

5-YEAR REVIEW

Tiburon Paintbrush (*Castilleja affinis* subsp. *neglecta*)

GENERAL INFORMATION:

Species: Tiburon paintbrush (*Castilleja affinis* subsp. *neglecta*)

Date listed: February 3, 1995

Federal Register (FR) citation: Service 1995 (60 FR 6671)

Classification: Endangered

State Listing:

The Tiburon paintbrush was listed as threatened by the State of California in 1990.

BACKGROUND:

Most recent status review:

[Service] U.S. Fish and Wildlife Service. 2012. *Castilleja affinis* spp. *neglecta* (Tiburon paintbrush) 5-Year Review: Summary and Evaluation. Sacramento, California. 26 pp.

We did not recommend a status change in the 2012 status review.

FR notice citation announcing this status review:

[Service] U.S. Fish and Wildlife Service. 2020. Endangered and threatened wildlife and plants; initiation of 5-year status reviews of 66 species in California and Nevada. Federal Register 85:4692–4694.

We did not receive any information from the public regarding Tiburon paintbrush in response to the FR notice.

ASSESSMENT:

Information acquired since the last status review:

This 5-year review was conducted by the U.S. Fish and Wildlife Service's (Service) Sacramento Fish and Wildlife Office. Data for this review were solicited from interested parties through a Federal Register notice announcing this review on January 27, 2020 (Service 2020, p. 4692). We used personal communications with species experts, obtained reports from partners, performed a literature search, reviewed information from our own files, and obtained data from an occurrence search of the California National Diversity Database (Diversity Database) maintained by the California Department of Fish and Wildlife.

Since the previous 5-year review, new information regarding Tiburon paintbrush has become available, as described below.

Distribution

The Diversity Database defines an Element Occurrence as a location record that includes populations, individuals, or colonies located within 1/4 mile of each other (sometimes with multiple parts), and includes seven Tiburon paintbrush Element Occurrences (Diversity Database 2020). In this document we use the term occurrence with the same definition, although from here on we use the terms occurrence and population interchangeably throughout this document.

We consider the current distribution to include seven populations: four in Marin County, one in Napa County, and two in Santa Clara County. Populations from the listing document (Service 1995, p. 6673), Recovery Plan (Service 1998, p. II-24), last 5-year review (Service 2012, pp. 5–6), and as of 2021 are listed in Table 1. The range is approximately 50 kilometers (30 miles) from east to west, and 112 kilometers (70 miles) from north to south. The range is the same as described as listing (Service 1995, p. 6673) and in the last 5-year review (Service 2012, pp. 5–6), although there have been several specific changes to populations within the range coinciding with revisions in the Diversity Database (Table 1). Since the last 5-year review, one Diversity Database occurrence in Marin County (Element Occurrence 6) has been deleted. This change in the Diversity Database occurred because Element Occurrence 6 was considered an incorrect mapping of Element Occurrence 8 (Diversity Database 2020). The Stinson Beach occurrence in Marin County (former Diversity Database Element Occurrence 10), not observed since 1965, is no longer included in the Diversity Database for this species. Doreen Smith (Rare Plant Committee Co-chair for the Marin Chapter of the California Native Plant Society) stated that the subspecies *Castilleja affinis* subsp. *affinis* in Bolinas (i.e., Stinson Beach) may have been misidentified as Tiburon paintbrush, but that it is unlikely that the listed species ever inhabited the area based on the lack of serpentine rock on the Point Reyes peninsula (Smith *in litt.* 2020).

Table 1. Tiburon paintbrush Diversity Database Element Occurrences (EO) from the final listing rule (Service 1995), Recovery Plan (Service 1998), last 5-year review (Service 2012), and currently.

County	Population	1995 EO #	1998 EO #	2012 EO #	2021 EO #	Notes
Marin	Old Saint Hilary's	2	2	2	2	
Marin	Middle Ridge	3	3	3	3	
Marin	Ring Mountain	4	4	4	4	
Marin	Nicasio Ridge	6	6	6	--	Former EO #6 was likely an incorrect mapping of EO #8.
Marin	Nicasio Ridge	--	8	8	8	
Marin	Stinson Beach	--	--	10	--	This is a historical record no longer considered to be the listed entity. Service (2012) notes inclusion of this occurrence in the Diversity Database, although it does not expand upon it in a description of Marin County occurrences.
Napa	American Canyon	5	5	5	5	
Santa Clara	Paintbrush Canyon	7	7	7	7	
Santa Clara	Paintbrush Hill	--	--	9	9	

Abundance

In this section of the status review we report monitoring information subsequent to that reported in the last 5-year review. See Appendix A for a summary table of abundance records, including those prior to the last status review.

Marin County

There are four Tiburon paintbrush occurrences in Marin County, three of which are on the Tiburon Peninsula: Middle Ridge, Ring Mountain, and Old Saint Hilary's Open Space Preserve (hereafter Saint Hilary's). The fourth Marin County occurrence is at Nicasio Ridge.

The Middle Ridge occurrence had approximately 850 plants in 2007, and over 200 in 2013 (Diversity Database 2020).

Ring Mountain Preserve had over 1,000 plants in 2013 (Diversity Database 2020). Marin County Parks and Open Space changed the monitoring protocol for Ring Mountain from plot-based surveys to a yearly census during peak bloom starting in 2015, and began including the adjacent Corte Madera Open Space parcel the following year. Over the 6 year period (2015-2020) that census data are available, counts ranged from 1,401 to 2,422 plants. However, additional monitoring is needed to ensure confidence in trends regarding the overall population and in smaller patches or subpopulations within the population (Marin County Parks and Open Space 2020, pp. 3–7).

There were no Tiburon paintbrush detected at Saint Hilary's during surveys in 2013 (Diversity Database 2020), but eight plants were observed within a 40-square-meter area in 2015 and five additional plants were detected in 2016 (Marin County Parks and Open Space 2020, p. 1). There is also potential habitat on a private property in the vicinity of Saint Hilary's (Magallanes and Minnick *in litt.* 2020).

Nicasio Ridge is bisected by the Golden Gate National Recreation Area park boundary line, with some plants occurring in the Golden Gate National Recreation Area but the majority occurring on private lands to the east. Abundance estimates for this population are taken from Chasse (2019, pp. 10–11), and are from the Golden Gate National Recreation Area only. From 2012 to 2018, annual surveys ranged from 139 to 210 plants. Most plants were found in what is referred to as the "Main Patch," but there are also two outlier patches noted.

Napa County

There is one Tiburon paintbrush occurrence in Napa County. The American Canyon occurrence is located mainly on private land, but a portion of occupied habitat is in the Newell Open Space Preserve, managed by the City of American Canyon. In the most recent survey count in 2013, about 170 plants were counted in the Newell Open Space Preserve (Diversity Database 2020). A site visit in 2020 documented presence of the species but did not include a count (Ruygt *in litt.* 2020). We are not aware of recent abundance estimates for the private portion of this occurrence.

Santa Clara County

Santa Clara County includes the Paintbrush Canyon and Paintbrush Hill occurrences.

Paintbrush Canyon had 1,000 plants in 2013 (Diversity Database 2020). The total population estimate in 2018 was $1,900 \pm 375$ plants. This total included an estimate of plant numbers from restrictive stratified sampling of an extended macroplot ($1,255 \pm 375$ plants), and census counts

of areas outside the macroplot (74 plants), experimentally seeded plots (517 plants), and targeted seeding areas (54 plants). The macroplot was established in 2006 as a 50 x 50 m plot and extended (doubled) in 2018 to encompass the majority of the population; sampling covered 20 percent of habitat in the extended macroplot, which was then extrapolated to yield an estimate of the entire extended macroplot (Niederer *et al.* 2018b, p. 13).

Paintbrush Hill is mapped as three separate polygons in the Diversity Database, and from 1997 through 2018 the species was only observed within the largest polygon; the last observations in the two smaller polygons were in 1994 and 1997, respectively. However, in 2019, 19 plants were observed in a location 250 feet south of the northern polygon. There were approximately 100 plants at Paintbrush Hill in 2013, 224 in 2018 (Creekside Science 2018a, p. 1), 222 in 2019 (including 46 seedlings; Valley Water 2020, p. 3), and 139 in 2020 (including 5 seedlings; Valley Water 2021, p. 3). In 2018, because of difficulty discerning individual plants, plants were counted as individuals if there were at least two centimeters of bare soil between emerging branches. This technique minimized guessing and increased repeatability, but may have led to overcounting in some cases (Creekside Science 2018a, p. 1). Counts in 2019 attempted to distinguish individuals using the best available information, including growth pattern, morphology, and distance from other plants (Valley Water 2020, p. 2). Counts following the Creekside Science (2018a, p. 1) two centimeter rule resulted in 340 reproductive adults compared to the Valley Water (2020, p. 3) estimate of 176, including a large individual with 46 stems that would have been counted as 12 individuals using the aforementioned rule. For this reason, Valley Water (2020, pp. 3–4) stated that they do not consider the two centimeter rule a viable metric, and counts in 2020 only used the best available information to differentiate individuals (Valley Water 2021, p. 2).

Morphological traits and measurements

Comparison of 15 morphological traits between Tiburon paintbrush and a closely related subspecies, *Castilleja affinis* subsp. *affinis*, found that Tiburon paintbrush was shorter or thinner for all traits. The yellow flower color, compared to red, was the most distinguishing trait of Tiburon paintbrush. More information about the traits measured and differences between the subspecies is described in Widener and Fant (2018, pp. 367–373). Within Tiburon paintbrush, there was significant variation in the floral traits as well, although less so than in *Castilleja affinis* subsp. *affinis*. The Tiburon paintbrush population at American Canyon was most different from the other populations within that subspecies. In another report, visual assessment of flower color in the Paintbrush Hill population noted variation ranging from yellow to peach to bright red (Valley Water 2021, p. 4).

Although Tiburon paintbrush flowers have been described as pollinated by hummingbirds (Kevin Bryant pers. comm. 2007, as cited in County of Santa Clara *et al.* 2010, p. 3), yellow coloration is often associated with bee pollinators (Widener and Fant 2018, p. 366). If the flower color of the *C. affinis* subspecies (yellow in Tiburon paintbrush vs. red in *Castilleja affinis* subsp. *affinis*) attracts a different suite of pollinators, this could reinforce reproductive isolation between the two despite overlapping ranges (Grant 1994, pp. 10409–10410; Kay and Sargent 2009, pp. 639–640). However, to our knowledge there have not been studies on Tiburon paintbrush pollination, and we again note the variation in flower color observed within putative Tiburon paintbrush. Niederer *et al.* (2018a, pp. 8–9) observed a hummingbird pollinating a Tiburon paintbrush at Paintbrush Hill and both bumblebees and hummingbirds pollinating the species in the Creekside Science nursery, suggesting that both insects and hummingbirds can be pollinators

for Tiburon paintbrush. Photos of pollination events from both pollinator types feature red flowers.

Genetics

The same morphological study described above found strong genetic differentiation between Tiburon paintbrush and *Castilleja affinis* subsp. *affinis* (Widener and Fant 2018, pp. 367–374). Overall, the study found similar levels of genetic diversity and genetic differentiation in six Tiburon paintbrush populations. The study included all known populations except the St. Hilary’s Church population in Marin County. While American Canyon and Nicasio Ridge did not have evidence of inbreeding, the other four populations had moderate to high levels, with Paintbrush Canyon being the highest. Potential non-mutually exclusive explanations for elevated levels of inbreeding include, but are not limited to: locally patchy habitat; low colonization ability; and low dispersal distances (discussed in Widener and Fant 2018, p. 376).

Additionally, two populations had lower differentiation than the others from the sister subspecies, which indicates potential historical or current low levels of hybridization. In particular, the American Canyon and Paintbrush Hill Tiburon paintbrush populations had some genetic overlap with *Castilleja affinis* subsp. *affinis*. The genetic overlap between the American Canyon Tiburon paintbrush and *Castilleja affinis* subsp. *affinis* is suggested to be related to differences in morphology in that population, which is described above.

There has been considerable discussion regarding genetic mixing and hybridization as it pertains to outplanting and population augmentation, which is discussed in [Seeding experiments](#) below. Valley Water (2021, p. 9) recommended continued study related to flower color and genetic variation at Paintbrush Hill.

Germination and seed viability analysis

Seeds from the Paintbrush Canyon and Paintbrush Hill populations were analyzed for viability by Ransom Seed Laboratory, Inc., using seeds sent from the University of California Botanical Garden. Seed counts resulted in 1.88 million to 3.17 million seeds per pound (Ransom Seed Laboratory, Inc. 2013). The seed viability analysis using seeds collected from Paintbrush Canyon in 2012 resulted in 74 percent germination and four percent dormancy in 21 day germination tests. Seeds collected from Paintbrush Hill in 2012 and 2013 resulted in 67 percent and 73 percent germination, and zero percent and three percent dormancy, respectively. Although the definition of seed dormancy can vary, it is generally described as the failure of an intact viable seed to germinate under favorable conditions (Bewley 1997, p. 1055). In this analysis, total viability was calculated for each sample by adding results from the percent germination and percent dormant (i.e., in 2012 the viability analysis added 74 percent germination and 4 percent dormancy to yield 78 percent viable) (Ransom Seed Laboratory, Inc. 2013). Seeds collected from Paintbrush Hill in 2019 had an 80 percent viability with no pre-treatment or stratification (Birker 2020, as cited in Valley Water 2021, p. 5). Note that these results pertain to immediate germination, not survival of seedlings for any period of time, which would likely be much lower.

There is also information about germination rates from plants grown in nursery or greenhouse settings. The highest rate of germination from nursery trials at the University of California Botanical Garden was 13.75 percent (Forbes *in litt.* 2018, as cited in Hillman and Johnston *in litt.* 2018). Creekside Science present germination rates of plants raised in their greenhouse during seed propagation efforts. Germination rates ranged from one to 37 percent. Observationally,

germination was higher in a shade structure than for outside plants; avoiding overwatering was also noted as important for greenhouse plants (Niederer *et al.* 2018a, p. 5). Some propagation problems were attributed to using pots that were too small; the latest information from Creekside Science points to higher success growing plants in raised beds and troughs (Niederer *in litt.* 2020).

Host plants

Tiburon paintbrush is hemiparasitic, meaning that it is able to produce its own chlorophyll but it is also known to form haustorial (rootlike structure that grows into or around another plant) root connections that absorb nutrients from host plants. Creekside Science used greenhouse experiments to test host plant success with three potential host plant species: purple needle grass (*Stipa pulchra*), tomcat clover (*Trifolium willdenovii*), and yarrow (*Achillea millefolium*). These potential host plants were chosen based on a vegetative associates survey in 2006, combined with information from various host plant studies from within the Tiburon paintbrush genus. Propagation faced several challenges, with attempts to grow plants sequentially occurring at the University of California Botanical Garden, Santa Clara University, and finally, Creekside Science Conservation University. Ultimately, yarrow was the preferred host plant based on health and survival (Niederer *et al.* 2018a, pp. 15–16). Methods for propagation, including less successful attempts, are included in Niederer *et al.* (2018a, pp. 16–20) and previous reports from Creekside Science. The authors suggest additional research on golden yarrow (*Eriophyllum confertiflorum*), which appears to be an important host plant based on results from seeding plots (Niederer *et al.* 2018a, pp. 31–39, 66).

Forty potential host plants were recorded within one foot of Tiburon paintbrush at Paintbrush Hill in 2019 and 2020, seven of which were recorded 30 times or more (Valley Water 2021, p. 7). The most common co-occurring species was soaproot (*Chlorogalum pomeridianum*), followed by lace parsnip (*Lomatium dasycarpum*), and yarrow. Golden yarrow (noted above as being potentially important at Paintbrush Canyon) is not common at Paintbrush Hill, but was recorded as a co-occurring species in both 2019 and 2020. Valley Water (2020, p. 8; 2021, p. 9) also noted that Tiburon paintbrush host selection on a plant that is desirable by herbivores may be detrimental to the species. Rooting disturbance that was consistent with gophers rather than pigs (based on observations inside and outside exclosures) appeared to include soaproot husks, raising the possibility that herbivores foraging for host plant bulbs could harm Tiburon paintbrush as a byproduct (see additional information about gophers in [Herbivory and Trampling/Rooting](#)).

Threats

At the time of listing, destruction of habitat through residential or recreational development was considered the greatest threat faced by Tiburon paintbrush (Service 1995, p. 6678). The listing rule also recognized the threats of grazing, soil erosion and slipping, foot traffic, the quarry near the American Canyon occurrence, restricted habitats/range, small population size, and competition with non-native plants (Service 1995, pp. 6678–6683). Additional threats noted in Service (2012, pp. 8–14) included nitrogen deposition from air pollution, which facilitates non-native grass invasions of serpentine soils, and climate change/drought. All of these threats continue to threaten the species, although development is no longer a threat for the occurrences that are on protected lands. At a meeting between Service staff and various partners in December

2020, fire retardant was also noted as a potential threat based on its potential to modify habitat by increasing the growth of invasive grasses (Service *in litt.* 2020).

Below, we focus on updated or new or updated information that relates to threats. Please see Service (2012, pp. 8–14) for a more comprehensive evaluation of the five factor threats affecting this species.

Recreation

Additional recreational opportunities continue to become available on or near Tiburon paintbrush populations. The Newell Open Space Preserve, which includes a portion of the American Canyon population, has trails that were opened to the public for hiking in 2014. However, these trails avoid the Tiburon paintbrush population and are not expected to impact the population. Coyote Ridge Open Space Preserve currently has trails that are open by reservation only, with docent-led hikes, which would avoid potential harm to Tiburon paintbrush. Planning is underway for expanded public access of the preserve to the public, with a sustainable trail network (as well as parking, signage, restrooms, etc.) slated to become open to the public in 2022 (Santa Clara Valley Open Space Authority 2020).

New efforts to reduce off-trail use at Ring Mountain have been implemented because signs signifying closure of unofficial trails in previous years had been somewhat ineffective. In 2019, new wayfinding signage was used to designate official trails, and staff are increasing efforts to raise awareness and educate visitors (Marin County Parks and Open Space 2020, p. 2).

Herbivory and trampling/rooting

Additional information about herbivory and trampling/rooting is available through camera trap and observational data from the Paintbrush Hill occurrence. Pig rooting has been noted as a threat consistently since at least 2006, and likely eliminated some Tiburon paintbrush plants in 2017 and 2018 (Weiss *et al.* 2018, pp. 8–10). Camera traps near Tiburon paintbrush at Paintbrush Hill recorded the presence of cattle, pigs, and deer in 2019 and 2020. Cattle were much more prevalent than the other two herbivores (1.3 vs. 0.2 and 0.02, respectively, per 24 hour period), and cattle were documented eating Tiburon paintbrush in two instances. Trampling was also noted to be a factor in addition to herbivory; broken stalks could lead to a reduction in seed set even if trampling does not directly kill the plants. Large herbivores were most prevalent at camera sites with the flattest topography in comparison to steeper camera sites. Pig rooting near the Tiburon Paintbrush population at that site was observed in 2020, and although not directly documented to kill the species in that year, coincided with the noted absence of 2 plants observed in 2019 but not after the rooting event (Valley Water 2021, pp. 5–6). Because pig rooting has been reported to kill the species in the past, Valley Water (2021, p. 6) suggests that pig rooting may have a greater negative effect than cattle herbivory, even though it is less common.

Impacts due to small mammal activity and insect herbivory could also be potential threats to Tiburon paintbrush. Damage consistent with gopher activity was observed at Paintbrush Hill in 2019 (Valley Water 2020, p. 6), and camera traps documented presence of Botta's pocket gopher (*Thomomys bottae*) in 2020 (Valley Water 2021, p. 7). Valley Water (2020, p. 6; 2021, p. 6) speculates that gophers may be foraging for soap root bulbs which led to collateral damage to Tiburon paintbrush when the two species occurred in proximity. A combination of camera trap data, observations of herbivory, and scat confirm that brush rabbits eat Tiburon paintbrush

(Valley Water 2021, p. 7), although at this time it is unclear if this threat is at the individual or population level. Insect herbivory is also noted in Valley Water (2021, p. 7), although it is not clear if it has an impact on flower or fruit production.

Fire retardant

Fire retardant adds resources to soil that creates a pulse in nitrogen and phosphorus that can fuel rapid growth by non-native invasive species. Observations in the field, combined with field and greenhouse experiments, all demonstrated that addition of fire retardant led to increased abundance of non-native annual plant species in intermountain grasslands (Besaw *et al.* 2011, pp. 1000–1001). Fertilization of serpentine soils with nitrogen and phosphorus allowed invasion by non-native annual plants within two years (Huenneke *et al.* 1990, pp. 481–487). By altering soil chemistry of normally nutrient-poor serpentine soils, the addition of fire retardant can similarly create an environment that promotes non-native annual plant species in Tiburon paintbrush habitat. Although we are not aware of specific research on Tiburon paintbrush regarding fire retardant, the available information suggest that fire retardant could be a threat to the species, and that post-retardant weed control may be important if fire retardant is dropped on the species' habitat.

Conservation

Conservation of Tiburon paintbrush has occurred in the form of land protection to protect extant occurrences, seed banking to preserve genetic diversity, and seeding experiments to investigate population augmentation options for the species.

Land protection

Although habitat loss and fragmentation still threaten the Tiburon paintbrush in areas that are unprotected, portions of all occurrences currently occur on protected land, land covered by Habitat Conservation Plans (California Protected Areas Database 2020), or land conserved through permittee-responsible mitigation. In Marin County, a portion of the Nicasio Ridge population occurs within the Golden Gate National Recreation Area, but the majority of individuals occur on private land to the east (Chasse 2019, p. 10). Most of the occupied habitat on the Tiburon Peninsula is protected: known individuals from the Old St Hilary's population occur in Old St. Hilary's Open Space Preserve, most of the Middle Ridge population occurs in Town of Tiburon Open Space Preserves, and most of the Ring Mountain population is on the Ring Mountain Preserve. In Napa County, approximately 5 to 10 percent of the American Canyon population is on the Newell Open Space Preserve (C. Cromer and J. Ruygt personal communication 2009 in Service 2012) (California Protected Areas Database 2020).

Permittee-responsible mitigation associated with the Kirby Canyon Landfill Development Project, reinitiated in 2013, has resulted in current protection of the two Santa Clara County Tiburon paintbrush occurrences. The biological opinion includes as a conservation measure the continued leasing and management of a 250-acre butterfly preserve (Kirby Canyon Butterfly Trust) until the landfill has been closed and revegetated with appropriate native vegetation, after which a conservation easement shall be placed on the restored landfill area (Service 2014, p. 7). The Butterfly Trust was sold to Valley Water with the stipulation that it is still being used as mitigation for the landfill (Service *in litt.* 2020). The Butterfly Trust includes the Paintbrush Hill occurrence, owned and managed by Valley Water, while Paintbrush Canyon is on land adjacent to the Kirby Canyon landfill and owned as mitigation by Waste Management, Inc.

Grazing enclosures

Grazing enclosures (also referred to as enclosures in some reports) were installed around a portion of the Paintbrush Hill occurrence by Creekside Science in 2012 in an attempt to reduce trampling, grazing by various herbivores, and rooting by feral pigs (Weiss *et al.* 2018, p. 1). Enclosure effect to date on population size and health has been unclear, and will continue to be evaluated (Valley Water 2021, pp. 4–5). Enclosures likely benefit Tiburon paintbrush by reducing herbivory, trampling and rooting, but may be susceptible to increased thatch and vegetative cover, including scrub encroachment. Early data suggested a pattern of more plants and more flowering stalks inside the cages, but analysis of counts from 2012 through 2018 indicated that plant numbers were not significantly different between caged and uncaged plots. Higher numbers of flowering stalks and fruits were reported for caged plants, but these numbers were not standardized by the number of plants and were significant for only one year (2016) (Weiss *et al.* 2018, pp. 2–4). Survey data from 2019 found that Tiburon paintbrush inside cages were taller, with more stems, more inflorescences, more infructescences (the fruiting stage of an inflorescence) per plant, and more capsules per infructescence. There were also more seedlings inside enclosures, despite a small amount of area available within the enclosures compared to total occupied habitat (Valley Water 2020, p. 5). These patterns persisted in 2020 with the exception of overall height, which was not different, and the location of seedlings, which were only found outside enclosures in 2020 (Valley Water 2021, pp. 4–5). The land manager’s most recent report recommends maintaining the enclosures while continuing to evaluate effectiveness (Valley Water 2021, p. 8).

Seed banking

Seeds from Tiburon paintbrush are stored at the California Botanic Garden and University of California Botanical Garden. The California Botanic Garden has over 60,000 seeds in their collection from 2018 through 2020, with the bulk collected in 2018 (California Botanic Garden 2020). Seeds from 2018 are first filial generation (first set of offspring from controlled or observed reproduction) mixed from both Paintbrush Hill and Paintbrush Canyon stock (Niederer *et al.* 2018a, p. 58) as opposed to genetically pure populations. The University of California Botanical Garden has seeds that were collected in 2007 (University of California Botanical Garden 2020). These include 8,490 wild-collected from Paintbrush Canyon and 565 wild-collected from Paintbrush Hill (Niederer *et al.* 2018a, p. 58).

Valley Water (2021, p. 3) notes that the California Botanic Garden deposits a backup sample with the National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado.

Seeding experiments

Attempted population augmentation at Paintbrush Canyon focused on seeding rather than outplanting, in part because of concerns revolving around water mold *Phytophthora* species. Although nursery stock poses a large *Phytophthora* risk (Swiecki and Bernhardt 2015), the risk approaches zero when using seeds in combination with best management practices (Swiecki and Bernhardt 2016; Niederer *et al.* 2018a, p. 23). Seeding experiments included plots with stratified seeds (cold treatment before seeding), unstratified seeds, and controls (no seeds) between 2013 and 2017. Irrigation methods also varied between plots. Out of 16,700 seeds planted, the plots had a high count in spring 2017 of 3,953, but by the summer reproductive period of that year were down to 37 plants. The authors speculate that a record heatwave in June likely contributed to the decrease in plants between those two time periods. Although success varied across years, it

was not clear which factors were most closely associated with reproductive Tiburon paintbrush. Factors considered included blocks (included to account for spatial variability), treatments (stratified/unstratified seed), and other independent variables (seeding rate, irrigation, and percent cover of host plants). The most important factor related to reproduction was plant age. Blocks were always significant, and seeding rate was a significant variable in most cases. The most successful seeding plots were in blocks that had high moisture (with and without irrigation), more shade (related to the block analysis), and high golden yarrow cover. Because of differences in threats, habitat, and topography across Tiburon paintbrush occurrences, these population augmentation results provide important lessons but are likely site specific (Niederer *et al.* 2018a, pp. 29–39, 52).

It is important to note that population augmentation at Paintbrush Canyon through 2017 used seeds from seed amplification in a growing facility that came from mixed stock (both Paintbrush Canyon and Paintbrush Hill). Seed tracking, lineage, and parentage were not recorded, and there is no documentation of where mixed seed was sown or how this mixed seed compares to the original population (Hillman and Johnston *in litt.* 2018). Further seeding using that stock was put on hold based on arguments both for and against seed mixing, summarized in Hillman and Johnson (*in litt.* 2018) and Weiss *et al.* (2017). Subsequently, Creekside Science removed all mixed stock and started a new cohort in their greenhouse using seeds from Paintbrush Canyon only. Seeding experiments have continued at Paintbrush Canyon using seeds from the new stock (Service *in litt.* 2020).

In 2013-2017 seeding experiments, yarrow had to be frequently trimmed to prevent it from outcompeting with Tiburon paintbrush (Niederer *et al.* 2018a, p. 50). In subsequent plantings, Creekside Science had higher success growing Tiburon paintbrush in raised beds, troughs, or large containers (Niederer *in litt.* 2020). In these larger containers, host plants no longer outcompeted Tiburon paintbrush (Service *in litt.* 2020).

Recovery criteria

The Recovery Plan for Serpentine Soil Species of the San Francisco Bay Area was issued on September 30, 1998. The Recovery Plan provides recovery criteria for 28 listed plants and animals, including Tiburon paintbrush, using a community-level approach that focuses on habitat protection and management, with specific criteria tailored based on the species (Service 1998). Recovery criteria for downlisting the Tiburon paintbrush have been partially met. A discussion crosswalking the species' status with downlisting recovery criteria in the last status review remains largely up to date (Service 2012, pp. 15–18), and is summarized with an updated assessment in Table 2. Because downlisting criteria have only been partially met, delisting criteria are not considered here.

Table 2. Status of Tiburon paintbrush populations relative to downlisting criteria. Summarizes status of Element Occurrences (EOs), numbered according to the Diversity Database and as shown in Table 1, in 2012 and 2021, with reference to other sections of this document for additional information when appropriate.

Recovery step	Downlist	2012 Status (see also Service 2012, pp. 15–18)	2021 Status
Secure and protect specified recovery areas from incompatible uses	Occupied habitat along with adjacent unoccupied habitat and a 150-meter (500 foot) buffer at six known sites	Partially met. EO 3/4/6/8 protected, but buffer not possible on EO 3/4 because of housing developments. EO 2/5/7/9 not protected.	Partially met. Most populations are protected (through mitigation or permanent protection); see <u>Land protection</u> for more details. Note that EO 6/8 (merged, as noted in <i>Distribution</i>), have some plants on federal land but most are on private land, contrary to the description in Service 2012.
Management plan approved and implemented for recovery areas, including survival of the species as an objective	For all sites and any adjacent occupied or unoccupied habitat identified as essential to continued survival	Partially met. EO 3/4 have Tiburon paintbrush mentioned in management plans, but other EOs do not (although some lands managed for other listed species.)	Partially met. EO 7/9 are also currently being managed for the benefit of the species through the Santa Clara Valley HCP.
Population monitoring in specified recovery areas shows:	Stable or increasing over 20 years that include the normal precipitation cycle (or longer if suggested by the results of demographic monitoring)	Not met. Population monitoring inadequate to establish long-term trends.	Not met, and varies by population. See <i>Abundance</i> and Appendix A.
Other actions	Seeds stored in at least two Center for Plant Conservation certified facilities; Seed germination and propagation techniques understood	Partially met. Seeds from EO 7/9 at one CPC certified facility; experimental propagation not attempted.	Partially met. Seeds from two populations at California Botanic Garden and University of California Botanical Garden; germination and propagation techniques partially understood from experimental plantings. See also <u>Seed banking</u> and <u>Seeding experiments</u> .

Conclusion:

After reviewing the best available scientific information, we conclude that the Tiburon paintbrush remains an endangered species. The evaluation of threats affecting the species under the factors in 4(a)(1) of the Endangered Species Act and analysis of the status of the species in our 2012 5-year review (Service 2012, pp. 8–18) remains an accurate reflection of the species current status.

RECOMMENDATIONS FOR FUTURE ACTIONS:

In this section we propose recommendations which will aid in the recovery and conservation of Tiburon paintbrush. Some of these recommendations have already been discussed in previous recovery documents (Service 1998; Service 2012) and have been carried over into this status review.

- 1) *Continue to control and eradicate non-native, invasive plant species.* Use existing management plans or develop and implement management plans including controlling non-native invasive species for all protected areas.
- 2) *In cooperation with willing landowners, target protection of Tiburon paintbrush occurrences on unprotected lands.* Continue to work with partners to protect occurrences on private lands, including buffers around them when possible. In particular, pursue opportunities to permanently protect land supporting Tiburon paintbrush in the former quarry area adjacent to the Newell Open Space Preserve in Napa County, and the area immediately east of the Golden Gate National Recreation Area land at Nicasio Ridge in Marin County.
- 3) *Monitor or continue to monitor all existing Tiburon paintbrush occurrences using a consistent protocol.* Implement survey protocols carefully to minimize damage to plants (e.g., if attempting to distinguish individual plants) or trampling from group visits.
- 4) *Survey additional serpentine habitats with the potential to support Tiburon paintbrush to determine whether undiscovered populations exist.* Habitat suitability studies or iterative niche modeling could be used to identify potential habitat areas. New populations should be noted with the appropriate County, California Department of Fish and Wildlife, the Service, and the California Native Plant Society.
- 5) *Seeds from Tiburon paintbrush should be collected from populations throughout the range and banked in Center for Plant Conservation certified gardens.* Priority should be placed on populations that do not yet have seeds banked.
- 6) *Conduct or continue research on Tiburon paintbrush.* Continue research on host plants, including testing additional species and host plant preference, and on seed germination. Identify environmental factors that might influence suitability of the habitat in occupied areas (and that might explain absence from other areas). Continue evaluation of exclosures to prevent grazing/rooting and, depending on results, consider additional exclosures or a fence around the Paintbrush Hill population.

Field Supervisor, Sacramento Fish and Wildlife Service

Approve _____ Date _____

LITERATURE CITED

- Besaw, L.M., G.C. Thelen, S. Sutherland, K. Metlen, and R. M. Callaway. 2011. Disturbance, resource pulses and invasion: short-term shifts in competitive effects, not growth responses, favour exotic annuals. *Journal of Applied Ecology* 48: 998-1006.
- Bewley, J.D. 1997. Seed germination and dormancy. *The Plant Cell* 9: 1055–1066.
- Birker, C. 2020. Processing, storage, accession, and germination test summary.
- California Botanic Garden. 2020. Online seed list, current through January 2020. Available online at <https://www.calbg.org/conservation/seed-conservation>. Accessed August 5, 2020.
- California Protected Areas Database. 2020. Available online at <<https://data.cnra.ca.gov/dataset/california-protected-areas-database/resource/2f5efcf0-dffa-4890-92a8-3df3589f0e50>>. Accessed March 1, 2021.
- Chasse, M. 2019. Threatened and endangered vascular plant species activities within the Golden Gate National Recreation Area, calendar year 2018. Unpublished comprehensive annual report for recovery permit TE-036499-9.
- County of Santa Clara, City of San Jose, City of Morgan Hill, City of Gilroy, Santa Clara Valley Water District, and Santa Clara Valley Transportation Authority. December 2012. Tiburon Indian Paintbrush species account within Santa Clara Valley Habitat Plan Volume 4, Santa Clara County, California.
- Creekside Science. 2018a. Creekside Science plant survey report: Paintbrush Hill. Unpublished report including California Natural Diversity Database field survey form.
- Creekside Science. 2018b. Creekside Science plant survey report: Paintbrush Canyon. Unpublished report including California Natural Diversity Database field survey form.
- Grant, V. 1994. Historical development of ornithophily in the western North America flora. *Proceedings of the National Academy of Sciences*. 91: 10407–10411.
- Huenneke, L.F., S.P. Hamburg, R. Koide, H.A. Mooney, and P.M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in California serpentine grassland. *Ecology* 71: 478-491.
- Kay, K. M, and R. Sargent. 2009. The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics* 40:637–656.
- Marin County Parks and Open Space. 2020. Unpublished report containing Tiburon paintbrush Marin County Parks and Open Space Conservation Actions provided to Sacramento Fish and Wildlife Office.
- Niederer, C., S.B. Weiss, J. Quenelle, M. Kent, and C. Schwind. 2018a. 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 for Central Valley Project Conservation Program and Central Valley Project Improvement Act Habitat Restoration Program R12AP20023.

- Niederer, C., S.B. Weiss, and J. Quenelle. 2018b. Tiburon paintbrush projects. Unpublished report prepared for Santa Clara Valley Habitat Agency.
- [Service] 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. Final Rule. Federal Register 60:6671–6685.
- [Service] U.S. Fish and Wildlife Service. 1998. Recovery plan for serpentine soil species of the San Francisco bay area. California/Nevada Operations Office, Region 1. September 30, 1998.
- [Service] U.S. Fish and Wildlife Service. 2012. *Castilleja affinis* spp. *neglecta* (Tiburon paintbrush) 5-Year Review. Sacramento, California.
- [Service] U.S. Fish and Wildlife Service. 2014. Reinitiation of formal consultation on the Kirby Canyon landfill development project in Santa Clara County, California. Service file number 2013-F-0612. Sacramento, California.
- [Service] U.S. Fish and Wildlife Service. 2020. Four Endangered Serpentine Plants in Santa Clara County Meeting Notes. Meeting held virtually via Teams on December 10, 2020.
- Swiecki, T., and E. Bernhardt. 2015. Phytophthora in nursery stock and restoration plantings. Phytosphere Research. Available online at <http://phytosphere.com/soilPhytophthora/Propagation.field.material.htm>. Updated February 5, 2021. Accessed February 25, 2021.
- Swiecki, T., and E. Bernhardt. 2016. Best management practices for producing clean nursey stock. Available online at <http://phytosphere.com/BMPsnursery/Index.htm>. Accessed February 25, 2021.
- University of California Botanic Garden. 2020. Plant collection database. Available online at <https://webapps.cspace.berkeley.edu/botgarden/search/search/>. Accessed August 5, 2020.
- Valley Water. 2020. Population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*). Unpublished report.
- Valley Water. 2021. Population monitoring, seed collection and banking, and habitat assessment for the Paintbrush Hill occurrence of Tiburon paintbrush (*Castilleja affinis* ssp. *neglecta*). Unpublished report.
- Weiss, S. B., J. Fant, and L. Widener. 2017. Seed mixing between the Paintbrush Hill and Paintbrush Canyon populations of *Castilleja affinis neglecta*. Unpublished report.
- Widener, L., and J. B. Fant. 2018. 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*). Conservation Genetics 19: 365–381.

In litteris:

- Forbes, H. Curator, University of California Botanical Garden. Email correspondence cited in Hillman and Johnston *in litt.* 2018.

Hillman, J., and D. Johnston. 2018. Senior Biologist/Plant Ecologist, Environmental Mitigation & Monitoring Unit, Watershed Stewardship & Planning Division, Santa Clara Valley Water District; and California Department of Fish and Wildlife (retired). Attachment entitled Summary of Genetics Issue and Tiburon Paintbrush (*Castilleja affinis* ssp. *neglecta*) to email dated September 25, 2018.

Niederer, C. 2013. Senior Biologist, Creekside Center for Earth Observation. Email with California Natural Diversity Database forms, shapefiles, and notes for Tiburon paintbrush observations in 2013, sent to multiple federal and state organizations and academic institutions on July 30, 2013.

Ruygt, J. 2020. Email correspondence to Samantha Lantz on August 8, 2020 regarding Newell Preserve Tiburon paintbrush occurrence.

[Service] U.S. Fish and Wildlife Service. 2020. Four endangered serpentine plants in Santa Clara County meeting notes. Meeting held virtually via Teams on December 10, 2020.

Smith, D. 2020. Rare Plant Committee Co-chair, Marin Chapter of the California Native Plant Society. Email correspondence to Samantha Lantz on August 7, 2020.

Personal communications:

Bryant, K. 2007. President, Santa Clara County Chapter of the California Native Plant Society. Phone call cited in Santa Clara County 2010.

Chasse, Michael. 2013. Biologist, National Park Service. Personal communication cited in Niederer *et al.* 2018a.

Cromer, Carolyn. 2009. Ecologist, Land Trust of Napa County. Telephone conversation with Maral Kasparian, U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office.

Magallanes, Amanda and Sarah Minnick. 2020. Ring Mountain Stewardship Coordinator and Vegetation and Fire Ecologist at Marin County Parks. Phone call with Samantha Lantz and Amber Aguilera, Sacramento Fish and Wildlife Office on October 6, 2020.

Niederer, C. 2020. Senior Biologist, Creekside Center for Earth Observation. Email to Samantha Lantz, Sacramento Fish and Wildlife Office, dated December 14, 2020. Subject: Paintbrush canyon update.

Ruygt, Jake. 2009. Botanist, J. Ruygt Consulting. E-mail to Maral Kasparian, U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office.

Smith, Doreen. 2013. Rare plant co-chair, Marin County Chapter of the California Native Plant Society. Personal communication referenced in Niederer *et al.* 2018a.

APPENDIX A

Table A1. Summary of abundance counts or estimates for all current Diversity Database element occurrences.

County	Site name	Diversity Database element occurrence	Year	Abundance	Source
Marin	Saint Hilary's	2	1961	3	Diversity Database 2020
Marin	Saint Hilary's	2	1981	10	Diversity Database 2020
Marin	Saint Hilary's	2	1986	25	Diversity Database 2020
Marin	Saint Hilary's	2	1988	101	Diversity Database 2020
Marin	Saint Hilary's	2	1989	not found	Diversity Database 2020
Marin	Saint Hilary's	2	1996	not found	Diversity Database 2020
Marin	Saint Hilary's	2	1997	13	Diversity Database 2020
Marin	Saint Hilary's	2	1999	present	Diversity Database 2020
Marin	Saint Hilary's	2	2013	not found	Diversity Database 2020
Marin	Saint Hilary's	2	2015	8	Marin County Parks and Open Space 2020
Marin	Saint Hilary's	2	2016	13	Marin County Parks and Open Space 2020
Marin	Middle Ridge	3	1983	100+	Diversity Database 2020
Marin	Middle Ridge	3	1986	~25	Diversity Database 2020
Marin	Middle Ridge	3	1988	191	Diversity Database 2020
Marin	Middle Ridge	3	1990	31	Diversity Database 2020
Marin	Middle Ridge	3	1997	228	Diversity Database 2020
Marin	Middle Ridge	3	2007	~850	Diversity Database 2020
Marin	Middle Ridge	3	2013	200+	Diversity Database 2020
Marin	Ring Mountain	4	1983	<100	Diversity Database 2020
Marin	Ring Mountain	4	1986	>50	Diversity Database 2020
Marin	Ring Mountain	4	1987	454	Diversity Database 2020
Marin	Ring Mountain	4	1988	661	Diversity Database 2020
Marin	Ring Mountain	4	1989	821	Diversity Database 2020
Marin	Ring Mountain	4	1996	50-100	Diversity Database 2020

County	Site name	Diversity Database element occurrence	Year	Abundance	Source
Marin	Ring Mountain	4	1997	63	Diversity Database 2020
Marin	Ring Mountain	4	2007	~500	Diversity Database 2020
Marin	Ring Mountain	4	2013	1000+	Diversity Database 2020
Marin	Ring Mountain	4	2015	1555	Marin County Parks and Open Space 2020
Marin	Ring Mountain	4	2016	2159	Marin County Parks and Open Space 2020
Marin	Ring Mountain	4	2017	1401	Marin County Parks and Open Space 2020
Marin	Ring Mountain	4	2018	1262	Marin County Parks and Open Space 2020
Marin	Ring Mountain	4	2019	1946	Marin County Parks and Open Space 2020
Marin	Ring Mountain	4	2020	2422	Marin County Parks and Open Space 2020
Marin	Nicasio Ridge	8	1991	~50	Diversity Database 2020
Marin	Nicasio Ridge	8	1994	28	Diversity Database 2020
Marin	Nicasio Ridge	8	1995	25	Diversity Database 2020
Marin	Nicasio Ridge	8	2000	84	Diversity Database 2020
Marin	Nicasio Ridge	8	2001	68	Diversity Database 2020
Marin	Nicasio Ridge	8	2004*	4	Chasse 2019
Marin	Nicasio Ridge	8	2008*	18	Chasse 2019
Marin	Nicasio Ridge	8	2009*	39	Chasse 2019
Marin	Nicasio Ridge	8	2010*	56	Chasse 2019
Marin	Nicasio Ridge	8	2011*	55	Chasse 2019
Marin	Nicasio Ridge	8	2012*	140	Chasse 2019
Marin	Nicasio Ridge	8	2013*	144	Chasse 2019
Marin	Nicasio Ridge	8	2014*	139	Chasse 2019
Marin	Nicasio Ridge	8	2015*	179	Chasse 2019
Marin	Nicasio Ridge	8	2016*	204	Chasse 2019
Marin	Nicasio Ridge	8	2017*	210	Chasse 2019
Marin	Nicasio Ridge	8	2018*	176	Chasse 2019
Marin	Nicasio Ridge	8	2019*	238	Chasse 2019
Napa	American Canyon	5	1986	>186	Diversity Database 2020

County	Site name	Diversity Database element occurrence	Year	Abundance	Source
Napa	American Canyon	5	1988	588	Diversity Database 2020
Napa	American Canyon	5	1990	present	Diversity Database 2020
Napa	American Canyon	5	2013	~170	Diversity Database 2020
Napa	American Canyon	5	2020	present	Ruygt <i>in litt.</i> 2020
Santa Clara	Paintbrush Canyon	9	2002	~5	Diversity Database 2020
Santa Clara	Paintbrush Canyon	9	2006	~1000	Diversity Database 2020
Santa Clara	Paintbrush Canyon	9	2009	1100	Diversity Database 2020
Santa Clara	Paintbrush Canyon	9	2013	1000	Diversity Database 2020
Santa Clara	Paintbrush Canyon	9	2018	1900 ± 375	Creekside Science 2018b
Santa Clara	Paintbrush Hill	7	1993	17	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	1994	9	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	1997	27	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	1999	80	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	2006	>140	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	2009	103	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	2013	~100	Diversity Database 2020
Santa Clara	Paintbrush Hill	7	2018	224	Creekside Science 2018a
Santa Clara	Paintbrush Hill	7	2019	222**	Valley Water 2020
Santa Clara	Paintbrush Hill	7	2020	139***	Valley Water 2021

*since 2004, counts have only included plants on Golden Gate National Recreation Area property (not private lands)

**count includes 46 seedlings

***count includes 5 seedlings