

Annual Report
Population monitoring, seed collection and banking, and habitat assessment for the
Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*)
January 2023

Background and Introduction

Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*; CAAFNE; State Threatened, Federally Endangered, CNPS RPR 1B.2) is a perennial root hemiparasite in the broomrape family (Orobanchaceae). It differs from its common sister taxon, *Castilleja affinis* ssp. *affinis* (CAAFAF), in having yellow to peach-colored floral bracts, rather than orange to red-colored, smaller inflorescences (15-25mm wide in CAAFNE vs. 30-50mm wide in CAAFAF), and in its habitat restriction to serpentine soils.

At the time of its listing as federally endangered in 1995 (USFWS 1995), only 1500 plants in six populations were known to exist, including one population in Santa Clara County (Paintbrush Hill, CNDDDB occurrence 7). Another nearby population, called Paintbrush Canyon, was subsequently discovered (2002, CNDDDB occurrence 9). Population sizes for this taxon range from 20 to 1000 plants depending on the occurrence (CNDDDB, Widener & Fant 2017), which suggests that a target population size of 2000 individuals (which is the non-taxon-specific recovery goal stated in the Santa Clara Valley Habitat Plan) may not be appropriate or attainable for this taxon and location.

The Paintbrush Hill occurrence, located on property owned and managed by the Santa Clara Valley Water District (Valley Water), has historically been documented as three separate subpopulation clusters, or polygons (Figure 1). No plants were observed in the two smaller polygons (North and South) from 1997 and 1994, respectively (see Table 1), until 2019 when plants were rediscovered near the north polygon. The number of plants in the Paintbrush Hill occurrence has fluctuated over time, with the number of plants counted per year ranging from a low of 9 individuals in 1994 to 208 or 224 in 2018, depending on count method (Creekside Science 2018a). Concerns over the small population size at Paintbrush Hill, coupled with its inclusion as a covered, no-take species in the Santa Clara Valley Habitat Plan (SCV-HP), a joint HCP/NCCP covering most of Santa Clara County, has led to increased focus on the development of recovery actions for the taxon and augmentation of the two known populations in Santa Clara County.

Polygon Location	1993	1994	1997	1999	2006	2009	2013	2018
<i>North</i>	NA	4	27	NA	NA	NA	NA	NA
<i>Central</i>	17	NA	0	80	>140	103	~100	208/224
<i>South</i>	NA	5	0	0	NA	NA	NA	NA

Table 1. Historical reports of number of plants at Paintbrush Hill, 1993-2018. Data sources: CNDDDB (1993-2013), Creekside Science (2018)

A baseline assessment of demography of the Paintbrush Hill occurrence is needed prior to developing a comprehensive plan for population augmentation or recovery or drafting any prescriptive management actions. In 2019, Valley Water committed to the following near-term tasks for the Paintbrush Hill CAAFNE occurrence: 1. Population monitoring; 2. Limited seed collection and banking; 3. Basic assessment of habitat quality and threats.

This fourth annual report complies with the agreement made in April 2019 (Valley Water 2019). It describes field activities that took place in 2022, assessments of potential threats to the CAAFNE population at Paintbrush Hill, planned future activities, and initial management recommendations for the population. Next year will be the final year of population monitoring under this agreement. Proposed management recommendations are discussed here and proposed for further discussion among the larger group.

Methods

Population Monitoring

CAAFNE individuals were identified based on the best available information for each plant. Factors taken into account included growth pattern, morphology, and distance from other plants.

Growth pattern: Plants that are close together can sometimes be distinguished because the stems all radiate from separate common centers or root crowns. Stems that angle towards a common center, even if the exact center point is obscured by rocks, can be inferred as belonging to a single plant.

Morphology: Often different plants may have different phenology (one is blooming slightly later than the other), stature (one may be taller), or coloration (plants may display differences in leaf or flower color). When plants have more than one flowering stem, those stems tend to be more like one another than stems from separate plants. After counting, plants were marked with durable metal plant markers and assigned a number, to facilitate tracking of individual plants across years to the extent possible.

Seedlings were defined as single unflowering stems less than 10cm tall. Seedlings were tallied only (no other metrics measured).

In addition to the plant count, additional information was collected during the population monitoring for all flowering CAAFNE plants. Information collected included:

- number of stems per plant
- number of inflorescences (flowering stalks) per plant
- width of inflorescences (in cm)
- dominant flower color (3 categories: pale peach/yellow, moderate orange-red, bright red)
- maximum plant height (in cm)
- potential host plant species within 12 inches
- evidence of herbivory on inflorescences (present/absent, putative vector if available (e.g., insect, cattle, rabbit))

This information was collected during peak flowering season in 2022, on 4/4 and 4/13/22. After peak flower, monitoring visits occurred approximately every four weeks to assess fruiting phenology and determine the best time for seed collection and collection of fruiting information. Seed and fruiting information were collected on 6/22 and 7/6/22. This information included:

- number of infructescences per plant
- number of inflorescences remaining per plant on that date, if any
- number of capsules per infructescence

Thatch and Ground Cover

In 2021, a pilot assessment was conducted of the relative components of ground cover inside and outside of the temporary paintbrush protection cages, for 60 one-foot square quadrats. This year, a more comprehensive assessment of these ground cover components was performed. The information types collected were:

- percent grass cover

- percent forb cover
- percent thatch or litter cover
- percent bare ground
- percent rock (>6 cm)
- Tiburon paintbrush present/absent in quadrat

Percent cover was estimated by eye using the California Native Plant Society reference plots for cover estimation (CNPS 2001). A one-by-one foot square of PVC pipe was used to delimit each quadrat.

Thatch quadrats were assessed on 3/29 and 4/4/2022. Outside of enclosures, parallel transects approximately 5 feet apart were taken across the north and middle polygons where Tiburon paintbrush is extant, including unoccupied areas between patches, with quadrats dropped approximately 5 feet apart along each transect line. Cover within enclosures was sampled completely. This resulted in a total of 304 quadrats outside enclosures and 259 inside enclosures.

Seed Collection and Banking

California Botanic Garden (CBG, formerly Rancho Santa Ana Botanical Garden)'s seed collection guidelines for California native plant species recommends collecting seed from no more than 5% of a population per year (Wall 2009). Five percent of our 2022 population count of 116 (seedlings excluded) would be six plants. However, as of July 6, only 51 plants had developing infructescences, so we reduced the number of plants for seed collection to two.

Fruit phenology was monitored approximately every four weeks after peak flower to track fruit development and determine the best time for seed collection. Fruits representing less than 5% of a plant's output were collected when the capsules were dry but not yet dehisced, as recommended by the Seed Conservation Program at CBG (Wall 2009). Fruits were allowed to air-dry in paper bags, after which seed was cleaned of vegetative material and deposited with the CBG conservation collection.

Seeds collected by Valley Water over the five years of this population monitoring effort will be stored permanently at CBG, which deposits a backup sample with the National Laboratory for Genetic Resources Preservation in Fort Collins, CO. The seeds collected by Valley Water will not be combined with the seed collection made by Creekside Science in 2018.

Results and Discussion

Population Monitoring

We counted 116 reproductively mature plants and 2 seedlings in the 2022 monitoring season, for a total population size of 118 individuals. This included 100 plants and 2 seedlings in the main (central) polygon, and 16 plants in the northern polygon (Figure 1).

The number of CAAFNE reported at Paintbrush Hill over time is illustrated in Figure 2. Please note that survey methods and locations have not been consistent throughout the entire period for which data are available. Although the available population numbers show for the most part a relatively stable or increasing trend in this population, the lower counts in 2020 – 2022 highlight that this small population may need additional protection if it is to persist or increase in size (Figure 2).

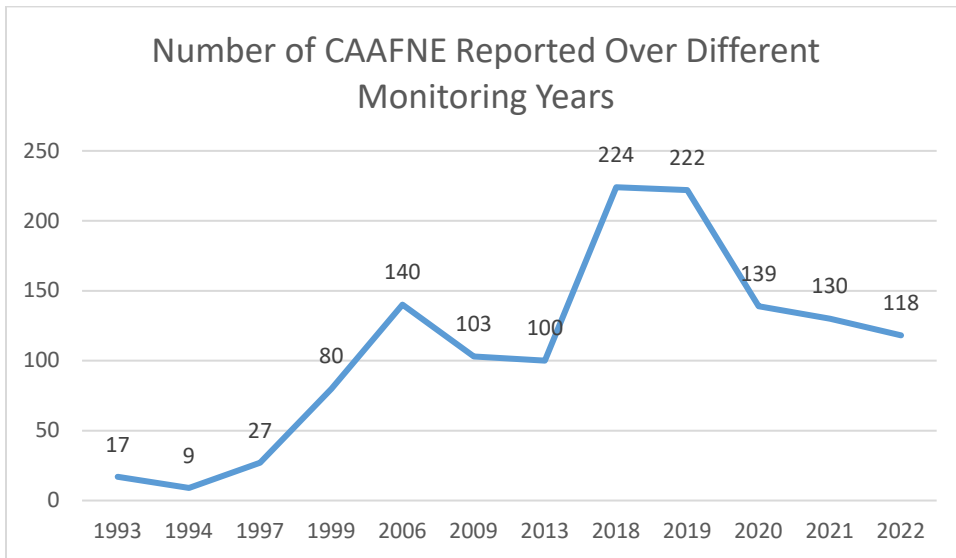


Figure 2. Number of CAAFNE plants at Paintbrush Hill, 1993-2022. Data sources: CNDDDB (1993-2013), Creekside Science (2018), Valley Water (2019-2022)

Population summary statistics (average plant height, average number of stems, inflorescences, and infructescences per individual, average number of capsules per infructescence) are listed in Table 2 (seedlings are excluded from these metrics because they were defined as single non-flowering stems less than 10cm tall). Average height per plant in 2022 was 15.0cm (n=113), with an average number of stems per plant of 6.0 and average number of inflorescences per plant of 2.0. 2022 plants were shorter and less branched than 2021 plants, but with more inflorescences per plant.

Average width of inflorescences was 18.3mm (n=215), with a range of 5mm to 36mm (described range is between 15 and 25mm). Of the 215 inflorescences that had flowers available for color scoring in April 2022, 96 (45%) had pale (yellow to peach) flowers, 107 (50%) had orange to red flowers, and 10 (5%) had bright red flowers. Last year's flower color range leaned more heavily to the pale (80% pale, 14% medium, 6% bright). Previous visual assessment confirms that variation in flower color is common in this CAAFNE population.

Phenology

Year	Peak Flowering Date	Fruit Maturity Date
2019	May 9	June 12
2020	May 5	July 6
2021	April 19	July 15
2022	April 13	July 6

Table 3. Key phenological dates for Paintbrush Hill Tiburon paintbrush population over time.

	All Years			2019			2020			2021			2022		
In enclosure?	All *	In	Out	All*	In	Out	All*	In	Out	All*	In	Out	All*	In	Out
Average height per plant (cm)	18.6	23.7	17.5	22.1	30.7	19.6	20.0	17.9	20.4	16.8	12.2	17.6	15.0	21.0	13.9
Average # of stems per plant	6.6	6.2	6.6	5.5	5.9	5.6	7.8	8.9	7.6	7.0	6.2	7.2	6.0	6.2	6.0
Average # of inflorescences per plant	2.2	3.0	2.1	2.7	4.4	2.1	2.3	0.8	2.5	1.8	1.4	1.8	2.0	2.3	1.9
Average # of infructescences per plant	1.3	2.8	0.8	1.9	3.7	0.6	0.6	1.5	0.5	0.7	3.3	3.7	1.4	2.2	1.2
Average # of capsules per infructescence	3.9	4.8	2.3	5.3	6.0	4.1	2.9	3.2	2.1	3.2	3.8	2.4	2.6	2.6	2.7

Table 2: Population summary statistics of CAAFNE at Paintbrush Hill in 2019-2022

* Reproductive plants only; seedlings not included

Thatch

In order to attempt to quantify potentially different growing conditions inside and outside of the protective cages installed around some of the CAAFNE patches, which were intended to be temporary but have now been in place for ten years, in 2021 we undertook a pilot thatch assessment project and in 2022 followed it up with a larger effort. The following tables (Tables 4 and 5) summarize the 2022 data.

In addition to addressing questions related to enclosures (see below), these data allow us to begin to quantify some of the habitat variables that characterize Tiburon paintbrush's preferred growing conditions, and compare areas that support paintbrush to neighboring areas that do not. See Table 4 below for a summary of the 2022 data.

Tiburon paintbrush present?	Average % Grass	Average % Forb *	Average % Rock (>6cm) *	Average % Thatch/litter *	Average % Bare ground *
No	15.1	15.4	21.7	19.0	29.0
Yes	14.8	23.4	33.4	13.2	15.0

Table 4. Average percent cover by cover type on Paintbrush Hill. Asterisks denote variables with significant differences ($p < 0.05$) between values where Tiburon paintbrush plants were or were not present in the quadrat.

On average, cover of non-grass plants (forbs) and rocks larger than 6cm were higher where paintbrush plants were present. Grass cover, thatch cover, and bare ground were higher where paintbrush plants were not present. The differences were significant for all variables except grass ($p < 0.05$, two-sample t-test assuming unequal variance). These data are consistent with those reported in 2021, except that in 2021 grass had a statistically significant effect difference but bare ground did not.

With regard to enclosures, results of the 2022 assessment indicate that thatch/litter and bare ground cover were greater inside them, although the effect is significant only for bare ground ($p < 0.05$, two-sample t-tests assuming unequal variance), and cover of grass, forbs, and rock were greater outside, difference significant only for grass cover (Table 5). These effects are not consistent with the results from the 2021 pilot study, which found the opposite effects with the exception of increased thatch inside enclosures which is consistent between the two years. The 2021 pilot assessed only one of five enclosures, which has heavy grass cover (see photo, Appendix D).

In enclosure?	Average % Grass *	Average % Forb	Average % Rock (>6cm)	Average % Thatch/litter	Average % Bare ground *
Yes	11.7	14.7	20.6	19.7	33.3
No	17.0	17.1	24.2	17.7	24.2

Table 5. Average percent cover by cover type within and outside of protective enclosures on Paintbrush Hill. Asterisks denote variables with significant differences ($p < 0.05$) between values inside and outside enclosures.

Number of plants inside enclosures

The number of plants observed inside cages declined over the first three years of our observations, but ticked up this year (Figure 3). The proportion of total population within enclosures is consistent with the number of plants, declining from 19% in 2019 to 14% in 2021 and ticking up to 17% in 2022.

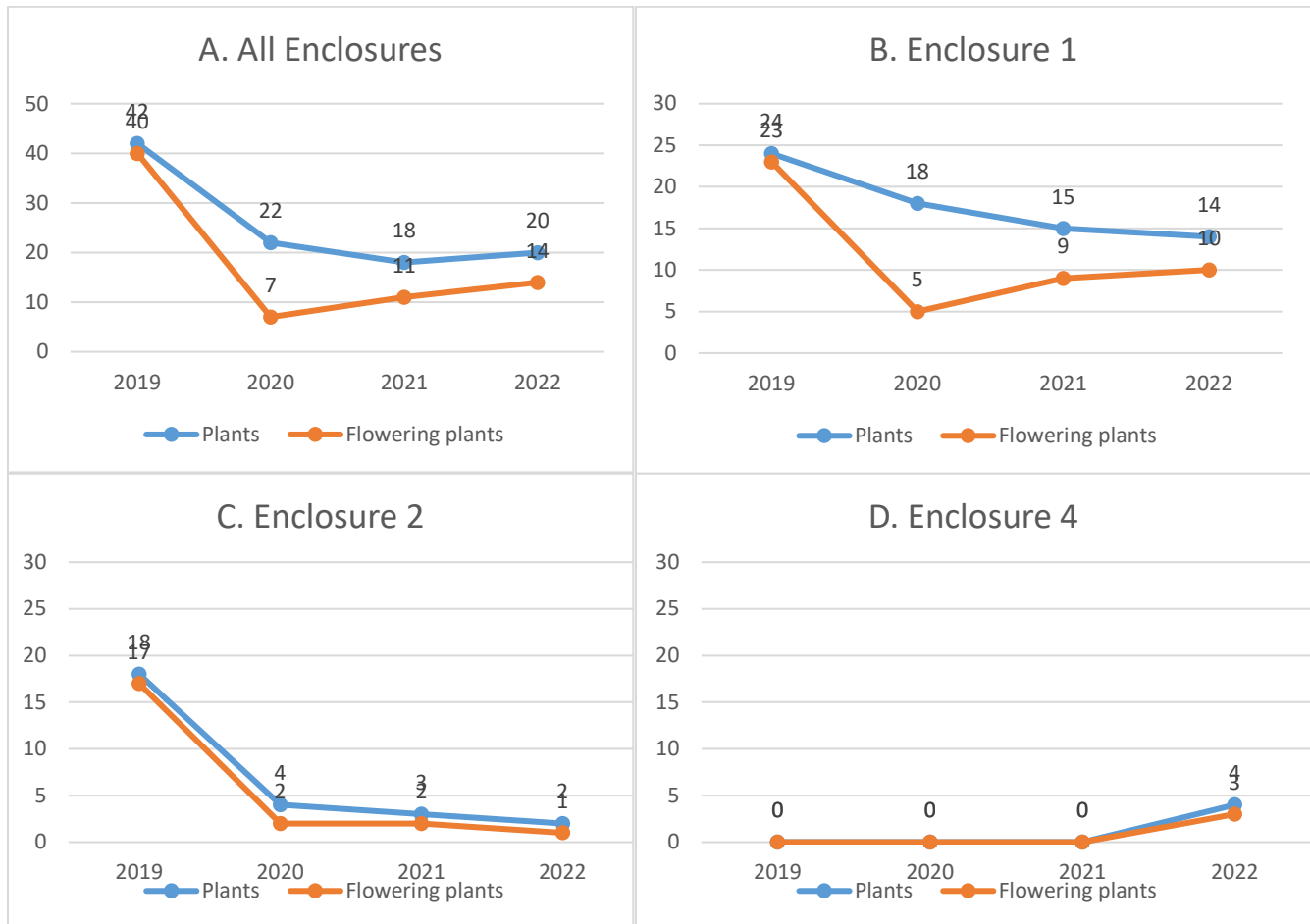


Figure 3. Number of Tiburon paintbrush plants observed inside protective cages from 2019-2022.

Of the five existing enclosures, only two have consistently supported Tiburon paintbrush plants during the period of Valley Water's population monitoring effort. Two have been empty since the start of the monitoring effort. A third has been empty until this year, when four plants reappeared. The size, extent of branching and inflorescences present on these plants suggest that these plants have been dormant during previous monitoring years rather than being new plants this year, which is an important life history consideration for the population. These enclosures were assessed carefully each year, so although it's possible some non-flowering above-ground plant material was missed, it is more likely there was underground root mass but no visible shoots in the intervening years, which is possible due to the plants' hemiparasitic life form. The plants growing within one foot of these individuals that this study noted as potential host plants were perennials golden yarrow (*Eriophyllum confertiflorum*), purple needlegrass (*Stipa pulchra*), Italian rye grass (*Festuca perennis*), blue-eyed grass (*Sisyrinchium bellum*) and bindweed sp. (*Calystegia* sp.), and annuals yarrow (*Achillea millefolium*), Q-tips (*Micropus californicus*), soft chess (*Bromus hordeaceus*) and foxtail chess (*Bromus madritensis*). The short-lived annuals (all annuals except yarrow) are less likely to be host plants, due to their small body size and short life history. These hypotheses are based on observations and life history traits and would need to be confirmed with experimental work on CAAFNE host preference in order to be definitive.

Seed collection and banking

Seed from two plants (5% of plants that set fruit in 2022) was collected on 7/6/22 and sent to CBG. The 2022 collection was so small that germination tests will not be done on that lot (C. Birker, 2022c). Germination was 80% for seeds tested from the lot collected from Paintbrush Hill in 2019, with no pre-treatment or stratification (C. Birker, 2020). Germination for the 2020 lot was 50% (C. Birker, 2022a), and 70% for the 2021 lot (C. Birker, 2022b).

Possible Host Plants

Over the four monitoring years to date, fifty-six potential host plant species have been recorded within one foot of CAAFNE individuals. Of these, six species were recorded one hundred times or more over all years and are reported here (all data available on request). By far the most common co-occurring species was soap plant, recorded 382 times, followed by lace parsnip (*Lomatium dasycarpum*), recorded 203 times. In 2022 soft chess, recorded 168 times, overtook yarrow, recorded 164 times, as the third most commonly recorded species. Other frequently co-occurring species were Italian rye grass, recorded 163 times, and melic grass (*Melica* sp.), recorded 155 times. As mentioned above, although soft chess is increasing in cover at the site, it does not seem likely that it is a prime host plant for CAAFNE due to its short life history and small stature.

Creekside Science has suggested that likely host plants for CAAFNE include yarrow, golden yarrow, and “possibly *Corethrogyne filaginifolia* and other perennial forbs and grasses” (Creekside Science 2018c). They have used three species in their host plant experiments at the Paintbrush Canyon occurrence: yarrow, purple needlegrass, and tomcat clover (*Trifolium willdenovii*), which is an early senescing annual (Creekside Science 2017), but state that one of the factors associated with successful seeding efforts was high cover of golden yarrow (Creekside Science 2018b). Golden yarrow is not widespread at the Paintbrush Hill occurrence, though it is present in small numbers: it was recorded five times within one foot of a CAAFNE individual during 2019 monitoring, seven times in 2020, and six times each in 2021 and 2022.

Threat Assessment

Herbivory and Trampling

Please see earlier reports for additional background information and data. To summarize, CAAFNE inflorescences appear to be very brittle. They are easily broken and can incur damage in the period between flowering and fruiting (approximately mid-April to mid-July) that prevents plants from completing their reproductive output for the year. Over time, because the plants are perennial, this means this population may persist but not increase, or may decline if reproductive success is not sufficient for replacement. In all four years of our study most plants, particularly those outside cage enclosures, experienced some level of damage over the course of the growing season, apparently from a combination of herbivory and animal trampling. Broken or missing inflorescence stalks are common (see reduction from an average of 2.1 inflorescences to only 0.8 infructescences per plant outside cages, with smaller reduction inside cages, Table 2). In order to minimize any human component to this phenomenon, sampling techniques should be implemented very carefully to avoid damaging plants, and group field visits during flowering periods should be limited. And in order to protect the plants, some measures to protect from large mammal impacts is likely to be needed.

In 2019, 41 of the total 222 plants (18%) were noted as having obvious signs of herbivory at the time of flowering (flowering stalks predated). Five more were marked as having most stems with multiple branches shorter than 5 cm, which may be a sign of herbivory followed by later season regrowth, and 2 were marked as trampled (22% affected in total). In 2020, 30 of 139 plants (22%) were noted as having obvious signs of herbivory at the time of flowering (flowering stalks predated), and twenty-six more were marked as having most stems with multiple branches shorter than 5 cm (40% affected in total). In 2021, after having observed the style of herbivory by cattle and rabbits, we began marking what animal was the likeliest culprit. Of the 43 plants (33%) where obvious signs of herbivory were noted on phenology data sheets in 2021, 19 were denoted as likely cattle (ragged/near complete herbivory), 24 likely rabbit (neat, clean nibbled stalks), 15 beetle (beetles present, only 1 with obvious damage to fruits), and 2 no vector imputed. Three were marked completely trampled by cattle and an additional 28 were marked as having most stems with multiple branches shorter than 5 cm, for a total of 74 of 130 plants affected this year (57%). In 2022, the area around the Tiburon paintbrush had bulls only, no cow/calf pairs, which significantly reduced the trampling and grazing pressure from cattle. As such, only one instance of cattle-specific damage was noted. Of the 32 plants noted to have some signs of herbivory in 2022 (28%), 11 were marked as likely rabbit, 1 likely cattle, 12 insect, and 9 unspecified. Multiple possible vectors were allowed to be noted per plant. Please note that these data are incidental observations and should not be considered an exhaustive survey of herbivory and trampling effects on the population.

Fencing

A site visit with representatives from the Habitat Agency to explore fencing alternatives took place in April 2022. Some of the logistical difficulties with the patch location were discussed, including the proximity to chaparral, the existence of a game trail alongside the plant population that would be diverted along a fence with likely detrimental effect to the habitat in the new location of the game trail, and the steepness of the site, especially in lower portion of the population. Two alternatives were discussed: fencing the population plus the adjacent chaparral patch, which would require more fence miles but would be easier to install due to easier equipment access and would avoid impacts to sensitive chaparral habitat. Valley Water is to follow up with assessing conditions around the chaparral patch for feasibility. A second alternative would be using temporary electric fencing in strategic locations. This might be limited logistically to the flatter top portion of the site where enclosures 1 and 2 currently stand, but since this is where the greatest cattle pressure has been noted, this could be a good compromise.

Invasive Plant Species

Yellow star thistle (*Centaurea solstitialis*) and tocalote (*Centaurea melitensis*) are present at the northern polygon, including directly adjacent to CAAFNE plants, and also occur scattered in moderate numbers in the grassland between the main central polygon and the road. Scattered black mustard (*Brassica nigra*) plants occur throughout both areas. A purple star thistle patch on the road approaching the northern polygon population was discovered and removed in 2021 and resprouts removed in 2022. Soft chess and red brome increased in frequency in proximity to paintbrush plants in 2021 and 2022. A limited number of yellow star thistle, tocalote and black mustard plants in the immediate vicinity of the CAAFNE patches have been removed during population monitoring activities as time allowed, but a concerted effort over time is recommended in order to prevent the spread and increase in density of these plants.

ATV Use

No evidence of ATV travel (active ATV use or tire tracks) has been observed during monitoring in 2019-2022.

Conclusions and Next Steps

Fluctuations are normal for plant populations, but with that said 2020 - 2022 have been unencouraging years for the Paintbrush Hill population. The region has been in a severe drought, and pressure on the population from trampling and herbivory appear significant. 2022, with decreased trampling and grazing pressure from cattle (bulls only on site), but continued drought conditions, appears to have provided a small respite. Spring 2023, after the significant rain events we've received, will be an excellent opportunity to see whether this environmental variable allows for a population rebound. The nearest precipitation gage (UTC) reports that the precipitation for the water year to date (17.8 inches since October 1 2022) already exceeds the total for water years 2020 and 2021.

We propose the following next steps for the Paintbrush Hill CAAFNE population:

1. Continue to discuss and pursue possibilities for fencing this population. Temporary electric fencing for the top portion of the population may be the best short to medium term solution.
2. Continue annual population monitoring in order to establish population trend. Population monitoring will occur in spring and summer 2023.
3. Continue limited annual seed collection and banking at an accredited institution. Collecting small amounts of seed annually will help build a reserve of seed which can be banked and used in the future for possible population augmentation as more is learned about the demographics of this taxon.
4. Develop a vegetation management plan for the area that takes into account encroaching invasive species such as yellow star thistle as well as invasive annual grasses such as soft chess whose cover is increasing at the site and may degrade site quality over time.

Time permitting, additional future research objectives for Valley Water include a literature review of effects of host plants on conservation of hemiparasitic plants, best practices for reintroduction of hemiparasitic plants, and further information relevant to the non-destructive identification of single individuals in challenging field settings.

Recommendations for further study (not within Valley Water's current scope of work):

1. Identify likely CAAFNE host plants. Host selection on a plant that is tasty or nutritious to burrowing mammals (such as soap plant) could be detrimental to CAAFNE persistence. Amplifying the presence of potential hosts without belowground storage organs (like golden yarrow for example) might improve outlook. Further research on host preference in this taxon is needed.
2. Identify factors that might influence suitability for CAAFNE of the habitat in the northern and southern polygons (and could explain absence from these areas). This could include an analysis of percent vegetation cover and/or community species composition.
3. Collaborate with Santa Clara University or other interested entity to elucidate flower color and associated genetic variation in this population.

Jenny Hazlehurst, CSU East Bay, is continuing her pollination studies on three endangered plant species on the ridge, including the Paintbrush Hill Tiburon paintbrush population. She will be collecting specimens for identification this year, which will provide more data than 2021's catch and release study, on possible pollinators of CAAFNE and a pollination network.

As the monitoring effort continues over the next year, we expect to be able to provide additional recommendations for critical research to be conducted in order to proactively manage this occurrence and the taxon as a whole.

Literature Cited

Birker, Cheryl (December 2, 2019). Email to Laura Garrison.

Birker, Cheryl (February 6, 2020). Processing, Storage, Accession, and Germination Test Summary.

Birker, Cheryl (January 12, 2022). Email to Laura Garrison.

Birker, Cheryl (February 2, 2022). Email to Laura Garrison.

Birker, Cheryl (December 20, 2022). Email to Laura Garrison.

California Native Plant Society, Field Protocols and Guidelines, "Percent Cover Diagrams from two sources", <https://www.cnps.org/plant-science/field-protocols-guidelines>, most recently accessed 1/18/2022. Source cited on document: CalPhase 3 Field Guide - Vegetation Diversity and Structure, April 10, 2001

Creekside Science 2017. Research facilitating recovery of the endangered serpentine endemic Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*) at Coyote Ridge in southern Santa Clara County. Final Report and Adaptive Management Plan, September 6, 2012 to December 31, 2017, written for the Central Valley Project Conservation Program and Central Valley Project Improvement Act Habitat Restoration Program (R12AP20023)

Creekside Science (2018a). Creekside Science Plant Survey Report, Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*)

Creekside Science (2018b). Castilleja Exclosures Summary: *Castilleja affinis* ssp. *neglecta* on Paintbrush Hill 2018-2019

Creekside Science (2018c). Tiburon Paintbrush Projects

Jameson, E.W., Jr. and H. J. Peeters (2004). Mammals of California. Berkeley, University of California Press, 2004.

Kaye, Tom (May 2, 2019). Email to Janell Hillman and Laura Garrison.

Santa Clara Valley Water District (2019). Proposal for population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush.

U. S. Fish and Wildlife Service (1995). Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for Ten Plants and Threatened Status for Two Plants from Serpentine Habitats in the San Francisco Bay Region of California. Federal Register vol. 60, no. 23: 6671-6685

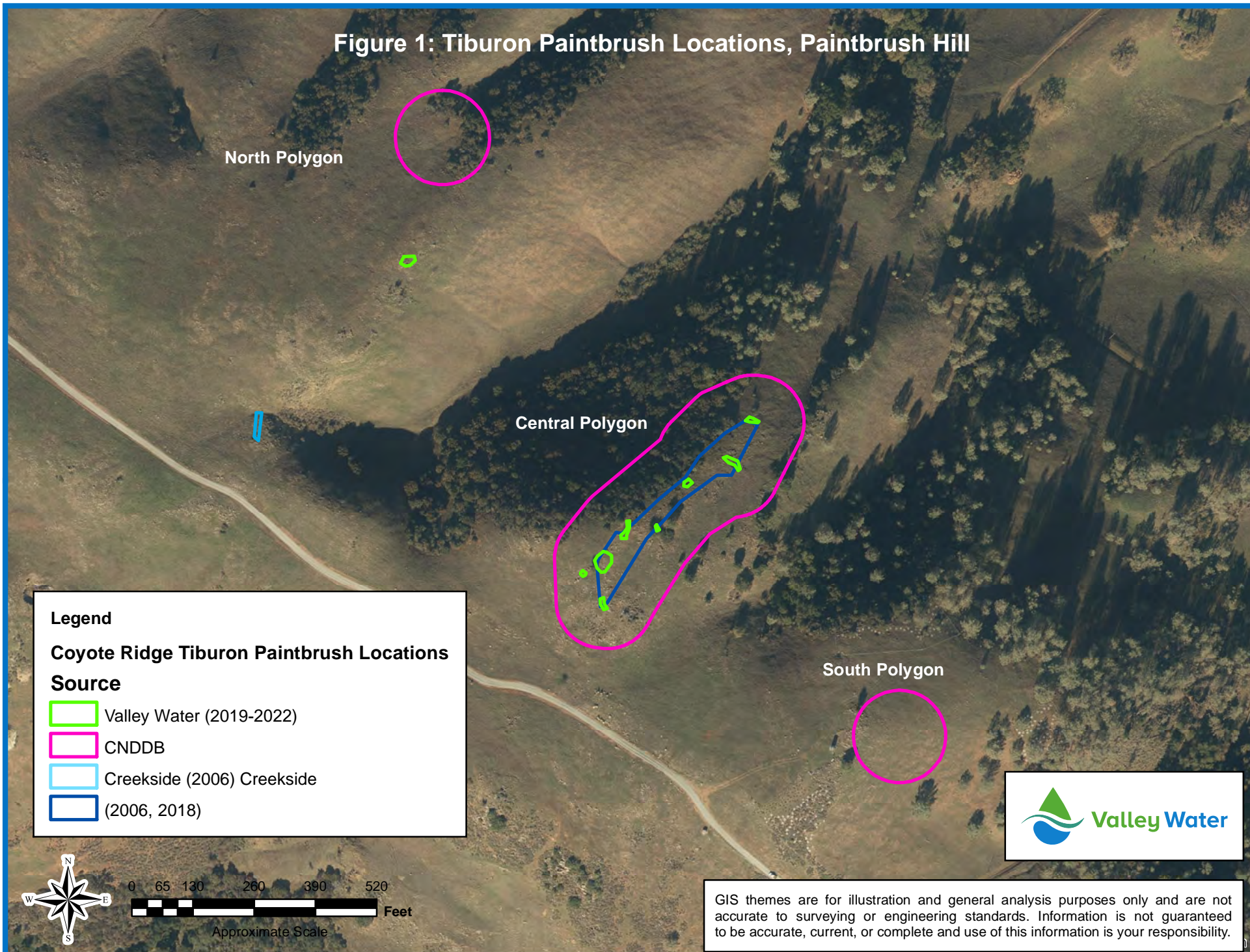
Valley Water (2019). Annual Report: Population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*). January 2020

Valley Water (2020). Annual Report: Population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*). January 2021

Wall, M. (2009). Seed collection guidelines for California native plant species. Seed Conservation Program Rancho Santa Ana Botanic Garden

Widener, L. & J.B. Fant (2017). Genetic differentiation and diversity of two sympatric subspecies of *Castilleja affinis*; a comparison between the endangered serpentine endemic (ssp. *neglecta*) and its widespread congener (ssp. *affinis*) Conserv. Genet. 19 (2): 365-381





Figure 1: Tiburon Paintbrush Locations, Paintbrush Hill

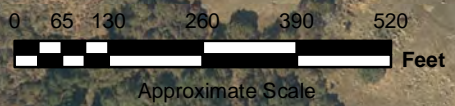


Legend

Coyote Ridge Tiburon Paintbrush Locations

Source

-  Valley Water (2019-2022)
-  CNDDDB
-  Creekside (2006) Creekside
-  (2006, 2018)



GIS themes are for illustration and general analysis purposes only and are not accurate to surveying or engineering standards. Information is not guaranteed to be accurate, current, or complete and use of this information is your responsibility.

Appendix A Foraging Event Photos

Event 1: Before



Event 1: After



Event 2: Before



Event 2: During (note cow tongue curled around CAAFNE inflorescence)



Event 2: After



Event 3: January 2020

Photo 1: CAAFNE metal markers with soap root bulb husk, disturbed earth. Located outside enclosure.



Photo 2: Disturbed earth inside enclosure



Photo 3: Partially uncovered soap plant with bulb husk visible. Located outside enclosure.



Event 4: June 2019

Event 5: May 2020

Soap plant husk and Tiburon paintbrush plants visible next to disturbed earth from rooting/burrowing event



Event 6: May 2020

Brush rabbit, nibbled flower stalks, scat



05-23-2020 06:41:17



Appendix B
Camera Trap Photos



Bushnell MP2CO

84 F 28 °C

05-08-2020 08:05:15

Cow grazing



Bushnell MP2

50 F 10 °C

05-29-2020 06:02:19

Pigs grazing



Bushnell MP1

55°F 12°C

06-02-2020 03:52:02

Deer



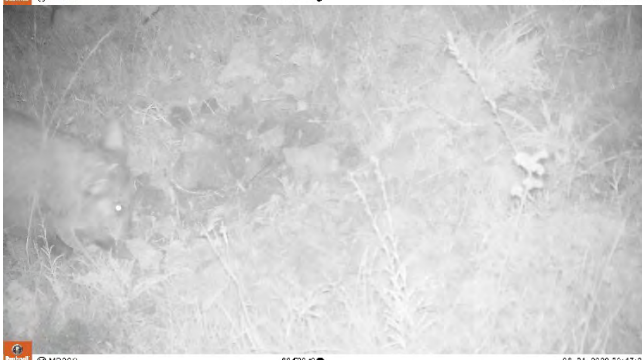
Bushnell MP1CI

46°F 7°C

05-01-2020 04:24:26

Western harvest mouse, inside cage enclosure

Appendix C
Rooting Event Photos
Near Enclosure 2, June 21, 2020



Appendix D
Enclosure Photos



Enclosure 2, showing grass and thatch buildup inside (3/8/2021)



Enclosure 1, showing one of several plants growing intertwined with fencing (3/30/2021)