

**CHAPTER 4: *HOLOCARPHA MACRADENIA* RESPONSE TO EXPERIMENTAL
DISTURBANCE REGIMES**

Introduction

Maintaining species richness in ecosystems can depend upon the maintenance of the disturbances with which the component species evolved (Denslow 1980, Collins and Glenn 1988). Because humans have altered disturbance regimes, fragmented landscapes, and destroyed populations of species, determining appropriate disturbance regimes requires research at various spatial, ecological, and temporal levels. With increasing alteration of ecosystems by exotic species, research must consider the effects that restoring evolutionary disturbance regimes may have on the dynamics between native and exotic species (D'Antonio 1993, Kotanen 1997). Because of their sensitivity to disturbances, annual forbs native to grasslands may be the best group of organisms for studies to explore these questions (Schaal and Leverich 1982, Watt and Gibson 1988).

There are critical stages in a plant's life history that may be particularly vulnerable to disturbance (Watkinson and Harper 1978, Guerrant 1995, Shea and Kelly 1998, Hunt 2001). In plant communities, groups of species may share vulnerable life history stages because of the consistency of disturbance regimes across large geographic areas (Axelrod 1985). For example, numerous studies in productive grasslands suggest that germination may be the primary limiting life history stage for a number of plant species (Grubb 1977, Rice 1985). With canopy closure, competition is intense, so species have evolved highly complex germination cues keyed to safe sites created by grazing, fire, and soil disturbances (Bazzaz 1979, Tilman 1993). In grasslands with highly variable disturbance or climate, long-term seed dormancy and longevity allow some species to forestall germination until appropriate conditions arise. Such species may be more limited by seed

predation than by germination itself (Klemow and Raynal 1983). But, with the influx of exotic invasive species in grasslands, the evolved germination cues may now place species in jeopardy when later life history stages are attained. Some research suggests that in weedy grasslands competition with exotics makes survival the seedling stage the primary limiting constraining factor (Brown and Rice 2000). For some species, competition with exotics may have the greatest impact on seed production, affecting seed bank size and, ultimately, population persistence (Pavlik et al. 1993, Carlsen et al. 2000). The limiting life history stages of a plant, therefore, are a product of not only the climate and evolutionary history of the ecosystem, but also the degree of invasion by exotic species.

Little is known about the extent and frequency of historic disturbance regimes of California's grasslands, which may have included fire, grazing, and soil disturbance (Bartolome 1989, Painter 1995). There are several components within each of these disturbance regimes that affect plants; for instance, litter reduction, alteration of canopy structure, nutrient cycling changes, and soil compaction (Collins and Barber 1985). With grazing, litter reduction and soil disturbance combine with alteration of canopy structure to change species composition. Litter accumulation can affect plant communities at many levels, from altered nutrient regimes to changes in species composition (Facelli and Pickett 1991, Reynolds et al. 2001). Soil trampling disturbance can also affect communities in a number of ways from reducing nitrogen-fixing soil crusts to reducing species diversity (Belnap 1993, McIntyre and Lavorel 1994). In grasslands, shorter

canopies increase light levels and change microclimates, increasing the survival of shorter statured species (Mitchley and Willems 1995).

Proponents of the intermediate disturbance hypothesis have suggested that species diversity is maintained when the disturbances occur at intermediate frequencies and intensities (Connell 1978). More recently, work in grasslands suggests that species diversity is maintained when disturbance regimes mimic the multiple disturbance types and frequencies with which species have evolved (Collins and Barber 1985).

Regardless of the specific disturbance regime of the past, humans have severely altered ecosystems through fire suppression and various management regimes of domestic ungulate grazing (Burcham 1975, Callaway and Davis 1993). California's grasslands have also been impacted by the invasion of exotic grasses and forbs (Huenneke and Mooney 1989). These grasslands have been severely reduced from their historical extent and fragmented due to urban and agricultural development (Noss et al. 1995). As a result of these impacts, many grassland species are now threatened with extinction (CDFG 2001). With many remaining natural grasslands being conserved in public ownership, a current priority is to determine appropriate management regimes for the restoration of this ecosystem and its many endangered species. Detailed understanding of life history traits of the threatened plant species is critical to this effort (Fink and Zedler 1989, Pavlik and Enberg 2001). By identifying vulnerable life history stages of the endangered grassland species it may be possible to develop management recommendations for the

restoration of appropriate disturbance regimes monitored for key components of disturbance (Pavlik and Enberg 2001).

The present study is paired with another study that documented a positive response of native annual forbs to cattle grazing in California coastal prairie. In the previous study, we focused on the response of the entire guild of native annual forbs to grazing throughout the geographical extent of California coastal prairie. We focused on native annual forbs because they are particularly sensitive to disturbance in these productive grasslands and contain a high number of species that are presently threatened with extinction (Tilman 1993, CDFG 2001). We then concluded that the diversity and abundance of native annual forbs increases in response to the wide array of cattle grazing regimes in comparison to the paired ungrazed sites (see Chapter 2), but we were unable to elucidate the effects of grazing frequency and the different components of grazing.

In this study, we seek to determine appropriate disturbance regimes to facilitate transitions through critical life history stages of one species of annual forb endemic to California coastal prairie. In a factorial experiment replicated at three natural coastal prairie sites, we tested hypotheses about the response of the endangered annual forb, *Holocarpha macradenia*, to disturbance. First, we hypothesized that this species requires some disturbance in order to persist and that increased frequencies of disturbance would increase the persistence of populations. Second, we hypothesized that individual components of grazing – litter removal and soil disturbance – act differentially to affect the survivorship of the species. With both hypotheses, we designed experiments to

examine disturbance effects at specific life history stages: germination, seedling survival, flower production, seed predation, and seedling recruitment. It was necessary to study these stages separately because survivorship is generally low and highly variable at early stages (particularly seed predation and germination), making it difficult to analyze effects at later stages. Recruitment data, however, integrate across these processes and suggest trajectories for future population viability. Finally, we discuss the potential impacts of associated abiotic and biotic variables (e.g., rainfall, soil moisture, plant community composition) on these processes.

Methods

Site Selection

Our study system was California coastal prairie – the species rich, mesic grasslands found on deep soils within a maritime fog belt that moderates the otherwise extreme summer drought of the Mediterranean climate. We conducted research at three coastal prairie sites, all within 4 km of the coast, in central California, USA, near the city of Santa Cruz. In choosing research sites, areas had to meet the following criteria. All sites 1) had been actively grazed by cattle, 2) contained a relatively intact coastal prairie community, 3) had slopes of <10% facing south to south east, 4) had sandy loam soils, deeper than 1 m, and 5) did not have a previously known population of *Holocarpha macradenia*, increasing the chance that plants we measured originated from our experimental propagules. The final selection of sites depended on the ability to obtain permission both for regular and long-term access to the site and to establish experimental populations of our focal species that is a regulated, endangered species.

The three sites we chose were separated by 25 kilometers from northwest to southeast. The names of the sites reflect place names from areas in close proximity: “Elkhorn” (near the Elkhorn Slough), “UCSC” (on the University of California, Santa Cruz campus), and “Swanton” (near the town of Swanton) (Fig. 1). During the four years of our research, annual rainfall ranged from 29 – 84 cm; the Elkhorn site received intermediate rainfall amounts to the moister UCSC and the drier Swanton sites (see Chapter 3 for climate data). Seasonal rainfall was close to average in 1999, 2000, and 2002 and 5 cm below the 20-year average in 2001. Mean winter (rainy season) temperature for the sites was 12.1 C° and summer mean was 16.4 C°. Soils were sandy loams and were deeper than 1 m (see Chapter 3 for data).

Species description

Holocarpha macradenia E. Greene (Asteraceae: Madiinae) is a tall-statured (1-5 dm) annual forb endemic to central California. The flowers appear from July-October (Munz and Keck 1968) and are generally most abundant in August and September. The species germinates following the first significant rain event (15-30 mm) in late fall and grows a rosette that increases in size until late June when plants approach reproductive age. At that time, plants bolt, producing a stem from the center of the rosette, which eventually reaches height of 0.3-1 m. The reproductive plant produces from one to 60 flower heads (inflorescences) averaging 1 cm in diameter. These flower heads produce two types of “seeds” (actually achenes) with different weights, shapes, and germination requirements. The seeds from the ray flowers have a thick, waxy seed coat and long-term dormancy.

The seeds from the disk flowers are lighter weight, more narrow, and may not maintain viability in the soil in the long-term. Neither type of seed has any evident structure for dispersal, although entire seed heads are sometimes removed when ripe and dispersed up to 15 cm, probably by small mammals. Preliminary data suggest that the seeds disperse no more than 45 cm from the plant by gravity alone (G. Hayes, unpublished data).

Historically, the species was found from Monterey through Contra Costa counties at elevations below 300 m (Hickman 1992). Now, however, the species is limited to nine natural populations in Santa Cruz and northern Monterey County and one introduced site in Alameda County. The species is listed as endangered by the State of California and as threatened under the federal Endangered Species Act.

We chose this species because it is part of a large guild of native annual forbs that begin as basal rosette-forming seedlings, but are relatively tall statured when mature, and reach maturity in the summer. Past research has shown that short-statured plants respond positively to grazing, but that the effect of grazing is less clear for species with varying heights at different life history stages (Noy-Meir et al. 1989, McIntyre et al. 1995). Over 200 taxa in this guild are listed as rare and endangered and include species in the genera *Amsinckia*, *Madia*, and *Hemizonia* (Carlsen et al. 2000).

Experimental Design

We used the same experimental design and plots described in Chapter 3 (Table 1).

Focal Species Measurements

Germination

Just prior to the onset of the winter rains, in early October 1999 and 2000, we placed 36 *Holocarpha macradenia* disk seeds, 10 cm apart, in a grid on the soil surface in each plot. We used disk seeds as opposed to ray seeds because the latter do not germinate until after at least one year in the seed bank, probably because they require complex cues of varying temperature and light (Gregory et al. 2001), S. Bainbridge, pers. comm.). We recorded individual seeds that germinated in December and January of each and summed the values to calculate the total number of germinated seeds for each year.

Seedling survival

In January 1999, 2000, and 2001, we planted 25 seedlings of *Holocarpha macradenia* 10 cm apart in a 5 _ 5 cm grid. The seedlings were grown in a greenhouse from seed sown in late October the prior year. Seedlings were transferred outdoors and fertilizer was withheld in late December to prepare the seedlings for survival under field conditions. When planted, the seedling roots had filled the 3.5 cm deep _ 1.5 cm wide planting cell; growing medium was 50% peat moss, 25% perlite, and 25% bark. When planted, each seedling had approximately four secondary leaves and resembled seedlings in natural populations. We monitored the survival of these seedlings at the end of the spring of each year (~May), prior to flower production. Because of issues concerning the genetic origin of the 2001 seedlings, we removed them from plots at UCSC and Swanton after the final monitoring period in May 2001.

Flower production

We monitored the numbers of flower heads produced by planted seedlings and the seedlings that subsequently recruited from each cohort of planted seedlings. From July through September of each year, we monitored total numbers of seed heads on each plant and summed these totals for each year.

Seed predation

In August 1999 and October 2000, we placed 25 *Holocarpha macradenia* disk seeds in a small amount of site specific, sterilized soil and within 8 cm _ 8 cm mesh bags on the soil surface of each plot. This method allowed for re-locating seeds and the seeds to remain at ambient moisture while permitting most seed predators access to the seeds. We assumed seeds displaced from the mesh bags to be predated and recorded numbers of remaining seed at the onset of rains. Because we were uncertain when seeds would disperse at the outset of the experiment, they were subjected to predation a full month longer in 1999 compared with 2000.

Recruitment

Beginning in January in 2000, 2001, and 2002 we monitored the number and growth of seedlings recruited from prior years within a 1 _ 1 m grid centered on the area where seedlings had been originally introduced. In February through May, we monitored additional recruits, marking each new recruit with a colored toothpick and removing markers for recruits that had died. We calculated both the total number recruiting and the percent recruiting that survived to May, just prior to flowering.

Statistical Analysis

We initially analyzed data using a two way ANOVA with site and experimental treatments as fixed factors (10 levels; Table 1) and the following life history stages as response variables: predation, germination, seedling survival, recruitment number, and flower head number. Because data on germination and seedling survivorship were expressed as percentages, they were arcsine transformed prior to analysis. When phytomass removal and soil disturbances did not show significant effects, we pooled the data from these secondary treatments and focused on frequency of clipping. When treatment _ site interactions were significant, we conducted separate analyses by site and present the results on different graphs. For most analyses, the plots were considered the unit of replication and measurements for individual plants were averaged within plots. But, for flower head number, the plant was considered the unit of replication, as some plots had no plants surviving to the flowering stage. We used Tukey's LSD mean comparison procedures to separate specific treatment effects when treatment was significant in the ANOVA.

Results

Germination

In 1999, significantly fewer seeds germinated in control plots than in the 3x and 6x/year disturbed plots at all sites (Fig. 2a). In 2000, significantly fewer seeds germinated in control plots than in the 2x and 3x/year disturbed plots at all sites (Fig. 2b). Germination was not affected by the secondary treatments of phytomass removal or soil disturbance in either year. Total average seed germination was higher in 2000 than in 1999 (27% vs. 9%). Germination was highest at Elkhorn and lowest at Swanton in both years.

Seedling survival

Response of seedling survival to treatments varied depending on the year. In 1999, seedling survival increased with increased frequency of disturbance, but secondary disturbances of phytomass removal and soil disturbance did not have effects (Fig. 3a).

In 2000, there was a significant site _ treatment interaction, so sites were analyzed separately (Fig 3b). At Elkhorn and Swanton, the 2x/year disturbances had the lowest survival. At Elkhorn survival was highest in 3x/year and control plots whereas at Swanton, survival was highest in 6x/year treatments (Figs. 4a and 4b). Survival at UCSC was extremely low, and there was no effect of the primary disturbances (Fig. 4c). There were no effects of secondary disturbances of litter removal or soil disturbance at any site.

In 2001, survival was highest at all sites in the 6x/year phytomass removal plots and lowest in the control plots (Fig. 3c). The UCSC site had the lowest survival in all years, Swanton was intermediate, and the Elkhorn site had the highest survival.

Flower production

In 1999, there was a significant site _ treatment interaction, so sites were analyzed separately. The three sites showed similar trends of larger numbers of flower heads with increasing frequency of disturbance, though the strength of this effect varied between sites (Figs. 5a and 6a – 6c). In 2000, treatments did not affect the number of flower heads produced per plant (Fig. 5b).

There were no effects of secondary treatments of litter removal or soil disturbance in either year. Because of variable survival, sample sizes for flower head production per plant varied substantially between treatments and sites in both years sampled.

Seed predation

Seed predation was quite high in all treatments but was not affected by either frequency of disturbance or secondary disturbances of phytomass removal and soil disturbance in either year (Figs. 7a and 7b). Predation was higher in 1999 than in 2000, which was likely due to the fact that seed was subject to one additional month of predation in 1999

Recruitment Number

In 2000, the number of plants recruiting at all sites from the 1999 seedling cohort was significantly greater in the 6x/year plots compared to the control plots (Fig. 8). In 2001, all clipping treatments had significantly more recruits than the control plots at all sites. In 2002, there were treatment effects only at the Elkhorn site, with more recruits in the plots disturbed 2x and 6x/year than in the control plots. At the other sites, recruitment was extremely low. There were more seedlings recruiting from the 1999 seedling cohort than the 2000 seedling cohort.

There were significant site _ treatment interactions with seedling numbers from the 2000 seedling cohort, so sites were analyzed separately (Figs. 8d – 8f). Treatment effects were limited to the Elkhorn site, the only site with sufficient recruitment; there were fewer than 5 recruits in any plot at Swanton and UCSC, so it was impossible to analyze treatment effects at these sites. At Elkhorn, there were significantly more recruits in the plots disturbed 3x/year compared to the plots disturbed 2x/year in 2002. Otherwise, there were no other effects of either primary or secondary disturbances on numbers of seedlings recruited from the 2000 cohort at any other date or site.

Recruitment Survivorship

The Elkhorn site was the only site to have sufficient numbers of recruits to allow analysis of treatment effect on survivorship of these plants. Because there were no recruits in the

control plots, we dropped these from the model, but we found no significant differences between treatments (Fig. 9).

Discussion

Our analyses suggest that more frequent disturbances increase *Holocarpha macradenia* population viability through time. We found that disturbance by grass canopy removal (clipping) differentially affected various life history stages of the species, which had higher germination in more frequently clipped plots, but mixed responses at the seedling survival and flower production stages. Predation was not affected by our treatments. We found that secondary disturbances of phytomass removal and soil disturbance had minimal effects during the study period, but these may have increasing effects with additional time. Our results can be used to inform management strategies to help the conservation of this endangered coastal prairie forb.

Disturbance effects were most clear at the germination life history stage of *Holocarpha macradenia*. Two trends are evident in our data. First, germination was higher in 2000 than in 1999, perhaps as a result of a greater amount and earlier rainfall. Second, and more importantly, more seeds germinated in the clipped plots compared to the control plots. This effect appears both in our germination experiment and in our monitoring of the number of plants recruiting in years subsequent to the initial seedling plantings. A number of factors may explain this trend: increasing light increases germination (Tilman 1993) and litter may change temperature at the soil surface, affecting germination

(Fowler 1988). *Holocarpha macradenia*, in particular, may require a combination of light and temperature stratification to cue germination (Gregory et al. 2001) and Sue Bainbridge, unpublished data).

We can confirm thicker litter and biomass in control plots compared to most other treatments (Chapter 3). There may have been sufficient light and temperature fluctuations to cue germination in all of the clipped treatments because, at the onset of the rainy season, our clipping regimes had reduced canopies; the control plots were the only plots with phytomass above 5 cm in height. Although our secondary treatment of litter accumulation only increased litter levels a small amount (Chapter 3), there may have been insufficient time during the course of our experiments for the accumulation of enough litter to affect germination. Other researchers maintain that coarse leaf litter in coastal prairie may accumulate for up to 3.5 years (Heady et al. 1988a). Other experiments on another species of rare grassland forb in California (*Amsinckia grandiflora*) suggest that clipping and litter reduction does not effect germination rates (Pavlik et al. 1993). The different responses may be a result of varying competitive abilities. The *Amsinckia* studied is an earlier season species while the *Holocarpha* must persist throughout the growing season and bloom much later. Also, *Amsinckia* grows in dryer, less competitive grasslands where competition may be less intense. Safe sites may be more limiting for *Holocarpha*, which may have evolved more strict germination cues.

It is interesting to note that soil disturbance did not affect germination of *Holocarpha macradenia*. This species apparently has the ability to germinate in the absence of bare

soil, as, in 2000, the amount of bare soil in our experiments increased with increasing disturbance frequency, and there was no concomitant increase in germination in that year. On this subject, research results elsewhere have been mixed. Some researchers suggest that soil disturbance may create safe sites for germination of annual forbs (Rapp and Rabinowitz 1985, Hobbs et al. 1988), others suggest that soil disturbance has little effect (Kotanen 1996), and still others suggest a negative effect on rare species, such as ours (McIntyre and Lavorel 1994). It may be that soil disturbance could play a more important role were phytomass not to have been removed. Other research has found that, in productive systems like ours, trampling can increase cycling of organic matter while decreasing the canopy height and phytomass, thereby increasing diversity (van der Maarel 1971). Our treatments first removed phytomass and then disturbed the soil, so we were unable to test this idea. Finally, it should be noted that we placed seeds on the soil surface for the germination experiment; in natural situations, seeds may not so readily contact the soil surface in the absence of trampling.

There were less clear impacts of our disturbance regimes on seedling and flower production life history stages. In 1999, more frequent clipping resulted in higher numbers of planted seedlings surviving to adults at all sites. This is the expected trend as prior research has indicated that grazing disturbance favors low-statured annuals (Noy-Meir et al. 1989, McIntyre et al. 1995). In grasslands, a shorter canopy may alleviate light competition and allow these shorter species to survive as seedlings (Foin and Hektner 1986, Tilman 1993). Other researchers contend that plants from productive habitats, like our study system may have evolved mechanisms to cope with varied light

regimes (Rincon and Grime 1989). We may see evidence of this coping mechanism in our focal species during the second year. In that year, at two sites, there was low survivorship in the plots clipped 2x/year. Early and abundant rains allowed an early flush of exotic grasses, and by spring the abundance of exotic grasses was similar in the control plots as the plots disturbed 2x/year. In such light-competitive cases, *Holocarpha macradenia* seedlings etiolate and do not create basal rosettes (Hayes, pers. obs.). Plants with this morphology would have been especially vulnerable to clipping in the spring and appeared unable to recover after being cut.

In 2001, still another trend is evident with *Holocarpha macradenia* survivorship, when phytomass removal resulted in the highest seedlings survival in the plots disturbed 6x/year. This result is confusing because our data do not provide evidence for the plots disturbed 6x/year having accumulated less litter than other plots. Nor do our data present evidence for reduced nitrogen due to litter removal (Chapter 3). We must conjecture, then, on factors that we did not monitor. Taller canopies and denser litter are conducive to a moister microclimate, which fosters higher abundance of some herbivores, including snails, slugs, sow bugs, and earwigs, which are invasive, exotic species in California coastal prairie (Barry Roth, pers. comm.). There was evidence of frequent herbivory by these species on the *Holocarpha macradenia* seedlings in treatments with taller grass and/or more litter. The role of exotic invertebrates on grassland processes deserves further attention. But still, these explanations beg discussion of our result that suggest significantly fewer seedlings surviving in the plots disturbed 6x/year with soil disturbance, as these treatments also had low litter levels and short canopies. It may be

that the cumulative years of soil compaction along with actual trampling of the plants were beginning, in this last year of monitoring, to show treatment effects. Soils in this region are slow to de-compact, as there is no hard frost. A major factor in de-compacting soils, then, is water percolation; in our sandy loams, this might take as many as 7 years. Soil compaction is known to be higher in grazed compared to ungrazed grasslands (Bauer et al. 1987), and this effect will probably take more time to assess in our experimental treatments.

Our data on the survival of recruited plants add little additional insight into effects of clipping on survival because of the low number of plants surviving at all sites. With recruits from the 1999 seedling cohort at Elkhorn, there was no difference in survivorship with clipping frequency; but it is important to note that no plants had recruited in the control plots. Rainfall during the years monitored was slightly below average; higher rainfall years may foster higher survivorship, and so we plan to continue monitoring in 2003.

As with seedling survivorship, our data on flower head production per plant show mixed responses to treatments. In 1999, the results were site specific with an overall trend towards more flower heads per plant with increasing disturbance frequency. In 2000, there were no significant treatment effects or observable trends, but there were much smaller sample sizes. Others have noted that competition with exotic grasses in similar grassland can reduce flower production (Pavlik et al. 1993, Carlsen et al. 2000). While we found that the more frequent disturbances reduced densities of competitive exotic

grasses, this species may escape the impact of this competition on flower production because it flowers after these species have long since desiccated.

One life history stage was clearly not differentially affected by our experimental treatments: seed predation. Overall, we found seed predation to be quite high, but it did not differ by treatment. Some studies describe less seed predation in other grasslands (Howe and Brown 1999), but research in California's grasslands suggest high interannual variability but equally high predation rates in many years (Espeland and Carlsen 2002). It is likely that the patch size of our experiments was too small to significantly change the seed predator dynamics. Our methodology could also have been at fault in our assumption that seeds that disappeared were also predated. Others have described seed harvesting animals displacing seeds without actually predated them (Bakker et al. 1996). Although some seeds may be predated on the plant, and some seeds fall to the ground early in the season, it is possible that a great number of *Holocarpha macradenia* seeds avoid predation by remaining on the plant until the onset of rains, which displace seeds from the plants just prior to germination (Hayes, pers. obs.). Others have noted that seeds produced earlier in the season are subject to greater seed predation (Brody 1997). Our data confirm this as one of the two years of seed predation data spanned twice the amount of time and similarly increased seed predation rates. High rates of seed predation may be an important factor for the species as this species' seeds have no apparent means of dispersing should a population's seed bank be depleted.

The time scale of this study remains an overall weakness. California's climate Mediterranean climate is quite variable. Although the variation in abundance of the state's grassland forbs is well known, the specific climatic predictor variables have not yet been identified. Future studies may find that the infrequent years of abundant forbs may be the key to long-term population viability. Furthermore, the habitat requirements of *Holocarpha macradenia* may be more complex than can be mediated by changes in disturbance regimes, limiting the species to areas we do not yet understand how to characterize.

A major conclusion from our study is that long-term survival of the species is site specific. Moreover, it is difficult to predict based on initial composition, soil texture, or soil chemistry appropriate locations for the restoration of *Holocarpha macradenia*: all sites were identifiable as coastal prairie, the natural habitat of the species. First, this is important from both a scientific and a management perspective as many experimental ecological manipulations are only replicated at one site though results are applied widely (Hurlbert 1984). Second, our data suggest that two of our three experimental populations are declining despite a wide range of experimental management regimes. The more healthy, Elkhorn experimental population is only a few hundred meters from a native population, suggesting that there may be microclimatic or other factors contributing to population viability.

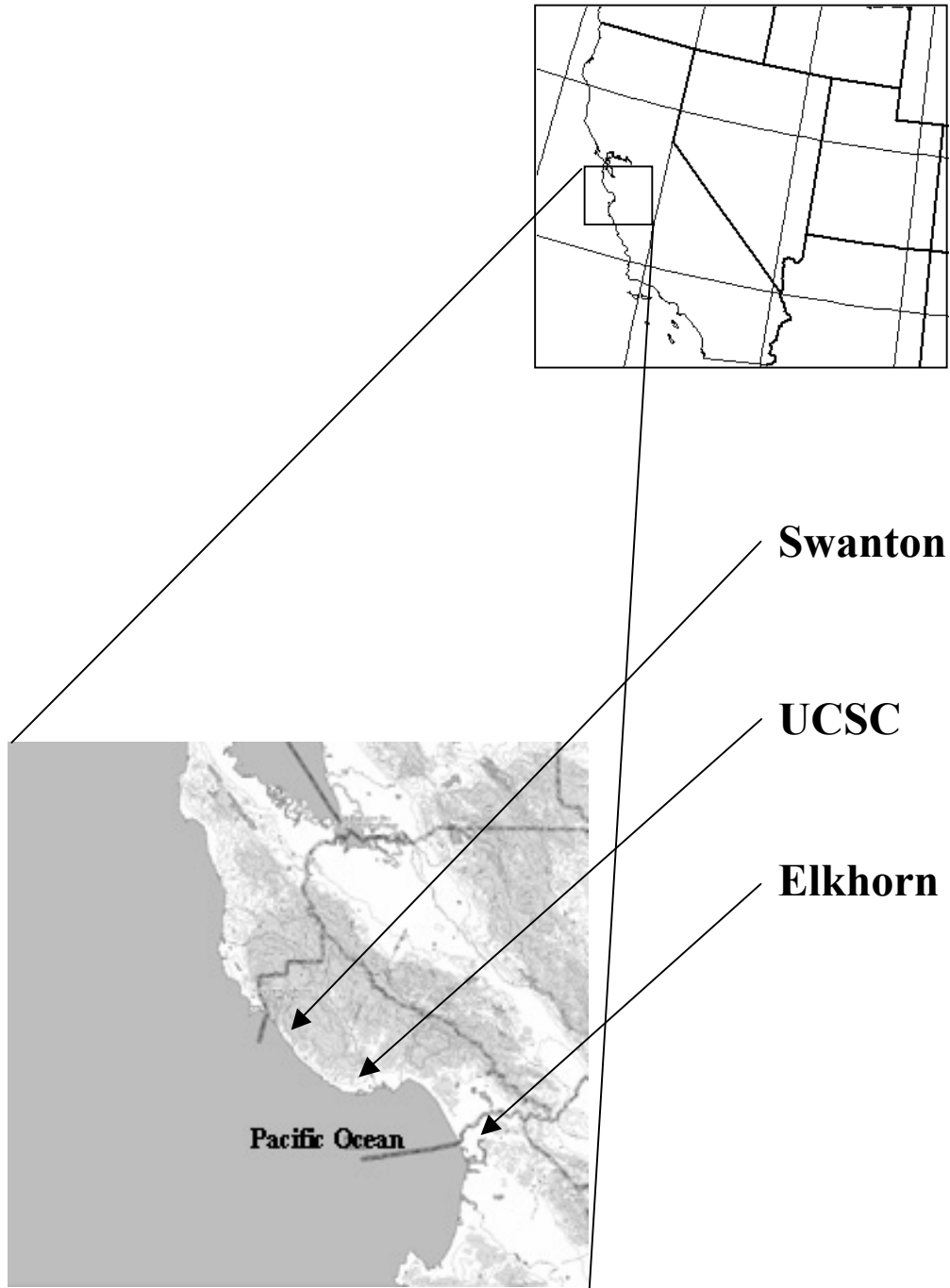
An important conclusion of our research for land managers is that, for *Holocarpha macradenia*, some disturbance is better than no disturbance. There is a trend towards

decreasing disturbance regimes in protected coastal prairie areas containing this and other rare annual forbs. Although our research does not show consistent trends for particular frequencies of disturbance in all years, we do suggest that managers maintain disturbances that decrease canopy height and reduce litter accumulation.

Our work suggests difficulties in establishing *ex situ* populations for further analysis, so more *in situ* experiments should be conducted. There are a limited number of populations (<10) of the species and limited spatial distribution at any one population. Therefore, it may be necessary to experiment in different populations simultaneously to further test hypotheses about responses to disturbance that can help inform restoration protocol. This will require cooperation from a number of private and public land managers as well as a number of regulatory agencies.

Figures

Figure 1: Geographical location of research sites



a. 1999

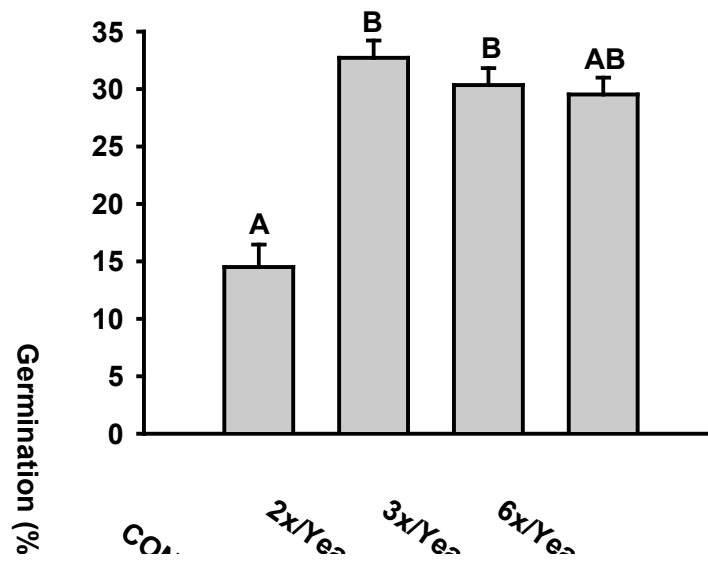
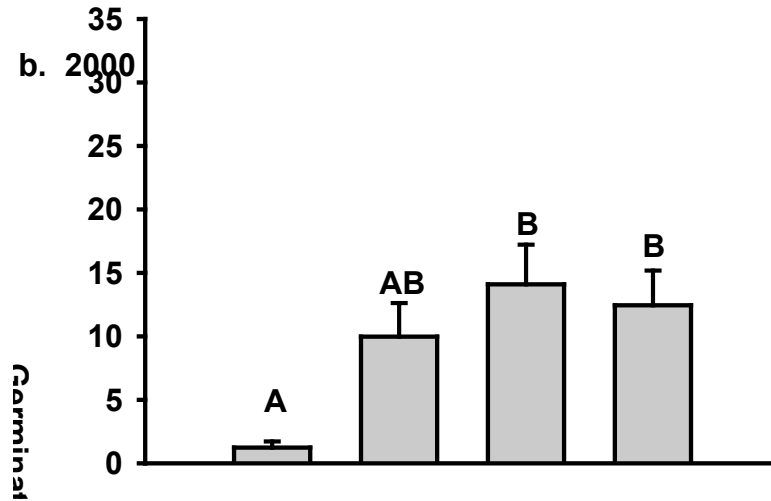
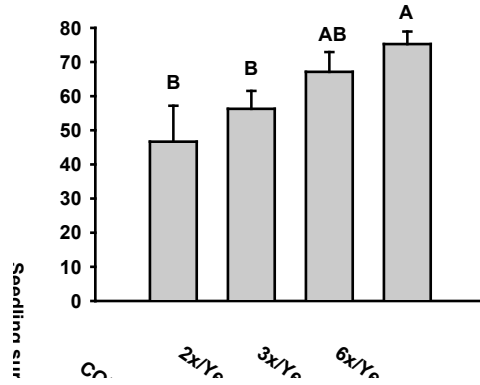
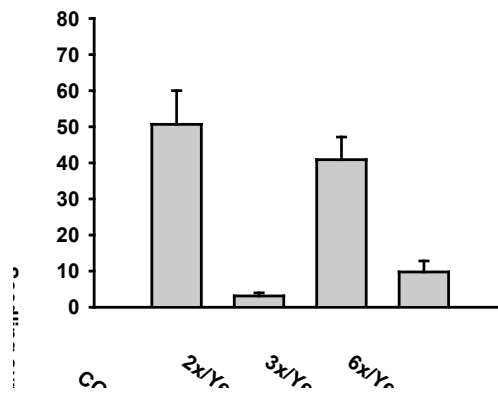


Figure 2: Percent germination of 36 disc seeds, averaged across all sites, in a) 1999 and b) 2000. Error bars indicate 1 se. Means with the same letter are not significantly different ($P < 0.05$) based on Tukey's LSD. $n = 9$, control; $n = 27$, all others.

a. 1999



b. 2000



c. 2001. all sites

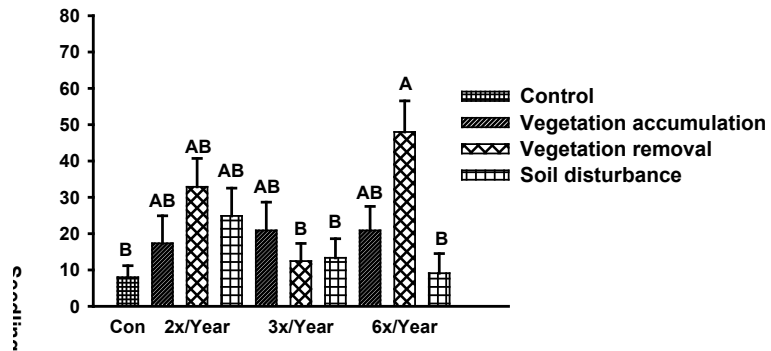


Figure 3: Seedling survival in a) 1999 ($n = 9$, control; $n = 27$, all others); b) 2000 ($n = 9$, control; $n = 27$, all others), and; c) 2001 at all sites ($n = 9$). Error bars indicate 1 se. Means with the same letter are not significantly different ($P < 0.05$) based on Tukey's LSD.

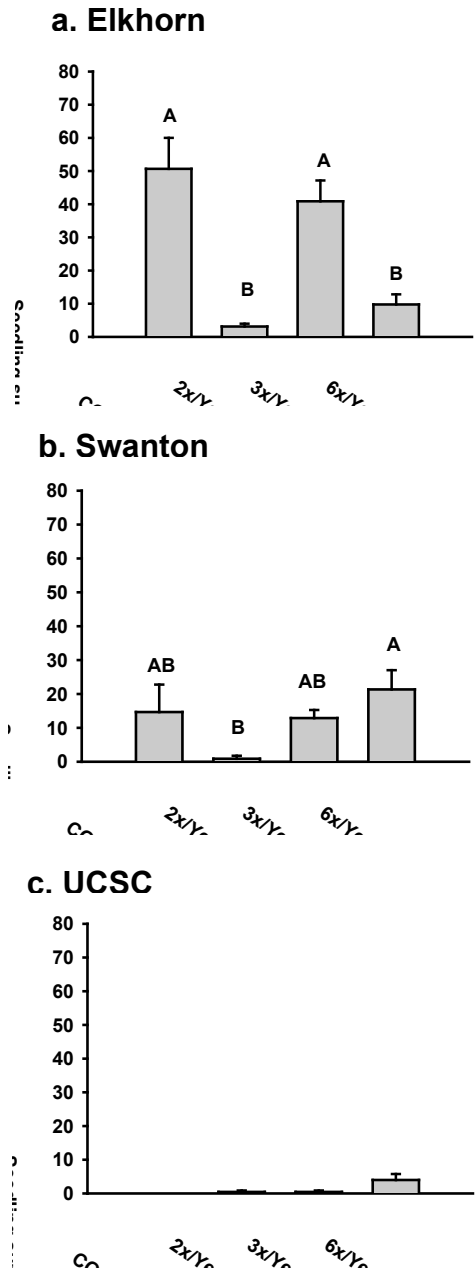


Figure 4: Seedling survival in 2000 at a) Elkhorn; b) Swanton, and; c) UCSC. Error bars indicate 1 se. Means with the same letter are not significantly different ($P < 0.05$) based on Tukey's LSD.

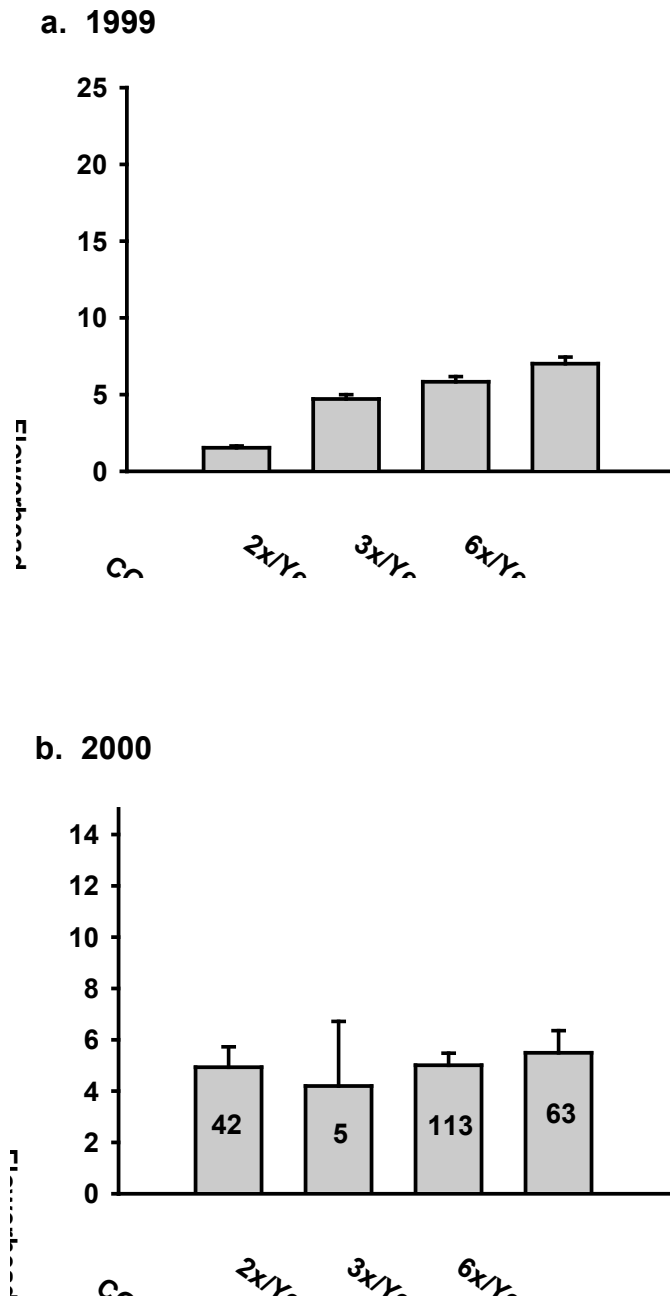
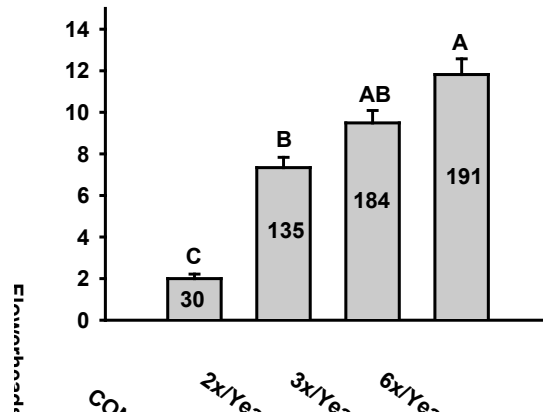
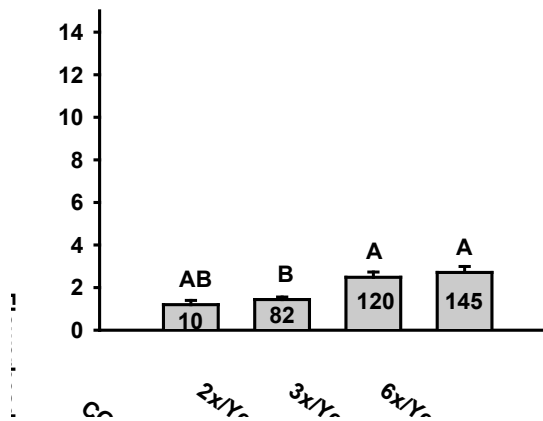


Figure 5: Number of flower heads per plant produced from seedlings planted in a) 1999, and b) 2000 at all sites. Significant site _ treatment interaction in 2000 required separate analysis per site (Fig. 6). Numbers on bars represent *n*. Error bars indicate 1 se. Means with the same letter are not significantly different ($P < 0.05$) based on Tukey's LSD.

a. Elkhorn



b. Swanton



c. UCSC

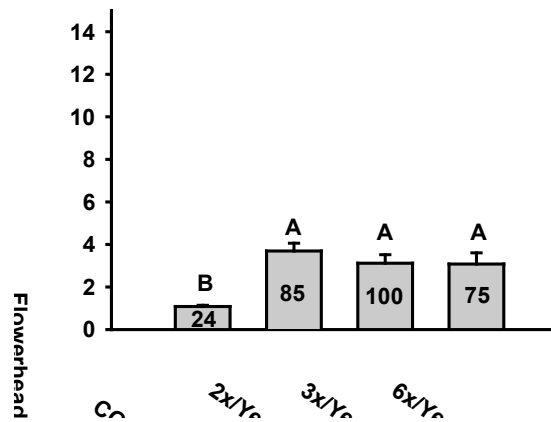


Figure 6: Flowerheads from seedlings planted in 1999 at a) Elkhorn b) Swanton and c) UCSC. Numbers on bars represent n . Error bars indicate 1 se. Means with the same letter are not significantly different ($P < 0.05$) based on Tukey's LSD.

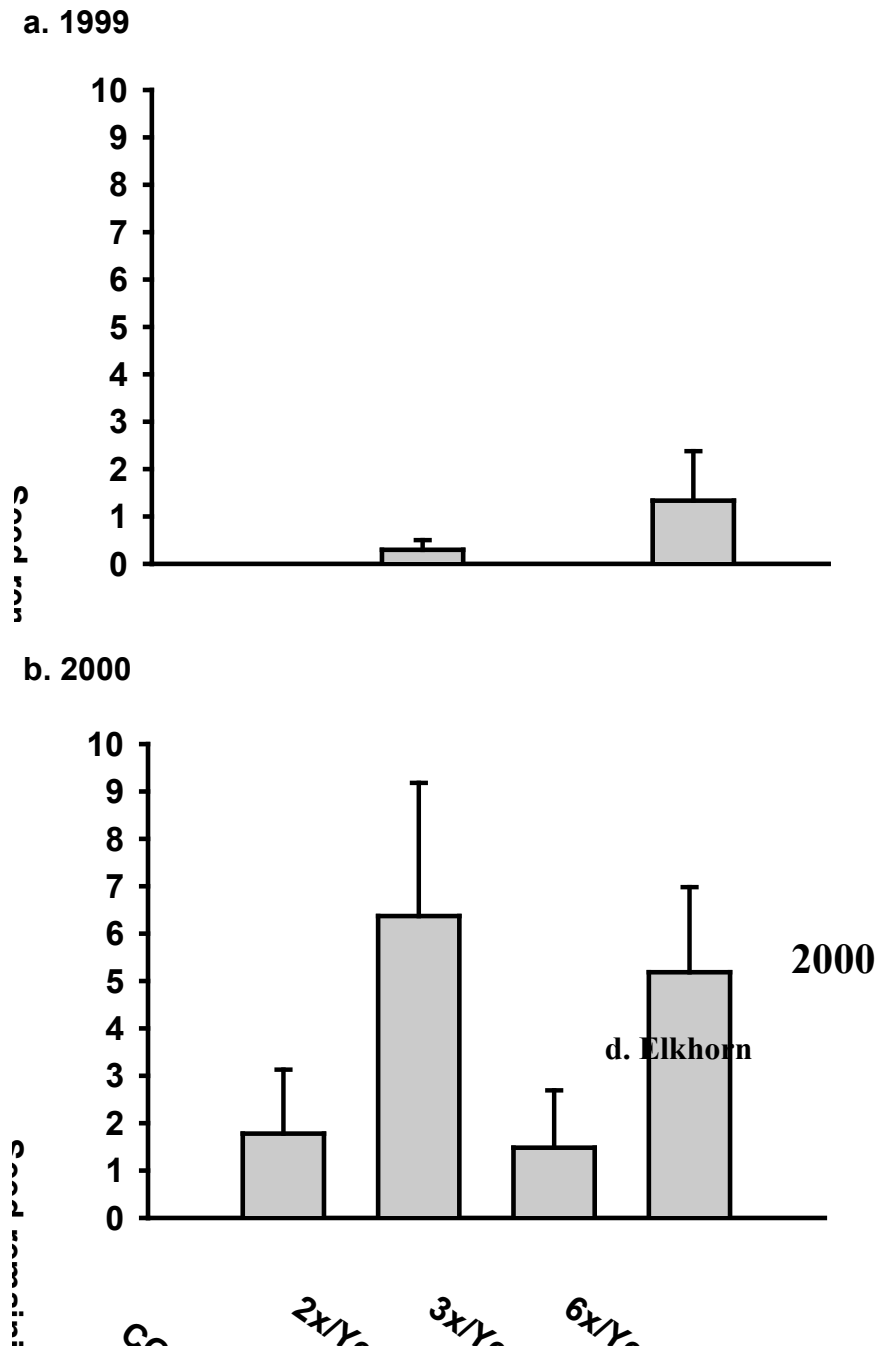
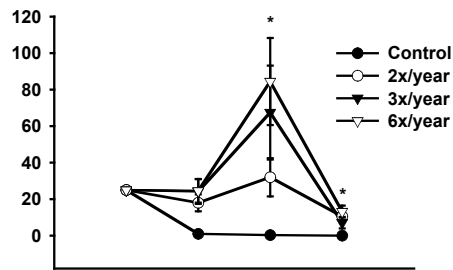


Figure 7: Results of seed predation experiment with percentage of seed remaining after: a) 2 months in 1999 and b) 1 month in 2000. Error bars indicate 1 se. $n = 9$ control; $n = 27$ all others.

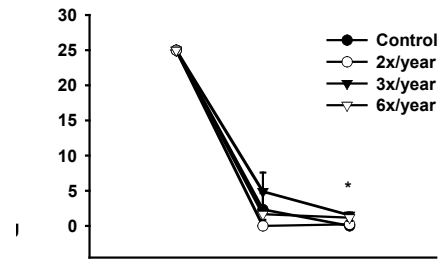
1999

2000

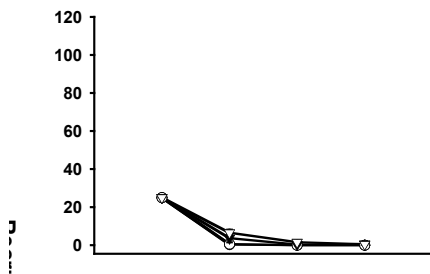
a. Elkhorn



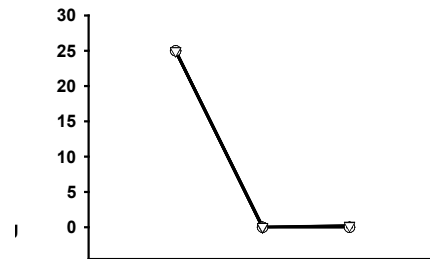
d. Elkhorn



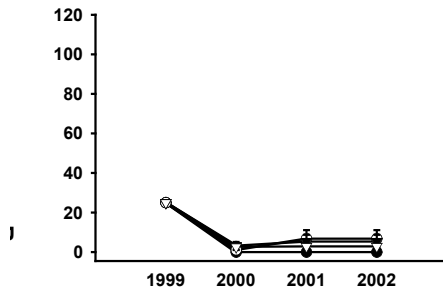
b. Swanton



e. Swanton



c. UCSC



f. UCSC

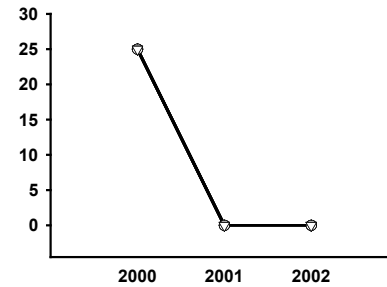


Figure 8: Recruitment from seedling plants: from the 1999 cohort at a) Elkhorn, b) Swanton, and c) UCSC, and; from the 2000 cohort at: d) Elkhorn, e) Swanton, and f) UCSC. Error bars are 1 se. * indicates significant differences ($P < 0.05$). $n = 9$ control; $n = 27$ all others.

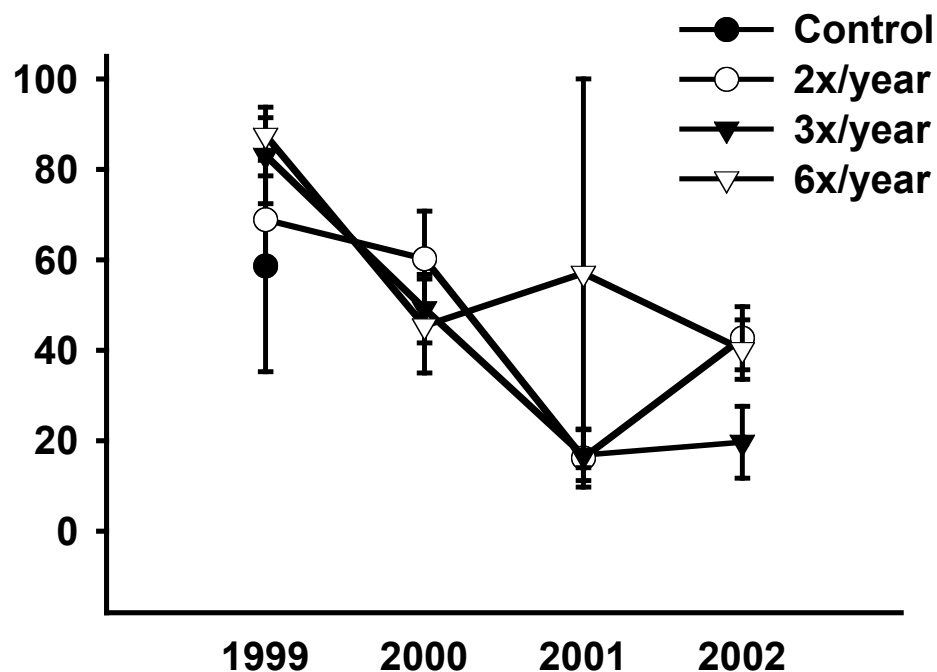


Figure 9: Seedling survivorship and survivorship of recruited plants: from the 1999 cohort at Elkhorn, the only site with sufficient recruiting plants for analysis. 1999 data are from planted seedlings; other dates are from recruited plants. Error bars are 1 se. * indicates significant differences ($P < 0.05$). Data are omitted from control plots in 2000, 2001, and 2002 due to insufficient recruiting plants (<5) in those years. $n = 9$ control; $n = 27$ all others.

Tables

Primary disturbance	Secondary disturbance		
	Litter accumulation	Litter removal	Soil disturbance
Clipped 2x/year	+	+	+
Clipped 3x/year	+	+	+
Clipped 6x/year	+	+	+
Control	na	na	na

Table 1: Experimental treatments.

