate population estimation. The effective capture radius of 77.3 m may be a meaningful distance to use for population estimation.

The streamflow analyses showed that abundance of Casey’s June beetles declined with increasing location upstream, a finding supported by the spatial pattern in abundance in 2018 where upstream locations had lower abundance. Hydrological processes that affect lower stream reaches more, can provide favorable sub-surface water table and soil moisture conditions and deposit more subsurface detritus and woody debris, which are food sources for overwintering larvae. The analyses also showed the effects from the February 2019, flood event. The estimated abundances in 2019 showed the opposite relationship (Harju 2021), such that middle and upper wash locations had higher abundances than the downstream sites, most likely a consequence of high hydrologic flows and large amounts of sediment deposition exerting a stronger effect on the downstream locations.

Removal of the large quantity of sediment in Palm Canyon Wash by the District to maintain flood control capacity after the February 14, 2019, flood event occurred from July 29, 2019, to February 28, 2020 (Service 2023). Levee repair in the mid-reach of Palm Canyon Wash, needed due to extensive scour from the flood event, occurred from September 1 to December 6, 2020 (Service 2023). The 2019 capture surveys were conducted prior to the sediment removal and levee repair and therefore, the estimated abundance in 2019 reflects the hydrological effects for that survey year, rather than a combined effect from hydrology and sediment removal and levee repair disturbance.

Harju’s analyses provide some estimate of rangewide abundance, given the challenges associated with the beetle’s spatially and temporally continuous and heterogeneous occurrence and emergence patterns. The estimate of the 2018 rangewide average daily emergence is 9,554.4 (95

percent CI=7,790.1–11,191.7) male Casey’s June beetles, based on data during the breeding season (April 26–May 24). There are several strict, and likely untenable, assumptions used to derive this estimate. It should be treated as an estimate with caveats and assumptions, elaborated on in Harju 2022, and not relied on as a statistically or biologically robust estimate of true abundance.

Survey and Capture Summary – Descriptive Statistics and Results

We provide a general summary of Rangewide and Sentinel survey frequency, timing, capture, and recapture results for all study years to accompany the abundance and distribution estimates. Interpretation of these summary results is more appropriately considered a Casey’s June beetle activity index because most insect sampling methods measure activity rates, not population abundance (Didham *et al.* 2020). Uncorrected (raw) male capture rates are correlated with survey effort and environmental covariates and are not corrected for detection probability. For example, uncorrected capture totals from Rangewide surveys in 2016–2019 were 2 to 12 times lower than abundance estimates corrected for detection probability (Appendix G, Table G1 and Appendix F, Table F1 to F4), a logical result given the low detection probability (approx. 1 percent). Here, we use the captures as an index of male activity within the effective trap survey areas for a simplified discussion of results and general comparisons.

Survey Frequency and Timing

Rangewide surveys were conducted on a total of 29 evenings and Sentinel surveys on 198 evenings between 2016 and 2022 (Table 4). Rangewide surveys began during the first week of April when weather conditions allowed and were conducted until the last week of May. Extremely high winds caused survey cancellation and a late start in April for the first three years. Annual survey effort ranged from 110 to 120 total hours for Rangewide surveys (Appendix G, Table G1) and 38 to 68 total hours for Sentinel surveys (Appendix G, Table G2).

Table 4. The number and timing of Casey’s June beetle Rangewide and Sentinel surveys by year, 2016–2022.

Survey Type	Year	Number of Survey Evenings	Frequency	Start Date	End Date
Rangewide	2016	4	every 2 weeks	April 14	May 26
Rangewide	2017	3	every 2 weeks	April 27	May 25
Rangewide	2018	3	every 2 weeks	April 26	May 24
Rangewide	2019	4	every 2 weeks	April 4	May 30
Rangewide	2020	5	every 2 weeks	April 2	May 28
Rangewide	2021	5	every 2 weeks	April 6	June 2
Rangewide	2022	5	every 2 weeks	April 5	May 31
Sentinel	2016	58	daily	April 1	June 2

Survey Type	Year	Number of Survey Evenings	Frequency	Start Date	End Date
Sentinel	2017	45	every 3 days	March 21	June 29
Sentinel	2018	35	every 3 days	March 20	June 28
Sentinel	2019	34	every 3 days	April 2	July 26
Sentinel	2020	26	every 3 days	March 25	June 17

Sentinel Surveys – Assessing the Flight Season

We began surveying at the Sentinel trap prior to the presumed start of the Casey's June beetle flight season, when feasible, to document as close as possible the date of first emergence. The first survey ranged from as early as March 20 to as late as April 2. From 2016–2018, first documented emergence was on April 1 or 2, as previously expected. The date of first emergence was noticeably delayed in 2019 (April 22) and 2020 (April 20) compared to previous years by approximately 20 days (Table 5).

The date of last emergence was more often documented in the third week of June (June 19–21; Table 5). The latest documented date was July 20 in 2019, which may be a result of the delayed emergence that year, an extended season compensating for a later start to breeding. In 2020, Sentinel surveys were prematurely stopped due to safety concerns in the field; therefore, the last documented date may not be representative of last emergence and should be interpreted cautiously.

Table 5. Casey's June beetle first and last emergence dates based on timing of first and last surveys during Sentinel surveys in 2016–2020.

Year	First Survey	First Emergence	Last Survey	Last Emergence
2016	April 1	April 1	June 2	May 29
2017	March 21	April 2	June 29	June 21
2018	March 20	April 1	June 28	June 19
2019	April 2	April 22	July 26	July 20
2020	March 25	April 20	June 17	June 17 ^A

^A Sentinel surveys in 2020 were prematurely stopped due to safety concerns in the field. Last emergence in 2020 was documented on June 17 at survey trap 10. The date of last emergence at the Sentinel trap was on June 3 in 2020. Supplemental trap sites were surveyed in conjunction with the Sentinel trap (trap 0) near the end of the breeding season to better document the presumed last beetle flight.

Rangewide and Sentinel Survey Capture Patterns

Casey's June beetle captures varied annually, as previously discussed for the detection probability-corrected abundance estimates. For Rangewide surveys in the Palm Canyon Wash focal area, the lowest capture rates occurred in 2016 (median=2; maximum=59) and the highest

in 2020 (median=5; maximum=238) (Appendix G, Table G1). Capture rates in Smoke Tree Ranch were consistent, with little annual variation, but lower than Palm Canyon Wash locations, throughout the study (Appendix G, Table G1). We found spatial variation in captures from upstream to downstream locations. In general, upstream traps had the lowest capture activities in most years (traps 1 and 3) and downstream traps the highest (traps 9, 10, and 11) (Figure 19). This pattern was described for the estimated mean nightly abundance (Figure 16) and the predicted site abundance (Figures 17 and 18) in Palm Canyon Wash, which may be a function of hydrological flows depositing greater amounts of debris and nutrients for immature stages of Casey's June beetle, and decreased scour impacts, further downstream. The exception to this pattern was in the mid-reach of Palm Canyon Wash (trap 7, trap 8 in some years) where captures were low, possibly related to sparse vegetation in that area. Smoke Tree Ranch is located on the upland floodplain terrace, separated from Palm Canyon Wash by a levee, and therefore is not subject to the same hydrology.

The capture pattern in the Tahquitz Creek focal area during Rangewide surveys in 2021 and 2022 differed from the Palm Canyon Wash focal area, with an overall lower relative activity and less annual variation (Figure 20). The upper Tahquitz Creek levee had relatively higher capture rates more similar to Palm Canyon Wash (median range=1–5) than the golf course. During all years surveyed, Casey's June beetles were not captured at one trap (trap 18) in upper Tahquitz Creek, likely indicating the extent of occurrence on the levee. Captures in the golf course were patchy and low. Casey's June beetles were consistently present and with relatively higher totals at traps near the edges of the golf course, including the upstream trap (trap 19) and the downstream areas near the confluence with Palm Canyon Wash (traps 23 and 24). Casey's June beetles were present at all traps throughout the golf course except one trap (trap 21) in the central area of the course.

The inter-annual fluctuation in Casey's June beetle activity was also apparent in the Sentinel survey data (Figure 21). With the more frequent survey interval, we documented the variable monthly capture activity which, for all surveys combined, demonstrates the non-linear capture pattern and peak in April (Figure 22), as described in the capture pattern and abundance estimates by Harju (2021). Capture activity at the Sentinel trap (trap 0) was lowest in 2019 (median=1; maximum=55). Assessment of the highest year of capture activity is affected by extremes in the data. Using median as the measure of central tendency, capture activity was highest in 2017 and 2018 (both years' median=3). However, the maximum total captures were in 2020 (maximum=190; CPUE=13.0 beetles per survey hour).

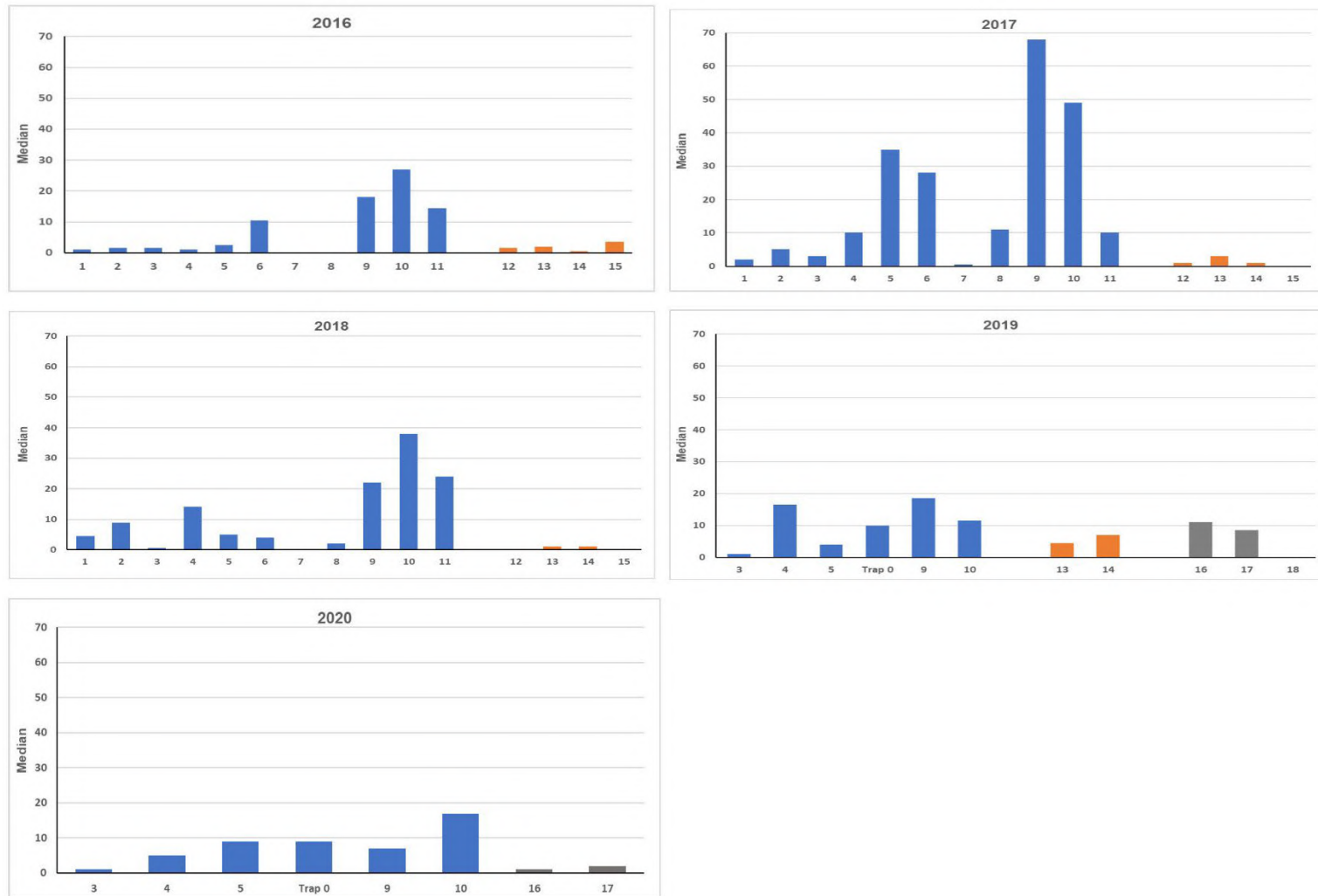


Figure 19. Median Casey's June beetles captured by trap (x-axis) during Rangewide surveys from 2016–2020 in Palm Canyon Wash (blue), Smoke Tree Ranch (orange), and Tahquitz Creek (gray). Palm Canyon Wash traps arranged from upstream (left) to downstream (right).

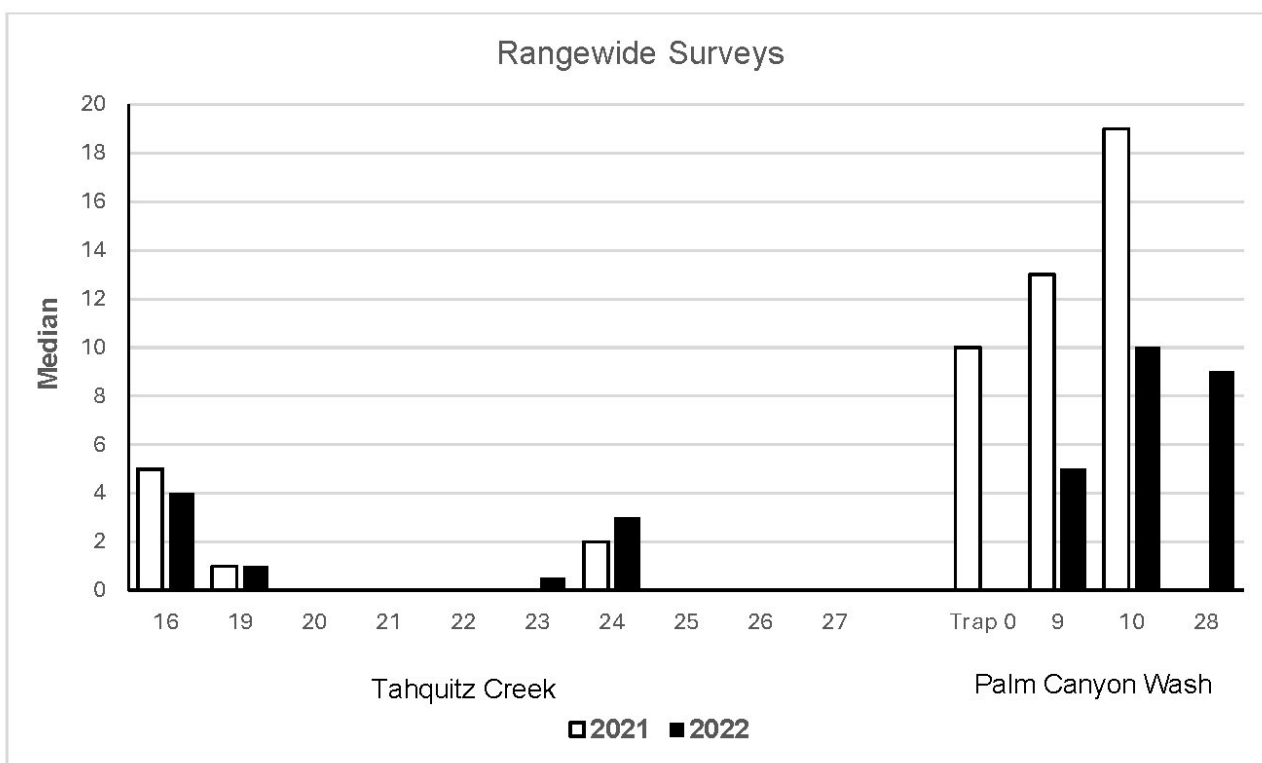


Figure 20. Median Casey's June beetles captured by trap during Rangewide surveys from 2021–2022 in Tahquitz Creek and Palm Canyon Wash. Traps arranged from upstream (left) to downstream (right). Tahquitz Creek trap 28 was not surveyed in 2021. Tahquitz Creek trap 25 and Palm Canyon Wash trap 0 were not surveyed in 2022.

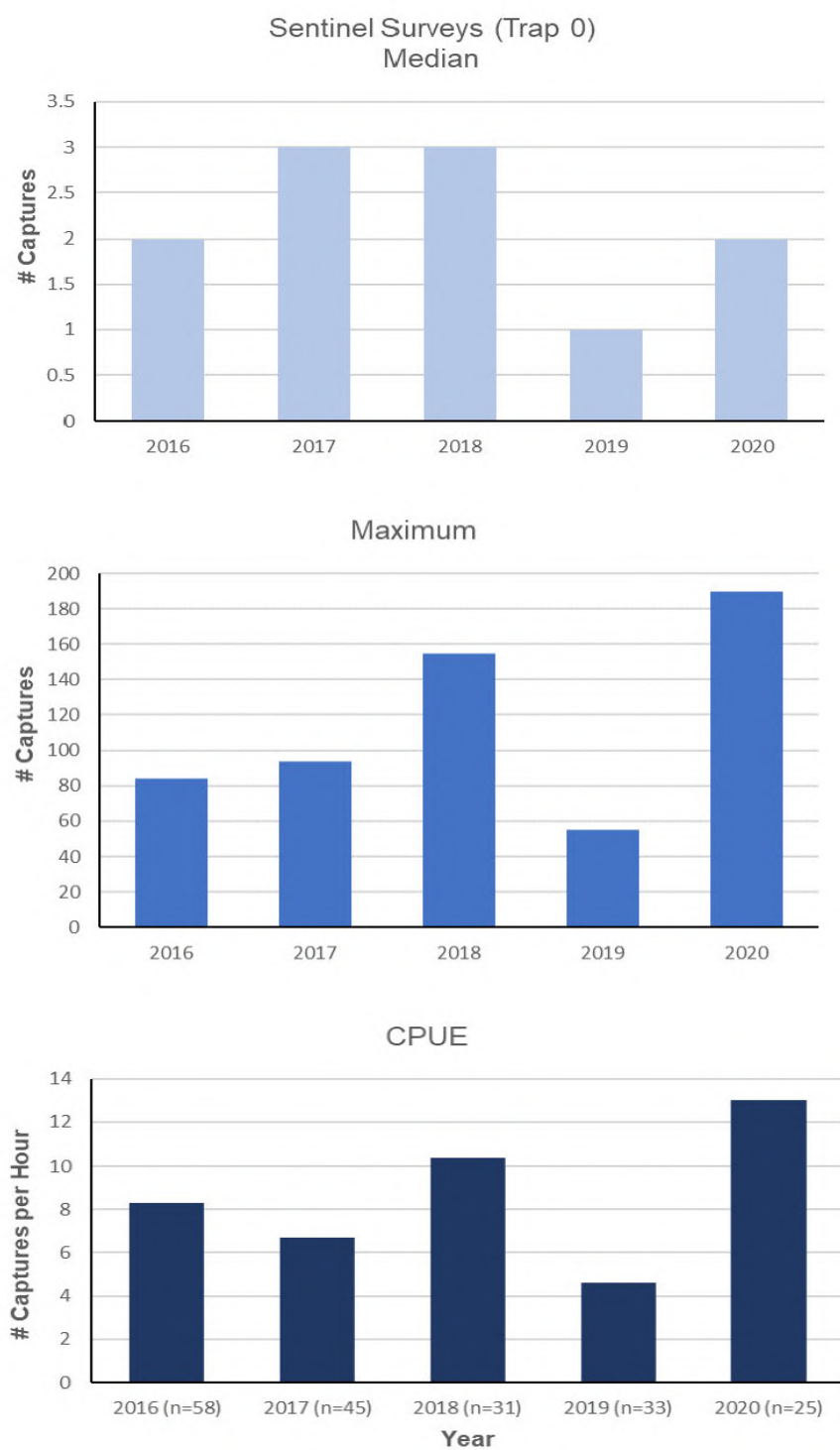


Figure 21. Median (top), maximum (middle), and CPUE (catch per unit effort; bottom) of Casey's June beetles captured during Sentinel surveys from 2016–2020. Note variable y-axis scales.

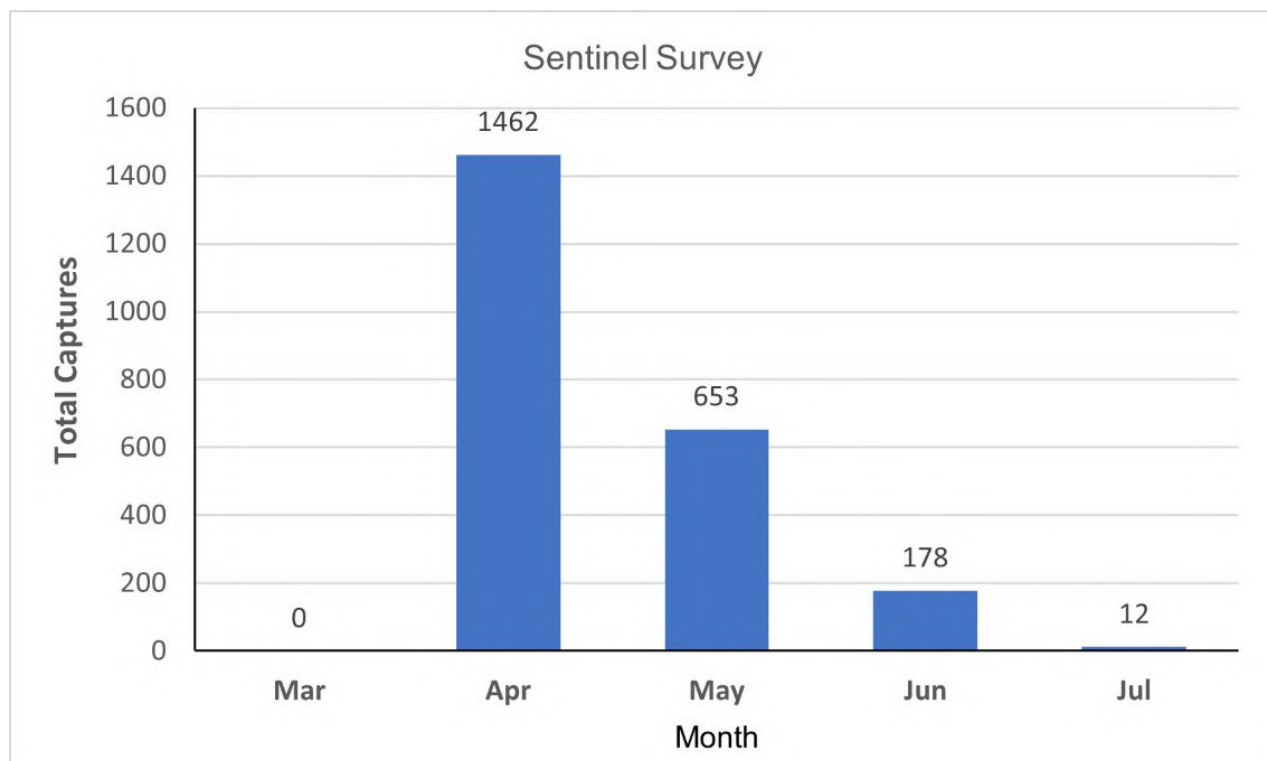


Figure 22. Total Casey's June beetle captures by month during Sentinel surveys for all years combined, 2016–2020.

Rainfall and Capture Variation

For the Palm Canyon Wash focal area, there was an apparent relationship with total annual rainfall in the previous year and total captures the following year (Table 6; Figure 23, using catch per unit effort as the index of relative activity). This relationship is unclear for the Tahquitz Creek focal area, due to insufficient years of data. Rainfall the previous year may presumably influence, either favorably or unfavorably, the conditions for immature stages, resulting in higher or lower adult emergence the following year, concomitant with rainfall totals; however, we did not statistically test this relationship using corrected relative abundance estimates.

Table 6. Casey's June beetle total captures and catch per unit effort (CPUE; survey hours) during Rangewide and Sentinel surveys with rainfall by year.

Focal Area	Year ¹	Total Rainfall ² - inches (cm) - Previous Apr–Mar	Total Rainfall ² - inches (cm) - Annual	Rangewide Total	Rangewide CPUE	Sentinel Total	Sentinel CPUE
Palm Canyon Wash	2015	-	2.09 (5.3)	-	-	-	-
Palm Canyon Wash	2016	3.15 (8.0)	5.15 (13.1)	432	2.4	506	8.3
Palm Canyon Wash	2017	8.05 (20.4)	7.17 (18.2)	624	5.7	454	6.7
Palm Canyon Wash	2018	3.68 (9.3)	4.31 (10.9)	746	5.1	477	10.4
Palm Canyon Wash	2019	8.87 (22.5)	9.5 (24.1)	632	5.2	217	4.6
Palm Canyon Wash	2020	5.78 (14.7)	3.87 (9.8)	961	8.1	488	13.0
Tahquitz Creek	2021	1.92 (4.9)	2.57 (6.5)	446	2.23	-	-
Tahquitz Creek	2022	2.09 (5.3)	1.18 (4.6)	286	1.67	-	-

¹ Rainfall reported in 2015 for comparison with 2016. No capture surveys were conducted in 2015.

² Rainfall data from NOAA 2020.

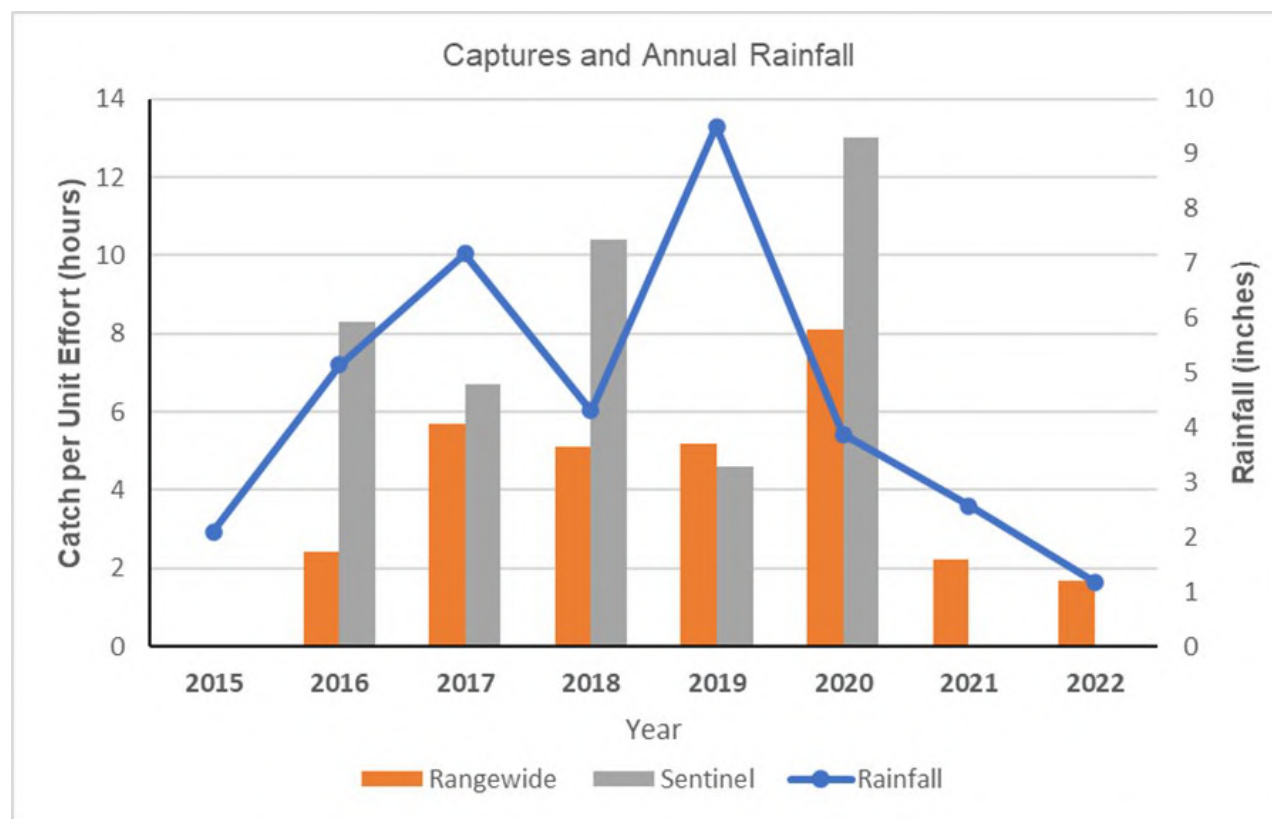


Figure 23. Casey's June beetle total catch per total survey effort (CPUE; hour) during Rangewide and Sentinel surveys with annual rainfall by year. Rainfall reported in 2015 for comparison with 2016. No capture surveys were conducted in 2015.

Recapture Patterns

We used mark-recapture and releases of Casey's June beetles at different distances from traps to test the black light attraction distance and effective survey area, assess recapture rates, and document movements among traps. Although recapture probability was low, we documented recapture rates, movements among traps, the frequency of recaptures according to release distance, and Harju (2022) used the release-to-recapture distances and a distance decay function to estimate an average recapture probability.

We found consistently low recapture rates across all trap locations and study years (Table 7, Table 8; Appendix G, Table G3). From 2016–2022, we recaptured a total of 224 marked Casey's June beetles during Rangewide surveys and a total of 72 marked beetles during Sentinel surveys (including supplemental trap sites; Table 8). Average recapture rates at traps with relatively low, moderate, and high capture activities ranged between 5.1 to 5.9 percent, and between 1.6 to 7.7 percent depending on study area location (Table 7). The highest average recapture rate was in the Tahquitz Creek golf course, which was influenced by improved recapture probability due to the closer trap spacing. Taken as a whole and based on all Rangewide surveys, the overall recapture rate was approximately 5 percent.

We documented male flight distances among traps, which provides an indication of minimum travel (flight) capabilities, but not specifically dispersal. During Rangewide surveys, the median recapture distance was 131 ft (40 m) for all years except 2016 (median=328 ft [100 m]) (Table 8). Based on recaptures among traps, the maximum documented distance ranged from 820 ft (250 m) to 2,539 ft (774 m). Most Casey's June beetles were recaptured at the same trap as their original capture but those that were not represent the farthest documented distances. Median and maximum recapture distances during Sentinel surveys were somewhat more variable but generally similar to the Rangewide survey results.

Because we surveyed daily in 2016 for Sentinel surveys, we could evaluate recaptures from different nights. Most Casey's June beetles were recaptured on the same night. A total of 5 beetles were recaptured at the Sentinel trap the following night after being marked. They were all released at the trap (release distance=0). The original capture times were within 1 hour after sunset and the recapture the following evenings were also within 1 hour after sunset. In 2020, the Sentinel trap was also included in Rangewide surveys. Because of this, and the timing of Rangewide surveys in relation to Sentinel surveys, we documented recaptures of Casey's June beetles at the Sentinel trap within 3 days after originally being marked on 13 occasions.

Table 7. Average recapture rate (percent of total captures) during Rangewide surveys by capture frequency category and location, 2016–2022 combined. Capture categories determined by the total captures at traps. Categories defined based on the frequency of captures in the dataset.

Capture Frequency Category ¹	Location	Average Recapture Rate (percent)
Low	-	5.5
Moderate	-	5.1
High	-	5.9
-	Palm Canyon Wash	6.1
-	Smoke Tree Ranch	1.6
-	Tahquitz Creek upper levee	5.7
-	Tahquitz Creek golf course	7.7

¹ Categories of total captures at traps for all years combined. Low=0 to 10 captures; Moderate=11 to 50 captures; High=51+ captures.

Table 8. Casey’s June beetle total captures and recaptures, and median and maximum recapture distances, calculated as the minimum straight-line distance from the release location to the recapture trap, 2016–2022. Results are summarized by year from the mark-recapture dataset for Rangewide, Sentinel, and supplemental surveys with all traps combined.

Survey ¹	Year	Number of Traps ²	Total Captures ³	Total Recaptures	Percent Recaptures ⁴	Median Distance ft (m)	Maximum Distance ft (m)
Rangewide	2016	15	399	19	4.8	328 (100)	656 (200)
Rangewide	2017	15	621	22	3.5	131 (40)	1,312 (400)
Rangewide	2018	13	762	41	5.4	131 (40)	820 (250)
Rangewide	2019	10	632	36	5.7	131 (40)	820 (250)
Rangewide	2020	8	794	65	8.2	131 (40)	2,539 (774)
Rangewide	2021	12	447	28	6.3	131 (40)	1,585 (483)
Rangewide	2022	9	286	13	4.5	131 (40)	820 (250)
Sentinel	2016	2	414	18	4.3	16 (5)	1,312 (400)
Sentinel	2017	4	475	18	3.8	98 (30)	328 (100)
Sentinel	2018	5	432	2	0.5	131 (40)	131 (40)
Sentinel	2019	4	295	11	3.7	131 (40)	328 (100)
Sentinel	2020	2	493	10	2.0	131 (40)	2,543 (775)
Other	2016	7	149	13	8.7	230 (70)	1,640 (500)

¹ Sentinel surveys included the primary Sentinel trap (trap 0) and supplemental traps to document additional information on length of the flight season. Other surveys included a pilot survey to evaluate different black light wattages on May 1 and 2, 2016, and a survey at trap 0 on April 30, 2016, serving as a reference site for a Casey’s June beetle presence/absence survey not associated with the study. We included all traps with mark-recapture data to maximize the small recapture sample that was available to evaluate flight distances.

² The number of traps with data in the mark-recapture analysis dataset. The number of traps may differ from the complete survey dataset because not all traps recaptured marked Casey’s June beetle.

³ Four capture records in 2017 (Sentinel surveys) were omitted due to missing information.

⁴ Percent recaptures of the total marked beetle captures in the dataset.

VEGETATION COMMUNITIES

Dudek botanists inventoried plant species and mapped vegetation communities throughout the Palm Canyon Wash and Smoke Tree Ranch study area on March 11, 12, and 13, 2015, documenting the overall composition of habitat communities at a macroscale (Dudek 2016; see Appendix D). To collect detailed habitat and vegetation community information, Dudek botanists conducted a plant inventory using the relevé method within 16 long-term survey plots in 2016 (April 27 and 28, May 12) and 8 survey plots in 2019 (May 2 and 30, June 7) (Strittmater and Amoaku 2019; Appendix D).

The 2015 and 2016 vegetation community mapping and sampling is the baseline quantitative and qualitative data for most of the range of Casey's June beetle. Prior to the February 2019 flood event, we collected three years of Casey's June beetle capture data and two years of habitat data. In 2019, we repeated the vegetation sampling, and continued Casey's June beetle capture surveys to capitalize on the opportunity to collect data after the large storm, a presumably significant occurrence for a narrow endemic species dependent on hydrological regimes.

A total of 167 native [127 (76 percent)] and non-native/naturalized [40 (24 percent)] plant species were recorded within the study area during the 2015, 2016, and 2019 surveys. There were 11 associations within nine vegetation alliances and three land cover types [Table 3 in Dudek (2019; Appendix D); Figure 24 below]. The vegetation alliances, land cover types, and total acres include:

- Cheesebush – 141.8 ac
- Creosote bush – 15.9 ac
- Creosote bush (disturbed) – 3.3 ac
- Creosote bush-brittle bush – 4.0 ac
- Creosote bush-white bursage – 6.7 ac
- Desert willow – 73.5 ac
- Desert willow (tamarisk) – 1.7 ac
- Desert willow (disturbed) – 1.3 ac
- Desert willow-smoke tree – 20.9 ac
- Fourwing saltbush (disturbed) – 1.7 ac
- Smoke tree – 24.4 ac
- Smoke tree (disturbed) – 5.4 ac

- Tamarisk thickets – 5.4 ac
- Disturbed habitat – 8.9 ac
- Unvegetated channel – 7.2 ac
- Urban/Developed – 137.1 ac
- Not mapped (no access) – 134.7 ac

Of the 459.2 total acres mapped, 314.9 ac (68.6 percent) was vegetated, 29.8 percent was developed or urban, and 1.6 percent was unvegetated channel in 2015. Within the vegetated area, cheesebush, desert willow, and smoke tree were the most prevalent.

Dudek quantified the vegetation communities and land cover types within the 16 survey plots in 2016 (Table 9) and assessed percent vegetative cover in 2016 and 2019 (Table 10). The survey plots in the active channel of Palm Canyon Wash had generally low vegetation cover, which is indicative of the open canopy of the habitat. Of the eleven survey plots in the active channel sampled in 2016, most (91 percent) had vegetation cover less than 40 percent (range=10–38 percent). For all survey plots sampled in 2016, six contained greater than 40 percent plant cover. Of these, all but one (Plot 11) were located in or abutting the upland terrace floodplain of Smoke Tree Ranch (Plots 12–15 and Plot 3, respectively). The remaining plots located within Palm Canyon Wash are susceptible to storm events and scouring, such that vegetation cover is likely to vary annually. This likelihood is evident in the 2019 vegetation data, which showed low plant diversity as well as lower overall percent cover in the survey plots susceptible to flood scour (i.e., not located in the upper terrace floodplain). This is attributable to the February 14, 2019, large flood event that caused high amounts of sediment deposition and scour in Palm Canyon Wash, which washed out many smaller trees and shrubs. In 2019, total plant cover did not change from 2016 at two survey plots located in the upland terrace floodplain (Plot 3 and Plot 14) which were protected from the flood scour and sediment deposition. One survey plot (Plot 13) contains acreage within both upland terrace and the active channel of Palm Canyon Wash, which likely influenced the 20 percent decrease in total plant cover due to flood-related vegetation loss. The remaining plots (Plot 0, 4, 5, 9, and 10), all located in the active channel of Palm Canyon Wash, showed a decrease in total percent plant cover. This decrease is the result of vegetation loss in all of the plant stratum (tree, shrub, and herbaceous; Table 10), likely due to wash out from the 2019 flood event.

While total percent vegetation cover was influenced by the 2019 flood event, there was no apparent relationship with the median number of Casey's June beetles. There also was no apparent relationship between vegetation cover and the median number of Casey's June beetles among traps in the same year and between 2016 and 2019 (Table 10; Appendix G, Figure G2). Plant species richness rather than percent cover was found to be more influential on the observed variation in Casey's June beetle numbers among traps (Harju 2022).

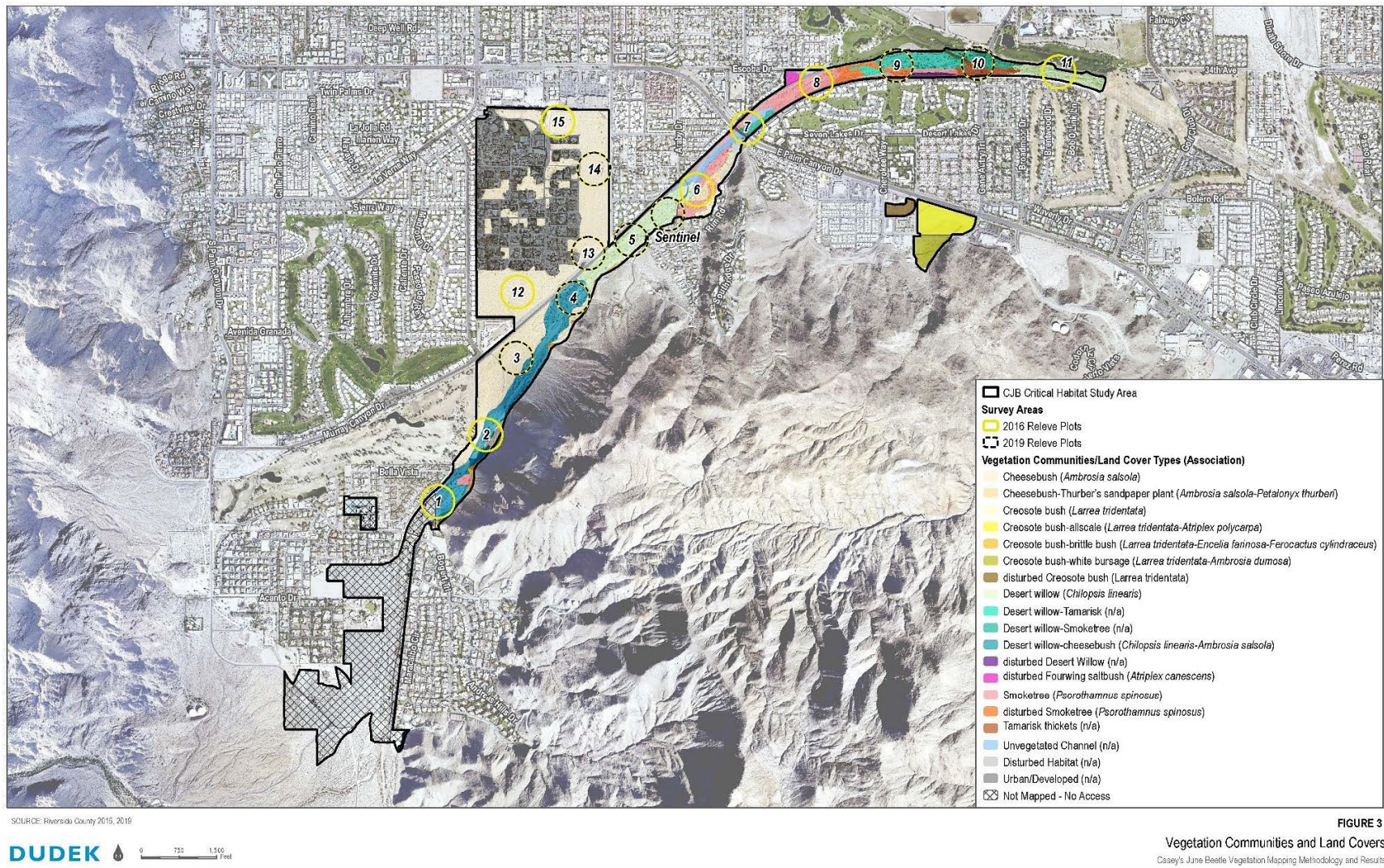


Figure 24. Vegetation communities and land cover types mapped within Casey's June beetle critical habitat in the study area. Figure and mapping from Strittmatter and Amoaku 2019 (Figure 3, Appendix D).

Table 9. Vegetation communities, land cover types, and acres within long-term survey plots sampled in 2016 using the relevé method. Reproduced from Table 4 in Strittmater and Amoaku 2019.

Plot	Vegetation Alliance/Land Cover ¹	Association ³	Acreage
0	Desert willow	<i>Chilopsis linearis</i>	5.27
0	Smoke tree	<i>Psorothamnus spinosus</i>	0.68
0	Urban/developed	N/A	0.41
0	Outside CJBCH ² study area	N/A	1.4
1	Creosote bush-brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	0.02
1	Desert willow	<i>Chilopsis linearis</i>	4.12
1	Unvegetated channel	N/A	0.28
1	Not mapped (no access)	N/A	2.22
1	Outside CJBCH study area	N/A	1.11
2	Cheesebush	<i>Ambrosia salsola</i>	2.94
2	Creosote bush–brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	0.07
2	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	3.36
2	Outside CJBCH study area	N/A	1.38
3	Cheesebush	<i>Ambrosia salsola</i>	6.74
3	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	0.98
3	Disturbed habitat	N/A	0.03
4	Cheesebush	<i>Ambrosia salsola</i>	0.97
4	Creosote bush–brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	0.11
4	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	6.09
4	Disturbed habitat	N/A	0.15
4	Urban/developed	N/A	0.43
5	Cheesebush	<i>Ambrosia salsola</i>	0.67
5	Desert willow	<i>Chilopsis linearis</i>	4.77
5	Outside CJBCH study area	N/A	1.8
5	Urban/developed	N/A	0.52
6	Cheesebush	<i>Ambrosia salsola</i> – <i>Petalonyx thurberi</i>	1.53
6	Creosote bush	<i>Larrea tridentata</i>	0.09
6	Desert willow	<i>Chilopsis linearis</i>	2.24

Plot	Vegetation Alliance/Land Cover ¹	Association ³	Acreage
6	Smoke tree	<i>Psorothamnus spinosus</i>	2.24
6	Unvegetated channel	N/A	1.34
6	Urban/developed	N/A	0.32
7	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psorothamnus spinosus</i>	1.49
7	Smoke tree	<i>Psorothamnus spinosus</i>	0.96
7	Disturbed habitat	N/A	0.18
7	Unvegetated channel	N/A	1.36
7	Outside CJBCH study area	N/A	2.65
7	Urban/developed	N/A	1.11
8	Fourwing saltbush (disturbed)	<i>Atriplex canescens</i>	0.02
8	Smoke tree	<i>Psorothamnus spinosus</i>	4.22
8	Disturbed habitat	N/A	0.79
8	Outside CJBCH study area	N/A	1.81
8	Urban/developed	N/A	0.91
9	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psorothamnus spinosus</i>	4.28
9	Desert willow (tamarisk)	N/A	0.15
9	Smoke tree	<i>Psorothamnus spinosus</i>	0.32
9	Smoke tree (disturbed)	<i>Psorothamnus spinosus</i>	1.61
9	Outside CJBCH study area	N/A	1.03
9	Urban/developed	N/A	0.35
10	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psorothamnus spinosus</i>	3.99
10	Tamarisk thickets	N/A	2.71
10	Outside CJBCH study area	N/A	1.03
10	Urban/developed	N/A	0.03
11	Desert willow	<i>Chilopsis linearis</i>	3.36
11	Outside CJBCH study area	N/A	4.24
11	Urban/developed	N/A	0.16
12	Cheesebush	<i>Ambrosia salsola</i>	7.75
13	Cheesebush	<i>Ambrosia salsola</i>	4.72
13	Desert willow	<i>Chilopsis linearis</i>	0.95
13	Disturbed habitat	N/A	0.76
13	Urban/developed	N/A	1.32
14	Cheesebush	<i>Ambrosia salsola</i>	7.24
14	Disturbed habitat	N/A	0.13

Plot	Vegetation Alliance/Land Cover ¹	Association ³	Acreage
14	Outside CJBCH study area	N/A	0.12
14	Urban/Developed	N/A	0.26
15	Cheesebush	<i>Ambrosia salsola</i>	6.13
15	Disturbed habitat	N/A	0.22
15	Outside CJBCH study area	N/A	0.47
15	Urban/developed	N/A	0.93
Total⁴			124.05

¹ Based on vegetation mapping in 2015.

² CJBCH=Casey's June beetle critical habitat.

³ N/A=not applicable.

⁴ Acreage may not sum precisely due to rounding.

Table 10. Percent vascular plant cover by stratum, change in percent cover between 2016 and 2019, and median number of Casey's June beetles captured at traps during Rangewide and Sentinel surveys in 2016 and 2019. Vegetation was sampled using the relevé method. Adapted from Tables 5 and 6 in Strittmater and Amoaku 2019.

Plot	Year	Overstory Hardwood Percent Cover	Low-Medium Tree Percent Cover	Shrub Percent Cover	Herbaceous Percent Cover	Total Vascular Percent Cover	Total Percent Cover Change ¹	Median (range) Casey's June Beetle
0	2016	-	15	10	10	35	-	2.0 (0–84)
0	2019	-	8	5	3	16	-19	1.0 (0–55)
1	2016	-	3	25	10	38	-	1.0 (0–2)
2	2016	-	5	15	5	25	-	1.5 (0–3)
3	2016	-	5	40	5	50	-	1.5 (0–5)
3	2019	-	5	40	5	50	0	1.0 (0–1)
4	2016	-	10	5	3	18	-	1.0 (0–15)
4	2019	-	5	3	2	10	-8	16.5 (0–28)
5	2016	-	15	10	5	30	-	2.5 (0–9)
5	2019	-	10	5	1	16	-14	4.0 (0–72)
6	2016	-	5	20	10	35	-	10.5 (0–48)
7	2016	-	1	3	6	10	-	0.0 (0–1)
8	2016	-	-	7	3	10	-	0.0 (0–6)
9	2016	-	5	15	5	25	-	18.0 (3–59)
9	2019	-	3	8	1	12	-13	18.5 (0–111)
10	2016	-	10	8	7	25	-	27.0 (6–58)
10	2019	-	12	1	1	14	-11	11.5 (0–119)
11	2016	-	10	25	15	50	-	14.5 (6–22)
12	2016	-	-	40	5	45	-	1.5 (0–2)
13	2016	3	12	35	15	65	-	2.0 (0–8)
13	2019	3	2	30	10	45	-20	4.5 (4–5)

Plot	Year	Overstory Hardwood Percent Cover	Low–Medium Tree Percent Cover	Shrub Percent Cover	Herbaceous Percent Cover	Total Vascular Percent Cover	Total Percent Cover Change ¹	Median (range) Casey's June Beetle
14	2016	-	1	40	5	46	-	0.5 (0–2)
14	2019	-	1	40	5	46	0	7.0 (1–13)
15	2016	-	1	35	10	46	-	3.5 (0–6)

¹ The change is total percent vegetation cover between 2016 and 2019 at plots sampled in both years.

UNDERGROUND SOIL TEMPERATURE

In 2017, we deployed 34 of the planned 36 temperature data loggers in Palm Canyon Wash at 12 long-term survey plots, underground at three depths [15 cm, 65 cm, 115 cm (5.9 in, 25.6 in, 45.2 in)]. We successfully retrieved data from a total of 8 traps in 2017 and 4 traps in 2019. The reduced dataset resulted from rocky soil prohibiting logger deployment, logger malfunction, and logger loss. At one trap, rocky soil prevented deployment of the mid and lower depth loggers. Data loggers malfunctioned at two traps. During 2018 and 2019, one logger malfunctioned at one trap, all loggers were stolen at another trap, and all loggers were washed out and lost at four traps during the large flood event (February 14, 2019). The resulting analyzed dataset included temperatures recorded hourly at all three depths for most traps with data from March 7 to August 15 in 2017 and January 31 to April 28 in 2019.

Monthly mean underground soil temperatures fit expected relationships with season and soil depth. Soil temperatures nearest the surface were higher on average in every month recorded compared to the lower depths in both years (Figure 25 and 26; Table H1 and H2 in Appendix H). This pattern was also evident in minimum and maximum temperature ranges among months and traps (Table 11). The variation among traps further demonstrates the microclimate variation documented during Rangewide capture surveys (see *Results, Rangewide and Sentinel Surveys*). Fluctuations in soil temperatures near the surface were greater compared to the increasingly stable temperatures at the mid and lower depths. The diel temperature fluctuations dampened with increasing soil depth, as exemplified by the noticeable pattern at trap 6 in March 2017 (Figure 27).

Because the underground soil temperature data represents the upper soil strata [within 3.8 ft (115 cm)] it is likely more influential on Casey's June beetle activity prior to emergence, and through the breeding season. Based on Sentinel survey data from 2016 to 2020, the dates of documented first emergence of Casey's June beetles are April 1, 2, 20, and 22 and the dates of documented last emergence are May 29, June 3, 17, 19, 21, and July 20 (Table 5). Based on the underground soil temperature, these first and last emergence periods coincide with monthly average soil temperatures (all traps combined) near the surface and at the lowest depth recorded that range from 26 °C at 15 cm to 22 °C at 115 cm in April (79 °F at 5.9 in to 72 °F at 45.2 in) and 34 °C at 15 cm to 29 °C at 115 cm in June (93 °F at 5.9 in to 84 °F at 45.2 in).

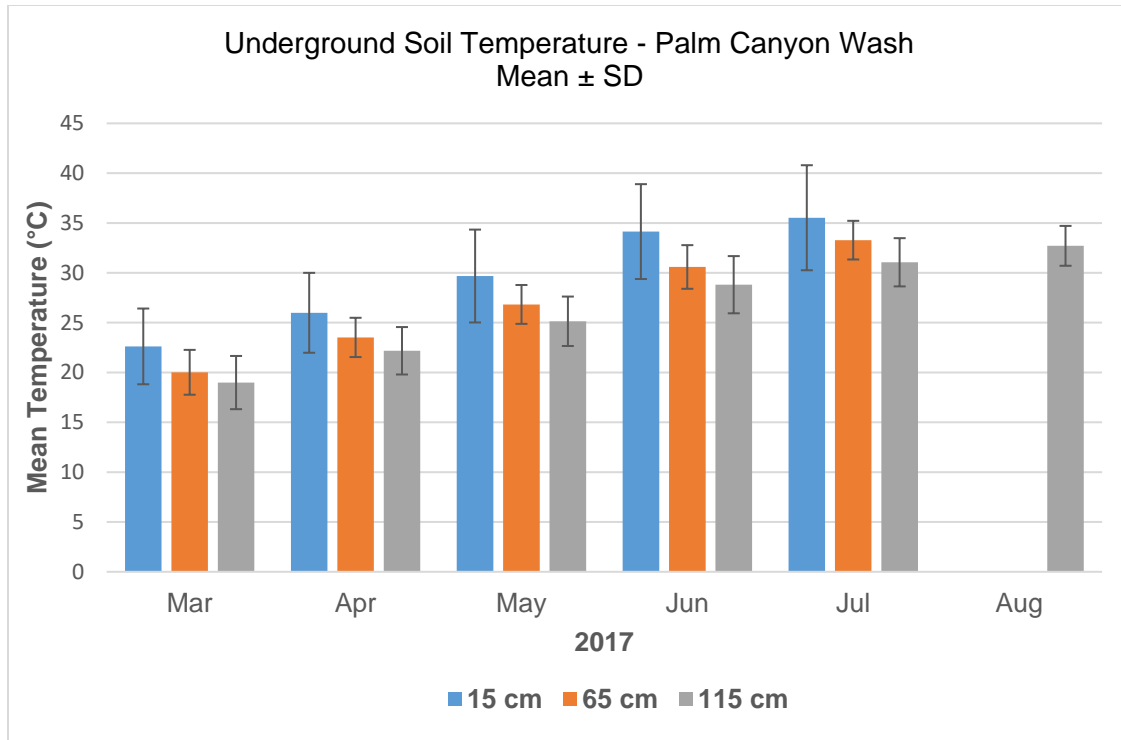


Figure 25. Mean and standard deviation (SD) of underground soil temperature (degrees Celsius) recorded at three depths at long-term survey plots ($n=8$ traps combined) in Palm Canyon Wash in 2017.

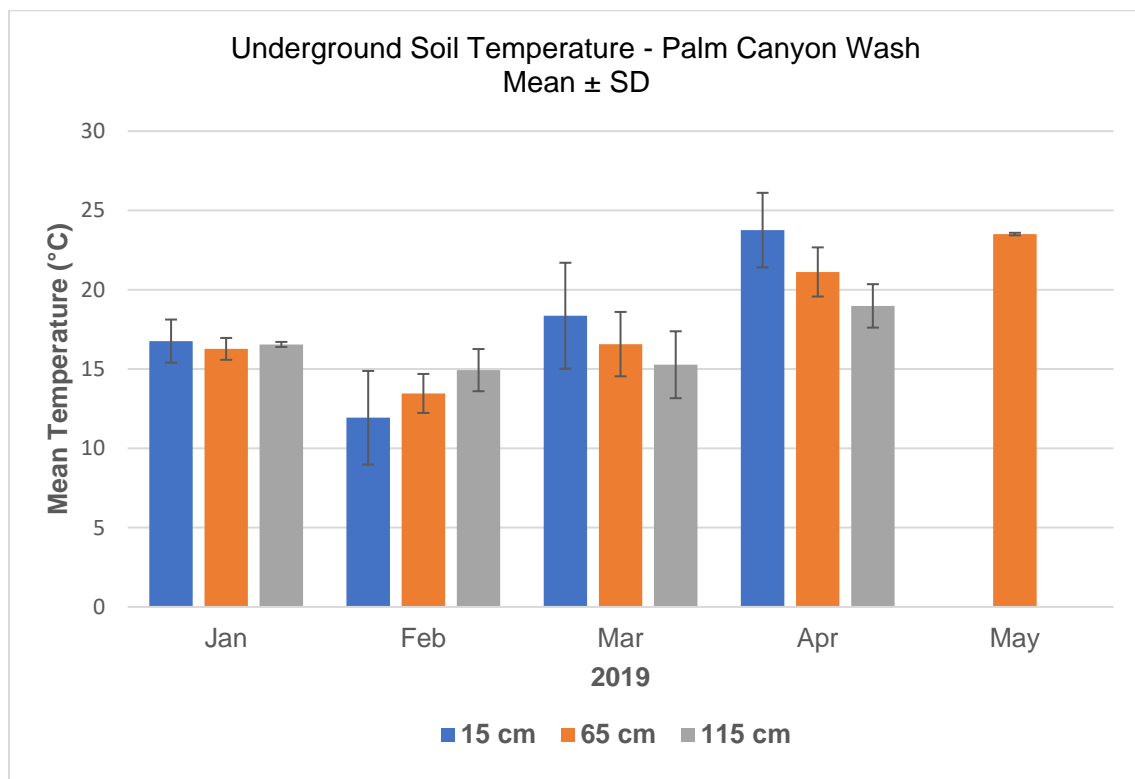


Figure 26. Mean and standard deviation (SD) of underground soil temperature (degrees Celsius) recorded at three depths at long-term survey plots ($n=4$ traps combined) in Palm Canyon Wash in 2019.

Table 11. Monthly mean underground soil temperature (degrees Celsius) recorded at 15 cm, 65 cm, and 115 cm depths at long-term survey plots, combined, in Palm Canyon Wash in 2017 and 2019.

Year	Month	Depth (cm)	<i>n</i>	Mean Temperature	SD Temperature	Minimum Temperature	Maximum Temperature
2017	Mar	15	4317	22.62	3.8	13	34.2
2017	Mar	65	4317	20.02	2.25	14.1	24.1
2017	Mar	115	4317	18.99	2.67	13.2	25.5
2017	Apr	15	5760	25.99	4.01	18.1	43.8
2017	Apr	65	5760	23.52	1.97	20.1	28.2
2017	Apr	115	5344	22.18	2.38	17.8	28.2
2017	May	15	5952	29.68	4.66	17.3	50.4
2017	May	65	5952	26.83	1.95	22.9	31.7
2017	May	115	4484	25.14	2.48	21	32
2017	Jun	15	4738	34.14	4.76	25.2	50.6
2017	Jun	65	5753	30.59	2.19	25.8	35.7
2017	Jun	115	4307	28.81	2.87	23.9	37.4
2017	Jul	15	885	35.53	5.27	22.8	45.1
2017	Jul	65	2727	33.28	1.94	29.4	37
2017	Jul	115	3300	31.06	2.42	27.5	38.6
2017	Aug	15	0	-	-	-	-
2017	Aug	65	0	-	-	-	-
2017	Aug	115	646	32.71	2	29.5	34.9
2019	Jan	15	10	16.76	1.36	14.9	18.9
2019	Jan	65	10	16.27	0.69	15.8	18
2019	Jan	115	10	16.55	0.16	16.4	17
2019	Feb	15	941	11.93	2.95	6	22
2019	Feb	65	941	13.46	1.23	8.8	17.7
2019	Feb	115	941	14.93	1.33	9	16.8
2019	Mar	15	2911	18.36	3.34	10.5	28
2019	Mar	65	2911	16.57	2.03	11.1	21.7
2019	Mar	115	2898	15.27	2.11	9.6	21.3
2019	Apr	15	1805	23.76	2.35	18.1	30.2
2019	Apr	65	1683	21.12	1.55	18.1	24.1
2019	Apr	115	2115	18.98	1.37	16.3	22.1
2019	May	15	0	-	-	-	-

Year	Month	Depth (cm)	<i>n</i>	Mean Temperature	SD Temperature	Minimum Temperature	Maximum Temperature
2019	May	65	29	23.51	0.08	23.4	23.6
2019	May	115	0	-	-	-	-

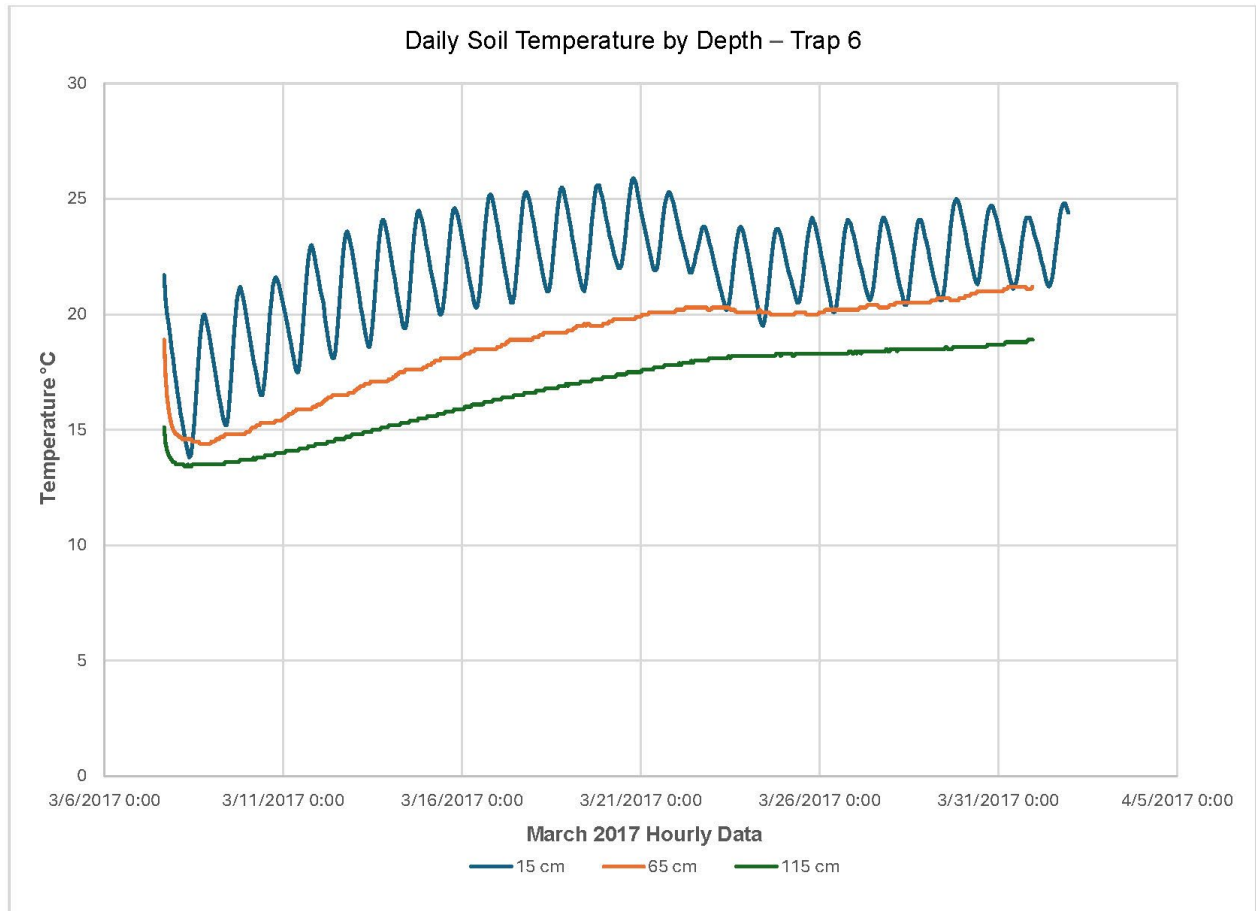


Figure 27. Daily underground soil temperature (degrees Celsius) recorded hourly in March 2017 (March 7 at 16:00 to March 31 at 23:00) for three depths at survey trap 6. The dip in temperatures after March 21 corresponds to a drop in ambient temperatures associated with a cold front in southern California at that time (<https://www.timeanddate.com/weather/usa/>).

EMERGENCE HOLES

We counted Casey's June beetle emergence holes as a relative index of activity. We documented a combined total of 7,572 Casey's June beetle emergence holes between 2015 and 2017 in the Palm Canyon Wash and upper reach of Tahquitz Creek survey area, combined (Table 12, Figures 28, 29, and 30; see Appendix I, Figures I-1 to I-3 for detailed map series). We achieved representative survey coverage as documented by the mapped surveyor tracks (Appendix I, Figures I-4 and I-5 for detailed map series; 2017 not shown due to missing survey track data). In 2015, there were 2,796 emergence holes within an estimated 225 ac (91 ha) in Palm Canyon Wash, counted during 10 days between June 13 and July 17. In 2016, there were 3,626 emergence holes within an estimated 78 ac (32 ha), counted during 7 days between July 5 and July 20 in Palm Canyon Wash at 12 long-term survey plots. In 2017, there were 1,150 emergence holes within an estimated 79 ac (32 ha), counted during 5 days between July 22 and July 26 in Palm Canyon Wash and the upper reach of Tahquitz Creek.

During 2015–2017, the percent of the survey area with compacted and cemented soils where emergence holes were visible varied among survey plots (Table 13). The percent compacted soils in the Palm Canyon Wash plots ranged between 10–85 percent, while 100 percent of the upper reach of the Tahquitz Creek survey area had compacted soils. This variation was consistent with differing hydrology and soil disturbance factors between Palm Canyon Wash and the Tahquitz Creek levee. Palm Canyon Wash has cemented soils that were deposited from upstream flows (see *Methods, Emergence Holes*) and varying types and degrees of animal and recreational activity including horseback riding, hikers, bikes, unauthorized off-highway vehicles, and wildlife that generally breaks down compacted soil. The top of the Tahquitz Creek levee does not experience hydrological sediment deposition but does receive regular irrigation that can compact soils. It also receives regular recreational activity including hikers and dogs that results in compacted soils due to foot traffic throughout the area on either side of the paved walkway.

To account for variation among survey plots in the areas where emergence holes were visible, we calculated an adjusted emergence hole count. The adjusted count total in 2015 was 531.9 emergence holes (range by trap=0.3–238.5), 1,737 emergence holes in 2016 (range by trap=1.5–882.8) and 631 in 2017 (range by trap=2.0–168.6) (Table 12, Table 13). Although emergence hole counts varied among years and traps, there was no significant difference among years (one-way ANOVA $p=0.165$) or among traps (one-way ANOVA $p=0.257$) due to high standard deviation around the means (Figure 31, Figure 32).

The location where low and high emergence hole counts occurred relative to the upstream to downstream gradient varied by year. Comparatively lower counts in the upper-most reach of Palm Canyon Wash around traps 1 and 2 was a consistent pattern for all survey years. In 2015, the highest counts were in the middle reach from around trap 3 to trap 6. In 2016, the middle reach continued to represent higher counts with the addition of the lower reach from around trap 9 to trap 11. Emergence hole counts were lower in 2017, and the areas of higher counts reduced to around traps 3 and 4 in the middle reach and traps 10 and 11 in the lower reach.

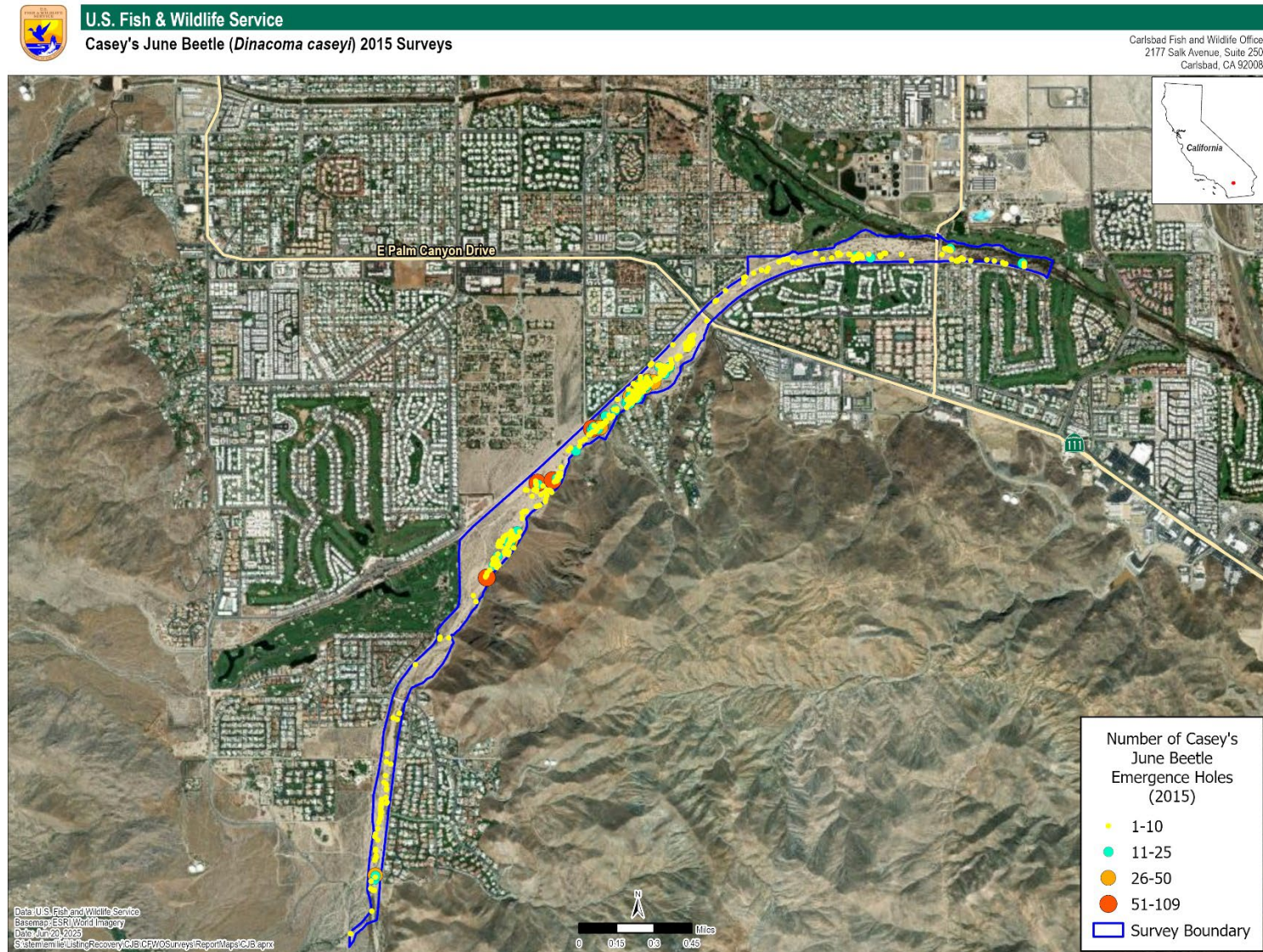


Figure 28. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash in 2015. The entirety of Palm Canyon Wash was surveyed. See Appendix I for a detailed map series.

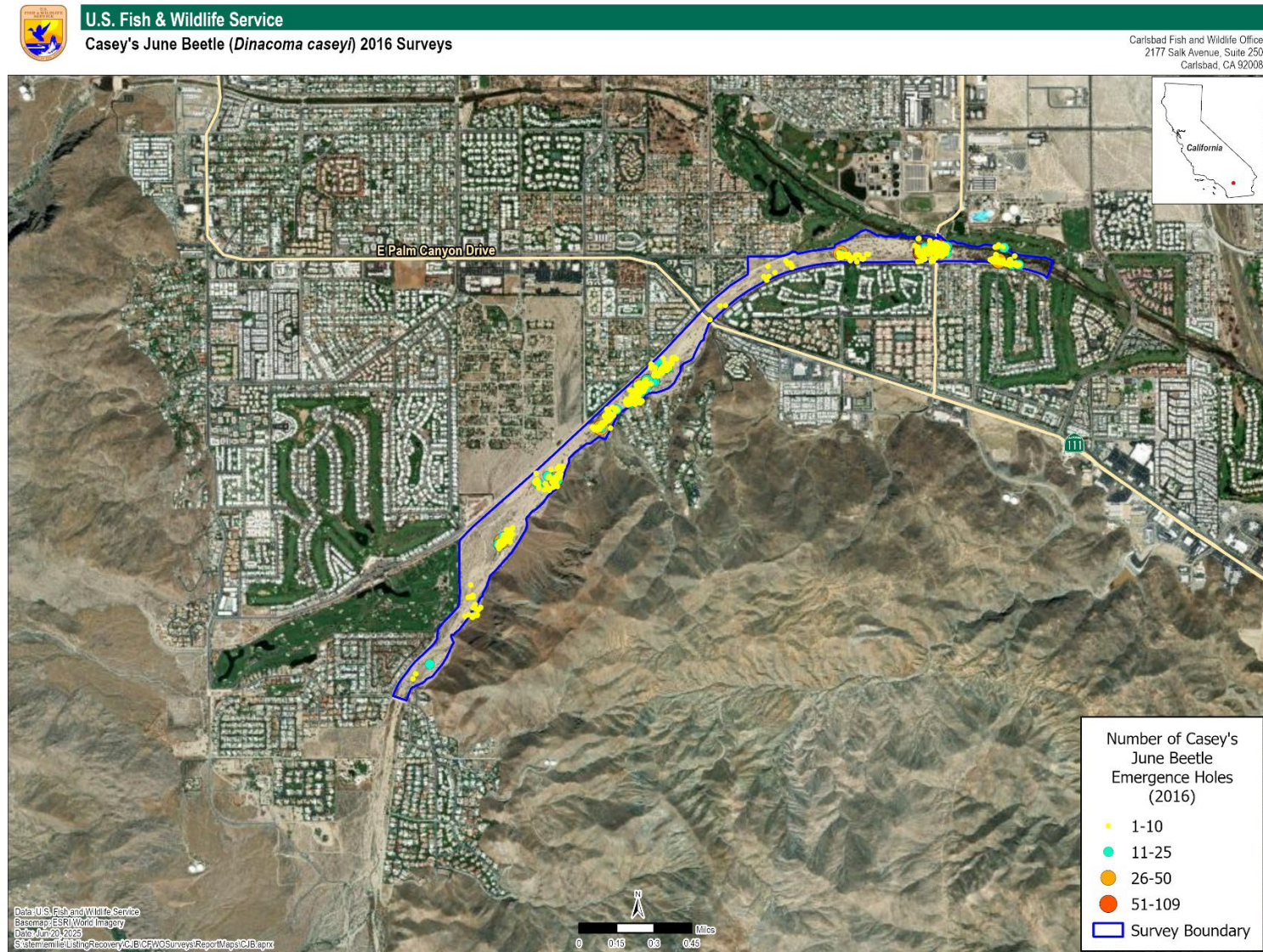


Figure 29. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash in 2016. The 100-meter radius area around traps was surveyed. See Appendix I for a detailed map series.

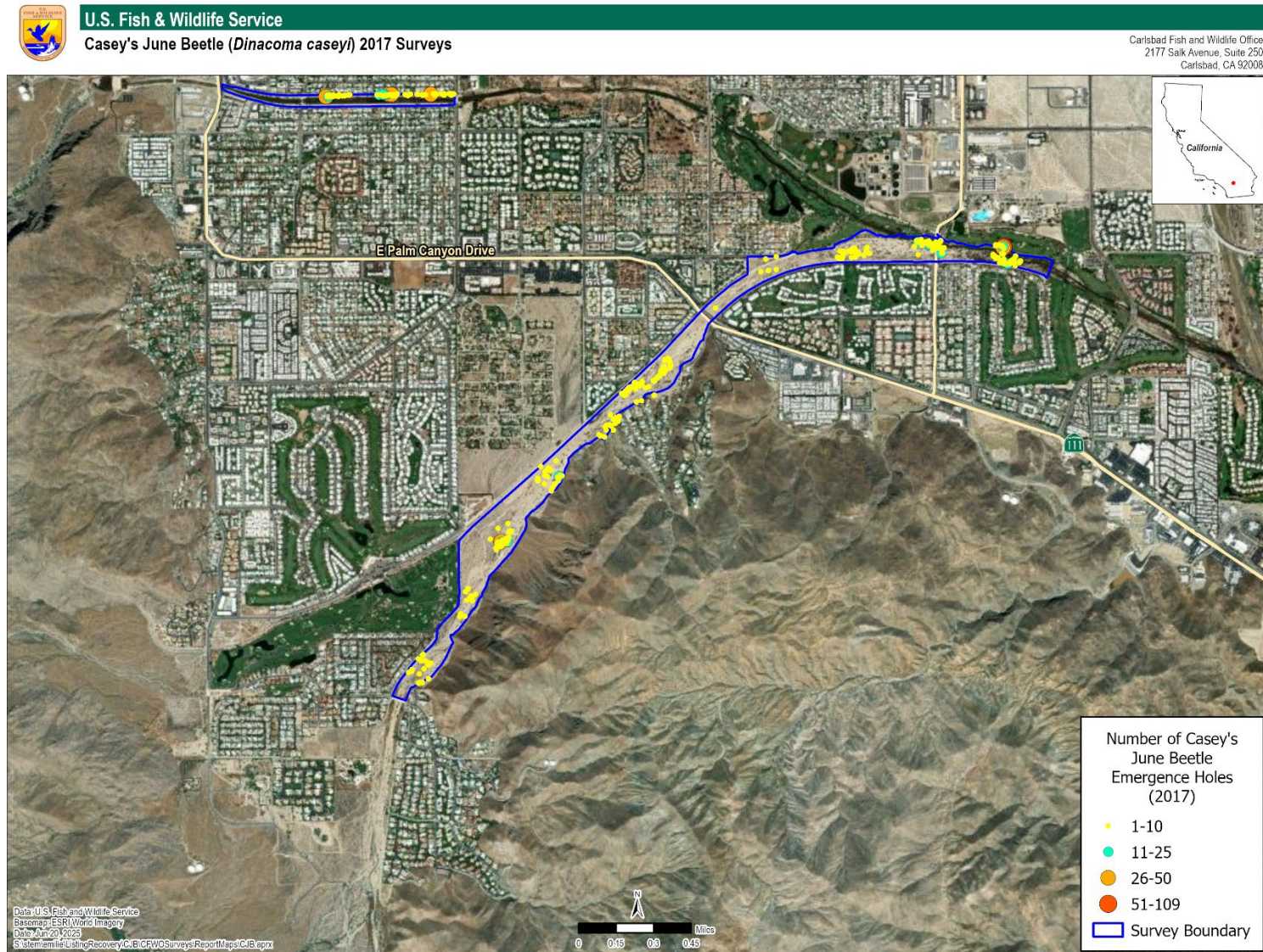


Figure 30. Overview of mapped locations of Casey's June beetle emergence holes in Palm Canyon Wash and the upper reach of Tahquitz Creek in 2017. The 100-meter radius area around traps was surveyed. See Appendix I for a detailed map series.

Table 12. Casey's June beetle emergence hole survey area and total emergence hole counts from 2015–2017.

Year	Survey Area	Number of Traps ¹	Survey Acres	Count	Adjusted Count ²
2015	Palm Canyon Wash – entire	12	225	2796	531.9
2016	Palm Canyon Wash – traps	12	78	3626	1736.95
2017	Palm Canyon Wash, Tahquitz Creek – traps	14	79	1150	631.15
Total				7,572	2,900

¹ In 2015, the entire Palm Canyon Wash study area was surveyed which included the 12 long-term survey plots established in 2016. For some analyses, we sampled the 2015 emergence hole dataset for counts within the long-term survey trap area (100-meter radius).

² The percent of compacted and cemented soils for the entire Palm Canyon Wash study area was not estimated in 2015. Therefore, the 2015 adjusted emergence hole count represents the sampled area of the 12 long-term survey plots (traps).

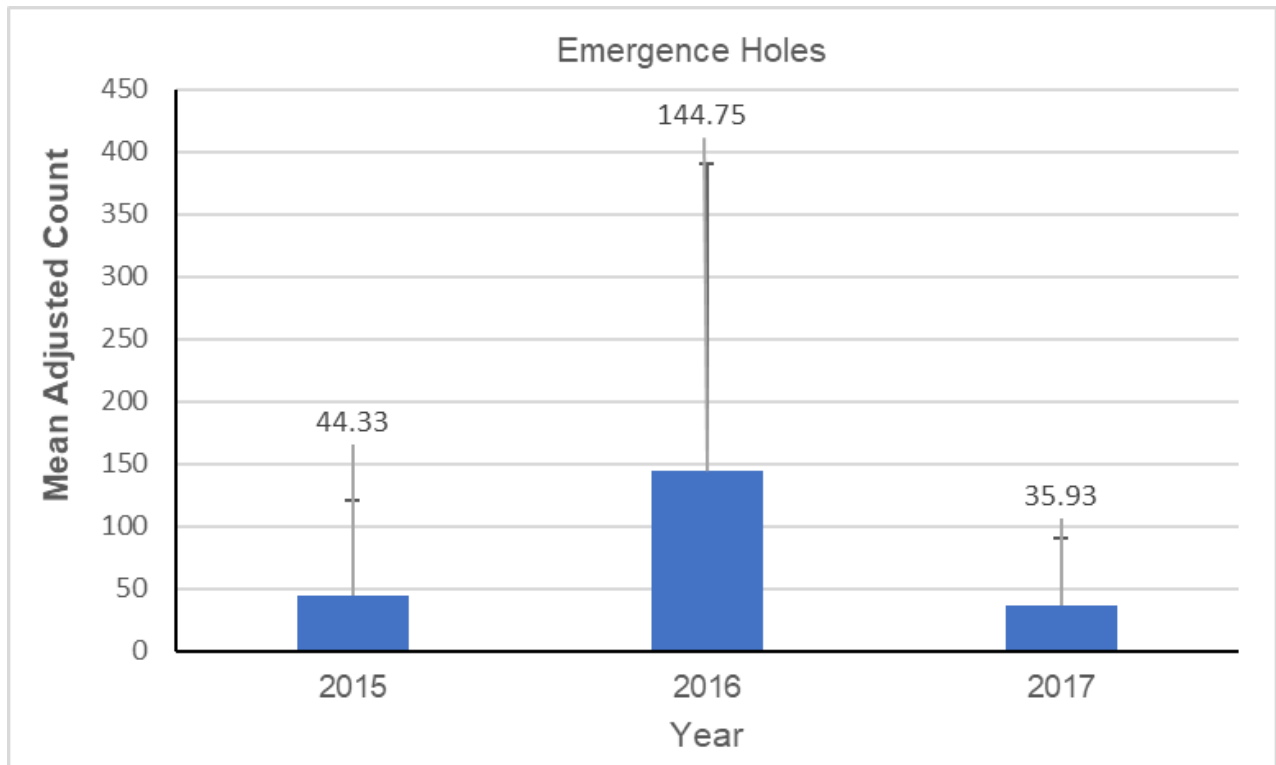


Figure 31. The mean and standard deviation (error bars) of Casey's June beetle adjusted emergence hole counts by year at long-term survey plots ($n=12$ traps) for 2015–2017 data combined. Traps 16 and 17 were excluded from analysis due to limited survey data (2017 only).

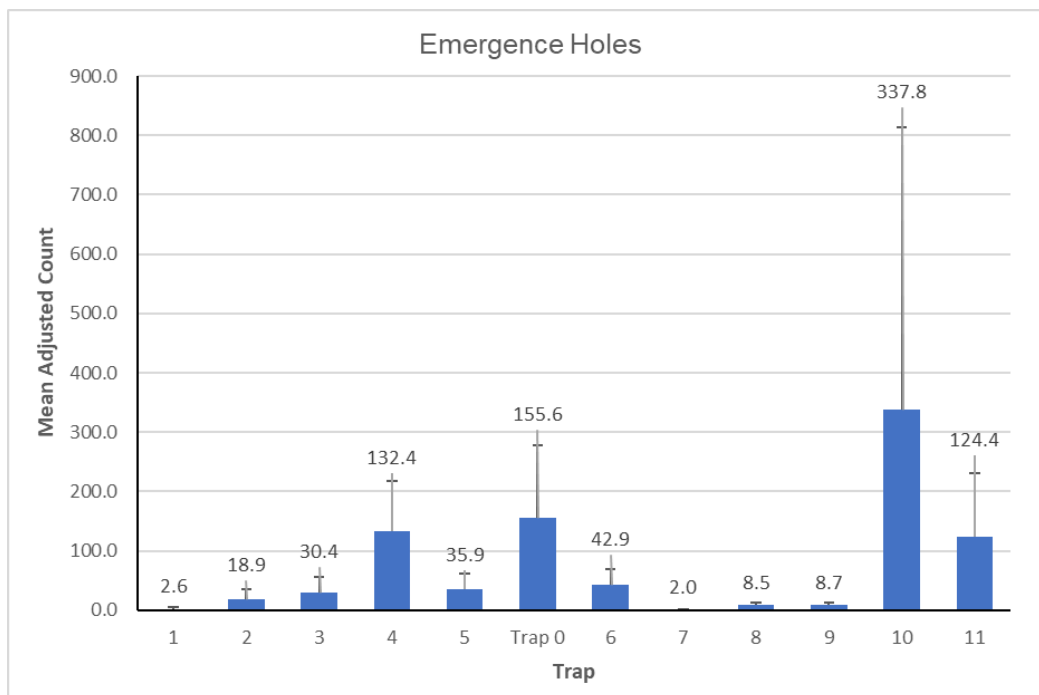


Figure 32. The mean and standard deviation (error bars) of adjusted emergence hole counts by long term survey plot ($n=12$ traps) for 2015–2017 data combined. Traps 16 and 17 were excluded from analysis due to limited survey data (2017 only).

We tested whether emergence holes and Casey’s June beetle captures were correlated using available data in 2016 and 2017 in Palm Canyon Wash. We found a low, positive correlation between the number of adjusted emergence hole counts and the median number of Casey’s June beetle captures (Spearman correlation $r=0.23$, $n=12$ survey plots), however this relationship was not statistically significant ($p=0.279$). Therefore, emergence hole counts are not shown to be a direct indicator of captures and vice versa, suggesting that other factors, as well as survey limitations, influence observed conditions.

Most emergence holes were in desert willow vegetation communities, ranging from 52.9 to 92.5 percent of the observations, depending on year (Table 14). Cheesebush represented the highest percentage of the total mapped area, which mostly occurred in Smoke Tree Ranch and the upland terrace in Palm Canyon Wash (Figure 24, Table 9). Emergence hole surveys were not conducted in Smoke Tree Ranch and the upland terrace areas had predominantly loose soil where emergence holes were not observable. Therefore, although desert willow was the third most prevalent of the entire mapped area, it was the most dominant vegetation community in the Palm Canyon Wash emergence hole survey area (Table 14, Figure 24).

Although we frequently found emergence holes within the drip zone of trees and shrubs, they also commonly occurred away from vegetation therefore, emergence activity was evident throughout the channel and was not exclusively associated with proximity to vegetation. Because we could only observe emergence holes in compacted soils, the true distribution of emergence holes throughout the wash and between the upland terrace, where soils tended to be loose, compared to the fluvially active portion of the channel is confounded by the occurrence and percentage of area covered by compacted soils and thus unknown.

Table 13. Casey's June beetle emergence hole counts, captures, vegetation, and percent compacted soil in long-term survey plots (trap), 2015–2017.

Trap	Percent Compacted Soil ¹	Total Percent Plant Cover ²	Dominant Vegetation Alliance ³	Year	Total Emergence Holes	Adjusted Total Emergence Holes ⁴	Total Captures ⁵	Median Captures ⁵
0	30	35	desert willow	2015	795	238.5	-	-
				2016	710	213	506	2
				2017	51	15.3	454	3
1	15	38	desert willow	2015	2	0.3	-	-
				2016	20	3.0	4	1
				2017	29	4.35	4	2
2	70	25	desert willow	2015	3	2.1	-	-
				2016	49	34.3	6	1.5
				2017	29	20.3	11	5
3	15	50	cheesebush	2015	42	6.3	-	-
				2016	389	58.35	8	2
				2017	176	26.4	6	3
4	65	18	desert willow	2015	253	164.45	-	-
				2016	302	196.3	17	1
				2017	56	36.4	20	10
5	25	30	desert willow	2015	192	48	-	-
				2016	212	53	14	2.5
				2017	27	6.75	76	35
6	25	35	desert willow	2015	185	46.25	-	-
				2016	269	67.25	70	10.5
				2017	61	15.25	64	28

Trap	Percent Compacted Soil ¹	Total Percent Plant Cover ²	Dominant Vegetation Alliance ³	Year	Total Emergence Holes	Adjusted Total Emergence Holes ⁴	Total Captures ⁵	Median Captures ⁵
7	50	10	unvegetated channel	2015	5	2.5	-	-
				2016	3	1.5	1	0
				2017	4	2	1	0.5
8	85	10	smoke tree	2015	12	10.2	-	-
				2016	14	11.9	6	0
				2017	4	3.4	27	11
9	10	25	desert willow-smoke tree	2015	52	5.2	-	-
				2016	146	14.6	98	18
				2017	64	6.4	173	68
10	75	25	desert willow-smoke tree	2015	6	4.5	-	-
				2016	1177	882.75	118	27
				2017	168	126	165	49
11	60	50	desert willow	2015	6	3.6	-	-
				2016	335	201	57	14.5
				2017	281	168.6	54	10
16	100	Not mapped	Not mapped	2017	84	84	-	-
17	100	Not mapped	Not mapped	2017	116	116	-	-

¹ Estimated during vegetation community surveys in 2016 (Strittmater and Amoaku 2019).

² Percent plant cover from 2016 vegetation surveys conducted by Dudek (Strittmater and Amoaku 2019). Includes vascular plant cover of all strata combined.

³ Vegetation alliance from 2016 vegetation surveys conducted by Dudek (Strittmater and Amoaku 2019). Alliance with the highest acreage in the survey plot is shown.

⁴ Total emergence hole counts adjusted for percent compacted soil within each survey plot.

⁵ Capture surveys were not conducted in 2015 or at traps 16 and 17 in 2017.

Table 14. Percent of Casey's June beetle emergence holes observed in dominant vegetation communities, 2015–2017.

Dominant Vegetation ¹	Percent of Total Mapped Vegetation ²	Percent of 2015 Emergence Holes ³	Percent of 2016 Emergence Holes ³	Percent of 2017 Emergence Holes ⁴
Cheesebush	30.9	1.4	6.3	8.7
Creosote	5.8	0.0	0.1	0.0
Desert Willow	20.9	92.5	81.7	52.9
Disturbed (various)	4.5	2.6	2.3	1.0
Smoke Tree	5.3	2.5	1.4	1.0
Tamarisk thickets (<i>Tamarix</i> spp.)	1.2	0.3	5.5	0.1
Un-vegetated	1.6	0.2	0.7	1.7
Urban Landscape	29.9	0.0	0.1	0.0
Not Mapped	-	0.3	1.6	34.8

¹ Vegetation communities were mapped in 2015 in the critical habitat area only (Strittmater and Amoaku 2019).

² Percent of the total mapped vegetation is included for area coverage comparison.

³ Casey's June beetle emergence hole observations in Palm Canyon Wash within the 328-ft (100-m) radius area of traps, excluding Smoke Tree Ranch where emergence hole surveys were not conducted.

⁴ Casey's June beetle emergence hole observations in Palm Canyon Wash and Tahquitz Creek within the 328-ft (100-m) radius area of traps, excluding Smoke Tree Ranch.

BODY SIZE, BODY CONDITION, AND EMERGENCE HOLE SIZE

Body Size

We measured male Casey's June beetle length, width, and weight in the field for a subset of beetles captured during Sentinel and Rangewide surveys in 2017 (Table 15). We recorded body dimensions from April 7 to June 21 and body weights from May 4 to June 21. Most samples were from Palm Canyon Wash at the Sentinel trap ($n=405$ [length], $n=404$ [width], $n=190$ [weight]). Only two individuals were sampled from Tahquitz Creek. For Palm Canyon Wash, beetle length and width averaged $16.42 \text{ mm} \pm 1.09 \text{ SD}$ and $7.80 \text{ mm} \pm 0.57 \text{ SD}$, respectively. The range of lengths are consistent with Hardy (1974), but the widths are slightly larger. Size variation as seen in the minimum and maximum measurements (Table 15) was noticeable in the field (Figure 8). For Tahquitz Creek, beetle length and width averaged $17.0 \text{ mm} \pm 1.41 \text{ SD}$ and $8.50 \text{ mm} \pm 0.71 \text{ SD}$, respectively, although conclusive results for Tahquitz Creek cannot be inferred due to the very small sample. We saw an apparent slight trend of decreasing mean body length from April to June, with progression through the breeding season, however, overlapping standard deviation of the means shows a non-significant relationship (Figure 33). Body width remained constant through the breeding season.

Mean Casey's June beetle weight was $0.22 \text{ g} \pm 0.05 \text{ SD}$ in Palm Canyon Wash. Mean weight in Tahquitz Creek was $0.28 \text{ g} \pm 0.11 \text{ SD}$, although this sample ($n=2$) was too small for conclusive results. Body weight sampled in May (mean= $0.23 \text{ g} \pm 0.05$, $n=155$) and June (mean= $0.20 \text{ g} \pm 0.03$, $n=35$) in Palm Canyon Wash showed a stronger relationship of decreasing weight, possibly

due to water loss during the life of individual beetles in the arid desert environment, with progression of the breeding season (Figure 34).

Table 15. Casey's June beetle body measurements from Palm Canyon Wash and Tahquitz Creek in 2017.

Measurement ¹	Location	<i>n</i>	Mean	SD	Minimum	Maximum
Length (mm)	Palm Canyon Wash	405	16.42	1.09	14	19
Length (mm)	Tahquitz Creek	2	17.00	1.41	16	18
Width (mm)	Palm Canyon Wash	404	7.80	0.57	5	9
Width (mm)	Tahquitz Creek	2	8.50	0.71	8	9
Weight (g)	Palm Canyon Wash	190	0.22	0.05	0.1	0.36
Weight (g)	Tahquitz Creek	2	0.28	0.11	0.2	0.35

¹ Body length, width, and weight was measured in the field.

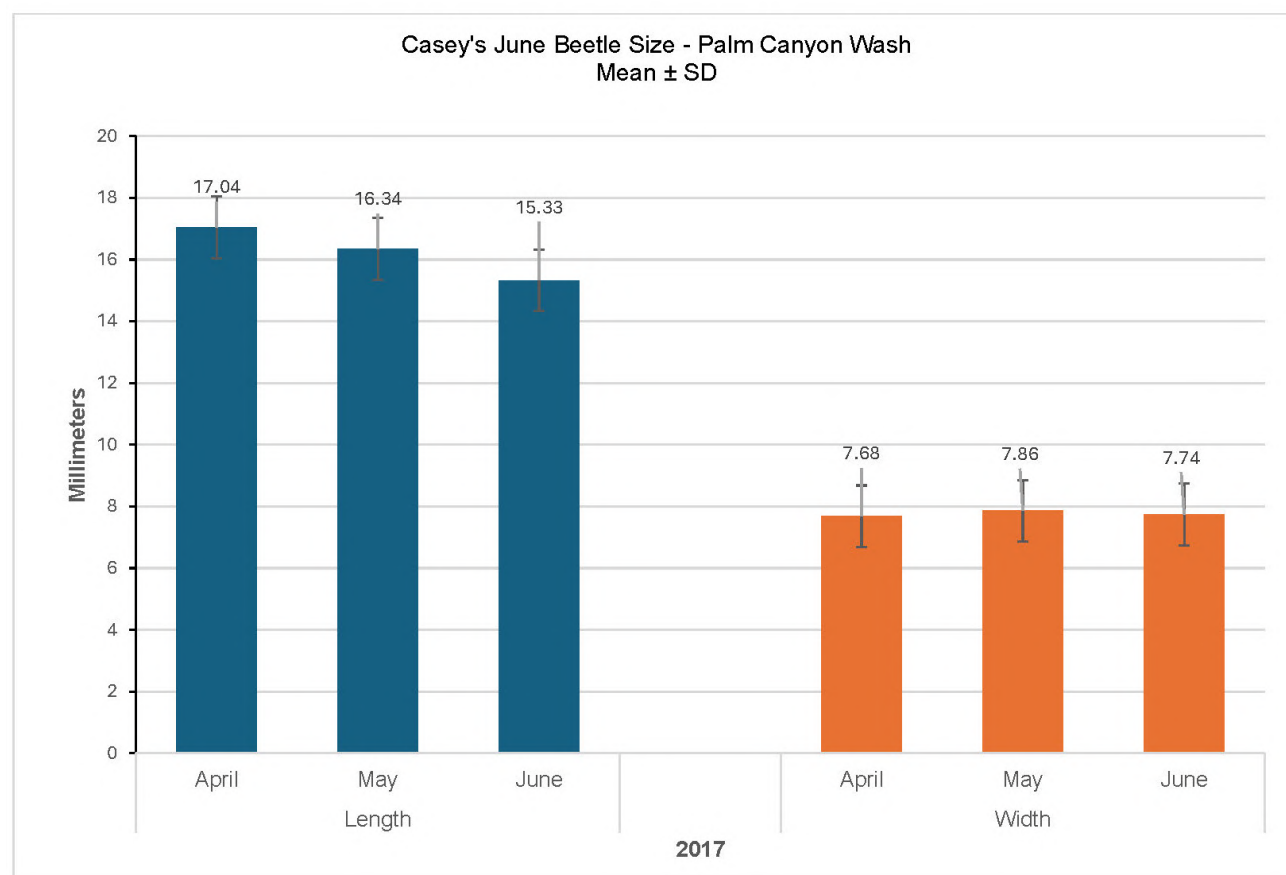


Figure 33. Casey's June beetle body measurements from April to June in Palm Canyon Wash in 2017. Body length and width was measured in the field.

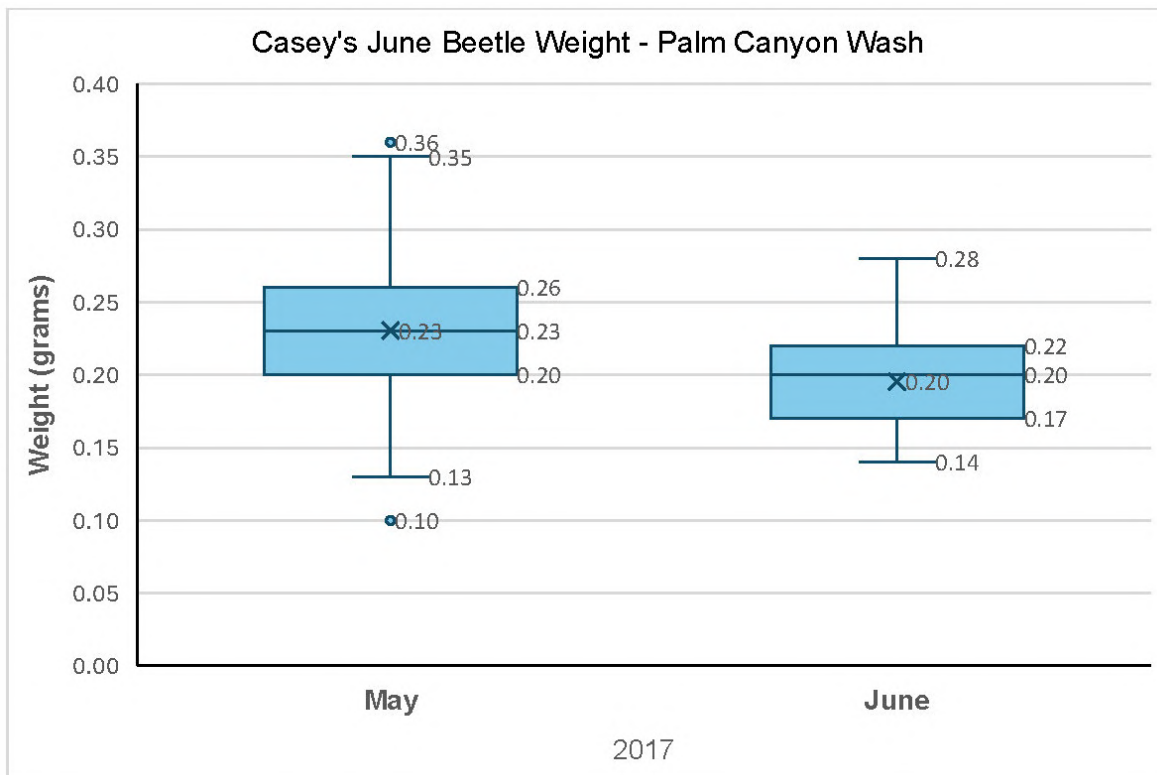


Figure 34. Casey's June beetle body weight from May to June in Palm Canyon Wash in 2017. Body weight was measured in the field. Mean, lower and upper quartiles, and minimum and maximum weight are shown. Outliers are shown as points outside the vertical box lines.

Emergence Hole Size

We recorded Casey's June beetle emergence hole physical characteristics and measurements in 2016 and 2017. We found Casey's June beetle emergence holes to have physically distinctive features, some of which were corresponding to the characteristics of the beetles. The emergence holes exhibited a uniformly circular shape, straight edges with no slant or slope to the exit trajectory (i.e., vertical shaft), and no soil spoils around the entrance (i.e., clean edges at the hole entrance). These features helped distinguish Casey's June beetle holes from other species, particularly the clean entrance edges and vertical shaft.

On April 2, 2016, and May 3 and 4, 2017, we collected size measurements of 47 Casey's June beetle emergence holes from Palm Canyon Wash ($n=31$) and the upper reach of Tahquitz Creek ($n=16$). The measurements demonstrated the uniformly round shape (Table 16). Based on Palm Canyon Wash, emergence holes averaged 9.6 mm (0.38 in.) wide. Emergence hole sizes were significantly larger in Palm Canyon Wash compared to Tahquitz Creek (Table 16) for both measurements (measurement 1 Mann-Whitney U-test $p<0.001$, measurement 2 Mann-Whitney U-test $p<0.045$).

Table 16. Casey's June beetle emergence hole measurements sampled from Palm Canyon Wash and the upper reach of Tahquitz Creek.

Measurement (mm) ¹	Location	<i>n</i>	Mean	SD	95 Percent CI	Min.	Max.
1	Palm Canyon Wash	31	9.6	0.9	9.3–9.9	7.0	11.0
1	Tahquitz Creek	16	7.6	1.7	6.8–8.5	4.0	10.0
2	Palm Canyon Wash	31	9.6	1.1	9.3–10.0	7.0	12.0
2	Tahquitz Creek	16	8.5	2.0	7.5–9.5	4.0	11.0

¹ Measurement 1 and Measurement 2 are equivalent to length and width, measured to the nearest 1.0 mm.

Comparisons with *Dinacoma marginata*

On May 29, 2016, Osborne measured the size of *D. marginata* emergence holes ($n=76$) and individual adult males ($n=70$) from Bautista Canyon. The *D. marginata* emergence holes ranged from 7.0 to 12.0 mm in diameter (mean=8.84 mm; SD=1.11 mm). Comparatively, Casey's June beetle emergence holes in Palm Canyon Wash were similar and averaged 9.6 mm wide, ranging from 9.3 to 10.0 mm.

The adult male *D. marginata* beetle size ranged from 7.0 to 10.0 mm wide (mean=8.26 mm; SD=0.63 mm). Only one male measured 10.0 mm in width; all other male *D. marginata* were 9.0 mm or smaller. Comparatively, Casey's June beetle males were generally smaller than *D. marginata* in body size. Mean width of male Casey's June beetles measured during the 2017 season from the Palm Canyon Wash Sentinel site was 7.80 mm ($n=404$; SD=0.57), ranging from 5.0 to 9.0 mm.

Relative Body Condition

We recorded the relative body condition of adult male Casey's June beetles as a proxy for age composition of male beetles alive during the flight season in 2016 and 2017. In 2016, we sampled a total of 157 Casey's June beetles from Palm Canyon Wash ($n=152$) and Smoke Tree Ranch ($n=5$) between April 19 and May 1. Most beetles were from the Sentinel survey site ($n=146$), and six were from trap 4 in Palm Canyon Wash. In 2017, we collected body condition data on a total of 437 individual males from the Sentinel site in Palm Canyon Wash and 2 adult males from Tahquitz Creek between April 7 and June 21.

The frequency of observed newly emerged beetles compared to very worn beetles (Table 17) assessed using a categorical scale of relative body wear as a proxy for days emerged was fairly consistent between years with an expected biological pattern of greater frequency of newer beetles (2016= category 1; 2017=category 1 and 2) early in the breeding season, and more beetles showing moderate wear during the peak month of May (2016=category 2; 2017=category 2 and 3). We observed a more even distribution of worn beetles (2016=category 3; 2017=category 4 and 5) during the peak and late breeding season (Figure 35, Figure 36). Generally, older beetles predominated later in the flight season. Though the categories varied between years in scale (course vs. fine), the observed frequency distribution performed as a general approximation to days since emergence.

Table 17. Relative body condition of Casey's June beetles in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek, 2016–2017, summarized as the frequency of each category. Body condition is an index using categories to describe the relative wear of white scales and hairs on the elytra and body as a proxy to days since emergence.

Year ¹	Location	<i>n</i>	Frequency of Category 1	Frequency of Category 2	Frequency of Category 3	Frequency of Category 4	Frequency of Category 5
2016	Palm Canyon Wash	152	24	81	47	-	-
2016	Smoke Tree Ranch	5	0	0	5	-	-
2017	Palm Canyon Wash	437	24	97	199	87	30
2017	Tahquitz Creek	2	0	0	1	0	1

¹ In 2016, categories were 1 to 3 (1=no or little wear, 2=moderate wear, and 3=heavy wear). In 2017, categories were 1 to 5 (ranging from 1=newly emerged, clean white to 5=very worn, no, or little white remaining).

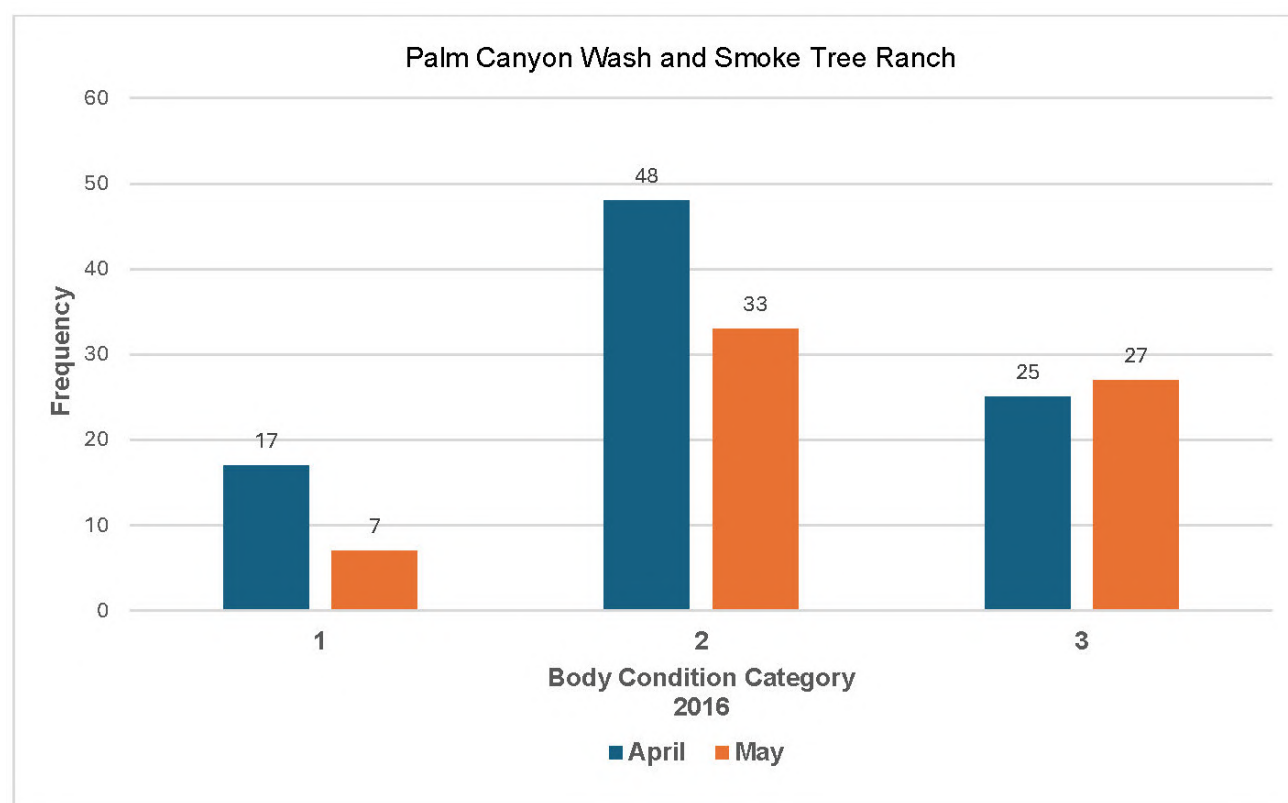


Figure 35. Frequency of relative body condition categories of Casey's June beetles in Palm Canyon Wash and Smoke Tree Ranch by month in 2016. Body condition is a categorical scale of 1 to 3, representing no or little wear (category 1), moderate wear (category 2), and heavy wear (category 3).

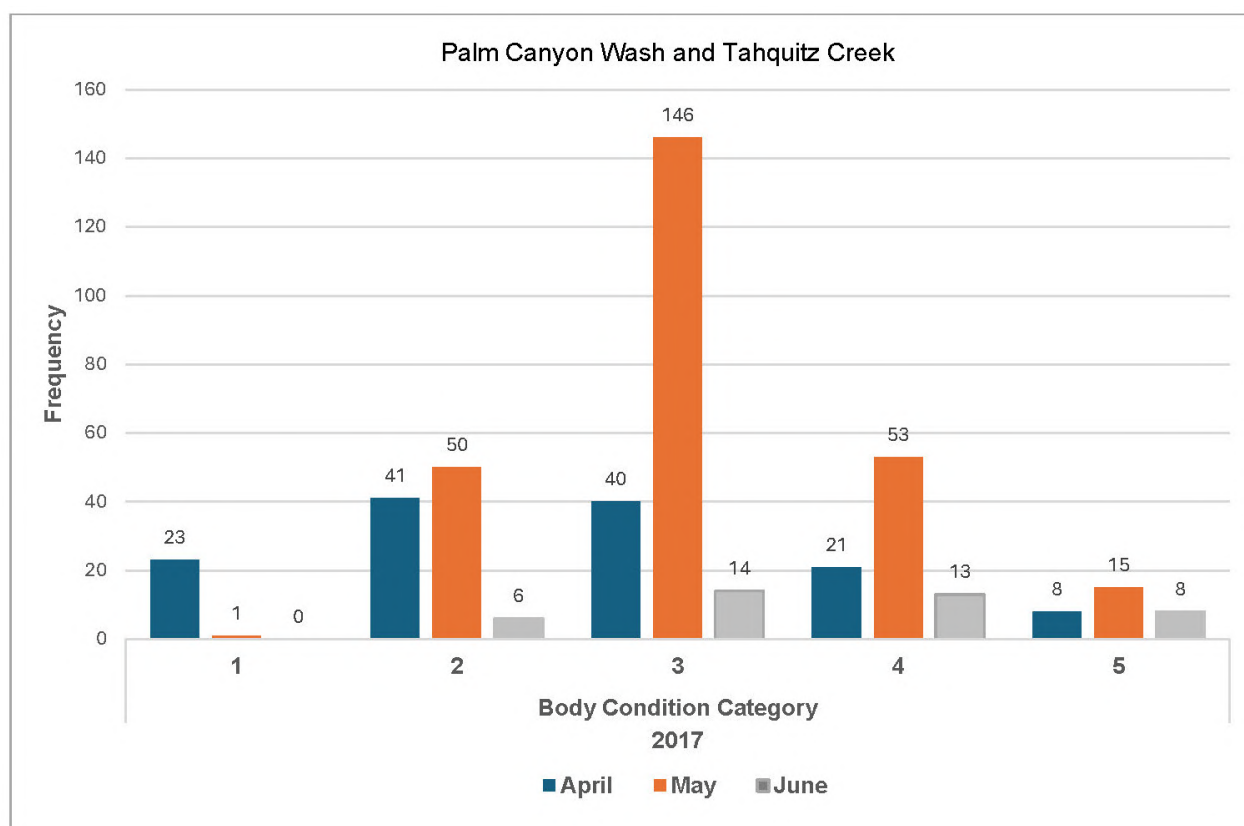


Figure 36. Frequency of relative body condition categories of Casey's June beetles in Palm Canyon Wash and Tahquitz Creek by month in 2017. Body condition is a categorical scale of 1 to 5, representing newly emerged, clean white beetles (category 1) to very worn beetles, with no or little white remaining (category 5).

FIELD AND LABORATORY OBSERVATIONS

Male Observations

Several important observations resulted from the 2016 small-scale experiment evaluating adult male longevity under mild outdoor captive conditions, and Sharpie® marker durability and effects on beetles. The general findings are (1) behaviorally, the males burrow back into the sand after evening flights; (2) the Sharpie® marks wear off within about 3 days; (3) most of the beetles survived for a week and were dead during the second week; and (4) the Sharpie® marks do not appear to cause obvious, deleterious effects on the beetles (Figure 37). We provide a brief summary of the observations during the 3-week experiment, by day and time (24-hour, when recorded).

1. April 20: Eleven live males collected in the early evening from Palm Canyon Wash and placed in a covered terrarium. We marked five males.
2. April 22 (12:00): All beetles are alive.
3. April 23 (13:00): All beetles are alive. The marks on the five marked individuals are becoming very indistinct. After being disturbed during this status check, two individuals begin flight, and the others burrow into the sand.

4. April 27 (10:00): One male is active in flight and a fence lizard is at the glass outside of the terrarium in pursuit. The other beetles are burrowed in the sand.
5. April 27 (12:00): One unmarked Casey's June beetle has died, while the others remain alive.
6. May 7: Only one Casey's June beetle remains alive. This is one of the marked individuals, but the mark is barely discernable. This single remaining male survived for another few days.

In general, through the course of observations, the beetles became active (in flight) in the early evening and late in the evening they burrowed back into the soil where they remained until the following evening. The artificial conditions were mild compared with the more extreme climatological conditions of Palm Springs, including moderate temperatures and modest humidity, and protection from predators. Therefore, we expect that under the more rigorous climatological conditions in the wash in Palm Springs, and exposed to daily predation risk, that adult male longevity is likely to be shorter than observed in this captivity arrangement.

In addition to observations of male Casey's June beetle burrowing under the soil for cover in the laboratory and the field, we documented males "resting" or taking cover in vegetation during the early evening on several occasions (Figure 38). In 2020, Sarah Greely incidentally observed beetles in vegetation during two Sentinel surveys (April 29, May 17) and one Rangewide survey (April 30). On April 29, three unmarked beetles were found in shrubs near the Sentinel trap at approximately 8:30 pm. On April 30, during the Rangewide survey at the Sentinel trap the next evening, male beetles were observed on the same shrub seen the previous evening. Greely observed a total of 7 unmarked males resting in vegetation during that survey. Although Greely marked these beetles, none were recaptured. The last observation on May 17 was of one unmarked male on vegetation approximately 40 meters from the Sentinel trap at 8:40 pm. Male Casey's June beetles may take cover in vegetation during the evening while waiting for calling females, and potentially during the day until the next evening breeding flight.

We documented the presence of mites on Casey's June beetle adult and immature stages during surveys. Mites are known to occur on beetles, and some are symbiotic while others parasitic (Nehring, Müller, and Steinmetz 2017, Sun and Kilner 2020). During a Sentinel survey on April 23, 2018, Greely documented one male Casey's June beetle with a mite on the upper elytron near the pronotum. Osborne found abundant mites (possibly of a different form) on the Casey's June beetle larvae and the pupa excavated from Palm Canyon Wash in 2022 (see *Larvae and Pupae* below). We have not obtained identification of these mites, and it remains unknown to us how they interact with or effect Casey's June beetles.

Female Observations, Collection and Laboratory Studies

Female Observations in the Field

We did not conduct systematic, female-specific surveys and our field information is limited and anecdotal. We incidentally observed a total of 22 adult female Casey's June beetle over the duration of the study, including one observation during study planning in 2014 (0–7 incidental observations per year). Observations occurred in April ($n=14$) and May ($n=8$) on 17 evenings.

Most of the observations were of females mating ($n=10$) or emerging and resting at the surface ($n=10$). Two females were observed attacked, predated, or being scavenged by ants on the same evening and location in the upper reach of Tahquitz Creek in 2021. Females were mostly discovered by chance, with only a few found by observing surrounding cues. For example, during an April 7, 2016, Sentinel survey a female was discovered by biologists observing a plant shaking on the surface as the female burrowed upward through the roots. Also, although there were few observations of females, we found that following males attracted to above ground calling females relatively reliable in contrast to finding females by random chance.

The observations of female behavior pre-and-post-mating suggest they remain above ground for only a few minutes to mate and then burrow underground quickly thereafter. Multiple males compete to mate with a single female, evidenced by one observation of 4–5 males mobbing a female. We observed females burrowing back underground where they emerged, in either the same emergence hole or a new burrow nearby. Their overall lack of extensive travel and slow movement at the surface is also suggestive of limited surface area dispersal, although females are capable of burrowing underground quickly, disappearing from view within minutes.

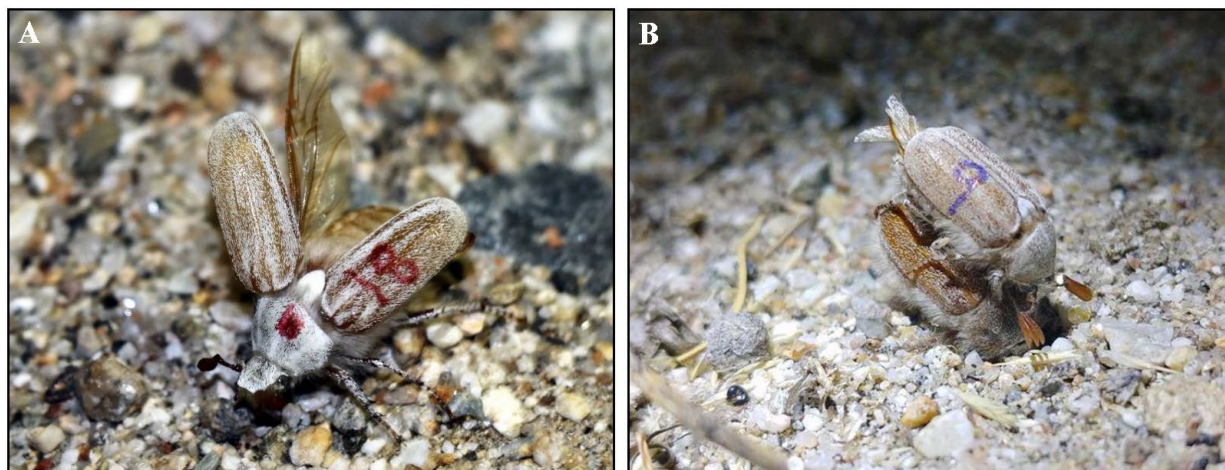


Figure 37. No obvious negative effects on individual Casey's June beetles from marking were observed. **(A).** Marked Casey's June beetle taking flight after capture and marking. **(B).** A marked male mating.¹⁹

¹⁹ Photos: Joanna Gilkeson, U.S. Fish and Wildlife Service (A), Wood Environment and Infrastructure Solutions, Inc. (B; Wood 2020).

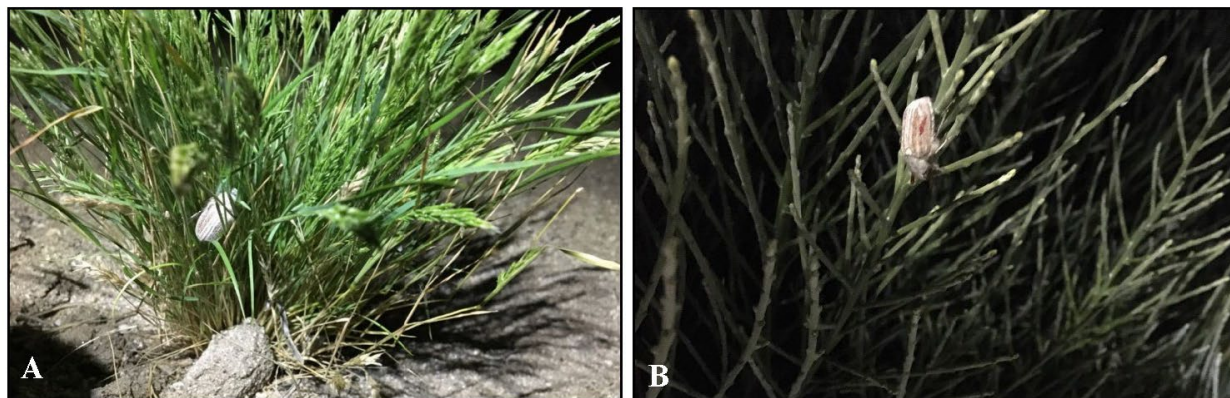


Figure 38. Male Casey's June beetles incidentally observed in grass vegetation (A) and in shrubs (B) in April and May 2020, during surveys in Palm Canyon Wash.²⁰

Collection and Laboratory Study – Females and Eggs

Osborne collected a total of nine gravid adult female Casey's June beetle between 2016 and 2021 for observation and laboratory study ($n=4$ in 2016; $n=1$ in 2017; $n=2$ in 2019; $n=1$ in 2020; $n=1$ in 2021; Table 18). A gravid female placed into a 24-ounce jar with damp sand on April 5, 2016, had produced ten eggs by April 20. Three of these very fragile eggs were preserved in Ethyl alcohol whereupon their clear liquid contents were denatured and turned white. The remaining seven eggs were replaced into the damp sand in hopes of obtaining early instar larvae; this attempt ultimately failed.

The April 8, 2016, placement of a mated female within the wood-framed, clear plexiglass viewing stadium showed burrowing to a depth of 14 inches (35.5 cm) within the first 11 hours overnight. After an additional 10.5 hours, the beetle had burrowed to a depth of 5.6 ft (1.7 m), and by 48 hours was found at the bottom of the stadium at a depth of 7.4 ft (2.25 m). Disturbed sands within the column indicated the generally straight path (downward) taken by the beetle. These movements may have resulted in the female repeatedly crossing through previous burrow pathways, unintentionally destroying fragile eggs. Osborne dissected the other gravid female that was collected in 2016, which revealed 10 eggs that were elliptically shaped and relatively large. The egg size was comparable to about a grain of rice, approximately 0.16 inches by 0.10 inches (4.0 by 2.3 mm), with no noticeable variation in size (Figure 39). The eggs were fragile and ruptured easily.

With females placed into 24-oz jars of damp sand in 2016 and 2019, observations showed that their movements likely resulted in the female repeatedly crossing through previous burrow pathways, unintentionally destroying fragile eggs. For the female placed with potted *Chilopsis* in 2017, examination of the contents the following year showed the effort was unsuccessful. In 2019, Osborne placed two females in small glass containers to rear laid eggs however, this attempt was unsuccessful, likely due to the small container size.

In 2020, the larger bucket container that Osborne used to house one gravid female collected on April 16, resulted in the successful propagation of three larva from this single female (Figure

²⁰ Photos: Sarah Greely, The Living Desert Zoo and Gardens.

39). On August 25, 2020, approximately four months after the female was placed into the rearing bucket, Osborne sifted the contents through a ¼-inch screen, followed by a 1/16-inch screen. The top few inches of sand were relatively dry, with damp sand below. Two larva approximately the diameter of a pencil were recovered using the ¼-inch screen. Of these larvae, one was found burrowed into and feeding on rotten wood. Both larvae were small enough to crawl through the ¼-inch screen. A third, smaller larval instar was recovered using the 1/16-inch screen. It was not possible to observe when the female died, and no remains were found during the content sifting. Osborne returned all of the larvae to the bucket and made periodic checks to record growth. During the larva propagation, Osborne observed the larvae feeding on detritus and rotten wood. The three larvae were later placed in separate 60-dram vials with moist sand and organic debris to continue rearing however, they did not pupate. Because the larvae were reared from eggs of a female Casey's June beetle housed in a separate container from other beetle species, the larvae are known to be Casey's June beetle.

Table 18. Results of gravid female Casey’s June beetle and beetle larvae study under small-scale captive rearing laboratory conditions, 2016–2022.

Study or Observation ¹	Year	Specimen ²	Survey	Number Collected	Collection Date (Placement Date)	Collection Location	Result
Burrowing	2016	female	Sentinel	1	April 7 (April 8)	Trap 0	Female burrowing observed over 48-hour observation
Egg Laying	2016	female	Sentinel	1	April 4 (April 5)	Trap 0	3 eggs
Egg Laying	2016	female	Incidental	1	April 27	Trap 0	Unsuccessful
Egg Laying	2017	female	Incidental	1	May 12	Trap 9–10	Potted <i>Chilopsis</i> container; Unsuccessful
Egg Laying	2019	female	Incidental	2	May 30	Trap 9	Unsuccessful
Egg Laying	2020	female	Incidental	1	April 16	Trap 10	Successful; 3 larvae
Egg Laying	2021	female	Incidental	1	April 30	Trap 10	Successful; 1 larva
Dissection	2016	female	Incidental	1	April 6	Trap 0	10 eggs
Larvae Rearing	2019	larvae	Soil Excavation (deep)	20	Nov. 17, Dec. 15	Trap 9–10	<i>Cyclocephala</i> beetles
Larvae Rearing	2022	larva, pupa	Soil Excavation (upper)	1, 1	March 27	Trap 0	Casey’s June beetle

¹ Dissection under microscope to study egg production.

² Gravid females, collected after observed mating in the field.

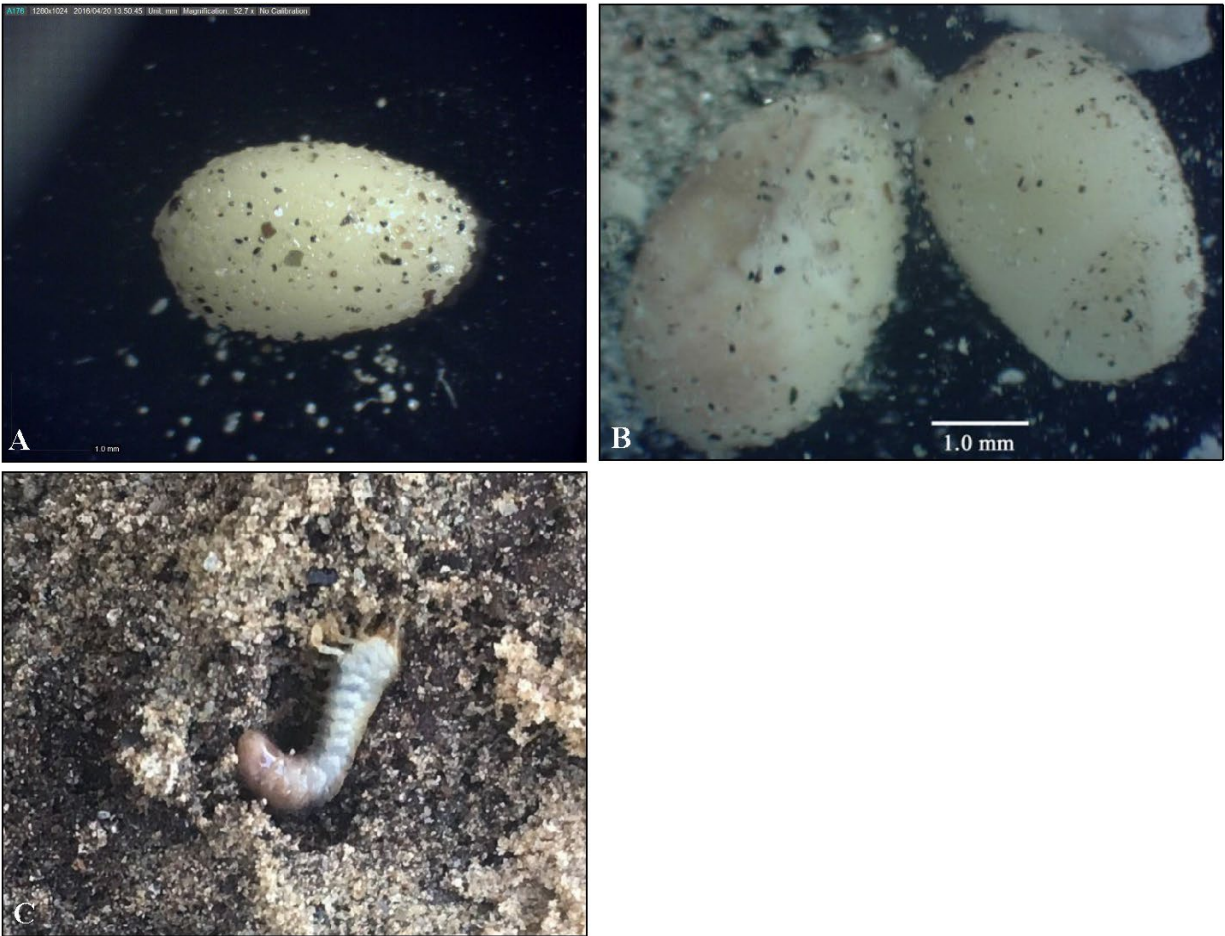


Figure 39. Casey's June beetle eggs and larvae from laboratory study in 2016 and 2020, respectively. **(A).** Casey's June beetle egg found by dissection of a female in 2016, viewed under microscope magnification. **(B).** Ruptured eggs, viewed under microscope magnification. **(C).** One of three Casey's June beetle larvae propagated from eggs laid by a female Casey's June beetle under captive rearing conditions in 2020.²¹

²¹ Photos: Ken Osborne, Osborne Biological Consulting.

In 2021, one gravid female was housed for captive egg rearing in a 3-ft (0.9-m) PVC drainpipe. We sifted the drainpipe soil on January 2, 2022, and found one live larvae (Figure 40). Under microscopy, Osborne documented and photographed its head anatomy including antenna, labium, mandibles, maxilla, maxillary palpus, and detail of the legs. Osborne also discovered two mites on the surface of the larva's integument. As with the larvae reared in 2020, this larva was known to be Casey's June beetle due to its separate housing from other beetle species.

Larvae and Pupae

From July 29, 2019, to February 28, 2020, the District removed 270,000 yd³ (206,430 m³) of sediment from a 42-ac (17-ha) area in Palm Canyon Wash (Service 2023). The District excavated sediment at depths ranging from approximately 1 to 9 ft (0.3 to 2.7 m), with greater sediment depths in the downstream portions of the removal area near the bridges at Palm Canyon Drive (Highway 111) and Gene Autry Trail.

In conjunction with the sediment removal in 2019, we searched for Casey's June beetle larvae on five days: November 13, 14, and 17, and December 15 and 21. We surveyed four sites in Palm Canyon Wash between approximately 390 ft (119 m) and 2,900 ft (884 m) upstream of the Gene Autry Trail bridge (Figure 3, Table 19) where the greatest depth of sediment was removed. Tree and vegetation clearing was a component of the removal work and had occurred just prior to the sediment removal and the soil excavation surveys. Two soil excavation sites (sites 1 and 4) were on the edge of the sediment removal area and live vegetation that was not removed or impacted by construction was nearby. One excavation site (site 3) was in the center of the sediment removal site. Tamarisk had been present and alive before it was cleared for the sediment removal. Site 5 was located next to a group of salvaged, mature desert willow but sediment was removed from the surrounding area.

We found 23 scarabaeiform (Stehr 1991) beetle larvae at two sites: 6 larvae on November 17 and 17 larvae on December 15 (Figure 41). We did not find Casey's June beetle larvae (see *Collection and Laboratory Study* below). The scarabaeiform larvae were at depths ranging from 4.8 to 6.2 ft (1.5 to 1.9 m) below ground. The maximum depth surveyed ranged from 6.2 to 9.5 ft (1.9 to 2.9 m) however, surface soils had already been removed from the upper strata (3.8 and 4.6 ft [1.15 and 1.4 m]) at two survey sites (Table 19) by the District as part of the sediment removal operations prior to the survey. Three larvae were found dead or dying during the December 15 survey, likely from damage incurred during digging. The larvae ranged in size (0.20–0.91 g [$n=20$ live larvae]) and most were found within an underground detritus layer and/or next to or among decaying tree roots. Most of the larvae were actively moving and feeding during the collection process.

On March 27, 2022, we successfully found Casey's June beetle during a soil excavation survey (Table 18, Table 19). We focused the survey on the upper soil strata and the period just before spring emergence to maximize the probability of detection. We found a total of two larvae, one pupa, and five larval exuvium (exoskeleton remains) within a soil excavation area totaling 5.8 ft long, 3.3 ft wide, and 2.0 ft deep (1.8 by 1.0 by 0.6 m). One larva was found dead, possibly from damage incurred during digging, at 14 inches (36 cm) below ground. The one live larva was 12 inches (36 cm) below ground. This larva was large and amber colored, and appeared ready to pupate (Ken Osborne, pers. comm.; Figure 42). The one live pupa was found 12 inches (30 cm)

below ground and was a male based on the presence of grasping organs (Figure 42; see *Immature Morphology of Casey's June Beetle* below). We found full or partial larval exuvium between 11 and 15 inches (28 and 38 cm) below ground (Figure 42). There was no vegetation detritus present in the upper soil strata.



Figure 40. Casey's June beetle larvae propagated from an egg laid by a female Casey's June beetle collected in 2021 and maintained under captive rearing conditions. (A). The 4-ft (1.2-m) PVC drainpipe container used to rear eggs and larvae, just prior to searching the soil for larvae on January 2, 2022. The dark bands in the soil column are detritus layers. (B, C). Casey's June beetle larvae found in a detritus layer in the soil column of the drainpipe housing container.²²

²² Photos: Ken Osborne, Osborne Biological Consulting (A). Noelle Ronan, U.S. Fish and Wildlife Service (B, C).



Figure 41. Beetle larvae found during soil excavation surveys in 2019 and 2022, Palm Canyon Wash. **(A).** *Cyclocephala* (masked chafer) beetle larvae from Site 3 on the sifting screen in 2019. **(B).** *Cyclocephala* (masked chafer) beetle larvae from Site 4 on the scale in 2019. **(C, D).** Casey's June beetle larvae from the Sentinel survey plot in March 2022, viewed on the sifting screen **(C)** and in a collection vial **(D)**.²³

²³ Photos: Noelle Ronan, U.S. Fish and Wildlife Service.

Table 19. Soil excavation locations surveyed for beetle larvae in 2019 and 2022 in Palm Canyon Wash.

Location	Site ¹	Latitude ²	Longitude ²	Date Surveyed	Nearest Vegetation	Distance to Vegetation ft (m)	Maximum Depth ³ ft (m)	Depth of Surface Soil Removed ft (m) ⁴
Gene Autry Bridge	1	33.801376	-116.499291	11-13-2019, 11-14-2019	desert willow	9.8 (3)	6.6 (2.0)	1.0 (0.3)
Gene Autry Bridge	3	33.801170	-116.499065	11-17-2019	tamarisk sp.	-	6.6 (2.0)	3.8 (1.15)
Gene Autry Bridge	4	33.801346	-116.496374	12-15-2019	tamarisk sp.	0	9.5 (2.9)	4.6 (1.4)
Escoba Drive Access	5	33.800830	-116.504598	12-21-2019	desert willow	2.0 (0.6)	6.2 (1.9)	0
South Araby Drive	0	33.793764	-116.515272	3-27-2022	desert willow	4.9 (1.5)	2.0 (0.6)	-

¹ Sites 1 and 3–5 were in the sediment removal area where removal operations were occurring using heavy equipment. Tree and vegetation clearing was a component of the removal work and had occurred just prior to the sediment removal. Sites 1 and 4 were on the edge of the sediment removal area and were near live vegetation not removed or impacted by construction. Site 3 was in the center of the sediment removal area. Tamarisk had been present and alive before it was cleared for the sediment removal. Site 5 was located next to a group of salvaged, mature desert willow, with sediment removed in the surrounding area. Site 0 is the Sentinel survey plot where no sediment removal occurred.

² Datum=NAD83

³ Maximum depth excavated, including the depth from the surface where surface soils were removed by the District for the sediment removal operation, prior to the survey.

⁴ The depth of soil removed from the surface by the District prior to the survey for the sediment removal operation. For Site 1, this is the depth at which we started surveying (no soil had been removed from the surface prior to the survey).

Collection and Laboratory Study – Larvae and Pupae from Soil Surveys

In 2019, we maintained the 20 live larvae found during the November and December soil survey (described above) under captive rearing conditions until they pupated to adults (Table 18). On June 19, 2020, these live larvae developed to *Cyclocephala* beetles (Figure 41), a species whose larvae superficially appear similar to Casey's June beetle larvae. During the captive rearing, different food items were offered to individual larvae to understand feeding behavior (see *Methods*). The only food item that showed evidence of feeding damage, and resulted in larvae weight gain and gut contents was the rotten wood.

Cyclocephala are scarab beetles attracted to lights and are a species commonly observed in the field and frequently captured in black light traps during the study. Despite selecting an area of previously high relative abundance/activity to maximize detection probability for this limited sampling effort, we found no Casey's June beetle larvae at depths up to 9.5 ft (2.9 m). However, we were not able to survey the upper soil strata at all survey sites due to the sediment removal activities.

In 2022, we collected the live larva and pupa found during the soil survey in March for captive rearing and study for species identification (Table 18). On April 3, 2022, Osborne confirmed both the larva and pupa to be Casey's June beetle under a dissecting microscope by anatomical comparison with a live, captive reared, known Casey's June beetle larva from 2020 (Osborne 2022b). By mid-June 2022, the larva was still alive and had not pupated, suggesting that Casey's June beetle life cycle development is capable of extending beyond 1 year. Given the larva and pupa species identification as Casey's June beetle and based on the proximity in the upper soil layers, we presume that the larval exuvium we found were likely Casey's June beetle. The larva died and was preserved in alcohol and the pupa failed to emerge to adult.



Figure 42. Casey's June beetle pupa and larval exuvium found during a soil excavation survey at the Sentinel plot in March 2022, in Palm Canyon Wash. **(A).** Pupa identified by anatomical comparison under a dissecting microscope by Ken Osborne, Osborne Biological Consulting. **(B).** Pupa collected from the field. **(C).** Presumed Casey's June beetle larval exuvium found during the soil excavation survey.²⁴

²⁴ Photos: Ken Osborne, Osborne Biological Consulting (A), Noelle Ronan, U.S. Fish and Wildlife Service (B, C).

Immature Morphology of Casey's June Beetle

Osborne describes the morphological features of Casey's June beetle eggs, larva, and pupa from detailed microscopic examination in the laboratory of the specimens collected during surveys (description format is consistent with entomological publication standards).

Egg: Eggs are oval shaped, 4.0 mm length and 2.5 mm width. The chorion is thin and fragile to the extent that any mechanical disturbance, such as an attempt to handle with forceps, would rupture the egg.

Larva: A final instar larva we recovered on March 27, 2022, measures 3.7 cm length by 1.0 cm width. The head width is 5.37 mm. The sclerotized head is dark reddish brown with the highly sclerotized mandibles blackish. Thoracic and abdominal segments are weakly sclerotized with the transparent cuticle showing orangish-ivory coloration throughout the body. The eighth abdominal segment (to some extent) and the ninth abdominal segment (greatly) are expanded as compared with the anterior segments, and partially present blackish color (viewed through the transparent cuticle) owing to the dark gut contents. Spiracles are rust color and conspicuous (*ca* 0.5 mm across). The thoracic and abdominal segments are profusely clothed with short (0.1 mm length) rust-colored setae dorsad of the spiracles. Longer rust colored setae (up to 0.7 mm) generally ventrad of the spiracles, also on dorsal thoracic segments, and on legs. Legs (particularly femoral segments) are progressively longer toward the posterior (prothoracic leg length < mesothoracic leg < metathoracic leg). Leg segments are swollen on distal ends, especially for the tibial and tarsal segments, so that these latter appear bulbous in shape. Tarsal segments, especially distally, bear prominent spines and heavy setae. Well-developed mandibular and maxillary palpi are present. Mandibles heavily sclerotized with a distal incisor lobe with concavity on the posterior surface. The maxillary galea (Snodgrass 1935) bears many (*ca* 18) medial sclerotized spines. A Casey's June beetle larva we reared in captivity, and exposed on January 2, 2022, was smaller than those found in the wilds later that year, with a head capsule width of 2.80 mm. This larva was morphologically similar to the final instar described above, having similar swollen tibial and tarsal segments with abundant spines and heavy setae, but had a more whitish body color and the head capsule with a slightly lighter colored reddish-brown. Figure 43 is photographs showing aspects of the larvae (Photos by Ken Osborne and Gregory Ballmer).

June beetle larvae we recovered during our excavations in November and December 2019, in Palm Canyon Wash, which were exposed to feeding tests whereby only organic detritus was accepted, were ultimately reared to maturity and found to be *Cyclocephala melanocephala*. The *Cyclocephala* larvae are morphologically differentiated from Casey's June beetle larvae on the basis that the tarsal segments of *Cyclocephala* terminate into an approximately conical shape, are not swollen, bulbous in shape, and bearing profuse spines and stout setae as with Casey's June beetle larvae. Setation on the dorsal abdominal segments of *Cyclocephala* is sparse, with longer setae as compared with the short, dense setation of dorsal aspects in Casey's June beetle.



Figure 43. Morphological description of Casey's June beetle larvae. (A). Lateral view of final instar collected from Palm Canyon Wash, March 2022. (B). Lateral view of larva (estimated to be third instar) reared from captive female and extracted from rearing medium on January 2, 2022. Note the bulbous tips (tarsal segments) of legs. (C). Anterior view of head of larva (individual in photo B). Missing maxillary palpus and dark spot the on head (right) suggest injury during rearing. (D). Lateral view of head of larva (individual in photo B) showing uninjured site with complete maxillary palpus. Note the bulbous shape of tarsal segments (lower right). (E). Close view of bulbous tarsal segments with many terminal spines for hind leg (center left) and mid leg (center right) of larva (individual in photo B). (F). Posterior view of mouthparts (head) of larva (individual in photo B) anesthetized with CO₂, mandibles spread by pins, showing anatomy of the maxilla. Photos: Ken Osborne and Gregory Ballmer.

Pupa: The pupae recovered in March 2022, measures 2.20 cm length and 0.99 cm width. Due to its size, the proportions of its antennal case, the size and position of its developing compound eyes, and context with three Casey's June beetle larvae (the only scarabaeoid larvae found during this excavation), and its location amidst an area long known for high Casey's June beetle abundance, Osborne concluded the pupa to be that of Casey's June beetle. Consistent with coleopterans, the pupa is exarate (appendages are not fused with the body surface). Abdomen and thorax have a light yellow-tan coloration while head, terminal abdominal segment, and appendages tend to a darker reddish brown-tan color. Given its size and male terminalia, Osborne determined it to be a male Casey's June beetle. Antennae are prominent (again a male trait). This pupa was recovered at such a stage of development that the compound eyes, and to some extent, the antennal lobes have developed to almost black coloration. Spiracles are conspicuous with their lateral position along abdominal segments 1 through 8. Dorsally, the abdominal segments stand in sharp relief, with the posterior edges contrasting with anterior portions of next successional segments giving an aspect serrated texture. The abdomen terminates with a pair of 1.0 mm length, opposing, curved (cremaster) spines. Figure 44 is photographs showing aspects of the pupa (Photos by Ken Osborne and Gregory Ballmer).

Incidental Observations of Predation

From 2016 to 2021, we noted incidental predation observations of Casey's June beetle. Most (95 percent) of the observations were recorded during Sentinel surveys. The greater frequency of Sentinel surveys throughout the beetle's flight season provided more opportunity to document natural history information observed incidentally during the black light surveys. On some evenings we observed more than one predator species, and sometimes more than one individual of those species, hunting and feeding on Casey's June beetle in the same area during the sunset hours.

All but one of the observed predation events were of male Casey's June beetle. We documented 110 predation events on 71 evenings (Table 20). Birds were the most commonly observed predator ($n=102$ predation events, 93 percent). Of the 10 bird species we observed hunting and feeding on Casey's June beetle, northern mockingbirds (*Mimus polyglottos*) were the most frequently seen, followed by greater roadrunner (*Geococcyx californianus*) and lesser nighthawk (*Chordeiles acutipennis*; Table 20). Other species observed hunting, feeding, or scavenging on Casey's June beetle on only one evening include coyote (*Canis latrans*), desert spiny lizard (*Sceloporus magister*), black caterpillar hunter (*Calosoma sayi*), desert stink beetle (*Eleodes* spp.), and sun spider (*Eremobates* spp. [Solfugidae]). Predation and attack of two male and two female Casey's June beetle by cone ants (*Dorymyrmex bicolor*), a native desert ant species, was observed by Michael Wilcox during Rangewide surveys on April 20 and May 4, 2021, along the upper reach of Tahquitz Creek (Wood 2021). One female was alive and being attacked when discovered; the remaining three individuals were already dead when found being attacked by cone ants. Wilcox collected a sample of the ants for identification to species by Doug Yanega, University of California, Riverside, Entomological Research Museum Collections Manager. Only one other possible ant (fire ant [*Solenopsis* spp.]) predation of male Casey's June beetle was documented and photographed by Ken Osborne on May 19, 2017, in the lower reach of Palm Canyon Wash; Osborne also documented a sun spider predating a male Casey's June beetle on the same evening and location.

We observed bats foraging around the black light traps, including a few observations of pallid bats (*Antrozous pallidus*) landing on the ground, and on the sheet around the trap to forage; pallid bats are known to grab prey from the ground (CDFW 2021). Since several bat species that occur in southeastern California forage on beetle species, it is possible they were consuming Casey's June beetle, however we could not confirm that by direct observation.

Although our observations were incidental, we noted some general patterns among years. On survey nights when Casey's June beetle were not captured and not observed flying, either due to poor weather or it was early or late in the Casey's June beetle breeding season, predator species were not congregated in the wash and hunting around the survey site. There also was a general trend of more predation observations early in the Casey's June beetle breeding season when their abundance was higher, with a gradual decline in predation activity as Casey's June beetle numbers waned through the breeding season.

GENETICS

The analysis of the phylogenetic relationship of Casey's June beetle to its sister taxa *Dinacoma marginata* confirmed the taxonomy of Casey's June beetle as a unique species. Evaluation of the population structure of Casey's June beetle from samples in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek indicated there is no substructure, despite the sampling locations being isolated by development. Therefore, there was no evidence of genetic divergence among the three locations sampled within the species' range. Some evidence of inbreeding was found. A thorough description of the results can be found in Rubinoff *et al.* (2020) and Rubinoff and San Jose (2021; Appendix E), which are briefly summarized here.

Of 48 *Dinacoma* samples collected in 2016, Sanger sequencing of the 1480 base pair, mtDNA amplicon was successful for 38 samples. For the ddRAD analyses, 6 *Dinacoma* samples (4 *D. caseyi*; 2 *D. marginata*) were excluded from the dataset due to low quality, resulting in a final dataset containing 42 individuals (Palm Springs: $n=16$, Bautista Canyon: $n=14$, San Felipe Valley: $n=12$). All 11 Casey's June beetle samples collected in 2019 and 2020 were added to the 2016 *Dinacoma* dataset and successfully analyzed for both Sanger sequencing of mtDNA from COI data and the ddRAD genomic data to match the preexisting 2016 samples.

Rubinoff *et al.* (2020) observed relatively low nucleotide and haplotype diversity in the mtDNA data from the COI dataset. A maximum likelihood phylogeny tree²⁵ generated from the COI data divided *Dinacoma* into three distinct clades²⁶: one representing Casey's June beetle in Palm Springs, and two representing *D. marginata* from the San Felipe Valley and Bautista Canyon (see Figure 2 in Rubinoff *et al.* 2020). The TCS haplotype network²⁷ constructed for the

²⁵ A phylogenetic tree is a branching tree diagram that represents a hypothesis about how organisms in the tree are related to one another. The branch length of the tree describes the proportional or relative evolutionary distance of a group of organisms; the length of the branch reflects the changes in a DNA sequence in a lineage (Campbell and Reese 2005).

²⁶ Within a cladogram or phylogenetic tree, a clade is a branch that includes a group of species with a common ancestor and all its descendants (Campbell and Reese 2005).

²⁷ A haplotype network is used for analyzing and visualizing the relationships among DNA sequences within a population or species. The TCS method (Templeton, Crandall, and Sing; often referred to as statistical parsimony) is based on a model of evolution of the genetic characters measured on each individual that can be applied to DNA sequences (Paradis 2018).

mitochondrial data also supports a clear separation of Casey's June beetle and the two distinct clades of *D. marginata* indicating the isolation of these populations. Measures of observed and expected heterozygosity and inbreeding from the ddRAD dataset were relatively uniform across populations. The maximum likelihood phylogeny generated by RAXML, used to assess population structure, also clearly distinguished the three *Dinacoma* clades that were identified with COI data. The clades appear to be evolutionarily nearly equidistant from each other. There was no substructure in the mitochondrial haplotype network and in the hierarchical analyses for the genomic data. Estimates of inbreeding were higher for *D. marginata* from Bautista Canyon, but lower in the Casey's June beetle population and the San Felipe Valley *D. marginata* population.

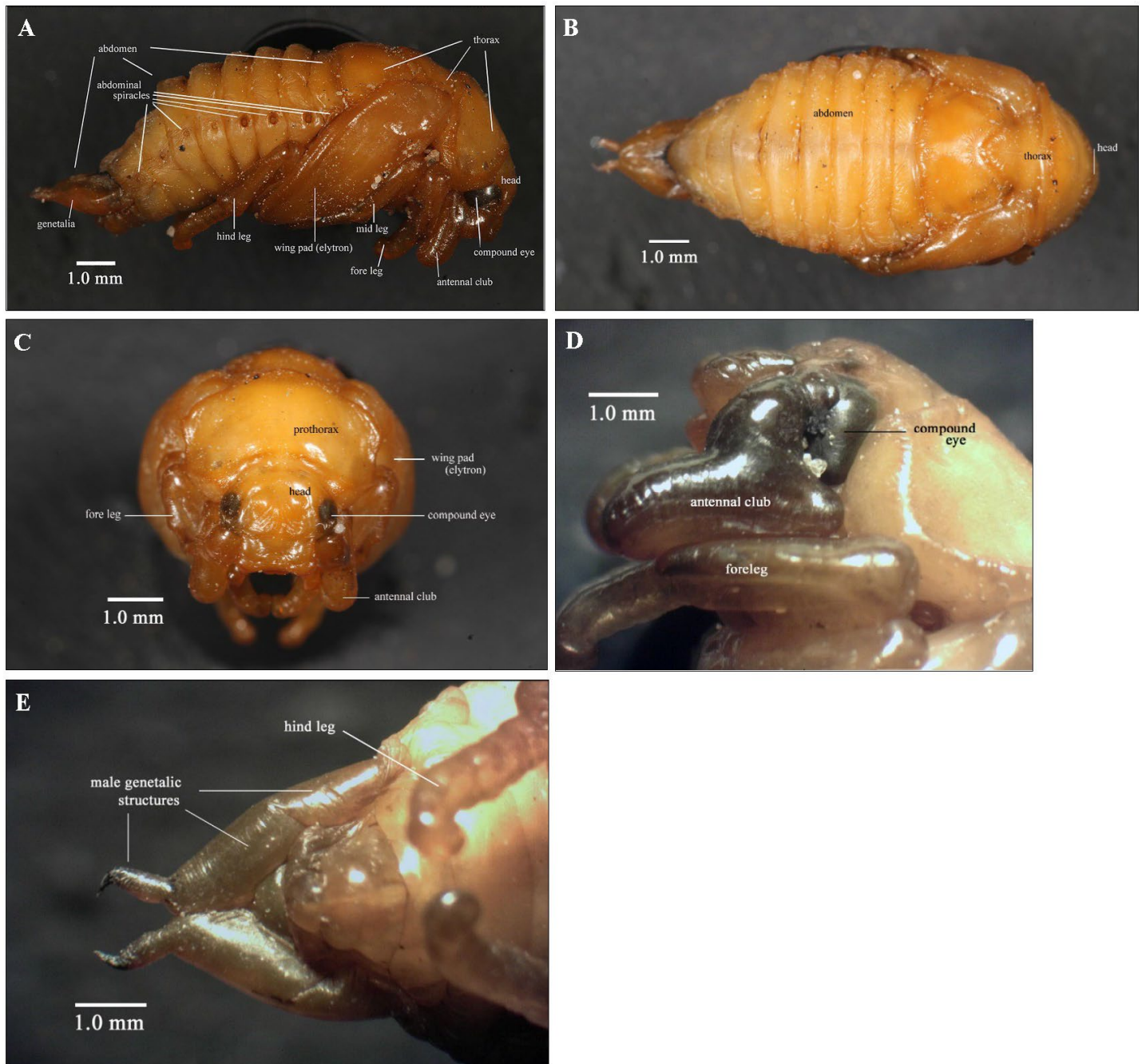


Figure 44. Morphological description of male Casey's June beetle pupa collected from Palm Canyon Wash, March 2022. (A). Lateral view of pupal male Casey's June beetle. (B). Dorsal view of pupal male Casey's June beetle. (C). Anterior view of pupal male Casey's June beetle. (D). Close, lateral view of head (head at top of image with dorsal aspect to the right) of pupal male Casey's June beetle. Note the large (diagnostic) antennal case enclosing the antennal clubs which are proportionally larger for Casey's June beetle than for other Scarab species present on our study sites. (E). Close ventral view of terminalia (genital structures) of pupal male Casey's June beetle. Photos: Ken Osborne and Gregory Ballmer.

Table 20. Incidental predation observations of Casey's June beetle during surveys from 2016–2021. The total number of observation events by species and year are summarized.

		Observation Events ¹						
Common Name	Scientific Name	2016	2017	2018	2019	2020	2021	Total
Birds								
American kestrel	<i>Falco sparverius</i>	1			1			2
Black phoebe	<i>Sayornis nigricans</i>				1	3		4
California thrasher	<i>Toxostoma redivivum</i>	1						1
Common raven	<i>Corvus corax</i>	2	1		3			6
Greater roadrunner	<i>Geococcyx californianus</i>	16						16
Lesser nighthawk	<i>Chordeiles acutipennis</i>	3	3	2	2			10
Northern mockingbird	<i>Mimus polyglottos</i>	22	17		10	4		53
Say’s phoebe	<i>Sayornis saya</i>	5				2		7
Scott’s oriole	<i>Icterus parisorum</i>		1					1
Western bluebird	<i>Sialia mexicana</i>	2						2
Other ²								
Black caterpillar hunter	<i>Calosoma sayi</i>		1					1
Cone ant	<i>Dorymyrmex bicolor</i>						2	2
Fire ant	<i>Solenopsis</i> spp.		1					1
Coyote	<i>Canis latrans</i>	1						1
Desert spiny lizard	<i>Sceloporus magister</i>					1		1
Desert stink beetle	<i>Eleodes</i> spp.				1			1
Sun spider	<i>Eremobates</i> spp.		1					1

¹ An observation event is defined as an incidental observation of one or more individuals of a predator species hunting and feeding on Casey's June beetle on a single evening. It is not the total number of individuals of the species predating Casey's June beetle.

² Desert stink beetles are saprophytic (feed on dead organisms or decaying organic material), therefore this observation was of a beetle feeding off an already dead Casey's June beetle rather than a predation event. Cone ants and fire ants are predators and scavengers and therefore may not have predated these beetles. However, one female was alive upon discovery of being attacked by cone ants and therefore this was presumably a predation event.

DISCUSSION

ABUNDANCE AND DISTRIBUTION

This study is the first to examine Casey's June beetle ecology and population dynamics. The relative abundance estimates represent an initial modeling of the capture results and provides an estimate of Casey's June beetle relative abundance and inter-annual fluctuations during a 6-year time span. Deriving a statistically robust estimate of abundance or a rangewide population estimate is challenging, given Casey's June beetle spatially and temporally continuous and dynamic population. The abundance estimates presented here are from a short time series. They should be interpreted cautiously and not considered a statistically or biologically robust estimate of true abundance, due to the many assumptions and caveats underlying the analyses (see Harju 2021).

Insect population abundance is notoriously difficult to estimate, and inter-annual fluctuation in insect abundance is the norm (Didham *et al.* 2020). We have mentioned assumptions and caveats underlying the data throughout this report with the intent of framing the survey results in the appropriate context. Drawing inference of population trends (declines, increases, stable) from insect abundance sampling is challenging and requires careful interpretation. Didham *et al.* (2020) provide an informative overview of these challenges, which include establishing the historical baseline, representativeness of site selection, robustness of time series trend estimation, detection bias effects, and potential artifacts of density dependence, phenological shifts and scale dependence.

We saw wide inter-annual fluctuation in the capture data, and variation in scale and covariates influencing Casey's June beetle population dynamics. Our surveys were designed to examine Casey's June beetle abundance and distribution, without the benefit of baseline data. Without historical baseline knowledge and lack of sufficient long-term data for trends, we make no inferences about population estimation or trends at this time. We assume no specific historical reference for Casey's June beetle abundance aside from the general presumption that the current population is lower than historical numbers, based solely on the extensive (97 percent) loss of habitat due to development. Given that sampling artifacts in trend estimation decrease in time series with more than 10 time-points, and that statistical power increases in time series with more than 15 time-points (Didham *et al.* 2020), our 5-year dataset for Palm Canyon Wash and 2-year dataset for Tahquitz Creek are still in progress. Additional surveys are needed to develop the baseline and the long-term time series data needed to understand population trends.

We placed the Rangewide survey plots systematically throughout the species range to achieve representativeness for estimates of relative abundance and distribution. In years when we surveyed a subsample of the Rangewide traps, the sample was spatially representative and encompassed the range of capture activities (i.e., low to high) we had already documented. We non-randomly sampled at higher activity areas only to facilitate examining select, specific questions (e.g., soil surveys). Random and/or systematically representative sampling should continue for future study directions. Surveys in unoccupied or low abundance sites should not be overlooked. Information about currently unoccupied sites in suitable habitat is an important component to understanding population dynamics, particularly in the context of conservation where there is opportunity to study future recolonization of unoccupied sites, recolonization of

sites after habitat restoration, and documenting shifting ranges due to climate change (Didham *et al.* 2020).

Capture and Recapture Patterns

We documented delayed first emergence in 2019 and 2020 by approximately 20 days during Sentinel surveys in Palm Canyon Wash. The delay in 2019 may be an effect from channel scour and heavy sediment deposition in the wash from the 2019 flood event that occurred in February prior to the beetle's breeding season. The sediment removal and levee repair to maintain flood control capacity had not yet occurred. However, the sediment removal and levee repair disturbance did occur prior to the 2020 Casey's June beetle breeding season, which may have influenced emergence timing in 2020. However, this relationship is unclear because sediment removal and levee repair did not occur within the Sentinel trap effective survey area and sediment removal amounts in the general vicinity were no more than 1 ft in depth. Similarly, levee repair impact acres were relatively small and spatially removed from the Sentinel trap area.

The capture pattern in the Tahquitz Creek focal area during Rangewide surveys in 2021 and 2022 differed from the Palm Canyon Wash focal area, with an overall lower relative activity and less annual variation. We suspect the activity difference between Palm Canyon Wash and Tahquitz Creek is due to the greater habitat disturbance and disrupted hydrology in Tahquitz Creek that contrasts with Palm Canyon Wash which has, comparatively, retained more of its historical hydrological function and the habitat is less disturbed.

We found consistently low recapture rates across all trap locations and study years. Given the consistency of the recapture rate, we presume that for future surveys conducted using the same trap equipment but without mark-recapture methods, total captures of Casey's June beetles would include approximately 5 percent recaptured individuals.

Although recapture observations are a small sample, the more frequent Sentinel surveys provided opportunities to observe behaviors and additional natural history details. We hypothesize that Casey's June beetles may remain close to or associated with an area, venturing near or far during nightly forays, and returning to the same area to burrow in soils until emerging again the following evening. This behavior may be a function of density-dependence. For an insect with a short adult life span, more frequent, consecutive survey nights can provide finer scale data, increase the probability of recaptures, and shed light on activity and behavior patterns.

VEGETATION COMMUNITIES

Our findings provide some clarification on the association of Casey's June beetle with vegetation and, for the hydrologically active portion of the channel, the subsurface root environment. The well-known phytophagous habit of another root-feeding (Evans and Hogue 2006) June beetle, *Polyphylla*, a melolonthine genus closely related to *Dinacoma*, supported the hypothesis, if not a general expectation, that Casey's June beetle would be feeding only on plant roots. However, our finding that in the hydrologically active channel Casey's June beetle larvae are saprophytic on organic debris, and the general association of the beetles with areas of heavier woody tree and shrub cover within the wash, indicated that these woody plants might be relevant on the basis of mechanical or structural function, as snags accumulating the wood and other organic flotsam

during flood events. The plant species (other than their size and efficiency in trapping driftwood) might be immaterial to the beetle's biology.

The large population of *Dinacoma marginata* (another *Dinacoma* presumably with similar biology to Casey's June beetle) within the Bautista Canyon wash system near Hemet, California, has soil substrates similar to those in Palm Canyon Wash (Osborne, pers. obs.). However, the vegetative community of Bautista Canyon is completely different from that of Palm Canyon Wash (Osborne, pers. obs.). Instead of *Chilopsis*, one finds *Populus* (Freemont's cottonwood) and *Baccharis* (mulefat), two examples of woody riparian species that can similarly trap organic debris during flood events. Furthermore, *Populus*, *Baccharis*, *Salix* (willow), *Prosopis* (mesquite), *Acacia* (catclaw), and (in lower elevations) *Chilopsis* are all dominant plants in habitats supporting *Dinacoma sanfelipe* (Gillett *et al.* 2020., Osborne, pers. obs.). Such plants (and many other species) may function to trap organic debris in floods, suggesting that specific vegetation species may not be necessary to *Dinacoma* existence, so long as the organic materials are deposited, one way or another, within the wash sediments. This hypothesis is also supported by our finding that Casey's June beetle is associated with both native and non-native vegetation and there is no significant association with any specific plant species.

Our data does not provide information on subsurface conditions and larval feeding ecology within the upland terrace habitat which may differ according to the reduced hydrological functions of scour and sediment and debris deposition. Given the reduced deposition of organic debris, it is possible that plant roots serve as food sources for Casey's June beetle larvae in the upland terraces.

HYDROLOGY

The overall gradational increase in Casey's June beetle numbers across the upstream to downstream continuum of the Palm Canyon Wash system came as some surprise given the apparent uniformity of vegetation along with the consistent sandy, gravelly soils seen throughout the wash. This variability in Casey's June beetle numbers makes more sense considering erosional and depositional dynamics of fluvial channels, along with our revelation of Casey's June beetle larval development occurring on organic debris transported at depth within the alluvial sediment of the wash. Erosional (scour) conditions are prevalent over upstream reaches of drainage systems such as Palm Canyon. Within middle reaches, erosion occurs during periods of high-water flow while deposition of alluvium occurs during periods of lower flow so that both these processes occur during each water flow event as water volumes crest and wane. Sediment deposition occurs in the middle and lower portion of drainage systems, resulting in the need for periodic sediment removal to maintain flood control capacity and prevent flooding in the surrounding communities.

Within alluvial systems typical of southwestern desert regions of North America, drainages terminate over alluvial fans with flood-borne sediments and organic debris such as wood, distributed across wide fan-shaped depositional areas by shifting channels (Alluvial Fan Task Force 2010). The Palm Canyon drainage, to which the Tahquitz Creek drainage makes some contribution, drains a watershed encompassing an area of more than 100 square miles (259 square kilometers; Corps 1971, District 1982) and has been the source for a large alluvial fan extending over the surrounding Palm Springs area, approximately 10 square miles (25 square

kilometers). The alluvial fan system and the organic debris on which the Casey's June beetle population relies has been renewed by these processes over evolutionary and ecological time scales. These natural processes have been arrested in the past century with the progressing development of Palm Springs, and the associated flood control infrastructure ranging from paved roads, gutters, culverts, concrete channels, and the prominent flood control levees constructed in the 1950's along Palm Canyon Wash and later, Tahquitz Creek. Active deposition is now confined to these leveed washes while other remaining undeveloped portions of the alluvial fan are rendered dynamically static.

It remains unclear whether Casey's June beetle can persist in upland terrace floodplains that are separated from hydrological processes. The Smoke Tree Ranch area, where we found Casey's June beetle numbers to be consistently low, is one of these hydrologically static alluvial benches; separated by the levee and cut off from future replenishment of alluvial materials with their associated organic debris. Without historical baseline knowledge of abundance and inter-annual fluctuations, and the current baseline still under development, we can only speculate based on information gained from this study as to how Casey's June beetle may or may not persist. One expectation would be that Casey's June beetle in this area should eventually, over decades, decline as the non-replenished buried woody detritus larval food resource is consumed. Similarly, the upstream portions of Palm Canyon Wash show lower Casey's June beetle numbers, which may be due to the diminished rate of sediment deposition and organic resources for the beetle. It is interesting to consider that the fluvial dynamics of this constrained wash may also have been altered (i.e., depositional tendencies diminished, and scour increased), after the surrounding urban development and construction of the levees, such that even the Casey's June beetle population in the upstream area may be a declining relictual shadow of what it may have once been. However, our data does not provide information on larval food habits in upland terraces therefore, we speculate that plant roots may function as a food source for Casey's June beetle larva in upland terraces, similar to other root-feeding June beetles, allowing for the species persistence throughout the wash system.

The highest observed Casey's June beetle numbers were in the lowest reaches of the study area which correspond to the portion of the wash that now essentially receives much of the sediment from the headwaters and Palm Canyon drainage area. Alternatively, Casey's June beetle may persist only in the active portion of the Palm Canyon Wash and Tahquitz Creek channels that remain connected to hydrological processes, with a lower population abundance that represents a new normal. Continuing surveys to understand population abundance and trends, and connectivity will be that much more pivotal for Casey's June beetle given these uncertainties.

BIOLOGY AND ECOLOGY

The results of field observations and small-scale, pilot laboratory investigations enable us to provide an overview of Casey's June beetle biology. Mating at the surface is brief, lasting a few minutes at most. Immediately after mating, female Casey's June beetles burrow back into the soil substrate (escaping being the predatory meal at the surface). They burrow rapidly downward, achieving depths of more than a meter or two (3 to 6 ft) within two or three days. At depths below 8 or 12 inches (20 or 30 cm), soils become somewhat damp and cohesive. Eggs are deposited singly or in small numbers, likely in context, if not direct contact, with decaying organic materials such as rotten wood. The female continues burrowing through organic

substrates laying eggs along the way, as due to the fragility of the eggs, females loitering in a small area to lay more eggs are likely to mechanically destroy these eggs. Based on limited data, we suspect that approximately a dozen eggs are produced (fecundity estimated after dissection of one female Casey's June beetle), after which the female dies.

Obtaining larvae derived from captive gravid Casey's June beetle females has been a significant event in the course of our studies. It allowed unequivocal association of larval morphology as known Casey's June beetle, confirmed its fundamental biology, and informed more broadly on its ecology. This understanding of biology and ecology in turn provides critical direction for habitat management and minimizing flood control and other disturbance-related impacts through science-driven mitigation and conservation. The larva that was derived from the captive gravid female morphologically matched (size, overall body proportions, bulbous tarsal segments with profuse spines) the larvae we subsequently recovered with our excavation in March 2022, thus allowing the determination of the excavated larvae to be Casey's June beetle. We have observed wild (putative) final instar larvae (March 2022) and reared larvae (extracted January 2022) apparently two instars removed from final as estimated by Dyer's Law (Chapman 1982, Triplehorn and Johnson 2005) and so these may not apply to earlier instar larvae (i.e., first and second instars), which remain undocumented.

Larval development may transpire over the course of one or two years (perhaps longer depending upon food availability). Timing of the captive larva suggested its development to be insufficiently advanced to complete its development in time for maturity after only one year. Upon full larval development, the larvae leave their organic nursery and ascend through the soil substrates, ultimately up to a depth of approximately 12 inches (30 cm) below the surface. The timing of initiation of this larval ascension from depths may immediately follow finalization of development, when there is no need to continue feeding, but since there are likely no temperature or moisture cues at depth to indicate the time of year or season, the question remains as to whether these larvae immediately pupate below the surface or remain as larvae awaiting near the surface for seasonal cues to initiate pupation (just preceding the flight season). Our small sample of three individual (larval and pupal) observations from March 27, 2022, does not allow an estimate of variation with respect to pupal depth, however, the abundance of pupal exuvial material encountered at this approximate depth strongly indicates this to be the typical pupal depth. At this shallow depth, pupae awaiting eclosion to the adult could easily detect diel temperature fluctuations (dampened with increasing soil depth) which provide cues as to the correct time of emergence. Observations of adults found at the soil surface interface in the hour before their flight began, suggested that adults may also emerge from the pupa, and await concealed within the soil surface for some time before initiating flight (or female calling) at the appropriate hour. On occasions, females began calling and successfully mating, within loose sand while remaining the whole time just below the surface, the male intromittent organ being of such length allowing it to probe through the sand and reach the female.

Based on our sample of excavations of wash substrates in the hydrologically active portion of the channel, it appears that rotten wood and other organic materials represent a very small proportion of the sediments. They were not ubiquitous. Considering the observations of abundant driftwood caught amidst the branches and roots of large trees and shrubs such as *Chilopsis*, we hypothesize that these structural features within the desert wash environment are important to Casey's June beetle population densities, as well as for other Scarabaeidae (with which Casey's June beetle

likely compete) utilizing organic materials within the alluvial setting. Since we did not survey the subsurface environment in the upland terrace floodplain for larvae, we are uncertain whether the relationship of Casey's June beetle larvae with buried woody debris and detritus is similar to that of the hydrologically active channel and cannot rule out larvae feeding on plant roots.

Male Observations

The fading of the Sharpie® marks observed over the course of a few days during the 2016 small-scale laboratory experiment was unsurprising given the beetle's nature of burrowing through sand. The result that marks were indistinct and difficult to read by day 3 helps verify the mark-recapture results within this time period. It also informs the capture pattern data from the Sentinel surveys analyzed by Harju (2021) and provides additional context. From those analyses (see *Results, Capture Patterns [Sentinel Survey]*) only 1.2 percent of beetles were recaptured three days after original capture and marking and no beetles were recaptured more than three days after initial marking. This result may be confounded based on our inability to reliably detect and read marks after day 3; even if those marked beetles had been recaptured after day 3, we would not have been able to reliably identify them as recaptures. However, it does support meeting the assumption that the marks remained intact during the survey interval for the Rangewide surveys, and in part, for the Sentinel surveys. We assumed that Casey's June beetles did not survive between Rangewide surveys that were conducted every 2 weeks, therefore mark retention beyond one day is not a consideration. The marks only needed to remain intact for that one evening. For the Sentinel surveys which were conducted every 1–3 days, depending on year, it's important to understand the limitations of mark retention using Sharpies®. When using this marking method, future surveys should be designed to meet the assumption of loss of marks by day 3.

The finding of no discernable effect from the marks on beetle longevity supports previously reported information from marking techniques for insects and meets the assumption of not adversely affecting beetles for mark-recapture (see *Methods, Mark-Recapture*). Importantly, not only for the study's data but for a narrow endemic endangered species, is to document no obvious negative impact on individual Casey's June beetles from marking for this study.

Body Size and Emergence Holes

The distribution of size classes for emergence holes and adult beetle measurements appears to be closely matched, indicating that individual emerging Casey's June beetles, when coming through a firm soil substrate, leave emergence holes nearly identical with their own dimensions. The size distribution for emergence holes appears to be influenced by the corresponding larger size of the females, which few specimens are collected due to the relative, and very substantial, difficulty of their sampling.

Any cursory examination of pinned series of *Dinacoma* males from museum reference collection reveals that Casey's June beetles are generally smaller than those of *D. marginata* or *D. sanfelipensis* and also, that the females for all *Dinacoma* are generally larger than the males, sometimes considerably so, as with Casey's June beetle.

We found emergence holes to be plentiful and readily identifiable by the trained observer where compacted or cemented soils were present. Within the compacted soil areas, the distribution of emergence holes was indicative of general Casey's June beetle occurrence and a relative index of activity. While emergence hole surveys can complement black light surveys or be useful during initial habitat assessments, their viability is limited by the spatially and temporally inconsistent prevalence of compacted soils. Further, the method's reliability is crucially dependent on knowledgeable observers able to identify Casey's June beetle emergence holes, which is acquired with field time and experience.

Soil Excavation Surveys and Larvae

Within the hydrologically active channel where we excavated soil to a depth up to approximately 9 ft (2.7 m), we found no Casey's June beetles. These results may be an artifact of the small sample size and spatially restricted survey area, such that Casey's June beetles were simply not in the locations surveyed. Another consideration is that we were not able to survey the upper soil strata at some survey sites, therefore we cannot rule out the possibility that larvae occupied these upper layers. Alternatively, despite the limited sample, our results are suggestive that Casey's June beetles are capable of occurring at greater depths than previously thought. Given the early winter timing of the surveys in November and December, and the typical life cycle of other scarab beetles, we expect that Casey's June beetle larvae would have been detectable (i.e., of sufficient size), similar to the *Cyclocephala* larvae we were able to find within this depth range. Further, the depths and speed at which female Casey's June beetles housed in captivity burrowed through soil indicates the species' adeptness at moving through soil. A contributing factor also influencing larval depth is the warmer and less stable soil temperatures we documented in the upper strata in early summer, which are likely not suitable for larval development when temperatures are high, probably above some prohibitive or detrimental threshold. We suspect that the soil temperatures, as well as the moisture gradient, are more favorable for Casey's June beetle larval growth and development at greater depths. Occurring at depth during the underground portion of their life cycle would account for the population's ability to withstand flood events, an evolutionarily adaptive trait for a species reliant on a dry wash hydrological system with sporadic, and sometimes large stream flows.

We caution against drawing inferences to larval ecology in the upland terrace floodplain where we did not survey. We also did not excavate soil around living trees and shrubs *in situ* such as desert willow and smoke tree and therefore data on the association of Casey's June beetle larvae feeding on plant roots is lacking.

Genetics and Connectivity

We confirmed the previously unknown taxonomy of Casey's June beetle as a unique species. Although differentiation within the Casey's June beetle population was considered possible due to isolation from habitat loss and fragmentation, poor dispersal capability of the flightless females, and the previously unknown flight capabilities of the males, there was no population substructure. The lack of differentiation may be the result of relatively recent isolation or male dispersal may be sufficient to maintain gene flow. Some evidence of inbreeding was also observed, as individuals are more closely related than would be expected based on random

mating, however inbreeding was also found in the sister taxa *Dinacoma* and therefore may be typical of a narrow endemic insect, reflecting geographical isolation and small population size.

Male Casey's June beetles had been documented to traverse urban environments because they are attracted to artificial light sources (e.g., streetlights; Service 2011). We documented a maximum flight distance of 2,543 ft (775 m) within a single evening, which are the first flight distances recorded for Casey's June beetle. The flight distance data provides insight into mechanisms of genetic exchange through male travel, which may occur across non-habitat (i.e., developed areas), thus providing indications of functional habitat connectivity. However, developed areas are likely population sinks. In the absence of habitat connectivity, close proximity to suitable habitat is likely required within the average distance traveled by a male per evening. Dispersal of both sexes also potentially occurs during flood events within hydrologically connected habitats allowing for gene flow and colonization of unoccupied habitat (Service 2025). Establishing habitat connectivity can ensure long-term genetic exchange within the Casey's June beetle population (Rubinoff *et al.* 2020).

Incidental Observations

Casey's June beetle life history and ecology is not well understood or studied. Much of its basic ecology is undocumented or presumed based on similar species. We found the incidental observations to be revealing and fascinating, and they spurred ideas about Casey's June beetle's niche in this dynamic ecological system, as well as concepts for future research and avenues for conservation and management.

Among the fascinating were our observations of Casey's June beetle predation. The list of documented predation events from incidental observations does not quite convey the magnitude of activity. During the height of the Casey's June beetle flight season, and at locations with higher beetle numbers, one might simultaneously observe several bird species and individuals loitering and hunting along the wash. At times, this could appear as a loose, mixed species flock, with birds hunting from perches, flying near or just above the ground, or landing on the ground. For example, during the peak of the Casey's June beetle flight season, amidst greater roadrunners hunting the beetles, we frequently observed small groups of lesser nighthawks flying inches above the wash and landing on the ground hunting, swarming the area as the beetles emerged in the evening. Meanwhile, passerines such as northern mockingbirds, western bluebirds, and phoebes could be seen plucking the beetles from the ground and pounding them against branches or a hard surface before eating them. An American kestrel could fairly reliably be seen hunting from the same perch near the Sentinel trap around sunset to hunt the emerging beetles.

Some bird species rely on abundant insect prey. For example, nighthawks and other nightjars may selectively target Coleoptera (beetles) due to their larger size and higher energy content (Knight *et al.* 2018), which is consistent with our observations of lesser nighthawks apparently capitalizing on the seasonal abundance of Casey's June beetle. The spring emergence of Casey's June beetle coincides with the energetically demanding seasonal timing of migration and early breeding for many bird species. These observations suggest that Casey's June beetle is an integral part of the desert wash ecosystem and inspired the fitting analogy of Casey's June beetles as the "salmon of the desert" by Osborne during our discussions.

The predation observations lend themselves to hypothesize that the mass emergence of Casey's June beetle during the breeding season demonstrates reproductive synchrony, an adaptive anti-predator strategy (Ims 1990, Yu 2010–2011). Reproductive synchrony is seen in a variety of organisms and is considered to function by means of predator satiation, predator swamping, group defense, and predator avoidance (Yu 2010–2011). Although Casey's June beetle mass emergence undoubtedly serves multiple functions to ensure successful reproduction, it's conceivable that reproductive synchrony is a beneficial tactic for Casey's June beetles, which are swamped by a myriad of predator species and individuals during their breeding season.

Hydrological processes in Palm Canyon Wash intermittently bring debris and sediment into the system from the drainage headwaters, in accordance with rainfall, snowmelt from the San Jacinto and Santa Rosa Mountains, and runoff intensity. During the study period, there was a layer of cemented sediment that was more than a foot thick in some areas resulting from headwater deposition after a fire. We incidentally observed male and female Casey's June beetles burrowing and digging through this cemented soil layer on several occasions. Although we had seen how adept Casey's June beetles were at burrowing through softer sandy soils, we were surprised by their digging capabilities through the cemented soil. For a crude hardness test, we attempted to hammer a nail through the cemented soil; the nail bent and made little headway into the soil layer. The beetles' digging while turning in a circular motion proved to be a more effective technique. The females we found in cemented soil areas burrowed back through the same hole they emerged from rather than digging a new one from the surface.

The incidental observations of ants predating or scavenging Casey's June beetles was previously undocumented and provide insight into another potential anthropogenic habitat disturbance effect on Casey's June beetle. Cone ants are a desert species found in Central and South America and the southwestern United States. They were documented to predate, and possibly also scavenge, Casey's June beetles in the upper reach of Tahquitz Creek which is in a residential area of Palm Springs. This ant species may be able to unnaturally thrive in association with the irrigated landscaping and cover adjacent to the sidewalk in this area (Wilcox 2021, pers. comm.).

CONSERVATION

Seasonal Restriction for Species Protection

The larvae and pupae studies, adult male longevity observations, and the Sentinel surveys, support a seasonal restriction on soil disturbance activities during the breeding season, which is currently April 1–May 31. We documented a longer breeding season than the current seasonal restriction window encompasses. Larvae are easily damaged with soil disturbance, as we experienced during the soil surveys, despite careful and deliberate methods intended to accurately document the conditions larvae were found while avoiding or minimizing mortalities. This informs our understanding of larvae susceptibility to soil disturbance. Based on a late-March soil survey, we found that Casey's June beetle larvae and pupae occur in the upper soil strata; immature stages likely begin to move toward the surface earlier in March. Immature stages near the surface awaiting eclosion to adult and adult males burrowed just under the soil surface during the day over their short adult life span, are susceptible to disturbance through crushing. Further, the Sentinel survey data shows a longer flight season than previously reported. We recommend that the seasonal restriction window be extended to better protect Casey's June

beetle during the vulnerable breeding season. The data reasonably supports a seasonal restriction window of early March through June. A seasonal restriction from March 1 to June 30 is more protective compared to the current seasonal restriction dates. A more moderate seasonal restriction window of March 15 to June 15 can also be considered, if needed.

Effective Survey Area

The Service's Casey's June beetle presence/absence survey guidelines are based on an assumed attraction distance to 15-watt black lights to be a 328-ft (100-m) radius, according to the current understanding of beetle attraction to black lights at the time. We tested this assumption and found a lower effective survey area of a 254-ft (77-m) radius, which provides statistical support for a more accurate effective survey area around the 15-watt standardized black light traps typically used to survey for Casey's June beetle. Revising the Service's survey protocol to include this tested effective survey and capture distance around black light traps would improve detection probability, an important consideration when assessing a proposed development's potential to impact Casey's June beetle habitat.

Prospects for Population Augmentation

Prospects of successful captive propagation of Casey's June beetle appear remote for the time being. Larval development on organic debris would initially suggest that Casey's June beetle could easily be reared on ordinary compost with rotting wood. However, suspected low fecundity of females indicates that many females would be required to acquire many offspring in the rearing. The fragility of eggs and the rapidity with which females burrow suggests that the females could easily destroy their own eggs if confined in too small a container. In two trials given a medium of 0.57 square feet (0.053 square meter) volume, we obtained only one Casey's June beetle larva. Our preliminary results suggest that a very large container may be required, such as a metal trash dumpster. The objective of such rearing is likely to be the production of gravid females which may be easily mated in the wild before release on an intended site, since release of males is not likely to have any consequential impact on establishment or population enhancement and harvesting of larvae would necessitate their being insinuated into the field at depth and associated with their appropriate food resources. Even if successful in obtaining many offspring within a large container, there comes the question of how to harvest the resulting female beetles as they emerge, since they don't fly or make themselves readily apparent.

We anticipate that future Casey's June beetle reintroduction or augmentation efforts may simply become an exercise of field collecting gravid females (just after mating in the wild) from population sites with high densities and immediately transporting them to be released at the soil surface on a recipient site (i.e., translocation). The sparsity of old wood and organic materials within typical wash sediments informs that simply releasing gravid Casey's June beetle females at random may generate poor results, such that females would be unlikely to find satisfactory substrates for eggs and larvae. Preparation of recipient sites over which females would be released, with targeted burial of rotting wood, compost, and other organic materials (perhaps allowed to age within these soils for some years), to a depth of one to two meters may prime the recipient sites for successful Casey's June beetle introduction. Such introductions should be undertaken on depositional environments within wash systems. Given the apparent low fecundity of females, release of many would be appropriate.

Habitat Management and Conservation Measures

Information from this study has been valuable for understanding habitat management and conservation measures that would benefit the population. Related to augmentation efforts are measures to restore or enhance portions of Casey's June beetle habitat showing low numbers or densities, perhaps due to past impacts. For example, the mid-to-lower reach in Palm Canyon Wash in the vicinity of traps 7 and 8 showed a gap in Casey's June beetle relative abundance. This area has been lacking substantial or large individual *Chilopsis* plants and other tree and shrub species are scarce. As habitat enhancement, we suggest planting rooted cuttings of *Chilopsis* in areas of low Casey's June beetle abundance, allowing them to grow to mature size, and avoiding them, to the extent practicable, during future flood control sediment removal activities.

Maintaining flood control capacity in Palm Canyon Wash is necessary for the Palm Springs community, a continuing effort since the levee was constructed in the 1950s. It exemplifies the common scenario of seeking species conservation amidst human development. Following sediment removal and levee repair in Palm Canyon Wash from the 2019 flood event, the Service consulted with the Corps and, as the applicant, the District, to offset impacts to Casey's June beetle (Service 2023). The Service (Ronan) coordinated with the agencies to implement Casey's June beetle avoidance, minimization, and offsetting measures based in part on information learned during this study. Along with avoiding the Casey's June beetle emergence and breeding season during an extended seasonal restriction window (see *Seasonal Restriction for Species Protection*), there were new measures that included 1) avoidance of (preserving in place) mature desert willow and smoke tree (typically over 6 ft [1.8 m] in height); and 2) salvage and burial of woody vegetation that could not be avoided to serve as a food source for immature Casey's June beetles. These measures were beneficial because direct impacts (e.g., mortality from crushing) to Casey's June beetles during their breeding season were avoided with the extended seasonal restriction, preservation of small, scattered areas of mature desert willow and smoke tree maintained patches of quality habitat upon which new plants could regenerate around, and buried salvaged woody debris (buried approximately 3–7 ft [1–2 m] deep) seeded the sediment removal and levee repair area with substantial organic substrates serving as nursery grounds for immature beetles. Importantly, the vegetation burial occurred in multiple locations throughout the sediment removal and levee repair area so that the subsurface food source was not clumped in one area. Additionally, the burial site coordinates were recorded and mapped, which will be extremely informative for future population monitoring. Also, of relevance to Casey's June beetle population dynamics is that sediment removal to maintain flood control capacity in Palm Canyon Wash has occurred periodically, about every 5–7 years, depending on rainfall, and within the same footprint, thus impacts are spaced over time and spatially constrained, allowing for areas to regenerate in the interim and other areas to remain undisturbed. The sediment removal does not occur throughout the entire Palm Canyon Wash but rather in the mid and lower reaches only. Future flood control maintenance activities will include a suite of offsetting measures, commensurate with the degree of disturbance, such as those described above (Service 2023).

The successful implementation of the measures discussed here serves as a model for offsetting impacts from soil disturbance activities, and we consider them to be recommended conservation measures beneficial to the Casey's June beetle population, particularly in the context of yet-unknown impacts from climate change. While repeated disturbance in Palm Canyon Wash

through time has likely suppressed population abundance, maintaining it at an unknown threshold, the population has persisted. The abundance estimates derived from the relatively short 6-year span of the data is a snapshot of abundance within a fluctuating low to some upper threshold continuum. Information from this study should support beneficial actions such as habitat enhancement and population augmentation, to ensure future persistence of the species.

Over the duration of the study, we observed an increase of homeless encampments in Casey's June beetle habitat, particularly in the downstream reach in Palm Canyon Wash near the Gene Autry Trail bridge. The encampment near the bridge had gradually grown, along with obvious amounts of trash, and affected survey efforts from 2020 to 2022, due to equipment theft and safety concerns for surveyors. The homeless encampment occurred²⁸ where the highest estimated abundance and capture activity of Casey's June beetle was documented anywhere within its limited range. Homeless encampments such as this increase the chance of direct habitat and soil disturbance, which can affect individual Casey's June beetle survival during the breeding season from March, when immature stages are near the surface, through June, when adults are breeding and females laying eggs. There is also the potential indirect effect from pollution due to trash. Results from this study support the previous recommendations from the Service for landowners and conservation easement land managers to reduce the impacts from homeless encampments on Casey's June beetle habitat through continued management.

Suitable habitat for Casey's June beetle is limited and dwindling, as are opportunities for conservation through habitat acquisition. Along with funding recovery actions, such as long-term monitoring and translocation, species conservation will require unconventional approaches to preserve and enhance habitat and maximize remaining options. For example, to offset impacts from a development project, non-turf areas within the operative Tahquitz Creek golf course are being conserved and habitat restored (Service 2018). During this study, we documented that Casey's June beetle are persisting in low numbers within the golf course. Creating a conserved corridor and patchwork of suitable, restored habitat islands is anticipated to improve connectivity with Palm Canyon Wash and expand suitable habitat in Tahquitz Creek.

Best Practices for Herbicide Use in Casey's June Beetle Habitat

Casey's June beetle conservation areas in Palm Canyon Wash are managed to protect the beetle and its habitat. Control of the establishment and proliferation of nonnative invasive plant species using mechanical (VCS Environmental 2023) and limited chemical methods (CNLM 2020a, CNLM 2020b) is necessary to maintain healthy vegetation communities suitable for Casey's June beetle. However, herbicides can directly and indirectly harm insects in varied ways. The diversity of results in the literature suggests that the effects of herbicides on insect mortality are dependent on the active ingredient, formulation, and insect species (The Xerces Society 2024, Smith *et al.* 2021, Albanese 2019, Kraus and Stout 2019). For example, the commonly used herbicide glyphosate has broad physiological impacts on insects, other arthropods, and vertebrates (Smith *et al.* 2021). Glyphosate acts by translocating to the plant roots where it is released into the soil. In addition, about 88 percent of the sprayed glyphosate ends up in the

²⁸ The City of Palm Springs removed the encampment in 2024.

topsoil where it can remain for weeks to years, depending on environmental conditions, due to its stable half-life (Smith *et al.* 2021).

Immature stages of Casey's June beetle are susceptible to the negative effects of herbicides due to their underground life history and association with both native and nonnative vegetation. Based on emergence hole surveys, the soil excavation surveys, and field observations, Casey's June beetle occurs throughout the wash system at variable distances from vegetation and varied soil depths, likely depending on time of year. Unless determined otherwise, we assume that Casey's June beetle may feed on plant roots in some situations (e.g., upland terraces), along with feeding on subsurface detritus. We recommend using mechanical methods, such as hand tools and mowing with small equipment, as the primary approach to invasive plant removal. However, use of herbicides may be warranted when herbicides achieve the most effective invasive plant control necessary to maintain suitable Casey's June beetle habitat and when used in a limited and conservative approach such that the benefits outweigh the risk of negative effects. We recommend the following considerations for herbicide use in Casey's June beetle habitat:

1. **Time of year.** Applying herbicides during the nonbreeding season (July–March) when larvae are assumed to be deeper in the soil can avoid potential effects. Beginning in March, the exposure risk is likely to increase as there is a presumed mix of larvae and pupae near the surface (i.e., within 2 ft [0.6 m]) preparing to pupate and emerge as adults to breed. As larvae, they are feeding. During pupation, they are not feeding.
2. **Mode of action.** Some herbicides (e.g., Reward®) transfer only to the leaf surfaces, acting through foliar absorption to kill plants but do not translocate through the roots. Some herbicides translocate to the roots (e.g., Habitat®), which may be a more effective mode of control.
3. **Invasive plant root depth.** Applying herbicides that translocate to the roots for invasive plants with shallower roots likely poses a reduced exposure risk and reduced depth of herbicide in the soil. For example, fountain grass (*Pennisetum setaceum*) roots are shallow, growing only to a depth of 1.0 ft (0.3 m) (USDA 2014).
4. **Frequency of application.** Herbicides that require more frequent application to achieve effective control would increase exposure (e.g., herbicides such as Reward® that act by foliar absorption).
5. **Area extent.** Selection of the appropriate control method and herbicide should consider whether spot treatment for smaller areas is sufficient or if more extensive spraying is needed.
6. **Effectiveness.** Control of some invasive plant species is more effective or causes less ground disturbance when using herbicides. For example, Mediterranean grass (*Schimus* sp.) can result in thick ground cover and hand pulling can cause significant ground disturbance, therefore herbicide treatment may prove to be the most effective method of control (CNLM 2020b).

RECOMMENDATIONS AND FUTURE RESEARCH

Long-term monitoring using black light trap surveys should continue to develop the baseline dataset and assess relative abundance and distribution trends, to understand the annual population fluctuation over time, to detect abundance lows over a sufficient time series, and direct habitat conservation and restoration where it is most needed. Along with development of a long-term monitoring program including periodic survey sampling (e.g., every 1–2 years initially, until at least 10 time series points), we recommend follow up surveys where salvaged vegetation was buried in the sediment removal and levee repair area of Palm Canyon Wash (Service 2023; see Appendix J, Figure J1-1 and J1-2, Table J1 for buried salvaged vegetation locations) to assess the long-term effectiveness of providing nursery habitat for immature stages of Casey's June beetle.

We included two survey designs to balance study goals. Rangewide surveys provided a systematic and more spatially representative sample (spatially rich but compromised frequent sampling over time) and Sentinel surveys provided data from one location sampled frequently (temporally rich, but spatially restricted). Each contributed to better our understanding of the species. To derive a robust population estimate, more work is need to sample Casey's June beetle more extensively and over a longer time series. Along with continuing surveys using a refined, more targeted (i.e., spatially, and temporally intensive surveys on a finer scale) field sampling design, further analyses using the existing data can identify additional spatio-temporal correlates of abundance, such as bootstrapping simulations of the full corrected abundance estimates to improve the accuracy of the estimated rangewide abundance (Harju 2022).

This study focused on Casey's June beetle capture surveys from sunset through the first portion of the night until about 10:00 pm, in accordance with current literature and the Service's Casey's June beetle survey guidelines. Because we only surveyed during the first 3 or 4 hours from sunset, we could not detect fluctuations in activity throughout the entire night, identify patterns, peaks, or when nightly activity ends. We recommend conducting a subset of night-long (sunset to sunrise) capture surveys to better understand relative activity patterns and abundance and compare how well these surveys, which were conducted for a few hours from sunset, represent the full breadth of Casey's June beetle breeding season activity. This would also inform development of future monitoring schemes to ensure that limited staff time and funding is focused on the most informative and productive nightly survey timing.

Female Casey's June beetle ecology and demographics is a significant knowledge gap. Development of a repeatable and reliable female survey detection protocol is needed. The probability of detecting females in the field is extremely low, which is unsurprising given their behavior and other ecological and physiological characteristics. Traditional transect and, likely, pit fall trap survey methods may prove ineffective or inefficient at detecting females.

Information on larvae ecology is also lacking. Our small sample of soil excavation surveys provide some basic information to build from, but the results are not statistically robust and therefore, inferences to the larger population are not possible. The soil surveys were only conducted in the hydrologically active portion of the channel. We recommend that future soil excavation surveys include upland terrace habitats to provide information on larval ecology in this unexplored portion of the wash.

We recommend evaluating alternative detection methods for all life stages, tailored to cryptic and rare species such as acoustic monitoring (Luff, Molyneux, and Ball 1979, Harvey *et al.* 2011, Lampson *et al.* 2013), x-ray microtomography to track larval movements (Johnson, Read, and Gregory 2004), pseudo-acoustic optical sensors that detect and classify insect flight sounds (Chen *et al.* 2014), eDNA methods (Rishan, Kline, and Rahman 2023, Roger *et al.* 2022, Valentin *et al.* 2020), and detection dogs (Bennett *et al.* 2022, Navarrete 2021).

Our study generated a rich suite of datasets on varied aspects of Casey's June beetle ecology and demographics. This report compiles an array of descriptive statistics, analyses estimating male abundance, and information on population dynamics, ecology, and habitat associations. However, the Service is limited by staff time and funding to complete a more detailed analysis of every ecologically relevant, and potentially interactive component of the data (e.g., climatological interactions). We recommend future research expand on the existing data and analyses as well as generate new data that builds upon learned information and explores hypotheses that have arisen as a product of this study, an effort that would be suitable for a university graduate or Ph.D. candidate research assistantship or USGS-led research.

Finally, we recommend that conservation is focused on acquiring and conserving as much remaining suitable Casey's June beetle habitat as possible is prioritized. Suitable habitat is limited and at some point, will not be available. At that time, recovery actions will be solely reliant on habitat enhancement, restoration, and population augmentation, along with monitoring population abundance, distribution, connectivity, and genetic diversity.

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PERSONAL COMMUNICATIONS, PERSONAL OBSERVATIONS AND UNPUBLISHED DATA

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APPENDIX A – Biologist, Staff, and Volunteer Participants

Table A1. Biologists, staff, and volunteers that participated during the Casey's June beetle study from 2015 to 2022.

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
A. Marchant	Ecological Conservation and Management						x		
Alec Williams	Amec Foster Wheeler/Wood						x	x	x
Alex Ocanas	The Living Desert Zoo and Gardens						x	x	
Alfredo Chavez	College of the Desert			x					
Alicia Thomas	U.S. Fish and Wildlife Service					x			
Alison Anderson	U.S. Fish and Wildlife Service		x						
Alma Kelang	College of the Desert		x						
Amanda Swaller	U.S. Fish and Wildlife Service		x						
Andrea Goodnight	The Living Desert Zoo and Gardens				x				
Anna Cassady	Dudek				x	x			
Athena Compton	Center for Natural Land Management				x				
Bailey Bingham	Riverside County Flood Control					x			
Betty Villalobos	College of the Desert		x						
Bianca De Lara	Public			x					
Bonnie Smith	The Living Desert Zoo and Gardens				x	x			
Brett Marshall	The Living Desert Zoo and Gardens					x			
Britney Schultz (Strittmater)	Dudek		x	x	x	x	x		
Brock Ortega	Dudek	x	x	x	x	x	x		
C. Magana	The Living Desert Zoo and Gardens					x			
Callie Amoaku	Dudek		x	x	x	x			
Candace McKenzie	Friends of the Desert Mountains				x				
Carla Sanchez	Amec Foster Wheeler/Wood						x		
Carol Thompson	Riverside County Flood Control					x		x	x

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Cassandra Strizak	The Living Desert Zoo and Gardens					x			
Charles Greely	Public				x	x	x	x	
Charles Land	California Department of Fish and Wildlife			x					
Christopher Gregory	U.S. Fish and Wildlife Service	x	x	x					
Clayton Suttles	College of the Desert			x					
Clinton Christensen	The Wildlands Conservancy		x						
Colin Barrows	Friends of the Desert Mountains			x					
Dale Hameister	Amec Foster Wheeler/Wood							x	x
Daniella Stage	The Living Desert Zoo and Gardens				x	x			
Danielle Ortiz	Bureau of Land Management			x					
Dave Clark	The Living Desert Zoo and Gardens				x				
David Dunn	The Living Desert Zoo and Gardens				x				
Dawn Petrick	The Living Desert Zoo and Gardens				x				
Deborah Rogers	Center for Natural Land Management				x				
Eddy Konno	California Department of Fish and Wildlife			x					
Emily Urguidi	Wood							x	x
Erin Bergman	Dudek			x					
Felicia Sirchia	U.S. Fish and Wildlife Service		x	x					
Frank Sterrett	Public		x						
Gabby Tolley	Riverside County Flood Control								x
Gabriele Cassell	The Living Desert Zoo and Gardens				x				
Gene Jennings	Riverside County Flood Control					x			
Ginny Short	Center for Natural Land Management		x	x	x	x			
Hal Fedora	Friends of the Desert Mountains				x	x			

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Helena Santiana	College of the Desert		x						
Isabella Anguiano	Public				x				
James Danoff-Burg	The Living Desert Zoo and Gardens				x				
James Thiede	U.S. Fish and Wildlife Service		x	x					
Jane Garrison	Public				x				
Jeff Petrick	The Living Desert Zoo and Gardens				x				
Jennifer Lopez	College of the Desert			x					
Jerry Aguirre	Riverside County Flood Control							x	x
Jill Vega	Save Oswit Canyon				x				
Joanna Gilkeson	U.S. Fish and Wildlife Service			x					
Joanna Ortega	Public				x	x			
Jody Fraser	U.S. Fish and Wildlife Service		x						
Joe Thompson	Save Oswit Canyon				x				
John Green	Wood								x
John Taylor	U.S. Fish and Wildlife Service		x	x					
Jon Rishi	Public		x						
Juan Eason	Amec Foster Wheeler/Wood							x	
Juanita Garner	Public			x					
Julie Simonsen	Ecological Conservation and Management		x	x		x			
Junkal Lhang	College of the Desert		x						
Justin Conley	Agua Caliente Band of Mission Indians			x	x				
Kara Akers	The Living Desert Zoo and Gardens				x	x			
Kara Clevinger	Amec Foster Wheeler/Wood						x		
Karen Mullen	Dudek		x	x					

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Kathleen Dayton	Dudek			x					
Kathy Craig	The Living Desert Zoo and Gardens				x				
Kathy Lorenzini	The Living Desert Zoo and Gardens				x	x			
Ken Corey	U.S. Fish and Wildlife Service				x				
Ken Osborne	Osborne Biological Consulting	x	x	x	x	x	x	x	x
Kevin Salgado	Riverside County Flood Control					x			x
Kiara Marshall	College of the Desert			x					
Kristine Hansele	The Living Desert Zoo and Gardens				x				
Kurt Broz	Amec Foster Wheeler/Wood								x
Kyle Mulroe	The Living Desert Zoo and Gardens						x	x	
Kyra Martinez	College of the Desert		x						
Lauren Duoto	Amec Foster Wheeler/Wood								x
Leslie Levy	Riverside County Flood Control					x		x	
Linda Gregory	Public			x		x			
Lloyd Shiganaga	The Living Desert Zoo and Gardens				x	x			
Mackenzie Forgey	Dudek				x				
Mana Lopez	College of the Desert			x					
Marco Ramos	Public			x					
Mariana Alarcon	College of the Desert			x					
Mark Kendrick	Save Oswit Canyon				x	x			
Mary McKay	Public		x						
Matthew Wilcox	Public		x						x
Melanie Bukovac	Amec Foster Wheeler/Wood								x
Melanie Davis	University of California, Riverside				x				

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Mendel Stewart	U.S. Fish and Wildlife Service			x					
Michael Alexander	Public			x					
Michael Flores	California Department of Fish and Wildlife			x					
Michael Wilcox	Amec Foster Wheeler/Wood		x	x			x	x	x
Michelle (Misha) Brown	The Living Desert Zoo and Gardens				x				
Mindy Beohm	Public		x						
Monique O'Conner	Dudek			x					
Nancy Rader	The Living Desert Zoo and Gardens				x				
Nathan Moorhatch	Amec Foster Wheeler/Wood		x				x	x	x
Nick Stage	The Living Desert Zoo and Gardens				x				
Noelle Ronan	U.S. Fish and Wildlife Service	x	x	x	x	x	x	x	x
Oscar Barcenos	College of the Desert		x						
Osiris Enriquez	College of the Desert		x						
Paulette Greenfield	The Living Desert Zoo and Gardens				x				
Peri Lee Pipkin	University of California, Riverside				x				
Pete Sorensen	U.S. Fish and Wildlife Service		x						
Peter Sanzenbacher	U.S. Fish and Wildlife Service		x	x					
Philip Clevinger	Amec Foster Wheeler/Wood		x					x	x
Quinn Greely	The Living Desert Zoo and Gardens				x	x	x		
Randy Sheppheard	Riverside County Flood Control					x		x	
Rebecca Gordon	U.S. Fish and Wildlife Service		x						
Rick Rogers	Biological consultant		x						
Robert Williams	Amec Foster Wheeler		x	x					
Ryan Fitch	San Diego Zoo Global			x					

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Sam Wilcox	Public		x						
Samantha Haynes	California Department of Fish and Wildlife			x		x			
Sarah Greely	The Living Desert Zoo and Gardens		x	x	x	x	x	x	
Scott Gressard	Dudek			x					
Scott Hoffmann	U.S. Fish and Wildlife Service		x						
Sean Berriman	Riverside County Flood Control							x	x
Shana Carey	Dudek				x				
Sharon Keeney	California Department of Fish and Wildlife			x					
Sharon Rogers	Public		x						
Shaunessy Johnson	College of the Desert		x						
Shawn Nicholson	U.S. Fish and Wildlife Service			x					
Sheri Shiflett	Public		x						
Skyler Greely	The Living Desert Zoo and Gardens				x	x	x		
Stan McKenzie	Friends of the Desert Mountains				x				
Stefan Awender	California Department of Fish and Wildlife					x			
Stephanie Ceja	Public			x					
Susan Forgrave	The Living Desert Zoo and Gardens				x	x			
Sybil Rivera	The Living Desert Zoo and Gardens					x			
Tamara Russell	Mt. San Jacinto College		x						
Tangerine Catacleesi	College of the Desert		x						
Tara Hanjan	Public			x					
Timothy (Detto) Kennedy	University of California, Riverside				x				
Tito Marchant	Ecological Conservation and Management		x	x		x	x		
Tom Forgrave	The Living Desert Zoo and Gardens				x	x			

Participant ¹	Affiliation ²	2015 ³	2016	2017	2018	2019	2020	2021	2022
Tony McKinney	U.S. Fish and Wildlife Service			x					
Tyler Goodearly	Center for Natural Land Management					x			
Vincent James	U.S. Fish and Wildlife Service		x	x					
Warner (Brett) Daniels	Coachella Valley Water District			x					
Zach Smith	Wood								x

¹ Participants included professional biologists and volunteers. Some participants contributed all volunteer hours: Coachella Valley Water District, College of the Desert, Friends of the Desert Mountains, Mt. San Jacinto College, San Diego Zoo Global, Save Oswit Canyon, The Living Desert Zoo and Gardens, University of California, Riverside, and the public. Some participants with professional affiliations contributed partial volunteer hours (e.g., travel, some evening surveys): California Department of Fish and Wildlife, Center for Natural Land Management, Dudek, Ecological Conservation and Management, Osborne Biological Consulting, The Living Desert Zoo and Gardens, U.S. Fish and Wildlife Service. Some participants only attended training sessions. Only participants trained, qualified, and permitted under U.S. Fish and Wildlife Service recovery permits handled and marked Casey's June beetles.

² Affiliations: Amec Foster Wheeler/Wood Environment and Infrastructure Solutions, Inc. is now WSP, Environmental Consulting Company; Public=local community members; Riverside County Flood Control=Riverside County Flood Control and Water Conservation District; San Diego Zoo Global is now San Diego Zoo Wildlife Alliance; Save Oswit Canyon is now Oswit Land Trust.

³ Study activities in 2015 were limited to planning and emergence hole surveys in Palm Canyon Wash.

APPENDIX B – Casey’s June Beetle Long-Term Survey Plots

Table B1. Description and location of Casey's June beetle long-term survey plots (traps) in Palm Canyon Wash, Smoke Tree Ranch, and Tahquitz Creek surveyed from 2016 to 2022.

Site	Trap ¹	Ecological Community	Latitude ²	Longitude ²	Years Surveyed
Palm Canyon Wash	0	Wash	33.793352	-116.515714	2016, 2017, 2018, 2019, 2020, 2021
Palm Canyon Wash	1	Upland Terrace	33.777570	-116.530740	2016, 2017, 2018
Palm Canyon Wash	2	Wash	33.781240	-116.527600	2016, 2017, 2018
Palm Canyon Wash	3	Upland Terrace	33.785450	-116.525630	2016, 2017, 2018, 2019, 2020
Palm Canyon Wash	4	Wash	33.788740	-116.521920	2016, 2017, 2018, 2019, 2020
Palm Canyon Wash	5	Wash	33.791930	-116.518100	2016, 2017, 2018, 2019, 2020
Palm Canyon Wash	6	Wash	33.794690	-116.513830	2016, 2017, 2018
Palm Canyon Wash	7	Wash	33.798120	-116.510510	2016, 2017, 2018
Palm Canyon Wash	8	Wash	33.800610	-116.506010	2016, 2017, 2018
Palm Canyon Wash	9a	Wash	33.801327	-116.500544	2018, 2019, 2020, 2021, 2022
Palm Canyon Wash	9	Wash	33.801550	-116.500740	2016, 2017
Palm Canyon Wash	10	Wash	33.801680	-116.495380	2016, 2017, 2018, 2019, 2020, 2021, 2022
Palm Canyon Wash	11	Wash	33.801230	-116.490040	2016, 2017, 2018
Palm Canyon Wash	28	Wash	33.800279	-116.506665	2022
Smoke Tree Ranch	12	Upland Terrace	33.789060	-116.525560	2016, 2017, 2018
Smoke Tree Ranch	13	Upland Terrace	33.791200	-116.520930	2016, 2017, 2018, 2019
Smoke Tree Ranch	14	Upland Terrace	33.795800	-116.520560	2016, 2017, 2018, 2019
Smoke Tree Ranch	15	Upland Terrace	33.798420	-116.522930	2016, 2017, 2018
Tahquitz Creek	16	Upland Terrace - levee	33.810735	-116.530472	2018, 2019, 2020, 2021, 2022
Tahquitz Creek	17	Upland Terrace - levee	33.810712	-116.536480	2018, 2019, 2020
Tahquitz Creek	18	Upland Terrace - levee	33.810729	-116.541061	2018, 2019
Tahquitz Creek	19	Wash - golf course	33.809258	-116.510050	2021, 2022
Tahquitz Creek	20	Wash - golf course	33.807928	-116.507673	2021, 2022

Site	Trap ¹	Ecological Community	Latitude ²	Longitude ²	Years Surveyed
Tahquitz Creek	21	Wash - golf course	33.806346	-116.505853	2021, 2022
Tahquitz Creek	22	Upland Terrace - golf course	33.803973	-116.503990	2021, 2022
Tahquitz Creek	23	Wash - golf course	33.803610	-116.501086	2021, 2022
Tahquitz Creek	24	Wash - golf course	33.402663	-116.498547	2021, 2022
Tahquitz Creek	25	Upland Terrace - golf course	33.802638	-116.491301	2021
Tahquitz Creek	26	Upland Terrace - golf course	33.806829	-116.509943	2021, 2022
Tahquitz Creek	27	Upland Terrace - golf course	33.802281	-116.503092	2021, 2022

¹ Trap 0 is the Sentinel survey plot. Trap 9 was moved due to a homeless encampment. Trap 9 and trap 9a are in close proximity. It is referred to collectively as trap 9 throughout the text.

² Datum=NAD83.

APPENDIX C – Casey’s June Beetle Marking Scheme

Table C1. The marking scheme used to individually identify male Casey's June beetles captured at traps during surveys from 2016 to 2022.

Trap ¹	Survey Location	Color ²	Number Placement Elytra Side ³	Pronotum Dot
0	Palm Canyon Wash	Red	Left	Yes
1	Palm Canyon Wash	Black	Right	No
2	Palm Canyon Wash	Black	Left	No
3	Palm Canyon Wash	Red	Right	No
4	Palm Canyon Wash	Red	Left	No
5	Palm Canyon Wash	Blue	Right	No
6	Palm Canyon Wash	Blue	Left	No
7	Palm Canyon Wash	Brown	Right	No
8	Palm Canyon Wash	Brown	Left	No
9	Palm Canyon Wash	Purple	Right	No
10	Palm Canyon Wash	Purple	Left	No
11	Palm Canyon Wash	Green	Right	No
12	Smoke Tree Ranch	Green	Left	No
13	Smoke Tree Ranch	Black	Right	Yes
14	Smoke Tree Ranch	Black	Left	Yes
15	Smoke Tree Ranch	Red	Right	Yes
16	Tahquitz Creek (upper reach)	Black	Right	Yes
17	Tahquitz Creek (upper reach)	Black	Left	Yes
18	Tahquitz Creek (upper reach)	Red	Right	Yes
19	Tahquitz Creek (golf course)	Black	Left	No
20	Tahquitz Creek (golf course)	Red	Right	No
21	Tahquitz Creek (golf course)	Blue	Right	No
22	Tahquitz Creek (golf course)	Brown	Right	No
23	Tahquitz Creek (golf course)	Blue	Left	No

24	Tahquitz Creek (golf course)	Green	Right	No
25	Tahquitz Creek (golf course)	Brown	Left	No
26	Tahquitz Creek (golf course)	Green	Left	No
27	Tahquitz Creek (golf course)	Red	Left	No
28	Palm Canyon Wash	Black	Right	Yes

¹ Trap 0 is the Sentinel survey plot.

² Due to limited well-performing marker colors, some marking schemes were reused in years that we surveyed a subset of Rangewide plots. The marking scheme was not duplicated between plots in the same year.

³ Sequential numbering (i.e., 1, 2, 3, 4, 5, etc.) placed on the right or left elytra.

APPENDIX D – Vegetation Communities

**Strittmater and Amoaku 2019 – Casey's June Beetle Vegetation Mapping Methodology
and Results**

MEMORANDUM

To: Noelle Ronan, U.S. Fish and Wildlife Service
From: Britney Strittmater and Callie Amoaku, Dudek
Subject: Casey's June Beetle Vegetation Mapping Methodology and Results
Date: November 15, 2019
cc: Brock Ortega, Dudek
Attachment(s): Attachment A, Figures
Attachment B, Plant Compendium
Attachment C, Vegetation Forms
Attachment D, Relevé Forms
Attachment E, Relevé Plot Spreadsheet
Attachment F, Relevé Plot Photos

Dudek conducted a botanical inventory, vegetation mapping, and relevé plot surveys for the Casey's June Beetle Project (Project). This memorandum summarizes the methodology and results for vegetation mapping and relevé plots and provides the overall plant inventory.

1 Introduction

Casey's June beetle (CJB), *Dinacoma caseyi*, was federally listed as endangered in 2011, due to a loss and modification of CJB populations and habitat (USFWS 2010). Subsequently, approximately 594 acres (most of the known range of CJB) of Critical Habitat within and along Palm Canyon Wash in eastern Riverside County was designated. Little is known about CJB and recovery actions are planned without suitable baseline information. This memorandum provides the methods and results of the botanical inventory, vegetation mapping, and vegetation relevé surveys as part of the effort to collect baseline information on habitat for CJB in the Palm Canyon Wash area (Figure 1, Attachment A).

2 Methods

Dudek conducted a botanical inventory, vegetation mapping, and vegetation relevé surveys from 2015 through 2019. The botanical inventory and vegetation mapping was completed in the 594-acre CJB Critical Habitat Study Area ("study area") shown on Figure 2 (Attachment A). Vegetation relevé surveys were completed within the 16 plots shown Figure 2. Table 1 lists the dates, personnel, survey type, and conditions of these surveys.

Table 1. Survey Information

Date	Personnel	Survey Type	Survey Area
3/11/15	BAS, CJA	Botanical Inventory; Vegetation Mapping	CJB Critical Habitat Study Area
3/12/15	BAS, CJA	Botanical Inventory; Vegetation Mapping	CJB Critical Habitat Study Area
3/13/15	BAS, CJA	Botanical Inventory; Vegetation Mapping	CJB Critical Habitat Study Area
4/27/16	BAS, CJA	Relevé plots	Sentinel and Plots 1–15
4/28/16	BAS, CJA	Relevé plots	Sentinel and Plots 1–15
5/12/16	BAS	Relevé plots	Sentinel and Plots 1–15
11/14/16	BAS, CJA	Soil Mapping; Vegetation Mapping Update	CJB Critical Habitat Study Area
5/2/19	APC, BAS, CJA	Subset Relevé plots	Sentinel and Plots 3, 4, 5, 9, 10, 13, and 14
5/30/19	APC, CJA	Subset Relevé plots	Sentinel and Plots 3, 4, 5, 9, 10, 13, and 14
6/7/19	BAS	Subset Relevé plots	Sentinel and Plots 3, 4, 5, 9, 10, 13, and 14

Personnel: APC = Anna P. Cassady; BAS = Britney A. Strittmater; CJA = Callie J. Amoaku.

Notes: CJB = Casey's June beetle.

2.1 Botanical Inventory

On March 11, 12, and 13, 2015, Dudek biologists Britney Strittmater and Callie Amoaku conducted a plant inventory within the study area. On April 27 and 28, and May 12, 2016, Dudek biologists Britney Strittmater and Callie Amoaku conducted a plant inventory within the 16 relevé plots. On May 2 and 30, 2019, and June 7, 2019, Dudek biologists Anna Cassady, Britney Strittmater, and Callie Amoaku conducted a plant inventory within eight relevé plots. Attachment B includes a cumulative plant compendium. Latin and common names for plant species with a California Rare Plant Rank follow the California Native Plant Society's Inventory of Rare and Endangered Plants (CNPS 2019). For plant species without a California Rare Plant Rank, Latin names follow the Jepson Interchange List of Currently Accepted Names of Native and Naturalized Plants of California (Jepson Flora Project 2019), and common names follow the U.S. Department of Agriculture's Natural Resources Conservation Service PLANTS Database (USDA 2018).

2.2 Vegetation Mapping

Vegetation communities and land uses within the study area were mapped in the field directly onto a 200-foot-scale (1 inch = 200 feet) aerial-photograph-based field map. Following completion of the fieldwork, all vegetation polygons were digitized using ArcGIS and a geographical information system (GIS) coverage was created. Once in ArcGIS, the acreage of each vegetation community and land cover present on site was determined.

Vegetation community classifications used in this report follow the Manual of California, Second Edition (Sawyer et al. 2009), where feasible, with modifications made to accommodate the lack of conformity of the observed communities using Oberbauer et al. (2008) and Holland (1986). Each natural community was mapped to the association level,

where feasible. Vegetation communities were mapped as the “disturbed” form if non-native vegetation covered approximately 20%–50% absolute cover. Vegetation mapping forms are included in Attachment C.

No access was allowed on the Tribal lands within the southwesternmost portion of the study area. These areas are described as “Not Mapped” in the vegetation layer.

2.3 Relevé Plots

Relevé plots were conducted in 2016 and 2019 in order to evaluate changes in species composition and structure of the washes vegetation over time. The methods used to conduct the relevé plots followed the most recent California Native Plant Society Relevé Protocol from 2016 (CDFW and CNPS 2016). This method provides for a visual assessment of vegetation communities used to classify and map large vegetation areas in a limited amount of time instead of more intrusive point-intercept transect methods. The relevé is a “semi-quantitative” method, relying on ocular (visual) estimates of plant cover rather than on counts of “hits” of a particular species along a transect line or other precise measurement techniques (CDFW and CNPS 2016). This method ensures that collection of vegetation data minimizes damage to vegetation in the spring and limits establishment of trails during monitoring visits. Relevé forms from 2016 and 2019 are included in Attachment D.

2.3.1 Relevé Plot Survey – 2016

Dudek biologists Britney Strittmater and Callie Amoaku conducted the relevé plot vegetation monitoring on April 27 and 28, 2016, and May 12, 2016 (Table 1). Twelve relevé plots, including the Sentinel site, were sampled within the Whitewater River, and four relevé plots were sampled within Smoke Tree Ranch. Each plot was a 100-square-meter (1,076-square-foot) circle, which resulted in some plots extending beyond the study area. For each plot, general information was collected, including date, location information, and observers; whether the plot is representative of the stand of vegetation; etc. A GPS coordinate was also taken at the center of each plot. A vegetation description was recorded, including the dominant layer, preliminary alliance name, dominant vegetation group, and phenology. Information regarding the wetland community type was also recorded. Photographs were taken in each cardinal direction from the center of the plot. Stand and environmental information was recorded, such as a description of impacts and site history. Surface cover and soil information was also recorded at each plot and included soil texture and parent material. The vegetation was assessed for each layer present (e.g., conifer/hardwood tree, regenerating tree, shrub, and herbaceous). A species list was created for each plot that included the layer in which the species was observed and an estimate of its cover class within that layer. In addition, surveyors determined an approximate total percent cover for each entire layer. Each plot's form is included as Attachment D, and photos for each plot are included in Attachment F.

2.3.1 Relevé Plot Survey – 2019

Dudek biologists Anna Cassady, Britney Strittmater, and Callie Amoaku conducted an updated relevé plot vegetation monitoring on May 2 and 30, 2019, and June 7, 2019 (Table 1). Relevé plot sampling was conducted on a subset of the 2016 plots: six relevé plots, including the Sentinel Site, were sampled within the Whitewater River, and two relevé plots were sampled within Smoke Tree Ranch. Each plot's form is included as Attachment D, and photos for each plot are included in Attachment F.

2.4 Survey Limitations

Access was not available within the 500-foot buffer from the project site because some of the properties are private and access was not granted and no access was allowed on the Tribal lands within the southwesternmost portion of the study area. Where access was unavailable, the 500-buffer was surveyed visually using binoculars. Therefore, vegetation mapping and habitat assessments were conducted from the project site or other public roads, in addition to being complemented with the use of aerial signatures of vegetation communities occurring within the study area.

The 2015 survey effort was conducted during the late winter/early spring season, which resulted in detection and identification of most annual and perennial plant species that may potentially occur in the area. Due to the timing of the surveys, certain later blooming spring and summer annuals and cryptic perennials may not have been detectable. The 2016 survey efforts were conducted in the spring and fall, resulting in detection and identification of most annuals and perennial plant species; however, due to the timing of the surveys, certain summer-blooming annuals and cryptic perennials may not have been detectable. The 2019 survey effort was conducted in the spring season, resulting in detection and identification of most annuals and perennial plant species; however, due to the timing of the surveys, certain summer-blooming annuals and cryptic perennials may not have been detectable. Additionally, a large storm event on February 14, 2019, deposited a high volume of sediment through the wash, eliminating evidence of annual species.

The Palm Springs area generally receives an average rainfall of approximately 5.49 inches per year (WRCC 2019). The 2016 survey efforts were conducted during an average rainfall year; however, the 2015 survey effort was conducted during a below-average rainfall year that may have resulted in a lower cover of annual species. January through October 2019 had rainfall measured as 7.33 inches. While 2019 is already an above-average rainfall year, the February 14, 2019, storm event resulted in 3.69 inches of rainfall within 24 hours, which caused high-velocity flows and sediment deposits through the Whitewater River (AgACIS 2019). Table 2 lists the monthly total precipitation from January 2014 through October 2019 (AgACIS 2019).

Table 2. Monthly Total Precipitation for Palm Springs, California

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2014	0.00	0.42	0.28	0.05	T	0.00	0.27	0.88	0.04	0.00	0.07	0.80	2.81
2015	0.22	0.17	0.83	T	0.12	T	0.42	T	0.14	0.15	T	0.04	2.09
2016	2.26	T	0.02	0.14	0.02	0.00	0.00	0.00	0.92	0.09	0.20	1.50	5.15
2017	3.53	1.65	0.00	0.00	T	0.00	0.08	0.71	1.20	0.00	T	0.00	7.17
2018	M	T	0.24	0.00	T	0.00	1.11	T	0.00	0.91	0.07	0.53	M
2019	1.45	4.38	0.42	T	0.10	0.00	T	T	0.98	0.00	M	M	M
Mean	1.49	1.10	0.30	0.03	0.04	T	0.31	0.26	0.55	0.19	0.07	0.53	4.31

Notes: T=Trace; M=Missing data.

3 Results

3.1 Botanical Inventory

A total of 167 species of native or naturalized plants, 127 native (76%) and 40 non-native (24%), were recorded within the study area during the 2015, 2016, and 2019 survey efforts. Portions of the study area were surveyed from public roads, which inherently constrains the ability to inventory all plant species. Plant species observed within the study area are listed in Attachment B.

3.1 Vegetation Mapping

Eleven associations within nine alliances and three land cover types were documented within the study area; of these, four were mapped as the disturbed form of the association. Unless otherwise noted, the vegetation classifications follow the Manual of California, Second Edition (Sawyer et al. 2009), with modifications made to accommodate the lack of conformity of the observed communities using Oberbauer et al. (2008) and Holland (1986). Figure 3 illustrates the distribution of vegetation communities and land covers, and Table 3 provides a summary of each vegetation community and land cover's extent within the study area.

Table 3. Vegetation Communities and Land Covers within the Study Area

Vegetation Alliance/Land Cover	Association	Acreage
Vegetation Alliance		
Cheesebush	<i>Ambrosia salsola</i>	138.4
	<i>Ambrosia salsola</i> – <i>Petalonyx thurberi</i>	3.4
Creosote bush	<i>Larrea tridentata</i>	2.3
	<i>Larrea tridentata</i> – <i>Atriplex polycarpa</i>	13.6
Creosote bush (disturbed)		3.3
Creosote bush-brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	4.0
Creosote bush-white bursage	<i>Larrea tridentata</i> – <i>Ambrosia dumosa</i>	6.7
Desert willow	<i>Chilopsis linearis</i>	33.7
	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	39.8
Desert willow (tamarisk)	N/A	1.7
Desert willow (disturbed)	<i>Chilopsis linearis</i>	1.3
Desert willow-smoke tree	<i>Chilopsis linearis</i> – <i>Psoralea argemone</i>	20.9
Fourwing saltbush (disturbed)	<i>Atriplex canescens</i>	1.7
Smoke tree	<i>Psoralea argemone</i>	24.4
Smoke tree (disturbed)	<i>Psoralea argemone</i>	5.4
Tamarisk thickets	N/A	5.4
Land Covers/Other		
Disturbed habitat	N/A	8.9

Table 3. Vegetation Communities and Land Covers within the Study Area

Vegetation Alliance/Land Cover	Association	Acreage
Unvegetated channel	N/A	7.2
Urban/developed	N/A	137.1
Not mapped (no access)	N/A	134.7
Total^a		594.0

Notes: N/A = not applicable.

^a Acreage may not sum precisely due to rounding.

3.3.1 Cheesebush Shrubland Alliance

The cheesebush (*Ambrosia salsola*) shrubland alliance¹ is an open to intermittent shrub layer where cheesebush dominates or co-dominates the shrub layer at low cover. This species is typically less than 2 meters (approximately 7 feet) in height with sparse herbs in the understory (Sawyer et al. 2009). This alliance is commonly found in valleys, flats, intermittent channels, washes, and arroyos on alluvial, sandy and gravelly, and disturbed desert pavement (Sawyer et al. 2009).

Within the study area, cheesebush shrubland alliance is mapped within the Smoke Tree Ranch area, located near the central portion of the study area, north of the wash. On site, two associations are mapped: *Ambrosia salsola* and *Ambrosia salsola*–*Petalonyx thurberi*.

3.3.2 Fourwing Saltbush Alliance

The fourwing saltbush scrub (*Atriplex canescens*) shrubland alliance is an open to intermittent shrub layer where fourwing saltbush dominates the shrub layer at low cover. This species is typically less than 3 meters (10 feet) in height with variable herbs and grasses in the understory. This alliance is commonly found on alluvial fans, rolling hills, playas, shores, and lake deposits (Sawyer et al. 2009).

Within the study area there is one area near relevé plot mapped as the disturbed form of fourwing saltbush scrub. It is dominated by fourwing saltbush and cheesebush, with high disturbance from trash and litter, tire tracks, and trails.

3.3.3 Desert Willow Alliance

The desert willow (*Chilopsis linearis*) woodland alliance is an open to intermittent tree layer with desert willow as the dominant or co-dominant species in a two-tiered canopy. The tree and shrub species are typically less than 6 meters (20 feet) in height, with an open to intermittent shrub layer and sparse herb layer (Sawyer et al. 2009). For a stand to be classified as desert willow woodland alliance, desert willow must be present at greater than 2% absolute cover. This alliance occurs in well-drained sand and gravel within washes, intermittent channels, canyon bottoms, and arroyos, and along floodplains and wash terraces where flooding is infrequent but where subterranean water is available (Sawyer et al. 2009).

¹ The cheesebush scrub alliance is no longer recognized in the 2018 California Natural Community List; however, the initial vegetation mapping was done in 2015 according to Sawyer et al. 2009.

Within the study area, desert willow alliance occurs throughout the northern/eastern portion of the wash. Two associations occur on site: *Chilopsis linearis* association and *Chilopsis linearis*–*Ambrosia salsola* association. There is one polygon mapped as the disturbed form of desert willow, where *Tamarix* spp. and castorbean (*Ricinus communis*) were present. Additionally, while it does not conform to the Manual of California Vegetation, 2nd Edition (Sawyer et al. 2009), one area was mapped as desert willow–saltcedar (tamarisk) woodland due to the co-dominance of tamarisk (*Tamarix ramosissima*) in the tree layer. In these areas, desert willow and tamarisk were the dominant trees, with blue palo verde (*Parkinsonia florida*), smoketree, and castorbean present at lower cover.

3.3.3 Desert Willow–Smoketree Wash Woodland Alliance

The desert willow–smoketree wash (*Chilopsis linearis*–*Psoralea argemone*) woodland alliance² is an open to intermittent tree layer with desert willow and/or smoketree as dominant or co-dominant species in the tree or tall shrub canopy. The tree species are typically less than 8 meters (26 feet) in height, with an open to intermittent shrub layer and sparse herb layer (CDFW 2018). For a stand to be classified as desert willow–smoketree wash woodland alliance, both desert willow and smoketree must be present at greater than 2% absolute cover. This alliance occurs in well-drained sand and gravel within washes, intermittent channels, canyon bottoms, and arroyos, and along floodplains and wash terraces where flooding is infrequent but where subterranean water is available (CDFW 2018).

Within the study area, desert willow–smoketree wash woodland alliance occurs throughout the southern/western portion of the wash. Desert willow and smoketree dominate in the tree layer, with fourwing saltbush and black-stem rabbitbrush (*Ericameria paniculata*) dominant in the shrub layer.

3.3.4 Creosote Bush Alliance

The creosote bush scrub (*Larrea tridentata*) shrubland alliance contains creosote as the dominant or co-dominant species within the shrub canopy. This community contains an open to intermittent canopy. Emergent trees may be present at a low cover, and the herbaceous layer is variable and may include various grasses and seasonal annuals. This alliance occurs on alluvial fans, bajadas, upland slopes, and intermittent washes on well-drained soils (Sawyer et al. 2009).

Within the study area, creosote bush scrub shrubland alliance occurs near relevé plot 6 and in upland areas south of East Palm Canyon Drive. On site, two associations are mapped: *Larrea tridentata* and *Larrea tridentata*–*Atriplex polycarpa*. Additionally, one polygon was mapped as the disturbed form of creosote bush.

3.3.5 Creosote Bush–Brittle Bush Alliance

The creosote bush–brittle bush scrub (*Larrea tridentata*–*Encelia farinosa*) shrubland alliance includes creosote bush and brittle bush as co-dominant shrubs in the canopy. Creosote bush–brittle bush scrub has a two-tiered shrub canopy less than 3 meters (10 feet) in height with an open to intermittent ground layer in which annuals are seasonally present (Sawyer et al. 2009). For a stand of vegetation to be classified as creosote bush–brittle bush scrub, both creosote bush and brittle bush must be greater than 1% absolute cover in the shrub canopy. The

² This alliance was not recognized in the Sawyer et al. 2009 classification, but is now recognized on the California Natural Community List (CDFW 2018).

creosote bush–brittle bush scrub shrubland alliance occurs on upland slopes, alluvial fans, bajadas, and minor washes (Sawyer et al. 2009).

Within the study area, creosote bush–brittle bush scrub shrubland alliance occurs along the southern edge of the wash. One association is mapped on site: *Larrea tridentata*–*Encelia farinosa*–*Ferocactus cylindraceus*. This association included creosote bush, brittle bush, and California barrel cactus (*Ferocactus cylindraceus*) as dominant species in the shrub layer, and common Mediterranean grass (*Schismus barbatus*) in the herb layer.

3.3.6 Creosote Bush–White Bursage Alliance

The creosote bush–white bursage scrub alliance (*Larrea tridentata*–*Ambrosia dumosa* alliance) includes creosote bush and white bursage as co-dominant shrubs in the canopy. Creosote bush–white bursage scrub has a two-tiered shrub canopy less than 3 meters (10 feet) in height with an open to intermittent ground layer in which annuals are seasonally present (Sawyer et al. 2009). For a stand of vegetation to be classified as creosote bush–white bursage scrub, both creosote bush and white bursage must be greater than or equal to 1% absolute cover in the shrub canopy. The creosote bush–white bursage scrub alliance occurs on upland slopes, alluvial fans, bajadas, and minor washes (Sawyer et al. 2009).

Within the study area, one polygon of creosote bush–white bursage scrub shrubland alliance occurs in an upland area south of East Palm Canyon Drive. One association is mapped on site: *Larrea tridentata*–*Ambrosia dumosa*.

3.3.7 Smoke Tree Alliance

The smoke tree (*Psoralea argophylla*) woodland alliance includes smoketree as the dominant or co-dominant species in the tree or tall shrub canopy. The tree species are typically less than 8 meters (26 feet) in height, with an open to intermittent shrub layer and sparse herb layer (Sawyer et al. 2009). For a stand to be classified as smoke tree woodland alliance, smoketree must be present at greater than 2% absolute cover. This alliance occurs in well-drained sand and gravel within washes, intermittent channels, and arroyos (Sawyer et al. 2009).

Within the study area, smoke tree woodland alliance is mapped throughout the wash. One association is mapped on site: *Psoralea argophylla*. Additionally, there is one area mapped as the disturbed form of smoke tree woodland alliance, which has *Tamarix* spp. present in the shrub and sapling layer.

3.3.8 Tamarisk Thickets Alliance

The tamarisk thickets (*Tamarix* spp.) semi-natural alliance includes tamarisk species as the dominant species in the shrub canopy with a continuous to open canopy cover less than 8 meters (26 feet) in height. The herbaceous layer is sparse and emergent trees may be present at a low cover. This community occurs along lake margins, ditches, wash, rivers and other watercourses (Sawyer et al. 2009).

Within the study area, one area in the northeast portion of the wash is mapped as tamarisk thickets semi-natural alliance. with both Athel tamarisk (*Tamarix aphylla*) and tamarisk (*Tamarix ramosissima*).

3.3.9 Unvegetated Channel

Although not recognized by Sawyer et al. 2009, unvegetated channel refers to portions of the wash where there is a lack of vegetation cover.

3.3.10 Urban/Developed

Although not recognized by Sawyer et al. 2009, urban/developed is defined by Oberbauer et al. (2008) as areas that have been constructed on or disturbed so severely that native vegetation is no longer supported. Urban/developed lands includes areas with permanent or semi-permanent structures, pavement or hardscape, landscaped areas, and areas with a large amount of debris or other materials.

Urban/developed land includes the residential development of Smoke Tree Ranch, the top of the concrete berm, and paved roads.

3.3.11 Disturbed Habitat

The classification of disturbed habitat is based on the predominance of bare ground, non-native plant species, and other disturbance-tolerant plant species. Oberbauer et al. (2008) describes disturbed habitat as areas that have been physically disturbed by previous human activity and are no longer recognizable as a native or naturalized vegetation association, but that continue to retain a soil substrate. Typically, vegetation, if present, is nearly exclusively composed of non-native annual plant species.

Within the study area, disturbed land encompasses the dirt access roads and barren upland lands.

3.1 Relevé Plots

Table 4 provides a summary of each vegetation community and land cover's extent within the 16 relevé plots and Figure 3 illustrates the distribution of vegetation communities and land covers within the 16 relevé plots. The relevé forms from the 2016 and 2019 surveys are included as Attachment D, data recorded is provided in Attachment E, and photos for each plot are included as Attachment F.

Table 4. Vegetation Communities and Land Covers within Each Relevé Plot

Relevé Plot	Vegetation Alliance/Land Cover ^a	Association	Acreage
Sentinel Site	Desert willow	<i>Chilopsis linearis</i>	5.27
	Smoke tree	<i>Psoralea argemone</i>	0.68
	Urban/developed	N/A	0.41
	Outside CJBCH study area	N/A	1.40
Plot 1	Creosote bush-brittle bush	<i>Larrea tridentata</i> - <i>Encelia farinosa</i> - <i>Ferocactus cylindraceus</i>	0.02
	Desert willow	<i>Chilopsis linearis</i>	4.12
	Unvegetated channel	N/A	0.28
	Not mapped (no access)	N/A	2.22
	Outside CJBCH study area	N/A	1.11

Table 4. Vegetation Communities and Land Covers within Each Relevé Plot

Relevé Plot	Vegetation Alliance/Land Cover ^a	Association	Acreage
Plot 2	Cheesebush	<i>Ambrosia salsola</i>	2.94
	Creosote bush–brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	0.07
	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	3.36
	Outside CJBCH study area	N/A	1.38
Plot 3	Cheesebush	<i>Ambrosia salsola</i>	6.74
	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	0.98
	Disturbed habitat	N/A	0.03
Plot 4	Cheesebush	<i>Ambrosia salsola</i>	0.97
	Creosote bush–brittle bush	<i>Larrea tridentata</i> – <i>Encelia farinosa</i> – <i>Ferocactus cylindraceus</i>	0.11
	Desert willow	<i>Chilopsis linearis</i> – <i>Ambrosia salsola</i>	6.09
	Disturbed habitat	N/A	0.15
	Urban/developed	N/A	0.43
Plot 5	Cheesebush	<i>Ambrosia salsola</i>	0.67
	Desert willow	<i>Chilopsis linearis</i>	4.77
	Outside CJBCH study area	N/A	1.80
	Urban/developed	N/A	0.52
Plot 6	Cheesebush	<i>Ambrosia salsola</i> – <i>Petalonyx thurberi</i>	1.53
	Creosote bush	<i>Larrea tridentata</i>	0.09
	Desert willow	<i>Chilopsis linearis</i>	2.24
	Smoketree	<i>Psoralea argophylla</i>	2.24
	Unvegetated channel	N/A	1.34
	Urban/developed	N/A	0.32
Plot 7	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psoralea argophylla</i>	1.49
	Smoke tree	<i>Psoralea argophylla</i>	0.96
	Disturbed habitat	N/A	0.18
	Unvegetated channel	N/A	1.36
	Outside CJBCH study area	N/A	2.65
	Urban/developed	N/A	1.11
Plot 8	Fourwing saltbush (disturbed)	<i>Atriplex canescens</i>	0.02
	Smoketree	<i>Psoralea argophylla</i>	4.22
	Disturbed habitat	N/A	0.79
	Outside CJBCH study area	N/A	1.81
	Urban/developed	N/A	0.91
Plot 9	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psoralea argophylla</i>	4.28
	Desert willow (tamarisk)	N/A	0.15
	Smoke tree	<i>Psoralea argophylla</i>	0.32

Table 4. Vegetation Communities and Land Covers within Each Relevé Plot

Relevé Plot	Vegetation Alliance/Land Cover ^a	Association	Acreage
	Smoke tree (disturbed)	<i>Psorothamnus spinosus</i>	1.61
	Outside CJBCH study area	N/A	1.03
	Urban/developed	N/A	0.35
Plot 10	Desert willow–smoke tree	<i>Chilopsis linearis</i> – <i>Psorothamnus spinosus</i>	3.99
	Tamarisk thickets	N/A	2.71
	Outside CJBCH study area	N/A	1.03
	Urban/developed	N/A	0.03
Plot 11	Desert willow	<i>Chilopsis linearis</i>	3.36
	Outside CJBCH study area	N/A	4.24
	Urban/developed	N/A	0.16
Plot 12	Cheesebush	<i>Ambrosia salsola</i>	7.75
Plot 13	Cheesebush	<i>Ambrosia salsola</i>	4.72
	Desert willow	<i>Chilopsis linearis</i>	0.95
	Disturbed habitat	N/A	0.76
	Urban/developed	N/A	1.32
Plot 14	Cheesebush	<i>Ambrosia salsola</i>	7.24
	Disturbed habitat	N/A	0.13
	Outside CJBCH study area	N/A	0.12
	Urban/Developed	N/A	0.26
Plot 15	Cheesebush	<i>Ambrosia salsola</i>	6.13
	Disturbed habitat	N/A	0.22
	Outside CJBCH study area	N/A	0.47
	Urban/developed	N/A	0.93
Total^b			124.05

Notes: CJBCH = Casey's June beetle critical habitat; N/A = not applicable.

^a Vegetation mapping based on the 2015 mapping effort.

^b Acreage may not sum precisely due to rounding.

3.1.1 Relevé Plot Surveys – 2016

In 2016, six relevé plots (Plot 3 and Plots 11 through 15) contained greater than 40% vascular plant cover. Of these six plots, only one (Plot 11) is located within the Whitewater River. The remaining plots are located in Smoke Tree Ranch (Plots 12 through 14) or along an upper terrace outside of the Whitewater River (Plot 3). The remaining plots were located within the Whitewater River and contained less than 40% vascular plant cover. These plots are susceptible to annual storm events and scouring, and vegetation cover may vary from year to year.

Table 5 provides a summary of the 16 relevé plots and the total vascular plant cover recorded for each stratum. The relevé forms provide a species list for each plot that includes the layer in which the species was observed and an estimate of its cover class within that layer (refer to Attachment D).

Table 5. 2016 Relevé Plot Percent Cover Summary

Relevé Plot	Total Percent Overstory Hardwood Cover	Total Percent Low-Medium Tree Cover	Total Percent Shrub Cover	Total Percent Herbaceous Cover	Total Percent Vascular Percent Cover
Sentinel Site	N/A	15%	10%	10%	35%
1	N/A	3%	25%	10%	38%
2	N/A	5%	15%	5%	25%
3	N/A	5%	40%	5%	50%
4	N/A	10%	5%	3%	18%
5	N/A	15%	10%	5%	30%
6	N/A	5%	20%	10%	35%
7	N/A	1%	3%	6%	10%
8	N/A	N/A	7%	3%	10%
9	N/A	5%	15%	5%	25%
10	N/A	10%	8%	7%	25%
11	N/A	10%	25%	15%	50%
12	N/A	N/A	40%	5%	45%
13	3%	12%	35%	15%	65%
14	N/A	1%	40%	5%	46%
15	N/A	1%	35%	10%	46%

Notes: N/A = not applicable.

3.1.2 Relevé Plot Surveys – 2019

Overall, the 2019 relevé plot survey resulted in low plant diversity in addition to an overall lower cover of species. These results are likely attributed to the February 14, 2019, storm event, which deposited a high amount of sediment within the wash and resulted in flows removing smaller shrubs and trees within the wash.

In 2019, the total vascular plant cover at Plot 3 and Plot 14 did not change from the recorded percent cover in 2016. Plot 3 is located along an upper terrace outside of the Whitewater River and Plot 14 is located within Smoke Tree Ranch. Plot 13 is located primarily within Smoke Tree Ranch; however, the southern portion of this plot overlaps the Whitewater River. This plot resulted in an overall decrease in total vascular plant cover, from 65% in 2016 to 45% in 2019. While vegetation cover remains stable within Smoke Tree, the portion within the Whitewater River showed a lower cover of annual species. The remaining plots (Sentinel Site and Plots 4, 5, 9, and 10) showed a decrease in total percent vascular plant cover, with the most prominent decrease, 19%, occurring at the Sentinel Site.

Table 6 provides a summary of the 16 relevé plots and the total vascular plant cover recorded for each stratum. The relevé forms provide a species list for each plot that includes the layer in which the species was observed and an estimate of its cover class within that layer (refer to Attachment D).

Table 6. 2019 Relevé Plot Percent Cover Summary

Relevé Plot	Total Percent Overstory Hardwood Cover	Total Percent Low-Medium Tree Cover	Total Percent Shrub Cover	Total Percent Herbaceous Cover	Total Percent Vascular Percent Cover
Sentinel Site	N/A	8%	5%	3%	16%
3	N/A	5%	40%	5%	50%
4	N/A	5%	3%	2%	10%
5	N/A	10%	5%	1%	16%
9	N/A	3%	8%	1%	12%
10	N/A	12%	1%	1%	14%
13 ^a	3%	2%	30%	10%	45%
14	N/A	1%	40%	5%	46%

^a This plot overlaps both Whitewater River and Smoke Tree Ranch and includes the total percent cover for the entire plot.

4 References

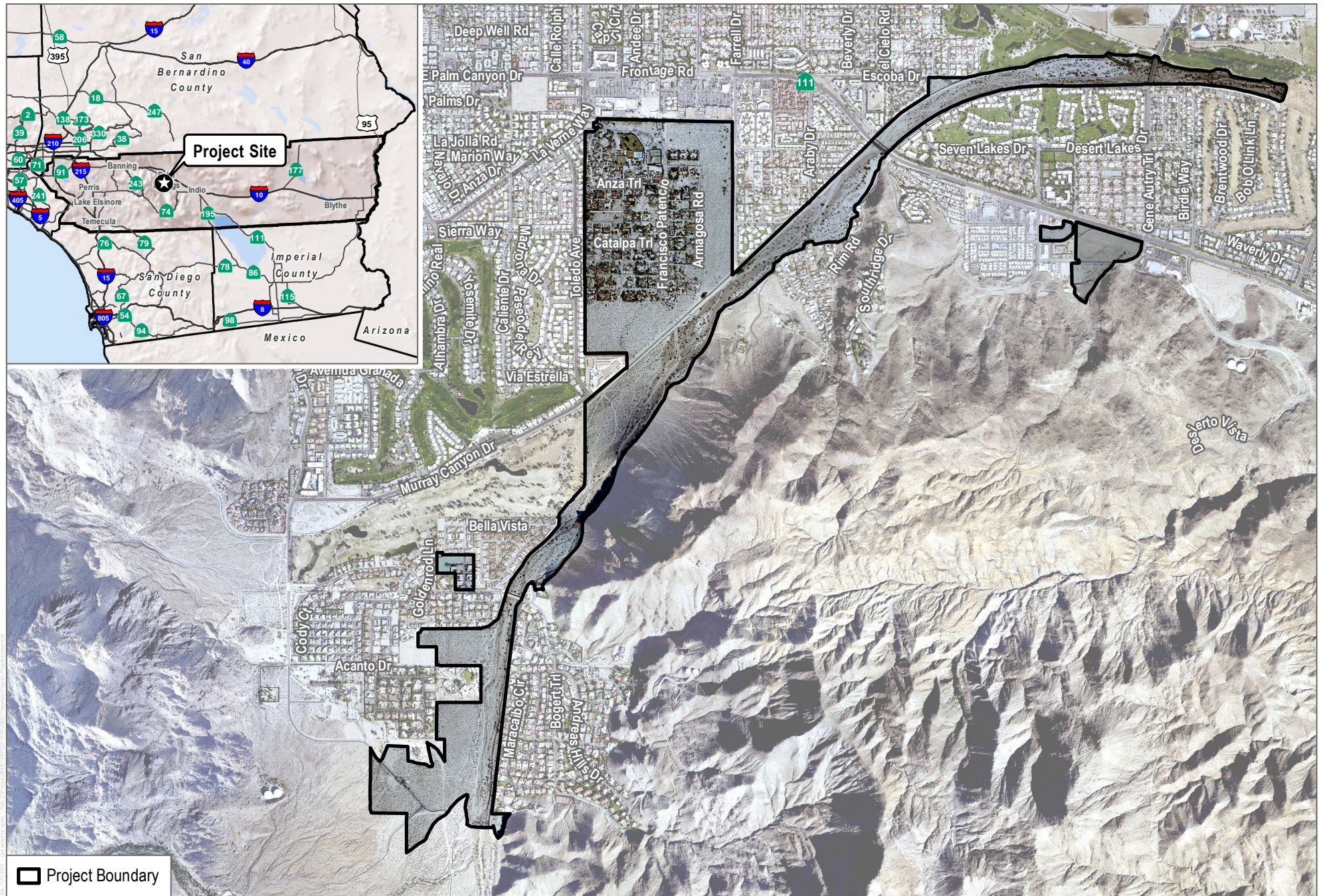
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Memorandum

Subject: Casey's June Beetle Vegetation Mapping Methodology and Results

USDA (U.S. Department of Agriculture). 2018. "California." State PLANTS Checklist. Accessed March 2018.
http://plants.usda.gov/dl_state.html.

WRCC (Western Regional Climate Center). 2019. "Palm Springs, California (046635)." <https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca6635>.



SOURCE: Riverside County 2016; 2019

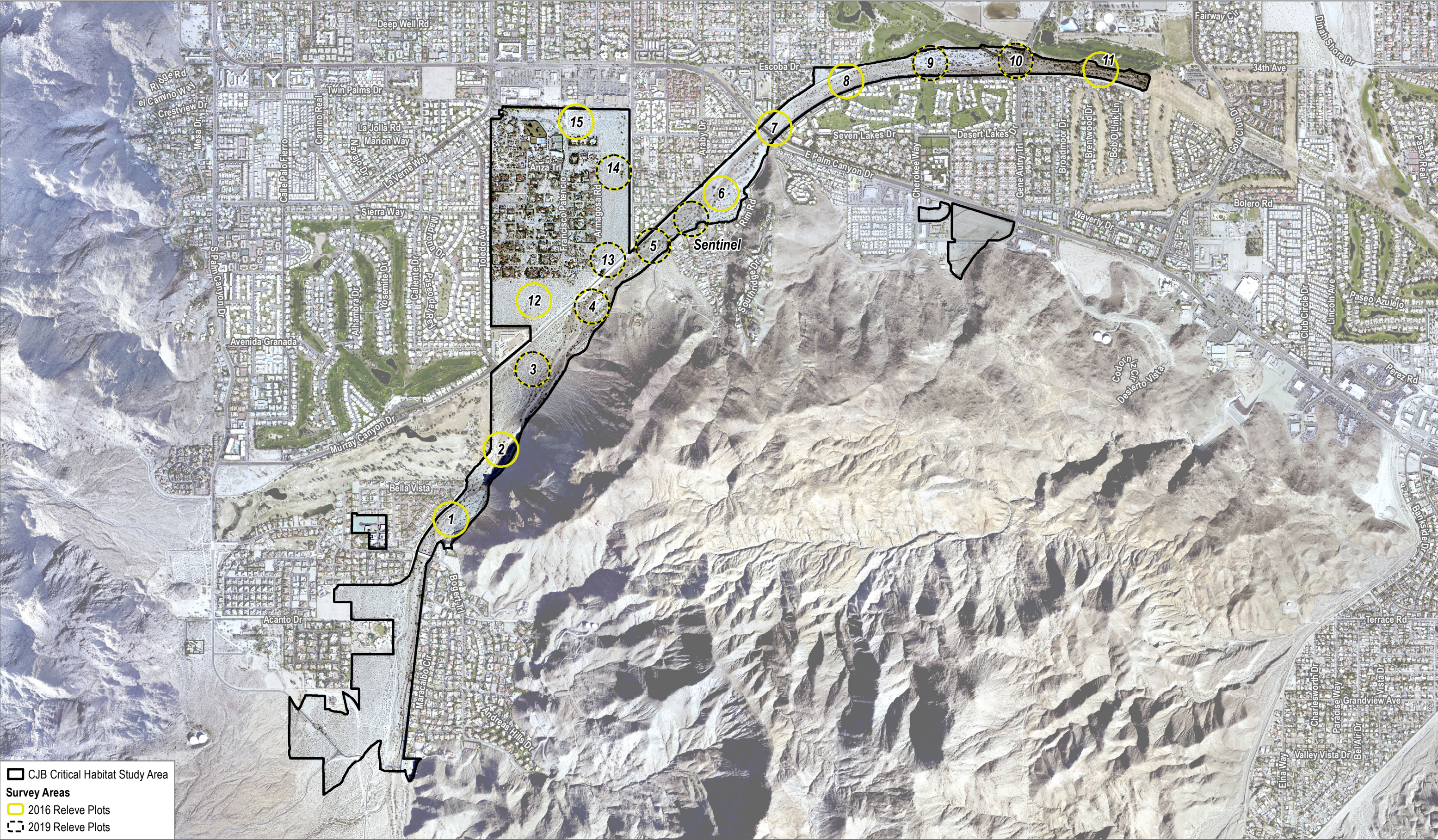
DUDEK



0 1,000 2,000 Feet

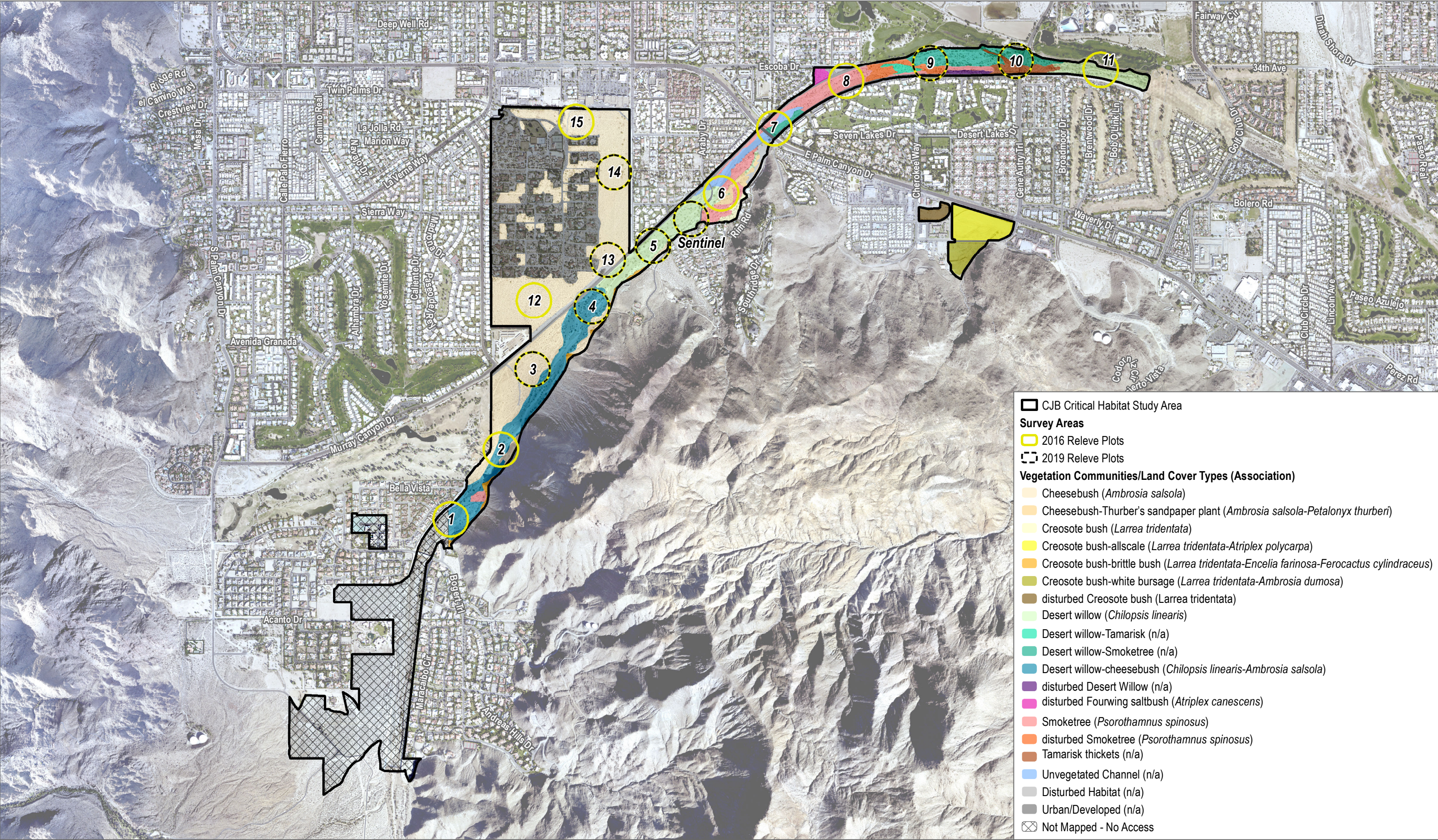
FIGURE 1

Project Location



SOURCE: Riverside County 2016, 2019

FIGURE 2
Survey Areas



SOURCE: Riverside County 2016, 2019

FIGURE 3
Vegetation Communities and Land Covers
Casey's June Beetle Vegetation Mapping Methodology and Results

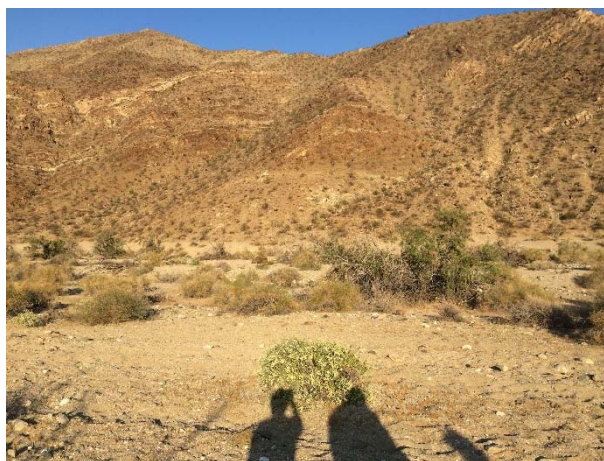
2016 Relevé Plot Photos

	
<p>2016 Photo from center of the Sentinel Relevé Plot Zero (0), facing North.</p>	<p>2016 Photo from center of the Sentinel Relevé Plot Zero (0), facing East.</p>
	
<p>2016 Photo from center of the Sentinel Relevé Plot Zero (0), facing South.</p>	<p>2016 Photo from center of the Sentinel Relevé Plot Zero (0), facing West.</p>

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 1, facing North.



2016 Photo from center of Relevé Plot 1, facing East.



2016 Photo from center of Relevé Plot 1, facing South.

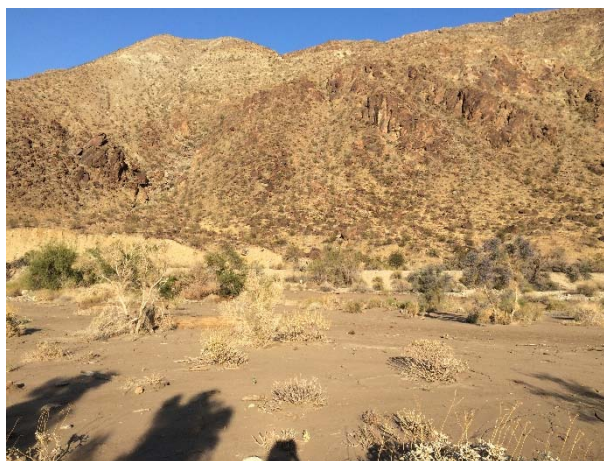


2016 Photo from center of Relevé Plot 1, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 2, facing North.



2016 Photo from center of Relevé Plot 2, facing East.



2016 Photo from center of Relevé Plot 2, facing South.



2016 Photo from center of Relevé Plot 2, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 3, facing North.



2016 Photo from center of Relevé Plot 3, facing East.



2016 Photo from center of Relevé Plot 3, facing South.



2016 Photo from center of Relevé Plot 3, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 4, facing North.



2016 Photo from center of Relevé Plot 4, facing East.



2016 Photo from center of Relevé Plot 4, facing South.



2016 Photo from center of Relevé Plot 4, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 5, facing North.



2016 Photo from center of Relevé Plot 5, facing East.



2016 Photo from center of Relevé Plot 5, facing South.



2016 Photo from center of Relevé Plot 5, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 6, facing North.



2016 Photo from center of Relevé Plot 6, facing East.



2016 Photo from center of Relevé Plot 6, facing South.



2016 Photo from center of Relevé Plot 6, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 7, facing North.



2016 Photo from center of Relevé Plot 7, facing East.



2016 Photo from center of Relevé Plot 7, facing South.



2016 Photo from center of Relevé Plot 7, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 8, facing North.



2016 Photo from center of Relevé Plot 8, facing East.



2016 Photo from center of Relevé Plot 8, facing South.



2016 Photo from center of Relevé Plot 8, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 9, facing North.



2016 Photo from center of Relevé Plot 9, facing East.



2016 Photo from center of Relevé Plot 9, facing South.



2016 Photo from center of Relevé Plot 9, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 10, facing North.



2016 Photo from center of Relevé Plot 10, facing East.



2016 Photo from center of Relevé Plot 10, facing South.



2016 Photo from center of Relevé Plot 10, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 11, facing North.



2016 Photo from center of Relevé Plot 11, facing East.



2016 Photo from center of Relevé Plot 11, facing South.



2016 Photo from center of Relevé Plot 11, facing West.

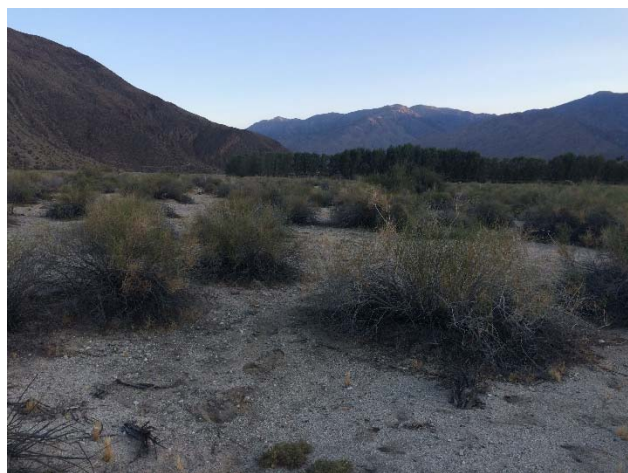
APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 12, facing North.



2016 Photo from center of Relevé Plot 12, facing East.



2016 Photo from center of Relevé Plot 12, facing South.



2016 Photo from center of Relevé Plot 12, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 13 (location a), facing North.



2016 Photo from center of Relevé Plot 13 (location a), facing East.



2016 Photo from center of Relevé Plot 13 (location a), facing South.



2016 Photo from center of Relevé Plot 13 (location a), facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 13 (location b), facing North.



2016 Photo from center of Relevé Plot 13 (location b), facing East.



2016 Photo from center of Relevé Plot 13 (location b), facing South.



2016 Photo from center of Relevé Plot 13 (location b), facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



2016 Photo from center of Relevé Plot 14, facing North.



2016 Photo from center of Relevé Plot 14, facing East.



2016 Photo from center of Relevé Plot 14, facing South.



2016 Photo from center of Relevé Plot 14, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS

	
<p>2016 Photo from center of Relevé Plot 15, facing North.</p>	<p>2016 Photo from center of Relevé Plot 15, facing East.</p>
	
<p>2016 Photo from center of Relevé Plot 15, facing South.</p>	<p>2016 Photo from center of Relevé Plot 15, facing West.</p>

2019 Subset Relevé Plot Photos

	
<p>2019 Photo from center of the Sentinel Relevé Plot Zero (0), facing North.</p>	<p>2019 Photo from center of the Sentinel Relevé Plot Zero (0), facing East.</p>
	
<p>2019 Photo from center of the Sentinel Relevé Plot Zero (0), facing South.</p>	<p>2019 Photo from center of the Sentinel Relevé Plot Zero (0), facing West.</p>

APPENDIX F
RELEVÉ PLOT PHOTOS



2019 Photo from center of Relevé Plot 3, facing North.



2019 Photo from center of Relevé Plot 3, facing East.



2019 Photo from center of Relevé Plot 3, facing South.




2019 Photo from center of Relevé Plot 3, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS

	
2019 Photo from center of Relevé Plot 4, facing North.	2019 Photo from center of Relevé Plot 4, facing East.
	
2019 Photo from center of Relevé Plot 4, facing South.	2019 Photo from center of Relevé Plot 4, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS

	
<p>2019 Photo from center of Relevé Plot 5 facing North.</p>	<p>2019 Photo from center of Relevé Plot 5, facing East.</p>
	
<p>2019 Photo from center of Relevé Plot 5, facing South.</p>	<p>A western directional photo is not available for this plot for 2019.</p>

APPENDIX F
RELEVÉ PLOT PHOTOS



2019 Photo from center of Relevé Plot 9, facing North.



2019 Photo from center of Relevé Plot 9, facing East.



2019 Photo from center of Relevé Plot 9, facing South.



2019 Photo from center of Relevé Plot 9, facing West.

APPENDIX F
RELEVÉ PLOT PHOTOS



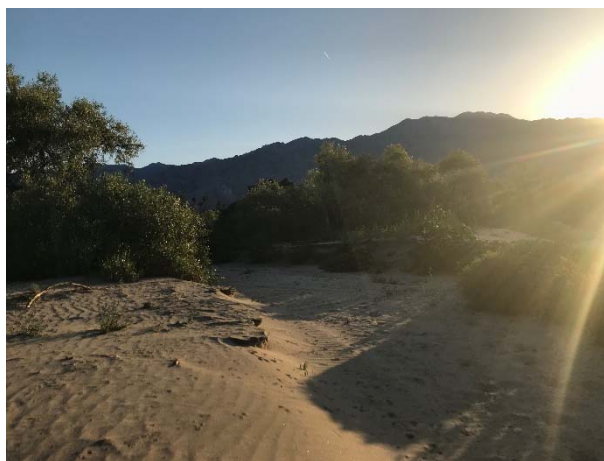
2019 Photo from center of Relevé Plot 10, facing North.



2019 Photo from center of Relevé Plot 10, facing East.



2019 Photo from center of Relevé Plot 10, facing South.







2019 Photo from center of Relevé Plot 10, facing West.





APPENDIX F
RELEVÉ PLOT PHOTOS

	
<p>2019 Photo from center of Relevé Plot 13 (location a), facing North.</p>	<p>2019 Photo from center of Relevé Plot 13 (location a), facing East.</p>
	
<p>2019 Photo from center of Relevé Plot 13 (location a), facing South.</p>	<p>2019 Photo from center of Relevé Plot 13 (location a), facing West.</p>

APPENDIX F
RELEVÉ PLOT PHOTOS

	
<p>2019 Photo from center of Relevé Plot 13 (location b), facing North.</p>	<p>2019 Photo from center of Relevé Plot 13 (location b), facing East.</p>
	
<p>2019 Photo from center of Relevé Plot 13 (location b), facing South.</p>	<p>2019 Photo from center of Relevé Plot 13 (location b), facing West.</p>

APPENDIX F
RELEVÉ PLOT PHOTOS

	
<p>2019 Photo from center of Relevé Plot 14, facing North.</p>	<p>2019 Photo from center of Relevé Plot 14, facing East.</p>
	
<p>2019 Photo from center of Relevé Plot 14, facing South.</p>	<p>2019 Photo from center of Relevé Plot 14, facing West.</p>

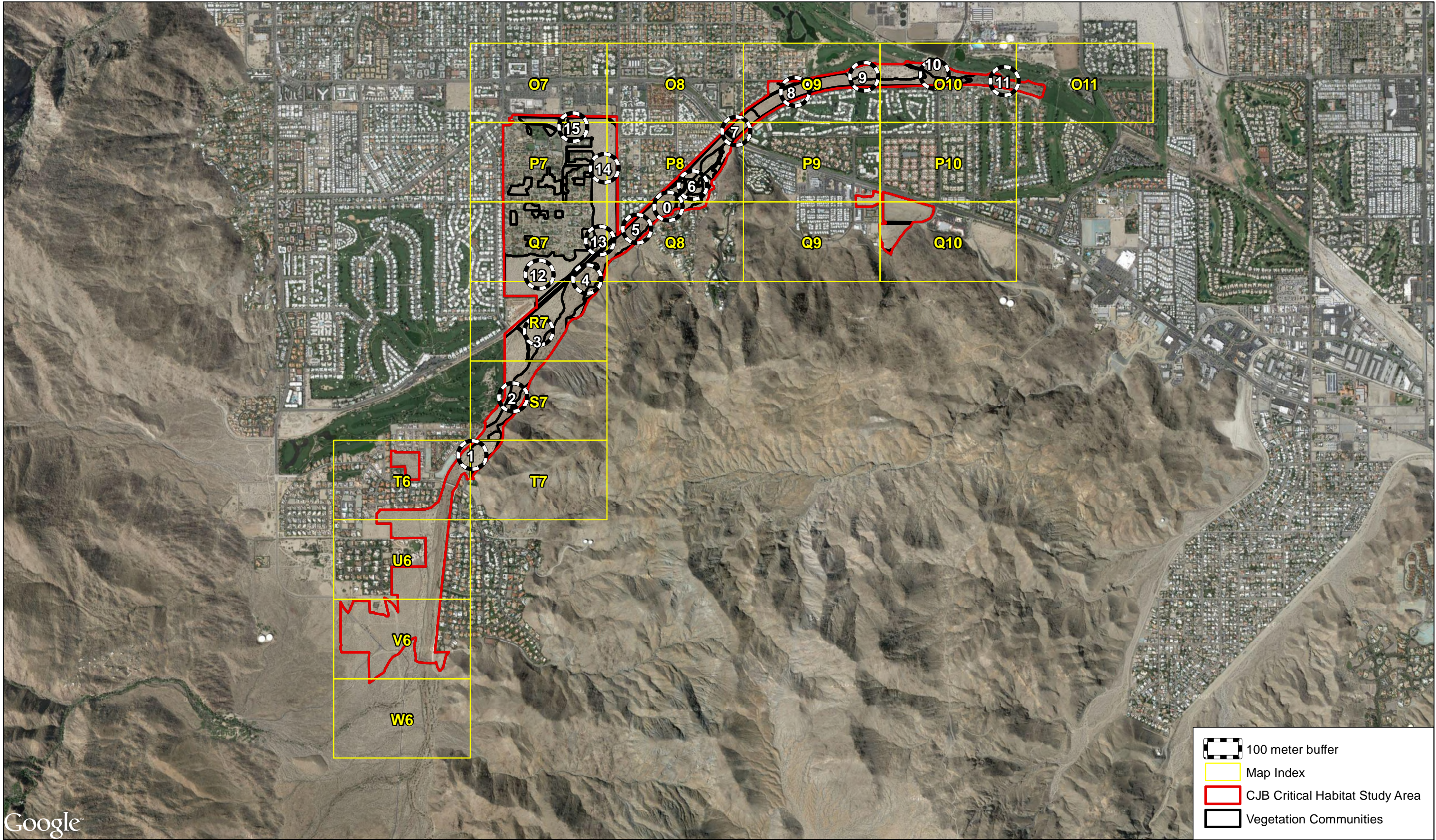
Supporting Information

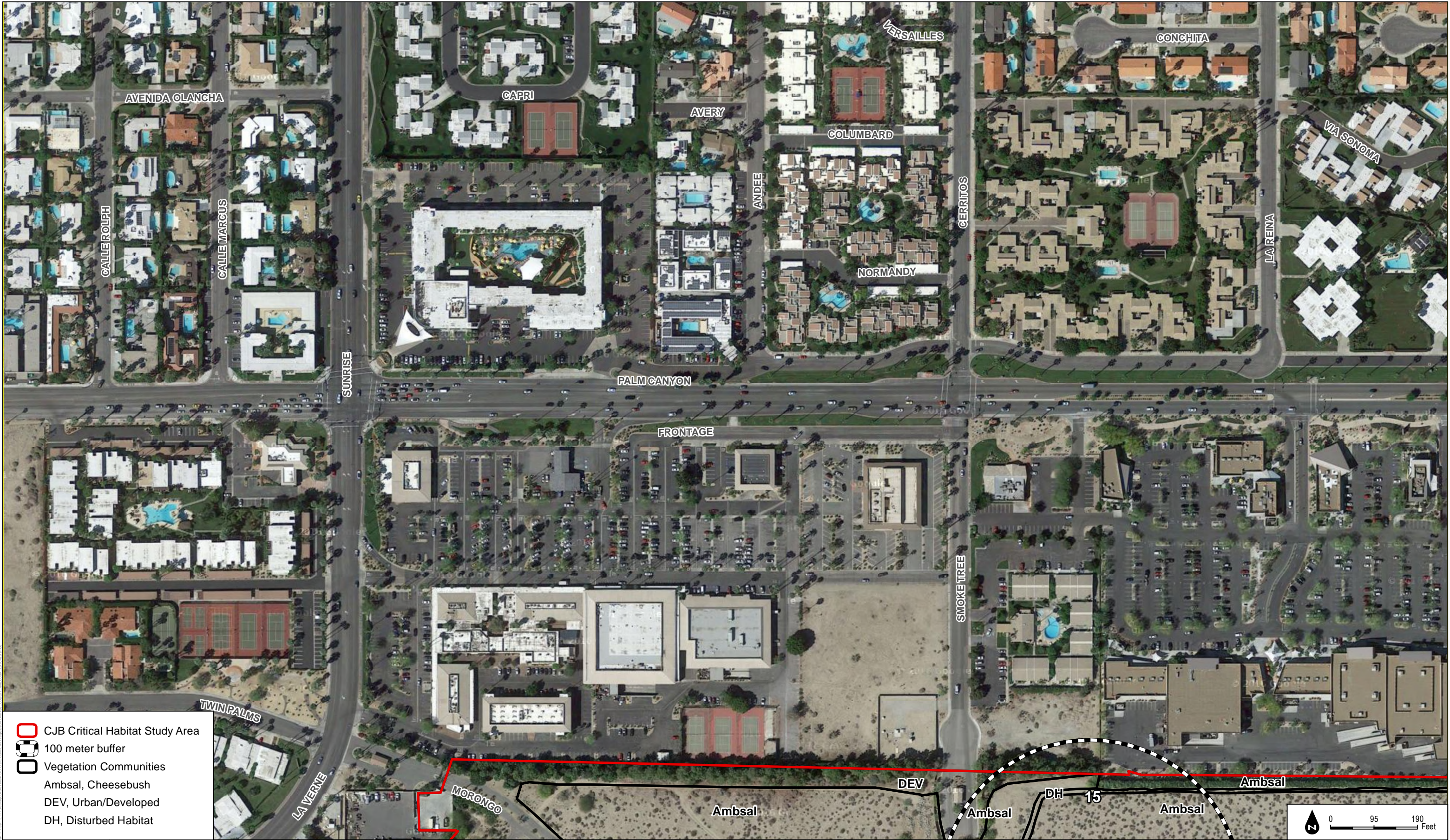
Appendices in Strittmater and Amoaku 2019, including the plant compendium (Appendix B), vegetation mapping forms (Appendix C), relevé survey forms (Appendix D), and relevé plot spreadsheet (Appendix E) from 2016 and 2019 are on file at the U.S. Fish and Wildlife Service, Palm Springs Fish and Wildlife Office, Palm Springs, California.

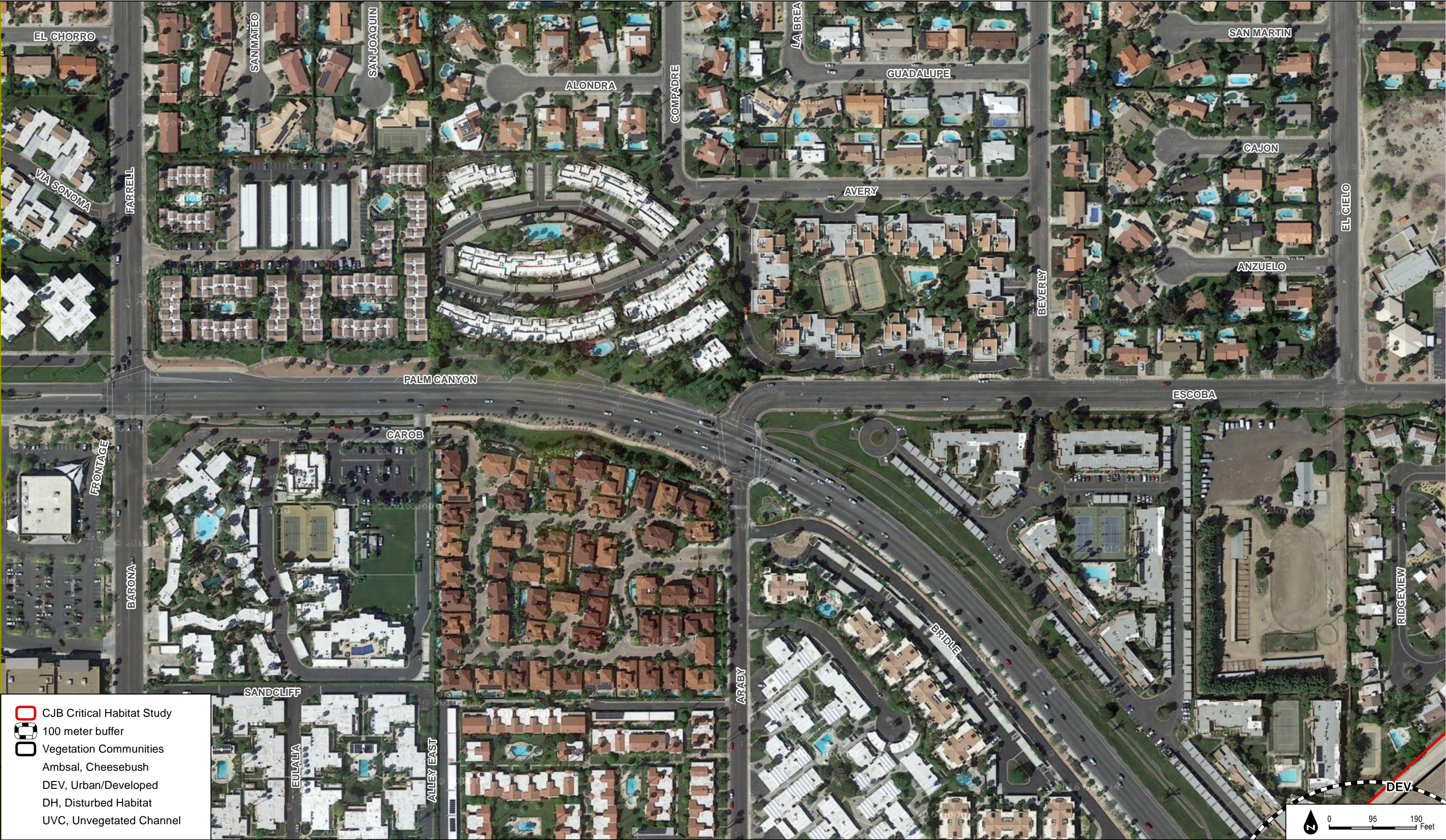
Plant Inventory 2015

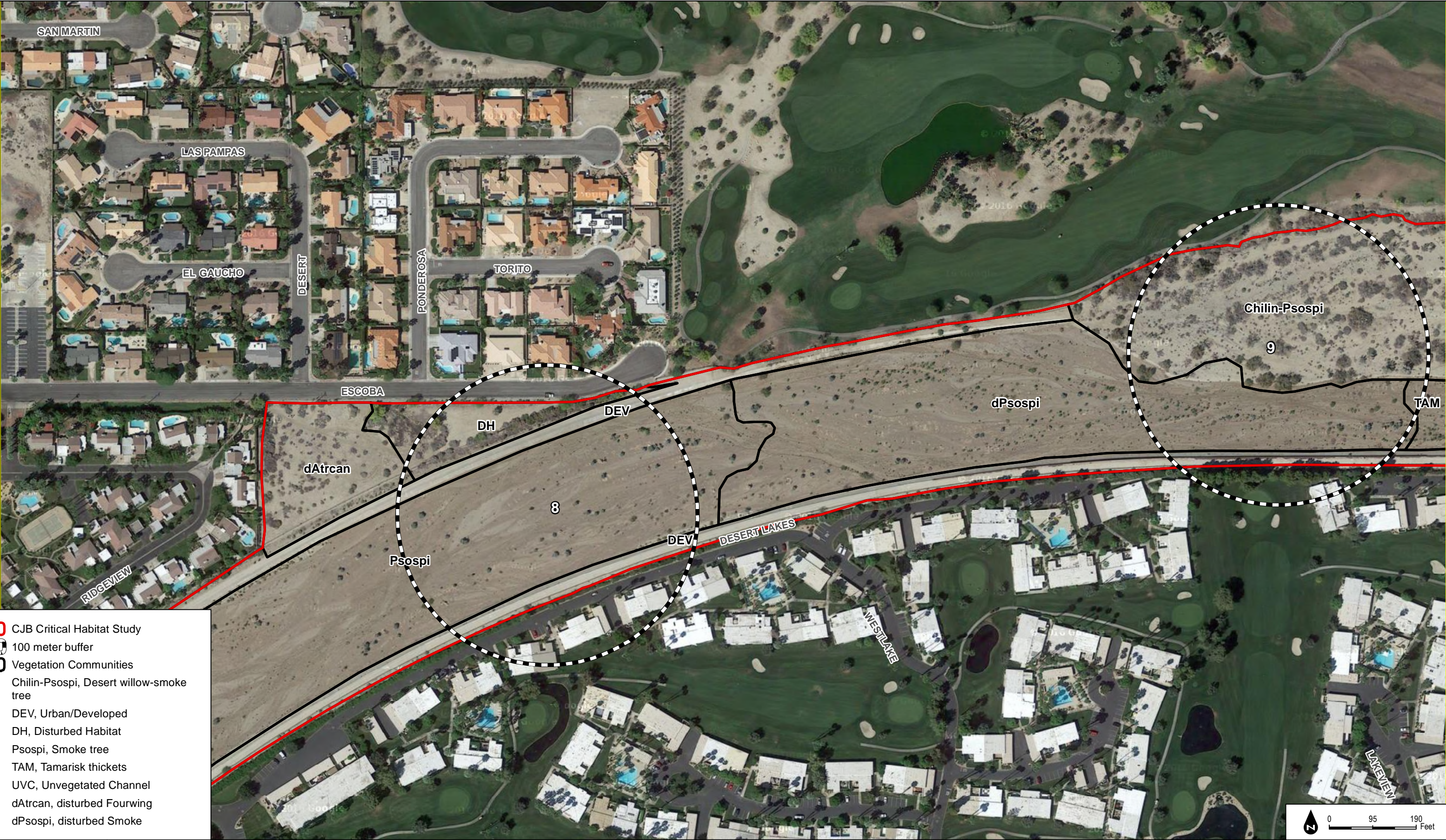
Georeferenced Vegetation Maps

Source: Dudek 2016









CJB Critical Habitat Study

100 meter buffer

Vegetation Communities

Chilin-Psospi, Desert willow-smoke tree

DEV, Urban/Developed

DH, Disturbed Habitat

Psospi, Smoke tree

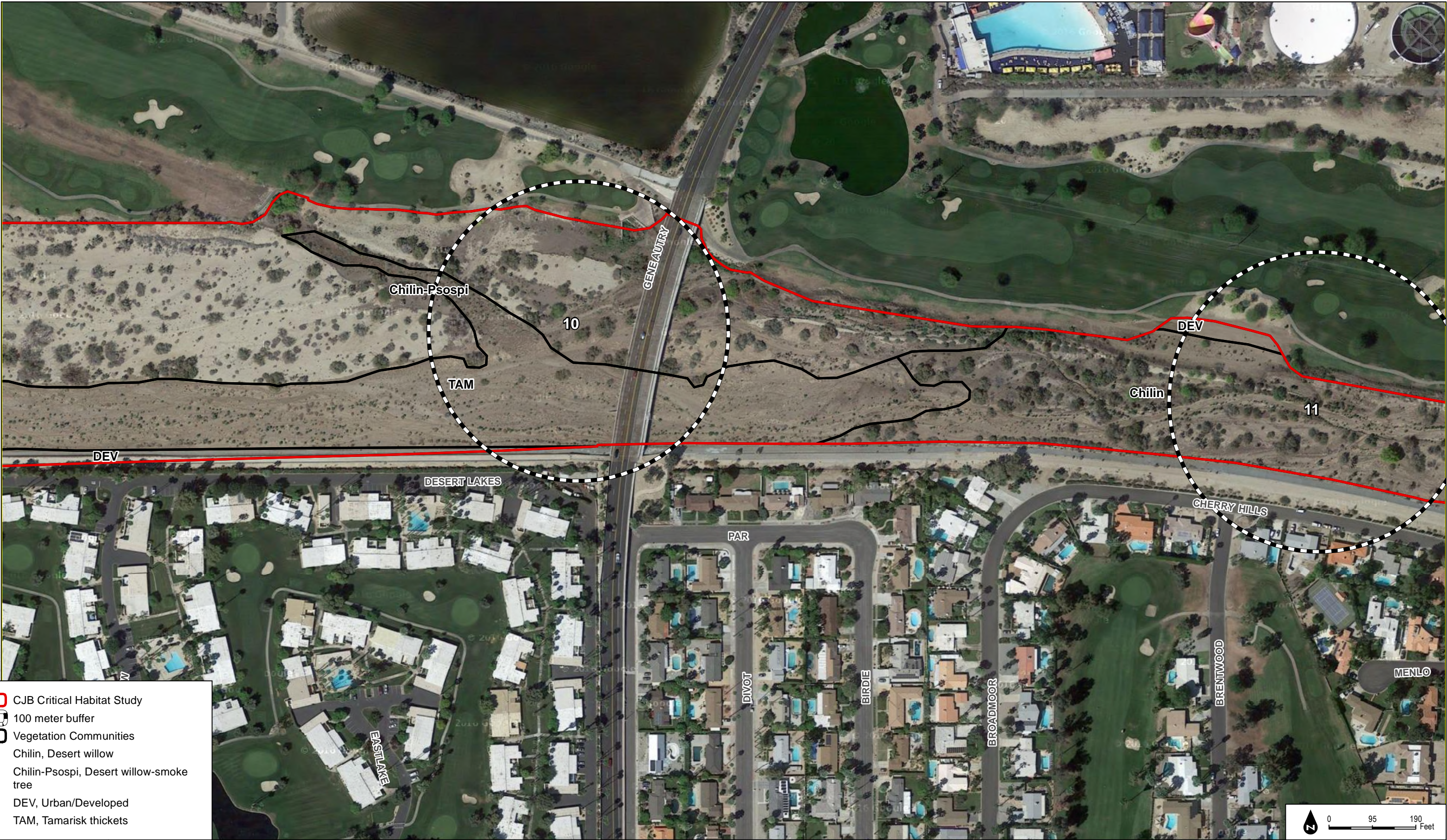
TAM, Tamarisk thickets

UVC, Unvegetated Channel

dAtrcan, disturbed Fourwing

dPsospi, disturbed Smoke

SOURCE: Google Imagery 2015; USFWS 2015.



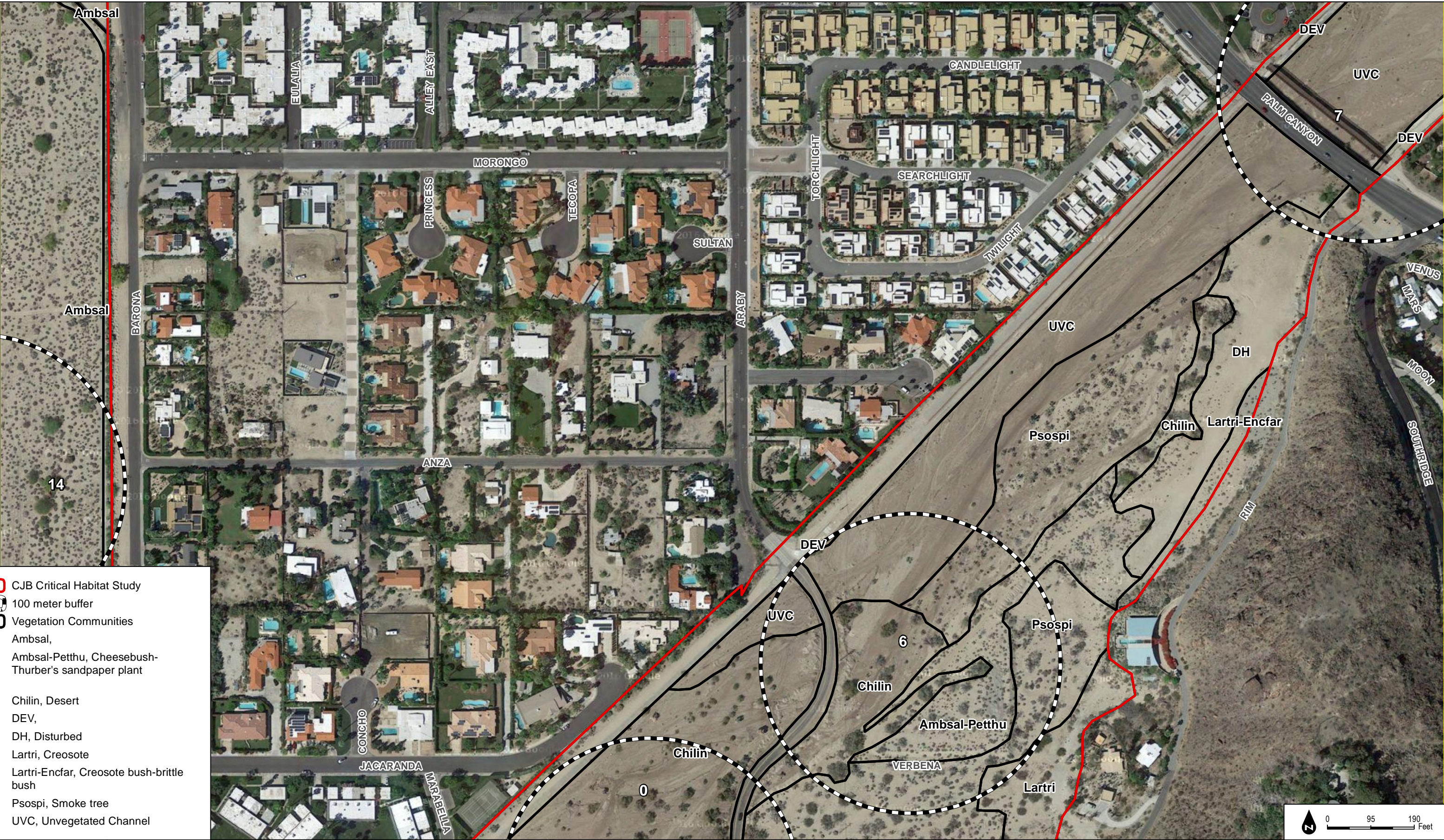


CJB Critical Habitat Study

100 meter buffer

Vegetation Communities

Chilin, Desert willow



CJB Critical Habitat Study

100 meter buffer

Vegetation Communities

Ambsal,
Ambsal-Petthu, Cheesebush-
Thurber's sandpaper plant

Chilin, Desert
DEV,
DH, Disturbed
Lartri, Creosote
Lartri-Encfar, Creosote bush-brittle
bush
Psospi, Smoke tree
UVC, Unvegetated Channel

SOURCE: Google Imagery 2015; USFWS 2015.



CJB Critical Habitat Study

100 meter buffer

Vegetation Communities

DEV, Urban/Developed

UVC, Unvegetated Channel

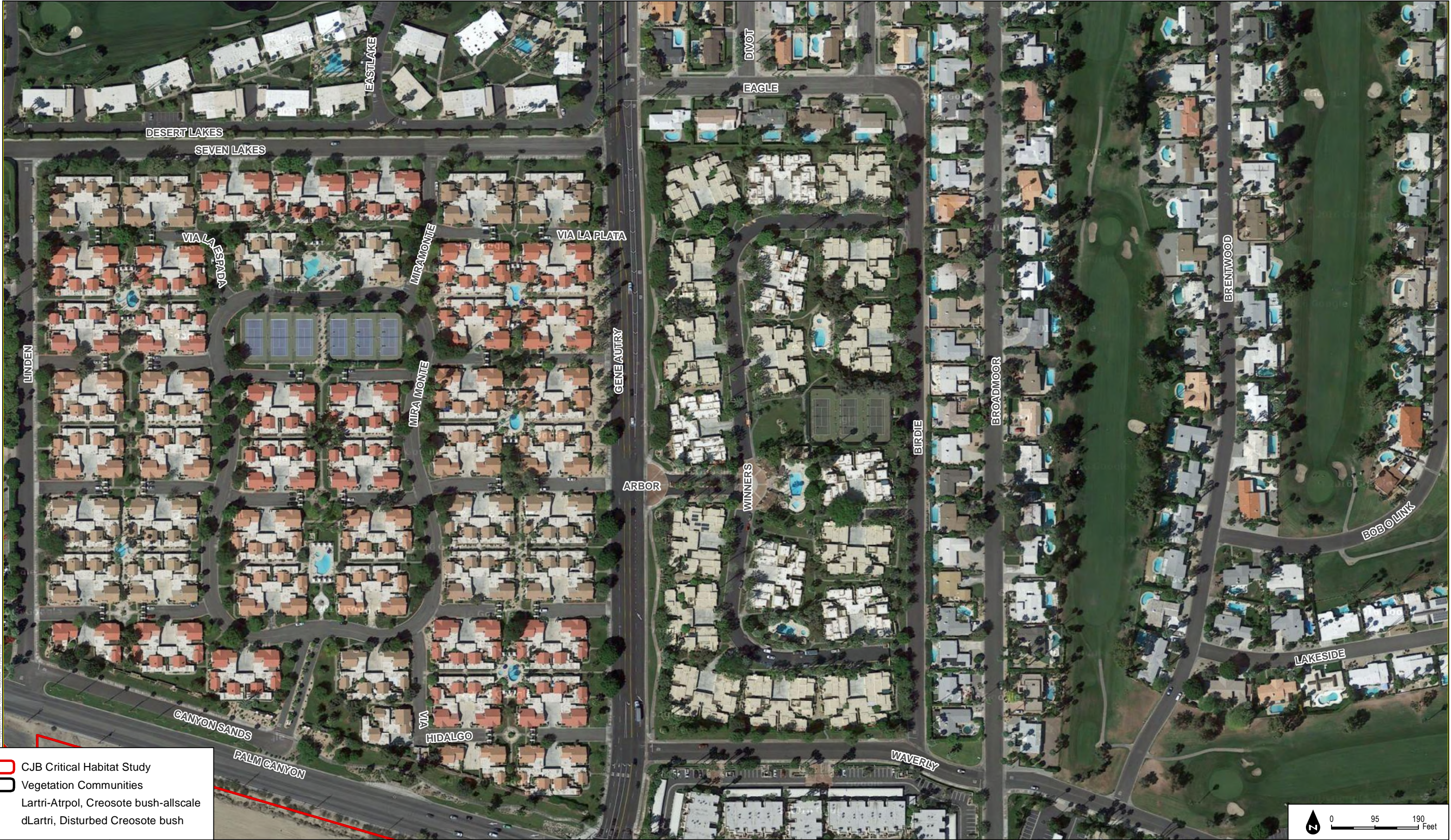
dLartri, Disturbed Creosote bush

SOURCE: Google Imagery 2015; USFWS 2015.

DUDEK

Casey's June Beetle - Vegetation Mapping

CJB Critical Habitat Study Area Vegetation Map - Page P9



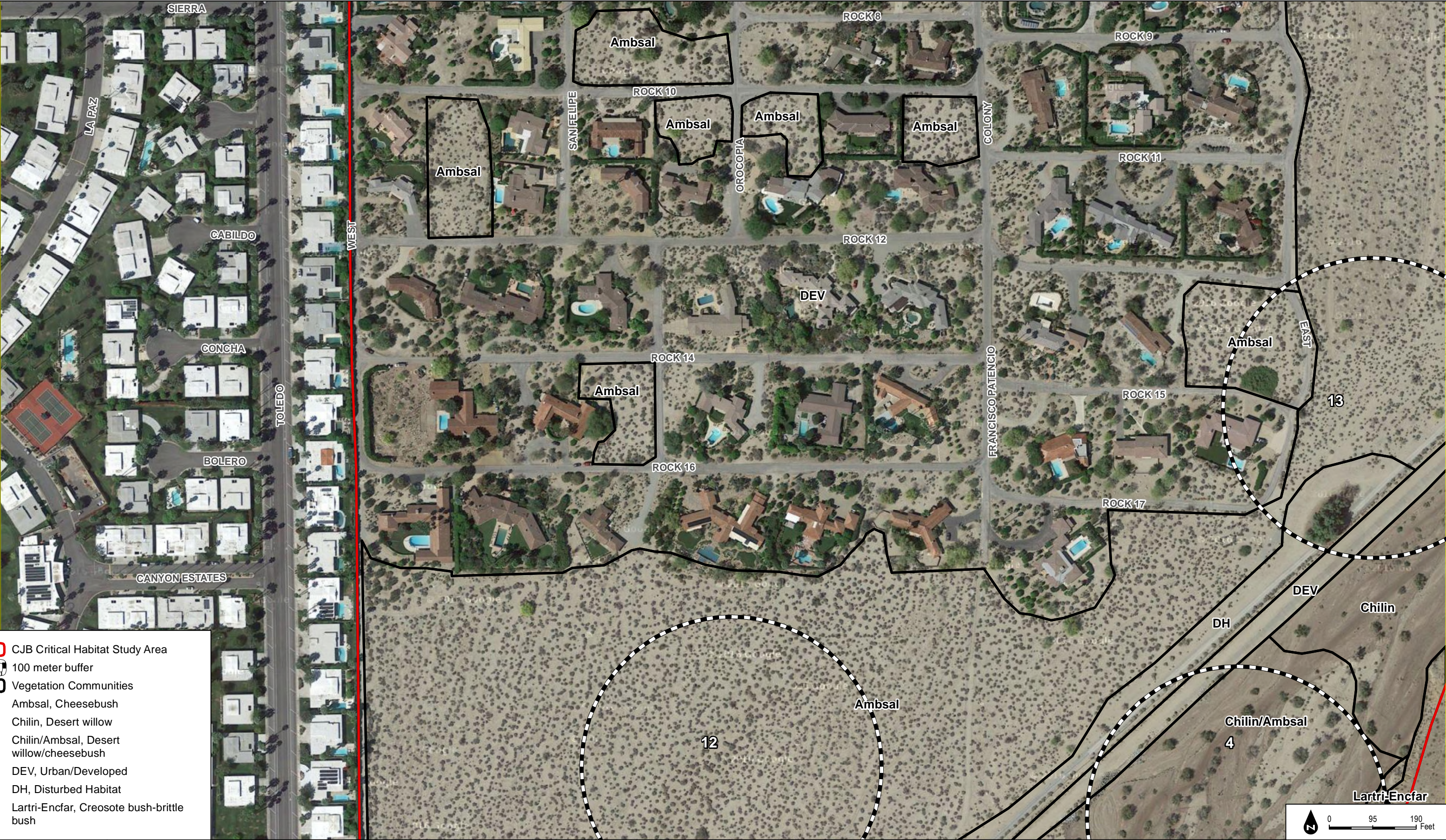
CJB Critical Habitat Study

Vegetation Communities

Larri-Atrpol, Creosote bush-allscale

dLarri, Disturbed Creosote bush

SOURCE: Google Imagery 2015; USFWS 2015.



CJB Critical Habitat Study Area

100 meter buffer

Vegetation Communities

Ambstal, Cheesebush

Chilin, Desert willow

Chilin/Ambstal, Desert willow/cheesebush

DEV, Urban/Developed

DH, Disturbed Habitat



Lartri-Encfar, Creosote bush-brittle bush

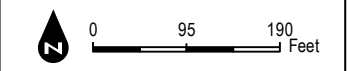


- CJB Critical Habitat Study Area
- 100 meter buffer
- Vegetation Communities
 - Ambsal, Cheesebush
 - Chilin, Desert willow
 - DEV, Urban/Developed
 - DH, Disturbed Habitat
 - Lartri, Creosote bush
 - Lartri-Encfar, Creosote bush-brittle bush
 - Psospi, Smoke tree

SOURCE: Google Imagery 2015; USFWS 2015.



-  CJB Critical Habitat Study
-  Vegetation Communities
 - Lartri-Ambdum, Creosote bush-white bursage
 - dLartri, Disturbed Creosote bush





CJB Critical Habitat Study Area

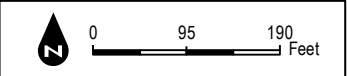
Vegetation Communities

DEV, Urban/Developed

Lartri, Creosote bush

Lartri-Ambdum, Creosote bush-white bursage

Lartri-Atrpol, Creosote bush-allscale





- CJB Critical Habitat Study Area
- 100 meter buffer
- Vegetation Communities
 - Ambsal, Cheesebush
 - Chilín/Ambsal, Desert willow/cheesebush
 - DEV, Urban/Developed
 - DH, Disturbed Habitat
 - Lartri-Encfar, Creosote bush-brittle bush

SOURCE: Google Imagery 2015; USFWS 2015.



CJB Critical Habitat Study

100 meter buffer

Vegetation Communities

Ambstal, Cheesebush

Chilin/Ambstal, Desert willow/cheesebush

Lartri-Encfar, Creosote bush-brittle bush

Psospi, Smoke tree

SOURCE: Google Imagery 2015; USFWS 2015.



CJB Critical Habitat Study Area

100 meter buffer

Vegetation Communities

Chilin/Ambasal, Desert willow/cheesebush

Lartri-Encfar, Creosote bush-brittle bush

Not Mapped

UVC, Unvegetated Channel

SOURCE: Google Imagery 2015; USFWS 2015.



CJB Critical Habitat Study Area

100 meter buffer

Vegetation Communities

Chilin/Ambasal, Desert willow/cheesebush

Lartri-Encfar, Creosote bush-brittle bush

Not Mapped

UVC, Unvegetated Channel

SOURCE: Google Imagery 2015; USFWS 2015.





CJB Critical Habitat Study Area

Vegetation Communities
Not Mapped



CJB Critical Habitat Study Area

Vegetation Communities
Not Mapped

APPENDIX E – Genetics Publications and Report

Rubinoff *et al.* 2020



Phylogenomics reveals conservation challenges and opportunities for cryptic endangered species in a rapidly disappearing desert ecosystem

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Abstract

There is growing evidence for the decline of cryptic species across the planet as a result of human activities. Accurate data regarding patterns of poorly known or hard-to-find species diversity is essential for the recognition and conservation of threatened species and ecosystems. Casey's June beetle *Dinacoma caseyi* is a federally listed endangered species restricted to the Coachella Valley in southern California, where rapid development is leading to habitat fragmentation. This fragmentation may be disproportionately impacting a wide-range of poorly-dispersing, cryptic species, including Casey's June beetle, which has flightless females. We characterized 1876 single nucleotide polymorphisms from across the genome along with 1480 bp of mitochondrial DNA of all confirmed extant *Dinacoma* populations. We found that *Dinacoma* is isolated into three distinct species, including evidence for a previously undescribed species revealed during this study. Each is restricted to a small part of the inland desert region. Our results suggest unappreciated and fine scale diversity, which may be reflected in the other cryptic species of the region. Patterns of diversity in non-vagile species should guide ongoing conservation planning in the region. These results show that genetic exchange within the one remaining island of beetle habitat (Palm Canyon Wash) is not limited. However, non-vagile species, such as Casey's June beetle, may not colonize suitable but fragmented habitat islands which presents risks to the species due to habitat loss and periodic natural events that may put the single population at risk of extirpation.

Keywords *Dinacoma* · DdRAD phylogenomics · Coachella valley · Southern California · MtDNA · DNA barcodes

Communicated by Anurag Chaurasia.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10531-020-01968-w>) contains supplementary material, which is available to authorized users.

Extended author information available on the last page of the article

Introduction

Although insects represent the majority of Earth's known and predicted biodiversity (Mora et al. 2011), they receive a disproportionately small fraction of conservation resources. There is overwhelming, growing evidence that many insect communities are in decline, and that these insect communities may be at least as vulnerable as associated vertebrate communities (Vogel 2017; Lister and Garcia 2018; Loboda et al. 2018; Sánchez-Bayo and Wyckhuys 2019). Yet with the majority of conservation resources dedicated to vertebrates, the implicit assumption is that habitat conservation plans relying on vertebrate umbrella species will preserve invertebrates, despite evidence to the contrary (e.g. Rubinoff 2001; Rubinoff and Sperling 2004; Régnier et al. 2009, 2015; Mckinney 1999). Conservation planning that continues to ignore threatened and less vagile invertebrates may leave them and similar species in peril, threatening biodiversity and ecosystem integrity. While it is not practical to intensively survey all threatened invertebrate populations, using appropriate invertebrate surrogates that reflect the population and landscape-scale challenges faced by less dispersive organisms will contribute to a more realistic and effective template for inclusive reserve planning and conservation of biodiversity.

California is a biodiversity hotspot and the subject of extensive conservation planning and development mitigation schemes for decades (e.g. CDFG 1993; CVAG 2016); fine scale patterns of endemism make it an ideal region for investigating how cryptic invertebrate diversity might be used to improve ongoing reserve design. The phylogeography of California species is complex, and variations in geology and microclimates have helped to generate the largest number of endemic species of any U.S. state (Stein et al. 2000); unfortunately, California also hosts more endangered animals than anywhere else in the nation (Dobson et al. 1997; USFWS 2019). In southern California, much of the conservation attention has focused on the coastal region which supports the highest levels of regional endemism (Stein et al. 2000) and development due to the expansion of the Los Angeles-San Diego metropolitan corridor. In recent decades, urbanization has pushed further east, and the desert valley regions are undergoing rapid conversion. The Coachella Valley, just east of Los Angeles, now hosts multiple federally listed endangered species prompting the creation of a Multiple Species Habitat Conservation Plan to balance human needs with the conservation of remaining natural areas critical to preserving endangered biological diversity (Alagona and Pincetl 2008). As usual, the focus has been on using vertebrate surrogates to conserve less-visible species. Conversely, little has been done to assess patterns of diversity for rare invertebrates and how they might serve as useful umbrella species for vertebrates and other, less vagile and more cryptic species that are also threatened by habitat destruction.

Unfortunately, data collection reflecting endangered invertebrate diversity and endemism in these inland desert areas has not kept up with the pace of development, possibly eliminating restricted species that needed conservation attention. A failure to recognize unique genetic diversity can lead to mismanagement and unnecessary extinction, since small populations with limited genetic exchange typically experience a loss of genetic diversity and face higher extinction risk (Avise 1989; Frankham 2005). Incomplete knowledge of species boundaries and cryptic diversity can impart an erroneously optimistic impression of connectivity among what are actually disjointed populations, or even taxa that represent distinct species or sub-species (Keogh et al. 2008; Murphy et al. 2011). Without such crucial information, effective conservation of diversity is difficult or impossible, undermining urgently needed action while squandering resources applied to ill-

informed mitigation. Phylogeographic research focused on fine-scale patterns of biodiversity and species boundaries are essential for effective conservation, and such intensive studies often reveal novel, cryptic species diversity (Holland and Hadfield 2002; Shaffer et al. 2004; Griffiths et al. 2010).

As a result of rapid development in the Coachella Valley, the region's biota is in urgent need of phylogeographic studies to better document patterns of diversity and endemism, and guide conservation strategies. Casey's June beetle (*Dinacoma caseyi* Blaisdell 1930) is a federally listed endangered species, restricted to the vicinity of Palm Springs, the largest city in the Coachella Valley; it has suffered dramatic population reductions due to development of its desert habitat (USFWS 2011). It is one of two described members of a genus confined to particular alluvial habitats in southern California, both species with flightless, fossorial, females occurring in highly localized populations. As such, the beetle represents a conservative model for understanding the impacts of fragmentation on population connectivity and isolation of the Coachella Valley's less vagile endemic species. Additionally, the genetic independence of *D. caseyi* and its sole congener, *D. marginata*, which occurs in isolated pockets to the south and west of *D. caseyi*, has never been phylogenetically examined and may be important in understanding broader patterns of biodiversity in other cryptic species in the region. While historically more widespread on the coast, *Dinacoma* now appears to be largely restricted to three isolated populations. Thus, the genus *Dinacoma* presents an ideal opportunity to investigate both local and regional patterns of cryptic species endemism among widely isolated populations in varied ecological contexts in southern California.

Nothing is known about systematic and genetic relationships between *Dinacoma* species, and both fine-scale and broader geographic assessments of relationships are needed. Further, since the taxonomy of *Dinacoma* has never been investigated at the molecular level, we also wanted to assess DNA-based support for the recognition of any unique populations within *D. caseyi* and species-level divisions across *Dinacoma*, and with respect to populations of the sister species *D. marginata*. We have sampled the full range of *D. caseyi*. *D. marginata* was described from coastal southern California but hasn't been recollected there in decades (likely due to obliteration of habitat). The other two populations we used, Bautista Canyon and San Felipe, are either newly discovered (San Felipe) or the only known source currently available (Bautista) for *D. marginata*. There may be other, unconfirmed, populations of *Dinacoma* isolated in pockets of southern California, or even Baja California, Mexico, but they are rare and were not available for this study.

Characterization of cryptic diversity and structure among *Dinacoma* populations and species is not only vital for management of *D. caseyi*, but, because the females are fossorial and flightless, also provides insight into broader phylogeographic patterns of endemism that may be shared by other poor-dispersing taxa in the threatened desert regions of southern California. Because mtDNA has historically been used to assess species and conservation status for a wide range of animals (e.g. Moritz 1994; Rubinoff 2006), comparing its utility to more data-rich but costly genomic techniques is relevant to future conservation genetic research. *Dinacoma* also provides an opportunity to understand how patterns of inheritance in the maternally transmitted mtDNA genome might be disproportionately impacted by extremely biased sex-based dispersal (in this case flightless females) as compared to patterns from nuclear markers. We used a combination of Sanger sequencing and double-digest restriction-site associated DNA sequencing (ddRAD) to investigate the population genetics, biogeography, and phylogenomics of *Dinacoma* and asked the following questions: Is there evidence for the reciprocal monophyly of the *Dinacoma* species and populations or do they show signs of recent genetic exchange? What

is the genetic diversity and distribution of the endangered *D. caseyi* as compared to *Dinacoma* populations outside of the Palm Springs area? How is development in the Coachella Valley affecting fine scale genetic diversity in *D. caseyi*, as reflected by genomic structuring? Has having flightless females led to more genetic isolation in maternally inherited mtDNA than nDNA across populations? And, what are the phylogeographic patterns in *Dinacoma* as they relate to current and future conservation planning? While this study is focused on a single genus, the focal species is a federally listed endangered species and, by virtue of its cryptic life history and flightless females, likely represents a more sensitive model species for habitat conservation plans that are intended to save a broader measure of biodiversity. By focusing on the most sensitive, rather than the more resilient endemic members of a threatened ecosystem, conservationists might have a better chance to preserve overall community structure and function.

Methods

We used both genomic sampling of genome-wide nuclear DNA and 1480 bp of mitochondrial (mt) DNA data from *cytochrome oxidase subunit I (COI)* to understand population structure and diversity within the remnant *Dinacoma caseyi* population. By comparing nuclear and mitochondrial datasets, we can not only explore the impacts of urbanization and inform conservation efforts for this recently listed, endangered beetle, but also examine the relative sensitivity and utility of nuclear vs. mitochondrial markers.

Sample acquisition and DNA extraction

Twenty adult *D. caseyi* and 28 *D. marginata* from three localities representing much of the range of the genus (Fig. 1) were collected into 70% + EtOH. *Dinacoma caseyi* were collected by hand at blacklights from their only known range in Palm Springs, Riverside County, CA. *Dinacoma marginata* were collected at blacklights from the two known extant population sites representing divergent parts of their inland range, from the San Felipe Valley in San Diego County, CA in the south, north to the Bautista Canyon region near Hemet in Riverside County, CA, only 40 km from where *D. caseyi* occurs. Two legs were dissected from adult beetles for DNA extraction. The remainder of each sample was placed into fresh 90–95% EtOH in separate, labeled, vials and deposited in the University of Hawaii Insect Museum (UHIM) as a voucher specimen at -80°C .

Separate DNA extractions were prepared for Sanger sequencing and for ddRAD in the labs of DR and SG, respectively. For Sanger sequencing, DNA was extracted from a single leg using the DNeasy™ Blood & Tissue kit (Qiagen). Tissue was manually ground in 1.5 mL Eppendorf safe-lock tubes (Eppendorf North America, Hauppauge, NY) using tube-fitted pestles. Proteinase-K digestion enzyme was added to tissue followed by 24 h incubation at 55°C . During this time, samples were constantly rotated using a VWR Tube Rotator (Avantor, Radnor, PA). All remaining extraction protocols followed Qiagen standards. For ddRAD, a single leg was homogenized in tissue lysis buffer using a 2010 Geno/Grinder Automated Tissue Homogenizer and Cell Lyser (SPEX SamplePrep, Metuchen, NJ, USA) for 30 s at 1500 rpm. The homogenate was then incubated in a 55°C water bath for 3 h. Incubation was followed by extraction on a Kingfisher Flex 96 automated extraction instrument (Thermo Scientific, Waltham, MA) using standard protocols with a NucleoMag Tissue Kit (MACHEREY-NAGEL, Düren, Germany). The quantity and quality of the extracted DNA samples were determined using a Quant-it Picogreen assay

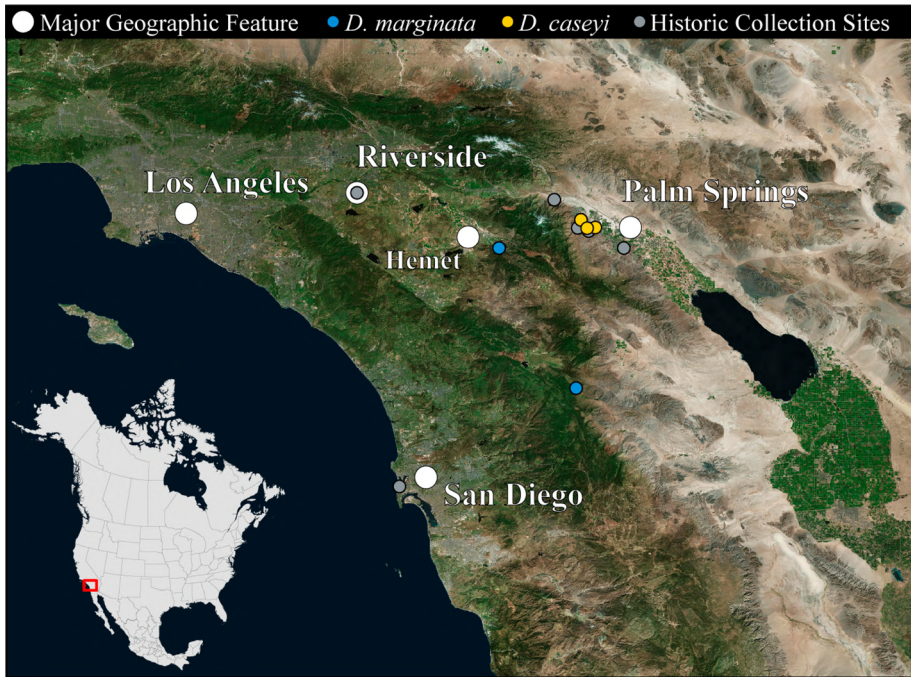


Fig. 1 *Dinacoma* sampling map. Colored markers represent collection localities from this study. *D. caseyi* all collected from the Palm Springs/Cathedral City area; *D. marginata* collected from Bautista Canyon near Hemet, CA (northern point) and the San Felipe Valley region (southern point). Grey markers indicate additional historic collection points for members of the *Dinacoma* genus. Dots encompass all known collection localities for *Dinacoma*

(Thermo Fisher, Waltham, MA) on a SpectraMax M2 Plate Reader (Molecular Devices, Sunnyvale, CA).

Sanger sequencing

Polymerase chain reaction (PCR) for *COI* was performed using either a BioRad T100™ or C1000 Touch™ thermal cycler. Primer pairs LCO-1490/HCO-2198 and Jerry/Pat2 were used to sequence 1480 base pairs of the *COI* (Folmer et al. 1994; Simon et al. 1994) under the following thermal regime: 3 min at 94 °C, 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 70 °C for 1 min, followed by a final 70 °C extension for 10 min and a 4 °C hold until termination. For those samples where both primer pairs failed to amplify, we paired primers LCO-1490/Pat2 to limit the potential for binding errors along the amplicon. This proved effective for a substantial portion of otherwise difficult samples. PCR products were purified using QIAquick® spin columns (Qiagen) following standard protocols. Sanger sequencing services were provided by either Eurofins Genomics (www.eurofinsgenomics.com) or the ASGPB Laboratory at the University of Hawaii Manoa (www.hawaii.edu/microbiology/asgpb). All sequences were aligned and manually checked for errors using GENEIOUS v7.1.9 (<https://www.geneious.com>, Kearse et al. 2012). Alignment was completed using the Geneious aligner algorithm with default parameters. Protein translation was conducted to identify a suitable reading frame and survey for internal stop

codons signaling potential pseudogenes. To identify an outgroup, we found the most closely related Scarab from the melolonthine subfamily using *COI* sequence data from the Barcoding of Life Database (BOLD), which was *Amblonoxia fieldi*, a species that occurs broadly in the southwestern U.S. and is found along the southern coast of California. *Amblonoxia fieldi* sequences were used to root analyses of *COI* data. *Amblonoxia* and *Dinacoma* are 2 of 11 genera within the tribe Melolonthini, including the more widespread genera *Polyphylla* and *Phyllophaga*. Unfortunately, no studies have included sampling of *Dinacoma* and *Amblonoxia*, however from preliminary analyses of all available sequences on BOLD, they appear to be more closely related to each other than any of the other genera in the tribe.

ddRAD library preparation

ddRAD DNA extractions were normalized to 4 ng/μL in 44.5 μL dH₂O and ddRAD libraries were prepared following Peterson et al. (2012). To prepare the library, 175 ng of DNA from each individual was digested using the restriction enzymes *Nla*III and *Mlu*CI. One of 48 unique barcode adapters was ligated to the restriction overhang, generating inline barcodes. Two subpools of samples containing 28 and 29 of these barcodes were generated and size-selected using a 1.5% agarose gel cassette on a Blue Pippin electrophoresis unit (Sage Science, Beverly, MA) with a target size selection of “narrow 400 bp”. The final PCR amplification step was run for 10 cycles, during which Illumina i7 barcodes were added for each sub-pool, and PCR products were cleaned using solid-phase reversible immobilization (SPRI) beads at a 1.5:1 ratio of PEG containing bead solution to sample volume (DeAngelis et al. 1995; Rohland and Reich 2012). The cleaned subpool libraries were analyzed for quantity and size distribution using the NGS Fragment Analysis Kit on a Fragment Analyzer and pooled at equal molar ratios to generate the final library (containing 57 individuals). This library was sequenced on a single lane of 100 bp single-end sequencing on an Illumina HiSeq 4000 sequencer.

Read clustering and SNP selection

Raw Illumina sequencing reads were processed using the STACKS v. 2.1 (Catchen et al. 2013) pipeline. In STACKS, the *process_radtags* function was used to clean and demultiplex data, removing reads with uncalled bases or low-quality scores and rescuing barcodes when possible. Given the lack of a reference genome for *Dinacoma*, de novo assembly of loci was conducted using the *denovo_map.pl* wrapper. The program was parameterized to allow 2 mismatches between loci within individuals and 3 mismatches between loci between individuals when creating the catalog, and other parameter set at default values. The STACKS core program *populations* was used for filtering and final SNP calling. The first SNP at each locus was retained, and SNPs found in < 15% of individuals were removed. The resulting dataset was then inspected, and a missing data threshold of 20% was used to identify and remove individuals with low quality data; the above procedures were then repeated without the low-quality individuals. The final dataset was subjected to additional filtering using VCFtools to exclude sites with greater than 25% missing data (Danecek et al. 2011). GENEIOUS v. 10.2.4 (Kearse et al. 2012) and PGDSPIDER v. 2.1.1.2 (Lischer and Excoffier 2012) were used to convert data between different file formats. To facilitate fine-scale investigations for *Dinacoma* populations and minimize intraspecific missing data and singleton/monomorphic SNPs, we also used these procedures to create three datasets using

only individuals collected from one of the three sampled populations (Palm Springs in Riverside County, $n = 16$; San Felipe Valley (SF) in San Diego County, $n = 12$; and Bautista Canyon (BC) near Hemet in Riverside County, $n = 14$).

Population genetics and phylogenetics

We calculated descriptive statistics and pairwise Nei's G_{ST} for the *COI* dataset using *pegas* v0.12 (Paradis 2010) and *strataG* v2.0.2 (Archer et al. 2017), and *mmmod* v1.3.3 (Winter 2012), respectively, in *R* v3.6.2 (R Core Team 2015). We assessed population structure with phylogenetic likelihood using *RAXML* (Stamatakis 2014). Analyses with *COI* mitochondrial data were completed using the *RAXML-HPC2 Workflow on XSEDE* implemented on the CIPRES Science Gateway (Miller et al. 2010) running *RAXML* v. 8.2.10. Analyses were parameterized to perform 25 alternative runs on distinct starting trees including bootstrapping calculations halted based on autoMRE criterion. Remaining parameters were left on default settings. Sanger data was used to construct two maximum-likelihood phylogenetic trees in *RAXML*: one that makes use of full sequences and a second with missing data from 5' and 3' ends removed (approximately 407 base pairs/individual retained). A *COI* haplotype network was also constructed for mitochondrial data in PopART (<https://popart.otago.ac.nz>) using TCS network inference (Clement et al. 2002).

Descriptive population genetic statistics for the ddRAD dataset were calculated in *GenoDive* v3.0.3 (Meirmans and Van Tienderen 2004). We also used *GenoDive* to calculate pairwise population differentiation based on G''_{ST} using 10,000 replicates, and test for deviations from Hardy–Weinberg equilibrium using 10,000 permutations of the least-squares method. The complete ddRAD data set (all individuals) was used to create a single, unrooted likelihood tree in *RAXML* with the same procedure used for mitochondrial data, except with the addition of a Lewis ascertainment bias correction (Lewis 2001). Lewis ascertainment bias correction requires that potentially invariable sites are removed from the data set. Removal was completed in *R* v. 3.4.1 (R Core Team 2015) with a custom script employing the *PHYRNOMICS* v. 2.0 (Banbury and Leache 2014) and *PHYLOTOOLS* v. 0.1.2 (Zhang et al. 2012) packages. We also completed *STRUCTURE* analyses to assess structure using a Bayesian clustering criterion (Pritchard et al. 2000). Using *STRUCTURE* v. 2.3.4, we ran one hundred simulations for each K value 1 to 15 with 150,000 repetitions and a 50,000-repetition burn-in under an admixture model with correlated allele frequencies among populations. Unspecified parameters were kept default. Evanno's ΔK (Evanno et al. 2005) and $\text{LnPr}(K|X)$ (Pritchard et al. 2000) were used to determine the optimum number of clusters for the data. *CLUMPP* was used to merge runs and generate a consensus plot (Jakobsson and Rosenberg 2007). The *POPHELPER* v. 2.2.1 (Francis 2017) package employed in *R* v. 3.4.1 was used to implement Evanno's method and *CLUMPP*, and to generate the *STRUCTURE* barplot. We also used the *CLUMPAK* web portal (clumpak.tau.ac.il/index.html) (Kopelman et al. 2015) to produce and inspect bar plots across the entire range of K -values ($K = 1$ –15) (Fig. S1).

Both principle component analysis (PCA) and Neighbor-Joining analysis (Saitou and Nei 1987) were conducted for the ddRAD dataset in *R* v. 3.4.1 and *SplitsTree4* v. 4.14.5 (Huson and Bryant 2006), respectively (Figs. S2, S3). Principal component analysis in *R* was completed using a custom script employing the package *SNPrelate* v. 1.16.0 (Zheng et al. 2012).

Within population substructure

Within-population substructure was assessed further using the locality-specific ddRAD datasets. For each population, an unrooted likelihood tree was constructed in RAXML following the above procedures used for the complete ddRAD dataset tree. We completed STRUCTURE analyses on these datasets using the previously described procedures with K values 1–6.

Results

Data properties

Sanger sequencing of the 1480 base pair, mtDNA amplicon was successful for 38 of 48 *Dinacoma* samples (see metadata). *Amblonoxia fieldi* COI barcode sequences from two individuals were used to root COI trees constructed in RAXML. Illumina sequencing generated 320,474,796 raw reads, with an average read count of 5,622,365 per individual. STACKS assembled raw reads into 4,630,270 catalog loci. Six of the original 48 *Dinacoma* samples (4 *D. caseyi*; 2 *D. marginata*) were low quality and excluded from our ddRAD analyses based on the percent-missing-data threshold. The final filtered dataset contained 699 SNPs for all individuals ($n = 42$), and the population-restricted datasets for Palm Springs ($n = 16$), Bautista Canyon ($n = 14$) and the San Felipe Valley ($n = 12$) samples contained 1568, 1253 and 1876 SNPs, respectively.

Sanger sequencing

We observed relatively low nucleotide and haplotype diversity in the COI dataset, ranging from 0.0017 to 0.0025 and 0.564 to 0.833, respectively (Table 1). Considering three “populations” (see below for rationale), estimates of Tajima’s D were negative for *D. marginata* in the San Felipe Valley and positive for the other two populations, although none of these estimates were statistically significant (Table 1). The two maximum likelihood phylogenies generated using COI data had identical topologies; the tree generated from all available sequence data is displayed in Fig. 2. The maximum likelihood phylogeny divided *Dinacoma* into three distinct clades: one representing *D. caseyi* from the Palm Springs region, and two representing *D. marginata* (Fig. 2). Within the *marginata* complex, samples from Bautista Canyon form a distinct clade from those from San Felipe Valley. The BC *D. marginata* clade is basal to the *D. caseyi* and SF *D. marginata*, clade, and this pattern was supported by pairwise distance measures of the COI dataset (Table 2). However, despite these geographic patterns, marginal to low bootstrap support values

Table 1 Descriptive statistics of the COI dataset, including nucleotide and haplotype diversity (and variance) and Tajima’s D (p -value)

Species (population)	#inds	nuc.div (var)	hap.div (var)	Tajima’s D (p)
<i>D. caseyi</i>	13	0.0017 (0.0000012)	0.564 (0.0117)	1.009 (0.17)
<i>D. marginata</i> (San Felipe Valley)	12	0.0025 (0.0000023)	0.833 (0.0094)	-0.481 (0.34)
<i>D. marginata</i> (Bautista Canyon)	13	0.0021 (0.0000016)	0.795 (0.0047)	0.775 (0.23)

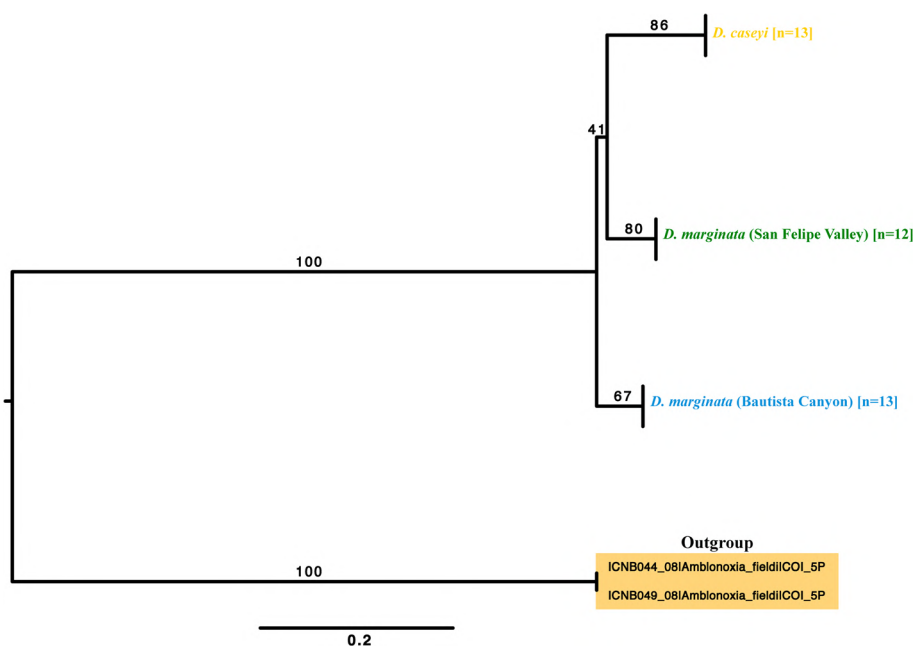


Fig. 2 Maximum Likelihood tree generated by RAXML using *COI* sequence data and public *COI* sequences available on BOLD for *A. fieldi* outgroup. Outgroup sequences of varying length nested within the 1480 bp amplicon targeted by this study. ML bootstrap values at corresponding nodes

Table 2 Pairwise population differentiation between species/regions

	(1)	(2)	(3)
<i>D. caseyi</i> (1)	–	0.0072	0.0245
<i>D. marginata</i> (San Felipe Valley) (2)	0.862	–	0.0105
<i>D. marginata</i> (Bautista Canyon) (3)	0.814	0.775	–

Lower triangle: G''_{ST} from ddRAD dataset, upper triangle: Nei's G_{ST} from COI dataset. For lower triangle, all comparisons were significant ($p < 0.05$) after Bonferroni correction. Numbers after species/region names correspond to columns

(> 80%) were obtained for these clades. The TCS haplotype network (Fig. S4) supports a clear separation of *D. caseyi* and two distinct clades of *D. marginata* reflecting the isolation of populations at different collection localities.

ddRAD sequencing

Measures of observed and expected heterozygosity and inbreeding from the ddRAD dataset were relatively uniform across populations, ranging from 0.045 to 0.083, 0.05 to 0.094, and 0.089 to 0.123, respectively (Table 3). *Dinacoma marginata* from Bautista Canyon displayed the highest heterozygosity and inbreeding compared to the other populations. Thirty-five loci of the 699 were observed to deviate from HWE, however

Table 3 Descriptive statistics of the ddRAD datasets

Dataset/region	Species (population)	Ind [#]	Loci [#]	H _O	H _E	G _{IS}
All individuals	<i>D. caseyi</i>	16	699	0.045	0.050	0.089
	<i>D. marginata</i> (San Felipe Valley)	12	699	0.055	0.061	0.105
	<i>D. marginata</i> (Bautista Canyon)	14	699	0.083	0.094	0.123
All individuals (HWE)	<i>D. caseyi</i>	16	664	0.044	0.045	0.024
	<i>D. marginata</i> (San Felipe Valley)	12	664	0.055	0.061	0.106
	<i>D. marginata</i> (Bautista Canyon)	14	664	0.080	0.086	0.069
Palm Springs region	<i>D. caseyi</i>	16	1568	0.213	0.224	0.051
San Felipe Valley region	<i>D. marginata</i> (San Felipe Valley)	12	1876	0.256	0.275	0.068
Bautista Canyon region	<i>D. marginata</i> (Bautista Canyon)	14	1253	0.191	0.225	0.154

Datasets include the full dataset with all individuals, the full dataset sans loci not conforming to HWE, and subset datasets for each of the three subregions

[#]ind/loci number of individuals/loci per dataset, H_O observed heterozygosity, H_E expected heterozygosity, G_{IS} inbreeding coefficient

descriptive statistics changed little when these loci were removed (Table 3), so we considered the entire dataset of 699 SNPs for downstream analyses. The unrooted maximum likelihood phylogeny generated by RAXML clearly distinguishes the three *Dinacoma* clades identified with *COI* data with high bootstrap support (Fig. 3). Clades appear to be evolutionarily equidistant from each other, or nearly so. While we lacked an outgroup to root the ddRAD phylogeny, pairwise differentiation measures of this dataset indicated a closer relationship between the two *D. marginata* populations, than compared to the *D. caseyi* population (Table 2). Analysis of STRUCTURE runs using ΔK and LnPr(KIX) supported K = 3 as the optimal cluster number (Figs. 4, S5). These three clusters correspond to *D. caseyi* from its range in the Palm Springs/Cathedral City area, BC *D. marginata*, and SF *D. marginata* and agree with the clades identified by RAXML (Fig. 4).

We looked for substructure in the mitochondrial haplotype network and through the use of hierarchical RAXML and STRUCTURE analyses for our genomic data, but found none. Figure 3 shows ML trees from population specific datasets with no branches having high support. Hierarchical STRUCTURE analyses all support K = 1 and are provided in the supplemental materials (Fig. S5). Descriptive statistics generated from the region-specific ddRAD datasets unsurprisingly showed higher heterozygosity estimates compared to the dataset generated from all populations (Table 3). Estimates of inbreeding were higher for *D. marginata* from Bautista Canyon, but lower in the other two populations (Table 3).

Discussion and conclusions

Our results strongly support the species-status of *D. caseyi* as a distinct, diagnosable, and isolated taxon from *D. marginata*. Further, our results strongly suggest the existence of an undescribed species of *Dinacoma* from San Felipe Valley which is equally isolated from the typical *D. marginata* population we sampled from Bautista Canyon. These results not only support the continued conservation of *D. caseyi* as a unique and isolated species, but also reveal a broader pattern of isolation among *Dinacoma* lineages. Because the isolation is supported by both the mtDNA and the more rapidly evolving nDNA from the ddRAD

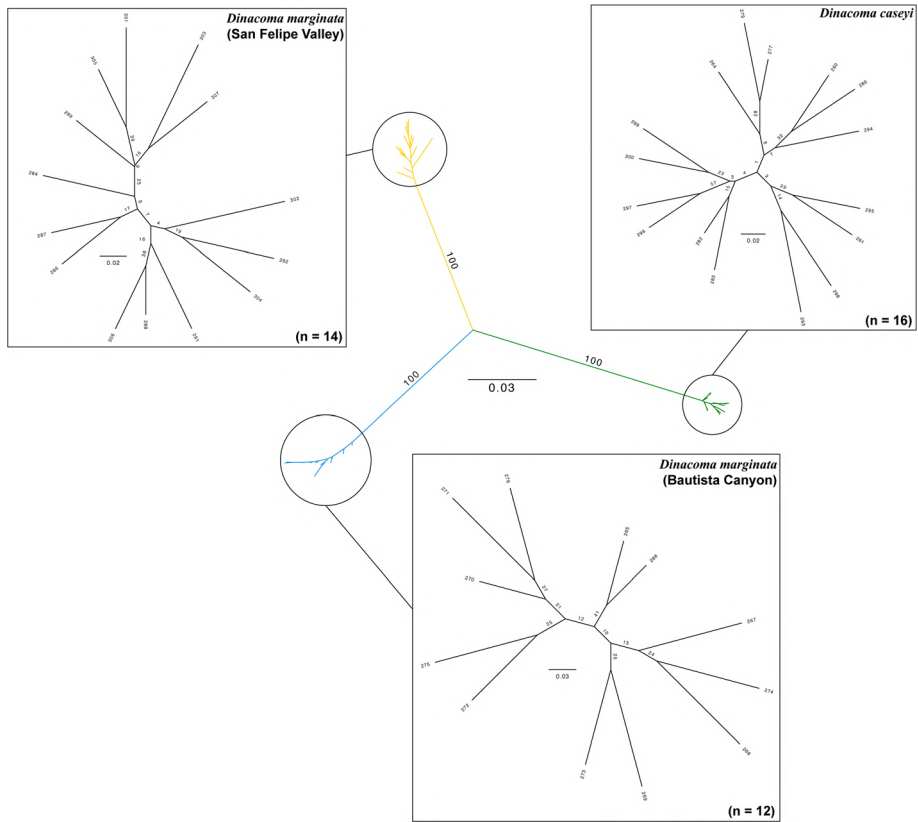


Fig. 3 Base tree is a maximum likelihood tree generated in RAXML for the STACKS dataset including all sampled individuals. ML Bootstrap support values displayed. Weak support (< 60) was obtained for all within-clade branching, with most receiving support values < 30 . Scale bar indicates mean substitutions per site. Boxes contain unrooted population specific RAXML trees for each of the localities sampled. All branches receive weak support (< 60) signifying an overall lack of within population substructure

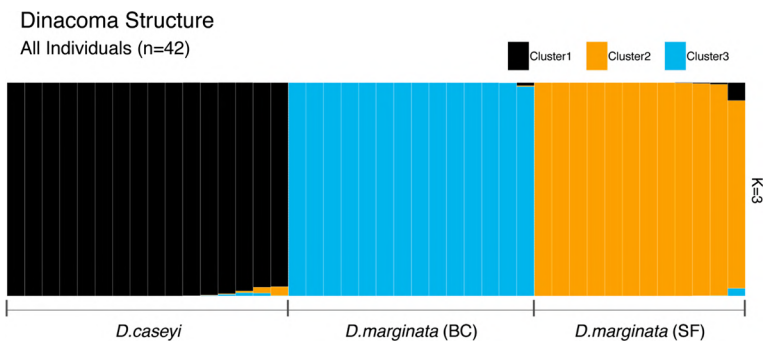


Fig. 4 Bar plot summarizing merged results for 100 STRUCTURE runs at the determined cluster optimum, $K = 3$

data, it suggests that there is very little, if any, active genetic exchange – even by the flighted male beetles – between these species. Estimates of inbreeding in these lineages also supported this conclusion. Conservation planning for the Coachella Valley should not assume that other poorly-known species all represent singular entities simply because they share a species name. Our results suggest that, for many groups, the region may represent a mosaic of unique, endemic species, and that additional assessment of cryptic species diversity is needed before conservation reserve planning proceeds further than it already has. If Multiple Species Habitat Conservation Plans continue to be designed with the assumption that most species occur region-wide, our results suggest endemic species will be lost.

Dinacoma's flightless females make the isolation of mtDNA across the region perhaps unsurprising, but the genomic nDNA data confirms that the males, which disperse through evening flights, are not traveling across the region between Bautista Canyon, San Felipe Canyon and the Palm Springs area. This suggests that other groups, even those with flight, may be naturally isolated into pockets of endemic species, worthy of conservation attention. Yet, within each population of *Dinacoma*, including *D. caseyi*, neither mtDNA nor nDNA show sub-structure. For *D. caseyi*, in particular, our sampling across the known distribution included many sites recently isolated by development, which was feared to be isolating this endangered species into unsustainable population fragments since roads are known dispersal barriers, even to flighted insects (Andersson et al. 2017; Muñoz et al. 2015). Our results provide indirect evidence for two important management phenomena in this regard. Firstly, nDNA indicates that not only are males flying freely within the *D. caseyi* population, but also that females are maintaining mtDNA genetic contact at some level, despite being flightless. Alternatively, this lack of local isolation may suggest that the habitat destruction and resultant isolation suffered by *D. caseyi* is too recent to reflect now-isolated groups of females on fragments of remaining habitat. Additional research to understand the dispersive abilities of female *D. caseyi* will be important in understanding the impact of roads and urbanization on population connectivity.

Evaluation of population fragmentation and inbreeding

One of the most important findings from this study is confirmation that *D. caseyi* is a distinct species, diagnosable from *D. marginata*. This not only justifies its protected status, but also reflects finer scale biodiversity patterns across a highly heterogeneous inland desert region. Within *D. caseyi*, there was no detectable structure in the population, even between samples from locations separated by housing developments and highways, possibly due to the recent nature of habitat fragmentation as discussed above. We suspect that *COI* is evolving too slowly to reflect the isolation that development has caused between females in the past few decades, and that the flighted males are dispersing between patches of habitat, reflecting the more sensitive genomic (nDNA) dataset's lack of sub-structuring. Effective conservation management planning may still involve establishing habitat connectivity between *D. caseyi* populations to ensure long term genetic exchange as has been suggested for preserving insect diversity in general (Samways 2007) and beetles specifically (Eggers et al. 2010). Estimates of inbreeding generated from the ddRAD dataset indicated that individuals in all populations were more related to each other than would be expected with random mating (i.e. positive G_{IS}). While *D. caseyi* appeared to have less inbreeding compared to *D. marginata* (Table 3), our limited sample sizes preclude strong comparative conclusions between populations of this species.

Evidence of deep isolation

A surprising result of this work is the robust support for the recognition of a new species of *Dinacoma*. The genomic divergence between the BC and SF populations is equivalent to that between *D. caseyi* and either *D. marginata* population, reflecting relatively deep divergence across the genus. Since the BC population is already recognized as *D. marginata* and is geographically closest to the type locality for *D. marginata* in coastal San Diego county, we continue to recognize BC as *D. marginata*. Unfortunately, the type locality is now heavily developed and *Dinacoma* has not been collected from the exact area in decades. Regardless of the taxonomy, the salient message from the data is that all three *Dinacoma* populations represent species-level divergence. This divergence is supported not only by mtDNA, which reflects the isolation of the flightless females, but also by the nDNA exchanged by the dispersive males. Isolation between *Dinacoma* species suggests the possibility of much finer scale patterns of biodiversity and speciation in a variety of other cryptic species across what might appear to be contiguous, homogenous and rapidly developing desert areas of southern California. Effective multi-species conservation planning should not assume that genetic exchange is occurring in what are currently considered widespread species in other cryptic groups. Each subregion may need to make independent investigations to save local and irreplaceable lineages that represent an unexpectedly rich and complex history of biodiversity; cryptic species diversity is essential in this regard (Bickford et al. 2007).

The cryptic nature of *Dinacoma* species presents a fascinating but ominous narrative for the future of many endemic species in southern California's western deserts. If *Dinacoma* is typical, and there is no reason to assume it is not, there are many, highly restricted species exhibiting fine scale patterns of speciation and isolation. The addition of genomic data to past and current studies will help reveal this hidden diversity. Unfortunately, efforts to identify and understand cryptic species are often neglected (Trontelj and Fišer 2009) in favor of management centered around charismatic taxa (eg. Rubinoff 2001). The implicit assumption is that they share similar diversification patterns and so current conservation plans will be adequate for the whole of the regional biota. Our findings suggest that this assumption is not accurate. In practice, a cryptic, sessile genus such as *Dinacoma* likely represents a more sensitive and detailed conservation model for the southern California desert community. Landscape-level changes have already irreparably reduced the span of the natural areas remaining in the region and may have extirpated species without them ever coming to light. We emphasize that the western deserts of southern California merit more intensive and careful biotic surveying to accommodate human use with as much endemic biodiversity as possible.

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Data availability Raw ddRAD reads are available on NCBI. BioProject: PRJNA552048; SRA: SRX9646661-SRR9646708. Sanger sequencing data is available on BOLD and GenBank MT324554-MT324591.

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Rubinoff and San Jose 2021

Report for supplemental Sequencing of Casey's June beetle (*Dinacoma caseyi*) samples from the Tahquitz creek drainage

Daniel Rubinoff and Michael San Jose

Background

Building on the results from Rubinoff et al. 2020, USFWS requested the addition of 11 new samples of *D. caseyi* from Palm Springs (four from Palm Canyon and seven from Tahquitz Creek) to be added to the pre-existing *Dinacoma* dataset. These samples were sequenced for both ddRAD genomic data and COI data to match preexisting samples.

Results

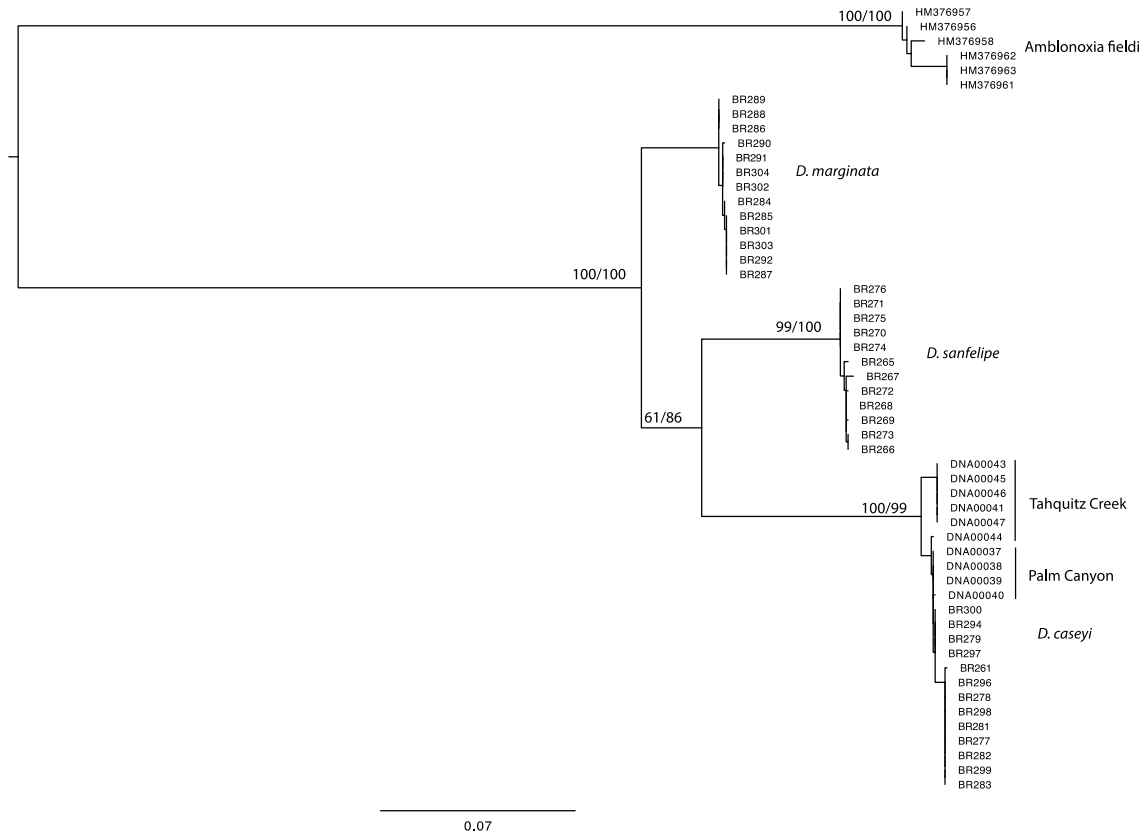
Progress was slowed by COVID pandemic since the same sequencing labs which we use for our work were being used to conduct COVID sequencing. Results were returned by the UC Berkeley Genomics facility in January 2021 and analysis was conducted following the same procedures as in Rubinoff et al. 2020.

Figures 1 and 2 demonstrate the phylogenetic affinities of the new Tahquitz Creek and Palm Canyon samples as they pertain to the preexisting dataset. Briefly, using both ddRAD genomic data including 5974 Single-nucleotide polymorphisms, or the mitochondrial gene COI (1480 bp), Tahquitz Creek and Palm Canyon are part of the greater *D. caseyi* population. Further, no diagnosable regions were revealed to distinguish these new samples from other areas of *D. caseyi*.

Conclusions

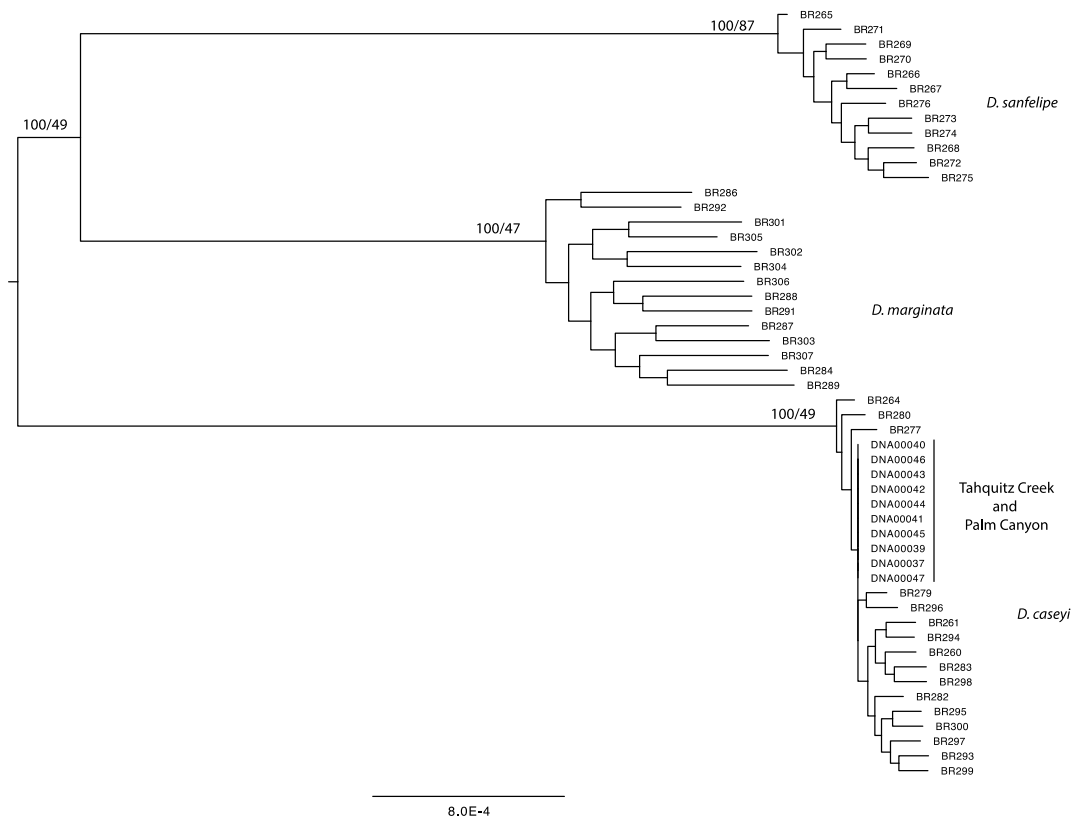
Because female *D. caseyi* are flightless and largely fossorial, there was some concern that development has isolated populations of this listed species. Despite the fact that we were not able to show any genetic evidence of this isolation in the current study, this may simply be a factor of the recency of the isolation of the areas. We caution that, for a species like *D. caseyi*, with only one generation per year (and possibly multiple years), it could take many years to reveal evidence of inbreeding depression and isolation. Therefore, given the recency of development in the Coachella Valley, our inability to find it does not demonstrate that there is current genetic contact between Tahquitz Creek and Palm Canyon samples and other parts of the *D. caseyi* range.

Figure 1)



Maximum Likelihood tree generated by Iqtree using COI sequence data and public COI sequences available on BOLD for *A. fieldi* outgroup. Outgroup sequences of varying length nested within the 1480 bp amplicon targeted by this study. ML and SH-ALRT bootstrap values at corresponding nodes.

Figure 2



Maximum Likelihood tree generated by Iqtree using 5974 SNPs. ML and SH-ALRT bootstrap values at corresponding nodes.

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<https://doi.org/10.12976/jib/2020.17.2.1>

<http://zoobank.org/urn:lsid:zoobank.org:pub:0B6ED543-EB48-4FFC-9847-E07D9A74C866>

A new species of melolonthine chafer in the endemic Californian genus *Dinacoma* Casey (Coleoptera: Scarabaeidae)

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Abstract

We describe *Dinacoma sanfelipe* **sp. nov.** from southern California, the first new species belonging to the melolonthine scarab beetle genus *Dinacoma* Casey, 1889 (Scarabaeidae: Melolonthinae: Melolonthini) to be described in 90 years, based upon examination of 141 specimens of that genus. Diagnostic comments, a dichotomous key, photographs of all species of *Dinacoma*, and a distribution map are presented to facilitate the identification of adult male specimens. One species in the genus is federally listed under the United States Endangered Species act, and all known species may be of conservation concern.

Key words: Scarabaeoidea, Melolonthini, scarab beetle, insect conservation, cryptic species

Introduction

The melolonthine scarab beetle genus *Dinacoma* Casey, 1889, belonging to the tribe Melolonthini, hitherto consisted of two species restricted to highly localized populations occurring in xeric habitats of southern California: the type species *Dinacoma marginata* (Casey, 1887), described from “California (San Diego Co.)”, and the federally listed *Dinacoma caseyi* Blaisdell, 1930, described from “Palm Springs, Imperial County, California”. Beyond the original generic description by Casey (1889), diagnostic characters distinguishing *Dinacoma*, together with keys to the two known species, were published by Blaisdell (1930) and Hardy (1974), who also each discussed the taxonomic history of the genus. Keys to distinguish *Dinacoma* from related Melolonthini genera were presented by Hardy (1974) and Evans (2002), and the genus was catalogued by Evans (2003) and Evans & Smith (2005).

Due to widespread human-induced degradation of their highly localized and restricted habitats, conservation of these endemic Californian chafers is of serious and ongoing concern, which is only exacerbated by the fact that female *Dinacoma* are fossorial and flightless, limiting their dispersal ability and hence capacity to respond to environmental modification (Wright *et al.* 2004, Rubinoff *et al.* 2020).

Recent phylogenomic analysis of *Dinacoma* concluded that the observed divergence between three extant populations is consistent with the existence of three species-level taxa: *D. caseyi*, *D. marginata*, and a third, cryptic species, occurring in the San Felipe Valley, east of the Laguna mountains in San Diego County, which we describe in this article (Rubinoff *et al.* 2020). We adhere to the Phylogenetic Species Concept, which defines species as the smallest aggregation of populations diagnosable by a unique combination of character states (Wheeler and Platnick 2000).

Material and methods

141 specimens of *Dinacoma* from the following institutional and private collections were studied:

KOCC - Kendall H. Osborne Collection, California, U.S.A.

LACM - Natural History Museum of Los Angeles County, California, U.S.A.

UCRC - Entomology Research Museum, University of California, Riverside, California, U.S.A.

UHIM - University of Hawai'i Insect Museum, Honolulu, Hawai'i, U.S.A.

Body length was measured using a millimeter scale, from the apex of the clypeus to the elytral apices; width was measured across the body at the level of the elytral humeri (i.e. the widest part of the body). Aedeagi were dissected out and the shape of the parameres were compared. Digital photographs of the dorsal and ventral habitus, and lateral and dorsal views of the aedeagi, of the holotype male of *D. sanfelipe* sp. nov., and of representative male specimens of *D. marginata* and *D. caseyi* were taken with a Nikon D-7100 DSLR camera mounted on an Olympus stereomicroscope. The image files were subsequently focus-stacked using Helicon Focus (Helicon Soft Ltd., Kharkiv, Ukraine), and edited on an Apple desktop computer.

Results

Description of new species

Superfamily Scarabaeoidea Latreille, 1802

Family Scarabaeidae Latreille, 1802

Subfamily Melolonthinae Leach, 1819

Genus *Dinacoma* Casey, 1889

Dinacoma sanfelipe sp. nov. Figs 1A–C, M; 2–3.

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Diagnosis

The new species can be distinguished from the two other species of *Dinacoma* by the following combination of morphological characters. Protibia with two distinct external teeth (with third distinctive basal tooth present in *D. marginata*). Anterior margin of clypeus weakly concave (deeply concave to moderately notched in *D. marginata* and *D. caseyi*). Anterior angles of clypeus sharply acute and outwardly divergent (less acute and divergent in *D. marginata* and *D. caseyi*). Scales covering elytra whitish colored medially, becoming yellowish laterally (scales all white in *D. caseyi*). Central elytral longitudinal stripe and juxta-sutural stripe composed of scales distinctly contrasting against remaining elytral ground color (these stripes are less distinct in *D. marginata* and *D. caseyi*). Whitish scales covering ventral surface of abdominal sternites not dense enough to obscure ground color of ventrites (scale covering very dense in *D. caseyi*, largely obscuring the ground color of ventrites except at the base of each ventrite).

Etymology

The new species' epithet is a nominative noun in apposition, indicating the unique locality in San Diego County, California, in which the new species has been collected.

Type Material

Holotype: ♂: UNITED STATES, California, San Diego County, San Felipe Valley; 33.14130°N, 116.54310°W, elev. 2574 ft; 3 June 2016; BL and MV light; Ken H. Osborne/ D. Wikle leg.; UHIM. **Paratypes:** 27 ♂♂: same data as for holotype.

The Holotype bears our red holotype designation label "HOLOTYPE, *Dinacoma sanfelipe* sp. nov., Gillett, Osborne, Reil, & Rubinoff 2020" and is deposited in the University of Hawai'i Insect Museum, Honolulu, Hawai'i. The 27 paratypes each bear our yellow paratype designation label "PARATYPE, *Dinacoma sanfelipe* sp. nov., Gillett, Osborne, Reil, & Rubinoff 2020" and are deposited in the following collections: UHIM (8), KOCC (14), LACM (1),

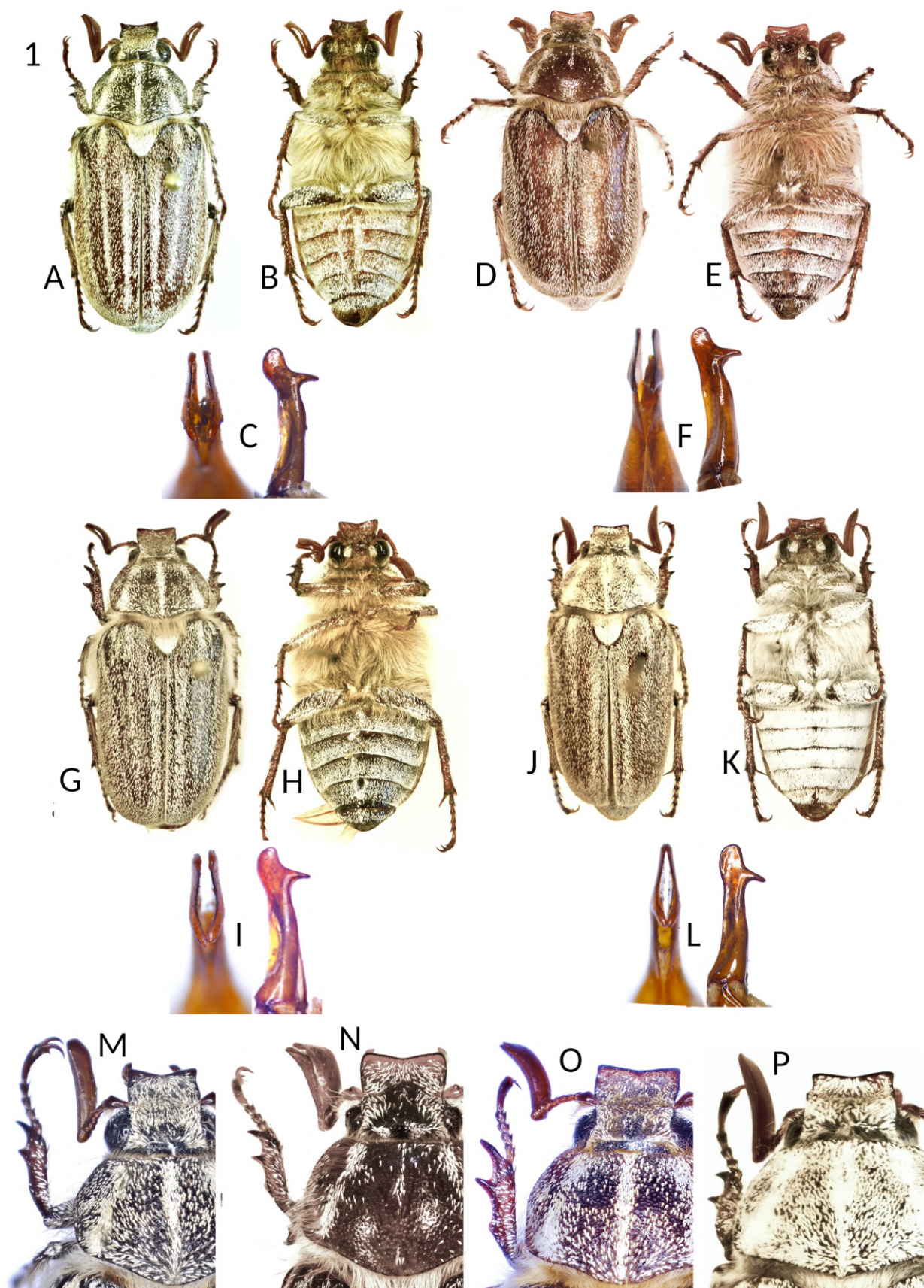


Figure 1. Dorsal and ventral habitus, lateral and dorsal views (showing parameres) of the aedeagus, and dorsal close-up view of right protibia and clypeus of male specimens of *Dinacoma*. **A–C, M**, Holotype of *Dinacoma sanfelipe* sp. nov. (length 23.0 mm); **D–F, N**, *D. marginata* (from Del Mar, San Diego) (length 20.0 mm); **G–I, O**, *D. marginata* (from Bautista Canyon) (length 21.0 mm); **J–L, P**, *D. caseyi* (length 17.5 mm).

UCRC (1), California Academy of Sciences, San Francisco CA (CAS) (1), Essig Museum of Entomology, Berkeley CA (EMEC) (1), National Museum of Natural History, Washington D.C. (USNM) (1), Muséum d'Histoire Naturelle de Genève, Switzerland (MHNG) (1).

Description of holotype (Figs 1A–C, M).

Dorsal habitus and ventral view shown in Figs 1A and B respectively. Male. Length 23.0 mm; width 10.0 mm. Body robust, elongate, and narrow. Head and pronotum ground color blackish-brown, clothed in long pale yellowish hairs and covered in scattered pale whitish backwardly pointing teardrop-shaped scales. **Thorax:** scales concentrated in three longitudinal stripes on the pronotum; one broad stripe that is narrowed medially on each side, and a narrower stripe along the center, which narrows posteriorly, reaching the base of the pronotum. Ventral surface of prosternum and metasternum clothed in a dense covering of long pale hairs. **Elytra:** lacking long hairs, moderately shining, ground color reddish brown, becoming darker brown basally; covered in scattered pale scales that are white medially and light yellowish-brown laterally. Scales forming a fine (two to three scales wide) sutural border running the length of the inner elytral suture. Each elytron bears a whitish juxta-sutural longitudinal stripe progressively narrowing from behind the scutellum to near the elytral apex. A second similar but slightly narrower longitudinal stripe runs distinctly approximately along the center of each elytron, from the base to about one fifth the elytral length from the elytral apex, where it becomes diffuse. Base of elytral humerus bearing a distinctive patch of whitish scales that largely mask the elytral ground color; the humeral callus itself is less densely clothed in scales, permitting the elytral ground color to show through. Lateral one third of each elytron more or less uniformly clothed in pale scales that reach the external elytral margin without forming further distinctive longitudinal stripe. **Head:** Excluding eyes, approximately as wide as long. Anterior margin of clypeus uniformly shallowly concave medially, lacking emargination, with anterior and lateral borders raised, and the anterior angles sharply acute and directed outwardly. Clypeus and vertex uniformly covered in erect long and pale yellowish-brown pilosity and scattered pale whitish-yellow hair-like recumbent scales. Base of head glabrous medially. Eyes large; interocular width equal to about 3 eye diameters. Antenna 10-segmented; scape equal in length to at least the next three segments combined; antennal club composed of 3 very long subequal segments, longer than all other segments combined. Ventral surface of head clothed in long pale hairs. **Abdomen:** Reddish brown in color. Abdominal ventrites almost uniformly covered in scattered whitish scales that permit the ground color of the ventrites to be clearly visible to the naked eye. A longitudinal area along the midline of the ventrites, and a fine margin along the base of each ventrite are less densely clothed in scales, contrasting against the more uniformly covered areas (Fig 1B). **Legs:** Femora dark brown, somewhat densely covered in whitish scales, but ground color is clearly visible. Tibiae lighter reddish-brown, sparsely covered in long pale hairs and whitish scales, and with a small dense patch of white scales at their base, adjacent to the femoral joint. Meta- and mesotibia bearing a tooth-like ridge a little more than halfway along the external surface; this ridge is fringed with short, stout bristles. Apex of meso and metatibiae each bearing two moderately long apical spurs; mesotibial apical spurs subequal in length, about as long as first tarsomere; metatibial spurs are unequal in length, with innermost spur longer than first tarsomere. Protibia laterally bidentate externally (including apical spur), and sinuous in basal half, but lacking a third distinct basal tooth (Fig 1M). Tarsi reddish brown, claws bearing an acute tooth at their base. **Genitalia:** aedeagus in dorsal view symmetrical, with a broad base and narrowing apically to a convexly rounded tip. In lateral view, the apical third is curved upwards, broadly rounded at the apex, and with ventral teeth almost vertical, only slightly posteriorly curved. Vertical length of teeth about one fifth of total length of aedeagus (Fig 1C).

Variation

The length of specimens in the type series varies between 16.5 – 23.0 mm (mean 19.0 mm; SD 1.36); ground color of pronotum and elytra varies between reddish brown to brownish black. The density of the scale covering varies on the pronotum and elytra such that in some specimens the longitudinal stripes are less discrete, but they are always distinct to the naked eye. The color of scales varies among individual specimens from a distinctly yellowish tone to almost purely white. Depth of clypeal anterior margin concavity is variable, as is the sharpness of the anterior angles of the clypeus and the degree to which they protrude laterally. Density of scale covering on abdominal ventrites variable but the reddish brown ground color of the ventrites is always clearly visible to the naked eye.

Distribution

The new species is known only from the San Felipe Valley in San Diego County, southern California (Figs 2 and 3).

Temporal Distribution

All 28 studied specimens were collected in early June.

Ecology

Nothing is known specifically about the immature biology of the new species. Adults emerge during early summer (late May and June - personal observation, KHO) and males fly about rapidly close to the ground, beginning approximately an hour after sunset, with flight activity tapering off after 2200 hours. They are attracted to electric lights after dark (beginning approximately 2030 hours). Females, which are rarely encountered in this genus, remain undescribed for the new species, but are presumably also flightless. The new species' habitat includes the valley bottom and gentle alluvial slopes, with vegetation ranging from riparian woodland (on San Felipe wash), consisting of Mesquite (*Prosopis glandulosa*), Fremont's cottonwood (*Populus fremontii*), Western willow (*Salix gooddingii*), and Desert willow (*Chilopsis linearis*), grading into desert transitional zone scrub adjacent to the valley bottom, dominated by Catclaw (*Acacia greggii*), Valley cholla (*Opuntia parryi*), and California buckwheat (*Eriogonum fasciculatum*). Photographs of the habitat at the type locality are shown in Fig 3.

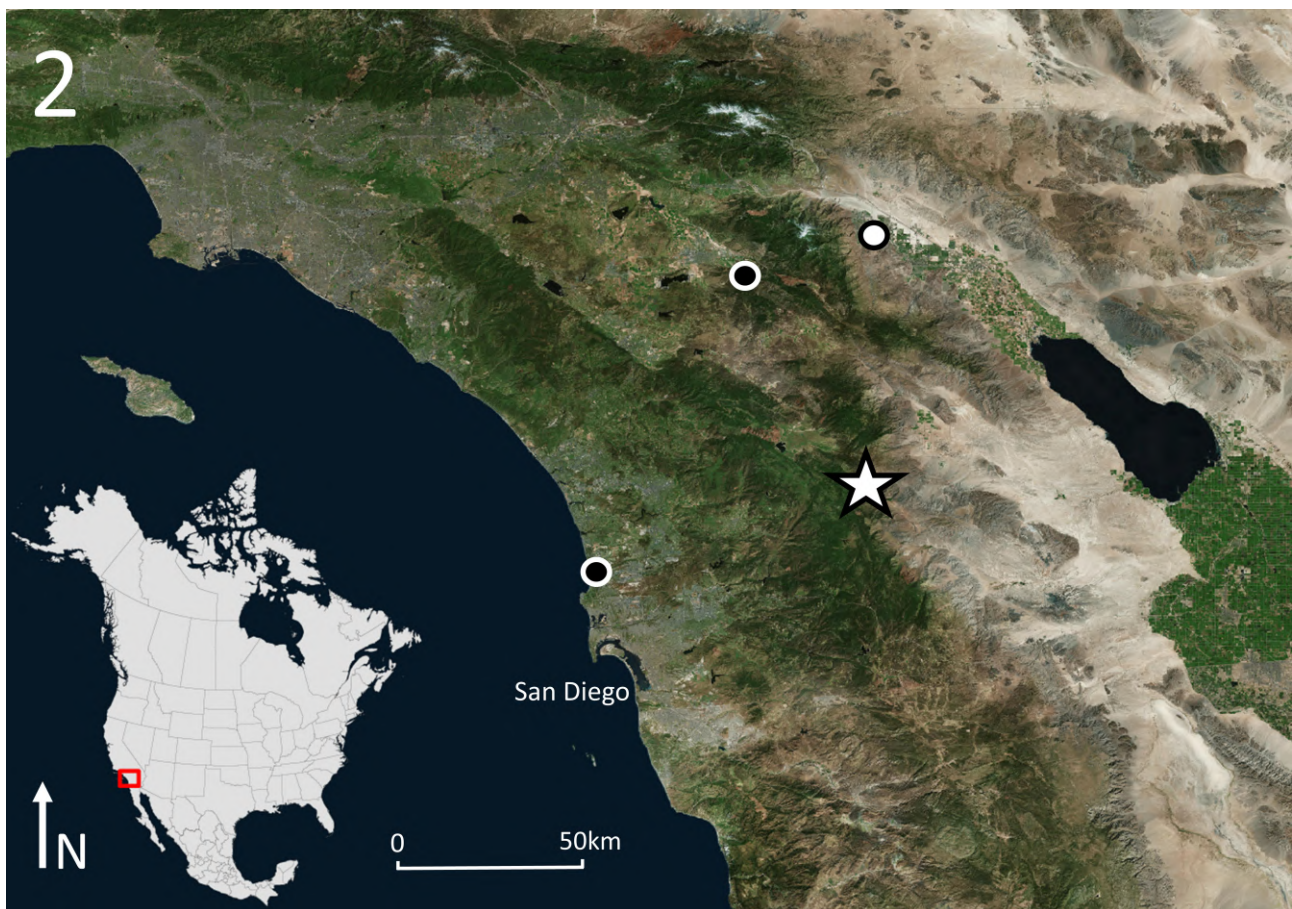


Figure 2. Map of southern California indicating collection localities for *Dinacoma sanfelipe* sp. nov. (white star), *D. marginata* (black-filled circles), and *D. caseyi* (white-filled circle).

Additional material examined

Dinacoma marginata (Casey, 1887) (2 ♀♀, 109 ♂♂). Figs 1D–I, N–O.

3 ♂♂: UNITED STATES, California, San Diego County, Del Mar; 10 August 1946; J. A. Omstook leg.; LACM ENT: 414867; 414869; 414870.

2 ♂♂: same collection data as for preceding, except 1 June 1947; LACM ENT: 414866/414868. 1 ♂: California, San Diego County, San Diego; 9 May 1934; UCRC. 56 ♂♂: California, Riverside County, Bautista Canyon; 33.6948°N, 116.8507°W; elev. 2223 ft; 27 May 2016; BL and MV light; Ken H. Osborne leg.; KOCC. 1 ♀: same collection data as for preceding, except, on ground; KOCC. 19 ♂♂: same collection data as for preceding, except, 29 May 2016; BL and MV light; KOCC. 1 ♀: same collection data as for preceding, except, on ground; KOCC. 8 ♂♂: California, Riverside

County; 33.759722°N, 116.880277°W; 14 June 2003; UCRC ENT 79604-11. 16 ♂♂: California, Riverside County; 33.671944°N, 116.927777°W, 5 July 1990; UCRC ENT 87625-6. 3 ♂♂: California, Riverside County; 33.696111°N, 116.851666°W; 15 June 1985; UCRC. 1 ♂: California, Riverside County; Hemet; 17 May 1984; UCRC.

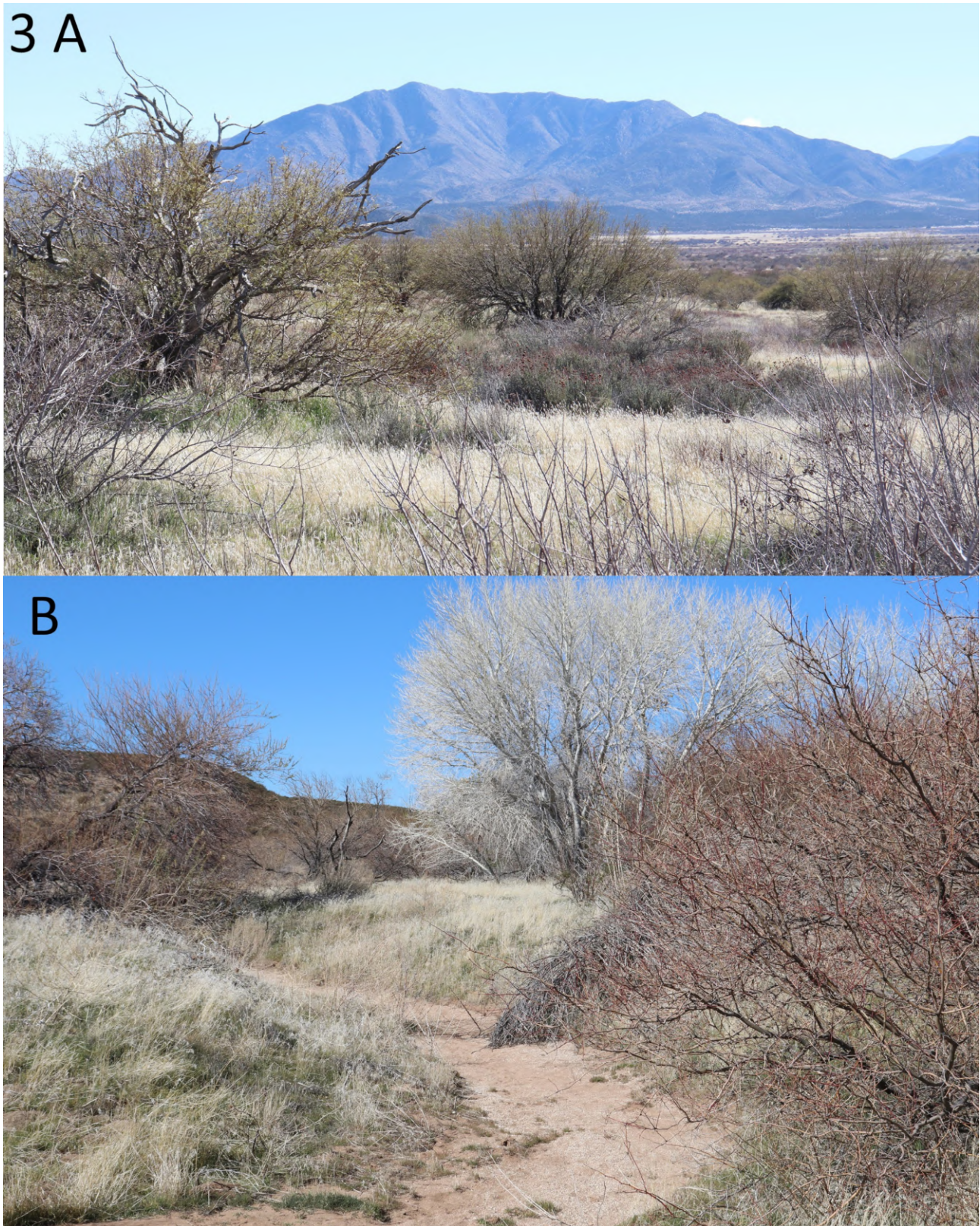


Figure 3. Photographs of the type locality of *Dinacoma sanfelipe* sp. nov. in San Felipe Valley, San Diego County, California, taken in February 2020. **A**, Desert transition scrub at the type locality, with *Prosopis*, *Acacia*, *Eriogonum*, and *Opuntia* (Granite Mountain lies in the far background); **B**, A wash near the collection site of the type series (where adult male *Dinacoma* were also observed in abundance), with *Prosopis*, *Populus*, *Salix*, *Chilopsis*, and *Bacharis*. Photographs by K. H. Osborne.

Dinacoma caseyi Blaisdell, 1930 (2 ♂♂). Figs 1J–L, P.

2 ♂♂: UNITED STATES, California, Riverside County, Palm Canyon Wash; 33.801°N, 116.497°W; at black light; 28 April 2016; K. H. Osborne leg, under USFWS permit #TE-837760; UCRC.

The approximate position of collection localities for examined specimens of all species is indicated in the map in Fig 2.

Key to adult males of *Dinacoma* species

The following dichotomous key is based in part upon that by Hardy (1974).

1. Elytra with all scales uniformly white in color; covering of scales on abdominal sternites extremely dense, obscuring reddish-brown ground color of ventrites (Fig 1K) *D. caseyi* Blaisdell
- Elytra with scales near suture white and those laterally yellowish-white; covering of scales on abdominal sternites less dense, not obscuring reddish-brown ground color of ventrites (Figs 1B, E, H) 2
2. Foretibiae bearing only two distinct external teeth, including apical tooth (Fig 1M). Anterior margin of clypeus weakly and uniformly concave, not weakly notched or with weak emargination. Anterior angles of clypeus distinctly acuminate and outwardly divergent (Fig 1M). Central and juxta-sutural longitudinal elytral squamous stripes distinct, clearly contrasting with remainder of elytral surface (Fig 1A) *D. sanfelipe* Gillett, Osborne, Reil, and Rubinoff sp. nov.
- Foretibiae bearing three distinct external teeth, including apical tooth (Figs 1N–O). Anterior margin of clypeus deeply concave, weakly notched or with weak emargination. Anterior angles of clypeus not so distinctly acuminate and not distinctly outwardly divergent (Figs 1N–O). Central and juxta-sutural longitudinal elytral squamous stripes less distinct, not clearly contrasting with remainder of elytral surface (1D, G) *D. marginata* (Casey)

Discussion

Based upon the specimens we examined, *D. caseyi* males are slightly smaller in mean length than those of the other two *Dinacoma* species (mean length = 17.25 mm; SD = 0.25; n = 2). No noteworthy difference in mean length was noted between males of *D. marginata* (mean length = 18.85 mm; SD = 1.10; n = 82) and *D. sanfelipe* sp. nov. (mean length = 19.0 mm; SD = 1.36; n = 28).

We did not detect any consistent diagnostic characteristics in the shape of the aedeagi or parameres to enable the three species to be distinguished.

When Casey described *D. marginata* (then in the genus *Thyce*), he specifically stated that “In *T. marginata* the anterior tibiae have two teeth exclusive of the exterior apical spur” (Casey 1887). Subsequently, Hardy’s (1974) brief diagnosis of the genus *Dinacoma* stated that the anterior tibiae in that genus are “bidentate, or at most with poorly developed third tooth”. Our study of *D. marginata* specimens from the type locality of San Diego (Figs 1D–F and N) and from Bautista Canyon, Riverside County (Figs 1G–I and O), suggests that there is some variation in the extent of development of the protibial teeth in that species. Specifically, whilst we recognize the basal (third) tooth as being distinctly present in both populations (in disagreement with Hardy’s (1974) statement above), it is clear that this tooth is most well-developed and distinctive in specimens belonging to the Bautista Canyon population. This population may prove to be distinctive from the (likely now extirpated) nominate San Diego population. However, because as yet no molecular sequence data is available for the topotypical San Diego population, we have decided to treat these two populations as conspecific.

In their genomic study, Rubinoff *et al.* (2020) found that each of the populations of *Dinacoma* (which did not include topotypical *D. marginata*) was isolated genetically, suggesting the possibility that *Dinacoma* evolve into extremely isolated and geographically restricted species, as is the situation for *D. caseyi*. Further research on the group may reveal additional populations that confirm the isolated nature of each species, reflecting the importance of incorporating some invertebrate-based research when planning conservation reserves. Further, *Dinacoma* distributions suggest historical dispersal, perhaps during wetter, glacial, periods, followed by isolation and speciation in pockets of suitable alluvial or coastal habitat. Such a distribution could be used to investigate for matching patterns of diversity in other groups, especially other insects, or even amphibians, to identify localized hotspots of species diversity across the southern California desert regions.

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APPENDIX F – Population Analysis

Harju 2021 – Population Analysis Report

Casey's June Beetle Population Analyses

Factors affecting emergence and relative abundance

Report

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INTRODUCTION

The Casey's June beetle (*Dinacoma caseyi*) is a small (1.4-1.8 cm long) scarab beetle with an extremely limited known range of 324 ha in southern Palm Springs, California (USFWS 2009). Habitat is gravelly sand soils associated with alluvial fans. Adults emerge from wintering burrows in April or May. Females are flightless and have only been observed on the ground. After emergence, adult males begin flying near dusk, in search of females crawling on the ground. Following mating, females re-enter their burrows or dig new burrows to deposit eggs. The larval beetles remain in the burrows until the following year when they emerge as adults. Casey's June beetles have been listed as Endangered under the U.S. Endangered Species Act since 2011 (USFWS 2011).

From 2016 through 2020 (and since ongoing), the U.S. Fish and Wildlife Service (USFWS) and partners have conducted extensive mark-recapture surveys within occupied range, along with measuring a suite of environmental covariates thought to influence Casey's June beetle emergence and abundance. In January of 2021 the services of Heron Ecological, LLC (Heron Ecological) were engaged to assist with analysis of the 2016-2020 data. The USFWS had already conducted initial mark-recapture modeling, but, due to extremely low recapture rates of individual marked beetles, requested that Heron Ecological assist with analysis of unmarked beetles. Here is the report of these analyses, with a goal of providing initial information on Casey's June beetle emergence and abundance patterns to better understand drivers behind these patterns. It is hoped that these results can help guide future analyses into Casey's June beetle populations and ecology.

METHODS

Sampling design

The Casey's June beetle capture data consist of two different sampling regimes. The first was the sentinel dataset, which was regular sampled (nightly in 2016 and every three nights 2017-2020), but only at a single site for the general duration of the emergence season each year. The sentinel site was generally sampled in April, May, and June, but in some years also Late March and/or early July. Sampling involved trapping already-emerged beetles, thus within this report I will refer to emergence rates as captures, assuming that captures rates are proportional to emergence rates. The second regime was the rangewide dataset, which involved traps usually spaced 500m apart spaced across the known range of Casey's June beetles on lands with available access (Figure 1). Rangewide sites were sampled in April and May of each year (2016-2020).

Survey methods consisted of turning on a 15-watt blacklight (which only attracts males) either a half hour or one hour before official sunset. Surveyor's then placed a 2 x 2 meter sheet under the trap bucket to collect and gather male beetles. Sheets were checked and traps were closed after one hour (for sentinel sampling) or three or four times each evening, approximately hourly (for rangewide sampling). Beetles were individually marked and released away from the trap site.

Additional data were collected and provided to Heron Ecological (e.g., emergence hole density, additional soil temperature data, vegetation data), but were not included in these analyses due to current time and budget constraints.

Figure 1. Generalized trap locations for Casey's June beetle, Palm Springs, California, 2016-2020.



Focal Datasets

I focused primarily on the two comprehensive capture datasets: sentinel and rangewide capture. The two datasets had different sampling schemes: sentinel sampling was spatially restricted (a single trap) but was temporally rich (every one or three nights throughout season), whereas rangewide sampling was spatially rich (nineteen traps, though not all surveyed every year) but temporally restricted (three to five surveys per season). Because of these differences, I used each dataset to focus on different information: sentinel sampling for patterns in capture and rangewide sampling for patterns in abundance.

Raw data

I summarized basic statistics on the raw capture data. I also created plots of sentinel captures and environmental covariates to evaluate basic patterns and associations.

Sentinel Sampling Statistical Methods – Capture patterns

I analyzed the sentinel survey data to answer four questions:

- 1.) What is the period within which population closure can be assumed?
- 2.) Are any weather or moonphase factors associated with whether any Casey's June beetles are captured?
- 3.) Are any weather or moonphase factors associated with how many Casey's June beetles are captured?
- 4.) What is the interannual temporal pattern in captures?

1.) What is the period within which population closure can be assumed?

For this question I calculated the maximum number of days between initial captures and subsequent recaptures of individually marked male Casey's June beetles. The goal was to determine a minimum time that individual Casey's June beetles are known to be above ground and within the emergence area prior to right-censoring (e.g., future recaptures not possible due to death, dispersal, failure to recapture, etc.). Given the fine temporal resolution of survey effort at the sentinel site (nearly every day in 2016, every three days 2017-2020), this may also approximate the maximum time that Casey's June beetles can be expected to remain alive and in the emergence area. I used a Poisson trend line to approximate the number of days at which zero beetles are expected to be recaptured following initial capture.

2.) Are any weather or moonphase factors associated with whether any Casey's June beetles are captured?

I used logistic regression to evaluate whether any (i.e., ≥ 1 captured male beetle) or no (i.e., 0 captured male beetles) Casey's June beetles were captured on a given survey night, using capture as a proxy that was likely proportional to true emergence. The logistic regression was constructed using a generalized additive mixed model (GAMM) to both account for a daily trend in capture rates as well as an autocorrelation term to adjust for the fact that probability of any beetles being captured on a given night was partially correlated with whether any beetles were captured on the previous survey night. I built two GAMMs, each with a Julian day smoothing term and predictor variables on probability of any captures of percent moonphase and humidity and maximum wind speed at sunset. The two models differed in their treatment of temperature, in that one also contained a predictor for air temperature (1 m aboveground) at sunset and the other contained a predictor for belowground temperature (10 cm) at sunset. Belowground temperature measurements were not collected in 2016, so the belowground model only contained data from 2017-2020.

3.) Are any weather or moonphase factors associated with how many Casey's June beetles are captured?

The number of beetles captured at the sentinel site on a given trapping night serves as an index on the true number of beetles emerging that evening. Further, because of the short interval in between sentinel trapping occasions, consecutive trapping nights could not be assumed to be independent of each other. To test for factors influencing variation in relative abundance of captured beetles on a given survey night, I used a quasi-Poisson GAMM with a smoothing term for Julian day and an auto-regressive error term for consecutive sampling occasions. I built a full model with predictors of year, air temperature, humidity, maximum wind speed, and percent moonphase for the total numbers of captured beetles.

4.) What is the interannual temporal pattern in captures?

Finally, I used the final submodel structure to predict expected numbers of captured male beetles, on average, as a function of Julian day to compare interannual variation in peak abundance.

Rangewide Sampling Statistical Methods

The general data structure of sampling Casey's June beetles lends itself to dynamic N-mixture abundance modeling of unmarked populations (Royle 2004, Dail and Madsen 2011). In N-mixture modeling, repeated surveys are conducted within a period of assumed population closure. Given that assumption, variation in counts are used to estimate the probability of detecting any individual animal, and thus to scale the observed number of animals to a corrected, or 'true', number of animals whose activity areas overlapped the survey location during the period sampled. Usually this means that a population is surveyed over multiple days within a closed period (e.g., breeding season). Casey's June beetles, however, appear very short lived, meaning that surveys conducted several days apart may be sampling new individuals and populations that were not emerged and active across the entire period. To accommodate this facet of Casey's June beetle life history ecology, I applied the N-mixture abundance modeling framework by treating hourly rangewide surveys as occurring within a closed period (i.e., a single evening), but allowing for open populations among 2-week separated sampling evenings. I did this separately for each year. The dynamic N-mixture abundance models were performed using the `pcountOpen` function in R package 'unmarked' (R v3.6.3). I tested multiple K values (i.e., theoretical higher-than-maximum true abundances) for the year with the highest observed abundances until reaching a K value with a stable corrected Akaike's Information Criterion (AICc) value. AICc is an index of the information content of a given model, with lower values equating to higher information content. Differences in AICc among models >4 (e.g., $\Delta AICc > 4$) indicate substantial differences with overwhelming support for the lowest AICc model. I set the dynamics setting to 'notrend' to allow for corrected abundance estimates within each sampling evening to be unconstrained by any population growth formula.

I then tested a series of null models, only varying the predictor variable for detection probability. In N-mixture modeling, detection probability is the probability of detecting an individual beetle given that it is emerged and present in the trapping area. Further, because

detection here was via capture, the detection probability estimates can be thought of as capture probability estimates. Once I determined the most meaningful detection probability predictor, I held that predictor constant and tested whether abundance on the first trapping night was better explained by a null model (i.e., constant across traps), differed by general habitat type within which each trap was located (i.e., wash or upland), or whether initial abundance was best explained by variation among traps regardless of general habitat.

I analyzed the rangewide survey data to answer three questions, separately for each year:

- 1.) What drives the probability of capturing an individual beetle, given that it is emerged and present in the trapping area?
- 2.) What drives initial corrected abundance of captures on the first survey night of the year?
- 3.) What is the true (corrected) abundance of male beetles over the course of sampling sessions within each year?

1.) What drives the probability of capturing an individual beetle, given that it is emerged and present in the trapping area?

For each year (2016-2020) I tested four models of factors driving detection probability of individual beetles: a null (i.e., constant) model, or varying as a function of aboveground temperature, belowground temperature, or wind speed at sunset.

2.) What drives initial abundance of captures on the first survey night of the year?

For each year I tested three models of factors driving initial abundance, each with the best detection probability predictor(s) identified in the previous question: a null model, or varying as a function of either general habitat or by individual trap site.

3.) What is the true (corrected) abundance of male beetles over the course of sampling sessions within each year?

Following identification of a meaningful model for detection probability, initial abundance, and no trend estimate, I used the best model within each year to estimate corrected abundance of male beetles with activity areas overlapping traps for each survey night within each year.

RESULTS

Raw data

Both sentinel and rangewide surveys for Casey's June beetles began in late March/early April and ended in June/July (sentinel) or late May (rangewide; Table 1). Sentinel traps had similar mean numbers of beetles captured per trap night (mean between 7.0 and 9.7) from 2016 through 2019, but markedly higher numbers of captures per trap night in 2020 (mean of 18.3; Table 2a). Rangewide traps had comparatively low captures on average per trap per night in 2016 (mean of 7.4) but markedly higher captures per trap per night in 2017 through 2020 (mean between 14.9 and 24.0; Table 2b).

Table 1. Beginning and ending sampling dates for sentinel and rangewide Casey's June beetle trapping surveys.

Year	Sentinel		Rangewide	
	Earliest	Latest	Earliest	Latest
2016	4/1	6/2	4/14	5/26
2017	3/21	6/29	4/27	5/25
2018	3/20	6/28	4/26	5/24
2019	4/2	7/26	4/4	5/30
2020	3/25	6/17	4/2	5/28

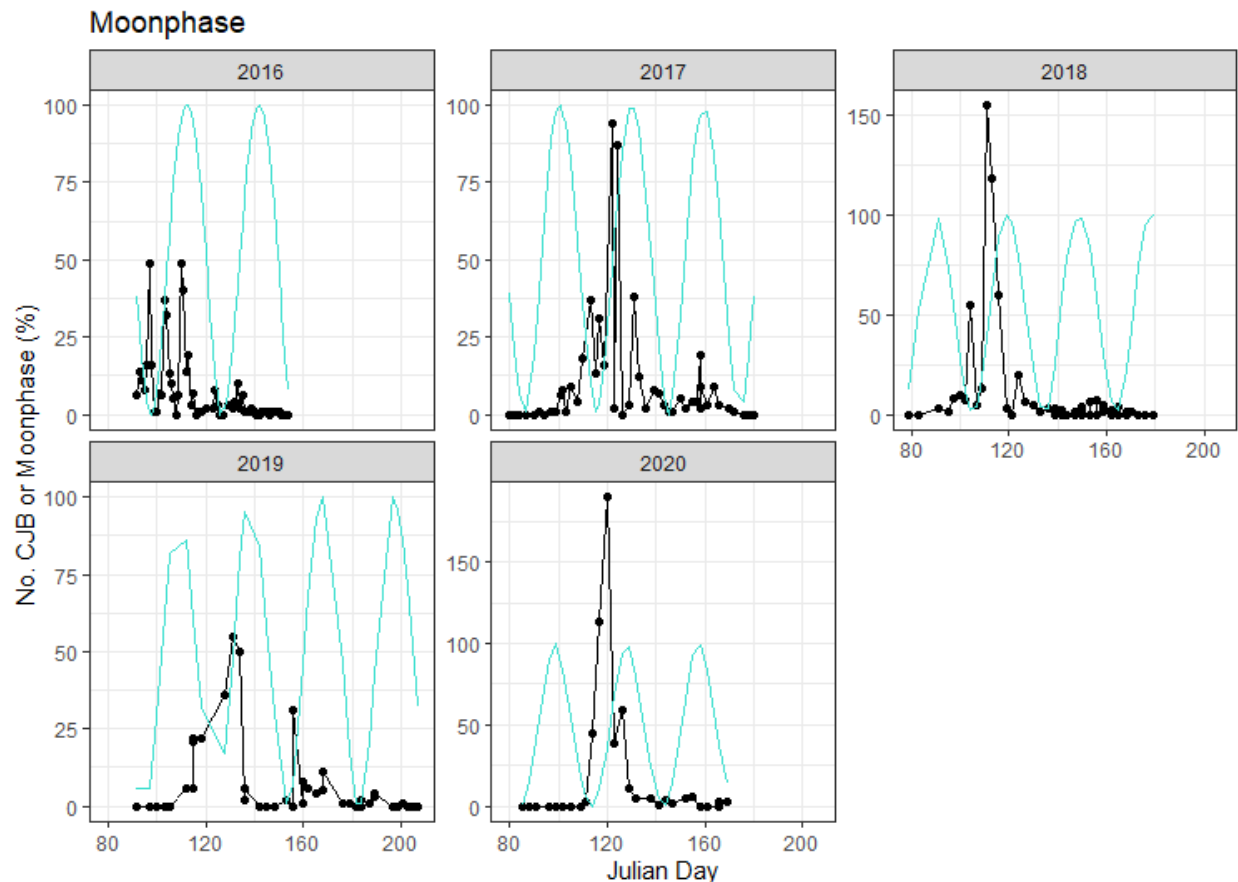
Table 2. Summary statistics for number of male Casey's June beetles captured at sentinel (a) and rangewide (b) traps.

a.) Sentinel					b.) Rangewide				
Year	Mean	SD	Min	Max	Year	Mean	SD	Min	Max
2016	7.41	11.63	0	49	2016	7.20	13.04	0	59
2017	9.71	18.89	0	94	2017	14.86	25.40	0	110
2018	8.14	25.44	0	155	2018	18.65	36.39	0	196
2019	7.00	13.01	0	55	2019	15.80	27.76	0	119
2020	18.30	41.78	0	190	2020	24.03	51.11	0	238
All years	9.21	22.64	0	190	All years	15.29	32.40	0	238

Plotting the raw data for sentinel captures and environmental covariates revealed some interesting patterns. Most notably, in four of the five years of sentinel trapping (2017-2020), there was a sharp increase and spike in the number of captured beetles occurring between Julian day ~100-140, depending on the year (Figure 2). The large spike in captures coincided with the waxing moonphase during this period, with the peak occurring at ~50% full (Figure 2). Even during 2016 which did not have a strong single spike, the height of beetle captures occurred

during the waxing moonphase, centered on 50% (Figure 2). Raw plots of beetle captures for air temperature at sunset (Figure A1), maximum wind speed (Figure A2), and percent humidity (Figure A3), did not show the same consistent association with the peak in each year's beetle capture, although see below for testing of weaker effects on capture rates.

Figure 2. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and percent moonphase (turquoise line).



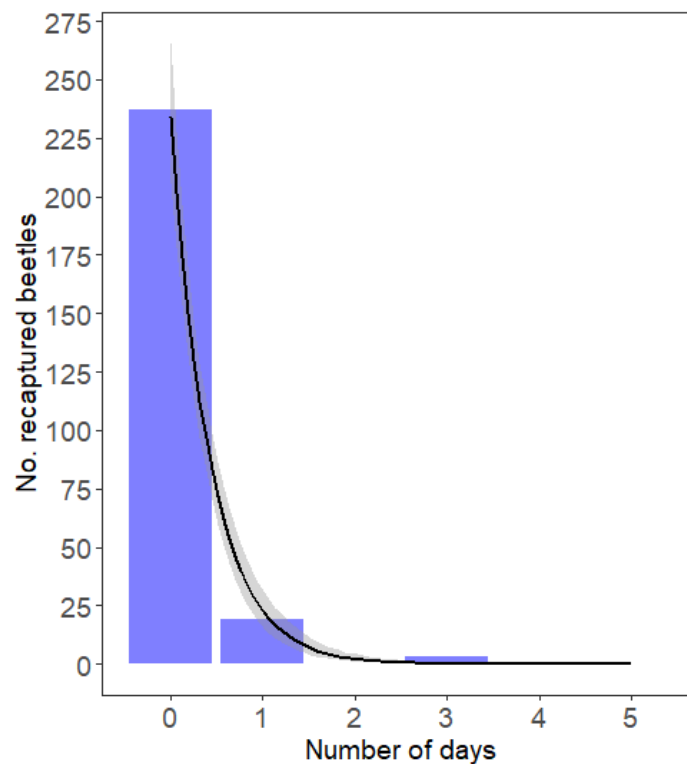
Sentinel survey data – Capture patterns

The sentinel dataset contained 5,471 unique marked male Casey's June beetles, captured 2016 through 2020. Of these, there were 259 recaptures of 255 unique beetles, meaning that only 4.7% of marked beetles were ever recaptured.

1.) What is the period within which population closure can be assumed?

Beetles that were recaptured were overwhelmingly recaptured on the same night as initially marked (237 of 259, 91.5%; Figure 3). Nineteen were recaptured the next day after initial capture (7.3%), and only three were recaptured three days after initial capture (1.2%). No beetles were recaptured more than three days after initial capture. This means that after ≈ 3 days trapping sessions may be considered to be independent.

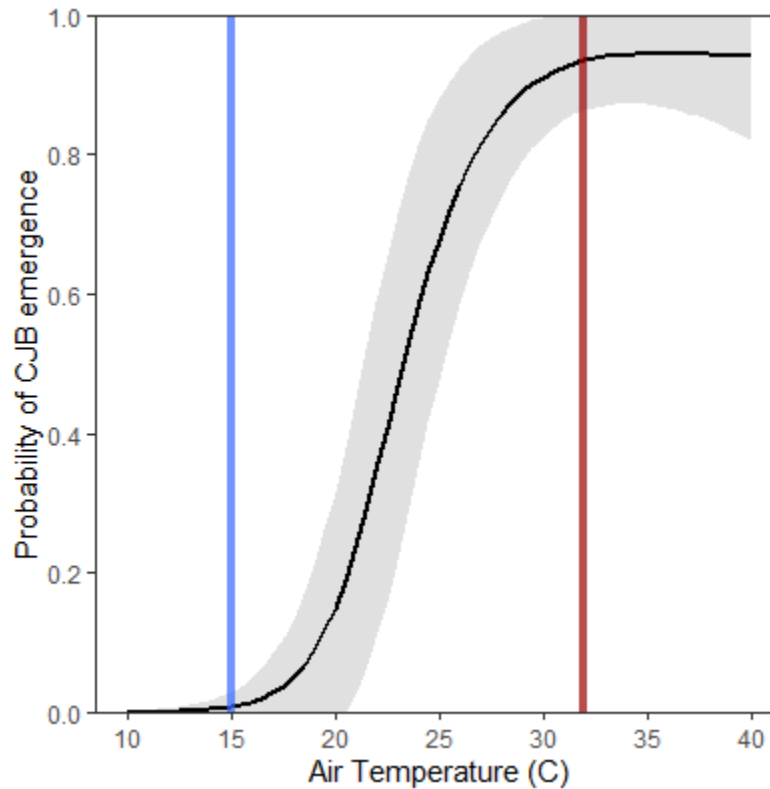
Figure 3. Days from first capture to subsequent recapture for male Casey's June beetles, 2016-2020. The x-axis is arbitrarily truncated after five days for display purposes; no beetles were recaptured more than three days after original capture. Trendline is Poisson regression line.



2.) Are any weather or moonphase factors associated with whether any Casey's June beetles were captured?

After accounting for the non-linear trend in beetle abundance over the course of the sampling season and autocorrelation between subsequent sampling events in whether any beetles were captured, the model with a nonlinear air temperature predictor performed better (R^2 0.30, air temperature F-value 10.37) than the model with belowground temperature (R^2 0.26, belowground temperature F-value 3.60). The final model found that percent moonphase (t-value -0.63, p-value 0.529) and humidity (t-value 1.58, p-value 0.116) were unrelated to captures, whereas maximum wind speed at sunset may have been positively related to capturing any beetles (β 0.11, se 0.05, t-value 2.16, p-value 0.032). There were also strong nonlinear patterns in probability of any captures over the course of the sampling season (F 4.69, p-value 0.003), with higher capture rates in the middle and lower capture rates at the beginning and the end of the survey season. The probability of any capture was also nonlinearly related to air temperature (F 10.37, p-value < 0.001), with capture probability rapidly increasing above 15° C and then becoming constant again at further temperature increases above ~32° C (Figure 4).

Figure 4. Probability of Casey’s June beetle emergence (i.e., any captures vs. zero captures) as a function of air temperature, with beetles increasingly likely to emerge as air temperature increases from 15 to 32° C. Blue and red lines mark lower and upper limits, respectively, for changes in the probability of emergence.



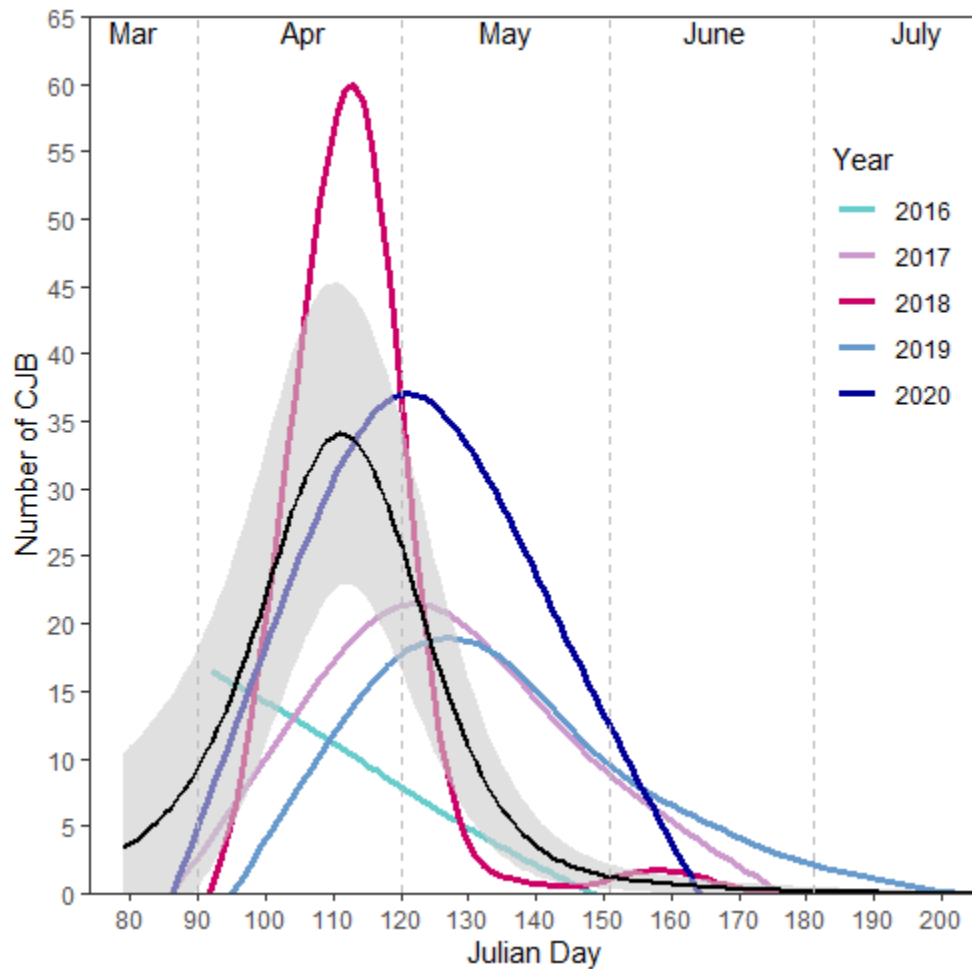
3.) Are any weather or moonphase factors associated with relative abundance of Casey’s June beetles?

Relative abundance of Casey’s June beetles was nonlinearly related to Julian day, with a peak in beetle abundance in late April/early May (F 19.79, p -value < 0.001). Relative abundance was positively associated with air temperature (β 0.23, se 0.03, t -value 7.35, p -value < 0.001), with more beetles being captured on warmer evenings. Relative abundance at the sentinel site also increased from 2016 through 2020, increasing by a factor of 1.17 (95% CI 1.00 – 1.39) every year. Humidity (t -value -0.16, p -value 0.873), max wind speed (t -value -0.65, p -value 0.517), and percent moonphase (t -value -1.44, p -value 0.152) at sunset were not related to relative abundance.

4.) What is the interannual temporal pattern in captures?

In most of the five years, the relative abundance of beetles caught at the sentinel trap showed strong nonlinear patterns with peaks in the number captured in late April or early May, depending on the year, aligning with the 50% full waxing moon at the same time period (Figure 2). Across all years, the model-adjusted smoothed trend in relative abundance of beetles captured at the sentinel trap peaked in late April due to 2016, when abundance started high and linearly decline over the sampling season (Figure 5).

Figure 5. Annual and across-year temporal variation in peak captures of Casey’s June beetles. Solid black line is across-year model-adjusted relative abundance, with gray 95% C.I. band.



Rangewide survey data – Abundance patterns

1.) What drives the probability of capturing an individual beetle, given that it is emerged and present in the trapping area?

The probability of capturing an individual beetle was related to belowground temperature, maximum wind speed, or both, depending on the year (Table 3). The relationships were always negative (Table 4), indicating that individual beetles were less likely to be captured as belowground temperature or maximum wind speed increased. At average observed wind speed or belowground temperatures, the probability of capturing an individual emerged male beetle was variable across years but was always low (e.g., between 0.02 and 0.14; Figure 6).

Figure 6. Probability of detecting (i.e., capturing) an individual male Casey’s June beetle, given that it has emerged and is present in the trapping area. Error bars are 95% confidence intervals.

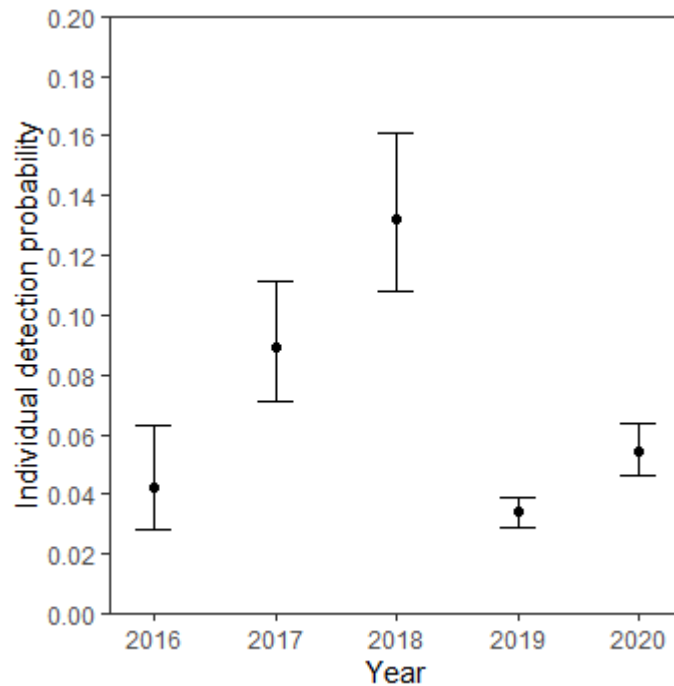


Table 3. Model selection results for detection (i.e., capture) probability of an individual beetle given that it is emerged and present in the trapping area. Lower ΔAICc values indicate stronger support for a given covariate.

Covariate	ΔAICc				
	2016	2017	2018	2019	2020
Ground temperature	72.76	149.83	312.39	334.57	175.79
Belowground temperature	0.00	0.00	246.57	0.00	120.37
Max. wind speed	0.22	61.13	0.00	219.10	0.00
Null (i.e., constant)	198.28	199.48	465.39	212.99	176.23

Table 4. Coefficients for individual detection probability (with standard errors) on the logit scale.

Covariate	Coefficient (se)				
	2016	2017	2018	2019	2020
Belowground temperature	-0.136 (0.014)	-0.236 (0.019)	-	-0.195 (0.016)	-
Max. wind speed	-0.415 (0.053)	-	-0.435 (0.025)	-	-0.204 (0.016)

2.) What drives initial corrected abundance of captures at the beginning of a trapping season?

Across years there was overwhelming support that allowing initial abundance to vary among trap sites was more informative than general habitat type (e.g., wash vs. upland) or assuming constant abundance across all traps (Table 5). That being said, habitat type outperformed a null model in three of the five years, indicating that habitat type is a better explanatory variable than constant abundance across sites, and was the best predictor in 2018 ($\Delta\text{AICc} = 0.00$, Table 5).

Table 5. Model selection results for general factors driving initial abundance (i.e., first trapping session within a year) for Casey's June beetles. Model selection overwhelmingly supported site-specific abundances. Each year contained a detection probability model as specified in Table 4.

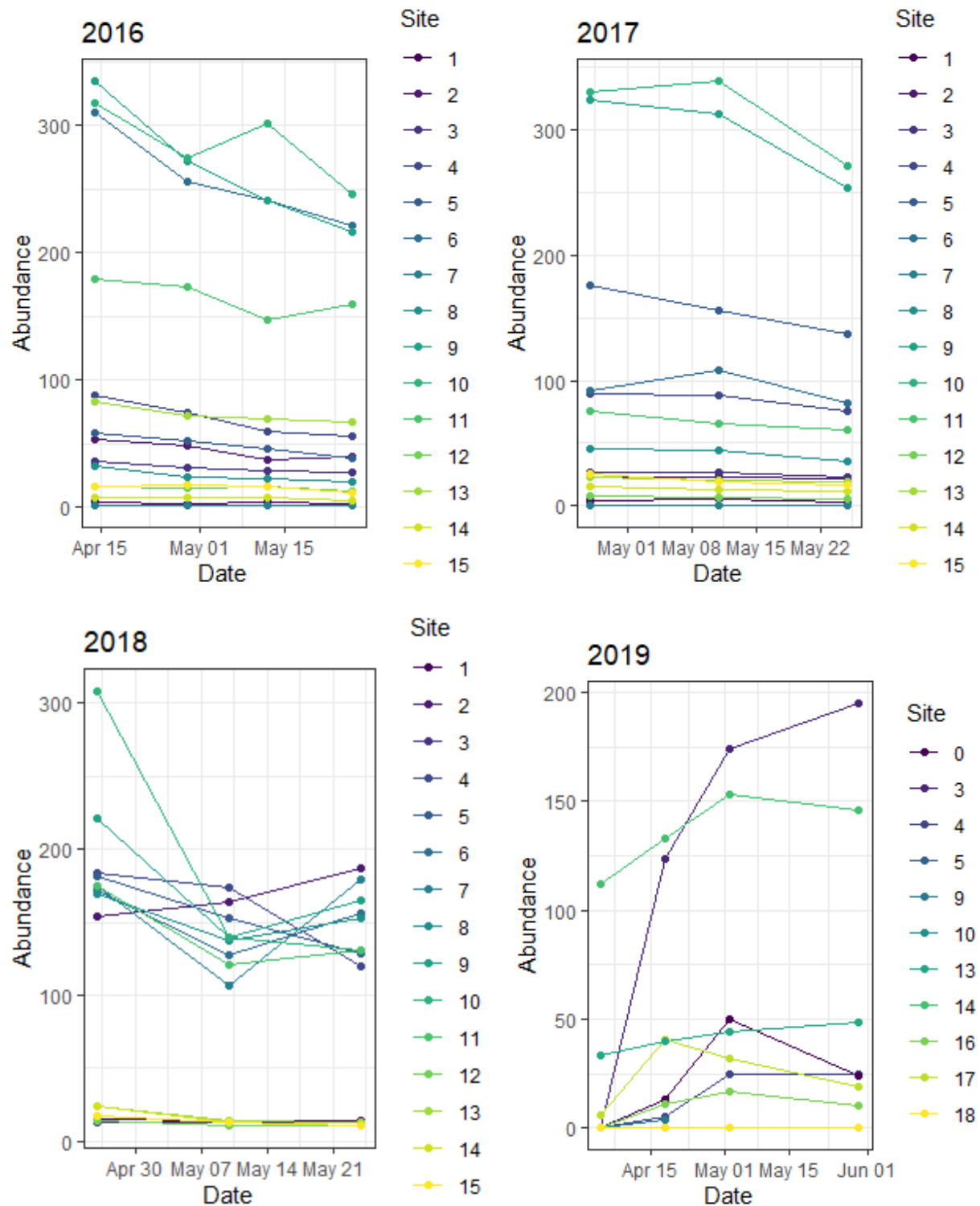
Covariate	ΔAICc				
	2016	2017	2018	2019	2020
Habitat type	299.38	394.16	0.00	188.85	192.05
Site-specific	0.00	0.00	738.08	0.00	0.00*
Null (i.e., constant)	524.69	819.64	682.10	177.87	138.26

*While the 2020 site-specific model converged, it returned NaN values for predictions of night-specific abundance estimates, so cautious inference is warranted.

3.) What is the true (corrected) abundance of male beetles over the course of sampling sessions within each year?

Corrected abundance estimates showed higher spatiotemporal variability than the sentinel trap data, with many sites showing constant estimated abundance across the rangewide trapping seasons and some sites showing temporal trends and peaks in estimated abundance (Figure 7). The spring peak in abundance was not evident in the rangewide trapping data, likely because the temporal resolution of the rangewide data (e.g., two weeks) was too low to capture the peak.

Figure 7. Within-year estimated corrected abundance at each trap site, after accounting for imperfect detection of individual male Casey's June beetles within a given evening. Abundance is the estimated number of male beetles with activity areas overlapping each trap site on a given night. Estimated abundance in 2020 is not provided because of convergence errors for the estimates. Note variable y-axes.



DISCUSSION

Casey's June beetles emerged over a several week period, but appeared to be alive and within the trapping area only for a short time, no more than three days. Beetle captures, and presumably emergence rates, tended to show a strong single peak in abundance at approximately 50% illumination of the waxing moon in late April/early May. The probability of capturing any beetles on a given night was strongly nonlinear over time, matching the peak observed in the raw data. Aside from the annual peak in capture rates, on a given night any beetles were more likely to be captured as air temperature increased from 15 to 32° C. The total abundance of captured beetles at the sentinel site also exhibited the late April/early May peak, but also increased with warmer air temperatures. At the sentinel site, there was a significant positive trend in the numbers of beetles captured from 2016 through 2020.

At the rangewide sites, the probability of capturing an emerged beetle was negatively related to belowground temperature, maximum wind speed, or both, depending on the year, and was always low. In other words, a beetle was less likely to emerge, be captured, and be recaptured as belowground temperature or maximum wind speed increased. At average observed belowground temperatures or wind speeds, the probability of capturing any given emerged male beetle was low. In four of the five years analyzed, a model with different abundances among sites on the first survey night performed overwhelmingly best, highlighting strong spatial variation in beetle abundances. Within years, unconstrained site and night-level abundances showed a range of temporal patterns, including constant abundance from late April to late May as well strong declines or increases over the rangewide trapping season.

Considering emergence patterns, there appeared to be two scales of factors at work. First, there was a dramatic spike in emergence rates and numbers of beetles captured around the 50% illumination date for the fourth or fifth waxing moon each year, whichever occurred in late April or early May. This event was clear in the data but was not apparent in statistical trends, as the beetles apparently keyed in on only the waxing moon in late April / early May and aside from this one-off event, emergence and abundance was unrelated to moonphase.

However, separate from the large moonphase-driven emergence event, weather or soil temperatures was related to emergence rates and abundances. On warmer evenings, beetles were more likely to emerge at all and the number captured increased. However, as belowground temp increased and/or wind speed increased, individual beetles were less likely to be recaptured. These findings indicate that temperature drives beetle emergence, but also modulates beetle behavior once emerged. It is possible that high wind speeds are associated with involuntary dispersal, or a behavioral preference for staying low on the ground in search of females and thus being less likely to be recaptured. It was interesting that belowground temperatures were more related to probability of recapture than ground temperatures, suggesting that possibly the physiological temperature of the beetles when they emerge matters more for behavior than the temperature of the air they emerge into.

Absolute abundance of Casey's June beetles also matters, particularly because they are an endangered species. The sentinel capture data highlighted several aspects of Casey's June beetle

population ecology and abundance. First, some beetles that emerge on a given night are still within the trap area on a subsequent night, possibly up to three nights apart. This suggests a rolling emergence window, whereby the male beetles captured on a given night may have emerged on a previous night. Second, this longevity was also quite rare in the dataset, as only 4.7% of captured beetles were ever recaptured, and of the recaptured beetles, only 8.5% percent were recaptured on a subsequent day (thus, 0.4% of all beetles were recaptured on a subsequent day). This indicates that a number of beetles remained uncaptured within each capture night, leading to the large estimated corrected abundances in some sites (Figures 6 and 7). The low estimated probability of capturing any given emerged beetle was corroborated by low recapture rates. If low recapture rates indicate high rates of population turnover, it may be that daily sampling of abundances and associated individual detection probabilities could be used to estimate nightly corrected abundances, generating full population size estimates.

The application of N-mixture modeling separate for each year of the rangewide trapping data, with each trapping evening being a primary sampling period and each hour within each evening being a secondary sampling period, provided a unique opportunity for temporal replication, as each year was treated independently. This means that the consistency in covariates among years provides additional information on the importance of those covariates to Casey's June beetle ecology. Here, we found that individual detection probability was consistently driven by belowground temperature, wind speed, or both. And further, that individual detection probability was always low. We also found that habitat type was often a useful predictor of abundance, but that individual site variation was usually a better predictor, indicating that microsite effects underlie abundance.

The results from this analysis should be interpreted along with several caveats and limitations. First, the methods for the rangewide sites assume a closed population within each evening, but it is possible that individuals emerge during the middle of the trapping event, and thus there is 'immigration' to the population available to be captured. Second, individual beetles are likewise assumed to remain available for capture during the trapping session and have not emigrated or died within the evening. Third, this analysis did not assess the spatial coverage of the rangewide sampling scheme, and thus the trap layout may be missing additional beetles that had emerged, were present within the range of Casey's June beetles, but were physically unavailable for capture due to being too far from a trap to be lured to the trap. Given the low rate of recaptures at and between traps, this is likely the case, and thus summing any individual night's corrected abundance is still underestimating true abundance. Fourth, this analysis has not identified micro-site factors that may drive site-level abundance, such as fine soils characteristics, above or belowground vegetation, or local hydrology. In particular, this fourth caveat is a topic worth considering for future analyses. Such information would be helpful for identifying what makes 'good' habitat and could guide future survey and conservation action efforts.

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U.S. Fish and Wildlife Service (USFWS). 2009. Listing Casey's June beetle (*Dinacoma caseyi*) as endangered and designation of critical habitat. *Federal Register* 74:32857-32875.

U.S. Fish and Wildlife Service. 2011. Determination of endangered status for Casey's June beetle and designation of critical habitat. *Federal Register* 76:58954-58998.

Appendix A. Annual plots overlaying total numbers of Casey's June beetles captured each night at sentinel traps and measured environmental data, plotted over Julian day within each year.

Figure A1. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and air temperature (C) at sunset (red line).

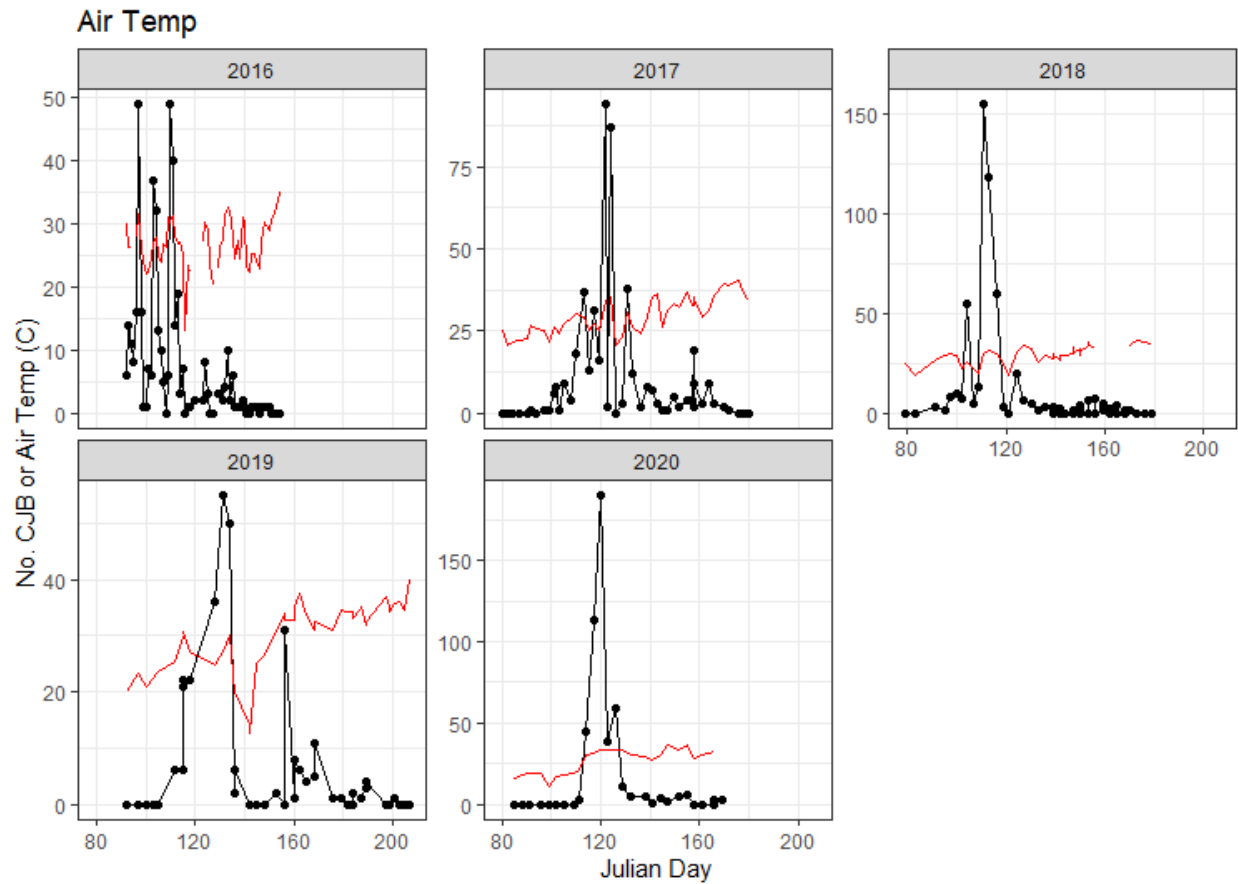


Figure A2. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and maximum wind speed (km/hr) at sunset (blue line).

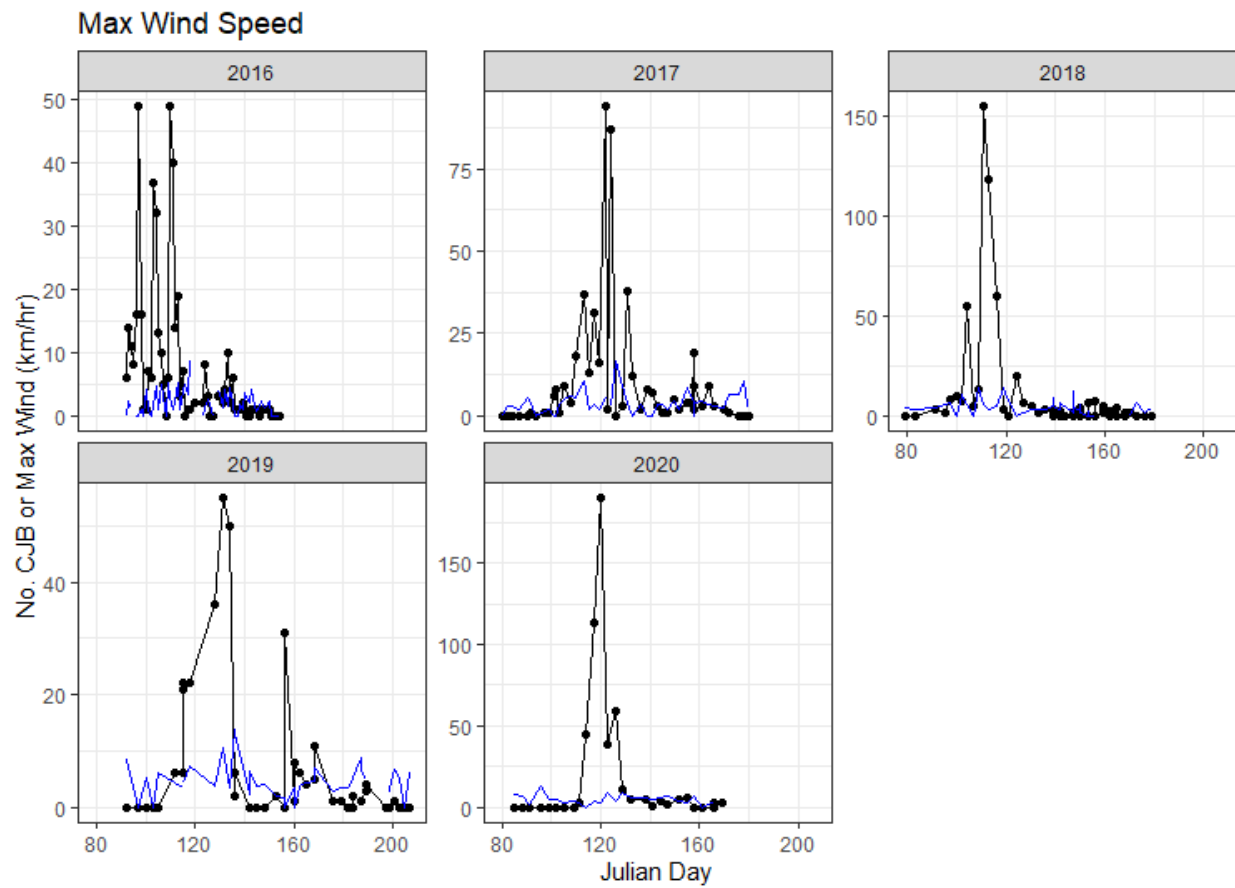
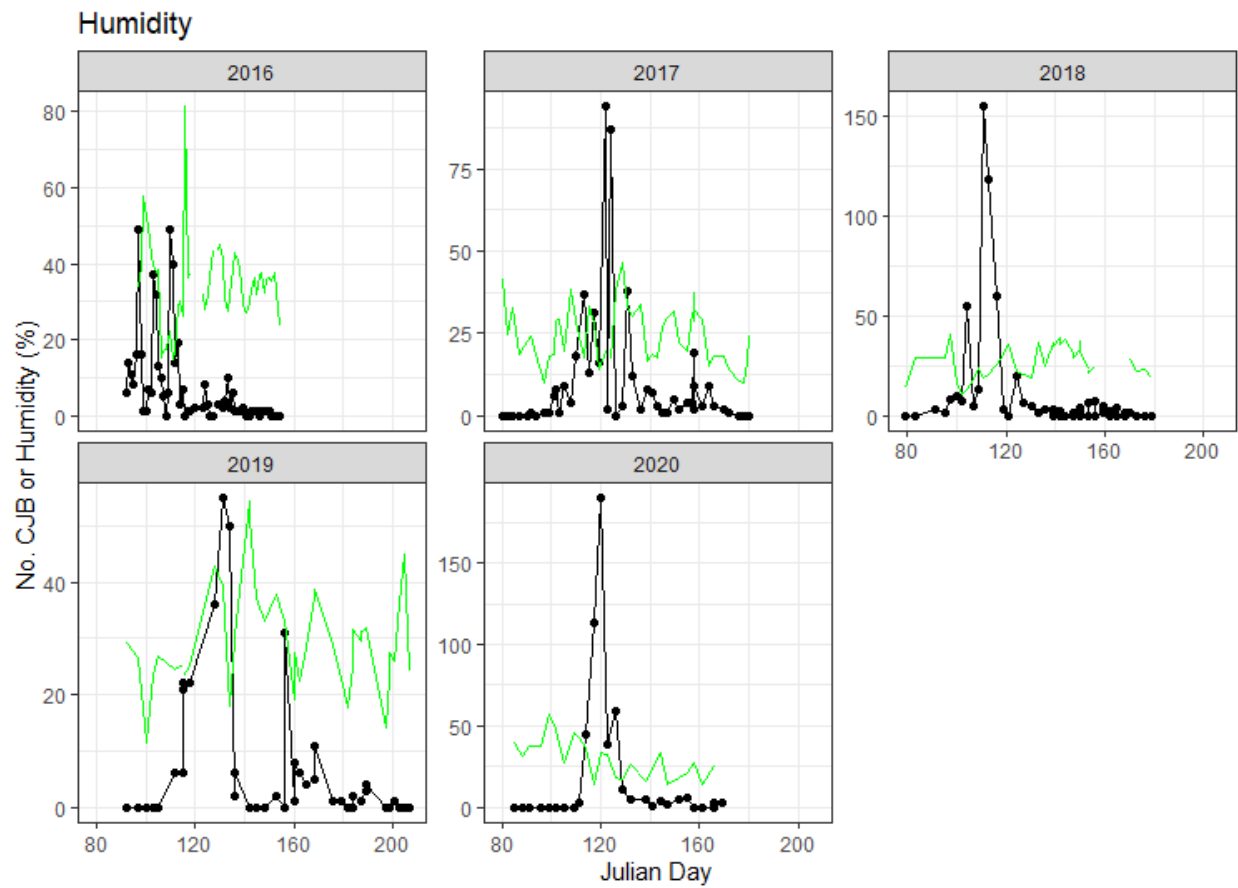


Figure A3. Number of captured Casey's June beetles at the sentinel trap (dots and black line) and percent humidity at sunset (green line).



Supporting Information

Data packages and R code (Harju 2021) are on file at the U.S. Fish and Wildlife Service, Palm Springs Fish and Wildlife Office, Palm Springs, California.

Predicted Estimated Abundance 2016–2019

Tables F1 to F4 (adapted from Harju 2021)

Table F1. Within-year corrected abundance (predicted abundance) of Casey's June beetles at Rangewide survey traps in 2016. Estimates from Harju 2021.

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
1	3.654993	1	7	4/14/2016	1
2	53.81084	40	68	4/14/2016	1
3	36.26706	26	48	4/14/2016	1
4	87.63773	71	105	4/14/2016	1
5	58.31007	45	73	4/14/2016	1
6	310.2733	279	341	4/14/2016	1
7	1.069612	0	3	4/14/2016	1
8	31.74311	22	42	4/14/2016	1
9	335.5278	310	350	4/14/2016	1
10	317.6671	287	346	4/14/2016	1
11	178.9866	155	204	4/14/2016	1
12	16.10962	9	24	4/14/2016	1
13	83.48284	67	101	4/14/2016	1
14	7.729523	3	14	4/14/2016	1
15	16.47465	9	25	4/14/2016	1
1	2.529836	0	6	4/29/2016	2
2	48.05075	35	62	4/29/2016	2
3	31.63355	21	43	4/29/2016	2
4	74.76657	58	92	4/29/2016	2
5	51.89951	38	66	4/29/2016	2
6	255.6237	225	287	4/29/2016	2
7	1.937009	1	4	4/29/2016	2
8	23.81952	15	34	4/29/2016	2
9	271.9149	241	303	4/29/2016	2

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
10	274.7808	244	306	4/29/2016	2
11	173.224	149	199	4/29/2016	2
12	15.52596	9	23	4/29/2016	2
13	71.40476	55	88	4/29/2016	2
14	7.500229	3	13	4/29/2016	2
15	16.97779	10	25	4/29/2016	2
1	3.559454	2	6	5/12/2016	3
2	36.99825	26	49	5/12/2016	3
3	29.14378	20	40	5/12/2016	3
4	59.90491	45	75	5/12/2016	3
5	46.34721	34	59	5/12/2016	3
6	241.3805	213	271	5/12/2016	3
7	1.130895	0	3	5/12/2016	3
8	21.84726	13	31	5/12/2016	3
9	241.0377	213	270	5/12/2016	3
10	301.1328	268	335	5/12/2016	3
11	146.8908	125	170	5/12/2016	3
12	16.14534	9	24	5/12/2016	3
13	69.74991	54	86	5/12/2016	3
14	7.092639	3	12	5/12/2016	3
15	16.65496	9	25	5/12/2016	3
1	2.202358	0	5	5/26/2016	4
2	39.38442	28	52	5/26/2016	4
3	27.46058	18	38	5/26/2016	4
4	55.83905	42	71	5/26/2016	4

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
5	39.0099	27	52	5/26/2016	4
6	221.4224	193	251	5/26/2016	4
7	0.834538	0	3	5/26/2016	4
8	20.23652	12	29	5/26/2016	4
9	216.415	188	245	5/26/2016	4
10	245.8581	216	277	5/26/2016	4
11	159.1848	136	184	5/26/2016	4
12	12.75489	6	20	5/26/2016	4
13	66.64247	51	83	5/26/2016	4
14	5.61598	2	11	5/26/2016	4
15	11.84705	6	19	5/26/2016	4

¹ LCL=Lower 95 Percent Confidence Limit. UCL=Upper 95 Percent Confidence Limit.

Table F2. Within-year corrected abundance (predicted abundance) of Casey's June beetles at Rangewide survey traps in 2017. Estimates from Harju 2021.

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
1	3.824249	2	7	4/27/2017	1
2	18.85961	12	27	4/27/2017	1
3	20.55987	13	29	4/27/2017	1
4	51.99283	41	64	4/27/2017	1
5	144.4385	124	166	4/27/2017	1
6	90.0393	75	106	4/27/2017	1
7	0.000239	0	0	4/27/2017	1
8	46.9611	36	59	4/27/2017	1
9	322.0518	294	347	4/27/2017	1
10	331.6879	304	349	4/27/2017	1
11	73.63581	61	87	4/27/2017	1
12	5.348824	2	10	4/27/2017	1
13	16.69492	10	24	4/27/2017	1
14	11.73048	6	18	4/27/2017	1
15	21.08832	14	29	4/27/2017	1
1	4.303339	2	7	5/11/2017	2
2	19.1087	12	27	5/11/2017	2
3	20.17276	12	29	5/11/2017	2
4	51.15225	38	65	5/11/2017	2
5	133.7856	115	153	5/11/2017	2
6	98.90828	84	115	5/11/2017	2
7	1.000193	1	1	5/11/2017	2
8	46.0941	35	58	5/11/2017	2
9	315.3201	286	343	5/11/2017	2

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
10	340.9953	322	350	5/11/2017	2
11	66.60723	53	81	5/11/2017	2
12	4.891449	2	9	5/11/2017	2
13	14.61995	9	22	5/11/2017	2
14	9.919504	5	16	5/11/2017	2
15	15.29195	8	23	5/11/2017	2
1	2.838784	0	6	5/25/2017	3
2	16.50613	10	24	5/25/2017	3
3	16.10186	9	24	5/25/2017	3
4	41.90935	30	55	5/25/2017	3
5	112.4278	93	132	5/25/2017	3
6	76.68368	61	93	5/25/2017	3
7	0.523069	0	1	5/25/2017	3
8	36.62774	26	48	5/25/2017	3
9	253.1701	224	284	5/25/2017	3
10	269.1623	240	299	5/25/2017	3
11	57.53694	44	72	5/25/2017	3
12	3.933341	1	8	5/25/2017	3
13	12.98618	7	20	5/25/2017	3
14	8.244716	3	14	5/25/2017	3
15	13.39275	7	21	5/25/2017	3

¹ LCL=Lower 95 Percent Confidence Limit. UCL=Upper 95 Percent Confidence Limit.

Table F3. Within-year corrected abundance (predicted abundance) of Casey's June beetles at Rangewide survey traps in 2018. Estimates from Harju 2021.

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
1	14.88283	9	21	4/26/2018	1
2	153.7031	132	177	4/26/2018	1
3	12.42947	0	21	4/26/2018	1
4	183.0783	160	207	4/26/2018	1
5	181.7762	159	205	4/26/2018	1
6	171.8986	152	193	4/26/2018	1
7	175.0153	0	217	4/26/2018	1
8	169.3878	145	195	4/26/2018	1
9	220.5486	198	244	4/26/2018	1
10	308.4286	284	333	4/26/2018	1
11	174.4736	155	195	4/26/2018	1
12	13.85355	8	20	4/26/2018	1
13	23.8943	18	31	4/26/2018	1
14	24.11644	19	30	4/26/2018	1
15	16.80596	11	23	4/26/2018	1
1	13.48277	8	20	5/10/2018	2
2	164.3184	140	190	5/10/2018	2
3	12.49902	6	20	5/10/2018	2
4	173.8325	150	199	5/10/2018	2
5	152.4807	129	177	5/10/2018	2
6	127.1766	106	149	5/10/2018	2
7	106.0887	86	127	5/10/2018	2
8	137.4072	115	161	5/10/2018	2
9	139.9984	119	162	5/10/2018	2

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
10	139.6325	120	160	5/10/2018	2
11	120.4428	102	140	5/10/2018	2
12	10.7691	5	18	5/10/2018	2
13	14.25137	8	22	5/10/2018	2
14	13.33128	7	21	5/10/2018	2
15	12.37612	6	20	5/10/2018	2
1	13.50668	7	21	5/24/2018	3
2	186.953	161	214	5/24/2018	3
3	12.53526	6	20	5/24/2018	3
4	119.7947	99	142	5/24/2018	3
5	128.7342	107	151	5/24/2018	3
6	155.8145	132	181	5/24/2018	3
7	179.5851	154	206	5/24/2018	3
8	153.0443	130	178	5/24/2018	3
9	165.2275	141	191	5/24/2018	3
10	131.2377	110	153	5/24/2018	3
11	131.1309	110	153	5/24/2018	3
12	11.18692	5	18	5/24/2018	3
13	13.14897	7	20	5/24/2018	3
14	10.7902	5	18	5/24/2018	3
15	10.22821	4	17	5/24/2018	3

¹ LCL=Lower 95 Percent Confidence Limit. UCL=Upper 95 Percent Confidence Limit.

Table F4. Within-year corrected abundance (predicted abundance) of Casey's June beetles at Rangewide survey traps in 2019. Estimates from Harju 2021.

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
3	1.58E-10	0	0	4/4/2019	1
4	4.46E-07	0	0	4/4/2019	1
5	1.97E-06	0	0	4/4/2019	1
9	9.35E-07	0	0	4/4/2019	1
10	3.10E-07	0	0	4/4/2019	1
0	0.009627	0	0	4/4/2019	1
16	2.87E-06	0	0	4/4/2019	1
17	6.326002	2	11	4/4/2019	1
18	1.64E-06	0	0	4/4/2019	1
13	33.39969	0	57	4/4/2019	1
14	111.7152	0	173	4/4/2019	1
3	123.3697	102	146	4/18/2019	2
4	5.000001	5	5	4/18/2019	2
5	2.95E-06	0	0	4/18/2019	2
9	4.000002	4	4	4/18/2019	2
10	4.70E-07	0	0	4/18/2019	2
0	13.01754	13	13	4/18/2019	2
16	11.00001	11	11	4/18/2019	2
17	40.62243	36	46	4/18/2019	2
18	2.21E-06	0	0	4/18/2019	2
13	39.77947	19	57	4/18/2019	2
14	133.0543	72	173	4/18/2019	2
3	174.1902	149	200	5/2/2019	3
4	25	25	25	5/2/2019	3

Trap	Abundance	LCL ¹	UCL ¹	Date	Visit
5	-	-	-	5/2/2019	3
9	-	-	-	5/2/2019	3
10	-	-	-	5/2/2019	3
0	50.0236	50	50	5/2/2019	3
16	17.00001	17	17	5/2/2019	3
17	32.25087	26	39	5/2/2019	3
18	2.22E-06	0	0	5/2/2019	3
13	44.16187	30	58	5/2/2019	3
14	152.8597	121	179	5/2/2019	3
3	195.0677	168	223	5/30/2019	4
4	24.46026	24	25	5/30/2019	4
5	-	-	-	5/30/2019	4
9	-	-	-	5/30/2019	4
10	-	-	-	5/30/2019	4
0	24.10436	18	31	5/30/2019	4
16	10.10349	7	13	5/30/2019	4
17	18.70635	12	26	5/30/2019	4
18	2.46E-06	0	0	5/30/2019	4
13	48.33089	36	62	5/30/2019	4
14	145.6842	122	170	5/30/2019	4

¹ LCL=Lower 95 Percent Confidence Limit. UCL=Upper 95 Percent Confidence Limit.

Harju 2022 – Supplemental Population Analysis Report

Casey's June Beetle Population Analyses
Geographic closure, 2018 rangewide abundance, and
hydrological and vegetation factors
driving site-level abundance

Supplementary Report

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Initial report: June 17, 2021

Additional analyses added: Nov 17, 2021

Revised analyses added: Apr 20, 2022

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INTRODUCTION

Heron Ecological previously submitted a report on factors affecting emergence and relative abundance of Casey's June beetle (*Dinacoma caseyi*) to the U.S. Fish and Wildlife Service (USFWS) and Dudek on June 17th, 2021 (Harju 2021). In August of 2021, the USFWS secured additional funding and requested additional analyses of the Casey's June beetle data, including new 2021 survey data. Following review of the November 17th report, USFWS requested re-analysis of some of the data to remove the impact of the 2019 flood and to update the layer for the expected range of Casey's June beetles. This report details those re-analyses. Please see Harju (2021a and 2021b) for background on Casey's June beetle ecology and life history and the sampling designs and methods for data collection for data used in this report.

NEW QUESTIONS

(Note: the methods and results for Questions 1, 2, and 5 are unchanged from Harju (2021b). Questions 3 and 4 are updated here, but Questions 1, 2, and 5 are retained so that this report can serve as a complete, corrected version of Harju (2021b).

I used existing datasets, and newly collected Casey's June beetle capture data from 2021, to analyze these new questions:

- 1.) What were the site-level corrected abundances of male Casey's June beetles on each trapping night in 2021?
- 2.) What is an effective distance within which to delineate population geographic closure?
- 3.) What was the detection probability-corrected estimated average nightly rangewide abundance of male Casey's June beetles in 2018?
- 4.) Was average corrected abundance related to preceding January-March streamflow?
- 5.) Were 2016 and 2019 average corrected abundances related to the site-level vegetation community?

METHODS FOR THE NEW QUESTIONS

1.) What were the site-level corrected abundances of male Casey's June beetles on each trapping night in 2021?

This question was an exact replication of the methods and results described in Harju (2021), but re-run on the new 2021 capture data. All capture locations except one (trap 16) were located on an active golf course in the lower portion of Tahquitz Creek which supported remnant patches of suitable Casey's June beetle habitat. See Harju (2021) for details on data preparation, N-mixture modeling (Royle 2004), and result interpretation. Results are 'corrected' abundance estimates, after imperfect detection of individual male Casey's June beetles within each trapping evening is adjusted for.

2.) What is an effective distance within which to delineate population closure?

Following capture, beetles were released at 0, 10, 30, 40, 50, 100, 150, 200, or 250 m from the trap of origin. Following release, some beetles were then recaptured, and the distance between release and recapture locations provides information on travel distances from the point

of release to the recapture site. I used distance detection functions in package ‘Distance’ in Program R (v4.0.5) to fit detection functions to the origin-to-recapture distance data to estimate the distance from release at which a beetle was as equally likely to be recaptured as not (i.e., the effective radius of the area of recapture surrounding an origin location). Theoretically, this radius can function as a general ‘boundary’, delineating an effective trapping radius around each rangewide trap because for any given location within Casey’s June beetle habitat, it represents the distance beyond which any emerging beetle is less likely to be captured in the trap than captured in it.

I truncated the farthest 5% of observed distances and then compared half-normal, hazard rate, and uniform distance detection key functions using Akaike’s Information Criterion (AIC) to select which performed best at explaining the decay in recapture probability over distance (Buckland et al. 2015). I then calculated the effective radius ρ as:

$$\rho = w * \sqrt{P_a}$$

where w is the truncation distance for recapture distances (i.e., 95% of observed distances = 250 m) and P_a is the average recapture probability within 250 m of any given point.

3.) What was the detection probability-corrected estimated average nightly rangewide abundance of male Casey’s June beetles in 2018?

Prior to estimating average nightly rangewide abundance I calculated the proportion of males present in the population that were newly emerged that same night. The proportion of newly emerged males per night (r) was determined using a Poisson regression model on days-since-released for all males that were captured and then subsequently recaptured. The model-based proportion of expected non-zero days since recapture was summed across days and the inverse was taken as the proportion of males expected to be captured on the day of emergence (e.g., day 0).

As an example of a simple way to estimate rangewide abundance of Casey’s June beetles, I performed some extrapolation calculations using the N-mixture detection probability-corrected results from 2018. First, I calculated the average corrected abundance and 95% lower and upper confidence intervals on abundance averaged across all three rangewide trapping nights for each sampled site in 2018. Second, I created a discretized hexagonal geographic closure grid overlapping all rangewide traps and ‘Expected CJB range’ provided by USFWS in March, 2022. The radius of each hexagon was determined using the results from Question 2 in Harju (2021b; e.g., the effective capture distance). Third, I joined the average abundance estimates and confidence intervals to individual 2018 rangewide traps and then extrapolated these across the extent of the hexagonal grid using the inverse distance weighted extrapolation tool in ArcGIS (v10.4) with a cell size equal to the effective capture distance (i.e., 77 m). This generated a continuous surface of predicted abundances. Fourth, I sampled the average interpolated abundance value within each hexagonal grid cell. Fifth, I created a new layer of the intersection between hexagonal grid cells and the ‘Expected CJB range’ and then multiplied estimated abundances within each hexagonal grid cells by the proportion of that hexagon cell that was within the ‘Expected CJB range’. Sixth, I summed the proportional estimated abundances across all cells within the CJB range. Seventh, I multiplied this value by the proportion of emerged male beetles that were expected to have emerged on a single day (based on the period of

population closure analysis for the sentinel data; Harju 2021a), such that the estimate of average Daily Emerged Males (DEM) was calculated as:

$$DEM = \sum_{i=0}^I (\widehat{N}_i * p_i) * r$$

where N was the average estimated abundance of males within each hexagon cell i as determined from the extrapolated abundance surface, p was the proportion of the survey potential boundary that fell within each hexagon grid cell i , and r was the proportion of males caught on a given night that were expected to be newly emerged males (e.g., not having emerged on a previous night).

Finally, I did these steps each for the mean corrected abundance as well as the average upper and lower 95% confidence intervals across sampling days. This yielded an estimate of the number of newly emerged male Casey's June beetles, rangewide, on any average night during the 2018 rangewide sampling window (i.e., April 26th – May 24th, 2018).

This calculation required a number of strict assumptions that were surely violated. First, beetle occurrence and emergence is a continuous process both over space (i.e., occupied range) and time (i.e., nonlinear temporal emergence patterns within days and over the emergence season). Thus discretizing ignores these continuums. Second, it assumes that the rangewide trap sites are representative samples of rangewide variation in abundance. Third, all assumptions inherent in the N-mixture abundance modeling are propagated here (Royle 2004). Fourth, inverse distance weighting assumes linear gradients in abundance over a two-dimensional surface, but true habitat and local abundances may be nonlinear over space. Fifth, by calculating the confidence intervals using all lowest or highest abundance confidence limits simultaneously, the nominal interval for nightly newly emerged male beetles may be overly wide. Sixth, true occupied range is unknown, and the use of 'Expected CJB range' as a value-modifier may not reflect the proportion by which the hexagonal estimates should be decreased. Nonetheless, this calculation may provide some information on potential total population size

4.) Was average corrected abundance related to preceding January-March streamflow?

I chose the window of January through March preceding each Casey's June beetle trapping season to link stream flows along Tahquitz Creek and Palm Canyon Wash to average site-level abundance estimates of Casey's June beetles as derived from N-mixture modeling (see Harju 2021). Only sites classified as wash sites were included in this analysis, as streamflow is not necessarily expected to directly influence Casey's June Beetles at upland sites. I chose this window because in several years there was a clear flow event in January that may affect underground beetle survival or emergence ability, because general pre-emergence flows may affect emergence, and because beetles generally began emerging in early April and thus abundance estimates used data beginning in April. The final reason ensured that there was no temporal overlap between stream flow measurements and abundance estimates, which would confound estimating relationships. Analyses were limited to 2016-2018 because of a site-wide scouring flood prior to sampling in 2019, site-level abundance being unestimable in 2020, and hydrologic data not having been provided for 2021.

First, I checked for correlations in average flow per day among the three U.S. Geological Survey stream flow gauge stations along Tahquitz Creek (Tahquitz) and Palm Canyon Wash

(Cathedral City and Headwaters). Then, after determining high correlations among stations and choosing one station, I tested for correlations among four daily measures of stream flow for January through March: average daily cubic feet per second (cfs), summed daily cfs, maximum daily cfs, and standard deviation in daily cfs. I also calculated the distance that each site was upstream from the Cathedral City (Cathedral) gauge station. Upstream distance from the Cathedral City gauge station to each trapping site was used as a predictor variable to represent potential spatial patterns in Casey's June beetle abundance driven by wash position, such that sites further upstream may be less affected by streamflow and sediment deposition or movement than sites downstream. I then used a linear regression model to evaluate whether average Casey's June beetle abundance was related to stream flow and/or distance from the Cathedral station.

Model fits were compared with each other using Akaike's Information Criterion corrected for small sample sizes (AICc). If the model with the lowest AICc value was > 2.0 units away from the second-lowest model, it was considered the best among the candidate set. Models within 2.0 AICc units of each other were considered 'competing', unless one of the competing models was a more complex version of the other, in which case I selected the simpler model for inference (Burnham and Anderson 2002). All models were compared to a null (i.e., intercept-only) model for goodness-of-fit. Model sufficiency was also assessed via examination of the model residuals.

5.) Were 2016 and 2019 average corrected abundances related to the site-level vegetation community?

Veg data were collected in 2016 and 2019 at the trap sites surveyed in those years (N. Ronan, pers. comm.). Data included species identification, percent cover by species, and native versus naturalized origin of each species. Species cover was assigned to percent cover classes ($<1\%$, 1-5%, 5-15%, 15-25%, or 25-50%). For analysis, I rounded each cover class up to its maximum value and then summed percent cover for all native species and then for all naturalized species at a site. For combined analyses, I then summed percent cover for both native and naturalized species at each site.

For statistical analysis, I first investigated potential effects of native versus naturalized vegetation on average site abundance of Casey's June beetles using linear regression and AICc. I evaluated potential relationships between abundance and native/naturalized species richness and native/naturalized percent cover. I then collapsed the dataset to combine native and naturalized plant species and evaluated whether average Casey's June beetle abundance was related to total percent plant cover, total plant species richness, whether a site was in a wash or upland, or a combination of those factors.

All spatial analyses were conducted in ArcGIS (v10.4) and all statistical analyses were performed in Program R (v3.6.3).

RESULTS FOR THE NEW QUESTIONS

1.) What were the site-level corrected abundances of male Casey's June beetles on each trapping night in 2021?

The April/May peak in Casey's June beetle abundance that was consistently observed in the sentinel data (Harju 2021) only appeared present at sites 16, 19, and 24 (Figure 1). The remaining sites showed gradual, consistent estimated abundance increases over the trapping season.

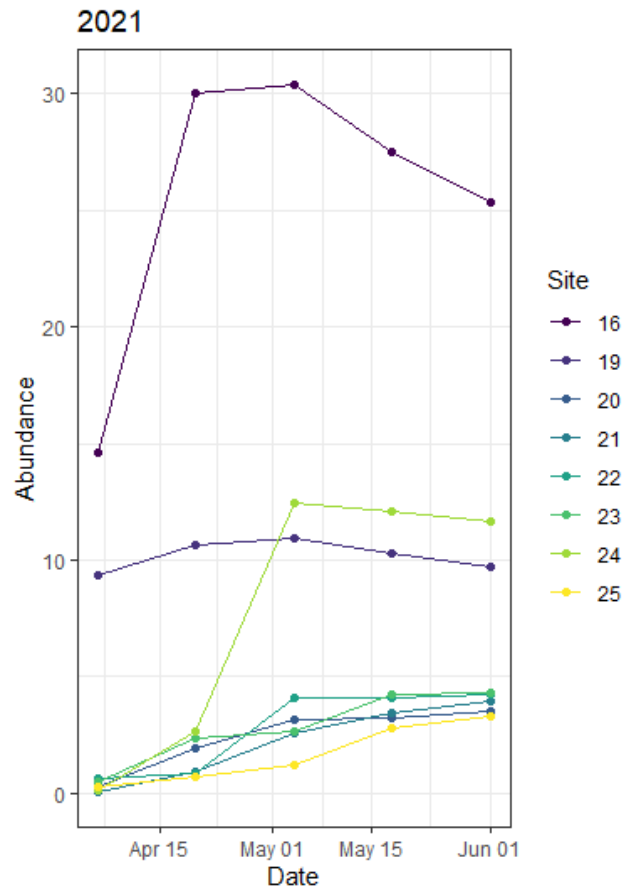


Figure 1. Corrected site abundance estimates of male Casey's June beetles on each of five trapping evenings during 2021.

2.) What is an effective distance within which to delineate population closure?

A uniform detection recapture distance decay function with fourth-order cosine adjustments performed best among alternative key functions for explaining distances of release-to-recapture (ΔAIC 100.1; Figure 2.). There was an average recapture probability of 0.096 (95% CI 0.082-0.109) and the effective capture radius around any given point was 77.3 m. This indicated that any beetle emerging < 77.3 m from a trap was more likely to be caught in that trap than not and any beetle emerging > 77.3 m from a trap was less likely to be caught in that trap than caught.

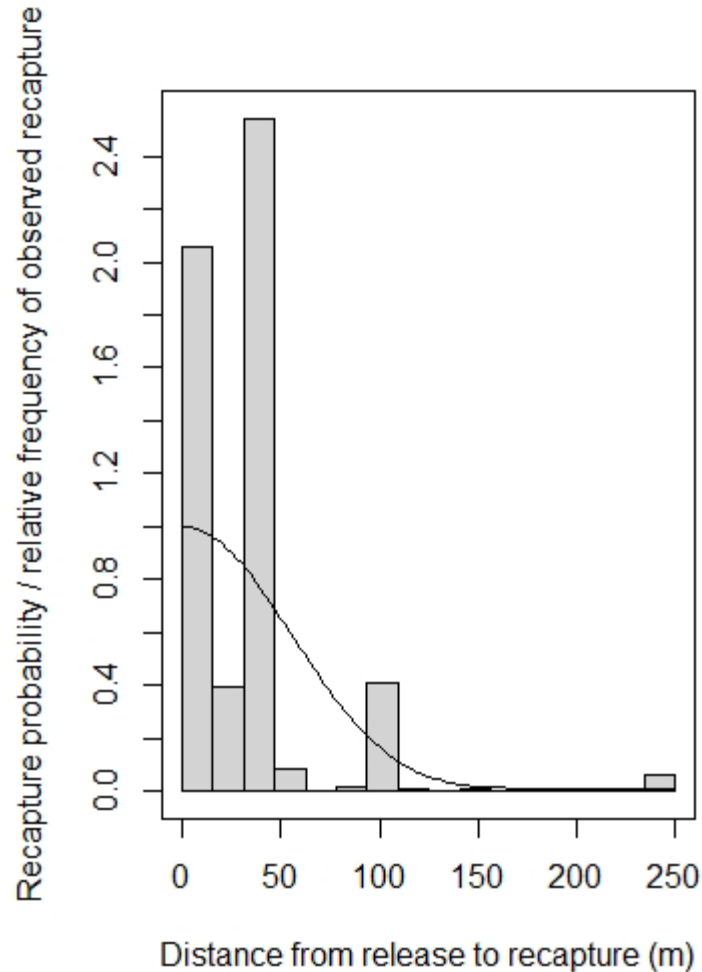


Figure 2. Recapture probability (line) and observed relative frequency of recapture (bars) for Casey's June beetles, California, USA, 2016-2021. Observed recapture distances truncated at the 95% of observed distance (i.e., 250 m, Buckland et al. 2015).

3.) What was the detection probability-corrected estimated average nightly rangewide abundance of male Casey's June beetles in 2019?

Based on sentinel site recaptures from 2017-2021, the Poisson regression model estimated that a proportion of 0.11 recaptured males were released on a previous day (Figure 3). This suggests that the inverse of this proportion, 0.89, is the proportion of males captured on a given day that had also emerged on that day. Thus 0.89 was used as the proportion of newly emerged beetles to discount the daily N-mixture abundance estimate for each grid cell to remove the estimated proportion of the N-mixture abundance estimate that were beetles from a previous day, thus deriving a single-day emergence estimate.

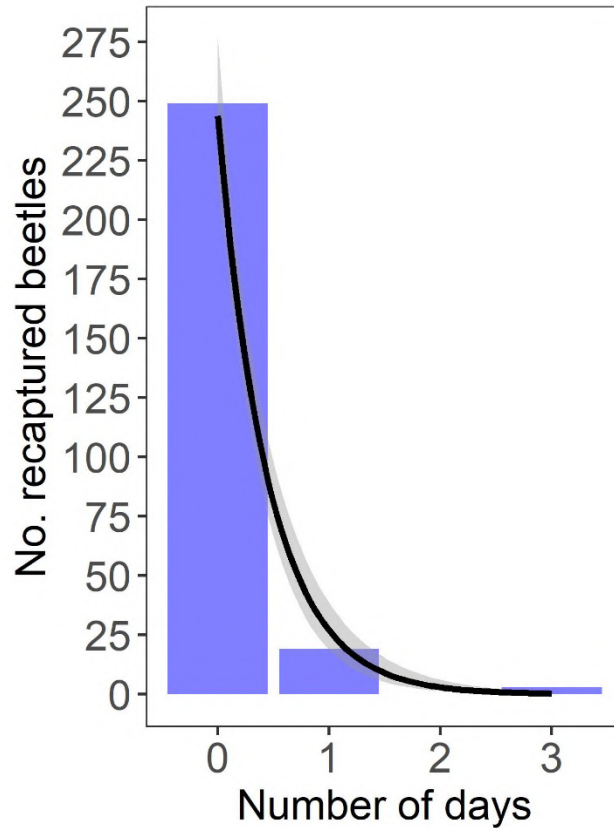


Figure 3. Poisson regression of expected number of days between when a male Casey's June beetle is first captured and released and then subsequently recaptured.

After averaging mean, lower 95% limits, and upper 95% limits across visits in 2019 within traps sites and conducting the calculation steps described in the Methods, there was an estimated average of 9,554.4 (95% CI 9,554.4 – 11,191.74) new male Casey's June beetles emerged rangewide per night between Apr 26th and May 24th, 2019 (Figures 4-6).

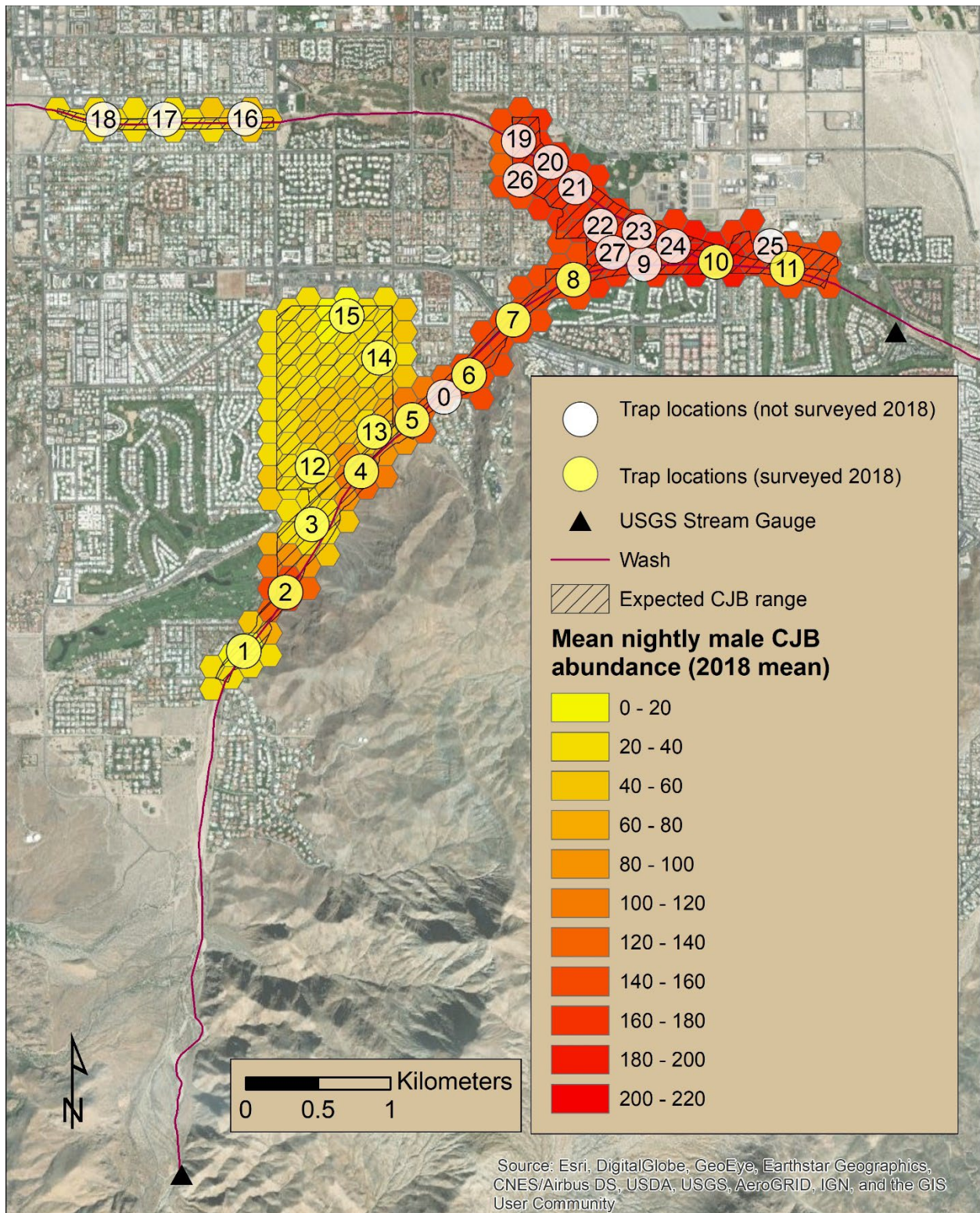


Figure 4. Estimated mean nightly abundance of male Casey's June beetles between April 26th and May 24th, 2018.

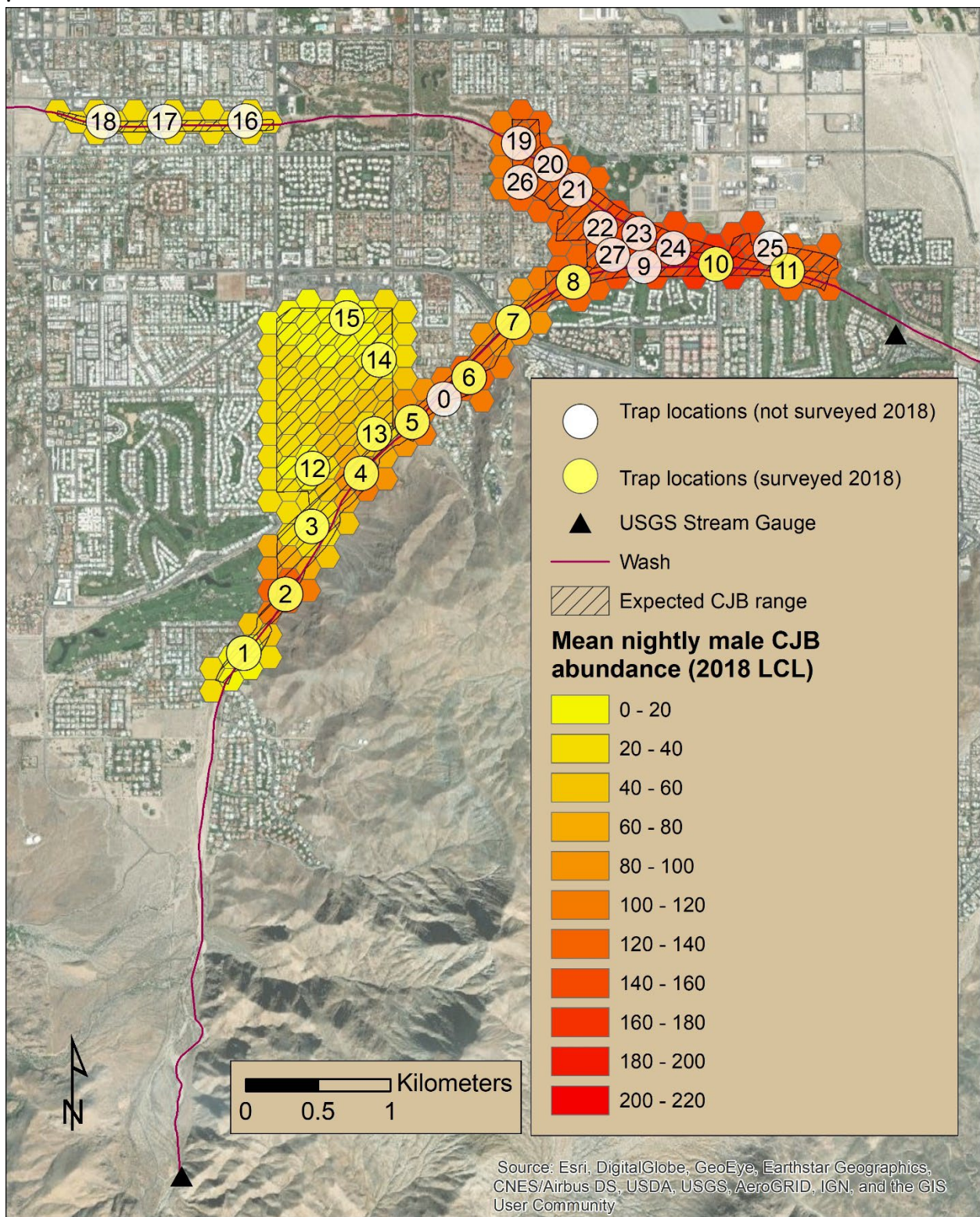


Figure 5. Mean nightly lower 95% confidence interval for estimated nightly abundance of male Casey's June beetles between April 26th and May 24th, 2018.

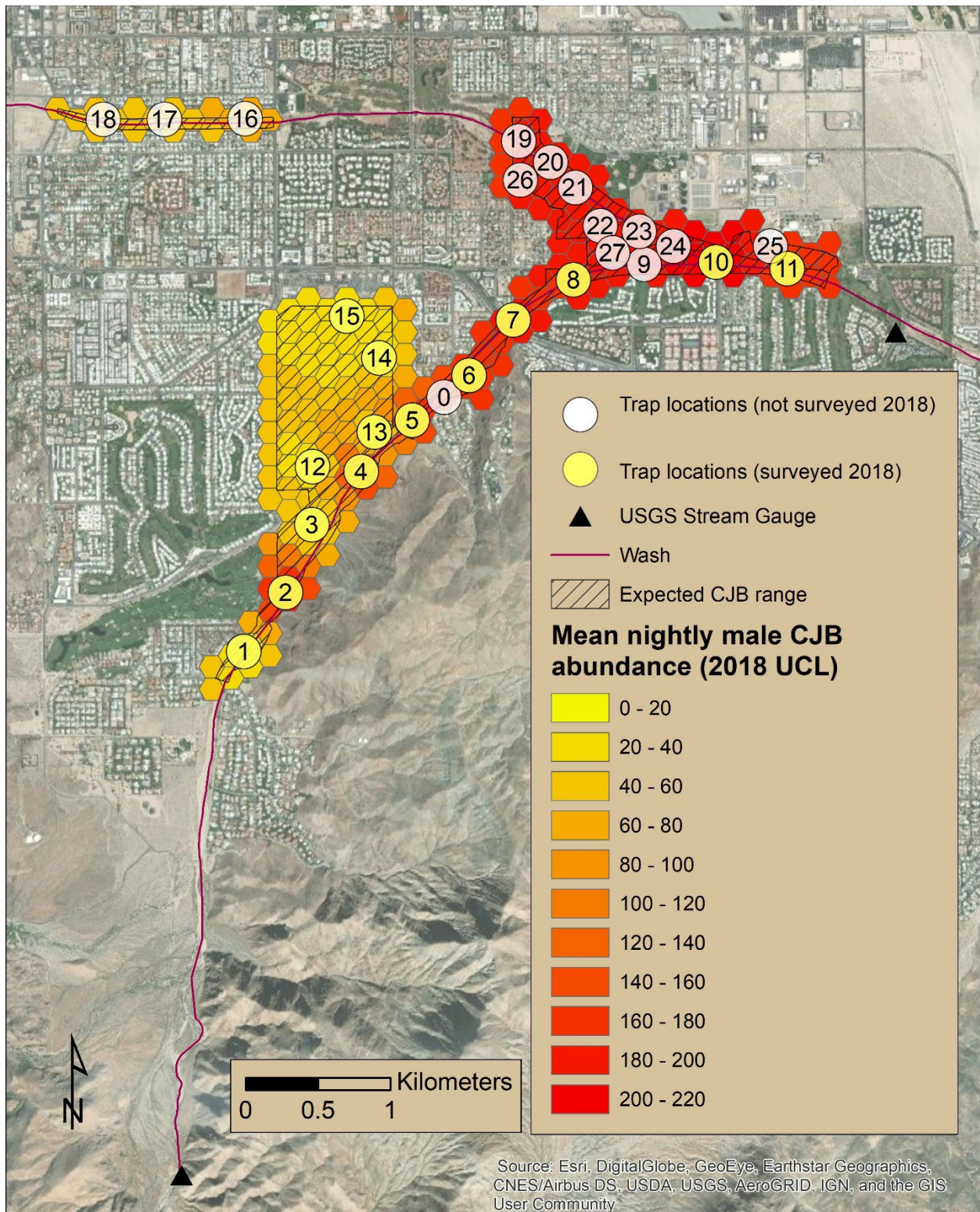


Figure 6. Mean upper 95% confidence interval for estimated nightly abundance of male Casey's June beetles between April 26th and May 24th, 2018.

4.) Was average corrected abundance related to preceding January-March streamflow?

The daily streamflow data from 2015 through 2020 (excluding 2019) were strongly correlated between the Cathedral City and Headwaters stations ($r = 0.92$), but only moderately correlated between the Headwaters and Tahquitz stations ($r = 0.44$), and the Cathedral City and Tahquitz stations ($r = 0.040$; Figure 7). Data from 2019 were excluded at the request of USFWS due to their anomalous nature and potential outsized and unrepresentative influence on the Casey's June beetle population. I chose to use data from the Cathedral City station only for further analyses, because this station was downstream from all sample sites, and thus was the most likely to capture hydrographic patterns that affected all sample sites. The 2015-2020 (excluding 2019) data from the Cathedral City gauge data were used to test for correlations among the different hydrographic measures prior to analysis. All four hydrographic measures (average daily cubic feet per second [cfs], total daily cfs, maximum daily cfs, and standard deviation in daily cfs) were perfectly correlated with each other ($r = 1.00$). I chose to use average daily cubic feet per second (cfs) for further analyses.

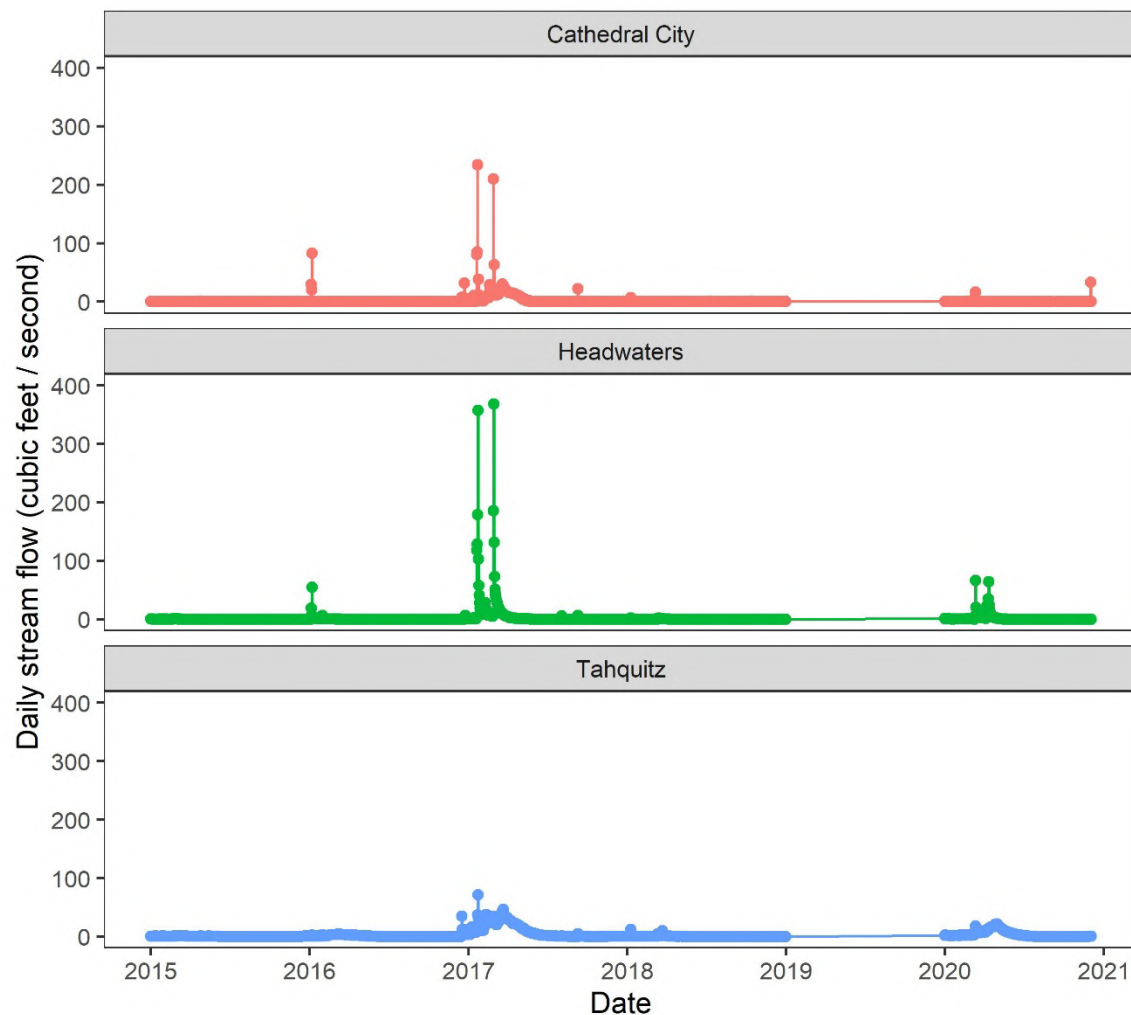


Figure 7. Daily streamflow (cfs) at three U.S. Geological Survey stream flow gauges in Tahquitz Creek and Palm Canyon Wash, in Palm Springs, California, from Jan 2015 to December 2020 (excluding 2019).

Model selection found that average cfs and distance upstream from the Cathedral City gauge was the best model at explaining variation in average site abundance of male Casey's June beetles (Table 2). The best model was > 2.0 AICc units lower than the second-best model, and was 59.2 AICc units below the null model, indicating good fit. Model residuals were also satisfactory.

Table 2. Model selection results for relationships between average site abundance of male Casey's June beetles and hydrographic measures, 2015-2020 (excluding 2019).

Model	AICc ^a	Δ AICc ^b	w^c
Avg. cfs ^d + Dist upstream ^e	327.4	0.0	0.73
Avg. cfs * Dist upstream	330.5	3.1	0.15
Avg. cfs	331.0	3.6	0.12
Dist upstream	386.8	59.4	0.00
Null ^f	388.5	61.1	0.00

^aSample size-corrected Akaike's information criterion

^bDifference in AICc from lowest AICc value

^cWeight of evidence in favor of a given model being best in the set

^dAverage daily flow in cubic feet per second

^eDistance upstream from Cathedral City stream flow gauge

^fIntercept-only model

Estimated average site abundance with zero stream flow and at the Cathedral City gauge station was 200.9 (95% CI 115.7 – 286.2) male Casey's June beetles. As a site was further upstream from the Cathedral City gauge station, average abundance declined by 32.3 (7.2 – 57.4; $t = -2.52$, $p = 0.019$) beetles for every one kilometer further upstream (Figure 8). Average abundance was predicted to increase by 0.5 (-2.2 – 3.2) beetles with every one cfs increase in average cfs, but this relationship was not significant ($t = 0.36$, $p = 0.721$).

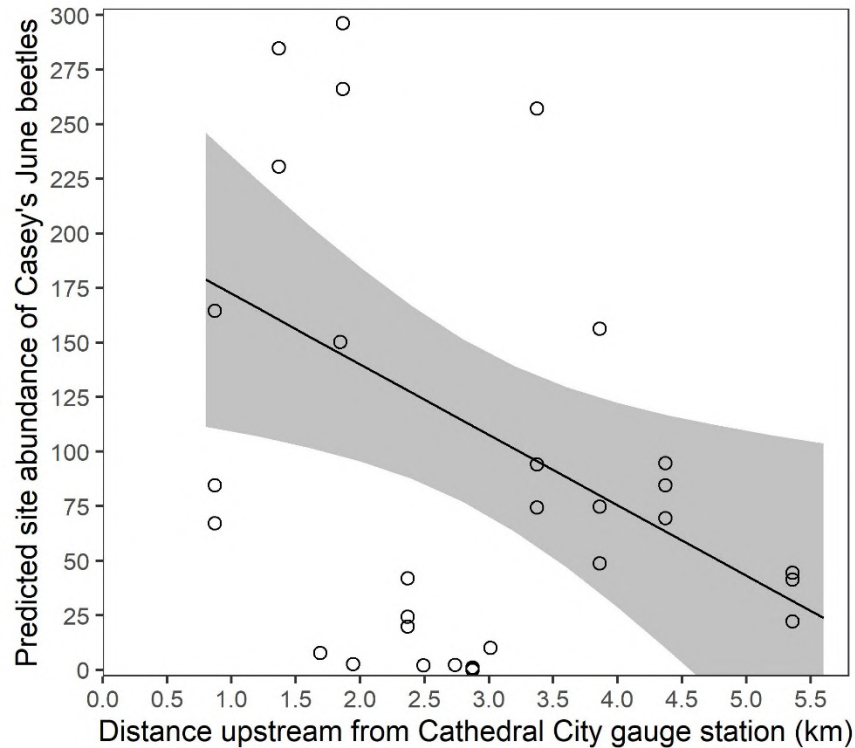


Figure 8. Predicted site abundance of Casey's June beetles as sites are located further upstream from the Cathedral City gauge station, Palm Springs, California, 2016-2018.

5.) Were 2016 and 2019 average corrected abundances related to the site-level vegetation community?

Total species richness was generally lower in 2019 than at the same sites in 2016 (Figure 9). When considering differences between native and naturalized plants, model selection results found that average Casey's June beetle site-level abundance in 2016 and 2019 was associated with species richness but not percent cover of native versus naturalized plants (Table 3). Average beetle abundance was predicted to increase with native species' richness, with an additional 5.4 (2.4 – 8.4) beetles at a site for every additional native plant present ($t = 3.61$, $p < 0.001$). For naturalized plants, there were predicted to be an additional 8.4 (2.0 – 14.8) beetles at a site for every additional naturalized plant present, but this stronger effect was not statistically different from the effect for native species richness ($t = 0.85$, $p = 0.398$; Figure 10).

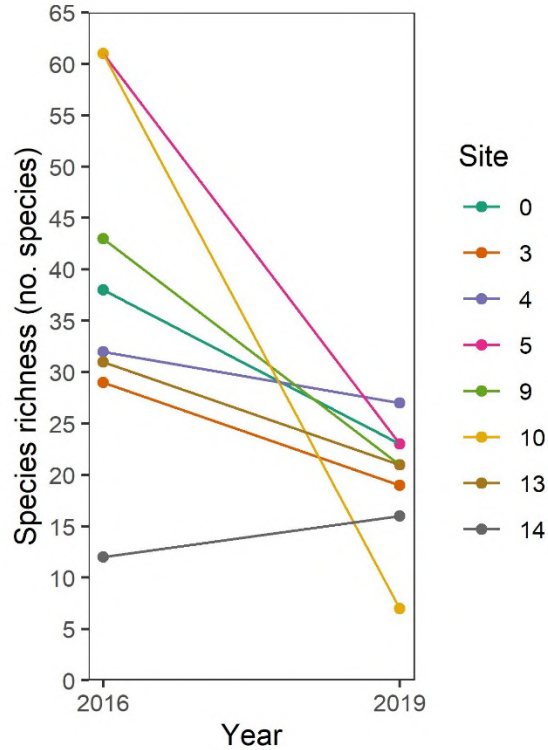


Figure 9. Total plant species richness at sites sampled in both 2016 and 2019.

Table 3. Model selection results for relationships between average site abundance of male Casey's June beetles and plant variables, depending on whether the plants were native or naturalized species.

Model	AICc ^a	Δ AICc ^b	w^c
Spp richness ^d * Native/Naturalized ^e	536.6	0.0	0.99
Percent cover * Native/Naturalized	547.3	10.7	0.00
Null ^f	547.3	10.7	0.00

^aSample size-corrected Akaike's information criterion

^bDifference in AICc from lowest AICc value

^cWeight of evidence in favor of a given model being best in the set

^dNumber of plant species

^eInteraction of whether a plant species was native or naturalized

^fIntercept-only model

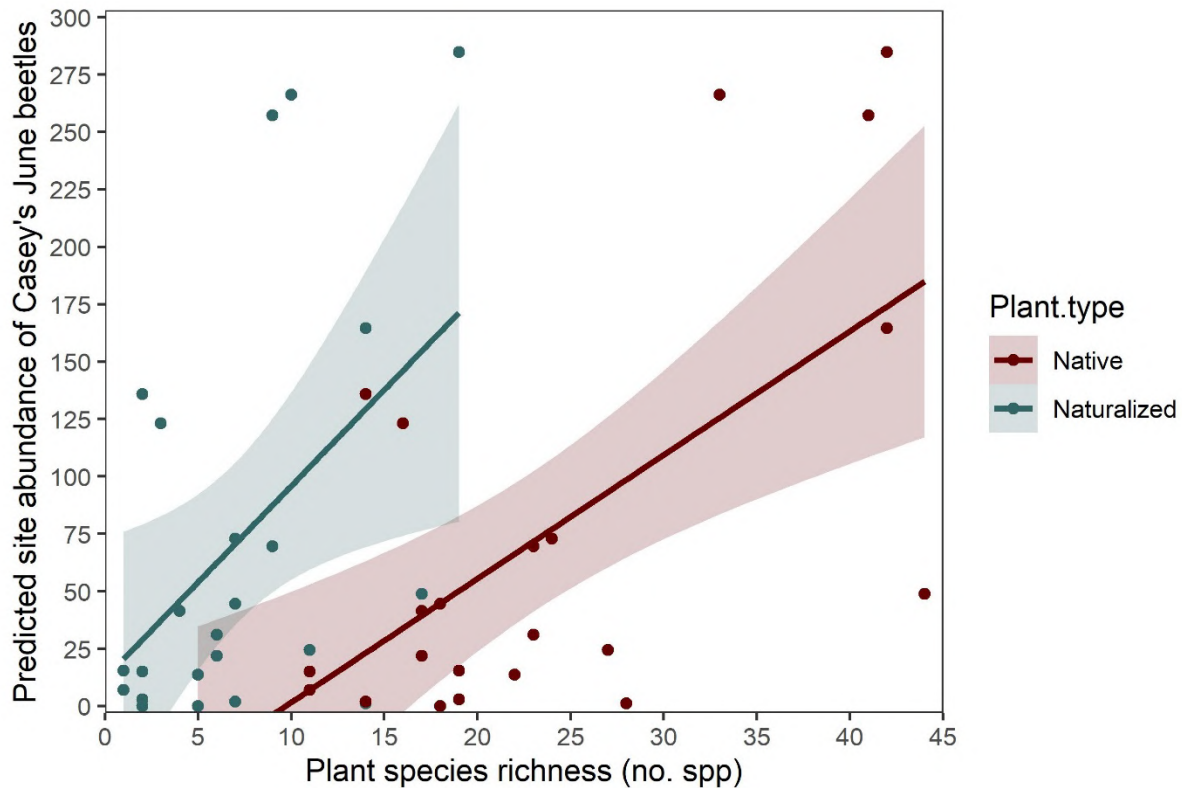


Figure 10. Predicted increase in average abundance of male Casey's June beetles as a function of native and naturalized plant species richness at a site, 2016 and 2019. Slope differences between native and naturalized plants were not significant ($p = 0.398$).

After pooling native and naturalized plant species (based on non-significant differences in the slope of effect on beetle abundance), two models were competing: percent cover + species richness and simply species richness. Because the first model was informationally equivalent to the second model, but contained more parameters, it was not further considered for inference (Burnham and Anderson 2002). Thus, considering only the species richness model, average abundance of male Casey's June beetles increased by 3.6 (1.5 – 5.7) beetles for every additional plant species at the site, regardless of whether it was native or naturalized plant ($t = 3.54$, $p = 0.002$; Figure 11). Model residuals were marginally satisfactory, but indicated some evidence of heterogeneity.

Table 4. Model selection results for relationships between average site abundance of male Casey's June beetles and plant variables and site location (i.e., wash versus upland).

Model	AICc ^a	Δ AICc ^b	w^c
Percent cover + Spp richness ^d	267.9	0.0	0.38
Spp richness	268.0	0.1	0.36
Spp richness + Wash/Upland ^e	270.3	2.4	0.11
Percent cover + Spp richness + Wash/Upland	271.2	3.3	0.07
Spp richness * Wash/Upland	272.7	4.8	0.03
Percent cover	273.9	6.0	0.02
Percent cover + Wash/Upland	274.4	6.5	0.01
Null ^f	276.1	8.2	0.01
Percent cover * Wash/Upland	277.5	9.6	0.00
Wash/Upland	277.8	9.9	0.00

^aSample size-corrected Akaike's information criterion

^bDifference in AICc from lowest AICc value

^cWeight of evidence in favor of a given model being best in the set

^dNumber of plant species

^eSite location: wash versus upland

^fIntercept-only model

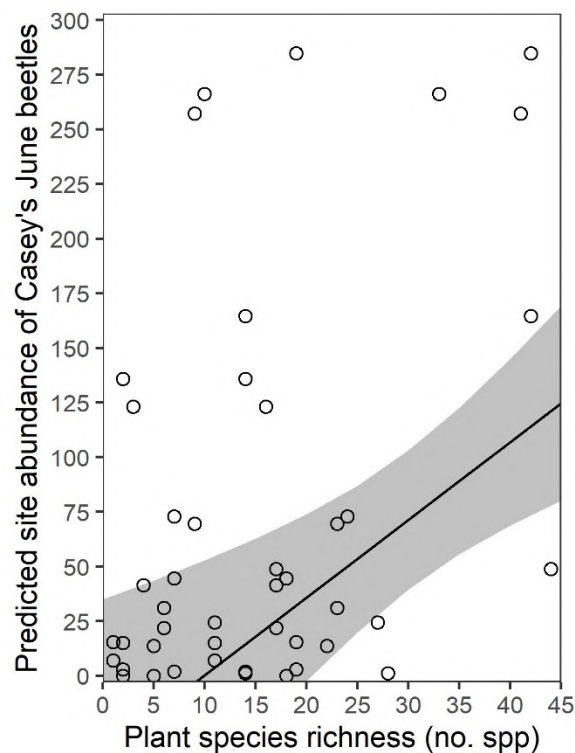


Figure 11. Predicted increase in average abundance of male Casey's June beetles as a function of plant species richness at a site, regardless of whether the species was native or naturalized, 2016 and 2019.

DISCUSSION

The addition of the 2021 survey data showed similar patterns as the 2016-2019 site-level abundance estimates previously analyzed (Harju 2021) in that there was notable variation in abundance across trapping events among sites. The 2021 data differed in that the total abundance estimated was among the lower-abundance sites from previous, with none in 2021 having estimated abundance over ~30 male beetles per evening. This may be due to inter-annual differences, but is perhaps more likely due to the fact that many of the sites in 2021 were new sites located on an active golf course that may reflect the 'normal' lower abundance sites sampled in previous years.

Across all years, the data on distance from release to recapture served as a useful proxy for emergence-to-capture distance. The distance decay model estimated a 50-50 capture distance of 77.3 m, suggesting that a beetle emerging < 77.3 m of a trap was more likely to be caught than not, and a beetle emerging > 77.3 m from a trap was less likely to be caught than to be caught. Casey's June beetles can exhibit continuous distribution within occupied habitat, albeit with high spatial variability in density. This presents numerous statistical challenges in population estimation and establishing linkages to exogenous variables driving population dynamics. Discretization of both beetle subpopulations and exogenous drivers can facilitate such estimation, and these data suggest that a radius of 77.3 m may be a meaningful distance to use for discretization.

Extrapolation of rangewide Casey's June beetle abundance from individual trap sites is inherently challenging due to the spatially and temporally continuous and heterogeneous nature of both beetle occurrence and the timing of beetle emergence. The steps I used here attempt to provide some estimate of rangewide abundance, given these challenges, and resulted in an estimate of 2018 rangewide average daily emergence of 9,554.4 (95% CI 7,790.1 – 11,191.7) male Casey's June beetles between April 26th and May 24th. Although there are several strict, and likely untenable, assumptions used to derive this estimate, standardizing the estimation procedures can provide a rough estimate of population size and its change over time. Nonetheless, it should be treated as an estimate full of caveats and assumptions, and not relied on as a statistically or biologically robust estimate of true abundance. To derive such an estimate, considerable work would need to be done to representatively sample Casey's June beetles more extensively to better understand the true extent of occupied range, the non-linear patterns of spatial heterogeneity in abundance, the non-linear temporal patterns in emergence within and among years, and the non-linear temporal patterns in emergence within a single day, among other factors. Although understanding these factors is likely beyond the current scope of field sampling and statistical methodology, further analyses using existing data to identify additional spatio-temporal correlates of abundance and using bootstrapping simulations of the full corrected abundance estimates to improve the accuracy of the estimated range-wide abundance may be useful first steps. Further, existing data could be used to better capture the non-linear abundance trends, partially driven by weather, and based on current field survey data to provide more accurate estimates of true abundance.

The streamflow analyses found that abundance of Casey's June beetles declined with a site's increasing location upstream. This finding was supported by the spatial patterns in estimated nightly abundance in 2018, whereby upstream habitat had lower abundances. This may be due to hydrologic and depositional forces associated with downstream locations, that may have higher or more persistent sub-surface water tables and/or more abundant subsurface food sources for overwintering grubs. These results were opposite those of the same rangewide

abundance estimate from 2019 (Harju 2021b), where higher abundances were estimated at middle/upper wash reaches than lower reaches. This may be explained by scouring flooding prior to Casey's June beetle emergence in 2019 (unpublished data), which may have had stronger negative impacts on abundance at lower wash reaches. Concomitantly, total plant species richness declined from 2016 to 2019 at all sites surveyed except upland site 14, also possibly due to flooding (Figure 9).

Future research and monitoring directions to understand spatio-temporal patterns in estimation of abundance and identification of factors driving abundance of Casey's June beetles could help elucidate the best ways to monitor population status and guide conservation actions for Casey's June beetles. Another future analysis path would be to develop moonphase-based emergence models within which annual surveys could be used to parameterize daily emergence counts and to develop rangewide abundance estimates for all surveyed years. Hopefully the results presented here help move knowledge of Casey's June beetle ecology and conservation towards that goal.

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APPENDIX G – Survey and Capture Summaries 2016–2022

Supplemental Figures and Tables

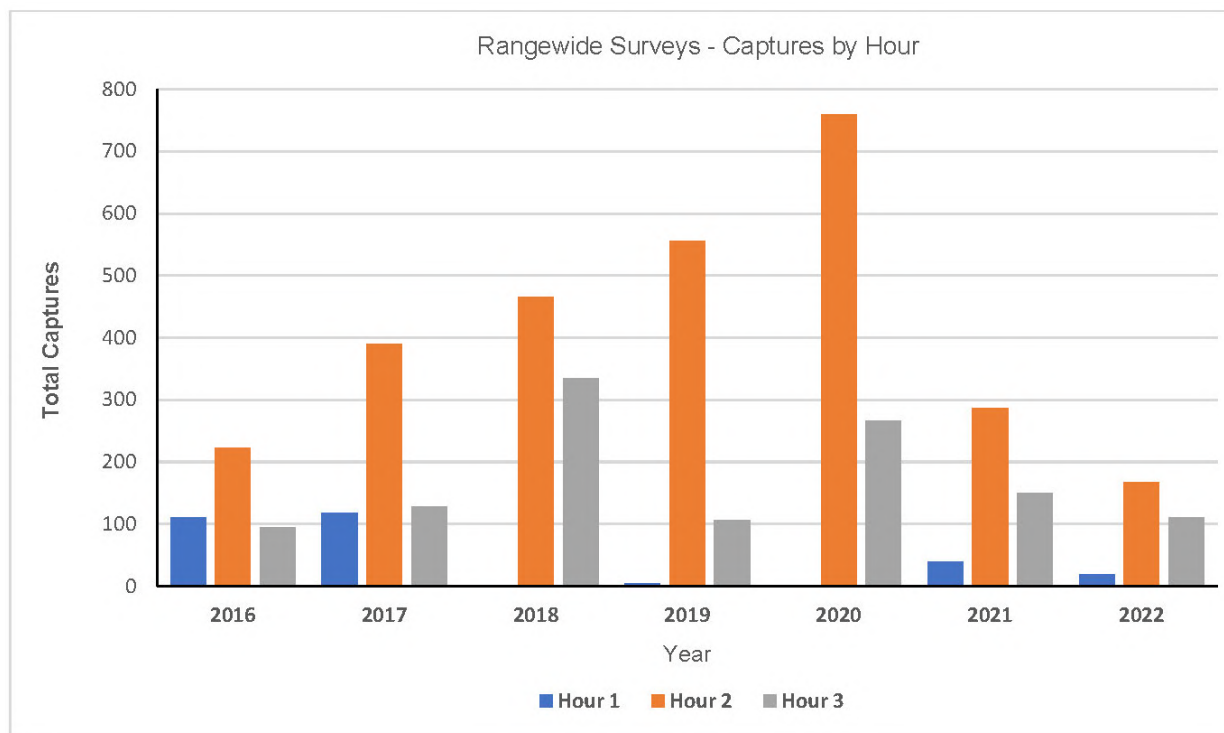


Figure G1. Total Casey's June beetle captures by hour during Rangewide surveys for all traps combined, 2016–2022.

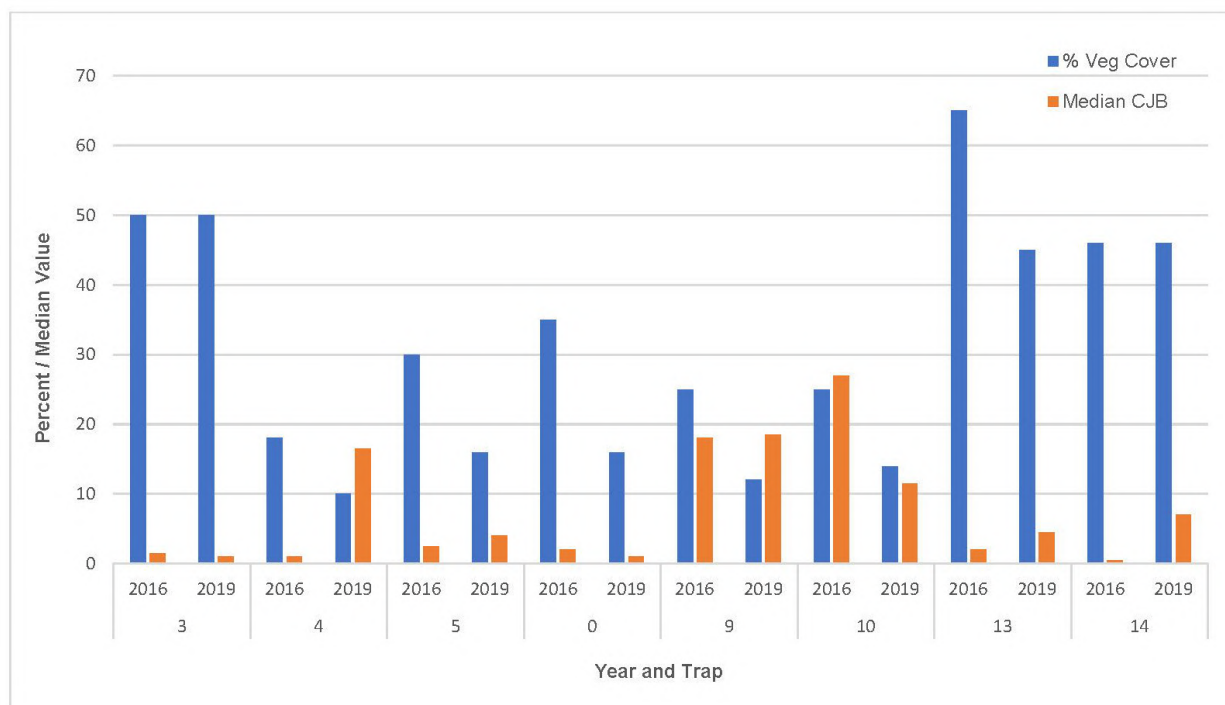


Figure G2. Percent vegetation cover and median Casey's June beetles at traps in Palm Canyon Wash and Smoke Tree Ranch in 2016 and 2019.

Table G1. Casey's June beetle (CJB) survey effort and capture summary from Rangewide surveys, 2016–2022.

Year	Location ¹	<i>n</i> surveys	<i>n</i> traps	Survey Effort (hours)	Total Unique CJB	Total Recaptured CJB	Mean	SD ²	Median	Min.	Max.
2016	All	4	15	178.8	432	21	7.2	13.2	2	0	59
2016	PCW	4	11	141.9	399	19	9.1	14.9	3	0	59
2016	STR	4	4	36.9	33	2	2.1	2.4	1.5	0	8
2017	All	3	15	110.2	624	22	14.9	25.7	4.5	0	110
2017	PCW	3	11	77.6	601	22	20.0	28.9	5.5	0	110
2017	STR	3	4	32.6	23	0	1.9	2.2	1	0	7
2018	All	3	15	146.0	746	42	18.7	36.9	4.5	0	196
2018	PCW	3	11	106.1	692	41	24.7	42.6	6.5	0	196
2018	STR	3	4	39.9	54	1	4.5	7.1	1	0	21
2019	All	4	11	120.5	632	36	15.8	28.1	4	0	119
2019	PCW	4	6	71.7	507	30	21.1	34.3	5	0	119
2019	STR	2	2	12.1	23	0	5.8	5.1	4.5	1	13
2019	TC (upper)	4	3	36.8	102	6	8.5	13.2	1	0	43
2020	All	5	8	118.8	961	64	24.0	51.8	5	0	238
2020	PCW	5	6	88.7	876	63	29.2	58.6	7.5	0	238
2020	TC (upper)	5	2	30.1	85	1	8.5	13.3	2	0	37
2021	All	5	13	199.8	446	28	6.86	24.01	1	0	186
2021	TC	5	10	154.8	68	7	1.36	3.47	0	0	21
2021	PCW	5	3	45.0	378	21	25.2	46.09	13	0	186
2022	All	5	12	171.5	286	13	4.93	12.86	0.5	0	83
2022	TC	5	9	129.5	58	1	1.35	3.09	0	0	18
2022	PCW	5	3	42.0	228	12	15.2	22.19	6	0	83

¹ PCW=Palm Canyon Wash; STR=Smoke Tree Ranch; TC (upper)=upper reach of Tahquitz Creek along the levee; TC=Tahquitz Creek, upper reach and golf course.

² Standard deviation.

Table G2. Casey's June beetle (CJB) survey effort capture summary from Sentinel surveys, 2016–2020.

Year	Trap	<i>n</i> surveys	Survey Effort (hours)	Total Unique CJB	Total Recaptured CJB	Mean	SD ¹	Median	Min.	Max.	CPUE ²	Density Index (ac) ³	Density Index (ha) ³
2016	0	58	60.7	506	20	8.7	15.5	2	0	84	8.3	110.0	266.3
2017	0	45	67.8	454	19	10.1	19.9	3	0	94	6.7	98.7	238.9
2017	16	1	1.0	2	0	-	-	-	-	-	-	-	-
2017	6	1	1.9	2	0	-	-	-	-	-	-	-	-
2017	9	1	2.2	9	0	-	-	-	-	-	-	-	-
2017	10	1	2.6	9	2	-	-	-	-	-	-	-	-
2018	0	31	45.9	477	1	15.4	35.7	3	0	155	10.4	103.7	251.1
2018	10	11	23.4	26	1	2.4	2.4	2	0	7	-	-	-
2018	11	7	17.2	9	0	1.3	2.2	0	0	5	-	-	-
2018	16	5	7.7	4	0	0.8	1.3	0	0	3	-	-	-
2018	17	5	8.5	5	1	1.0	1.0	1	0	2	-	-	-
2018	18	5	8.0	0	0	0.0	0.0	0	0	0	-	-	-
2019	0	33	47.0	217	8	6.6	14.2	1	0	55	4.6	47.2	114.2
2019	9	9	12.8	63	2	7.0	9.8	4	0	31	-	-	-
2019	13	1	1.3	6	0	-	-	-	-	-	-	-	-
2019	14	1	1.5	22	2	-	-	-	-	-	-	-	-
2020	0	25	37.5	488	9	19.5	44.1	2	0	190	13.0	106.1	256.8
2020	10	2	3.9	6	1	3.0	0.0	3	3	3	-	-	-

¹ Standard deviation.² CPUE=total catch per total survey hours.³ Density Index=calculated using effective survey area of 77.3-meter trap radius [4.6 acres (1.9 hectares)].

Table G3. Casey's June beetle (CJB) capture and recapture summary by trap and year from Rangewide surveys, 2016–2022.

Year	Location	Trap	Total Unique CJB	Mean	SD ¹	Median	Min.	Max.	CPUE ²	Density Index (ac) ³	Density Index (ha) ³	Recaptured CJB
2016	Palm Canyon Wash	1	4	1.0	1.2	1	0	2	0.4	0.9	2.1	0
		2	6	1.5	1.7	1.5	0	3	0.6	1.3	3.2	1
		3	8	2.0	2.5	1.5	0	5	0.7	1.7	4.2	1
		4	17	4.3	7.2	1	0	15	1.4	3.7	8.9	0
		5	14	3.5	4.4	2.5	0	9	1.2	3.0	7.4	2
		6	70	17.5	21.7	10.5	1	48	5.4	15.2	36.8	7
		7	1	0.3	0.5	0	0	1	0.1	0.2	0.5	0
		8	6	1.5	3.0	0	0	6	0.4	1.3	3.2	2
		9	98	24.5	24.7	18	3	59	4.8	21.3	51.6	1
		10	118	29.5	21.6	27	6	58	7.9	25.7	62.1	0
		11	57	14.3	7.5	14.5	6	22	4.9	12.4	30.0	5
	Smoke Tree Ranch	12	5	1.3	1.0	1.5	0	2	0.4	1.1	2.6	0
		13	12	3.0	3.8	2	0	8	1.2	2.6	6.3	1
		14	3	0.8	1.0	0.5	0	2	0.4	0.7	1.6	0
		15	13	3.3	2.5	3.5	0	6	1.7	2.8	6.8	1
2017	Palm Canyon Wash	1	4	1.3	1.2	2	0	2	0.5	0.9	2.1	0
		2	11	3.7	2.3	5	1	5	1.3	2.4	5.8	1
		3	6	3.0	4.2	3	0	6	1.1	1.3	3.2	0
		4	20	10.0	11.3	10	2	18	4.5	4.3	10.5	0
		5	76	25.3	17.6	35	5	36	9.4	16.5	40.0	0
		6	64	21.3	17.0	28	2	34	7.9	13.9	33.7	7
		7	1	0.5	0.7	0.5	0	1	0.2	0.2	0.5	0
		8	27	9.0	6.2	11	2	14	3.5	5.9	14.2	0

Year	Location	Trap	Total Unique CJB	Mean	SD ¹	Median	Min.	Max.	CPUE ²	Density Index (ac) ³	Density Index (ha) ³	Recaptured CJB
		9	173	57.7	49.3	68	4	101	21.4	37.6	91.1	3
		10	165	55.0	52.3	49	6	110	24.4	35.9	86.8	8
		11	54	18.0	18.4	10	5	39	6.5	11.7	28.4	3
	Smoke Tree Ranch	12	3	1.0	1.0	1	0	2	0.4	0.7	1.6	0
		13	9	3.0	2.0	3	1	5	1.1	2.0	4.7	0
		14	4	1.3	1.5	1	0	3	0.5	0.9	2.1	0
		15	7	2.3	4.0	0	0	7	0.8	1.5	3.7	0
2018	Palm Canyon Wash	1	9	4.5	2.1	4.5	3	6	0.8	2.0	4.7	0
		2	18	9.0	9.9	9	2	16	1.8	3.9	9.5	0
		3	1	0.5	0.7	0.5	0	1	0.1	0.2	0.5	0
		4	48	16.0	16.1	14	1	33	5.3	10.4	25.3	4
		5	53	17.7	25.5	5	1	47	6.0	11.5	27.9	4
		6	70	23.3	36.1	4	1	65	7.4	15.2	36.8	9
		7	0	0.0	-	0	0	0	0.0	0.0	0.0	0
		8	10	3.3	3.2	2	1	7	1.3	2.2	5.3	2
		9	128	42.7	55.0	22	1	105	14.4	27.8	67.4	1
		10	241	80.3	101.4	38	7	196	26.3	52.4	126.8	9
		11	114	38.0	33.3	24	14	76	12.4	24.8	60.0	12
	Smoke Tree Ranch	12	5	1.7	2.9	0	0	5	0.5	1.1	2.6	0
		13	17	5.7	8.1	1	1	15	1.7	3.7	8.9	1
		14	22	7.3	11.9	1	0	21	2.2	4.8	11.6	0
		15	10	3.3	5.8	0	0	10	1.0	2.2	5.3	0
2019	Palm Canyon Wash	0	73	18.3	23.9	10	0	53	6.3	15.9	38.4	8
		3	3	0.8	0.5	1	0	1	0.3	0.7	1.6	0

Year	Location	Trap	Total Unique CJB	Mean	SD ¹	Median	Min.	Max.	CPUE ²	Density Index (ac) ³	Density Index (ha) ³	Recaptured CJB
		4	61	15.3	13.5	16.5	0	28	5.3	13.3	32.1	1
		5	80	20.0	34.8	4	0	72	6.6	17.4	42.1	11
		9	148	37.0	51.5	18.5	0	111	11.9	32.2	77.9	7
		10	142	35.5	56.6	11.5	0	119	11.8	30.9	74.7	3
	Smoke Tree Ranch	13	9	4.5	0.7	4.5	4	5	1.5	2.0	4.7	0
		14	14	7.0	8.5	7	1	13	2.3	3.0	7.4	0
	Tahquitz Creek (upper)	16	41	10.3	9.2	11	0	19	3.3	8.9	21.6	1
		17	61	15.3	19.8	8.5	1	43	5.0	13.3	32.1	5
		18	0	0.0	0.0	0	0	0	0.0	0.0	0.0	0
2020	Palm Canyon Wash	0	171	34.2	65.5	9	0	151	11.6	37.2	90.0	20
		3	15	3.0	4.2	1	0	10	1.0	3.3	7.9	1
		4	86	17.2	30.8	5	0	72	5.7	18.7	45.3	8
		5	182	36.4	70.4	9	0	162	12.2	39.6	95.8	11
		9	133	26.6	48.1	7	0	112	9.1	28.9	70.0	1
		10	289	57.8	101.2	17	0	238	20.2	62.8	152.1	22
	Tahquitz Creek (upper)	16	15	3.0	3.9	1	0	9	1.0	3.3	7.9	0
		17	70	14.0	17.6	2	0	37	4.6	15.2	36.8	1
2021	Tahquitz Creek (upper and golf course)	16	32	6.4	8.6	5	0	21	2.1	7.0	16.8	6
		19	7	1.4	1.7	1	0	4	0.4	1.5	3.7	0
		20	2	0.4	0.6	0	0	1	0.1	0.4	1.1	0
		21	0	0.0	0.0	0	0	0	0.0	0.0	0.0	0
		22	1	0.2	0.5	0	0	1	0.1	0.2	0.5	1
		23	2	0.4	0.6	0	0	1	0.1	0.4	1.1	0
		24	17	3.4	4.4	2	0	11	1.1	3.7	8.9	0

Year	Location	Trap	Total Unique CJB	Mean	SD ¹	Median	Min.	Max.	CPUE ²	Density Index (ac) ³	Density Index (ha) ³	Recaptured CJB
		25	1	0.2	0.5	0	0	1	0.1	0.2	0.5	0
		26	1	0.2	0.5	0	0	1	0.1	0.2	0.5	0
		27	5	1.0	1.7	0	0	4	0.3	1.1	2.6	0
	Palm Canyon Wash	0	216	43.2	80.2	10	0	186	14.4	47.0	113.7	12
		9	78	15.6	14.5	13	2	36	5.2	17.0	41.1	5
		10	84	16.8	13.6	19	1	36	5.6	18.3	44.2	4
2022	Tahquitz Creek (upper and golf course)	16	29	5.8	7.1	4	0	18	1.9	6.3	15.3	1
		19	6	1.2	1.3	1	0	3	0.4	1.3	3.2	0
		20	4	0.8	1.1	0	0	2	0.3	0.9	2.1	0
		21	0	0.0	0.0	0	0	0	0.0	0.0	0.0	0
		22	0	0.0	0.0	0	0	0	0.0	0.0	0.0	0
		23	2	0.5	0.6	0.5	0	1	0.2	0.4	1.1	0
		24	16	3.2	2.9	3	0	7	1.0	3.5	8.4	0
		26	1	0.2	0.5	0	0	1	0.1	0.2	0.5	0
		27	0	0.0	0.0	0	0	0	0.0	0.0	0.0	0
	Palm Canyon Wash	9	62	12.4	18.9	5	1	46	4.1	13.5	32.6	5
		10	131	26.2	32.8	10	6	83	8.7	28.5	68.9	3
		28	35	7.0	6.8	9	0	15	2.9	7.6	18.4	4

¹ Standard deviation.² CPUE=total catch per total survey hours.³ Density Index=calculated using effective survey area of 77.3-meter trap radius (4.6 acres [1.9 hectares]).

APPENDIX H – Underground Soil Temperature 2017 and 2019

Table H1. Monthly underground soil temperature (degrees Celsius) recorded at 15 cm, 65 cm, and 115 cm depths at long-term survey plots in Palm Canyon Wash in 2017.

		15 cm				65 cm				115 cm			
Month	Trap	Mean	SD ¹	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
March	2	24.60	4.14	16.8	34.2	22.23	1.27	19	23.5	20.92	1.29	18.2	22.4
March	3	24.07	2.29	17.1	28.3	21.62	1.29	18.6	23	20.53	1.23	18.1	22
March	4	25.64	2.68	17.9	30.9	22.14	1.69	18.4	24.1	20.76	1.53	17.9	22.7
March	5	16.31	1.85	13	18.7	18.08	1.92	14.1	20.2	21.77	2.26	13.9	25.5
March	6	21.82	2.32	13.8	25.9	18.69	2.04	14.4	21.2	16.68	1.79	13.4	18.9
March	8	23.72	3.07	16.4	29.4	18.94	1.40	15.2	20.3	16.42	1.54	13.2	18.2
March	9	22.55	1.94	17.5	26.4	18.76	1.27	15.7	22.2	16.30	1.26	13.8	18
March	10	22.92	2.15	17.1	27	19.96	1.45	16.6	21.5	18.35	1.39	15.7	20.1
April	2	28.13	6.00	18.1	43.8	25.18	1.22	23.2	27	23.84	0.98	22.4	25.5
April	3	27.46	2.49	21.9	32.7	24.77	1.20	22.9	26.6	22.66	0.35	22	23.2
April	4	30.16	2.64	24.1	35.6	26.17	1.36	23.9	28.2	24.60	1.14	22.7	26.5
April	5	20.62	1.13	18.7	22.5	22.15	1.22	20.1	24.1	24.92	1.77	21	28.2
April	6	24.95	1.68	21.2	28.1	22.86	1.01	21.2	24.5	20.62	1.06	18.9	22.4
April	8	26.00	3.09	19.2	32.6	22.13	1.29	20.1	24.1	19.93	1.11	18.2	21.8
April	9	24.59	1.96	20	28.5	21.50	0.96	20.1	23	19.27	0.96	17.8	21
April	10	26.03	2.49	20.6	31.2	23.42	1.24	21.5	25.4	21.87	1.08	20.1	23.8
May	2	32.70	7.53	17.3	50.4	28.28	1.05	26.7	30.1	25.51	0.02	25.5	25.6
May	3	31.62	3.28	22.8	38.7	27.98	1.00	26.5	30	-	-	-	-
May	4	33.33	2.84	25.4	39.3	29.70	1.09	28.1	31.7	27.86	0.81	26.5	29.5
May	5	23.84	0.75	22.5	25.4	25.56	0.96	24	27.4	28.25	1.90	23.4	32
May	6	28.34	1.90	23.6	32.1	26.11	1.11	24.4	28.1	23.77	0.81	22.4	25.5
May	8	29.76	4.03	20	38.9	25.69	1.19	23.9	27.8	23.32	0.86	21.8	25.1

		15 cm				65 cm				115 cm			
Month	Trap	Mean	SD ¹	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
May	9	28.06	2.50	22	33.7	24.27	0.89	22.9	26	22.34	0.78	21	23.9
May	10	29.79	2.61	23.1	35.6	27.04	1.01	25.3	29	25.32	0.87	23.8	27.1
June	2	36.86	6.97	25.2	50.6	31.96	1.38	29.9	34.2	-	-	-	-
June	3	37.70	3.26	30.4	44.5	32.04	1.47	29.8	34.4	-	-	-	-
June	4	37.60	2.77	31.5	43.5	33.53	1.37	31.4	35.7	31.17	1.10	29.5	33.1
June	5	26.98	1.02	25.4	28.8	29.15	1.21	27.3	31.1	32.94	2.18	28.4	37.4
June	6	33.17	2.20	28.7	37.5	29.86	1.25	28	31.9	27.25	1.15	25.5	29.3
June	8	35.82	4.25	26.5	44.4	29.60	1.26	27.5	31.6	26.79	1.14	25.1	28.8
June	9	33.86	2.80	27.7	39.5	27.66	1.18	25.8	29.5	25.77	1.11	23.9	27.5
June	10	32.73	1.94	28.6	36.2	30.90	1.28	28.8	33	28.88	1.09	27.1	30.8
July	3	40.73	2.16	35.8	45.1	35.24	0.68	34.3	36.3	-	-	-	-
July	4	-	-	-	-	36.15	0.52	35.4	37	34.10	0.57	33.1	34.9
July	5	29.19	0.28	28.8	29.7	31.67	0.52	30.9	32.5	35.66	1.32	32.8	38.6
July	6	35.68	1.07	33.7	37.9	32.76	0.58	30.2	33.4	30.40	0.65	29.2	31.3
July	8	37.48	3.32	31.9	42.7	31.79	0.47	31.2	32.5	29.73	0.62	28.7	30.6
July	9	33.48	7.12	22.8	37.4	29.48	0.05	29.4	29.6	28.46	0.64	27.5	29.5
July	10	-	-	-	-	33.06	0.12	32.8	33.3	31.03	0.19	30.8	31.4
August	4	-	-	-	-	-	-	-	-	34.54	0.22	34.3	34.9
August	6	-	-	-	-	-	-	-	-	31.12	0.20	30.8	31.4
August	9	-	-	-	-	-	-	-	-	29.52	0.04	29.5	29.6

¹ Standard deviation.

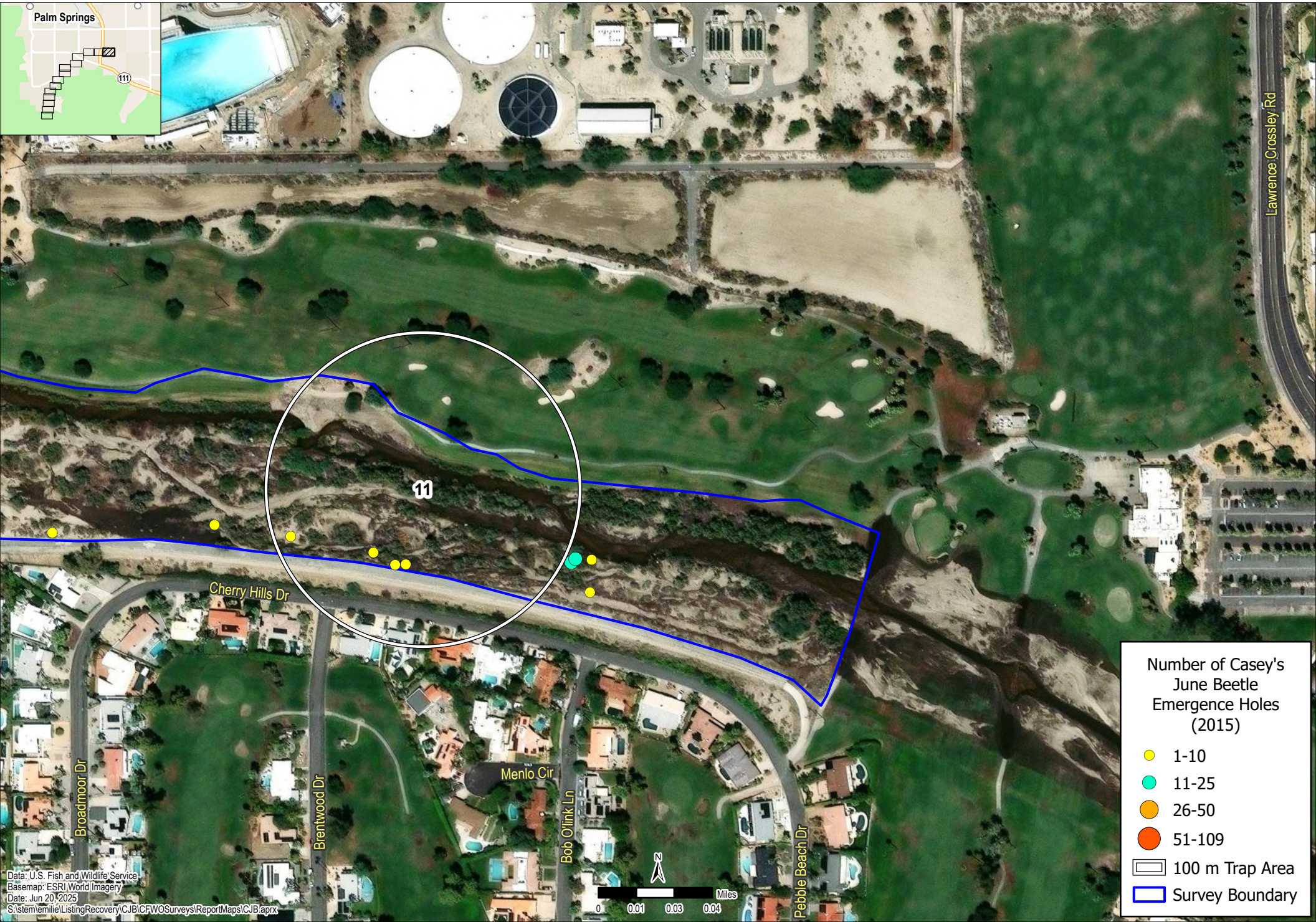
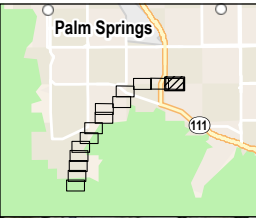
Table H2. Monthly underground soil temperature (degrees Celsius) recorded at 15 cm, 65 cm, and 115 cm depths at long-term survey plots in Palm Canyon Wash in 2019.

		15 cm				65 cm				115 cm			
Month	Trap	Mean	SD ¹	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
January	3	16.76	1.36	14.9	18.9	16.27	0.69	15.8	18	16.55	0.16	16.4	17
February	3	12.07	2.97	6	22	13.41	1.14	11.3	15.8	14.88	0.98	13.5	16.6
February	4	17.42	1.56	15.3	20.5	12.28	2.12	11.1	17.7	10.69	1.87	9.7	15.5
February	5	18.74	0.79	17.3	19.6	13.93	1.18	13.1	16.8	12.20	1.73	11.2	16.8
February	10	11.08	2.31	6.7	15.7	13.62	1.39	8.8	15.4	15.32	1.67	9	16.6
March	3	19.77	3.61	12	28	18.71	1.65	15	21.7	17.99	1.28	15	20.2
March	4	17.00	3.21	10.5	24.3	15.26	1.62	11.1	18.1	14.08	1.41	9.6	16.3
March	5	18.48	2.77	12.4	24.8	16.23	1.51	13	18.9	14.19	1.45	11.2	16.6
March	10	18.16	3.08	10.9	24.7	16.02	1.40	14.1	19.4	14.78	1.25	12.4	21.3
April	3	24.14	2.33	20.3	28.1	23.07	0.68	21.8	24.1	21.14	0.51	20.2	22.1
April	4	23.46	2.63	18.1	30.2	21.04	1.62	18.1	23.8	18.25	1.04	16.3	20
April	5	23.85	2.08	19.1	28.5	20.82	0.92	18.9	22.5	18.79	1.18	16.6	21
April	10	23.93	2.22	18.6	28.9	19.59	0.56	18.5	20.8	19.00	1.21	16.9	21.1
May	4	-	-	-	-	23.51	0.08	23.4	23.6	-	-	-	-

¹ Standard deviation.

APPENDIX I – Emergence Hole Map Series

Figure I1, Maps 1–14. Detailed map series of emergence hole counts, 2015.

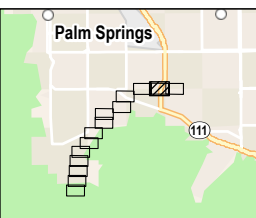


Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109

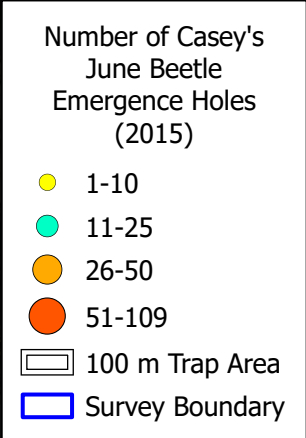
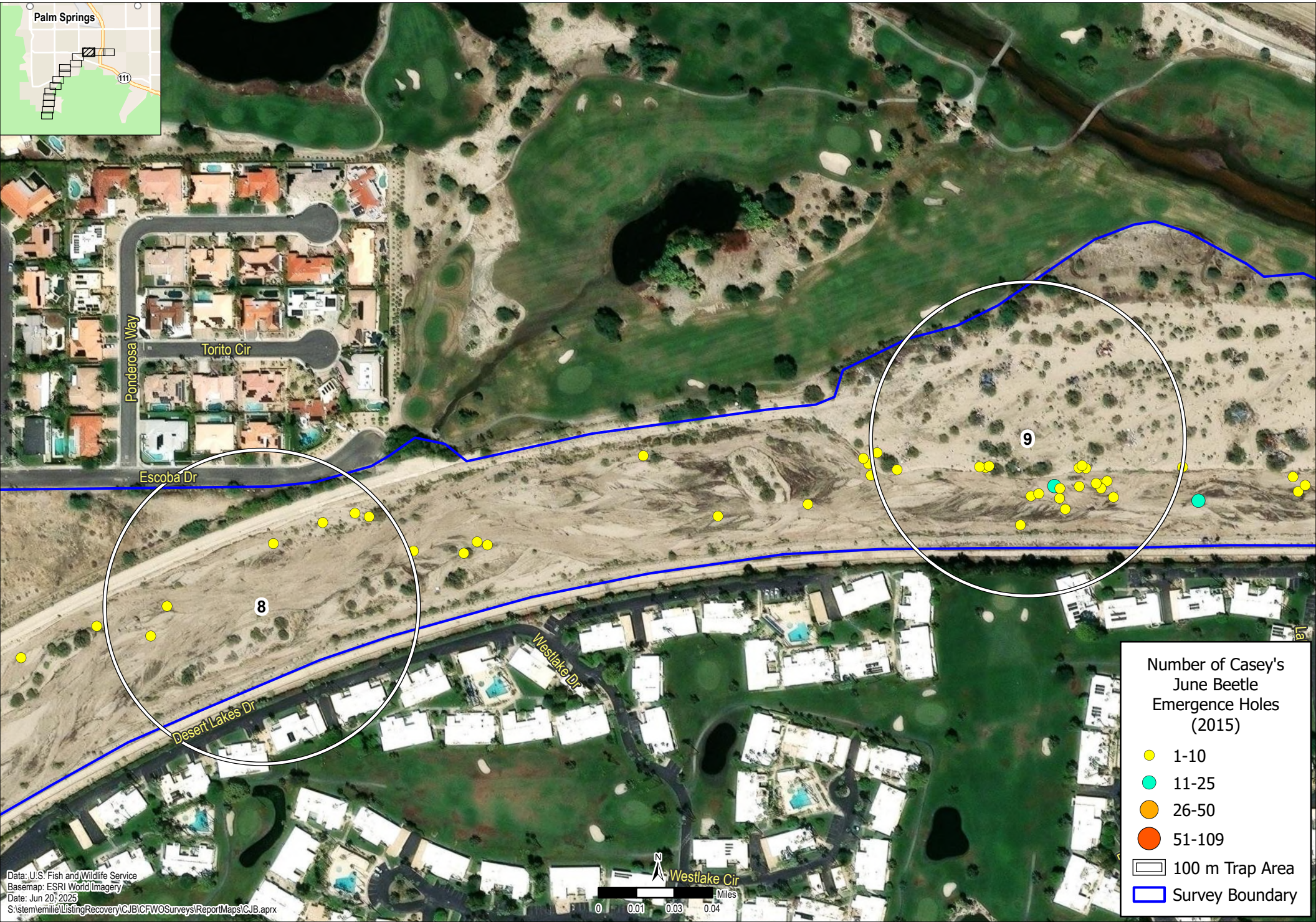
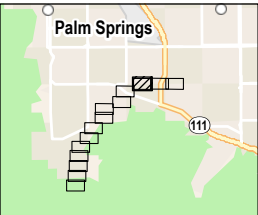
100 m Trap Area
Survey Boundary

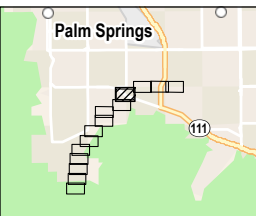


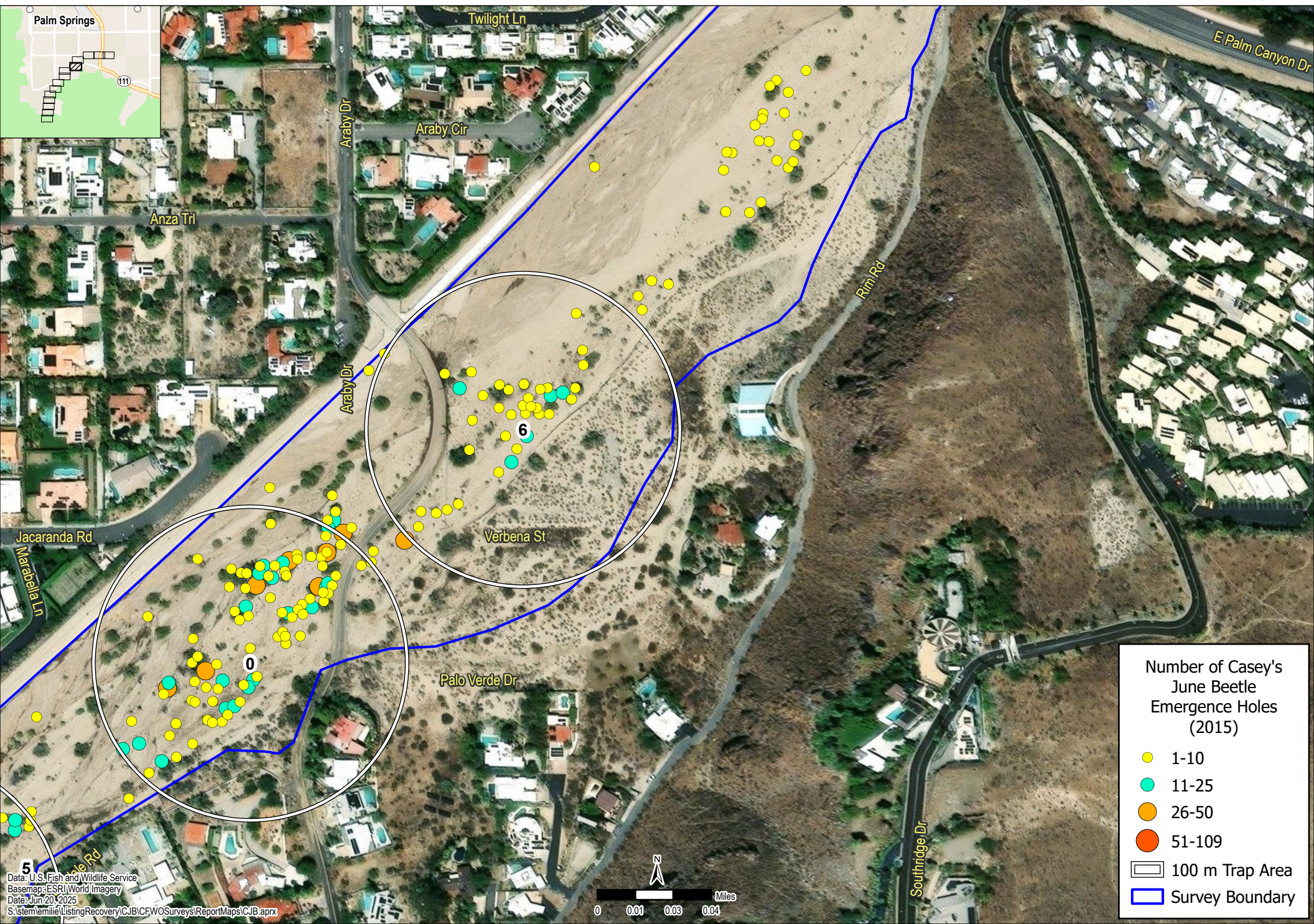


Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary

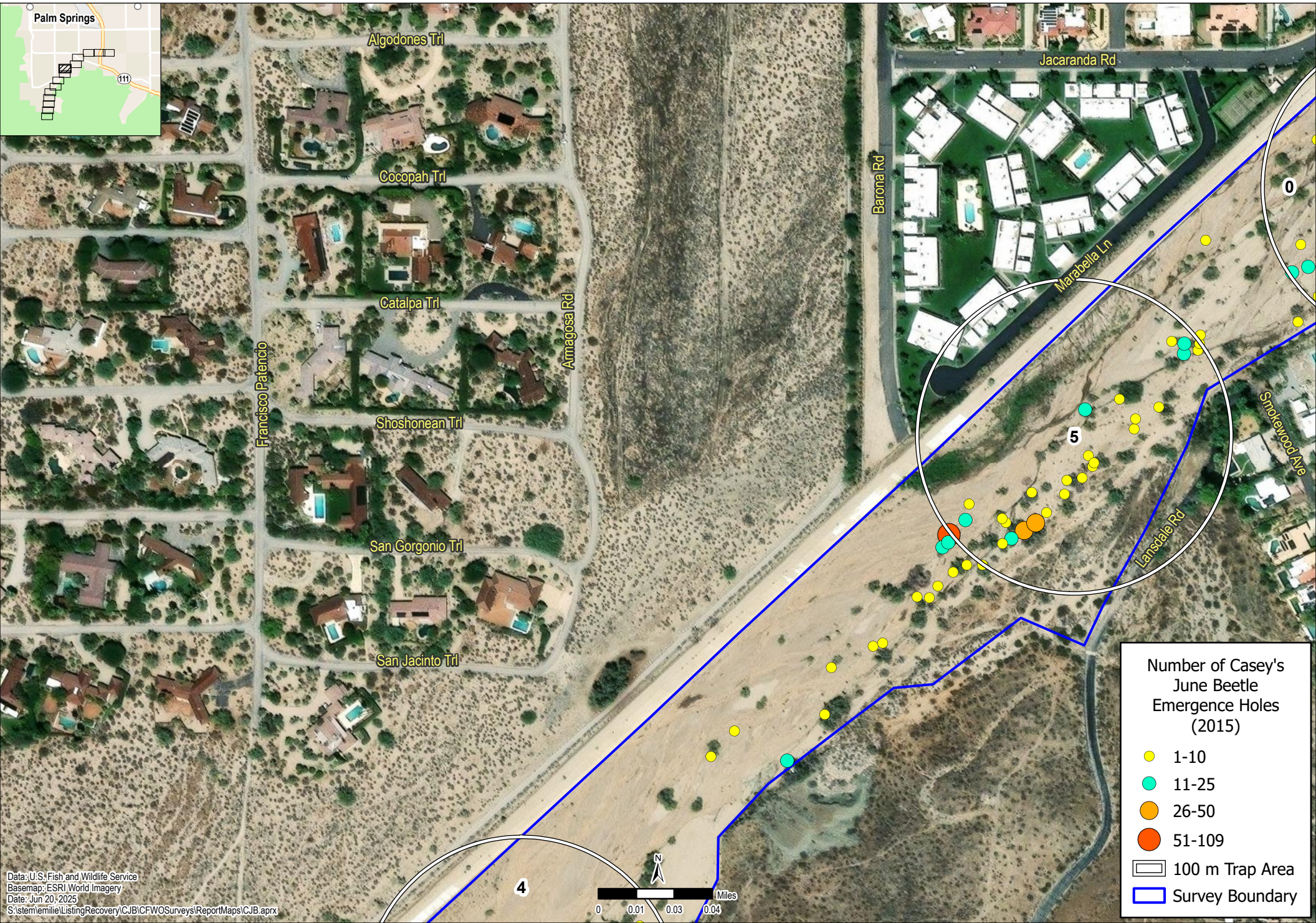


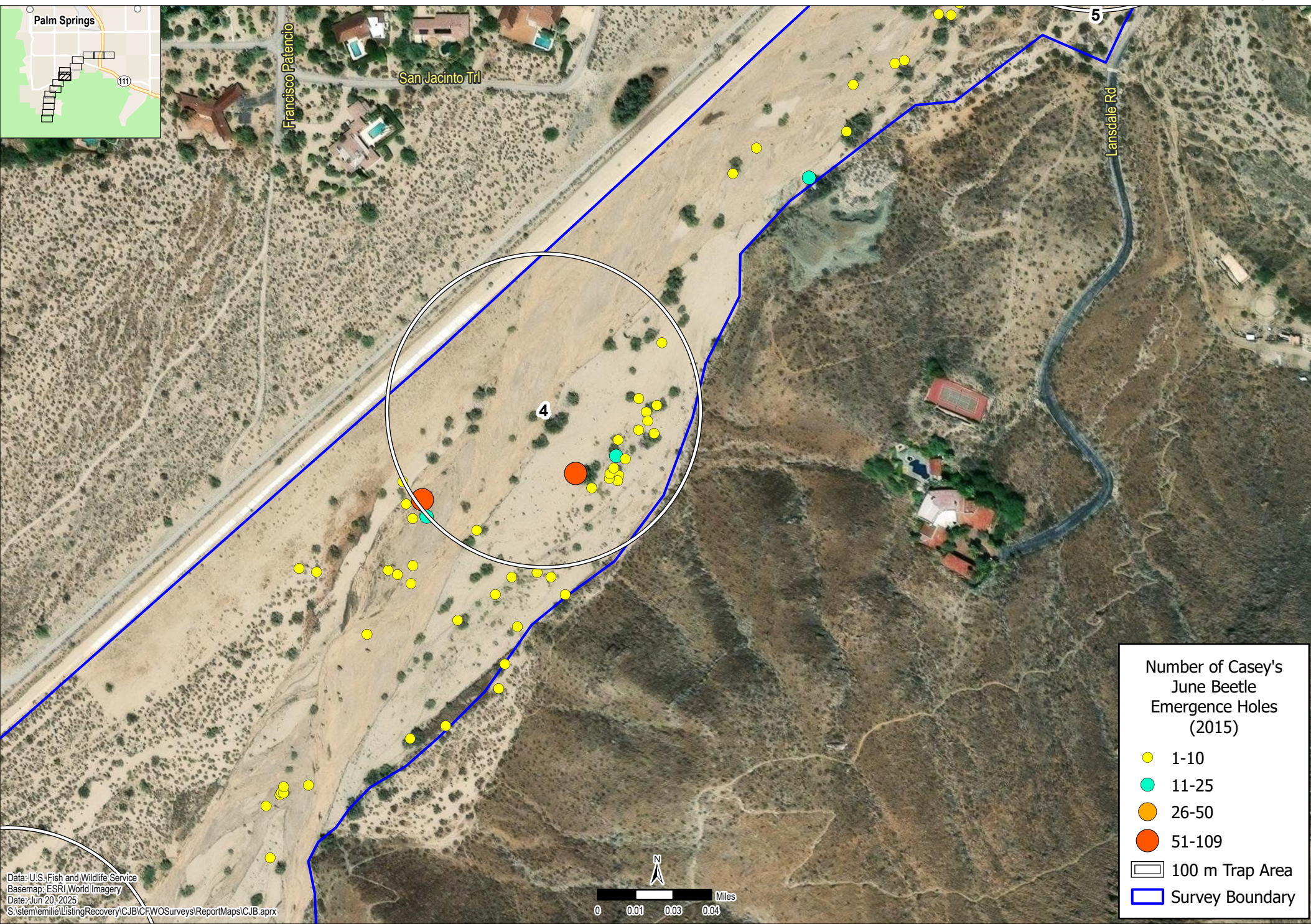
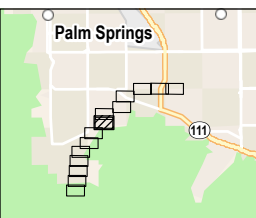




Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary





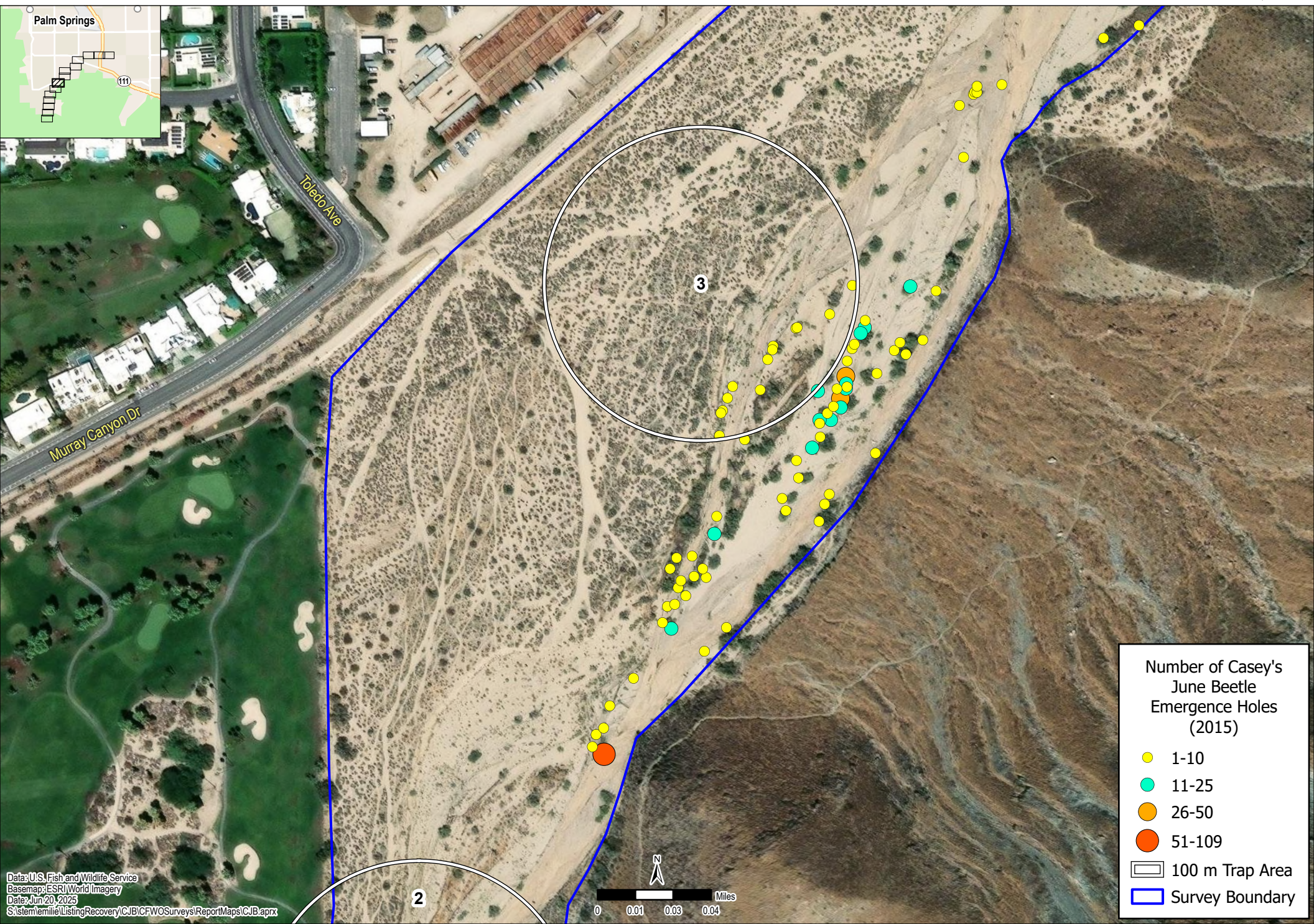
Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area

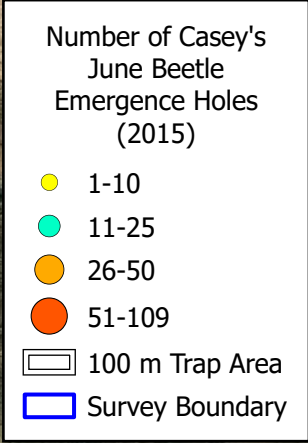
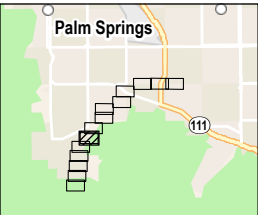
Survey Boundary

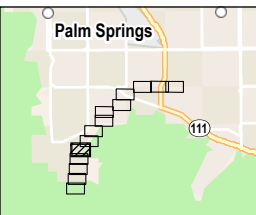




Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary

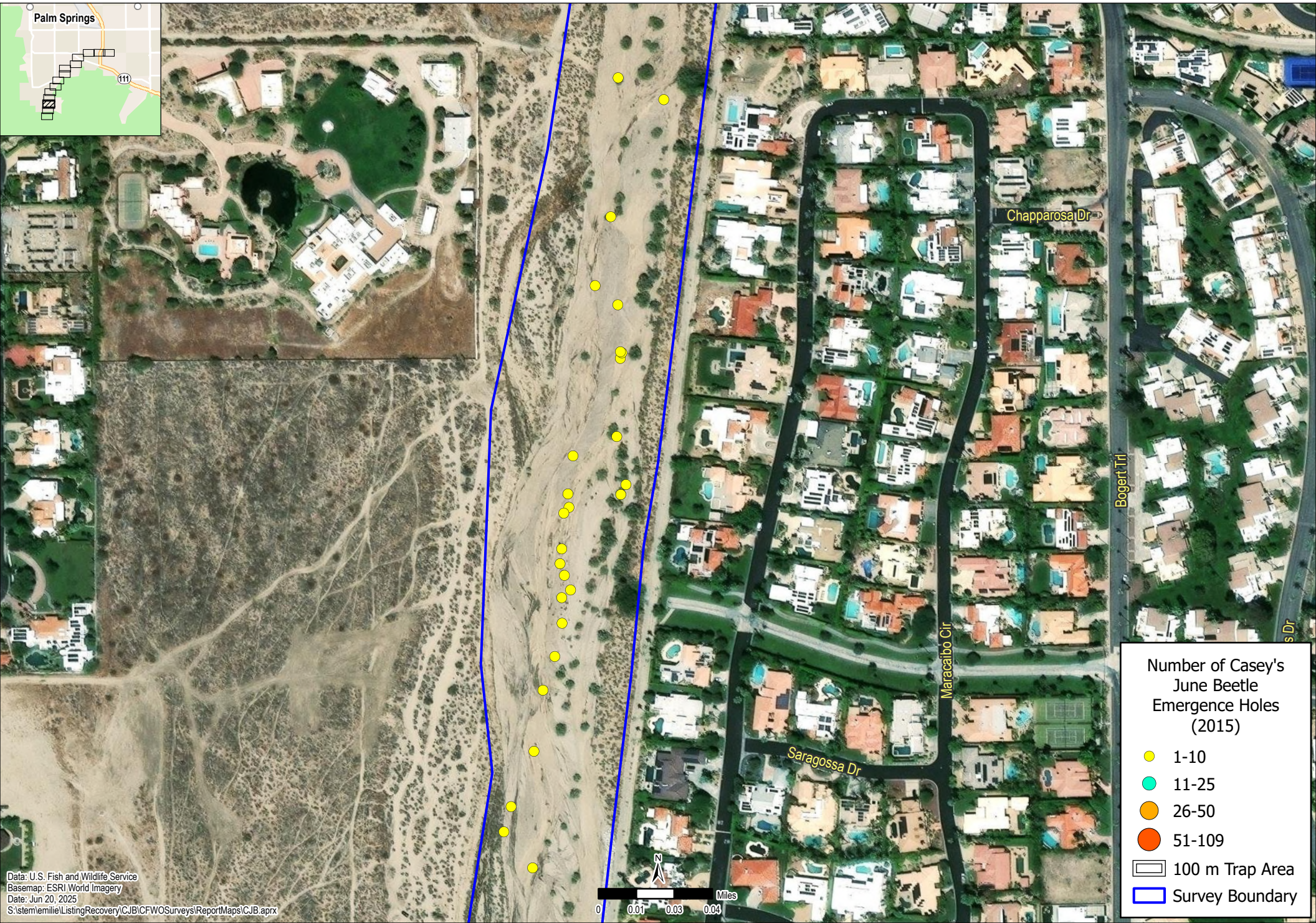
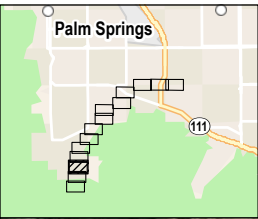




Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary

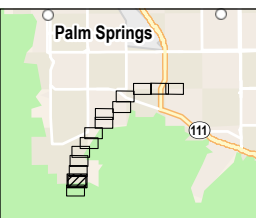




Number of Casey's
June Beetle
Emergence Holes
(2015)

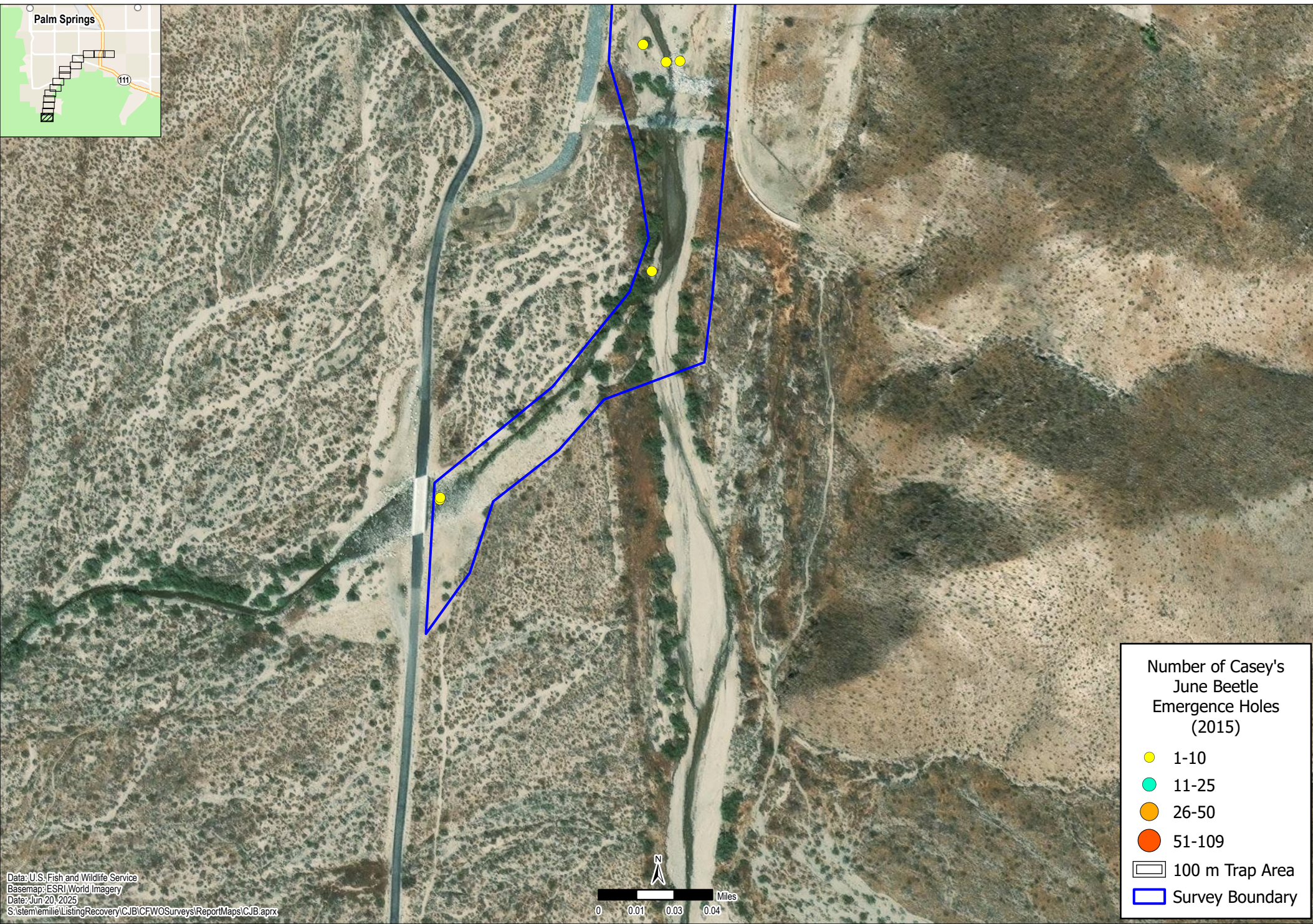
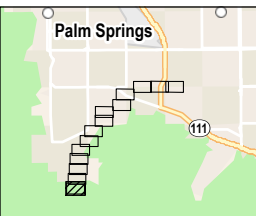
- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area
Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary



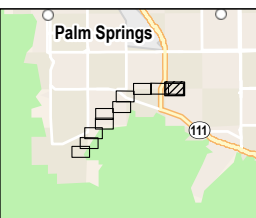
Number of Casey's
June Beetle
Emergence Holes
(2015)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area

Survey Boundary

Figure I2, Maps 1–10. Detailed map series of emergence hole counts, 2016.

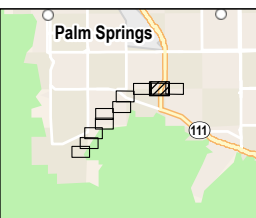


Number of Casey's
June Beetle
Emergence Holes
(2016)

- 1-10
- 11-25
- 26-50
- 51-109

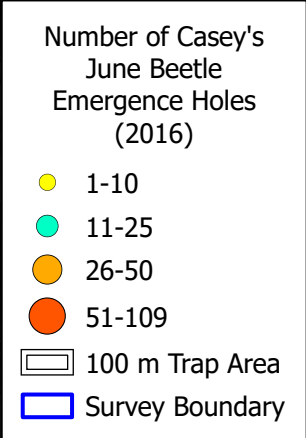
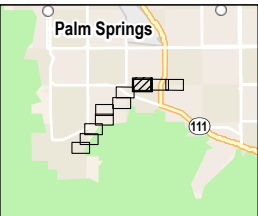
100 m Trap Area
Survey Boundary

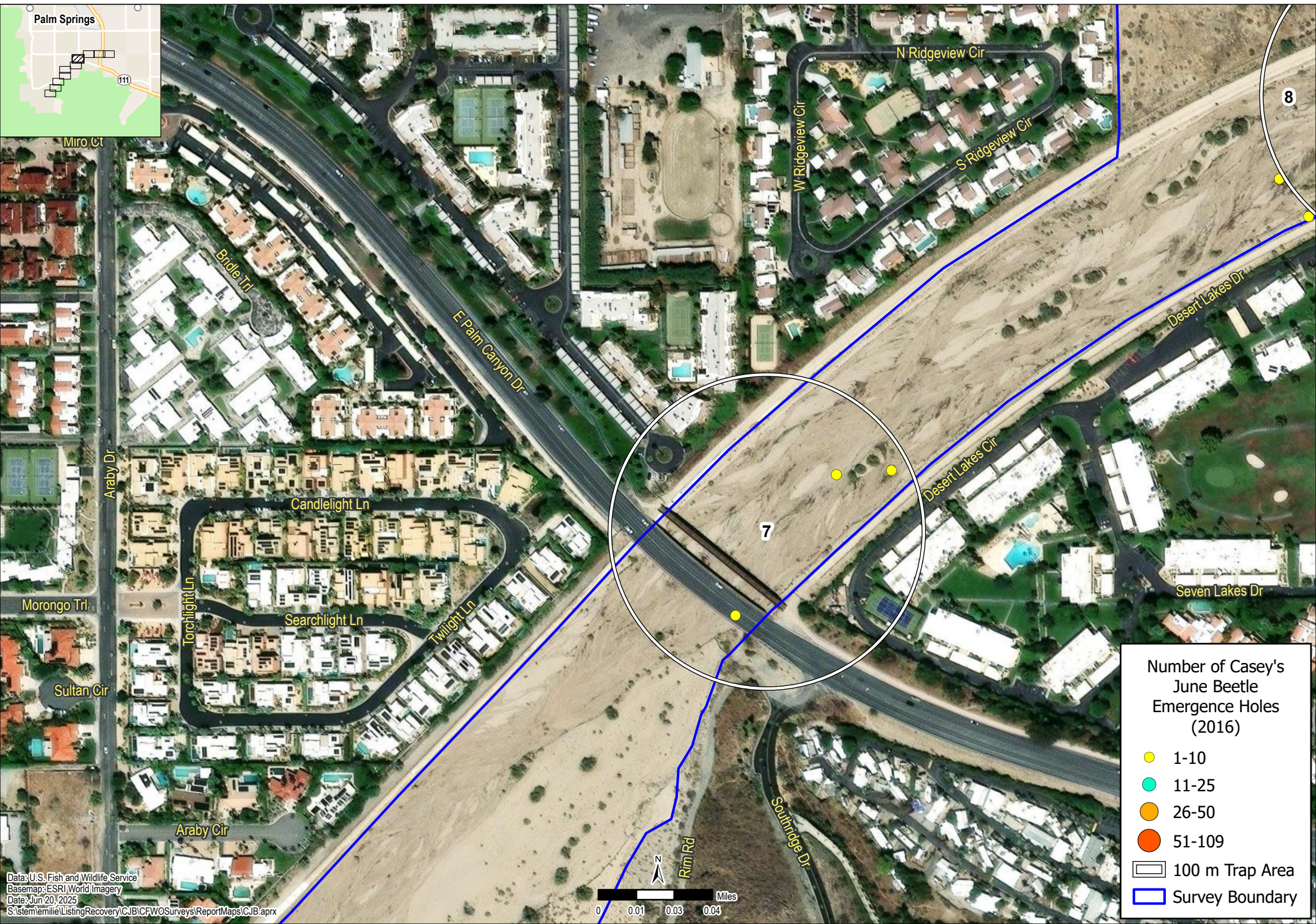


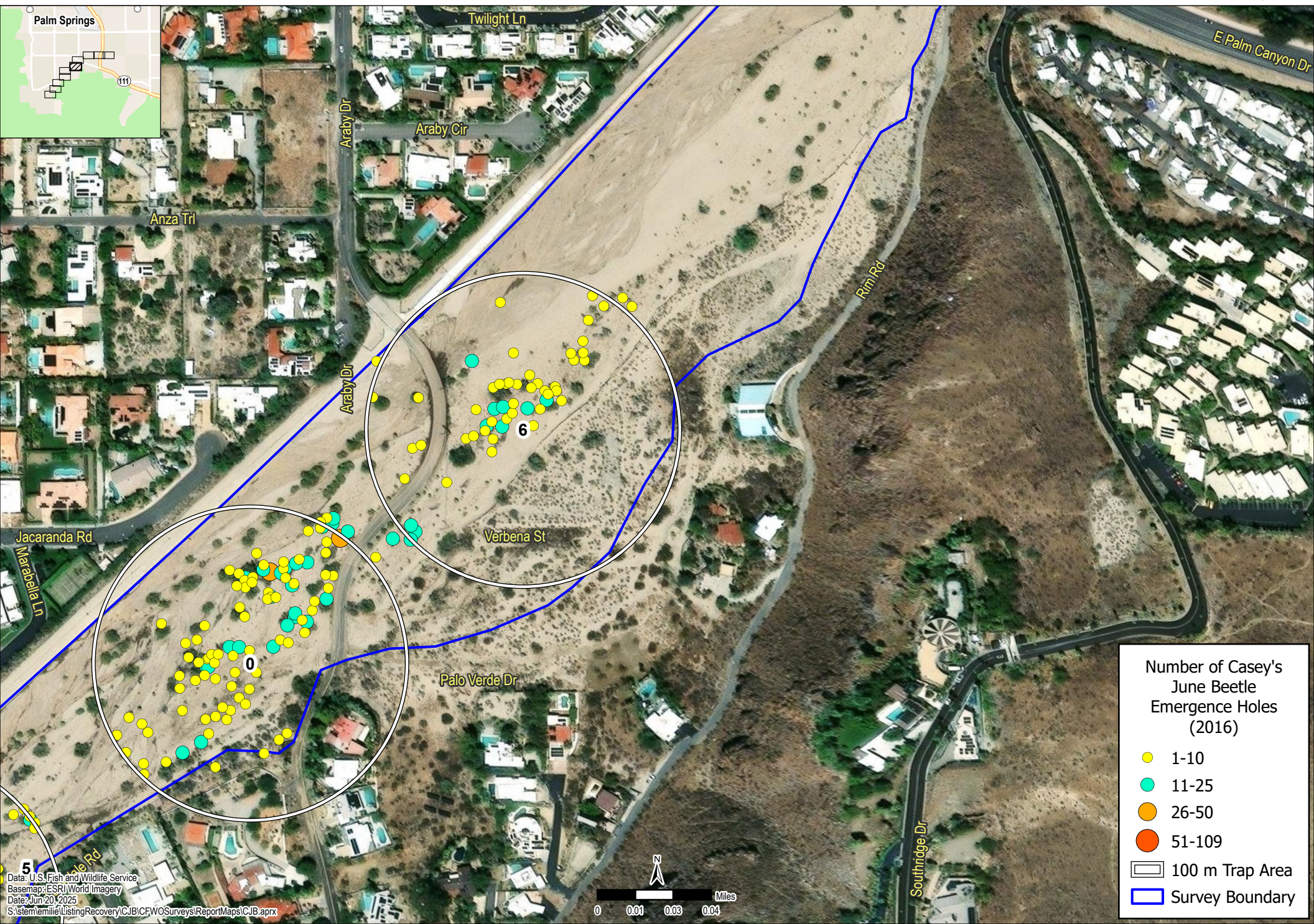


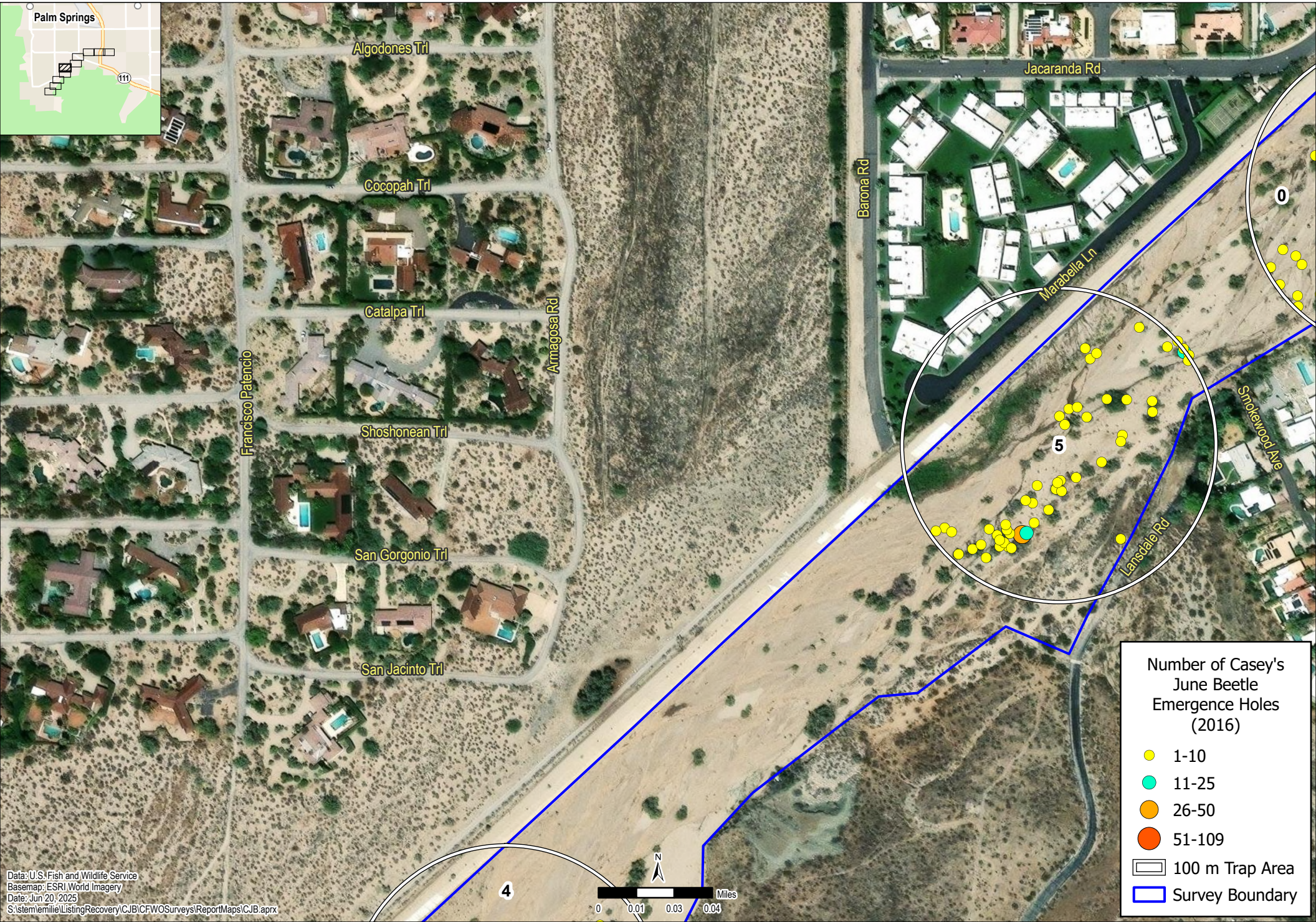
Number of Casey's
June Beetle
Emergence Holes
(2016)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary







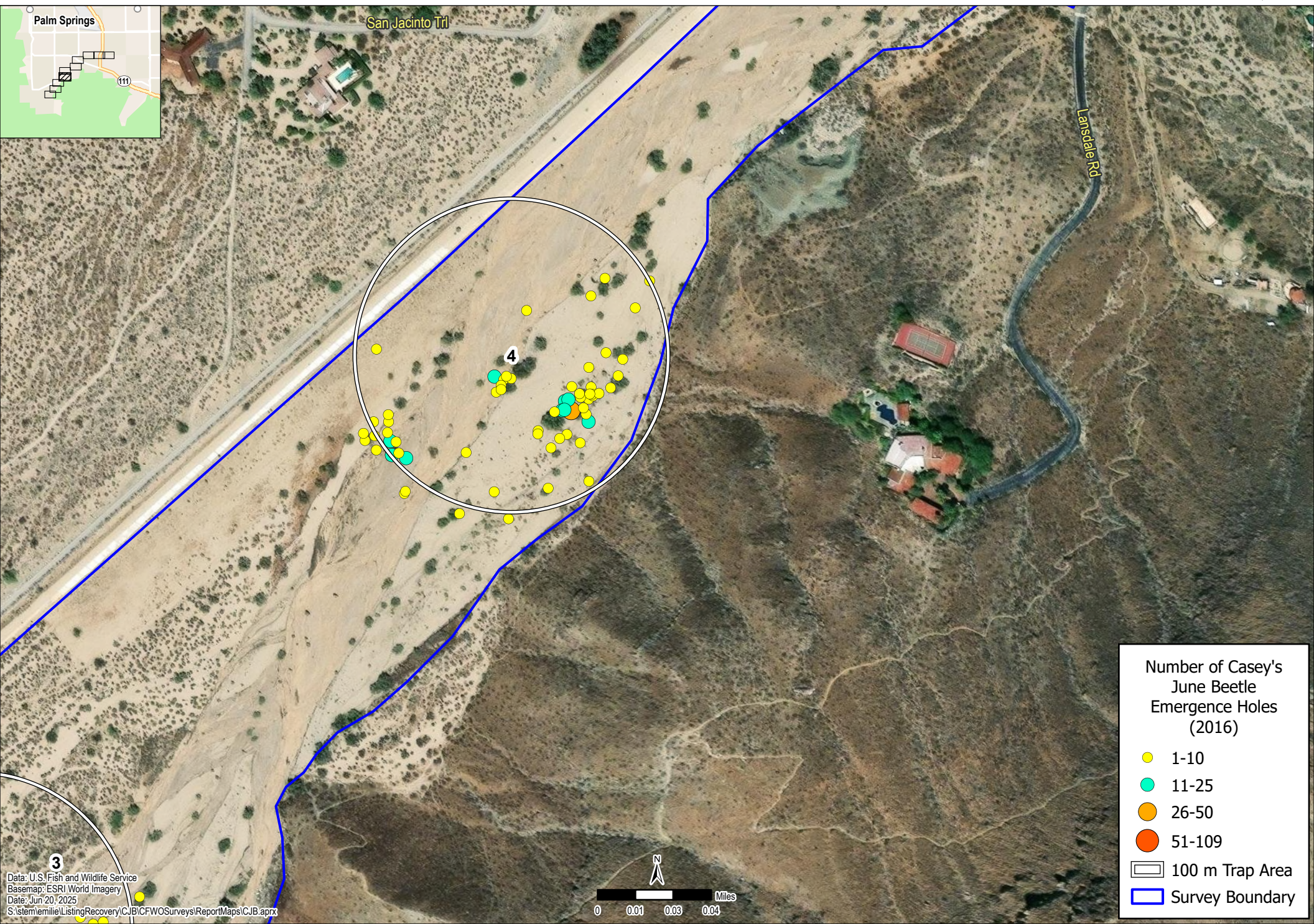
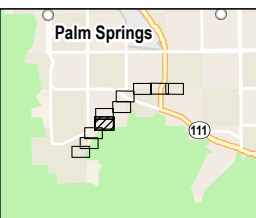


Number of Casey's June Beetle Emergence Holes (2016)

- 1-10
- 11-25
- 26-50
- 51-109

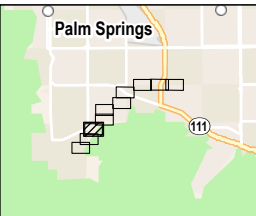
100 m Trap Area

Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2016)

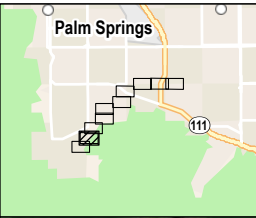
- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2016)

- 1-10
- 11-25
- 26-50
- 51-109

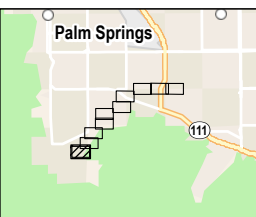
100 m Trap Area
Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2016)

- 1-10
- 11-25
- 26-50
- 51-109

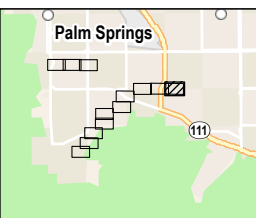
100 m Trap Area
Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2016)

- 1-10
- 11-25
- 26-50
- 51-109
- 100 m Trap Area
- Survey Boundary

Figure I3, Maps 1–13. Detailed map series of emergence hole counts, 2017.

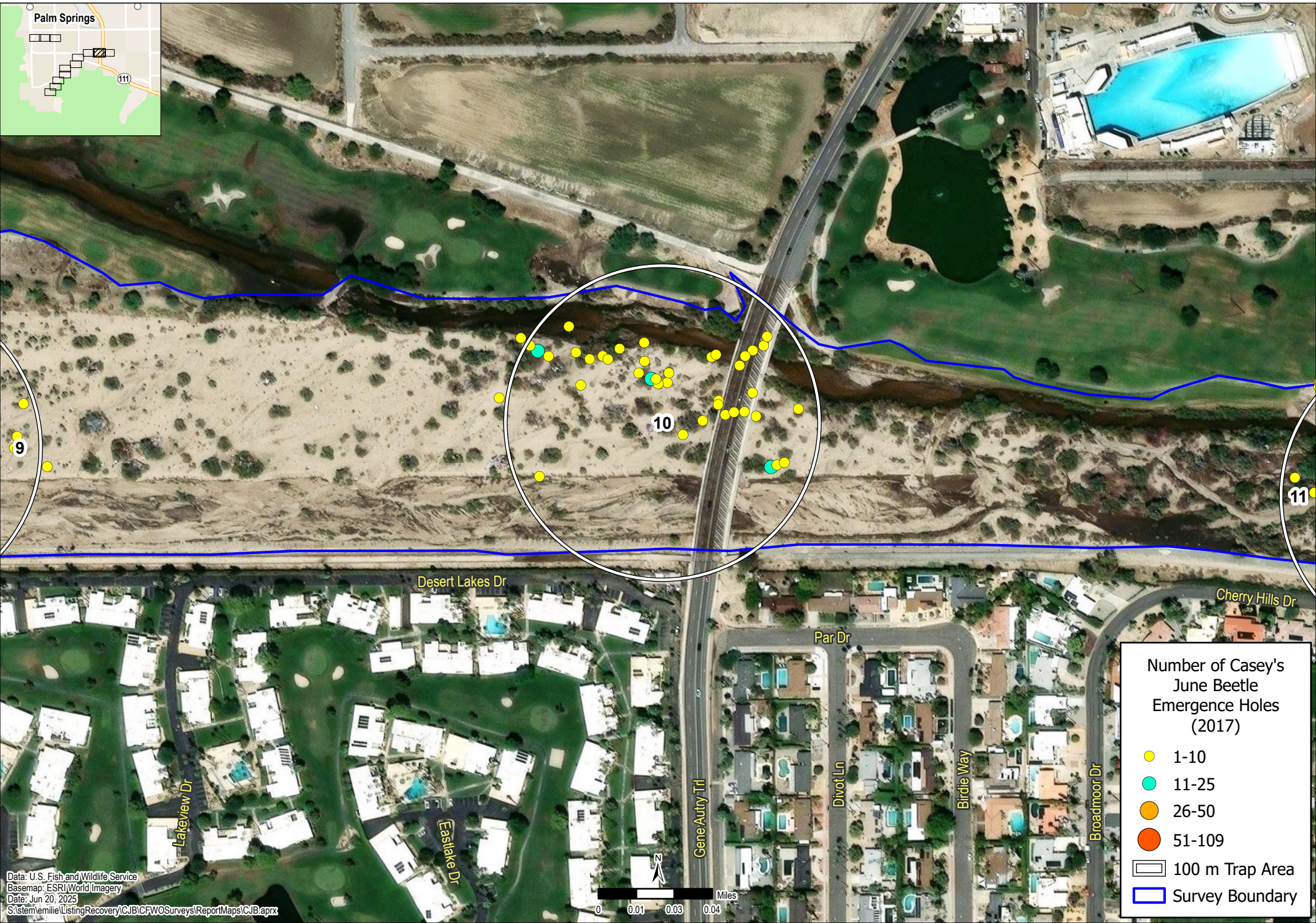
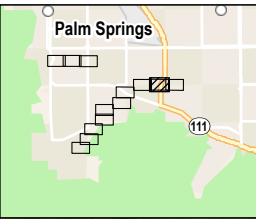


Number of Casey's June Beetle Emergence Holes (2017)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area

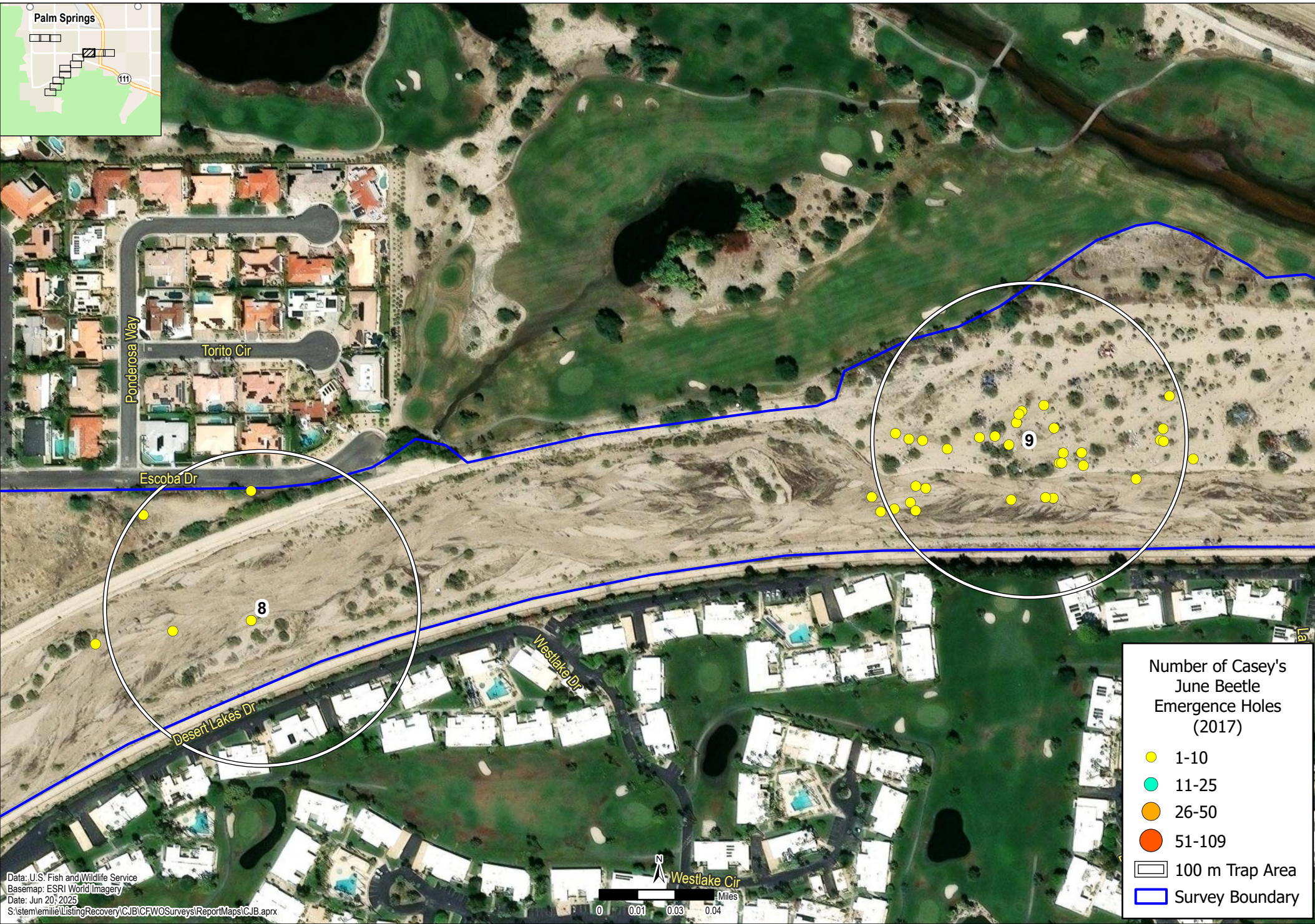
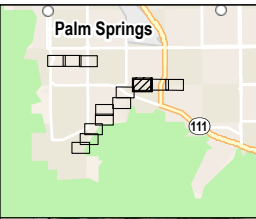
Survey Boundary



Number of Casey's
June Beetle
Emergence Holes
(2017)

- 1-10
- 11-25
- 26-50
- 51-109

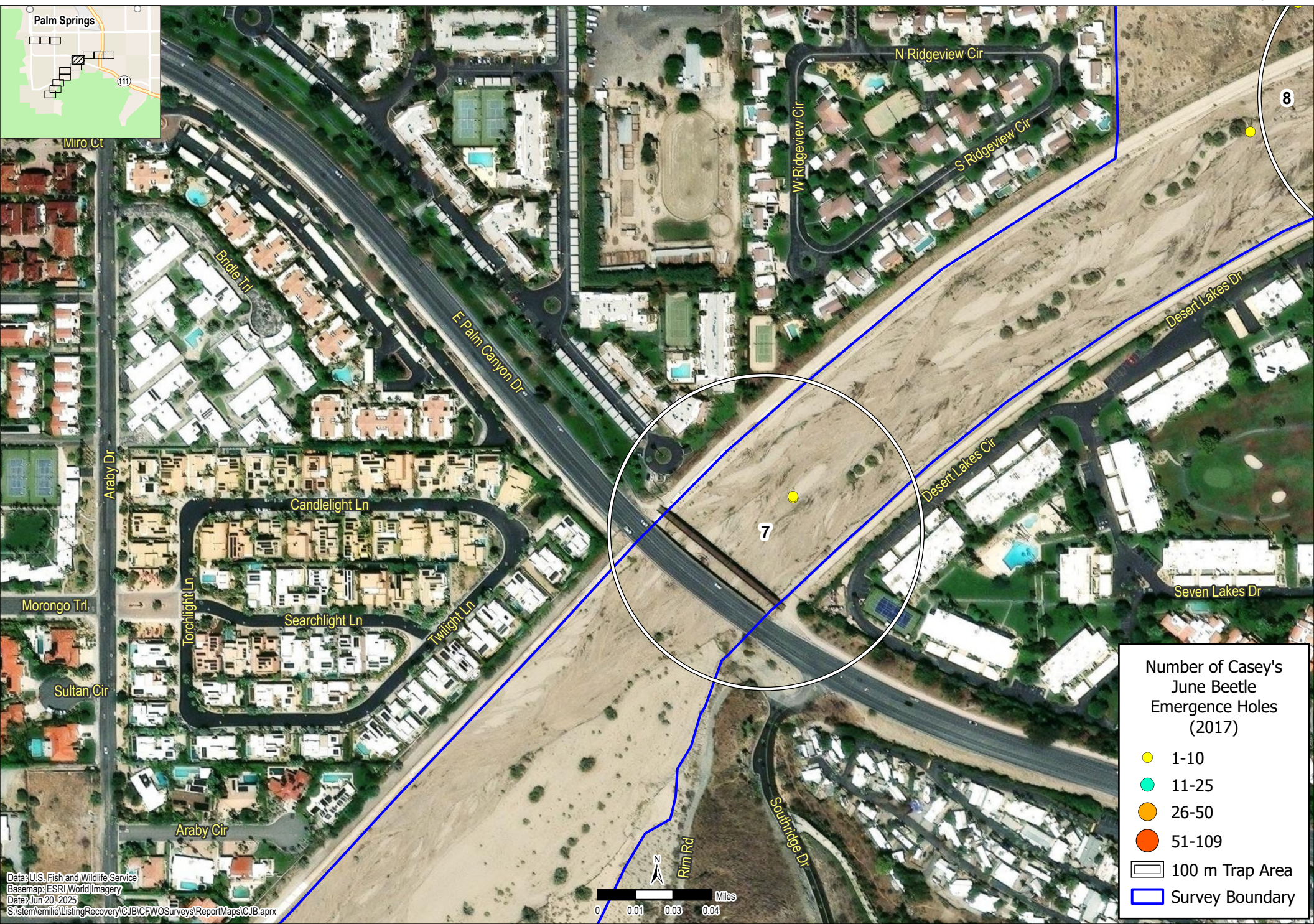
100 m Trap Area
Survey Boundary

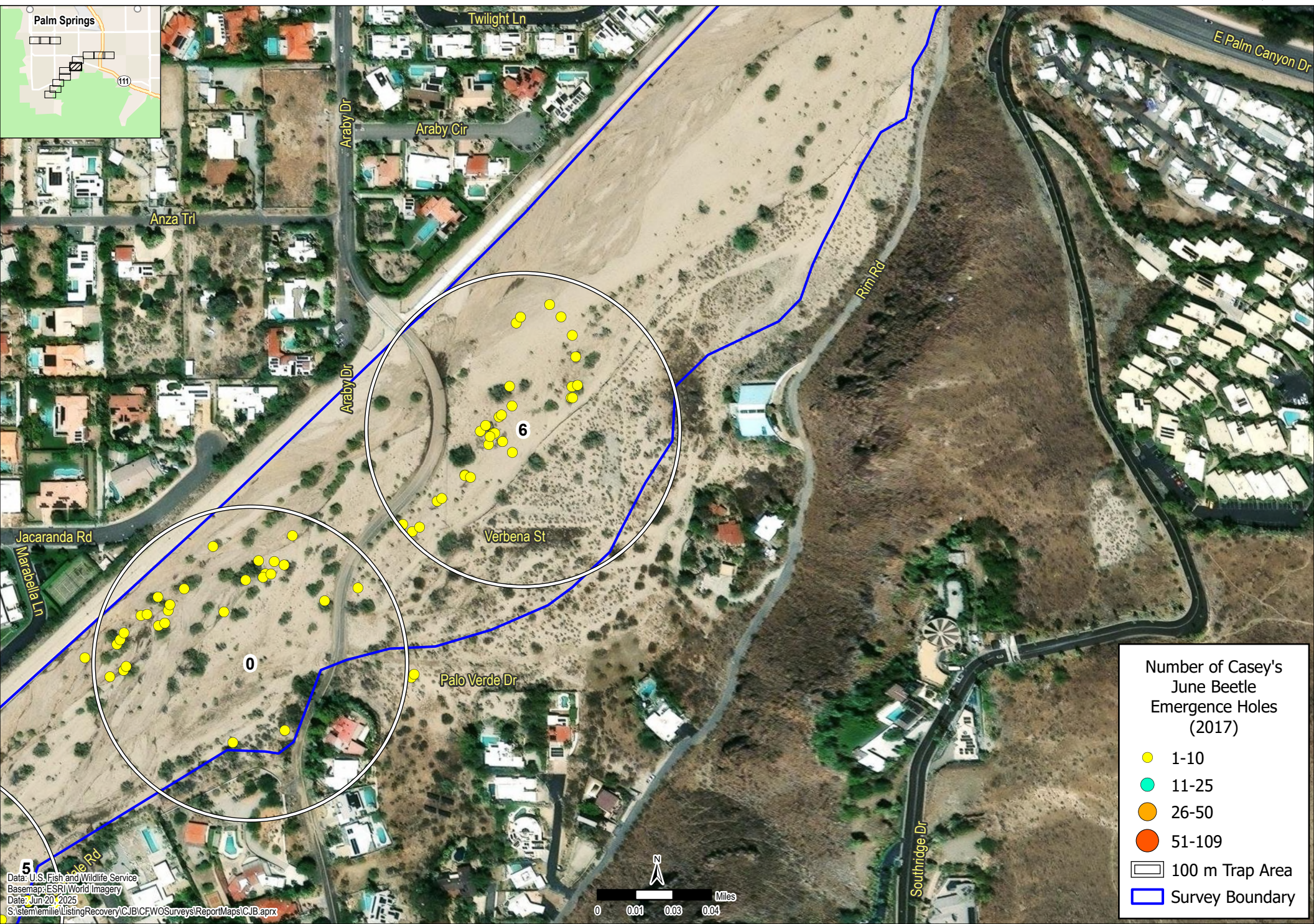


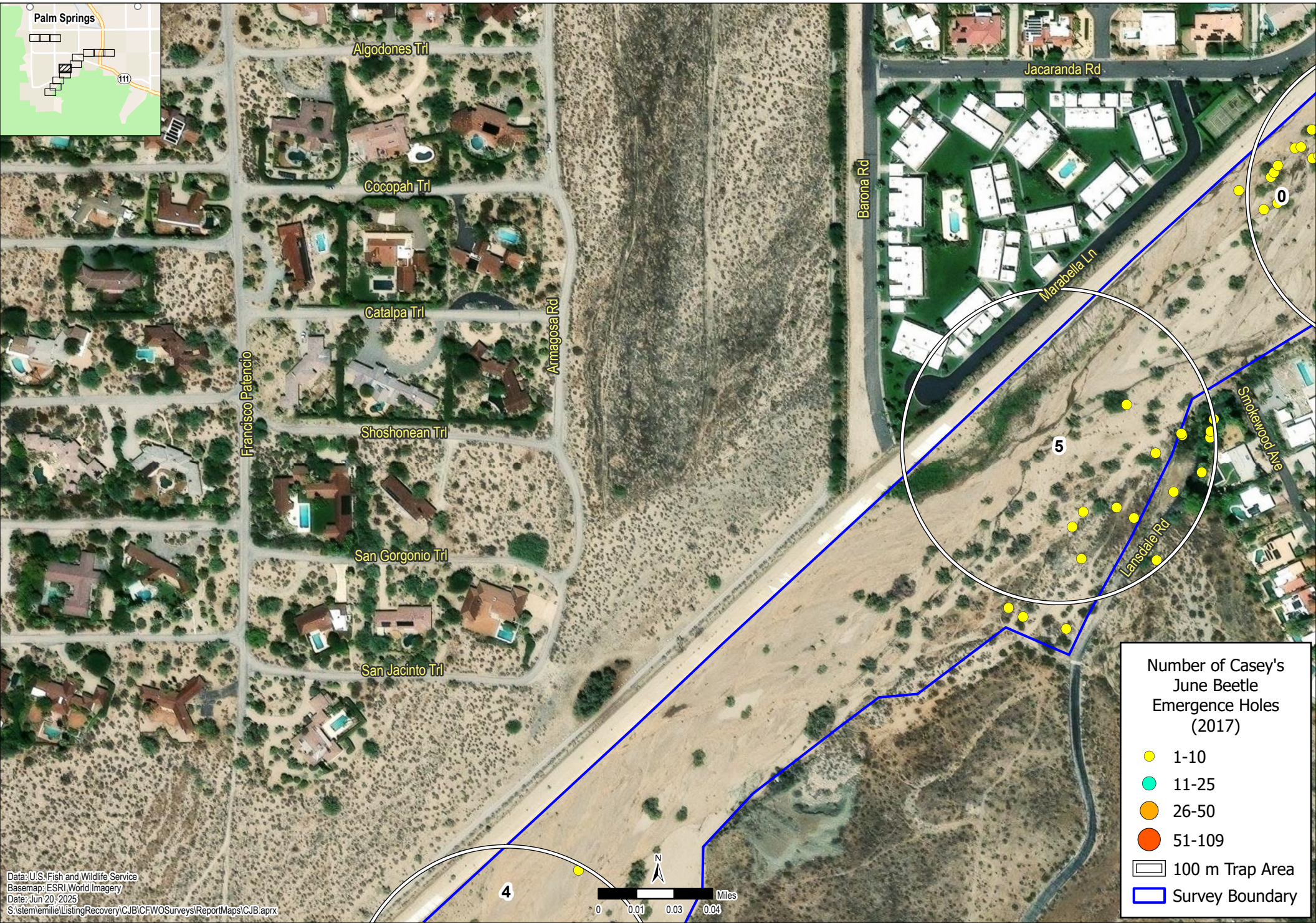
Number of Casey's
June Beetle
Emergence Holes
(2017)

- 1-10
- 11-25
- 26-50
- 51-109

- 100 m Trap Area
- Survey Boundary





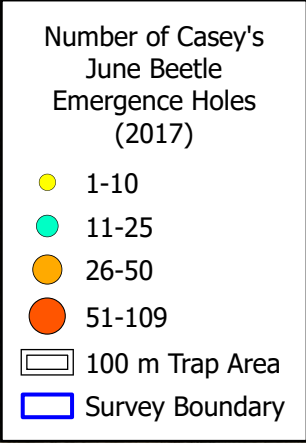
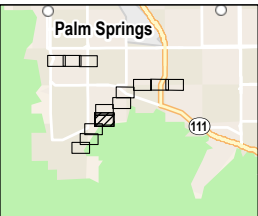


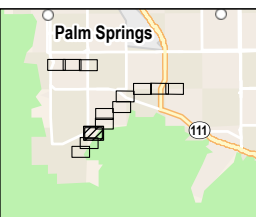
Number of Casey's June Beetle Emergence Holes (2017)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area

Survey Boundary





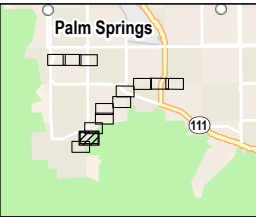
Number of Casey's
June Beetle
Emergence Holes
(2017)

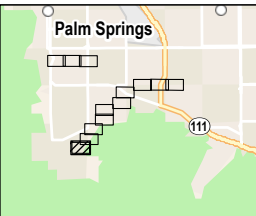
- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area

Survey Boundary



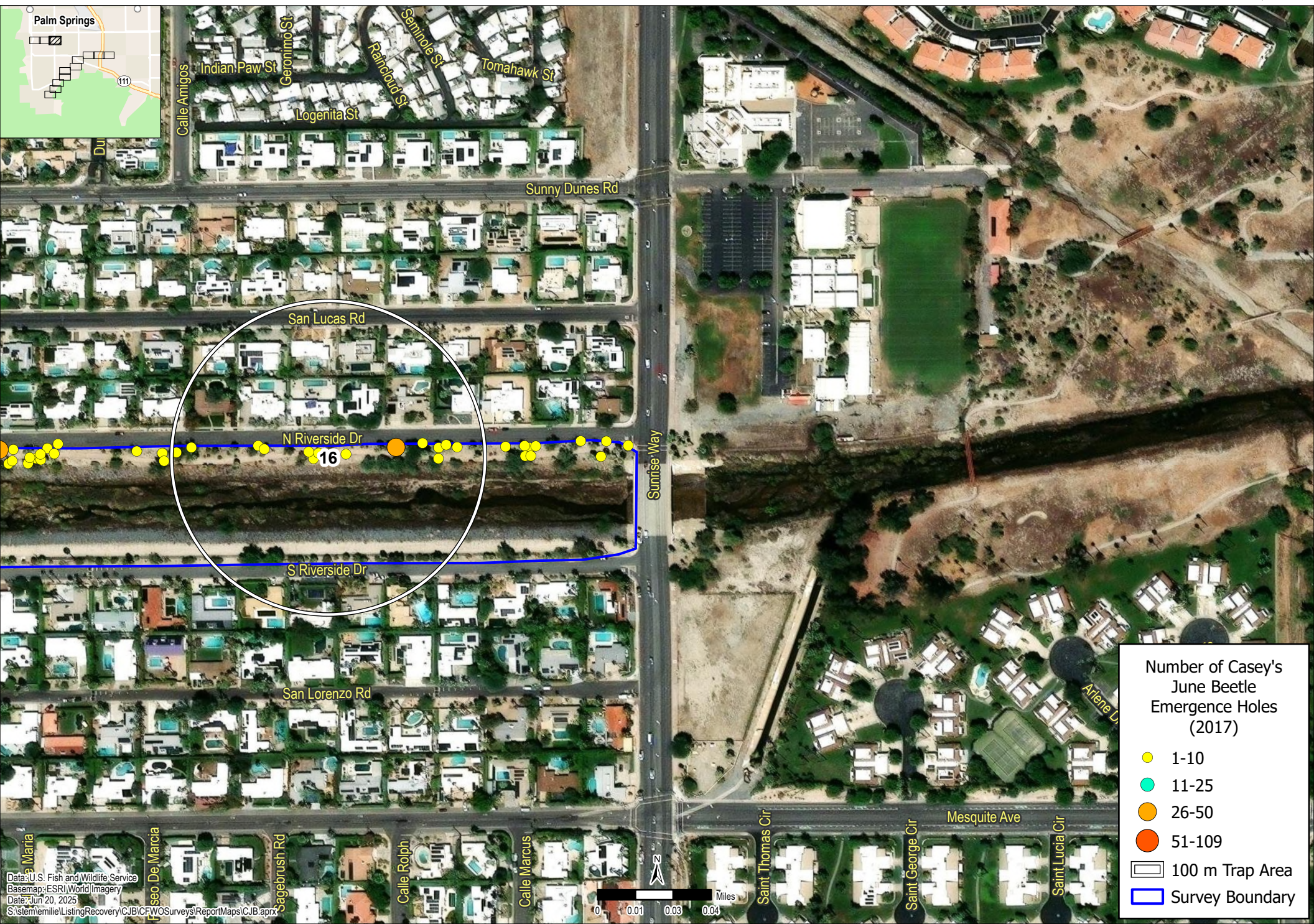


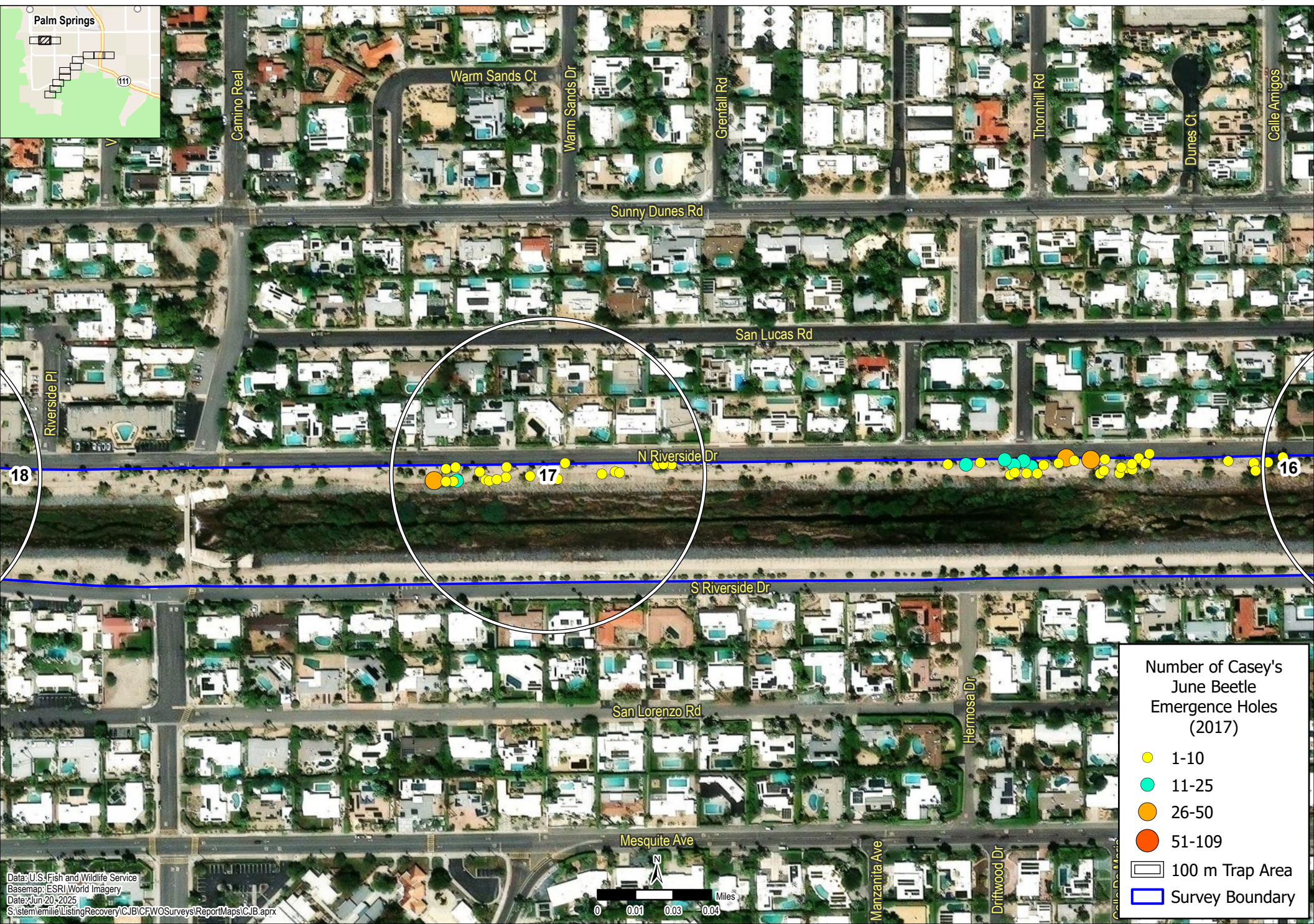


Number of Casey's
June Beetle
Emergence Holes
(2017)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area
Survey Boundary



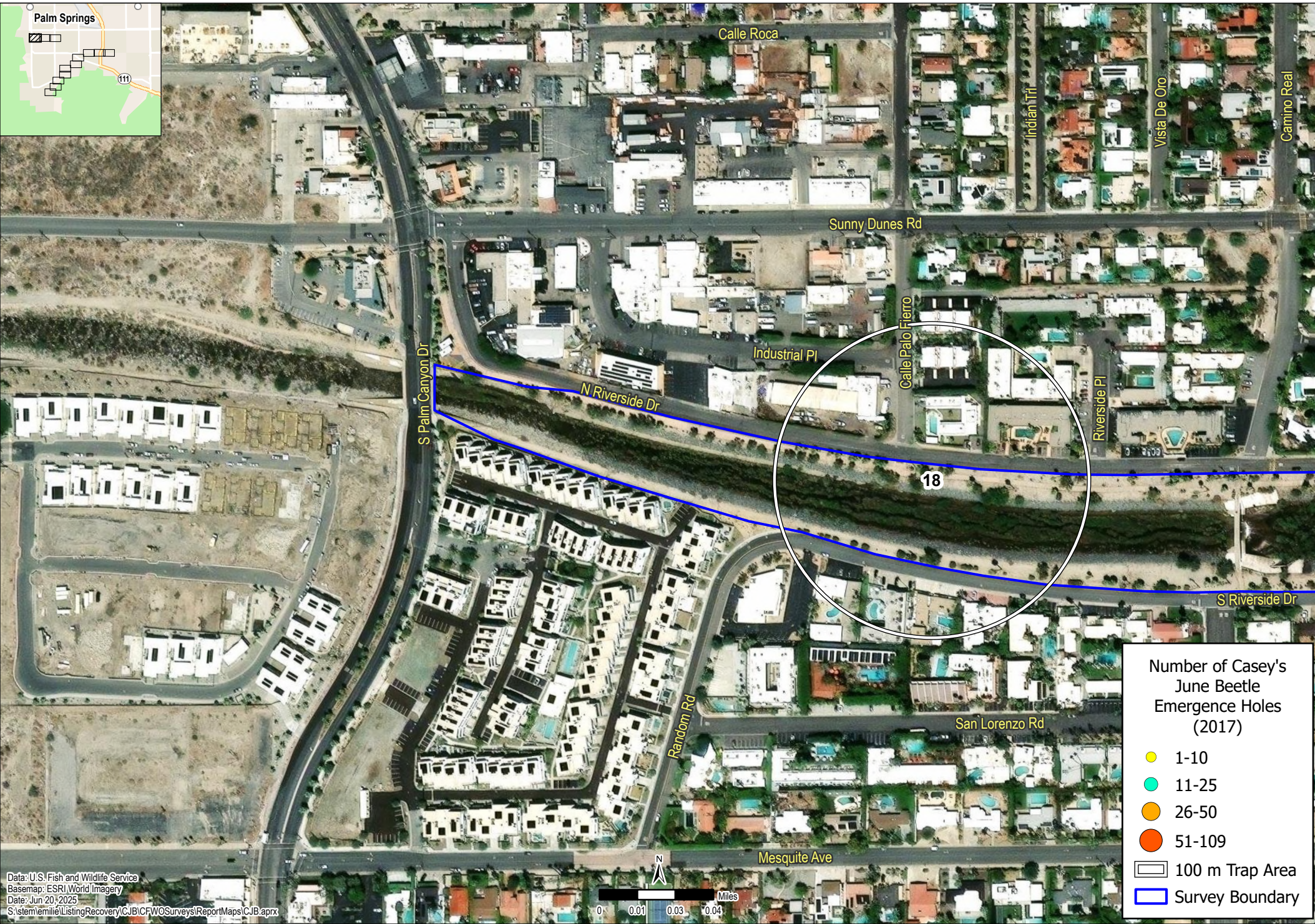
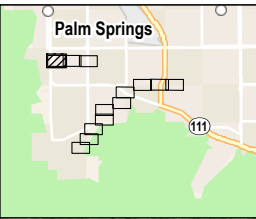


Number of Casey's
June Beetle
Emergence Holes
(2017)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area
Survey Boundary





Number of Casey's
June Beetle
Emergence Holes
(2017)

- 1-10
- 11-25
- 26-50
- 51-109

100 m Trap Area
Survey Boundary

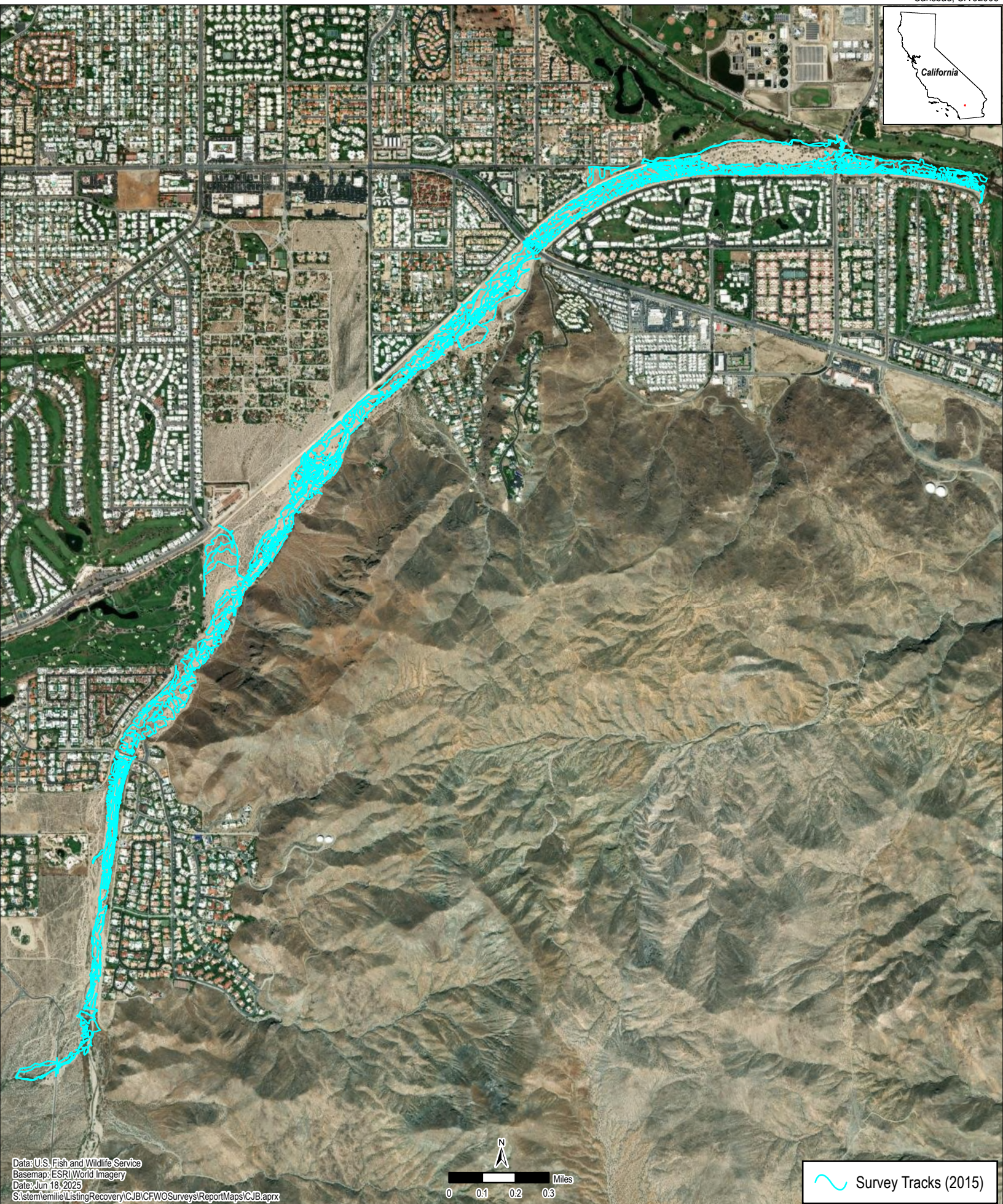


Figure I4. Overview and detailed map series (**Maps 1–4**) of surveyor tracks showing meandering transects and area coverage of emergence hole surveys in 2015. The entirety of Palm Canyon Wash was surveyed.

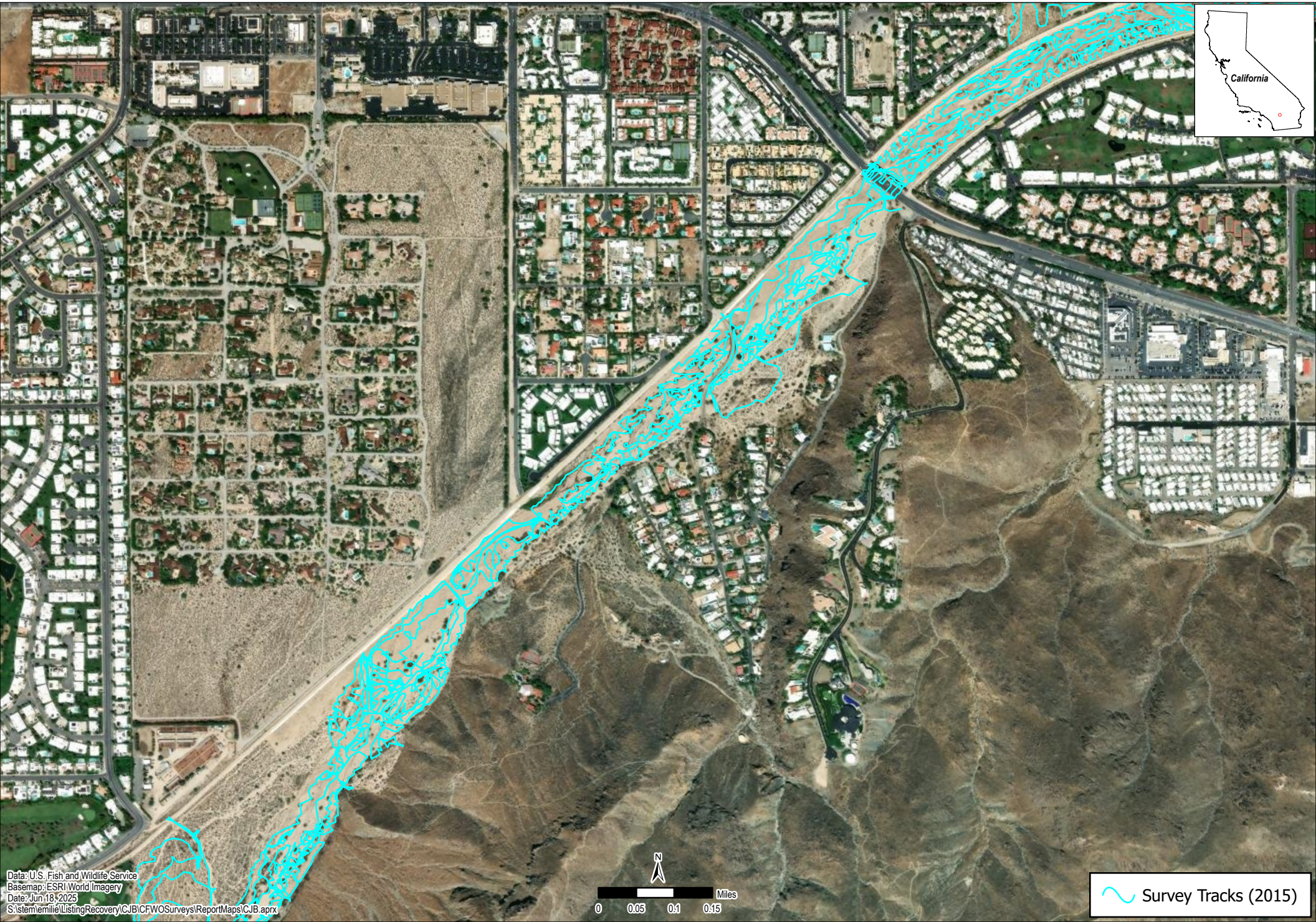


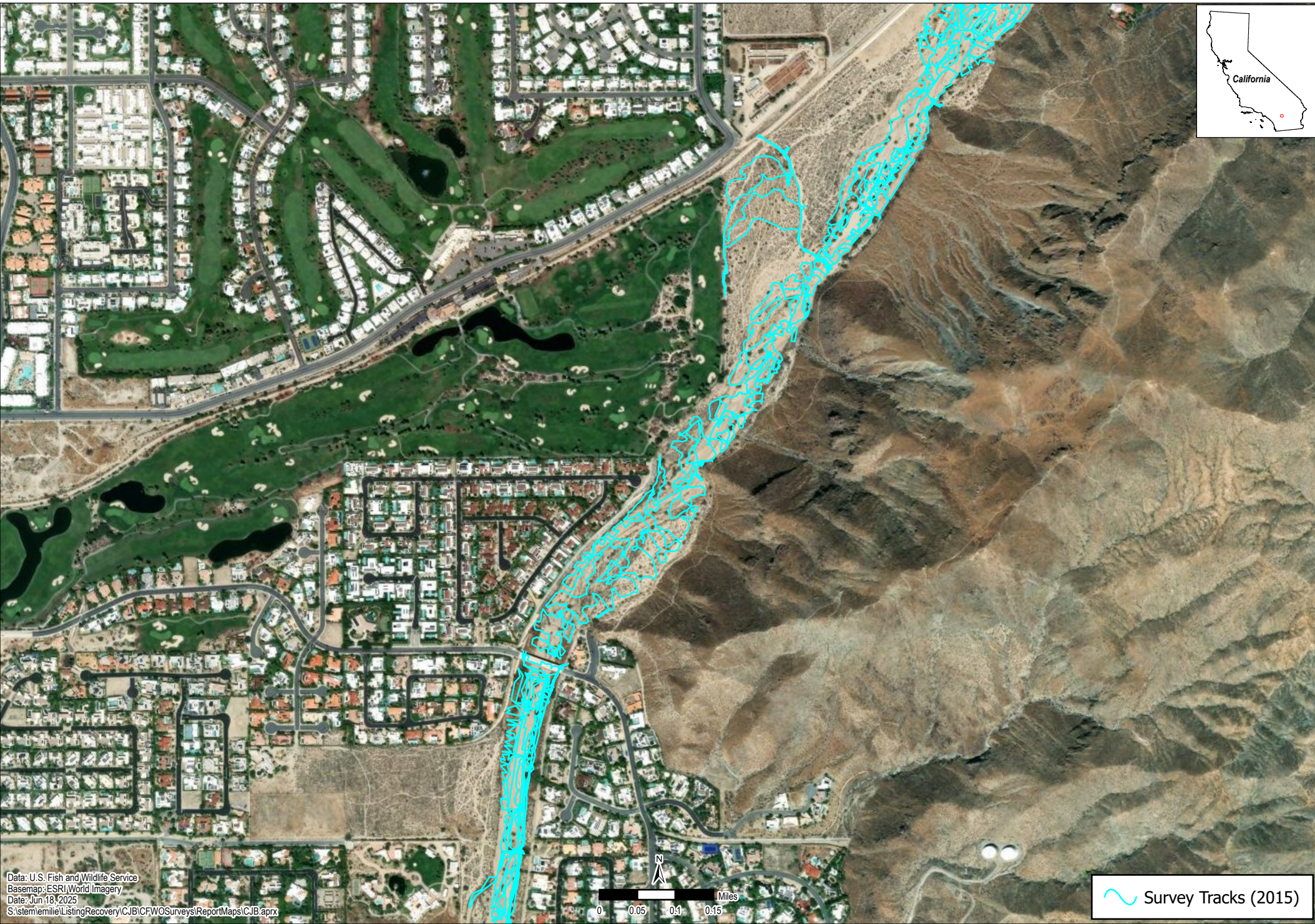
U.S. Fish & Wildlife Service
Casey's June Beetle (*Dinacoma caseyi*) 2015 Survey Tracks

Carlsbad Fish and Wildlife Office
2177 Salk Avenue, Suite 250
Carlsbad, CA 92008









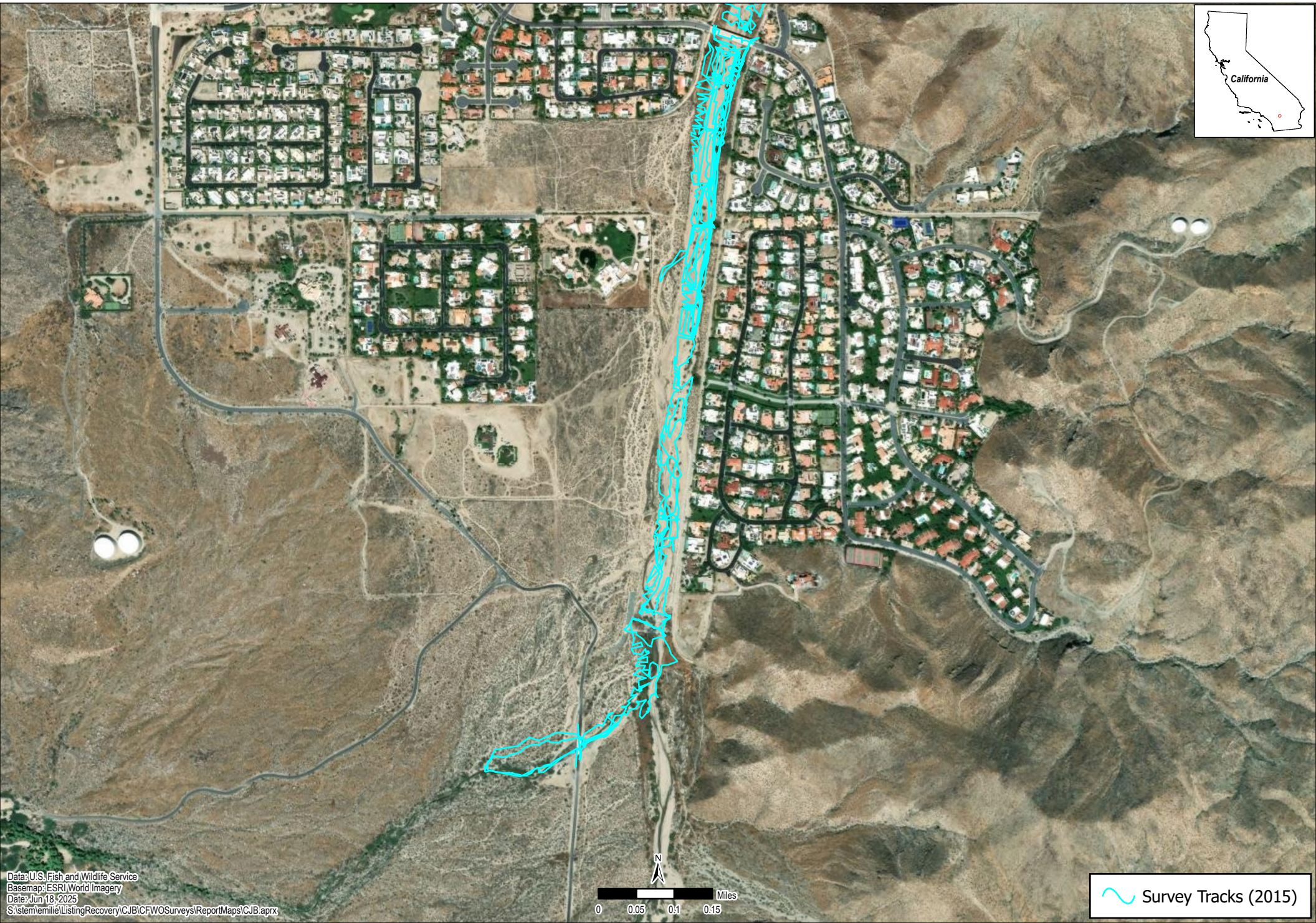
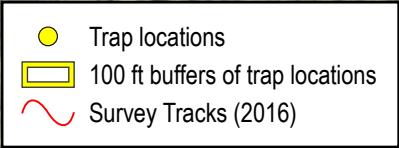
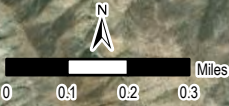
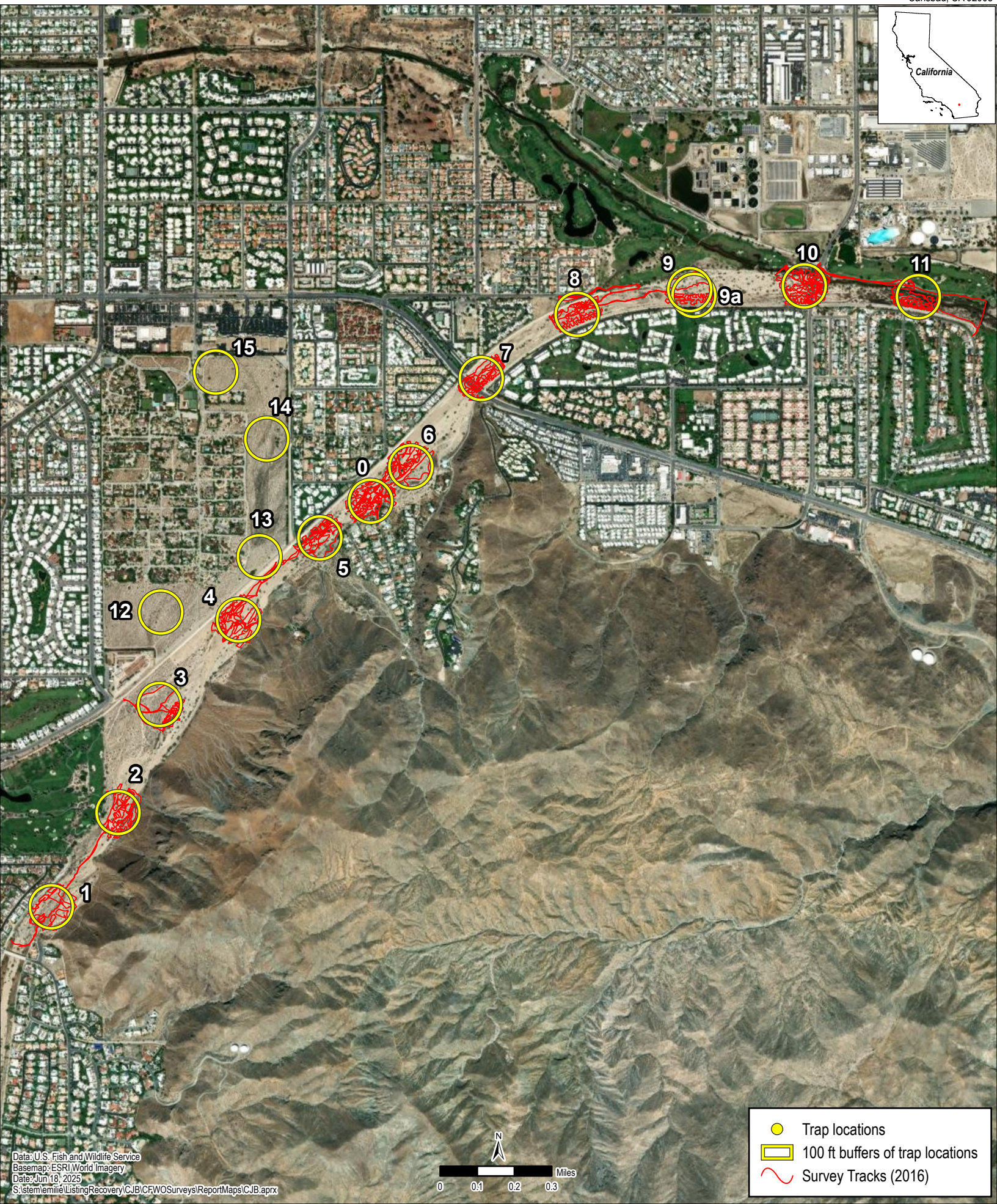
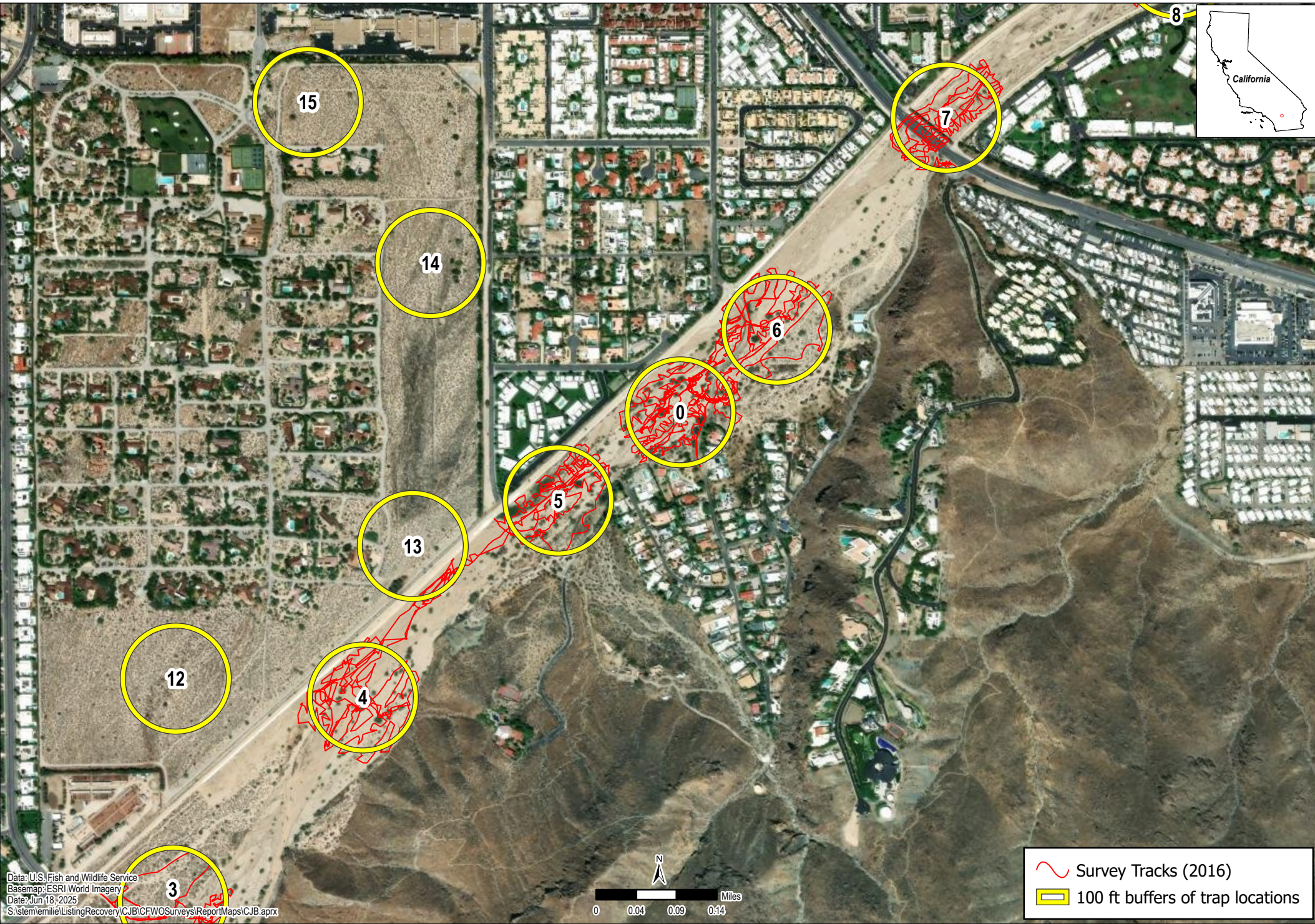
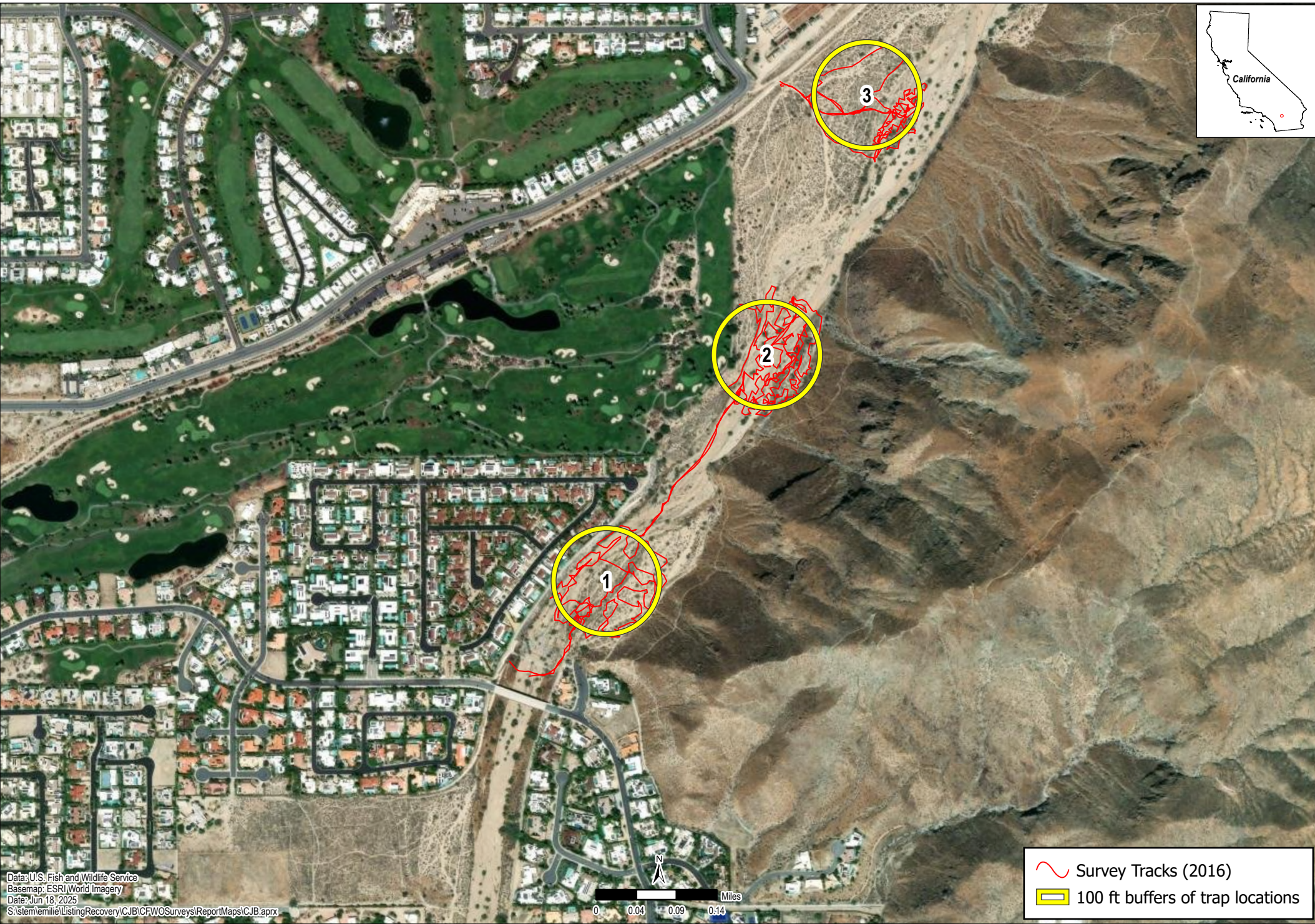


Figure I5. Overview and detailed map series (**Maps 1–3**) of surveyor tracks showing meandering transects and area coverage of emergence hole surveys in 2016. The 100-meter radius area around traps in Palm Canyon Wash was surveyed.









APPENDIX J – Buried Salvaged Vegetation in Palm Canyon Wash

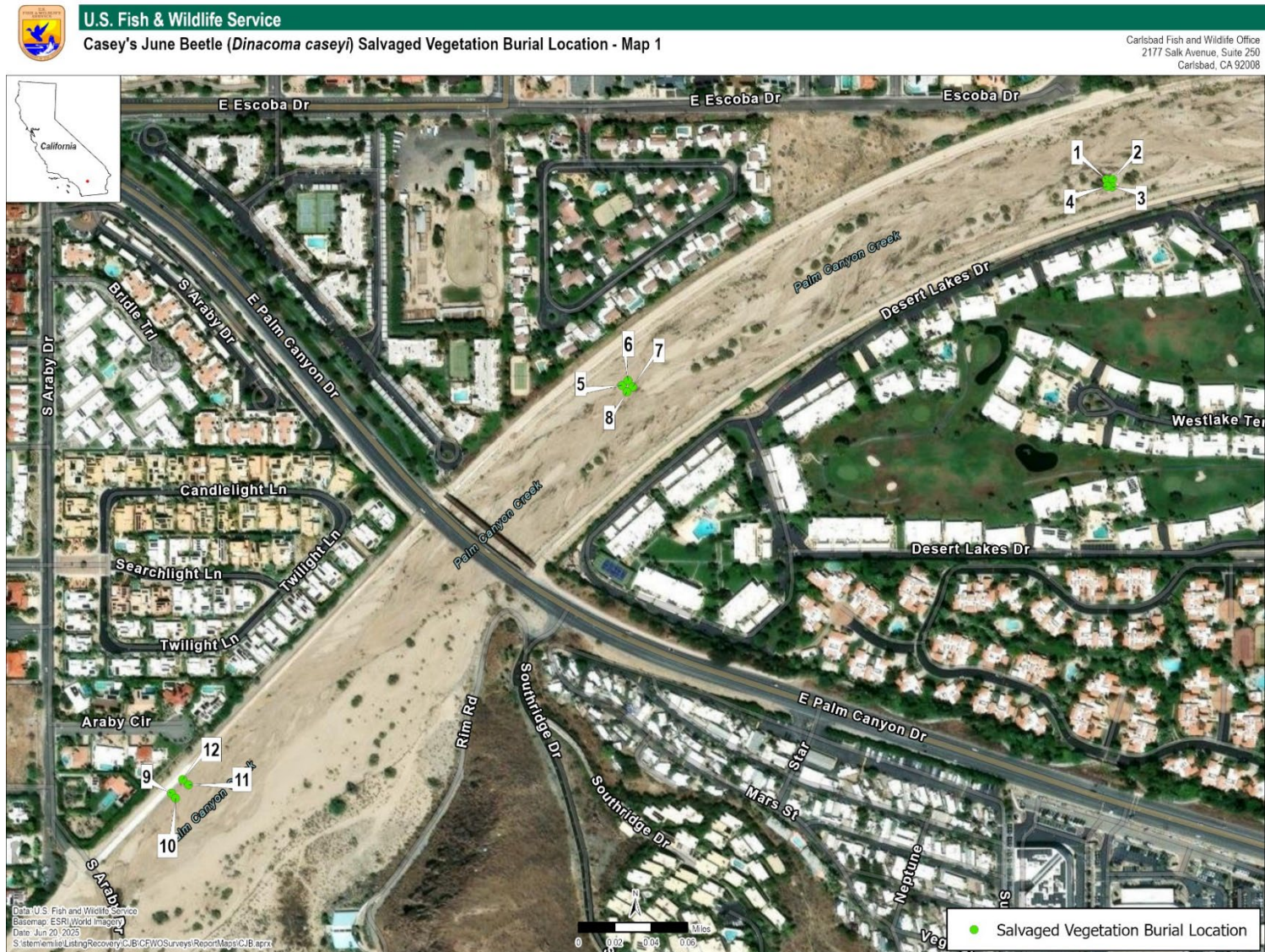


Figure J1-1. Locations of buried salvaged vegetation in the sediment removal area (locations 1–8) and the levee repair area (locations 9–12) of Palm Canyon Wash, 2019–2020. Location identification numbers correspond to the ID numbers in Table J1.



Figure J1-2. Locations of buried salvaged vegetation in the levee repair area of Palm Canyon Wash, 2020. Location identification numbers correspond to the ID numbers in Table J1.

Table J1. Salvaged vegetation burial locations in Palm Canyon Wash from sediment removal and levee repair maintenance activities, 2019–2020¹. Woody vegetation that could not be avoided was salvaged and buried to provide a larval food source.

Maintenance Area	ID ²	Cluster ²	Latitude	Longitude	Location Description
Sediment Removal	1	1	33.800757	-116.504922	southern most cluster, E. Escoba Dr. area
Sediment Removal	2	1	33.800759	-116.504863	southern most cluster, E. Escoba Dr. area
Sediment Removal	3	1	33.800698	-116.504868	southern most cluster, E. Escoba Dr. area
Sediment Removal	4	1	33.800703	-116.504916	southern most cluster, E. Escoba Dr. area
Sediment Removal	5	2	33.799133	-116.509561	mid-reach, downstream of E. Palm Canyon Dr. bridge
Sediment Removal	6	2	33.799172	-116.509506	mid-reach, downstream of E. Palm Canyon Dr. bridge
Sediment Removal	7	2	33.799121	-116.50946	mid-reach, downstream of E. Palm Canyon Dr. bridge
Sediment Removal	8	2	33.799081	-116.509516	mid-reach, downstream of E. Palm Canyon Dr. bridge
Levee Repair Reach 3	9	3	33.795896	-116.51389	mid-reach, downstream of S. Araby Dr.
Levee Repair Reach 3	10	3	33.795856	-116.513849	mid-reach, downstream of S. Araby Dr.
Levee Repair Reach 3	11	3	33.795962	-116.513725	mid-reach, downstream of S. Araby Dr.
Levee Repair Reach 3	12	3	33.796005	-116.513781	mid-reach, downstream of S. Araby Dr.
Levee Repair Reach 2	13	4	33.791532	-116.51899	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	14	4	33.791483	-116.518936	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	15	4	33.791451	-116.519012	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	16	4	33.791495	-116.51904	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	17	5	33.790684	-116.520193	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	18	5	33.790639	-116.520133	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	19	5	33.790601	-116.520205	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	20	5	33.790651	-116.52025	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	21	6	33.790124	-116.52096	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	22	6	33.790074	-116.520914	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	23	6	33.790018	-116.52099	adjacent to Smoke Tree Ranch

Maintenance Area	ID ²	Cluster ²	Latitude	Longitude	Location Description
Levee Repair Reach 2	24	6	33.790075	-116.521044	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	25	7	33.789744	-116.521331	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	26	7	33.789702	-116.521281	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	27	7	33.789648	-116.521334	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	28	7	33.789708	-116.521407	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	29	8	33.789453	-116.52174	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	30	8	33.789412	-116.521668	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	31	8	33.789373	-116.521709	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	32	8	33.789427	-116.521781	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	33	9	33.789184	-116.522239	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	34	9	33.789083	-116.522267	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	35	9	33.789097	-116.522335	adjacent to Smoke Tree Ranch
Levee Repair Reach 2	36	9	33.78917	-116.522333	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	37	10	33.788611	-116.523	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	38	10	33.788567	-116.522936	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	39	10	33.788514	-116.522974	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	40	10	33.788554	-116.52304	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	41	11	33.78842	-116.5233	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	42	11	33.788374	-116.523244	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	43	11	33.7883	-116.523364	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	44	11	33.788346	-116.523398	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	45	12	33.788046	-116.523671	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	46	12	33.787979	-116.523606	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	47	12	33.787957	-116.523662	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	48	12	33.788025	-116.523725	adjacent to Smoke Tree Ranch

Maintenance Area	ID ²	Cluster ²	Latitude	Longitude	Location Description
Levee Repair Reach 1	49	13	33.787716	-116.524128	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	50	13	33.787661	-116.524067	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	51	13	33.787589	-116.524163	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	52	13	33.787641	-116.524217	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	53	14	33.787352	-116.524749	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	54	14	33.787283	-116.524666	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	55	14	33.787244	-116.524732	adjacent to Smoke Tree Ranch
Levee Repair Reach 1	56	14	33.787296	-116.524809	adjacent to Smoke Tree Ranch

¹ Salvaged vegetation burial occurred between July 29, 2019, and February 28, 2020 (sediment removal area), and September 1 to December 16, 2020 (levee repair area).

² ID and Cluster identification numbers assigned by the Service.