Population trends, demography, and the effects of environment and disturbance on *Cypripedium fasciculatum* **in southern Oregon.** Final Project Report

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Preface

This report is the result of a cooperative Challenge Cost Share project between the Institute for Applied Ecology (IAE) and a federal agency. IAE is a non-profit organization dedicated to natural resource conservation, research, and education. Our aim is to provide a service to public and private agencies and individuals by developing and communicating information on ecosystems, species, and effective management strategies and by conducting research, monitoring, and experiments. IAE offers educational opportunities through 3-4 month internships. Our current activities are concentrated on rare and endangered plants and invasive species.

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Abstract

Cypripedium fasciculatum Kellogg ex S. Watson (clustered lady's slipper, Orchidaceae) is a rare woodland orchid that can be found in scattered populations throughout northwestern North America. Although several studies have explored its relationship with mycorrhizal fungi and habitat associates, there has been little information on the species' demography, including determining the likelihood that this species has a dormant life stage and population growth rates. Information is also limited on the effects of disturbance on this species. This report summarizes a ten-year demographic study of 28 *Cypripedium fasciculatum* populations in southwest Oregon that was established to address some of these information gaps.

The number of emergent stems and proportion of flowering plants varied significantly between sites and years. In general, 13% - 45% of emergent plants will become dormant the following year. The majority of dormant plants re-emerged after one year. Demographic analyses found that the growth rate for these populations was less than one, suggesting that they will eventually decline to zero.

Analyses of habitat variables found that the population size and stability in flowering and plant size were generally associated with higher basal area of trees and cover of low- to midlevel vegetation. However, it is important to note that our studies were conducted in relatively large populations and thus habitat characteristics are expected to be within the preferred range for this species. It is likely that there would be a negative response by *C. fasciculatum* if cover of any of these habitat layers were to significantly increase or decrease.

Poor replication and a lack of records prohibited making strong conclusions regarding the effects of tree thinning and fire on *C. fasciculatum*. We recommend future studies using replicated treatments (including thinning of the mid-story vegetation and low severity ecological burns) to determine if these management techniques could maintain *C. fasciculatum* habitat while having a neutral to positive effects on the species.

Although there was significant variability in population size and reproduction, we did not find any habitat nor environmental factors to explain this variation. As these populations have a declining growth rate, we recommend that future studies focus on determining the best methods for reintroduction of this species and methods to manage habitat that do not harm existing populations.

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Introduction

Cypripedium fasciculatum Kellogg ex S. Watson (clustered lady's slipper; Figure 1) is a rare woodland orchid that occurs in coniferous forests. Cypripedium fasciculatum is found in scattered locations throughout western North America. In Oregon, it occurs in the Blue Mountains in the northeastern part of the state and in the Klamath- Siskiyou and western Cascades ecoregions of Southwestern Oregon, where approximately 683 populations have been reported on federal land (Vance et. al. 2004). This species is a U.S. Fish and Wildlife Species of Concern, a Bureau of Land Management Sensitive Species in Oregon, Washington and



Figure 1. *Cypripedium fasciculatum* (clustered lady's slipper).

California, a U.S. Forest Service Region 6 Sensitive species, and an Oregon State Department of Agriculture Candidate Species for listing on the state threatened and endangered list. It is on List 2 (threatened with extirpation) of the Oregon Natural Heritage Information Center (ORBIC 2010).

Despite its extensive range, little is known about the demography of *C. fasciculatum*, in part because of its complex life history and cryptic life stages (Figure 2). *Cypripedium fasciculatum* is a long lived perennial that can enter dormancy for one or more years, then reemerge above-ground. Multiple aerial stems can arise from the rhizome of a single *C. fasciculatum* genetic individual creating some ambiguity as to identification of individual plants. These characteristics can also make it difficult to quickly assess the effects of environmental factors and disturbances on population dynamics.

There is also uncertainty about the best strategies for managing *C. fasciculatum* habitat. Seevers and Lang (1998) found that population threats included physical site disturbance and changes in forest canopy structure (loss of microclimate). The authors recommended maintaining canopy closure at or above 60%, maintaining litter layer to retain moisture, and avoiding prescribed fire. In contrast, Latham (2000) suggested that understory vegetation in *C. fasciculatum* habitats should be thinned to create more open habitat.

In 1998, a long-term demographic study of *C. fasciculatum* populations in southwest Oregon was initiated (Latham and Hibbs, 2001). The goals of this study were to (1) Assess the status and demographic structure of *C. fasciculatum* populations in southwest Oregon, (2) Describe habitat characteristics and relationship of the species population characteristics to major environmental variables, (3) Identify biological traits useful in monitoring, and (4) Evaluate the effects of disturbance on *C. fasciculatum* populations.



Figure 2. Cryptic life stages of *Cypripedium fasciculatum*. (a) Seedlings are very small and may be very easy to miss during population surveys. (b) This species may remain dormant for one or more years as with this excavated rhizome.

Methods

Species description

Cypripedium fasciculatum occurs in widely disjunct locations from north central Washington south through Oregon to central California and east to the mountains of Idaho, Montana, Colorado, Wyoming, and Utah. In Oregon, this taxon occurs predominantly in the Klamath Mountains in the southwest corner of the state.

While Cypripedium fasciculatum has a relatively large range, it is restricted to narrow habitats within this range. In southwest Oregon, it is most commonly found in sites below 2800', on a northwest to northeast aspect, and an average slope 75% (Latham 2000). This species is found in Pseudotsuga menziesii (Douglas-fir) forests where trees are at least 70 years old (Knecht 1996) and there is little understory vegetation (Latham 2000). Other species occurring with C. fasciculatum tend to be early- to mid-, or mid- to late-seral indicators, suggesting that this is a mid-successional species (Knecht 1996). Brown (2008) found that C. fasciculatum was more likely to occur in microsites with Cornus nuttallii cover, steep slopes, moist soils, and open overstories. He suggested the relationship with C. nuttallii was because C. *nuttallii* filters the light, whereas a uniform cover of conifers can allow in direct light. Other associated species included Acer glabrum, Adenocaulon bicolor, Calocedrus decurrens, Carex multicaulis, Chimaphila menziesii, Clintonia uniflora, Prosartes hookeri, Festuca occidentalis, Galium bolanderi, G. trifidum, Goodyera oblongifolia, Lithocarpus densiflorus, Pinus lambertiana, Quercus vacciniifolia, and Maianthemum racemosum (Brown 2008). Cypripedium fasciculatum can also be found in mixed successional forests in overstory openings where the shade is provided by shrubs, saplings, and large perennial forbs (Hollis et al. 2002). In a study of C. fasciculatum populations occurring throughout the Cascade range, Knecht (1996) found that

while some regional habitat trends were evident, none occurred range-wide. Soil properties also lacked regional trends, although pH ranged from 5.70 - 6.65, higher than expected in a coniferous forest.

Cypripedium fasciculatum is small, measuring less than 18 cm from the base to the apex. It has two opposite, elliptical leaves with a total leaf span up to 30 cm. The stem is conspicuously puberulent. In most cases, there is a single miniature bract between the leaves and the flowers. The flowers are small compared to most other *Cypripedium* species, only 4.5 cm from tip to tip. Flower color ranges from brown markings on a green or golden background to predominately reddish-brown. The flowers are found in clusters of two to ten at the end of the stem, often causing the stem to droop under their weight (Figure 1). The fruits are 2 cm oblong capsules that contain thousands of small, dust-like seeds. *Cypripedium fasciculatum* has a small, shallow rhizome with fibrous roots that produce a dormant bud during the current year's growing season (Harrod 1994). This bud remains inactive through the winter, then bolts in April to produce an aerial stem.

Pollinators of this species are probably stingless parasitic wasps (Family Diapriidae, Subfamily Belytinae, genus *Cinetus*; Ferguson and Donham 1999, Ferguson et al. 2000). Seed dispersal is limited to a little over 1m (Harrod and Everett 1993). Fruit set in out-crossed *C*. *fasciculatum* was higher than in self-pollinated plants, however, this species is capable of both mating systems (Knecht 1996, Lipow et al. 2002). This species is pollinated by Diapriidae wasps and cannot mechanically self-pollinate. Fruit set is positively correlated with leaf size, but there is no correlation with flower number (Knecht 1996, Lipow et al. 2002). Each fruit produces an extremely large numbers of seeds that are dispursed near the parental plant. Harrod and Everett (1999) found that no seeds were collected further than 190 cm from the parent plant, and 95% of all seeds were within 130 cm.

Cypripedium fasciculatum forms associations with numerous species of mycorrhizal fungi. This species requires the presence of *Rhizoctonia* for germination (Arditti 1967, Wells 1981). Whitridge (2004) found that *C. fasciculatum* formed associations with several ectomycorrhizal species in the genera *Russula, Tulasnella*, and *Tomentella*, and suggested that *C. fasciculatum* may parasitize fungi as an intermediate between non-photosynthetic, mycoheterotrophic orchid and non-orchid species. Shefferson (2005, 2007) similarly found association between *C. fasciculatum* and ectomycorrhizae and suggested that this association may contribute to survival during dormancy. However, recent isotope analyses suggest that this species does not dependent on ectomycorrhizae (S. Copeland, *personal communication*).

Study overview

Initially, 29 *C. fasciculatum* populations occurring on federal land in 5 environmental regions were selected for this study (Latham and Hibbs, 2001). A few of these sites had been monitored since 1996. A total of 892 *C. fasciculatum* plants were tagged and monitoring began for the majority of sites in 1999. Additional sites were added in subsequent years and the number of sites increased to 39. In 2003, the Medford BLM took-over management of the project and reduced the number of sites to 29, though these were not all the original 29 sites. Medford BLM partnered with the Institute for Applied Ecology to continue monitoring in 2004. In 2005, after four years without emergent plants, several locations where plants had previously been located were excavated. We found no living rhizomes and population monitoring was discontinued. Monitoring of the remaining populations continued until 2007.

In this report, we include data from a total of 29 sites, include demography data from 27 sites, count plots (five sites, 2 of which were not included in the demographic surveys), and the Murphy Gulch grid (which contained several demography plots). The sites represent five different ecological regions in Southwest Oregon that were classified by temperature, precipitation and soil type (Latham and Hibbs, 2001). Medford BLM owns the majority of study sites, but 3 sites are on Rogue River-Siskiyou National Forest land (Table 1, Figure 3). Site elevations range from 354 meters to 1360 meters above sea level.

Several of the sites were selected for small-scale treatments to determine the effects of disturbance and litter on *C. fasciculatum*. Wellington Butte #3 (a relatively large and scattered population) was selected for a thinning treatment (Latham and Tappeiner, 2000). Conventional thinning never occurred, but tree girdling and shrub removal treatments were conducted in the spring of 2002. Plans also included manual litter removal by hand raking half of the plots in each thinning treatment (Latham and Tappeiner, 2000); however it is uncertain whether or not ground disturbance treatments occurred. Tree girdling appeared to occur at Murphy Gulch prior to 2003, but there are no records of the extent or exact timing of this treatment. In order to explore the effect of fire on *C. fasciculatum*, Round Prairie A and B were burned in 2000 after an understory thinning. Round Prairie C and D were left untreated as controls. Finally, in order to determine if litter accumulation inhibited germination, litter removal and seed addition plots were established at Alexander Gulch A, B, and C, White Horse Park, and Taylor Creek.

From 2000 - 2004, surveys were conducted twice each year. Emergence, size class, flowering status, and herbivory were assessed from early April through May. Site with flowering plants were surveyed a second time to determine fruiting. From 2005 - 2007, each site was visited once, during their fruiting stage, from mid-May to late June.

Plot design and sampling

Demography plots

Demography plots were 2 x 2 m permanent plots in which all plants were mapped, tagged and measured ("demography plots"). The initial number of tagged plants at sites with demography plots ranged from 1 to 187 plants. 2m x 2m demography plots were dissected into four 1 m^2 quadrants. Plot centers were marked with a metal curly-que. Plots were generally oriented with the top uphill; the azimuth for most plots was documented. Cypripedium fasciculatum stems were marked with numbered aluminum tags and their location was mapped. When multiple stems emerged from the same rhizome, we tagged all stems, noting that they were from the same individual (Seevers and Lang, 1998, Hollis et al. 2002). Although the exact location of emergence varied some from year to year, it was generally possible to identify and distinguish individual plants. If a stem recorded in previous years was absent and a tag was missing, it was not replaced. If a plant occurred in close proximity to, but outside of a plot border, it was recorded in the "0" quadrant (Figure 2) and the position was carefully mapped. Many new plants were found in 2004 at Wellington Butte #3 around and between Plots 14 and 15. These plants were all recorded as associated with one of the established demography plots. Additional new plants were found in this area in 2005 and 2006. These plants were tagged and monitored individually and may provide useful information about this apparently dynamic site.

We noted presence and absence of all stems in each plot. When plants were present, we measured the leaf lengths, and the number of undeveloped buds, flowers, and capsules. The number of flowers was estimated by counting floral bracts, which subtend each flower. In

general, buds that did not mature were so small that they either did not have a bract or the bract itself was so tiny that it would not be confused with a functional flower bract. Most undeveloped buds were small, translucent, whitish nubs at the center of the leaf axil. Sometimes they were larger and identifiable as flower buds, but could be distinguished by their lack of color, small size, and lack of development.

Site name	Enviro. Region	Elevation (m)	on Demographic Cour Plots Plots		Additional plots or treatments
Alama a la a Carlah A	4	020	10	(
Alexander Gulch A	4	939	10	0	Cooding plat
Alexander Gulch D	4	927	11 o	0	Seeding plot
Revaluer Guich C	$\frac{4}{3(1)^2}$	927 500	0	0	Seeding plot
Emigrant Crack	5 (1) 5	1008	0	24	
Eningrant Creek	J 4	1098 720	4	0	
Keeler Creek	4	500	19	0	
Logan-Bridegview	$\frac{4}{3}(2)^2$	506	$\frac{2}{0}$	6	
Murphy Gulch	3 (2)	790	0	0	20 m x 28 m cansus grid.
Murphy Gulen	5	790	+	0	20 III X 28 III census grid,
D1 + V - 11	2	(92	(0	Tree girdning
Pleasant Valley	3 5	083	6	0	
Randecore Pass	$\frac{5}{2}$	1195	2	0	Construct because
Round Prairie A	$\frac{3(2)}{2(2)^2}$	396	8	0	Control burn
Round Prairie B	$3(2)^{-}$	365	15	0	Control burn
Round Prairie C	$3(2)^{2}$	335	14	0	
Round Prairie D	$3(2)^2$	335	13	14	
Sexton Mountain 2	$3(1)^2$	884	7	0	
Slick Rock	3	762	10	0	
Soda Creek 2A	5	930	1	0	
Soda Creek 2B	5	945	1	0	
Soda Creek 3	5	1042	13	6	
Sykes Creek	3	610	3	0	
Tallowbox	4	1360	3	0	
Taylor Creek ¹	$3(1)^{2}$	369	25	0	Wildfire
Taylor Ridge ¹	$3(1)^2$	610	3	0	
Tyler Creek	5	1042	1	0	
Wellington Butte #1	4	1067	3	0	
Wellington Butte #3	4	927	35	0	Tree girdling, possibly litter
					raking near plots 14 & 15
Wellington Butte #4	4	975	6	0	
Whitehorse Park	$3(1)^2$	354	9	0	Seeding plot

Table 1. Cypripedium fasciculatum monitoring sites and characteristics.

¹French Gulch is on the Rogue National Forest, Taylor Creek and Taylor Ridge are on the Siskiyou National Forest; all other sites are on the Medford District Bureau of Land Management.

²Due to similarity in climate and need for replication, Environmental regions 1 and 2 were combined with environmental region 3. The original environmental region is noted in parentheses.



Figure 3. Distribution of *C. fasciculatum* in Oregon and sites included in this study. Sites were grouped into three environmental regions.

Browsing and the presence of "neighbors" (any other *C. fasciculatum* plants with stems within 2.54 centimeters) were noted. Browsing was recorded in six categories based on ocular estimation of the percentage of plant material that has been consumed. Browsing categories were defined as 0= none, $1 \le 5\%$, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 75-100%.

Vegetation cover data were also collected within each plot. Variables included cover of mineral soil, litter and duff, moss, ferns, graminoids, forbs, shrubs, and trees. Ferns, graminoids, forbs, shrubs, and trees were each separated into two height categories, 0-15cm and >15-2m tall.

A number of additional variables (e.g. litter depth) were measured during the study, but are not included here in analyses due to redundancy with analyzed variables.

Count plots

Count plots were initiated in 2000. Prior to 2004, additional 2 x 2 m count plots were set up when new *C. fasciculatum* plants were found in the general vicinity of a monitored population. This occurred at three populations, Alexander Gulch A, Round Prairie D, and Soda Creek. Only count plots were installed at Brimstone and Logan-Bridgeview. Plants in the count plots were counted, but not mapped or tagged. At Murphy Gulch, a large grid the encompassed several demography plots was established to census to whole population.

Similar to the demography plots, count plots were $2m \times 2m$ plots dissected into four 1 m^2 quadrants. Plot centers were marked with a metal curly-que. Plots were generally oriented with the top uphill; the azimuth for most plots was documented. In each count plot, all *C*. *fasciculatum* stems in each size class were counted. The total number of flowers and fruits in each plot were also recorded.

Murphy Gulch grid

At Murphy Gulch, a 20 m x 28 m monitoring grid divided into 140 $2x2 \text{ m}^2$ cells was established to census the population. Plants in the cells were tallied by their vegetative or reproductive status. In addition, 4 demography plots were also established at this site. These plots were positioned to capture as many *C. fasciculatum* individuals as possible, and did not align with the 2m x 2m grid cells. These demography plots were tagged and tracked as above and included in the demographic analyses.

Tree plots

At each site a 8m radius, 0.02ha plot was established to characterize type of size of trees. Within the plot, the basal area of each tree over 1.37m was measured.

Disturbance treatments

To examine the response of this species to various forms of disturbance, experimental treatments, including seeding, forest thinning, scraping to remove litter, and controlled burns were established at several sites (Table 1).

Forest thinning

To evaluate the effects of forest thinning on *C. fasciculatum*, tree girdling was used to kill trees in *C. fasciculatum* habitat at Wellington Butte #3 and Murphy Gulch. At Wellington Butte, this treatment also included shrub cutting (shrubs were cut at the base but their debris was left in place). Tree girdling was only partly effective, killing some trees but not others. Raking of litter

had been planned at this site (Latham and Tappeiner 2000), but no clear records of that treatment were available. However, in and around plots 14 and 15, the litter layer was observed to be very thin or non-existent during site visits in 2004 and 2005. Tree girdling also occurred at Murphy Gulch; although the exact year of implementation is unknown, it was prior to 2003.

Fire

In fall 2000, two of the four Round Prairie sites (Round Prairie A and B) were burned during a controlled burn. Fire intensity was moderate (Mark Mousseaux, *personal communication*). Plant responses to these treatments were measured by comparing the change through time in the burned and unburned Round Prairie sites.

A small wildfire overlapping the Taylor Creek *C. fasciculatum* site burned through the area between the 2004 and 2005 visits. We mapped the area that appeared to be affected by the fire. The fire burned through the southeast corner, burning all of plot 15 and part of plots 2, 12, and 13. A fire line was constructed through plot 16, scraping down to mineral soil and removing organic debris from a strip in the upper half of the plot and depositing it into the lower area. We identified the plants that were burned in the area and recorded the survival of burned and unburned plants.

Seeding plots

Seeding plots were established at Alexander Gulch A, Alexander Gulch B, Alexander Gulch C, and Whitehorse Park. One half of each plot (one plot per site) was scraped to mineral soil prior to seeding; litter remained intact in the other half of each plot. An unknown quantity of seeds was distributed over all plots in 1999. Plots were monitored for *C. fasciculatum* each year through 2007.

Relationships between population characteristics

We used stepwise linear regression (SPSS 19, 2011) to test for a relationship between length of the longest leaf and total leaf length and the proportion of flowering and fruiting plants. Variables were averaged within sites and years prior to analysis. Analyses were conducted for plants in all demography plots from 1999-2007. 1998 was excluded from analyses due to insufficient information.

Evaluation of dormancy and mortality

We estimated the rate of dormancy using two methods. First, we used the demographic data to determine the fate of tagged plants which did not emerge for one or more consecutive years. Nine hundred tagged plants were missing for one or more years their initial observation. Plants which were never seen again aboveground were presumed dead. Plants which subsequently re-emerged were presumed to have been dormant for that time period. In this analysis, we assumed that plants that were absent in 2007 were dead; thus, mortality rates may be over-estimated (and dormancy rates underestimated).

Second, from 2005 to 2007, we haphazardly selected plants that had been noted as absent for 3 to 9 years for excavation to determine if they were dormant or dead. We excavated a total of 131 plants, 6 in 2005, 9 in 2006, and 116 in 2007. Plants were noted as dormant if a portion of living rhizome remained. If the rhizome was missing or decayed, the plant was determined to be dead.

Demographic models

Tagged plants in the demographic plots were classified as one of 4 stages: Small (S), Vegetative (V), Flowering (F), or Dormant (D) (Figure 4, Table 2). Dormant plants were those that were absent for 1 or more years and subsequently re-emerged. This means that survivorship of dormant plants in the model was always 100%; plants that were dormant and died without reemergence were included in mortality from their last above-ground stage. Plants that emerged in a plot within 2 years of the start of monitoring were assumed to be plants emerging from dormancy rather than new recruits. These stages were selected for three reasons. Flowering plants were



Figure 4. Life-cycle diagram of staged-based model for C. fasciculatum. Solid arrows represent survivorship and growth, dashed arrow indicates fecundity.

Table 2. Parameters used to classify tagged plants into each stage.							
Stage	Flowering	Number of stems	Length of longest leaf				
S (small)	No	1	<50 mm				
V (vegetative)	No	≥1	\geq 50 mm				
F (flowering)	Yes	≥ 1	Any				

likely to be larger than non-flowering plants (Figure 5) and had an increased probability of flowering again compared to other plants. Finally, small plants had an increased probability of mortality compared to vegetative and flowering plants.

Transition probabilities (solid arrows in Figure 4) were estimated for each year of the study, excluding 1998-1999 because of small numbers of tagged plants in 1998. Tagged plants were grouped by environmental region to obtain sufficient sample sizes. This makes the assumptions that transition probabilities do not vary predictably between sites within an environmental region (e.g., factors like site population size do not affect growth or survivorship of individuals). Sites affected by management actions (fire, tree girdling) were excluded from the data. A total of 11,490 plants were tagged throughout the study. Because the number of individuals entering or remaining in dormancy could not be estimated for the last year, these transitions were replaced with the mean value from previous years. This yielded 8 complete transition matrices for each environmental region (24 matrices in total).

Fecundity and Recruitment

Estimating fecundities for C. fasciculatum was problematic in that the linkage between fruit production and recruitment to the aboveground population is cryptic. Seedlings likely remain belowground for 1 or more years. Each year, large numbers of very small seed is produced; however recruitment is infrequent. Additionally, newly emerged recruits are indistinguishable from small plants emerging from dormancy, making estimates of the number of new recruits each year imprecise. For the purposes of this study, we used the following criteria to identify new recruits: plants newly identified in the plots 2 or more years after monitoring began in that plot, which were not flowering, had only 1 stem, with the length of the longest leaf < 50mm. This means recruitment could not be estimated for the first two years that a plot was monitored.



Figure 5. Number of stems vs. length of longest leaf for flowering (1, solid line) and vegetative (0, dotted line) plants.

Contrary to our expectations, the number of new recruits was not correlated with any variables related to reproduction (number of flowering plants, number of fruits) at any time lag. The strongest correlation was with the total number of plants in the same year. Given that seed is unlikely to be limiting in these populations, we decided to link recruitment to the number of plants in the current timestep rather than the number of plants in a previous timestep. We estimated the average number of new recruits per plant based on the slope of a linear regression of number of recruits vs. number of above ground plants, assuming a y-intercept of zero (Eq. 1).

$$\overline{NR}_t = n_{_{S+V+F}}(t+1) \times 0.03 \tag{1}$$

The mean number of recruits did not vary significantly by environmental region or year, so the same recruitment function was used for all transition matrices.

Environmental and demographic stochasticity

As we were concerned about extinction risk and dynamics of small population sizes, we felt it important to incorporate demographic stochasticity in our models. Traditional matrix models allow for fractional individuals; while this is not an issue for larger population sizes, it is crucial for small population sizes. Demographic stochasticity was incorporated into simulation modeling following Caswell (2001), using a multinomial distribution to generate the number of individuals in each stage based on the matrix transition probabilities.

We modeled each environmental region separately, using stochastic matrix projection, incorporating both environmental and demographic stochasticity. At each time step, one of the 8 projection matrices A_k was randomly selected to project the population to incorporate environmental variability. Demographic stochasticity was incorporated into projecting vital rates as well as fecundities. For each time step, we first calculated the contribution of individuals in each stage *j* to the population vector $\mathbf{n}(t+1)$ by generating a random vector from a multinomial distribution with parameters $\mathbf{a}_{\cdot j}$ and $n_j(t)$, where $\mathbf{a}_{\cdot j}$ is the vector per-capita transitions of stage *j* to all stages and death.

Fecundity was modeled as a Poisson distribution. The number of new individuals added to stage S is given by:

$$NR_{(t+1)} = \text{Pois}(n_{S+V+F(t+1)} \ge 0.03)$$
(2)

To summarize, the algorithm for projecting the population from $\mathbf{n}(t)$ to $\mathbf{n}(t+1)$ was:

- 1. Randomly select transition matrix A_k from k=1,2,...8.
- 2. For each stage *j*, generate a random vector from a multinomial distribution with parameters $\mathbf{a}_{\cdot j}$ and $n_{j(t)}$. This vector gives the number of individuals in each stage (including death) produced by transitions of the $n_{j(t)}$ individuals in stage *j* at time *t* (Caswell 2001).
- 3. Repeat step 2 for all stages and add the results to give the number of individuals at t+1 produced by survivorship and growth.
- 4. Calculate new recruits using eq. (2).
- 5. Add the individuals produced by transition (survivorship and growth) and recruitment to obtain $\mathbf{n}_{(t+1)}$.

Simulated population projections

We projected simulated populations from each region to estimate average annual growth rate (lambda), extinction risk, and forecast population sizes. Population projections make the basic assumption that conditions in the future will remain the same as the study period during which transition probabilities were calculated. Additionally, it is assumed that each projected population is an entire cohesive population of intermixing individuals, rather than a collection of small populations with randomly varying transition probabilities.

For each region, we set an initial above ground population size $n^{above}_{(0)}$ equal to 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 50, 75, or 100 individuals. Individuals were distributed to each aboveground stage according to the distribution of individuals in each stage across the environmental region. Additionally, the number of individuals in the dormant stage was calculated based on the same distribution. For each initial population size, we projected the population forward 30 years with 1,000 iterations. For each iteration, we recorded whether or not the population went extinct, the final population size, the final above-ground population size, and the distribution of individuals in each stage.

Demographic modeling data analysis

R 2.8.1 was used for all demographic modeling.

Community and environmental effects of C. fasciculatum populations

We used non-parametric multiplicative regression [NPMR; Hyperniche v. 2.0 (McCune and Mefford 2009)] to determine which community and environmental factors had the greatest impact on number and characteristics of *C. fasciculatum*. We analyzed both the average and coefficient of variation (CV) of four response variables (flowers, fruits, leaf area, and number of plants per site). The average was taken over time at each site. Although this metric allows comparisons of the factors contributing to 'success' (e.g. populations with a larger number of plants would tend to have a larger average N), an average masks variation over time. For example, a population with large fluctuations in population size a mean may have the same average N as a population that is much more stable. Thus, we also analyzed the CV of each response variable. The CV is calculated as

$$CV = \frac{s}{r} \tag{3}$$

The CV has the advantage that it is a dimensionless number and allows for comparisons of patterns between populations with different population size. We grouped predictors into community and environmental variables. Due to the low number of replication, we were limited in the number of variables that could be analyzed. Thus we grouped cover of different vegetation types into aggregate variables (Table 3).

NPMR uses a local multiplicative smoothing function with leave-one-out cross-validation to estimate the response variable. We used a Gaussian weighting function with a local mean estimator in a forward stepwise regression of our response variable against the predictors, then expressed fit as a cross-validated R^2 (or xR^2). The xR^2 differs from the traditional R^2 because each data point is excluded from the basis for the estimate of the response at that point. Consequently, with a weak model, the residual sum of squares can exceed the total sum of squares and thus xR^2 becomes negative. Rather than fitting coefficients in a fixed equation, NPMR fits 'tolerances', the standard deviations used in the Gaussian smoothers.

NPMR overcomes many of the problems associated with trying to describe habitat effects with statistical methods that are essentially linear and do not take into account complex interactions (McCune, 2006). Default settings were used for all factors. For each dependent factor the best model for a given number of habitat/environment factors (independent factors) are suggested. Each model includes a measure of fit (xR^2) and a tolerance value for each habitat factor. Tolerance is the breadth of values that a habitat factor can vary without invoking a response in the dependent factor (e.g. size of the plateau). As with multiple regression, a balance between increased precision and the inclusion of spurious factors is needed in selecting a model. Predictors were added only if improved model fit (xR^2) by at least 0.5. Once a model was selected, the sensitivity of the habitat factors was evaluated. Sensitivity indicates the size of the response in the dependent factor for each incremental change in the habitat factor. For example if the sensitivity of CV flowering to strata2 is 0.05, changing the value of strata 2 from 1 to 2 result in a 0.5 increase in moss richness.

Precipitation effects on C. fasciculatum

A regression analysis (SPSS 19, 2011) was used to evaluate the effect of precipitation on the proportion flowering and total number of emergent plants in each population from 1998-2007. We tested for effects of (1) total precipitation, (2) precipitation in the spring/summer

Table 3. Variables used in analyses of community and environmental effects of C. fasciculatum.

Community variables
(Exposed) mineral soil (cover %)
Litter and duff (cover %)
Moss (cover %)
Strata 1 (sum of % cover for graminoids, ferns, forbs, shrubs, and trees 0-15 cm tall)
Strata 2 (sum of % cover for graminoids, ferns, forbs, shrubs, and trees >15 cm $- 2$ m tall).
Basal area (total basal area of all trees in 0.02 ha plots)
Environmental variables
Elevation
Azimuth
Slope
Riparian (Ordinal code describing the relative moisture condition of the population)
$\frac{1}{2}$ Cover mass calculated in $2m^2$ relate

¹Cover was calculated in 2m² plots.

²Basal area was calculated from measurements taken on all trees within 0.02ha plots.

immediately prior to emergence, and (3) precipitation in the fall/winter immediately prior to emergence. Precipitation data was downloaded from the PRISM climate group (www.prism.oregonstate.edu). Data was taken from the approximate center of each environmental region, then used for all sites within that region (Appendix A).

Results and Discussion

Population trends

Demography plots

Cypripedium fasciculatum population sizes fluctuated substantially between years. Most populations increased in numbers from 1998 through approximately 2000, then remained stable or declined from then (Table 4; Figure 7). Over the entire study period (2002 to 2007), populations declined at 16 of the 27 demography plot sites. There was no change in population size at two of the populations (Soda Creek 2A and Tyler Creek) and no net change at Taylor Ridge (though the population increased slightly in 1999 and 2000). There was a net increase in population size at three populations (Sexton Mountain 2, Alexander Gulch A, and Tallowbox), however the increase in population size occurred primarily in 1999 and the populations have remained stable or have declined since. Only population (Soda Creek 2A) exhibited a recent increase in size.

The percent of flowering plants in each population was variable between sites within years and between years within a site (Table 5). While a few populations (Pleasant Valley, Wellington Butte 3, and Soda Creek 3) consistently had relatively few flowering plants, the percent of flowering plants changed dramatically between years at the remainder of the sites.

Env. Region	Site Name	Site Number	Treated	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
	Murphy Culch	01	Vaa	50	60	EC	FO	4.4	10	10	7	0	0
3	Nurphy Guich		res	52 15	03	00	50	17	12	10	10	10	0 7
	Pleasant valley	09	INO Vee	15	23	20	20	17	0	14	12		
	Round Prairie R	C2A C2B	Yes	10	10	17	14	14	12	11	10	0	0
	Round Prairie B	C2B	res	31	32	29	45	43	19	23	24	25	24
	Round Prairie C		INO N Io	29	40	37	42	44	18	20	24	21	23
	Round Prairie D	C2D	INO	45	38	46	49	53	38	40	44	35	35
		C7	No	10	21	23	24	23	21	21	21	17	21
	Slick Bock	C23	No	26	27	20	20	28	24	28	24	20	20
	Sykes Creek	C16	No	20	5	5	5	20	27	20	27	20	20
	Taylor Creek 2	C10	Voc	56	84	84	84	69	ے 12	46	40	36	31 2
	Taylor Bidge	C24	No	1	6	5	1	1	τ <u>ς</u> Λ	40	40	1	1
	Whitehorse Park	C6	No	17	18	20	23	+ 17	16	18	17	- 15	11
	Alexander Gulch	00	INU	17	10	20	20	17	10	10	17	15	
4	A	C3A	No	49	75	67	66	35	64	62	73	73	72
	Alexander Gulch		-	-	-	-			-	-	-	-	
	В	C3B	No	51	55	53	52	49	48	50	50	51	54
	Alexander Gulch												
	С	C3C	No	27	27	28	27	24	26	27	27	26	25
	French Gulch	C17	No	28	33	32	30	28	25	21	26	26	25
	Keeler Creek 1	C13	No	2	6	6	5	1	1	2	1	2	0
	Tallowbox	C5	No	3	11	8	7	7	6	10	6	6	6
	Wellington Butte 1	C20	No	2	2	4	1	2	1	2	1	1	3
	Wellington Butte 3	C22	Yes	64	96	110	59	7	33	52	68	80	77
	Wellington Butte 4	C30	No	5	5	20	15	0	0	0	0	0	0
5	Emigrant Creek	C4	No	15	28	27	27	22	21	21	20	21	24
	Randecore Pass	C18	No	19	26	21	20	17	17	17	19	21	15
	Soda Creek 2A	C19A	No	1	1	1	1	0	1	1	1	1	1
	Soda Creek 2B	C19B	No	6	6	5	6	6	5	5	5	5	9
	Soda Creek 3	C32	No	45	45	37	50	45	47	44	44	45	40
	Tyler Creek	C31	No	1	1	1	1	0	1	1	1	1	1

Table 4. Numbers of tagged plants in demography plots at each site over the study period. Numbers were corrected by sampling effort, as different numbers of plots were sampled in different years.

Cypripedium fasciculatum in southwest Oregon

Env.		Site									
Region	Site Name	Number	1999	2000	2001	2002	2003	2004	2005	2006	2007
3	Murphy Gulch	C1	48	41	58	9	25	20	29	50	38
	Pleasant Valley	C9	5	0	0	0	0	0	8	20	14
	Round Prairie A	C2A	0	12	21	29	33	27	40	67	17
	Round Prairie B	C2B	20	13	19	30	26	48	54	52	38
	Round Prairie C	C2C	15	3	15	7	0	8	13	33	43
	Round Prairie D	C2D	21	10	23	17	18	28	30	46	46
	Sexton Mountain 2	C7	82	83	83	78	86	86	76	82	90
	Slick Rock	C23	44	24	25	18	17	21	46	60	55
	Sykes Creek	C16	80	80	60	0	0	67	100	100	100
	Taylor Creek 2	C10	28	7	44	26	5	37	13	67	62
	Taylor Ridge	C24	50	40	75	50	50	75	100	75	100
	Whitehorse Park	C6	50	45	57	47	31	50	53	67	73
4	Alexander Gulch A	СЗА	43	43	51	3	38	61	60	68	72
	Alexander Gulch B	C3B	84	50	78	16	54	62	62	65	59
	Alexander Gulch C	C3C	89	75	89	42	77	78	81	88	76
	French Gulch	C17	36	17	22	0	20	33	19	42	44
	Keeler Creek 1	C13	67	50	40	0	0	0	0	0	-
	Tallowbox	C5	18	0	14	0	0	0	17	17	0
	Wellington Butte 1	C20	50	50	100	-	0	0	0	0	33
	Wellington Butte 3	C22	13	14	25	0	6	6	9	30	30
	Wellington Butte 4	C30	-	30	13	-	-	-	-	-	-
5	Emigrant Creek	C4	86	48	67	18	48	52	65	62	58
	Randecore Pass	C18	31	33	45	18	47	47	42	52	60
	Soda Creek 2A	C19A	100	100	100	-	0	100	0	0	100
	Soda Creek 2B	C19B	-	100	83	-	80	80	80	100	100
	Soda Creek 3	C32	-	5	12	11	19	16	14	22	28
	Tyler Creek	C31	-	100	100	-	0	100	100	100	100

Table 5. Percent of flowering plants in demography plots at each site over the study period. "-" indicates when known plants were present in the plot. 1998 is not shown due to insufficient data.



Figure 6. The proportion of flowering (a) and fruiting (b) plants is positively correlated with the average length of the longest leaf.

Both the proportion of plants flowering ($F_{1,226}=212.732$, P<0.0005, $R^2=0.485$) and fruiting ($F_{1,226}=114.773$, P<0.0005, $R^2=0.337$) in a population were positively related to the average length of the longest leaf in a population (Figure 6). Our analyses also show that with a few exceptions, most plants do not flower until the longest leaf is at least 18.75 mm and do not flower until the longest leaf is 37.19 mm.

Size-dependent flowering has been found in a number of orchid species, including *Tipularia discolor* (Whigham and O'Neille, 19991), *Liparis lilifolia* (Whigham and O'Neille, 1991), *Coeloglossum viride* (Willems, and Melser 1998), *Himantoglossum hircinum* (Carey et al. 2002), and *Cypripedium acaule* (Primack and Stacy 2009). Primack and Stacy 2009 found that in *C. acaule*, fruiting resulted in a cost to the plant such that although flowering plants tended to initially be larger, repeated fruiting eventually led to smaller plant size.

Count plots

Similar to the trends observed in demography plots, there was a decline in the total population size of *C. fasciculatum* in the count plots in four of the five sites (Figure 8). Over the same period of time, the proportion of flowering plants in the population increased from an average of 23% to 60%.

Murphy Gulch grid

The population of *C. fasciculatum* in the Murphy Gulch grid declined by more than 50% in the seven years it was monitored. The population was at its highest in 2001, the year it was established. This was approximately the same year that *C. fasciculatum* populations peaked at the other sites. From 2002 to 2007, the population size fluctuated from a low of 126 individuals in 2006 to a high of 292 in 2001 (Table 6). On average, 39% of the population was composed of flowering individuals.



Figure 7. The total number of *C. fasciculatum* plants in each three environmental regions in the Medford District BLM from 1999 - 2007.



Figure 8. The total number of C. fasciculatum plants in count plots at five sites in the Medford District BLM from 2000 - 2007.

Cypripedium fasciculatum in southwest Oregon

Year	Vegetative	Flowering	Total
2001	104 (36%)	188 (64%)	292
2002	119 (89%)	15 (11%)	134
2003	118 (66%)	61 (39%)	179
2004	131 (69%)	58 (31%)	189
2005	85 (56%)	68 (32%)	153
2006	65 (52%)	61 (39%)	126
2007	81 (58%)	58 (42%)	139

Table 6. Number (and %) of vegetative, flowering, and total C. fasciculatum at the Murphy Gulch Grid each year from 2001 - 2007.

Disturbance treatments

Populations at the forest thinning sites experienced a significant decline in emergent stems in 2002 (Figure 9), the year of treatment in Wellington Butte #3 and possibly Murphy Gulch. 2002 was a poor year for flowering and fruiting at all sites; however, the decline in the number of emergent stems was less severe at the other sites in this study.

Comparing the population trends between years and sites, fire appeared to have little effect on *C. fasciculatum* (Figure 10). Fire intensity at Taylor Creek was sufficient to kill the aboveground biomass of a *Lithocarpus densiflora* (tanoak) within the site. However, only the plant that was in the fireline failed to emerge after the fire One year post-fire at Round Prairie, the number of plants decreased by 18% at one burned site, but increased by 55% at another. In contrast, the number of plants increased by 14% and 7% at the other Round Prairie sites. Similar patterns continued throughout the study; the number of plants at the burned sites declined by 65% and 17% from 2000 (pre-burn) to 2007; in the unburned plots, the number of plants declined by 38% and 24% over the same time period.



Figure 9. Total number of plants from 1998 – 2008 at two thinned sites. Regions 3 and 4 are also graphed for comparison.



Figure 10. Total number of plants from 1998 – 2008 at three burned sites (Round Prairie A, Round Prairie B, and Taylor Creek) and the remaining sites in Region 3 that had not been burned.

The treated populations tended to have lower levels of strata 2 and higher levels of variability in flowering, fruiting and leaf area. This variability may be due in part to the treatments. In a comparison of a site where the duff layer had been removed by a fire and 3 unburned populations Harrod et al. (1997) found that the number of fruiting plants decreased by 33% at the burned site. The number of fruits per stem also significantly decreased. Excavation of the burned site produced no rhizomes where the duff layer had been removed, although a few rhizomes were found where this layer remained, indicating that *C. fasciculatum* can tolerate only low severity fire. Fire scars in areas occupied by *C. fasciculatum* in California's north coast range suggest that fire was once more common in areas occupied by orchids (Skinner et al. 2009). Frequent fires would be expected to be patchy and low in severity, consistent with our observations and similar results found after a fire in the Lolo National Forest in 1998 (S. Shelley *personal communication*).

Seeding plots

We did not observe *C. fasciculatum* in the seeding plots in any year of the study. Seeds of this species appear to be particularly difficult to germinate (S. Copeland, *personal communication*). Harrod et al. (*unpublished report*) observed seedlings in an area where the duff layer had been removed during a fire, thus future treatments may benefit from testing combinations of post-fire characteristics, such as improved by contact with mineral soil and nutrient addition (to mimic the flush of nutrients after a fire).

Evaluation of dormancy and mortality

We used two methods to estimate the rate of dormancy. First, we excavated 131 plants that had been absent for at least three years. Of these, only 4 living rhizomes (3% of total) were located (Table 8). However, 11% of rhizomes that had been absent for 7 years were still alive, suggesting the potential for longer dormancy. Second, using the demographic data, we determined the fate of tagged plants which did not emerge for one or more consecutive years. Using this method, we found there was a 70% chance that a plant that was absent would reemerge after one year (Table 7). The probability of re-emergence declined sharply after that. The probably that a plant absent for 8 or more years was 0. Thus, while it is possible for *C*. *fasciculatum* to remain dormant for a relatively long period of time, repeated observations of a decrease in population size are likely indicative of a declining population, not dormancy.

We acknowledge that our estimate of dormancy from the demographic data could be an overestimate due to several potential sources of error. The possibility exists that the original tagged plant died and a new individual emerged in the same location. On the other hand, an individual may have inadvertently missed during a survey, particularly if there had been significant herbivory. However, the similarity of our dormancy estimates from the demographic data and rhizome excavations suggest that our estimates are reasonably accurate.

Dormancy has been recorded in at least six species of *Cypripedium*, more often than in any other plant genus (Shefferson et al. 2007). Shefferson (2005) has suggested that dormancy may buffer a plant's survival against environmental stress. This may be the case in *C*. *fasciculatum* as the rate at which plants within each stage became dormant varied significantly between years (Table 9). While in most years 3%-10% of aboveground plants within each stage went dormant the following year, occasionally nearly 22% of a stage became dormant the following year (e.g. vegetative plants [V] in Region 4, 2001-2002).

Years Absent prior to excavation	Living rhizome found	Dead/no rhizome found	Total Excavated	% Found Alive/Dormant
3	1	3	4	2.5
4	0	4	4	0
5	0	17	17	0
6	1	59	60	1.7
7	2	17	19	10.5
8	0	22	22	0
9	0	5	5	0
Grand Total	4	127	131	3.1

Table 7. Fate of excavated plants (Tagged plants which did not emerge for \geq 3 years.)

Demographic models

Transition matrices were constructed for each year from 1999 to 2007 for each environmental region (Table 9). These matrices indicate the probability of an individual at each stage (small plant, vegetative, flowering, or dormant) at time 't' advancing to each stage at time 't+1'. For example, in region 3, the probability that a small plant in 1999 will remain a small plant in 2000 is 0.531; the probability that a small plant will become vegetative is 0.125, and the probability that it will flower is 0. In nearly all years and environmental regions, it was most likely that a plant would remain in the same stage from one year to the next. For example, there were only two transitions (from 2001 to 2002 in regions 4 and 5) when flowering plants had a higher chance of returning as vegetative plants. Similarly, four years of monitoring *C*. *fasciculatum* in the Swan Valley in Montana, showed that flowering plants tend to return in this stage each year (Greenlee 1997). While dormant plants most frequently emerged as vegetative plants, they occasionally emerged as flowering plants. Similar patterns have been observed in populations in the Clearwater National Forest (Lichthardt 1997 *in* Greenlee 1997).

Number of consecutive years absent	Number of plants which never re-emerge (presumed dead)	Number of plants which re-emerge	% Dormant
1	105	259	71.2
2	41	41	50.0
3	32	14	30.4
4	26	5	16.1
5	79	8	9.2
6	147	5	3.3
7	86	1	1.1
8	43	0	0
9	8	0	0

Table 8. Estimated rates of dormancy from plants which did not emerge for 1 or more consecutive years.

Fecundity and Recruitment

Contrary to our expectations, the number of new recruits was not correlated with any variables related to reproduction (number of flowering plants, number of fruits) at any time lag. We anticipated recruitment would be strongly correlated with fruit production 2 years prior, but this was not the case. Instead, we found the strongest correlation was with the total number of plants in the same year (Figure 12, Figure 11). A possible explanation is that the factors controlling the likelihood that a plant emerges from dormancy are also the factors that influence the likelihood that a seedling will send up its first aboveground leaf. The mean number of recruits did not vary significantly by environmental region or year.

The relationship between recruits and population size was fit with a simple regression, where the average number of new recruits is 0.03 * Aboveground Population Size. For the purposes of simulation modeling, at each time step the number of new recruits was calculated as follows:

- 1. Calculate N_{above} (sum of all above-ground stages)
- 2. Draw a random number from the poisson distribution with mean $0.03*N_{above}$
- 3. This is the number of new recruits, which is added to the 1st stage (S).

 $S_{t+1} = S_t + NR$

The Poisson distribution was used to ensure that the number of recruits used in the modeling were integers and not fractional individuals. Additionally, the poisson distribution fits the observed distribution of the numbers of new recruits – long-tailed, not a normal distribution.

Population projections

We projected simulated population s from each region to estimate average annual growth rate (lambda), extinction risk, and forecast population sizes. We found close agreement between observed and mean annual growth rates (Table 10). For all environmental regions, annual growth rates are slightly <1, indicating declining populations (Figure 13). Risk of extinction in 30 years is greatest for small populations, and is near zero in populations with greater than 10 individuals Although large populations (those with 100 or more individuals) are unlikely to go extinct in 30 years, they are projected to decline to nearly 1/3 of their current size in this time period (Figure 14, Figure 15). In an analysis of over 180 populations of C. fasciculatum in Oregon and California that were revisited 1 to 29 years after the previous site visit, Newton et al. (2010) found similar estimates of extinction risk. In this study, small populations (<10 plants) went extinct in 50% of the cases, and mid-sized populations (10-30 plants) went extinct in 30.5% of the cases, while only 2% of large populations (>30 plants) declined to zero. The majority of populations of this species are small. For example, 80% of populations in the Medford District BLM GeoBOB database are recorded as having 10 or fewer individuals (M. Mousseaux, *personal communication*). Thus, the chance of population extinction in this species is relatively high.

Table 9. Transition matrices for each environmental region. 1998-1999 was excluded because of small sample size (low numbers of tagged plants at most sites). S = small plant; V = vegetative; F = Flowering; D = Dormant. The stage that an individual was in at time 't' is along the top row. The stage that an individual will move to in time 't+1" is in the left column.

Region 3 tra	3 transition matrices Region 4 transition matrices				Region 5 transition matrices									
<u>1999-2000</u>	-		_	_	<u>1999-2000</u>	-		_	-	<u>1999-2000</u>	-		_	_
s	S	V	<u> </u>	0	s	0.630	0.025	<u>⊢</u>	0	sí	0.333	<u>v</u>	<u>⊢</u>	0
v	0.125	0.831	0.204	0.750	v	0.074	0.750	0.235	0.250	v	0.083	0.800	0.364	1.000
F	0	0.015	0.714	0.188	F	0	0.075	0.671	0.250	F	0	0.100	0.606	0.000
D	0.094	0.092	0.061	0.063	D	0.148	0.100	0.082	0.500	D	0.167	0.100	0.030	0.000
<u>2000-2001</u>	•	.,	_	_	<u>2000-2001</u>	•	.,	_	-	<u>2000-2001</u>	•		_	_
9	S	V 0.044	F	<u>D</u>	s	<u>S</u>	V 0.143	F	<u> </u>	s ľ	<u>S</u>	V 0.026	F	<u>D</u>
v	0.084	0.678	0.109	0.538	v	0.021	0.381	0.118	0.294	v	0.007	0.632	0.036	0.778
F	0	0.233	0.891	0.000	F	0	0.302	0.779	0.118	F	0	0.289	0.893	0.000
D	0.000	0.044	0.000	0.154	D	0.034	0.095	0.074	0.471	D	0.111	0.026	0.036	0.222
<u>2001-2002</u>					<u>2001-2002</u>					<u>2001-2002</u>				
	S	V	F	D		S	V	F	D		S	V	F	D
S	0.689	0.101	0	0	S	0.281	0.125	0	0	S	0.583	0.122	0	0
V E	0.067	0.674	0.286	0.500	V	0.094	0.375	0.513	0.350	V E	0.167	0.732	0.400	0.200
D	0.022	0.030	0.079	0.250	D	0.156	0.000	0.152	0.500	D	0.083	0.073	0.200	0.800
2002-2003					2002-2003					2002-2003				
2002-2005	S	v	F	D	2002-2003	S	v	F	D	2002-2003	s	v	F	D
S	0.321	0.068	0	0	S	0.214	0.031	0	0	s	0.600	0.000	0	0
V	0.054	0.614	0.200	0.273	v	0.357	0.406	0.000	0.558	v	0.150	0.615	0.000	0.471
F	0	0.045	0.733	0.000	F	0	0.510	1.000	0.116	F	0	0.269	1.000	0.235
D	0.179	0.193	0.067	0.727	ן ט	0.179	0.052	0.000	0.233	ן ט	0.100	0.096	0.000	0.294
<u>2003-2004</u>	•	v	-	_	<u>2003-2004</u>	•	v	-		<u>2003-2004</u>	•	v	-	
s	5	0.043		0	s	0 524	0.067		0	s [0 556	<u>v</u>		0
v	0.280	0.638	0.054	0.395	v	0.143	0.507	0.053	0.300	v	0.000	0.791	0.065	0.250
F	0	0.217	0.892	0.053	F	0	0.333	0.920	0.050	F	0	0.093	0.871	0.083
D	0.160	0.072	0.027	0.132	D	0.095	0.093	0.027	0.400	D	0.056	0.093	0.000	0.417
2004-2005					2004-2005					2004-2005				
	S	v	F	D		S	v	F	D		S	V	F	D
S	0.514	0.088	0	0	S	0.423	0.020	0	0	S	0.750	0.024	0	0
V E	0.143	0.676	0.078	0.333	V	0.308	0.667	0.103	0.263	V	0.063	0.707	0.156	0.300
ь р	0.057	0.029	0.059	0.200	D	0.192	0.235	0.007	0.316	D	0.125	0.098	0.063	0.300
0005 0000					0005 0000					0005 0000				
2005-2006	s	v	F	D	2005-2006	s	v	F	D	2005-2006	s	v	F	D
S	0.414	0.033	0	0	S	0.500	0.051	0	0	s	0.722	0.025	0	0
V	0.276	0.417	0.051	0.400	v	0.136	0.424	0.068	0.429	v	0.167	0.575	0.125	0.273
F	0	0.233	0.780	0.300	F	0	0.441	0.883	0.000	F	0	0.250	0.813	0.364
D	0.138	0.217	0.068	0.200	D	0.045	0.051	0.000	0.357	D	0.000	0.075	0.063	0.182
<u>2006-2007</u>					<u>2006-2007</u>					<u>2006-2007</u>				
c 1	S	<u>V</u>	F	<u>D</u>	~ 1	S	<u>V</u>	F	D	~ ^	S	<u>V</u>	F	D
S	0.294	0.049	0	0 355	S V	0.600	0.095	0	0 355	S V	0.476	0.061	0	0 355
F	0.176	0.390	0.091	0.355	F	0.120	0.548	0.076	0.300	F	0.093	0.576	0.075	0.355
D	0.093	0.097	0.052	0.258	D	0.121	0.094	0.051	0.258	D	0.092	0.080	0.046	0.258
										I				

Cypripedium fasciculatum in southwest Oregon

Population size and recruitment at each site

Total number of new recruits over study period (1999-2007)



Figure 11. The number of new recruits is most strongly correlated to the total above ground population size in the same year. This suggests that the factors controlling plant reemergence also influence the emergence of new recruits.



Figure 12. The total number of recruits observed over the study period as a function of the average population size at each site (excludes treated sites). The slope is similar to Figure 1 (0.21).

Table 10. Observed annual growth rate (λ) compared with the mean growth rate predicted by stochastic simulation modeling. A $\lambda > 1$ represents positive growth, <1 represents negative growth (declining population).

Region	Observed mean annual growth rate (λ)	Range of observed annual growth rate (λ)	Model mean predicted annual growth rate λ
3	0.955	0.69-1.17	0.956
4	0.982	0.71-1.17	0.967
5	0.977	0.86-1.14	0.971



Figure 13. Simulated population size and growth rate in each environmental region after 30 years.



Figure 14. Extinction risk after 30 years in populations of C. fasciculatum.



Figure 15. Starting from 100 individuals (90 above ground, 10 dormant), mean and 95% CI for projected populations in each region.

Response variable	Selected model xR ² , predictive factor/s (tolerance)
Community factors	
Average Proportion Flowering	0.0849; Strata 2 (8.97)
Average Proportion Fruiting	0.1904; Strata 1 (6.35)
Average Leaf area	0.3447; Mineral soil (5.87), Strata 1 (6.35), Strata 2 (13.45)
Average Number of plants	0.5791; Basal area (2260345)
CV Flowering	0.2022; Strata 2 (4.48), Basal area (6781033)
CV Fruit	0.07; Strata 2 (11.208)
CV Leaf area	0.2837l; Strata 2 (6.725), Basal area (6781033)
CV Number of plants	-0.0312; Moss cover (22.725)
Environmental factors	
Average Proportion Flowering	0.0617; Elevation (153.75), Slope (4.2)
Average Proportion Fruiting	0.1146; Elevation (51.25)
Average Leaf area	0.1001; Azimuth (16.9)
Average Number of plants	-0.0816; Slope (67.2)
CV Flowering	0.0831; Azimuth(135.2), Slope (4.2)
CV Fruit	-0.0789, Slope (16.8)
CV Leaf area	-0.0227, Azimuth(135.2)
CV Number of plants	-0.031, Elevation (102.5)

Table 11. Selected NPMR models explaining variation in *C. fasciculatum* population variables.

Community and environmental effects of C. fasciculatum populations

Strata 2 was the strongest predictive factor in models explaining the average proportion of flowering, CV flowering, CV fruit, and CV leaf area (Table 11). As the cover of strata 2 increased, the average proportion of flowering plants increased (Figure 16a), while the variability if flowering (Figure 17a), fruiting (Figure 17c), and leaf area (Figure 17e) decreased. Basal area was a secondary factor influencing CV flowering and leaf area; as basal area increased, the variation in the proportion of flowering plants increased (Figure 17b) while variation in leaf area decreased (Figure 17d). A model including basal area explained 58% of the average number of plants found in a population; as basal area increased, the number of *C. fasciculatum* also increased (Figure 16d). Strata 1 explained 19% of the average proportion of fruiting plants in a population (Figure 16b). While moss cover was the strongest predictor for the variation in number of plants in a population, this model was very weak ($xR^2 = -0.0312$; Figure 17f).

Environmental factors explained little of the average nor variation in population characteristics of *C. fasciculatum* (Table 11). The average proportion of fruiting plants and average leaf area were most sensitive to environmental variables, with elevation explaining 11% of the difference in average number of plants between populations and azimuth explaining 10% of the difference in leaf area between populations. The average proportion of fruiting plants increases between 700 – 1100m, with a peak near 900m (Figure 18a). The highest average leaf area is at north azimuth, declined moving to the east and west, with a smaller increase as aspect approaches south (Figure 18b).



Figure 16. NPMR results for *C. fasciculatum* characteristics, including (a) average proportion flowering plants, (b) average proportion of fruiting plants, (c) average leaf area, and (c) average N and main predictive factors.



Figure 17. NPMR results for variation in *C. fasciculatum* characteristics and predictive factors., including (a) CV proportion flowering and primary predictive factor Strata 2, (b) CV proportion flowering and secondary predictive factor basal area, (c) CV leaf area and primary predictive factor Strata 2, (d) CV leaf area and primary predictive factor basal area, (e) CV proportion fruiting and primary predictive factor Strata 2, and (e) CV N and primary predictive factor moss cover.



Figure 18. Non-parametric multiplicative regression results for (a) the average proportion of fruiting C. fasciculatum and the main predictive factor, elevation, and (b) average leaf area and azimuth.

Our data suggest that the most important habitat feature to manage for is tree basal area; increasing basal area should increase *C. fasciculatum* size and reproductive capacity and decrease the variability between years. Our data also suggest another mechanism for the neutral (thinning) to negative (fire) effects of disturbance treatments. *Cypripedium fasciculatum* was positively correlated with cover of low- and mid-statured vegetation. Thus, disturbances that reduce the cover of these strata would be expected to negatively impact *C. fasciculatum*. However, it is important to note that cover of these two layers did not exceed 40%. Thus, higher levels of cover of these vegetation layers may have negative effects on this species.

These habitat characteristics are consistent with the observation that *C. fasciculatum* is most commonly associated with mid-successional species (Knecht 1996) which tend to form a mid- to high-level canopy, with a low-to-moderate cover of short-statured plants. It has been suggested that these habitats may be on the only areas that support the symbiotic organisms with which *C. fasciculatum* is associated (Harrod et al. 1997).

Precipitation effects of C. fasciculatum populations

We found only week effects of precipitation on the proportion flowering and total number of *C. fasciculatum* (Table 12). Total precipitation was positively related to total number of plants in environmental region 3 and proportion of flowering plants in environmental region 5. Both spring /summer and fall/winter precipitation were positively related to the proportion of flowering plants in environmental region 3. However, the correlation coefficients for these relationships were less than 0.142, indicating the precipitation explains less than 15% of the observed variability.

While rainfall has been found to be related to population characteristics in many orchid species (Inghe and Tamm 1988, Wells et al. 1998;), not all species show these relationships studies (Falb and Leopold 1993, Coates et al. 2006). More complex analyses, including incorporating a time lag may reveal an effect of rainfall on *C. fasciculatum*. The absence of observed rainfall influences in this study may reflect the use of relatively simple climatic indices, since only seasonal and annual rainfall over the previous 12 months was investigated. Temperature affects phonological characteristics in some species or orchids (Shefferson et al. 2001, Brzosko, 2002) and could be incorporated in future analyses.

Environmental	df	MS	F	Р	\mathbb{R}^2				
Region	(regression, total)								
	Total number of emergent plants (N)								
Total precipitation	Total precipitation								
3	1,119	2446.663	8.069	0.005	0.064^{1}				
4	1,89	125.236	0.170	0.681	0.002				
5	1,59	1.470	0.006	0.939	0.000				
Spring/Summer, Fall/Win	iter								
3	2,119	335.775	1.046	0.355	0.018				
4	2,89	251.561	0.340	0.713	0.008				
5	2,59	5.611	0.022	0.978	0.001				
Proportion flowering									
Total precipitation	-								
3	1,119	0.025	0.303	0.583	0.003				
4	1,89	0.016	0.163	0.687	0.002				
5	1,59	1.293	9.615	0.003^{2}	0.142				
Spring/Summer, Fall/Winter									
3	2,119	0.707	9.699	$< 0.0005^{3}$	0.142				
4	2,89	0.236	2.514	0.087	0.055				
5	2,59	0.330	2.230	0.117	0.040				

Table 12. Regression results for the effect of total precipitation or spring/summer and fall/winter precipitation on the **total number of emergent** and **proportion flowering** *C. fasciculatum*.

 ^{1}N (Region 3) = 0.023*(total precip) +4.451

²Ppn Flowering (Region 5) = 0.001*(total precip) -0.079

³Ppn Flowering (Region 3) = 0.002*(Spring/Summer) + 0.005*(Fall/Winter) + 0.256

Conclusions

The ten-year demographic study of *C. fasciculatum* was initiated to address four areas where information was needed to improve management for this species:

1. Assess the status and demographic structure of *C. fasciculatum* populations in southwest Oregon.

- a. There was a decline in the number of plants in more than 2/3 of the sites included in this study.
- b. There was significant variability in the proportion of flowering plants both between sites and inter-annually.
- c. Both the proportion of flowering and fruiting plants were positively correlated with plant size. In general, plants did not flower until the length of the longest leaf was at least 18.75mm.

- d. *Dormancy*: On average, approximately one third of the emergent plants will become dormant the following year. Most dormant plants reemerged after one year and there was no evidence for dormancy lasting longer than seven years.
- 2. Describe habitat characteristics and relationship of the species population characteristics to major environmental variables.
 - a. Size and reproductive capacity were positively related to basal area of trees and cover of low- and mid-statured vegetation. However, it is important to note that the variability in the cover of these layers was relatively small.
 - b. Abiotic habitat variables (slope, aspect, elevation) had little influence on *C*. *fasciculatum* populations.
 - c. There was little evidence for an effect of previous year's precipitation on *C*. *fasciculatum*, suggesting either a lack of precipitation effects or a more complex relationship than that explored here.

3. Identify biological traits useful in monitoring.

- a. We recommend that future monitoring of this species include the number of flowering and vegetative individuals and length of the longest leaf.
- b. The length of time required for monitoring can be reduced by monitoring populations when they are fruiting and using floral scars to determine when plants have flowered, but not produced any fruits.

4. Evaluate the effects of disturbance on *C. fasciculatum* populations.

- a. Poor replication and a lack of records prohibit making strong conclusions regarding the effects of tree thinning and fire on *C. fasciculatum*.
- b. Population so *C. fasciculatum* declined at thinned sites; however, population declines were seen in other populations during the time period and thus may not be indicative of a negative treatment effect.
- c. Fire had no clear effect on the number of *C. fasciculatum*, but burned sites had higher levels of variability in flowering, fruiting, and leaf area. Low severity fires would be expected to maintain appropriate habitat characteristics while not damaging existing plants
- d. We recommend future studies using replicated treatments (including thinning of the mid-story vegetation and low severity ecological burn) to determine if these management techniques could maintain *C. fasciculatum* habitat while having a neutral to positive effect on the species.

The majority of populations included in this long-term study had starting populations of great than 10 individuals and thus where likely already in at least moderately good habitat. Despite this, we found declining population growth rates in all environmental regions. We recommend that future studies focus on determine the best methods for reintroduction of this species and methods to manage habitat that do not harm existing populations.

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Environmental Region	Flowering year	Total	Precipitation (mm) Spring/summer	Fall/winter
4	1998	813.05	248.28	512.45
4	1999	961.8	344.15	668.33
4	2000	607.58	176.49	424.62
4	2001	451.36	218.19	233.78
4	2002	603.7	157.92	451.13
4	2003	656.63	146.42	464.77
4	2004	704.67	266.06	486.81
4	2005	533.64	186.75	338.01
4	2006	970.08	262.82	690.4
4	2007	627.32	182.96	481.64
5	1998	818.05	347.79	843.91
5	1999	1246.29	444.88	1092.88
5	2000	1482.78	230.14	769.07
5	2001	987.11	267.84	357.21
5	2002	606.56	222.66	752.83
5	2003	997.05	197.62	725.48
5	2004	984.8	366.22	778.07
5	2005	1060.37	256.56	531.18
5	2006	848.71	486.12	1092.75
5	2007	1587.21	309.82	786.16
3	1998	1016.66	214.41	767.28
3	1999	919.97	280.51	882.35
3	2000	1044.86	146.91	652.99
3	2001	1097.25	176.22	239.61
3	2002	759.67	114.83	571.91
3	2003	419.17	104.93	663.28
3	2004	701.95	271.66	608.2
3	2005	835.51	169.55	445.79
3	2006	820.84	370.89	903.26
3	2007	675.26	311.75	748.97

Appendix A. Precipitation data used in regression analyses. Data from the PRISM Climate Group (www.prism.oregonstate.edu).