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

Background and Introduction

Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*; CAAFNE; State Threatened, Federally Endangered, CNPS RPR 1B.2) is a 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, CNDDB occurrence 7). Another nearby population, called Paintbrush Canyon, was subsequently discovered (2002, CNDDB occurrence 9). Population sizes for this taxon range from 20 to 1000 plants depending on the occurrence (CNDDB, 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 have been observed in the two smaller polygons (North and South) since 1997 and 1994, respectively (see Table 1). 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*
North	NA	4	27	NA	NA	NA	NA	NA
Central	17	NA	0	80	>140	103	~100	208/224
South	NA	5	0	0	NA	NA	NA	NA

Table 1. Historical reports of number of plants at Paintbrush Hill, 1993-2018. Data sources: CNDDB (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 second annual report complies with the agreement made in April 2019 (Valley Water 2019). It describes field activities that took place in 2020, assessments of potential threats to the CAAFNE population at Paintbrush Hill, planned future activities, and initial management recommendations for the

population. (A comprehensive list of recommended management activities will be provided after 3-5 years of population monitoring, but likely actions will be discussed as they come up.)

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 to belong 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 (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 (not labeled with metal markers, 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))

This information was collected during peak flowering season in 2020, on 5/5/20 and 5/6/20. After peak flower, monitoring visits occurred every 2-3 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 7/6/20. This information included:

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

Seed Collection and Banking

Rancho Santa Ana Botanical Garden (RSABG)'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 2020 population count of 134 (seedlings excluded) would be six plants. However, as of July 6, only 38 plants had developing infructescences, so we reduced the number of plants for seed collection to two.

Fruit phenology was monitored every 2-3 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 RSABG (Wall 2009). Fruits were allowed to air-dry in paper bags, after which seed was cleaned of vegetative material and deposited with the RSABG conservation collection.

Seeds collected by Valley Water over the next several years as part of this population monitoring effort will stored permanently at RSABG, 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.

Threat Assessment

Threat assessment for the Paintbrush Hill population took two forms during the 2020 field season: camera traps for observations of herbivorous mammals, and ad hoc observations during monitoring visits. Four automated passive infra-red motion sensor-activated wildlife cameras (Bushnell Trophy Cam HD Aggressor No-Glow) were installed on metal T-stakes about 3 feet off ground level. An additional five cameras were installed at about 1.5 feet off ground level attached to T-stakes or cage enclosures around CAAFNE plants, oriented both within and outside cages, intended to capture burrowing animal activity inside cages and small mammal herbivory outside cages. The cameras were set up in still photo mode to take a single image each time the motion sensor activated, with an interval of at least one second between images. To decrease the likelihood of the cameras' motion sensors being repeatedly triggered by vegetation swaying in the wind, the cameras' intensity setting was set to low. Cameras were active 24 hours per day and remained in place until the end of the growing season. (Most cameras were deployed April-June; two were deployed starting in February, one remained through July and one remained until September. The four cameras at 3-foot height were redeployed in November 2020 and will remain through approximately March 2021 to capture the wet season.) All images were reviewed for the presence and identity of potential herbivores by a qualified wildlife biologist.

Observations during monitoring visits included additional herbivory observations (digging animals, insects), observations of ground disturbance by cattle and pigs, and observations of presence and abundance of invasive plant species near CAAFNE.

Results and Discussion

Population Monitoring

We counted 134 reproductively mature plants and 5 seedlings in the 2020 monitoring season, for a total population size of 139 individuals. This included 117 plants and 5 seedlings in the main (central) polygon, and 17 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, this year's low count highlights that this small population may need additional protection if it is to persist or increase in size (Figure 2).

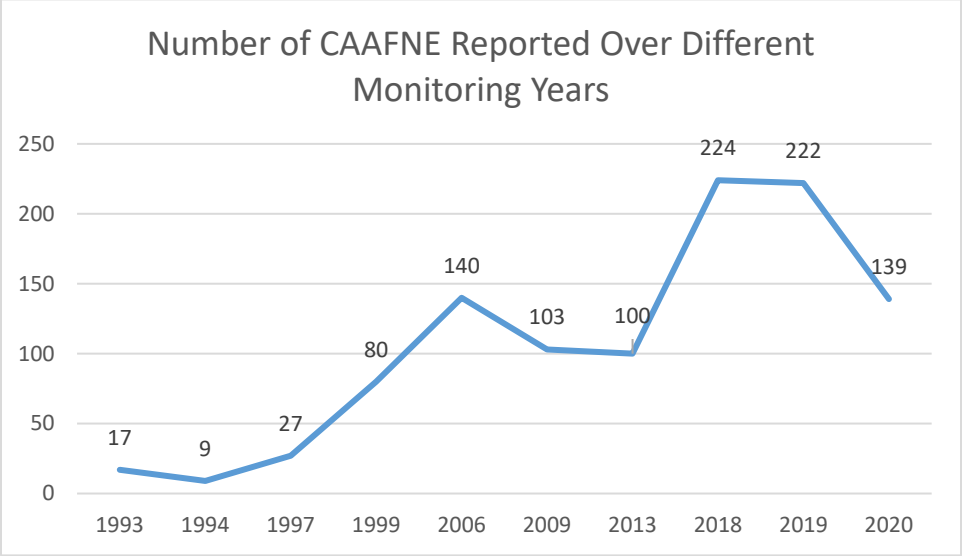

Figure 2. Number of CAAFNE plants at Paintbrush Hill, 1993-2020. Data sources: CNDDB (1993-2013), Creekside Science (2018), Valley Water (2019-2020)

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 2020 was 20cm (n=134), with an average number of stems per plant of 7.8 and average number of inflorescences per plant of 2.3.

2020 plants were shorter and more branched than 2019 plants, and had fewer inflorescences, which could be seen as consistent with a drier year where plant focus is on collecting nutrients rather than expending energy on reproduction.

Average width of inflorescences was 21.4mm (n=275), with a range of 5mm to 45mm (described range is between 15 and 25mm). Of the 275 inflorescences that had flowers available for color scoring on 5/6/20, 121 (44%) had pale (yellow to peach) flowers, 144 (52%) had orange to red flowers, and 17 (6%) had bright red flowers. Previous visual assessment confirms that variation in flower color is common in this CAAFNE population.

These traits indicate more morphological overlap with CAAFAF than expected. As mentioned in last year's annual report, genetic analysis performed in 2017 (Widener and Fant 2017) noted that two of the sampled populations of CAAFNE showed lower genetic differentiation from the CAAFAF populations than all others; Paintbrush Hill was one of those two populations. The authors could not rule out a potential hybrid element at these locations. More genetic work, sampling from the full range of flower color, might be prudent to evaluate this occurrence's genetic difference from or similarity to CAAFAF. Dr. Justen Whitall's lab at Santa Clara University has expressed interest in pursuing this line of research.

One of the outstanding management questions for this population is whether the cage enclosures (five approximately six- by eight-foot structures installed by Creekside Science in 2012) help or hinder the CAAFNE plants (Valley Water 2019, Creekside Science 2018b). Results from this field season continue to support the idea that the positive effect of protection from cattle herbivory and trampling outweigh any suppressive effect of thatch/increased cover of non-paintbrush vegetation within the cages (Table 2). Plants inside cages again had more stems, more inflorescences, more infructescences per plant, and more capsules per infructescence than plants outside enclosures. They were however not taller

this year. Also in contrast to last year, the only seedlings observed this year were found outside of an enclosure. The data summarized in Table 2 will be combined with data from forthcoming monitoring seasons to provide a statistically meaningful analysis of these metrics. This will inform future management recommendations regarding the use of enclosures at this occurrence.

	Both Years			2019			2020		
	All plants*	Inside cages	Outside cages	All plants*	Inside cages	Outside cages	All plants*	Inside cages	Outside cages
Average height per plant (cm)	21.3	26.7	19.9	22.1	30.7	19.6	20.0	17.9	20.4
Average # of stems per plant	6.6	6.8	6.5	5.5	5.9	5.6	7.8	8.9	7.6
Average # of inflorescences per plant (2019 first flush only)	2.5	3.2	2.3	2.7	4.4	2.1	2.3	0.8	2.5
Average # of infructescences per plant	1.0	3.0	0.5	1.9	3.7	0.6	0.6	1.5	0.5
Average # of capsules per infructescence	5.1	16.2	2.4	5.3	6.0	4.1	2.9	3.2	2.1

Table 2: Population summary statistics of CAAFNE at Paintbrush Hill in 2019-2020
* Reproductive plants only; seedlings not included

Seed collection and banking

Seed from two plants (5% of plants that set fruit in 2020) was collected on 7/8/20 and sent to RSABG. Viability information for these seeds should be available by the end of February 2021. Viability was 80% for seeds tested from the lot collected from Paintbrush Hill in 2019, with no pre-treatment or stratification (C. Birker, 2020).

Threat and Habitat Assessment

Herbivory and Trampling

CAAFNE inflorescences appear to be very brittle. They are easily broken and can incur damage in the the period between flowering and fruiting (approximately mid-May 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 over time if reproductive success is not sufficient for replacement. In both 2019 and 2020 most plants 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 were common (see reduction from an average of 2.1-2.5 inflorescences to only 0.5-0.6 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.

Large Mammals

Camera trap data recorded the presence of cattle, deer, and pigs in 2019 and 2020 (Table 3). Cattle were the most commonly observed large herbivore (1.3 individuals per camera day (24 hr period), as compared with 0.2 for pigs and 0.02 for deer), and the cameras captured two clear instances of cattle eating CAAFNE (Appendix A). In one event one inflorescence stalk was visibly consumed, in the other 9 flowering stalks were visibly consumed. Observations of large herbivores were much (20-60x) more common at the flattest camera site than the steepest. Herbivory does not appear to be the only vector of damage to inflorescences, as broken-off stalks were often observed hanging from the plants—trampling seems to be a significant vector as well and may contribute to a reduction in seed set and therefore potentially population viability. However, damage from cattle was not observed to kill plants. Pig rooting has been reported to kill plants and so rooting events may have a greater negative effect even though they are less frequent. In 2019, cattle were removed from the site on June 5. In 2020, cow/calf pairs were removed from the site May 15, but dry cows were put back on starting June 10 and remained until November.

A large pig rooting event occurred in mid-February 2020 very near the CAAFNE occurrence and overlapping it in one location at the bottom of the hill (Figure 3). Two plants that were in that area in 2019 could not be found in spring 2020, though it is not certain that they were destroyed by the rooting event. It seems luck prevented greater destruction, since the rooting was directly adjacent to the population.

In 2020 there were fewer cattle sightings than in 2019, likely because it was a drier year, so vegetation cover overall was lower in the serpentine grassland, and the cattle spent more time elsewhere on the preserve. However, combined herbivory and trampling pressure on CAAFNE was similar in the two years, as measured in the reduction in number of inflorescences to number of infructescences outside cages (Table 2). This may indicate that plants are more brittle or vulnerable to trampling in a dry year.

	Cattle	Pig	Deer
Events (N)	54.00	24.00	12.00
Event Mean Duration (seconds)	129.85	88.83	49.17
Trap Days	154.91	405.4	405.4
Events Per Trap Day	0.35	0.06	0.03
Individuals Per Trap Day	0.02	0.001	0.003

Table 3: Camera trap data summary over two years. Note that event is defined as a set of images with one or more animals present; a single event may include multiple individual animals.

Small Mammals

In addition to the stationary camera traps, observations of potential threats were collected during the monitoring period, including additional observations of animal presence or damage.

In 2019, damage to three plants in the central polygon from a digging animal occurred between May 8 and May 14. Two plants were apparently completely removed by this activity (no plant material observed May 14) and one sustained apparent damage from deposited soil but had one flowering stem that remained undamaged. This activity wasn't captured by the camera traps, so the identity of the animal was speculative, but the shape of the spoils pile was consistent with pocket gopher activity. The plants that were here were growing near soaproot (*Chlorogalum pomeridianum*), which is common at this site; one possibility is that gophers were foraging for soaproot bulbs and the damage to CAAFNE was collateral. (Gophers have been reported to forage on lily corms (*Brodiaea* spp. in that case) in serpentine areas with low plant cover, Jameson and Peeters 2004.) Because of this observation and

observations of “nibble” style flower stalk herbivory inconsistent with cattle, in 2020 we added four cameras positioned lower and pointed towards the ground to capture small mammals.

Presence of Botta’s pocket gopher was confirmed by camera trap data in 2020, though burrowing activity was not captured on camera. Other small mammals captured by the cameras included *Peromyscis* sp., Western harvest mouse/house mouse (confirmed inside enclosures), unidentified rat, brush rabbit, and black-tailed jackrabbit (Appendix B). With camera trap data, evidence of herbivory (clean nibbled stalks), and scat, we can confirm that brush rabbits eat Tiburon paintbrush (Appendix A, Event 6).

Insects

Insect herbivory was also observed during population monitoring: small beetles or weevils were frequently observed feeding on flower and fruit tissues of CAAFNE, as well as the occasional caterpillar (Appendix A, Event 5). It is not clear whether this had a significant effect on flower and fruit production (e.g., via reduced seed set or viable seed production), though it is possible that some information on this could come from the germination trials to be done by RSABG.

Invasive Plant Species

Yellow star thistle (*Centaurea solsitialis*) 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 limited number of yellow star thistle, tocalote and black mustard plants in the immediate vicinity of the CAAFNE patches were removed in 2019 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) was observed during monitoring in 2019 or 2020.

Host Plant Analysis

Over the two monitoring years to date, forty potential host plants were recorded within one foot of CAAFNE individuals. Of these, seven species were recorded thirty times or more over both years and are reported here (all data available on request). By far the most common co-occurring species was soap plant, recorded 192 times, followed by lace parsnip (*Lomatium dasycarpum*), recorded 101 times, and yarrow (*Achillea millefolium*), recorded 65 times. Other frequently co-occurring species were Italian rye grass (*Festuca perennis*; recorded 56 times), purple needlegrass (*Stipa pulchra*; recorded 45 times), melic grass (*Melica* sp.; recorded 32 times), and purple clarkia (*Clarkia purpurea*; recorded 30 times).

Creekside Science has suggested that likely host plants for CAAFNE include yarrow, golden yarrow (*Eriophyllum confertiflorum*), 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 and seven times in 2020.

Conclusions and Next Steps

Fluctuations are normal for plant populations, but with that said 2020 was not an encouraging year for the Paintbrush Hill population. A concerted effort to survey for seedlings was not possible due to COVID-19 related fieldwork restrictions. The population evidently narrowly avoided potentially heavy damage from pigs (Figure 3), underscoring the threat to the population posed by trampling and herbivory, which if removed or mitigated could result in a more robust population at this location.

Although we do not anticipate making formal management recommendations until 3 to 5 years of monitoring have been completed, it does seem prudent to suggest that a large enclosure be considered for this occurrence. This would prevent further rooting damage by pigs and trampling and herbivory by cattle. Although it would not protect against herbivory by insects or smaller mammals such as brush rabbits or burrowing animals, evidence of which has also been observed at the site, based on the extent of the pig rooting damage to areas near the plants and evidence of cattle trampling and herbivory from camera traps in 2019 and 2020, the larger animals are capable of causing significant damage to this small population, and a large enclosure could prevent a significant proportion of the reproductive structure damage that the population is experiencing, allowing more seed set to occur and thereby allowing for more new individuals to recruit to the population.

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

1. Continue annual population monitoring in order to establish population trend. Population monitoring will occur in spring and summer 2021.
2. 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.
3. Continue evaluation of enclosures which exclude large herbivores, and compare with non-enclosure areas to evaluate herbivory/trampling/other damage to plants. Consider quantifying plant cover inside and outside of cages, ideally in both a relatively wet and dry year, to address the concern that enclosures may cause a buildup of thatch detrimental to CAAFNE persistence. Consider adjusting grazing lease to protect plants from cattle grazing and trampling as an alternative, and/or during planning and implementation phases for a large enclosure. Another interim measure would be to use Liquid Fence to deter herbivory on plants currently outside enclosures.
4. Assessment of seedling recruitment and survivorship. COVID-19 field restrictions prevented this from occurring in 2020. If restrictions permit, we propose to scale our monitoring effort for this metric using early spring 2021 observations of the number and spatial distribution of seedlings over time. Small quadrats may be employed rather than repeated surveys of the entire occurrence.

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.

As the monitoring effort continues over the next 1-3 years, 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.

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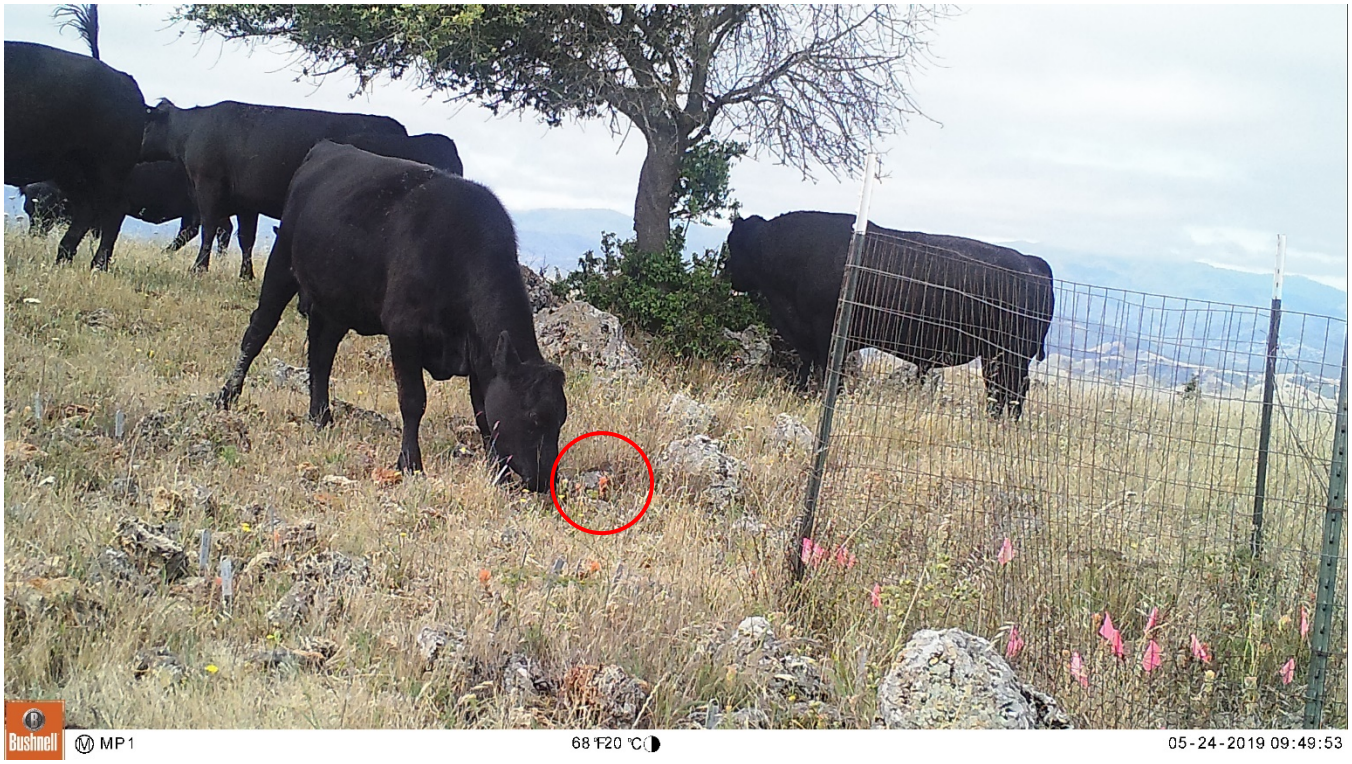
Appendix A
Foraging Event Photos

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

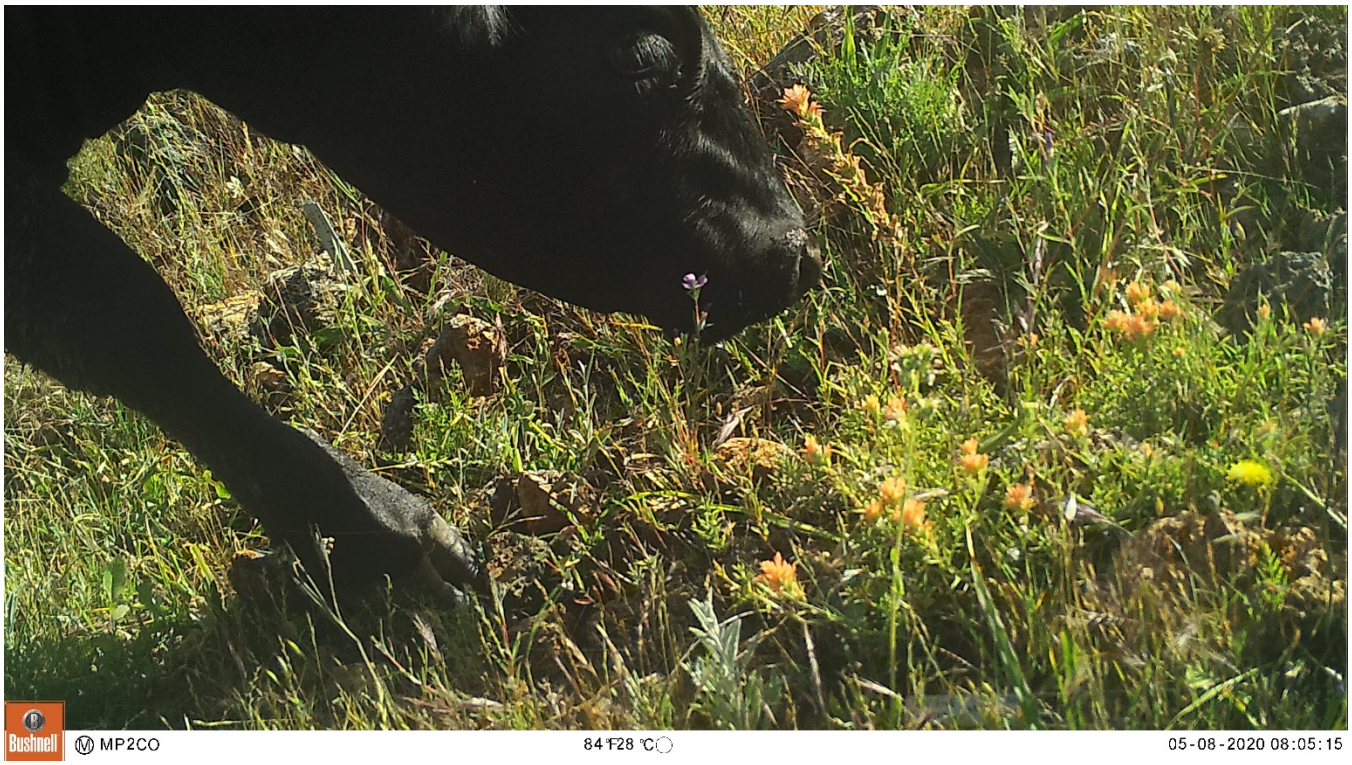
Soap plant husk and Tiburon paintbrush plants visible next to disturbed earth from rooting/burrowing event

Event 5: May 2020

Event 6: May 2020

Brush rabbit, nibbled flower stalks, scat

Appendix B
Camera Trap Photos



Cow grazing

Pigs grazing

Deer

Western harvest mouse, inside cage enclosure

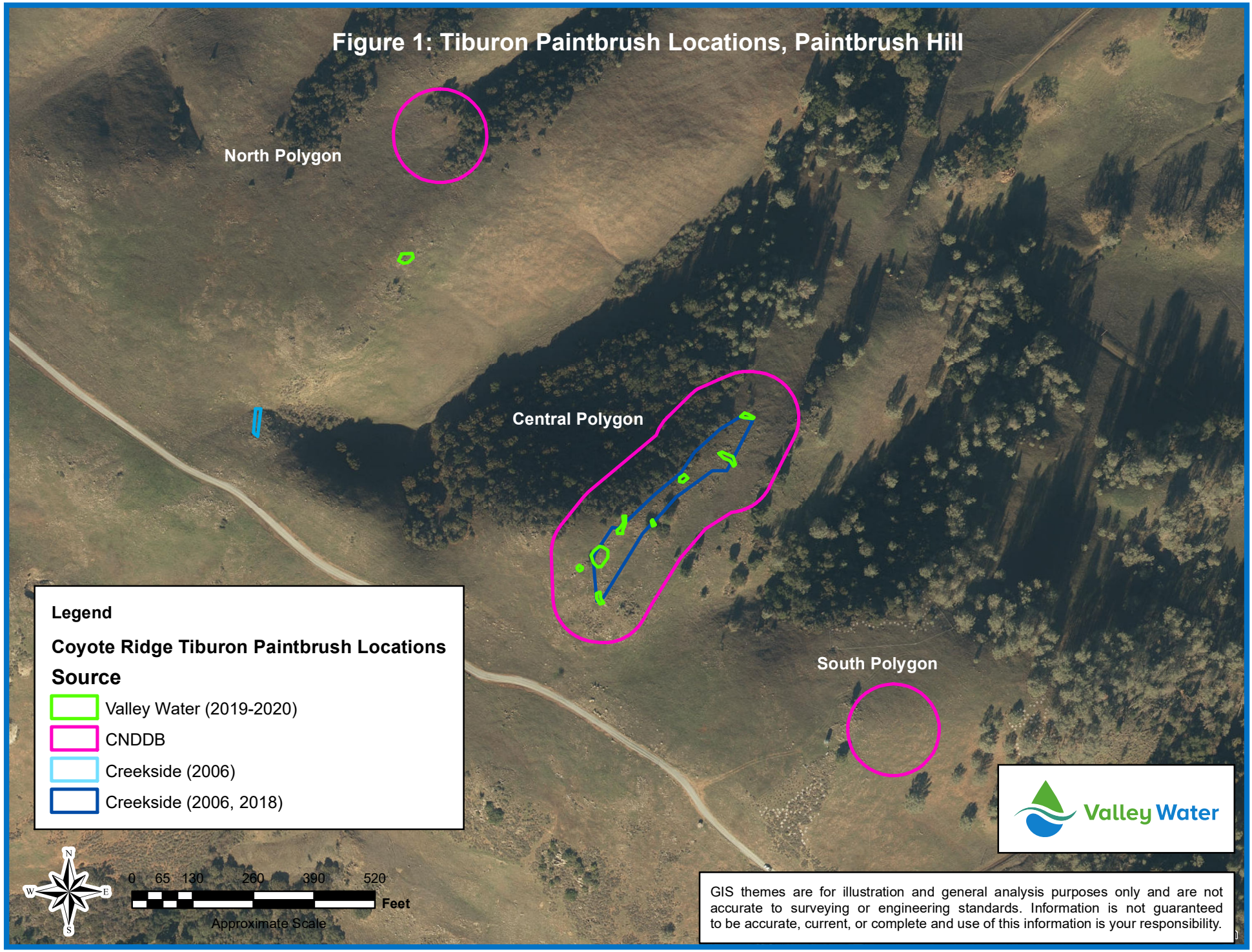


Figure 1: Tiburon Paintbrush Locations, Paintbrush Hill
North Polygon
Central Polygon
South Polygon
Legend
Coyote Ridge Tiburon Paintbrush Locations
Source
Valley Water (2019-2020)
CNDDB
Creekside (2006)
Creekside (2006, 2018)
N
W
E
S
0 65 130 260 390 520
Feet
Approximate Scale
Valley Water
GIS themes 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.

Figure 3: Ground Disturbance at Paintbrush Hill, February 2020