Annual Report
Population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*)
January 2024

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, 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.

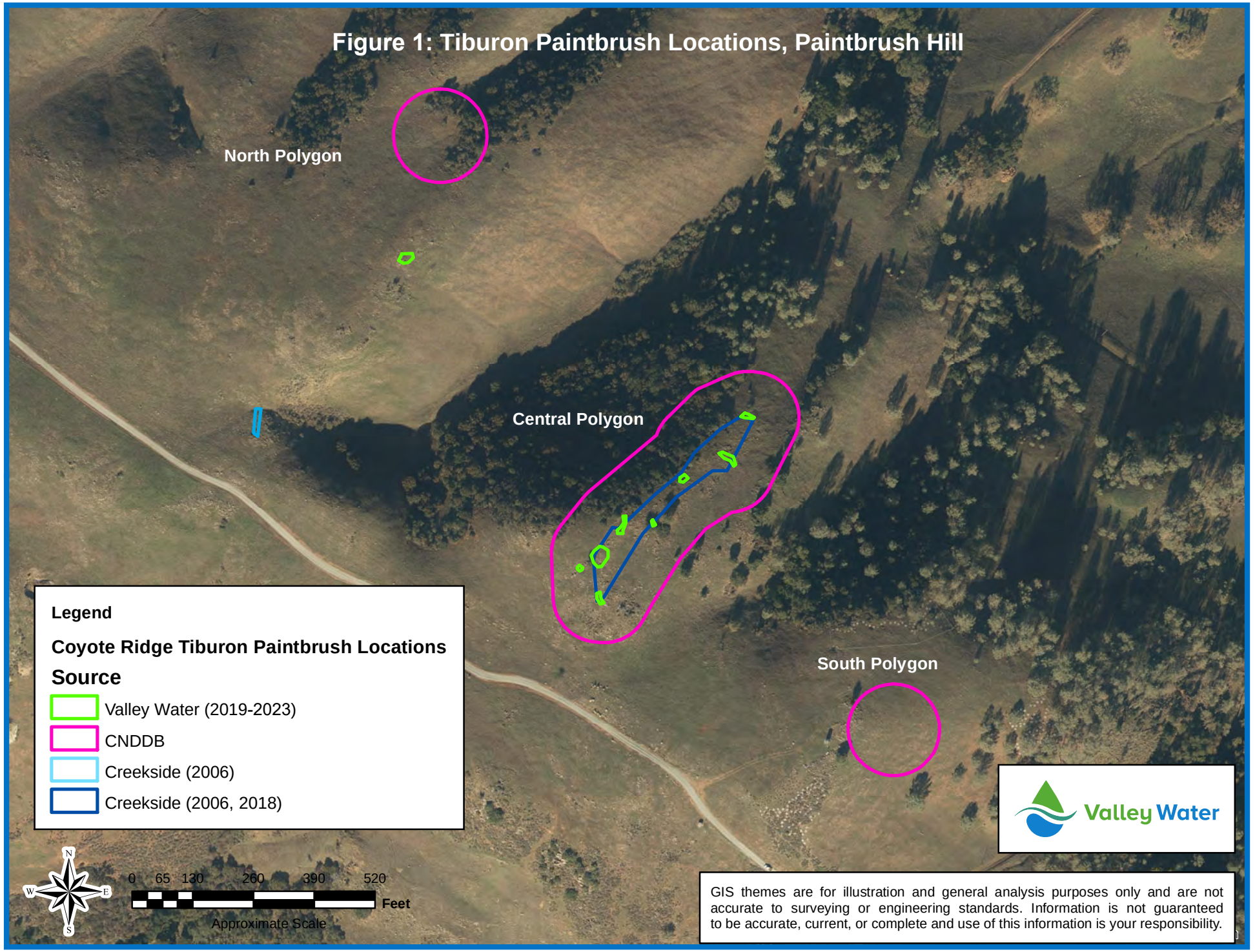
The Paintbrush Hill occurrence, located on property owned and managed by the Santa Clara Valley Water District (Valley Water), has historically been documented as three separate subpopulation clusters, or polygons (Figure 1). No plants were observed in the two smaller polygons (North and South) from 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*
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.

Figure 1: Tiburon Paintbrush Locations, Paintbrush Hill



This fifth annual report complies with the agreement made in April 2019 (Valley Water 2019). It describes field activities that took place in 2023. It summarizes the five years of population monitoring that have taken place over the course of this agreement, and presents a list of future management actions for discussion and decision-making among the larger interest group. The two most immediate recommendations are the instigation of a concerted invasive plant removal effort in the immediate vicinity of the population, and the implementation of either additional small to medium enclosures or trial electric fencing for partial protection of the population, potentially with the addition of an associated reduced-effort follow up monitoring program to assess effectiveness and inform decision making regarding a future permanent fencing decision.

Methods

Population Monitoring

As in previous years (2019-2022), 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 2023, on April 24 and May 11, and an additional round of data collection was done on June 29 during a fruit phenology check when it became clear that a late wave of flowering had occurred in a subset of plants. After peak flower, monitoring visits occurred approximately every three to 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 July 17 and August 3, with a final visit on August 17 for a single large plant that was phenologically the latest. Fruiting information collected 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

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 2023 population count of 105 would be five plants. However, as of July 19, only 59 plants had developing infructescences, so we reduced the number of plants for seed collection to three.

Fruit phenology was monitored approximately every three to 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 are stored in a permanent collection 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.

2023 Results

2023 Population Monitoring

We counted 105 reproductively mature plants and no seedlings in the 2023 monitoring season, for a total population size of 105 individuals. This included 88 plants in the main (central) polygon, and 17 plants in the northern polygon (Figure 1). No seedlings were observed in the 2023 monitoring season.

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 2023 was 20.0cm (n=105), with an average number of stems per plant of 5.4 and average number of inflorescences per plant of 3.7. 2023 plants were taller and less branched than 2022 and 2021 plants, with more inflorescences per plant.

Average width of inflorescences was 22.6mm (n=442), with a range of 5mm to 45mm (described range is between 15 and 25mm). Of the 425 inflorescences that had flowers available for color scoring in April and May 2023, 319 (75%) had pale (yellow to peach) flowers, 99 (23%) had orange to red flowers, and 7 (2%) had bright red flowers. Last year's flower color range leaned more heavily to the medium color range (45% pale, 50% medium, 5% bright), and the previous year more to the pale (80% pale, 14% medium, 6% bright). Previous visual assessment confirms that variation in flower color is common in this CAAFNE population.

Average number of infructescences per plant was 2.8 (5.9 for plants inside enclosures, the highest number recorded over the 5-year period), and the number of capsules per infructescence was 5.0 (7.0 for plants inside enclosures, again the highest number recorded).

Seed Collection and Banking

Seed from three plants (5% of plants that set fruit in 2023) was collected on 7/17, 8/3 and 8/17/23 and sent to CBG. Germination tests have not yet been completed on the 2023 seeds.

	All Years			2019			2020			2021			2022			2023		
In enclosure?	All†	In	Out	All†	In	Out	All†	In	Out	All†	In	Out	All†	In	Out	All†	In	Out
Average height per plant (cm)	18.8	***24.5**	*17.6	22.2	**30.7**	19.6	20.0	17.9	**20.4**	15.2	14.8	**17.3**	15.0	**20.1**	14.0	20.0	**28.5**	17.9
Average # of stems per plant	6.4	6.1	**6.4**	5.6	**5.9**	5.6	7.8	**8.9**	7.6	7.0	4.5	**7.5**	6.0	5.9	**6.0**	5.4	**5.5**	5.4
Average # of inflorescences per plant	2.5	***3.7**	*2.2	2.7	**4.4**	2.1	2.3	0.8	**2.5**	1.8	**2.8**	1.4	2.0	**2.2**	1.9	3.7	**7.4**	2.8
Average # of infructescences per plant	1.5	***3.3**	*1.1	1.3	**3.7**	0.6	0.6	**1.5**	0.5	1.8	**2.6**	1.6	1.4	**2.2**	1.2	2.8	**5.9**	2.1
Average # of capsules per infructescence	4.2	***5.4**	*3.4	5.0	**5.6**	4.0	4.7	4.0	**5.0**	3.2	**4.3**	2.9	2.6	2.6	**2.7**	5.0	**7.0**	3.6

Table 2: Population summary statistics of CAAFNE at Paintbrush Hill in 2019-2023

† Reproductive plants only; seedlings not included

* p-value for the difference between in/out of enclosure is significant at $p < 0.01$ (Welch's t-test). Tests not done for individual years.

Note: Higher value for in/out of enclosure is bold in each set of three columns

Possible Host Plants

In 2023, the six most common co-occurring plants within one foot of a CAAFNE individual included two native forbs (soap plant [*Chlorogalum pomeridianum*] and yarrow [*Achillea millefolium*], which have been in the top several species for all five monitoring years, and four non-native grasses (wild oat [*Avena fatua*], Italian rye grass [*Festuca perennis*], soft chess [*Bromus hordeaceus*], and foxtail chess [*Bromus madritensis*]). The non-native grass species' collective relative frequency in proximity to CAAFNE individuals has increased over the course of our monitoring program. See below, five-year results summary, for further discussion.

Five-Year Population Data and Discussion

Number of Plants

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 population size, the lower counts in 2020 – 2023 highlight that this small population may need additional protection if it is to persist or increase in size (Figure 2).

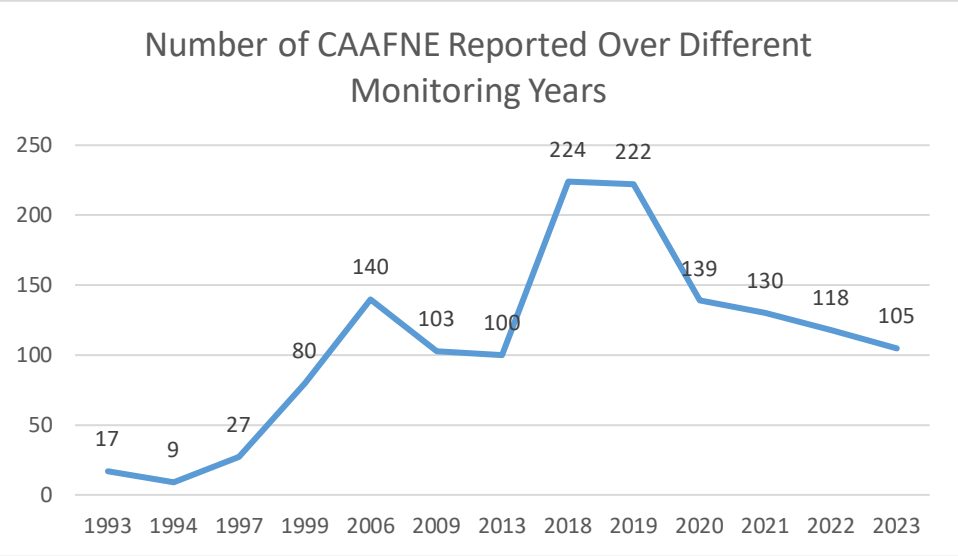


Figure 2. Number of CAAFNE plants at Paintbrush Hill, 1993-2023. Data sources: CNDDB (1993-2013), Creekside Science (2018), Valley Water (2019-2023)

The majority of the variability in plant numbers is to be found in the larger, central polygon (see Figure 1 for locations); the northern polygon hosted a range of 16-19 plants over the 5-year span, whereas the central polygon ranged from 203 plants in 2019 to 88 in 2023 (Figure 3).

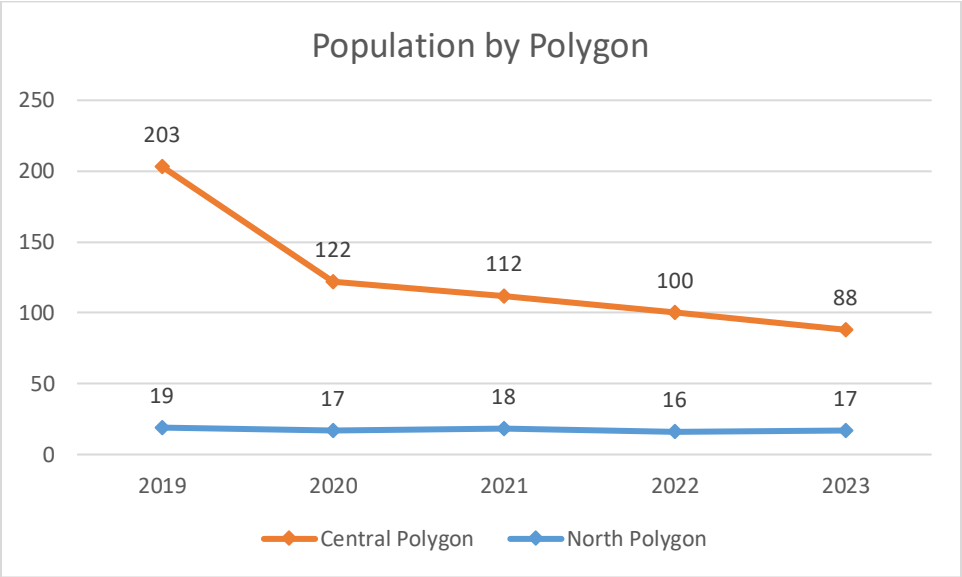


Figure 3. Number of CAAFNE plants at Paintbrush Hill by polygon, 2019-2023. Data source: Valley Water (2019-2023)

Number of Plants Inside Enclosures

The number of plants observed inside cages declined over the first three years of our observations, then increased again in 2022 and 2023 (Figure 4). The proportion of the total population within enclosures has remained within a close range, between 14% in 2021 and 20% in in 2023.

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 last 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 last 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, blue-eyed grass (*Sisyrinchium bellum*) and bindweed sp. (*Calystegia* sp.), and annuals yarrow, Q-tips (*Micropus californicus*), soft chess and foxtail chess. 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.

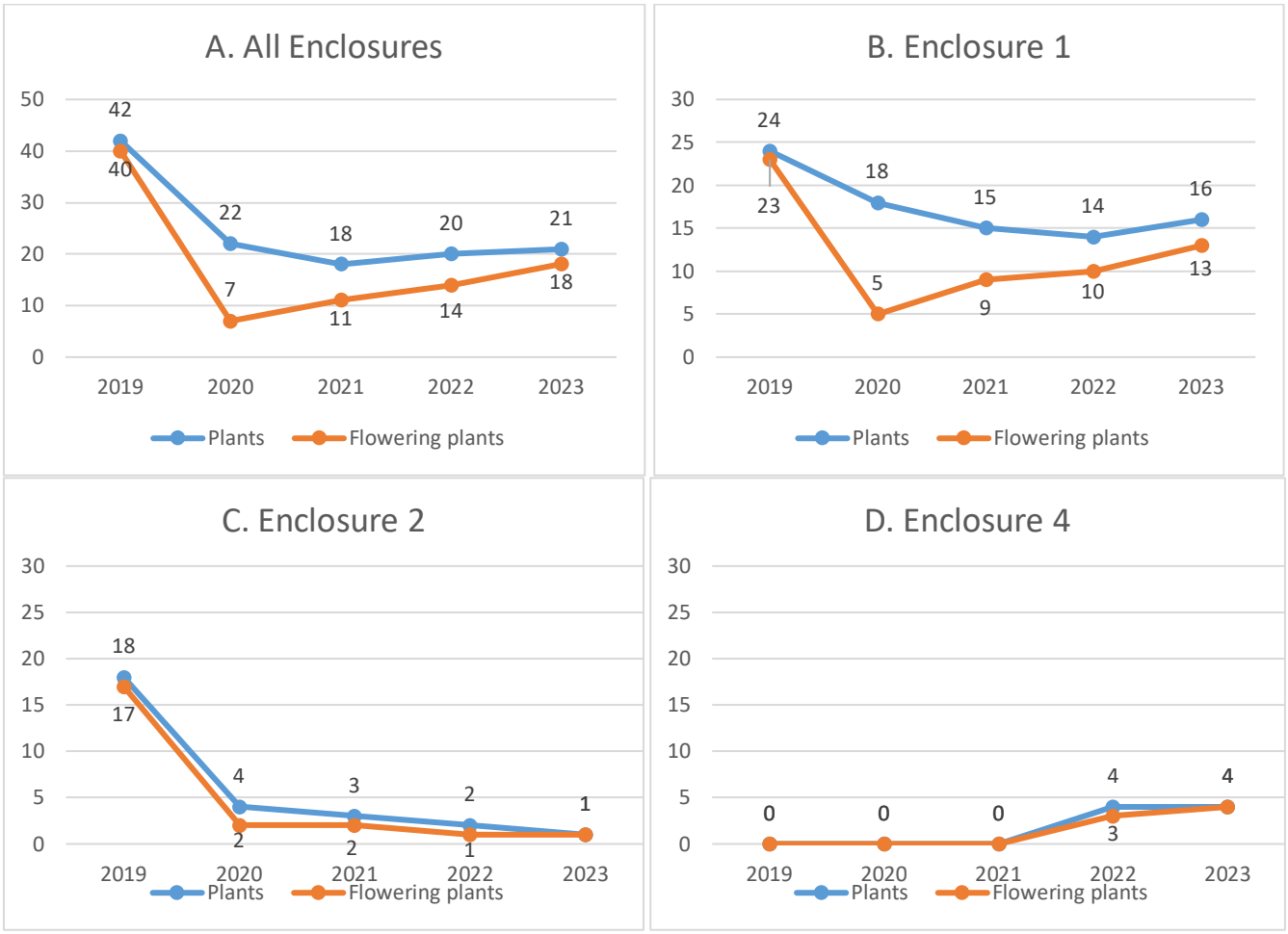


Figure 4. Number of Tiburon paintbrush plants observed inside protective cages from 2019-2023.

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
2023	May 11	July 17-Aug 17

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

Five-year Population Statistics

Across five years, a pattern emerges showing that enclosures make a difference to Tiburon paintbrush plants (Table 2). Statistically significantly higher numbers for plant height, number of inflorescences, number of infructescences, and number of capsules per plant occur inside enclosures when compared with plants outside them. The only value for which a difference was not statistically significantly was number of stems per plant. This is likely due to a confounding factor, which is that while larger, healthier plants (e.g. those inside enclosures) may have more stems for this reason, it appears that Tiburon paintbrush has an herbivory response to regrow in a low shrubby form with multiple short non-flowering stems when flowering stems are eaten (or broken) early in the season. As such, plants may have

multiple stems per plant for very different reasons. We tried to capture this with the notation of “multiple stems under 5cm?” on the datasheet, which is discussed in the section on herbivory.

Note that the numbers of capsules presented in Table 2 are standardized per infructescence, which mutes the apparent difference between reproductive output inside and outside enclosures. An average plant outside an enclosure would be expected to produce 5.1 capsules per year (1.1 infructescences X 3.4 capsules), whereas an average plant inside an enclosure would be expected to provide more than triple this output, at 17.8 capsules per year (3.3 X 5.4). This means that the plants inside enclosures are providing a high proportion of the functional reproductive output of this population, but at this time only a little less than 20% of the population is protected in this way.

Additional elements interesting to note for future studies include that flower size and color are not as cleanly delineated from CAAFAF as described, with CAAFNE inflorescences ranging up to as large as 4.5 cm across, instead of a maximum of 2.5 as described. Flower color fluctuates in the population between plants and years, and while on the whole the population has paler pink or yellow flowers than CAAFAF, any single plant might be more orange or closer to red than described in the literature.

Seed Collection and Banking

A small amount of seed has been collected in each of the five years of this monitoring program, representing approximately 5% of the reproductive output of the population in each year. 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), 70% for the 2021 lot (C. Birker, 2022b), and the 2022 collection was so small that germination tests were not done on that lot (C. Birker, 2022c). Germination tests have not yet been completed on the 2023 seeds. The seeds collected by Valley Water over the five years of this population monitoring effort are stored in a permanent collection 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.

Habitat Factors, Host Plants, and Threat Assessment

Thatch and Ground Cover Assessment

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 more than ten years, in 2021 we undertook a pilot thatch assessment project and in 2022 followed it up with a larger effort, which also allowed for an assessment of categories of ground cover associated with presence/absence of Tiburon paintbrush plants. A detailed description of methods and results for both the pilot and follow-up thatch & ground cover efforts can be found in the 2022 annual report (Valley Water 2022).

Of the data collected in those efforts, perhaps the most useful was the association of the presence of CAAFNE with higher average forb cover and rock cover, and the association of the absence of CAAFNE with higher average thatch cover and bare ground cover (Table 4). These were all significant associations. CAAFNE presence was also associated with lower grass cover, but this difference was significant only in the pilot. No distinction was made between native and non-native, or annual and perennial grasses in this effort.

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.

With regard to the thatch assessment, the results were inconclusive, with the pilot and the follow-up providing conflicting results with the exception of showing increased thatch cover inside enclosures, as expected.

Possible Host Plants

Over the five monitoring years, sixty-six potential host plant species have been recorded within one foot of CAAFNE individuals. Of these, 10 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 475 times, followed by lace parsnip (*Lomatium dasycarpum*), recorded 246 times. In 2022 and 2023 soft chess, recorded 233 times, overtook yarrow, recorded 232 times, as the third most commonly recorded species. Other frequently co-occurring species were Italian rye grass, recorded 227 times, and melic grass (*Melica* sp.), recorded 222 times. The other four species in the top ten were wild oat (recorded 182 times, 77 of which were in 2023), foxtail chess (138 times), blue-eyed grass (133 times), and imbricate Phacelia (*Phacelia imbricata*, 101 times). As mentioned above, although the Mediterranean annual grasses (soft chess, wild oat, foxtail chess) are increasing in cover at the site, it does not seem likely that they are prime host plants for CAAFNE due to their short life history, small stature, and phenology that is mismatched with that of CAAFNE.

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, six times each in 2021 and 2022, and three times in 2023.

If a future outplanting effort is desired at this location, additional research beforehand into host preference in CAAFNE is strongly advised.

Herbivory and Trampling

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, 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. In 2023, only 15 occurrences of clear herbivory were noted (14%), of which two were categorized as likely rabbit and four as likely cattle. An additional 12 plants were marked as having most stems shorter than 5 cm, for a total of 26% affected this year. For herbivory notations, 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.

In an effort to clarify the vectors of herbivory and quantify the type and degree of pressure on the population, wildlife cameras were deployed and camera trap data were collected over three seasons in 2019-2021 (two growing seasons and one winter wet season). The most salient facts are presented here. Additional camera trap methodology details and data can be found in the 2021 annual report (Valley Water 2021). Camera trap data recorded the presence of cattle, deer, and pigs in 2019, 2020, and 2021. Cattle were the most commonly observed large herbivore. 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. Most camera captures of cattle were of probable grazing and trampling events. Pigs and deer were less frequent visitors but were also present all year. No clear photos of pigs or deer grazing on paintbrush were captured. 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 5). It seems luck prevented greater destruction, since the rooting was directly adjacent to the population. A smaller rooting event was captured on camera in June 2020 (Appendix C). Pig rooting has been reported to kill plants and so rooting events may have a greater negative effect than regular grazing events even though they are less frequent.

Observations of large herbivores were much (20-60x) more common at the flattest camera site than the steepest.

Small mammals were also observed to eat and damage Tiburon paintbrush plants. The two most relevant species are Botta's pocket gopher and brush rabbit. Damage to plants from burrowing animals which appear to be eating soaproot (*Chlorogalum pomeridianum*) bulbs associated with CAAFNE plants has occurred in all years of this study (Appendix A, Events 3 and 4). Presence of Botta's pocket gopher was confirmed by camera trap data in 2020, though burrowing activity was not captured on camera. Gophers are resident at the site, burrows and mounds are in direct contact with paintbrush plants, and appear to have destroyed multiple plants over the five years of this study (plants in or adjacent to disturbed soil have disappeared and not resprouted). 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).

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), and they seem to do so fairly commonly at the site (see numbers above in first paragraph of this section).

Other small mammals captured by the cameras included *Peromyscus* sp., Western harvest mouse/house mouse (confirmed inside enclosures), unidentified rat, brush rabbit, and black-tailed jackrabbit (Appendix B). *Peromyscus* and Western harvest mouse/house mouse burrow and are omnivorous but it is unknown whether they impact paintbrush.

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. Compaction of soil by cattle may also be a relevant factor in flatter portions of the site, and erosion in the steeper portions.

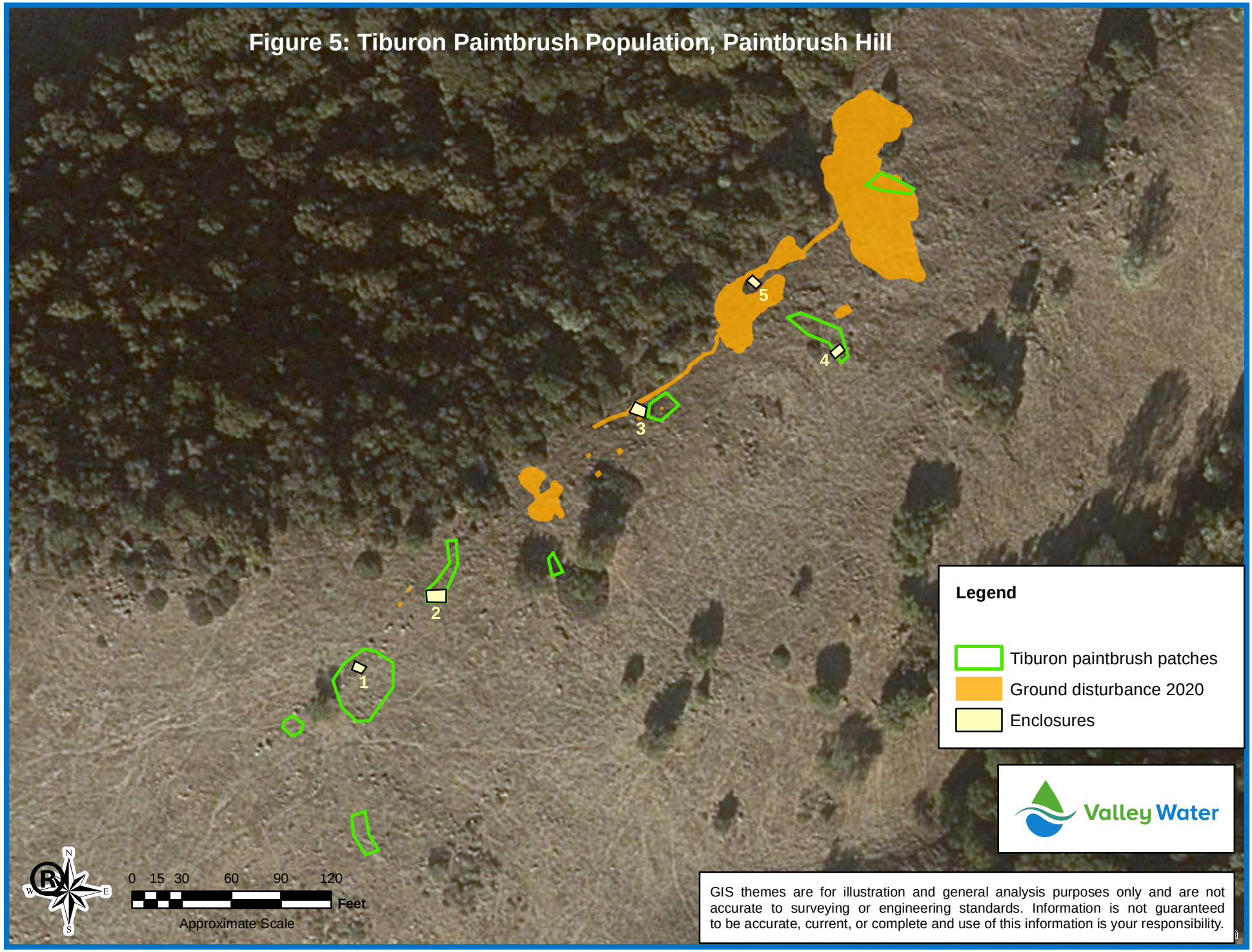


Figure 5: Tiburon Paintbrush Population, Paintbrush Hill
1
2
3
4
5
Legend
Tiburon paintbrush patches
Ground disturbance 2020
Enclosures
Valley Water
N
W
E
S
0 15 30 60 90 120
Feet
Approximate Scale
GIS themes are for illustration and general analysis purposes only and are not accurate to surveying or engineering standards. Information is not guaranteed to be accurate, current, or complete and use of this information is your responsibility.

Damage from cattle was not observed to kill plants in 2019 or 2020, but in 2021 plants in one area (below [N of] enclosure 3, see Figure 5) were trampled so thoroughly that they appeared to be destroyed and did not return in subsequent years.

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 years of our study some 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 2.2 inflorescences to 1.1 infructescences per plant outside cages, with smaller reduction inside cages (3.7 to 3.3), Table 2). This reduction was less pronounced in 2022 and 2023, corresponding with a change in cattle stocking in the area (David Mauk, pers. comm.). In these two years, cattle were removed in May, with no cattle returning to the site until the following November. This means there was no trampling or herbivory from cattle during most of the most sensitive portion of the development of CAAFNE fruits (late May-July), when inflorescence stalks become brittle and easily broken, resulting in lower production of mature seed.

In order to minimize any human component to this phenomenon, sampling and management techniques should be implemented very carefully to avoid damaging plants, and group field visits during flowering periods should be limited. But more importantly, in order to protect the plants, some measures to protect from large mammal impacts, such as fencing, is likely to be needed. The population number decline even in years with reduced trampling pressure suggests that if cattle stocking rates return to 2019-2021 levels, the pressure on the population may become unsustainable.

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 location were discussed, including the proximity to chapparal which could be impacted if the entire population were fenced; the steepness of the site, especially in lower portion of the central polygon; and the existence of a game trail alongside the plant population that would be diverted along a new fence, with likely detrimental effect to the habitat in the new location of the game trail. Two alternatives were discussed: fencing the population plus the adjacent chaparral patch, which would require more fence miles but could be easier to install due to easier equipment access and would avoid impacts to sensitive chaparral habitat. 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. A third alternative, proposed here, is to adjust the locations of the current aging small enclosures to better protect the current population. Creating two medium-sized enclosures where enclosures 1 and 2 currently stand to better protect the greatest number of plants where the greatest cattle pressure persists, moving or replacing enclosures 3 and 5 to capture current plant locations, and potentially installing additional small enclosures would protect a large proportion of the population without some of the drawbacks of a large fence enclosure. This strategy should be accompanied by invasive grass removal within enclosures, and potentially thatch removal in enclosure 2 where it is currently dense, given the data we have collected showing negative relationships between the presence of CAAFNE and increased grass and thatch cover (Table 4).

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 purple star thistle (*Centaurea calcitrapa*) patch on the road approaching the northern polygon population was discovered and removed in 2021 and resprouts removed in 2022 and 2023. 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 and their encroachment into the core CAAFNE area.

Soft chess, foxtail chess, and wild oat have increased in frequency in proximity to paintbrush plants since 2020 (Figure 6). The frequency of observations of Italian rye grass has stayed stable throughout this time. The reduction in soft chess reflected in 2023 numbers below reflects a 2023 weeding effort in enclosures 1 and 2 where soft chess density was especially high.

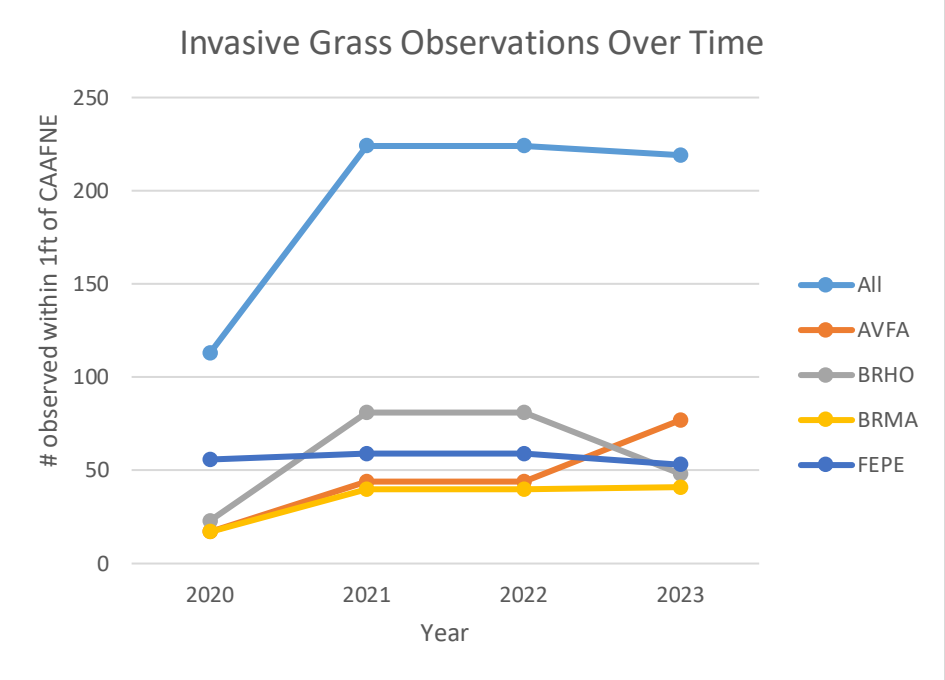


Figure 6. Number of invasive grass observations within one foot of Tiburon paintbrush plants from 2019-2023.

ATV Use

No evidence of ATV travel (active ATV use or tire tracks) has been observed during monitoring in 2019-2023. This does not appear to be an active threat to the population.

Conclusions and Next Steps

Fluctuations in number are normal for plant populations, but with that said 2020 - 2023 have been unencouraging years for the Paintbrush Hill population, especially in the central polygon. The region has suffered from a severe drought, and pressure on the population from trampling and herbivory appear significant. 2022, with decreased trampling and grazing pressure from cattle, but continued drought conditions, appeared to have provided a small respite for the habitat. The growing season of 2023, with its significant rain events, could have presented an opportunity for a population rebound, but this did not occur. One can conclude that rainfall alone does not appear to tightly control the population's numbers.

The habitat quality in the central polygon has declined over the last several years, likely due to a number of factors including environmental conditions (drought, depositional N), compaction, erosion, grazing and trampling from cattle and game, and encroachment by non-native plant species. Protecting this population from further habitat deterioration should be a high priority.

Given our observations over the last five years, we have the following recommendations to offer for the Paintbrush Hill CAAFNE population:

1. Continue to discuss and pursue protecting this population via fencing. Possibilities include adjusting the current aging small enclosures to better protect the current distribution of plants and habitat, temporary electric fencing for a portion of the population (the upper portion of the central polygon including enclosures 1 and 2, which is flat, attracting cows and calves and encompassing most plants, is the best candidate), or an eventual complete fencing option.
2. Develop and implement a vegetation management plan for the area that takes into account encroaching invasive species such as yellow star thistle and black mustard as well as invasive grasses such as soft chess and wild oat whose cover is increasing at the site and may degrade habitat quality over time.
3. If any elements of the above are implemented, then a reduced-effort monitoring program should continue, in order to assess whether the implemented measures are having their intended positive effects on the population.

   Recommended measurements for a reduced monitoring effort:
   -number of plants
   -plant height
   -number of inflorescences
   -number of infructescences
   -number of capsules

   Plant number is the most basic and required unit of monitoring (although as noted previously in this report, that doesn't mean it is always straight-forward to ascertain). Plant height is a proxy for plant vigor and can also indirectly capture some herbivory information. Number of inflorescences coupled with number of infructescences speaks to plant capacity for successful reproduction – in other words, how much of the reproductive effort expended by the plant results in mature seed? Number of capsules is the most direct corollary to reproductive potential. If a much reduced monitoring effort is desired, a plant count in spring followed by a capsule count in late summer would be another option, capturing only the most critical demographic and reproductive measures.

Additional possibilities for further study to increase our understanding of the population biology of this taxon and this occurrence:

1. Identify functional CAAFNE host plants through experimentation. 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 before the success of a potential outplanting augmentation program can be assured.
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, soil characteristics, community species composition and/or

other ecological factors in those areas as compared to that in the central polygon and other extant populations.

3. Collaborate with Santa Clara University or other interested entity to elucidate flower color and associated genetic variation in this population.

As a point of interest and reminder, 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 after obtaining her collecting permit, which will provide more data than 2021's catch and release study. Putative outcomes of the study may include a list of possible pollinators of CAAFNE and a pollination network.

We look forward to discussing these results and recommendations and hope to come to agreement regarding future protective actions for this important population of this endangered taxon.

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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

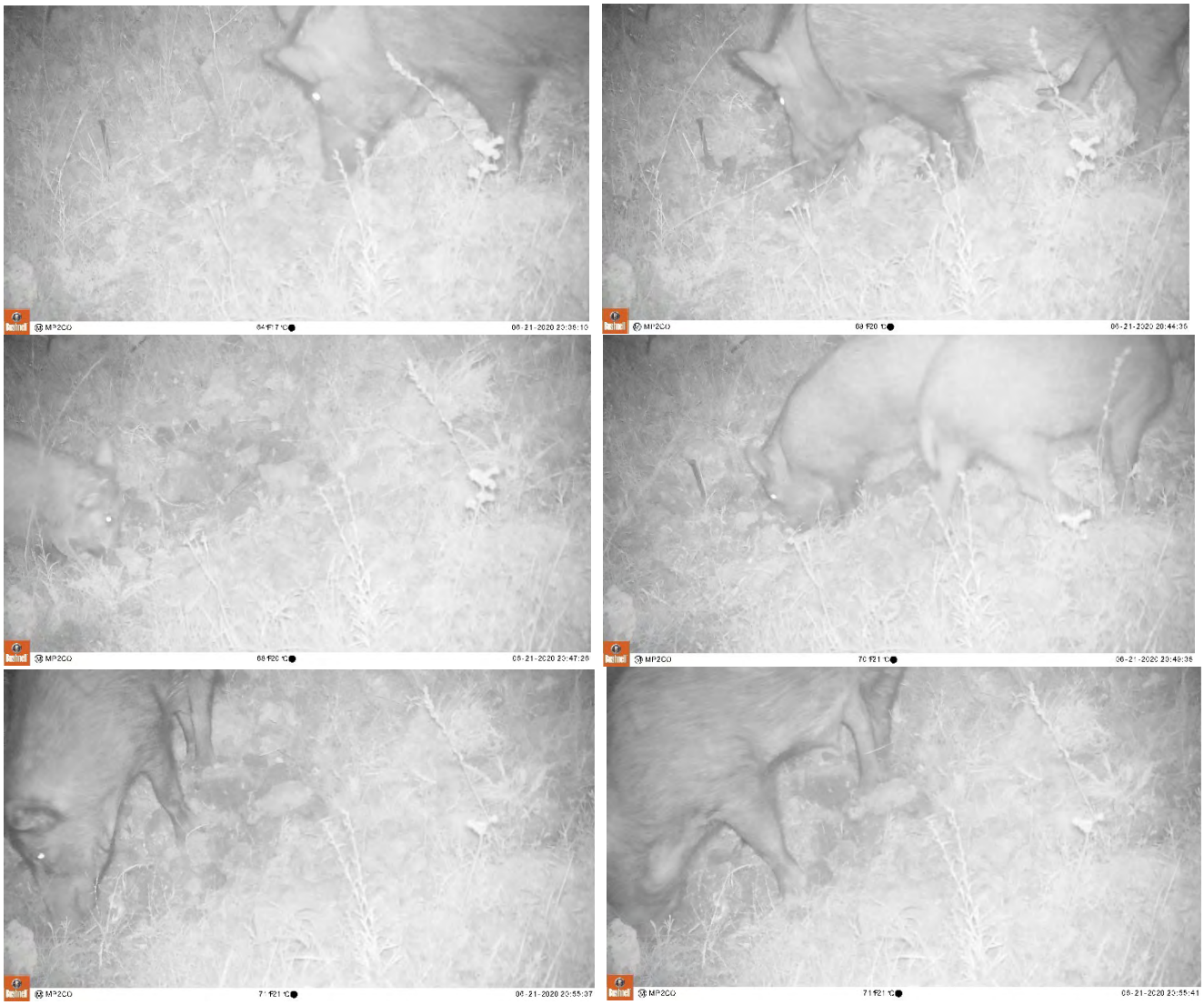
Cow grazing

Pigs grazing

Deer

Western harvest mouse, inside cage enclosure

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)