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FINAL PROJECT REPORT
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An Investigation of the Reproductive Ecology and Seed Bank Dynamics of Burke's Goldfields (*Lasthenia burkei*), Sonoma Sunshine (*Blennosperma bakeri*), and Sebastopol Meadowfoam (*Limnanthes vinculans*) in Natural and Constructed Vernal Pools

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1. Location of work: Santa Rosa Plain, Sonoma County, California

2. Background: Burke's goldfield (*Lasthenia burkei*), a small, slender annual herb in the sunflower family (Asteraceae), is known only from southern portions of Lake and Mendocino counties and from northeastern Sonoma County. Historically, 39 populations were known from the Santa Rosa Plain, two sites in Lake County, and one site in Mendocino County. The occurrence in Mendocino County is most likely extirpated. From north to south on the Santa Rosa Plain, the species ranges from north of the community of Windsor to east of the city of Sebastopol. The long-term viability of many populations of Burke's goldfields is particularly problematic due to population decline. There are currently 20 known extant populations, a subset of which were inoculated into pools at constructed sites to mitigate the loss of natural populations in the context of development.

Sonoma sunshine (*Blennosperma bakeri*), which is also known as Baker's stickyseed, is a small (up to 12 inches in height), annual herb in the aster family (Asteraceae). *Blennosperma bakeri* is found in grasslands and vernal pools. The species is restricted to the Laguna de Santa Rosa and Sonoma areas in Sonoma County. There are currently 23 known extant populations, a subset of which is populations at constructed sited. A number of other populations have been extirpated in recent years. Sebastopol meadowfoam (*Limnanthes vinculans*) is a small (up to 12 inch tall), multi stemmed herb of the false meadowfoam family (Limnanthaceae). It is found in seasonally wet meadows, swales and vernal pools in the Laguna de Santa Rosa, Sonoma County, and in one location in Napa Co. There are currently 37 known extant populations, a subset of which were inoculated into pools at constructed sites to mitigate the loss of natural populations in the context of development.

Many other, specialized and rare vernal pool plants, have co-evolved with specialized bee pollinators (Thorp & Leong 1998), and likely depend on a vast seed bank to assure long-term persistence at a given site (Griggs & Jain 1983). Viable seed production, deposited in the seed bank each flowering season is the long-term survival strategy of these species. To date, only information inferred from related congeners is available regarding their specific pollinator relationships and reproductive ecology. Other *Lasthenia*, *Limnanthes and Blennosperma* species rely on pollination from specialized bee pollinators, and Thorp (1969) describes two species of solitary bees *Andrena* (*Diandrena*) *submoesta,* and *A. (D.) puthua* as collecting pollen only from flowers of the genus *Lasthenia,* but there had been no distinct records for the pollinators in the range of *L. burkei*. *Limnanthes ssp.* pollinators include *Andrena* (*Hesperandrena*) *pulverea* , and known pollinators of *Blennosperma bakeri* are also in the genus *Andrena* with *Andrena blennospermatis* as the specialist pollinator (Dr. Robbin Thorp, pers com.). It is imperative to quantify the role these flower visitors play in successful reproduction of these endangered plants.

3. Need: To inform long-term management and support the recovery of vernal pool endemic annual plant species, key assessment metrics include yearly reproductive output, seed bank status, breeding system, pollination ecology, as well as status of closely associated pollinators. This project investigated the reproductive ecology of Burke's

goldfields (*Lasthenia burkei*), Sonoma sunshine (*Blennosperma bakeri*), and Sebastopol meadowfoam (*Limnanthes vinculans*), three State and federally listed endangered annual plants, endemic to natural vernal pool sites, and in recent decades inoculated into constructed vernal pools throughout the Santa Rosa Plain, Sonoma County, California. Confirmation of these species' breeding system, and an assessment of the average annual reproductive output in conjunction with pollinator identity, availability and visit frequency at natural and constructed vernal pool sites will help highlight specific management and long-term status monitoring needs essential to sustained population persistence at both pool types. The project also provides methods to estimate the site- or pool-specific status of the seed bank for Sonoma sunshine and Sebastopol meadowfoam, and offers a potential modeling framework to forecast pool- or site-specific population and seed bank dynamics to further inform management and decision-making. Data and methods obtained from this project in conjunction with recent conservation genetic studies will guide future management of these species on CDFG managed preserves identified in the Santa Rosa Plain Conservation strategy.

4. Objectives:

- Confirm whether Burke's goldfields (*Lasthenia burkei*), Sonoma sunshine (*Blennosperma bakeri*), and Sebastopol meadowfoam (*Limnanthes vinculans*) are obligate out crossing species (*in situ*) and so depend on insect pollinators for viable seed set *in situ*.
- Determine yearly seed set per study population in healthy natural, degraded natural and constructed pools.
- Identify main pollinator(s) and determine the location of their upland nest sites (if appropriate).
- Establish estimates of seed bank numbers through soil cores.
- Assess whether yearly seed set estimates suggest a substantial addition to the seed bank, or indicate a continual draw down from the seed bank without proper replenishment of seeds each year at natural healthy, natural degraded and constructed sites.

I. Breeding System of Burke's goldfields (*Lasthenia burkei*), Sonoma sunshine (*Blennosperma bakeri*), and Sebastopol meadowfoam (*Limnanthes vinculans*) and and Annual Seed Set in Natural and Constructed Vernal Pools

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Introduction

Vernal pool ecosystems are in decline throughout California, with only 10% of historic habitat remaining (Holland 1978, Griggs and Jain 1983). Loss and fragmentation of vernal pools have diminished many naturally rare and endemic plant species specializing in this unique habitat. Many extant populations of rare vernal pool plants have been fragmented and isolated within their range with implications for population and species persistence. Therefore, many vernal pool annual plant species are now State or federally listed as threatened or endangered, and recovery plans require information on their population ecological and genetic status to guide recovery and management efforts.

Annual seed set in vernal pool endemic plants is naturally variable from year to year due to inconsistency in environmental factors that influence germination, flowering, successful pollination and maturation of ovules (Bliss and Zedler 1998, Griggs and Jain 1983). The annual growth and density of flowers or inflorescences in vernal pool annual plants is dependent on the number of individuals that germinate and grow throughout the winter months, and will vary within and between pools in any given year. In some cases, natural variation may be extreme, with thousands of individuals present in one year, and only few or none present in another. Determination of whether or not a population is in decline therefore depends on evaluating long-term abundance trends as well as pool-specific long-term seed banks.

Population viability of vernal pool annual plants is ultimately dependent on 1) the potential of a population to set viable seeds each growing season, being closely linked to density of flower patches to attract pollinators, and pollinator availability, and on 2) long-term seed bank viability. Declining or lower-density plant populations can experience increased pollen limitation from lowered pollinator visits that result in decreased seed production (Jacquemyn et al. 2002; Knight 2003). A decrease in per capita reproductive rate with decreasing population density is called an Allee effect (Allee et al. 1949, Stephens et al. 1999, Dennis 2002). It may be the result of either reduced density or quality of compatible mates (Agren and Ericson 1996, Ashih and Wilson 2001, Wolf and Harrison 2001), or the scarcity of pollinators (Mustajarvi et al. 2001, Forsyth 2003), or both, with particular implications for co-evolved rare plant-specialist pollinator mutualisms. If one or the other declines, it can have consequences on the reproductive

output of the other, and may result in a negative feedback vortex towards extinction (Gilpin and Soulé 1986). As more than 90% of all angiosperms are pollen limited and rely on animal pollinators for pollen transfer (Buchmann and Nabhan 1996), Allee effects are particularly important in many animal-pollinated plants (Kunin 1997, Knight 2003), such as vernal pool annual plants know to have specialist bee pollinators (Thorp & Leong 1998).

Self-fertilization plays a role in many scenarios of plant population dynamics and offers an important way for plants to alleviate Allee effects (Lennartsson 2002). Yet, self-fertilization also poses the risk of inbreeding depression with negative consequences for population persistence from reduced fitness (Byers and Waller 1999). The assessment of the breeding system of annual plants can give insight into whether predominant self-fertilization or out-crossing is occurring. By implementing pollinator exclusion trials, preventing animal visitors' access to flowers or inflorescences *in situ*, the comparison of seed set of open-pollinated flowers with enclosed flowers can then show how effective both reproductive strategies are in a given taxon. Measurements of pool-specific average seed set per individual, coupled with annual plant abundance and density estimates will allow assessment of whether Allee effects might be occurring in any given population. Further investigations and adaptive management measures can so be implemented to address its potential negative impacts on population persistence. Such measures might include hand pollination of individuals, or introduction of specialist pollinators from nearby locations with a large enough source population.

Burke's goldfields (*Lasthenia burkei*), Sonoma sunshine (*Blennosperma bakeri*), and Sebastopol meadowfoam (*Limnanthes vinculans*) are three State and federally listed endangered annual plants, endemic to natural vernal pool sites, and in recent decades inoculated into constructed vernal pools throughout the Santa Rosa Plain, Sonoma County, California. To assess whether Allee effects, self-fertilization, and inbreeding depression exist in these three Northern California endangered vernal pool annual plant species, the objectives of this part of our investigation were 1) to confirm whether the three species are obligate out-crossing species and so depend on insect pollinators for viable seed set *in situ*, and 2) to determine average yearly seed set per study population in natural and constructed pools. We also estimated population abundance and percent cover of the sampled pools for each species to assess whether average seed set per individual was a function of population size and density.

We further investigated the respective animal pollinator communities of each endangered plant, and examined visitation rates of major pollinators in the following report section, to evaluate whether pollinator limitation existed across natural and constructed pools. In the last section, we report on methods to sample the long-lived seed bank of two of our target species, and discuss the potential for assessing population viability by combining various demographic parameters into a density-structured model to project plant population viability and dynamics into the future.

Methods

Study Species Background
Sonoma sunshine (*Blennosperma bakeri*), which is also known as Baker's stickyseed, is a small (up to 12 inches in height), annual herb in the aster family (Asteraceae). *Blennosperma bakeri* is found in grasslands and vernal pools. The species is restricted to the Laguna de Santa Rosa and Sonoma Valley in Sonoma County. There are currently 23 known extant populations, a subset of which were inoculated into pools at constructed sites to mitigate the loss of natural populations in the context of development.

Sebastopol meadowfoam (*Limnanthes vinculans*) is a small (up to 12 inch tall), multi stemmed herb of the false meadowfoam family (Limnanthaceae). It is found in seasonally wet meadows, swales and vernal pools in the Laguna de Santa Rosa, Sonoma County, and in one location in Napa Co. There are currently 37 known extant populations, a subset of which were inoculated into pools at constructed sites to mitigate the loss of natural populations in the context of development.

Burke's goldfield (*Lasthenia burkei*), a small, slender annual herb in the sunflower family (Asteraceae), is known only from southern portions of Lake and Mendocino counties and from northeastern Sonoma County. Historically, 39 populations were known from the Santa Rosa Plain, two sites in Lake County, and one site in Mendocino County. The occurrence in Mendocino County is most likely extirpated. From north to south on the Santa Rosa Plain, the species ranges from north of the community of Windsor to east of the city of Sebastopol. The long-term viability of many populations of Burke's goldfields is particularly problematic due to population decline. There are currently 20 known extant populations, a subset of which were inoculated into pools at constructed sites to mitigate the loss of natural populations in the context of development.

Breeding System
To prevent flower visitation to inflorescences we installed 1 mm mesh pollinator enclosures (Figure 1.1) on 171 *B. bakeri*, 72 *L. vinculans*, and 58 *L. burkei* inflorescences in two natural and two constructed pools per species, during the spring 2009 flowering season (Figure 1.2). We also marked open pollinated inflorescences on 562 *B. bakeri*, 363 *L. vinculans*, and 370 *L. burkei* inflorescences at the same pools. In the late spring/early summer, we collected all seeds from all enclosed and open pollinated flowers for each of the three species. We determined the number of viable vs. non-viable seeds from all enclosed and open-pollinated flowers by species.

Seed Germination
During the 2010 spring flowering season, we performed greenhouse germination trials (germination of seeds on sterile agar plates over a four week period) of enclosed and open-pollinated seeds to verify seed viability across treatments.

Seed Set by Pool Type
We marked open pollinated inflorescences of *B. bakeri, L. vinculans*, and *L. burkei* across natural and constructed pools during spring flowering seasons in 2009 and 2010 (Figure

1.2; Tables 1.1.1 & 1.1.2, Appendix 1.1). Pools sampled and numbers of samples collected per pool type (natural or constructed pool) per year are shown in Table 1.1.2. We collected all seeds from all marked inflorescences in both sampling years and determined the number of viable vs. non-viable seeds from all marked inflorescences. We performed Analysis of Variance of seed set results across sample years and pool types, and sampled pools.

Population Abundance Estimates

We utilized the standardized protocol developed by the "Adopt-a-Vernal Pool (AVP)" citizen science program (http://www.citizen-science.org/Laguna/rdPage.aspx) to estimate vernal pool annual plant population sizes per pool for each species in 2009 and 2010. The protocol includes population density estimates (# individuals/0.1 square meter quadrat) of all patches of various cover classes (see section below) and direct plant counts to estimate total population abundance. We entered our data into the AVP database for easy online access, and to compare to long-term term trends, available via the AVP program for some sampled pools (Table 1.1.1). We examined whether each sample population's estimated abundance was a predictor of seed set per individual, using regression analysis.

Seed Set by Cover Class

To test the effect of population density on seed set, we used the California Native Plant Society cover class guidelines (Appendix 1.2), visually estimating percent cover, and either counting all individuals in the sample patch (if small), or, for five replicates, counting individuals within a 0.1 square meter quadrat placed within the area containing sampled plants. For analysis, we further grouped cover classes 1, 5 & 10%; 15 & 25%; and 35, 50 & 75% and analyzed seed set by cover class group for each species via one-way Analysis of Variance (ANOVA).

Results

Breeding System

Blennosperma bakeri is a predominately out crossing species with a mean seed set of 4.06 ± 1.64 (s.d.) per open pollinated inflorescence as compared to only 0.82 ± 1.31 (s.d.) seeds set on average per enclosed inflorescence ($P < 0.0001$). A total of 2,282 seeds were set in 562 open pollinated inflorescences, while 141 seeds developed in 171 enclosed inflorescences. Of the tested 171 enclosed inflorescences, 104 or 61% did not set any viable seed, while in comparison, only 3%, or 14, of 561 open-pollinated inflorescences failed to set seed.

Limnanthes vinculans is a predominately out crossing species with a mean seed set of 2.09 ± 1.78 (s.d.) in open pollinated inflorescences as compared to 0.61 ± 1.21 (s.d.) in enclosed inflorescences ($P < 0.0001$). A total of 757 seeds were set in 363 tested open pollinated flowers, while only 44 seeds developed from 72 enclosed inflorescences. Of these 72 enclosed inflorescences, 51 or 71% did not set any viable seeds, while only 32%, or 115, of 363 open-pollinated inflorescences failed to set seed.

Lasthenia burkei is a predominately out crossing species with a mean seed set of 95.75 ±

64.37 (s.d.) in open pollinated inflorescences as compared to 37.90 ± 47.46 (s.d.) in enclosed inflorescences ($P < 0.0001$). A total of 35,428 seeds were set in 370 open pollinated flowers, while 2,198 seeds developed in 58 enclosed inflorescences, suggesting that *L. burkei* is able to self-fertilize, assuming the mesh enclosures effectively kept out pollinating agents. Of these 58 enclosed inflorescences, 11, or 19%, did not set any viable seeds, while only 4%, or 16 of 370, open-pollinated inflorescences failed to set seed.

Seed Germination

We tested germination of a total of 819 *B. bakeri* seeds from 241 inflorescences, collected in 2009 over a four-week period in spring 2010. We investigated germination success of 128 seeds from 63 enclosed inflorescences, and 691 seeds from 178 open pollinated inflorescences. We found that from open-pollinated inflorescences, 78%, or 539 of 691 tested seeds, germinated. For the few enclosed inflorescences that set seed, a slightly lower proportion of viable seeds, 75%, or 96 of 128 tested seeds, germinated. Comparing the average number of seeds germinating per inflorescence between treatments, about twice as many open-pollinated seeds germinated per inflorescence (mean = 3.07 ± 1.65 (s.d.)) than from enclosed inflorescences (mean = 1.52 ± 1.41 (s.d.); $p = 0.000$).

Over a four-week period in spring 2010, we tested germination success of a total of 501 *L. vinculans* seeds from 221 inflorescences, collected in 2009 and deemed viable from visual inspection of seed size and shape. Observed germination failure of 160 seeds visually pre-categorized as non-viable, verified our size and shape-based categorization technique. We investigated germination success of 44 seeds from 21 enclosed inflorescences, and 458 seeds from 152 open pollinated inflorescences. We found that from open-pollinated inflorescences, 55%, or 250 of 458 tested seeds, germinated. For enclosed inflorescences, 68%, or 30 of 44 tested viable seeds, germinated. The average number of seeds germinated per open-pollinated inflorescence (1.65 ± 1.48 (s.d.)) did not differ from enclosed inflorescences (1.43 ± 1.43 (s.d.); $p = 0.529$).

Over a four-week period in spring 2010, we tested germination success of a total of 3,087 *L. burkei* seeds from 107 inflorescences collected in 2009. We investigated germination success of 626 seeds from 22 enclosed inflorescences, and 2,461 seeds from 85 open pollinated inflorescences. We found that from open-pollinated inflorescences, 56%, or 1,366 of 2,461 tested seeds, germinated. For enclosed inflorescences, 64%, or 401 of 626 tested seeds, germinated. The average number of seeds germinated per open-pollinated inflorescence (16.07 ± 9.96 (s.d.)) did not differ from enclosed inflorescences (18.22 ± 10.11 (s.d.); $p = 0.369$).

Seed Set by Year and Pool Type

Average *Blennosperma bakeri* seed set per inflorescence was different across the two sampling years, showing a 1.3-fold increase from 2009 to 2010 (Figure 1.3; Table 1.2, $p = 0.0001$). Averaged over the two sampling years seed set was 1.5 times greater in natural versus constructed pools (Figure 1.4, Tables 1.3.1; $P < 0.0001$). A significant pool effect

(p = 0.0001), shown by extremely high 2010 average seed set at two natural pools at Haroutounian (HARO 1 mean seed set = 13.138; HARO 2 = 11.268) may explain the departure from the 2009 results of no difference in seed set between pool types. In 2009, only 2 natural and 2 constructed pools were compared, not including Haroutounian pools.

Our results showed that maximum seed set jumped from 10 seeds per inflorescence in 2009 to 32 in 2010. Accordingly, average seed set of *B. bakeri* across sample sites of ~4 seeds per individual also increased 3-fold to ~12 seeds per individual in 2010. We found seed set greater than 10 seeds/inflorescence only at the two pools at Haroutounian (Figure 1.5). To test whether this extraordinary seed set at these two natural pools likely caused the observed differences between pool type and years, we re-evaluated the data by excluding both Haroutounian pools from the analysis of variance (Table 1.3.2). Our second set of ANOVA results still supported differing average seed set across sampling years (p = 0.001), yet showed no difference between pool type (p = 0.421, Table 1.3.2).

Average seed set per flower of *L. vinculans* in 2010 was 1.7 fold that in 2009 (Table 1.4, Figure 1.6; p = 0.000). Average seed set across both years also differed between pool types, and was 1.3 times higher in four natural pools than four constructed pools (Figure 1.7, Tables 1.4, 1.5; P = 0.001). As in *B. bakeri*, average seed set of *L. vinculans* differed among individual pools (Figure 1.8; p = 0.000), yet no single pool or site had extremely divergent seed set, and maximum seed set was 5 seeds per flower across all sampled pools.

Average seed set of *L. burkei* was different across the two sampling years, being slightly lower by a factor of 1.15 in 2010 (Table 1.6, Figure 1.9; p = 0.001). Average seed set across sample years did not differ among natural and constructed pools (Figure 1.10; Table 1.7; P = 0.117). Average seed set differed among individual pools (Figure 1.11; p = 0.000), yet as with *L. vinculans*, no single pool or site showed seed set that drastically exceeded that of other pools. Maximum seed set was 281 seeds per inflorescence.

Seed Set Relative to Population Abundance by Pool

Pool-specific population abundance was a predictor of average seed set per individual for *B. bakeri*, only when including the two Haroutounian pools with extraordinary average seed set (R^2 = 0.56, P = 0.002; Figure 1.12). When excluding these two pools from the analysis, there was no predictive relationship between population abundance and average individual seed set (R^2 = 0.16, P = 0.22). There was also no such relationship in tested *L. vinculans* (R^2 = 0.06, P = 0.46; Figure 1.13), and *L. bakeri* (R^2 = 0.13, P = 0.23; Figure 1.14) pools.

Seed Set by Cover Class

In all three species cover class groups were a predictor of average seed set (Figure 1.15). Cover classes > 35% resulted in 1.32 times more average seed set in *L. burkei,* 1.14 times higher in *B. bakeri,* and 1.13 times more in *L. vinculans* than lower cover classes. In *L. vinculans*, cover classes 15- 25% increased average seed set of cover classes <10% by 1.43 fold (Figure 1.15).

Discussion

Annual vernal pool plant viability is related to the predominant breeding system and successful seed set *in situ*. In order to project population responses to changing environmental conditions into the future, as well as evaluate persistence of inoculated populations in constructed pools, annual seed set, dependence on and availability of animal pollinators, level of density-dependent pollen limitation, and seed bank viability are key metrics for long-term assessment.

The predominant breeding systems of *Blennosperma bakeri*, *Limnanthes vinculans*, and *Lasthenia burkei* are insect-mediated out-crossing, with some potential for self-fertilized seed set. Unless enclosures failed to keep out very small pollinators, we found that all three species had some ability to effect pollination within mesh enclosures under field conditions. In the greenhouse, three quarters of 171 *B. bakeri* viable closed-pollinated seeds, 68% of 44 viable closed-pollinated *L. vinculans* seeds, and 64% of 2,198 viable closed-pollinated *L. burkei* seeds, germinated successfully. This suggests a proportion of potentially self-fertilized seedlings per species are viable under ideal conditions.

Efficiency of pollination within *B. bakeri* enclosures was about half of that in open-pollinated flowers, indicating that self-fertilization is less effective than out-crossing in producing viable germinating seeds in this species, however, we may need to take into account a potential enclosure effect. Pollination efficiency was similar to that in open-pollinated flowers of *L. vinculans* and *L. burkei* inflorescences, showing that, even if infrequent, closed-pollinated seeds are as effective as those out-crossed in producing viable seeds that germinate under ideal conditions in these two species.

For all three focal species successful *ex situ* germination of seeds collected in 2009 ranged between 55-78 percent for both open-pollinated and closed-pollinated seeds. *In situ,* this range will likely vary upward or downward, depending on pool specific conditions in any given year. Inbreeding depression may have a negative effect on the success of self-fertilized seed germination *in situ*. However, under ideal conditions, a small proportion of closed-pollinated seeds are viable and so have at least the potential to help alleviate Allee effects in declining populations of all three species.

Pollen limitation occurs when plants produce fewer fruits and/or seeds than they would with adequate pollen receipt. We found evidence of pollen limitation in all three species. Average seed set of *B. bakeri* was different across two sampling years, showing a 1.3-fold increase from 2009 to 2010 (Table 1.2). While there was no difference in seed set across natural versus constructed *B. bakeri* pools in 2009, in 2010 an additional natural site with extraordinarily high seed set was sampled, causing average seed set in natural pools to be 1.5 times that in constructed pools. Maximum seed set per *B. bakeri* inflorescence was 32, while yearly averages ranged between 4.06 – 5.12 seeds set per inflorescence (Table 1.2).

For *L. vinculans*, average seed set in 2010 was 1.7 fold that in 2009, and across both years also differed between pool types. Being 1.3 times higher in natural versus

constructed pools suggests that pollen limitation is greater at constructed pools, most likely being a factor of reduced pollinator availability or efficiency. As in *B. bakeri*, average seed set of *L. vinculans* differed among individual pools, yet no single pool or site had extremely divergent seed set. Maximum seed set was 5 seeds per flower across all sampled pools, with annual averages ranging between 1.84 and 3.20 seeds per flower, again a likely factor of pollinator availability and/or efficiency.

Average seed set of *L. burkei* was different across the two sampling years, being slightly lower by a factor of 1.15 in 2010. Among natural and constructed pools average seed set did not differ across sample years (Figure 1.10). Average seed set differed among individual pools, yet no single pool or site showed seed set that drastically exceeded that of other pools. Maximum *L. burkei* seed set was 281 seeds per inflorescence, with annual averages ranging from 82.66 to 91.66 seeds/inflorescence.

For all three species, cover classes greater than 35% showed significant increase in average seed set, suggesting that pollen limitation is a factor of floral density, and so directly relevant to attracting pollinators (Figure 1.15). Floral display helps bees and other pollinators find flowers, and if floral density is high, pollinators can travel more efficiently between flowers, increasing seed set. Overall estimates of population abundance per pool were less of a predictor of seed set, but average seed set did differ across individual pools for all species (Figures 1.5, 1.8, 1.11 – 1.14).

Average seed set was higher in two species at natural versus constructed sites (Figures 1.4 & 1.7). At some constructed pools floral displays may have declined after initial pool inoculation, or may have never been large enough to attract the same level of pollinators as pools at natural sites. Pollinators, especially specialist solitary bee pollinators, may also not be as abundant at constructed sites as they are at natural sites. At natural sites, effective pollinator levels will likely fluctuate from year to year, or may be declining at some sites, while staying more stable at others. Section II of this report will shed more light on pollinator communities and visitation rates at sample sites.

Recommendations for Management

Since all targets are predominant out-crossing species, pollen limited, and so depend on specialist and generalist pollinators for annual seed set, we suggest the following management actions:

1. Continue monitoring of population abundance at a majority of natural and constructed pools (utilize and continue AVP model);
2. Monitor annual seed set for all three species at pools that indicate an overall decline in abundance for several years in comparison to reference sites that show long-term stability in abundance (this could be integrated into the AVP model at target sites);
3. At sites where consistently low floral displays are found, find out possible causes, such as invasive species (i.e. *Mentha pulegium, Agrostis avenatis, Glyceria declinata*). Implement and test exotic competitor removal and other management options within an adaptive management framework.

4. Assess the impact of grazing or mowing of the vernal pool uplands in this Coastal (wetter) vernal pool system and compare to studies in Central Valley pools (Marty 2005).

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Tables

Table 1.1.1: Population abundance by species per pool in 2009 & 2010, and available pool-specific AVP program records of minimum and maximum pool-specific population abundance (2007-2011).

Species	Site-Pool	Pool type	Sampling Year	Population abundance	Minimum abundance recorded (year)	Maximum abundance recorded (year)	Years pool was surveyed
B. bakeri	ALTN-D	constructed	2010	558,455	133,663 (2011)	558,455 (2010)	2
B. bakeri	ALTN-K	constructed	2010	440,021	80,400 (2011)	440,021 (2010)	2
B. bakeri	HARO-1	natural	2010	811,423	346,613 (2009)	1,038,772 (2008)	4
B. bakeri	HARO-2	natural	2010	1,844,706	160,049 (2008)	1844706 (2010)	3
B. bakeri	**MAGG-1**	**natural**	**2009**	**384,727**	**8,052 (2011)**	**384,727 (2009)**	**4**
B. bakeri	MAGG-1	natural	2010	51,267	8,052 (2011)	384,727 (2009)	4
B. bakeri	SLRO-7	constructed	2010	*not available*	*not available*	*not available*	0
B. bakeri	SLRO-17	constructed	2010	124,980	0 (2011)	173,107 (2008)	4
B. bakeri	**TCMB-36**	**constructed**	**2009**	**38,771**	**5,083 (2010)**	**38,771 (2009)**	2
B. bakeri	TCMB-36	constructed	2010	5,038	5,083 (2010)	38,771 (2009)	2
B. bakeri	**TCMB-87**	**constructed**	**2009**	**532,766**	**78,105 (2010)**	**532,766 (2009)**	**2**
B. bakeri	TCMB-87	constructed	2010	78,105	78,105 (2010)	532,766 (2009)	2
B. bakeri	**YCPA-1**	**natural**	**2009**	**31,163**	**10,290 (2008)**	**111,663 (2010)**	**4**
B. bakeri	YCPA-1	natural	2010	111,663	10,290 (2008)	111,663 (2010)	4
B. bakeri	YCPA-2	natural	2010	23,388	1,111 (2008)	23,388 (2010)	4
L. vinculans	ALTN-Na	constructed	2010	15,463	15,463 (2010)	800,946 (2009)	2
L. vinculans	**MARI-1**	**natural**	**2009**	**31,950**	**0 (2011)**	**307,117 (2008)**	**4**
L. vinculans	MARI-1	natural	2010	89,289	0 (2011)	307,117 (2008)	4
L. vinculans	SJAC-1	natural	2010	29,209	450 (2008)	20,269 (2009)	4
L. vinculans	SLRO-17	constructed	2010	536	140 (2007)	1,714 (2009)	3
L. vinculans	**TCMB-57**	**constructed**	**2009**	**25,927**	**25,927 (2009)**	**44,555 (2010)**	**1**
L. vinculans	TCMB-57	constructed	2010	44,555	25,927 (2009)	44,555 (2010)	1
L. vinculans	**TCMB-106**	**constructed**	**2009**	**21,381**	**1,340 (2011)**	**21,381 (2009)**	**2**
L. vinculans	TCMB-106	constructed	2010	*not available*	1,340 (2011)	21,381 (2009)	2
L. vinculans	WRIG-7	natural	2010	17,200	3,061 (2011)	22,353 (2009	3
L. vinculans	**WRIG-18**	**natural**	**2009**	**76,788**	**17,873 (2010)**	**76,788 (2009)**	**2**
L. vinculans	WRIG-18	natural	2010	17,873	17,873 (2010)	76,788 (2009)	2
L. burkei	ALTN-D	constructed	2010	624,316	150,360 (2011)	624,316 (2010)	2
L. burkei	ALTN-L	constructed	2010	833,376	8,297 (2009)	833,376 (2010)	3
L. burkei	PIMA-1	natural	2010	76,443	37,347 (2011)	137,385 (2009)	5
L. burkei	**WILK-7**	**natural**	**2009**	**213,191**	**77,077 (2008)**	**213,191 (2009)**	**4**
L. burkei	WILK-7	natural	2010	105,125	77,077 (2008)	213,191 (2009)	4
L. burkei	**WILK-8**	**natural**	**2009**	**92,501**	**25,887 (2010)**	**233,383 (2008)**	**4**
L. burkei	WILK-8	natural	2010	25,887	25,887 (2010)	233,383 (2008)	4
L. burkei	**WOFU-5**	**natural**	**2009**	**29,676**	**150 (2007)**	**29,676 (2009)**	**5**
L. burkei	WOFU-5	natural	2010	13,757	150 (2007)	29,676 (2009)	5
L. burkei	WOFU-6	natural	2009	51,516	40 (2007)	51,516 (2009)	2
L. burkei	WRIG-1	constructed	2010	10,982	0 (2011)	28,212 (2009)	4
L. burkei	**WRIG-6**	**constructed**	**2009**	**259,495**	**59,479 (2010)**	**259,495 (2009)**	**3**
L. burkei	WRIG-6	constructed	2010	59,479	59,479 (2010)	259,495 (2009)	3

Table 1.1.2: Sample sizes and number of pools per sites sampled per species in 2009 and 2010 sampling years across pool types (constructed vs. natural)

	Species	2009		2010	
		Natural	Constructed	Natural	Constructed
Sample size	*B. bakeri*	301	261	437	318
Pools sampled	*B. bakeri*	2	2	5	6
Sample size	*L. vinculans*	224	211	328	217
Pools sampled	*L. vinculans*	2	2	4	4
Sample size	*L. burkei*	221	145	335	392
Pools sampled	*L. burkei*	3	1	5	4

Table 1.2*:* Sample size and average seed set of *B. bakeri* per inflorescence by pool type and year

Year	Pool type	n	Average seed set	St. Dev.	p-value
2009	all	562	4.06	1.64	0.0001
2010		754	5.12	4.45	
2009/2010	natural	618	5.66	4.66	0.0001
	constructed	698	3.79	1.77	

Table 1.3.1*:* Analysis of Variance results for *B. bakeri* seed set by year and pool type

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
YEAR	568.593	1	568.593	53.083	0.0001
POOL TYPE	996.377	1	996.377	93.019	0.0001
YEAR * POOL TYPE	1,030.546	1	1,030.546	96.209	0.0001
Error	14,053.478	1,312	10.711		

Table 1.3.2. Analysis of Variance results for *B. bakeri* seed set by year and pool type without Haroutounian pools

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
YEAR	33.324	1	33.324	11.710	0.001
POOL TYPE	1.847	1	1.847	0.649	0.421
YEAR*POOL TYPE	3.440	1	3.440	1.209	0.272
Error	3,366.566	1,183	2.846		

Table 1.4: Seed set per inflorescence of *L. vinculans* by pool type averaged across samples from 2009 & 2010

Year	Pool type	n	Average seed set	St. Dev.	p-value
2009	all	435	1.84	1.78	0.0001
2010		545	3.20	1.42	
2009/2010	natural	552	2.89	1.64	0.001
	constructed	428	2.22	1.77	

Table 1.5*:* Analysis of Variance results for *L. vinculans* seed set in 2009 & 2010

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
YEAR	353.886	1	353.886	155.866	0.0001
POOL	198.824	7	28.403	12.510	0.0001
POOL TYPE	23.813	1	23.813	10.488	0.001
Error	2,202.334	970	2.270		

Table 1.6: Seed set per inflorescence of *L. burkei* by pool type averaged across samples from 2009 & 2010

Year	Pool type	n	Average seed set	St. Dev.	p-value
2009	all	556	91.66	59.69	0.001
2010		537	82.68	51.82	
2009/2010	natural	221	95.56	67.39	0.117
	constructed	145	95.19	59.97	

Table 1.7*:* Analysis of Variance results for *L. burkei* seed set by pool type in 2009 & 2010

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
YEAR	32,845.825	1	32,845.825	10.584	0.001
POOLTYPE	7,657.482	1	7,657.482	2.467	0.117
YEAR*POOL TYPE	6,694.342	1	6,694.342	2.157	0.142
Error	3,379,695.826	1,089	3,103.486		

Figures

Figure 1.1: Mesh enclosure on *B. bakeri* (left), *L. vinculans* (middle), *L. burkei* (right).

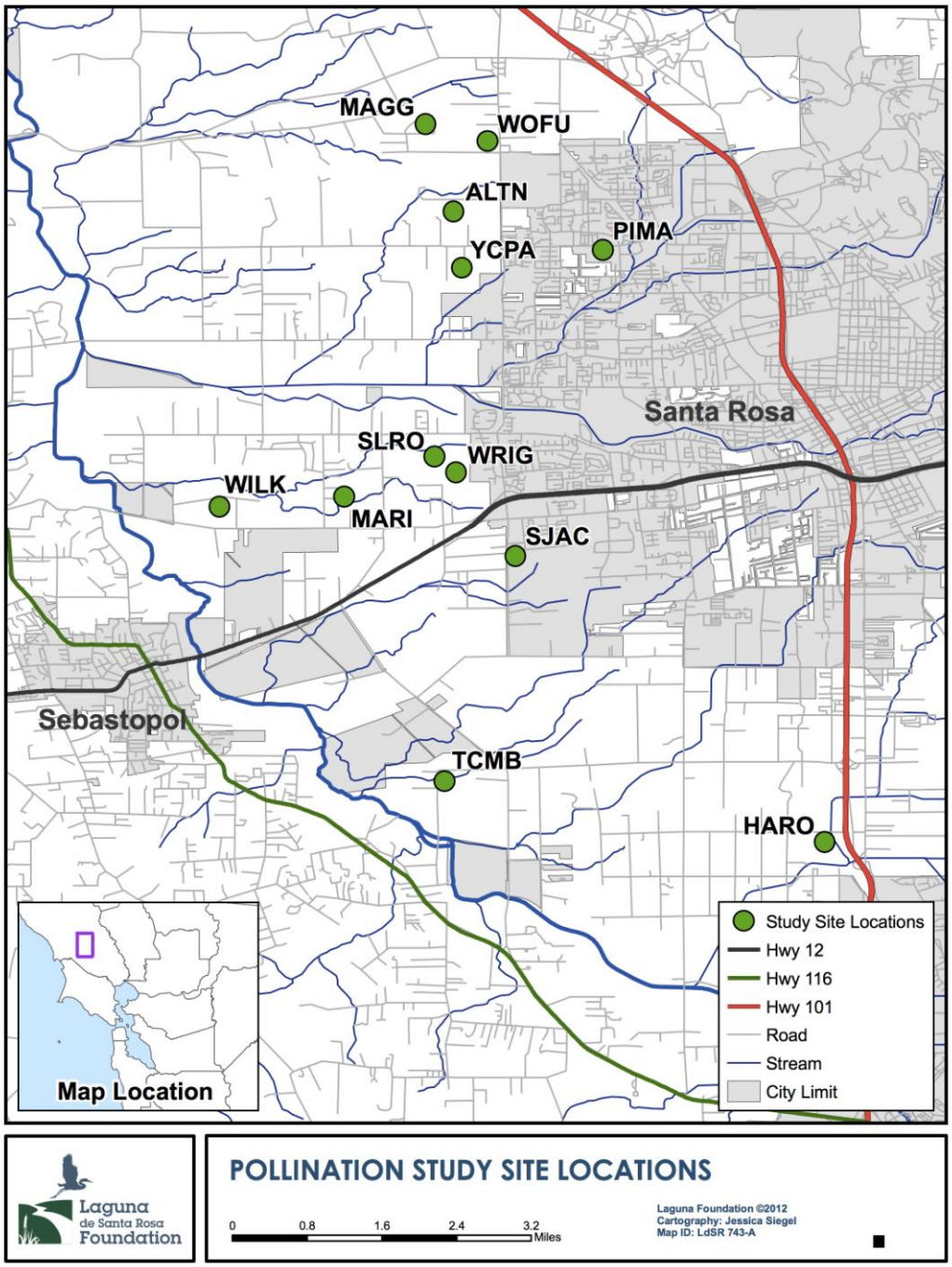


Figure 1.2: Pollination study site locations. *Blennosperma bakeri*: ALTN (c), TCMB (c), SLRO (c), YCPA (n), HARO (n), MAGG (n)*; Limnanthes vinculans:* ALTN (c), TCMB (c), SLRO (c), MARI (n), SJAC (n), WRIG (n); *Lasthenia burkei:* ALTN (c), WRIG (c), PIMA (n), WILK (n), WOFU (n). [c = constructed pool, n = natural pool]

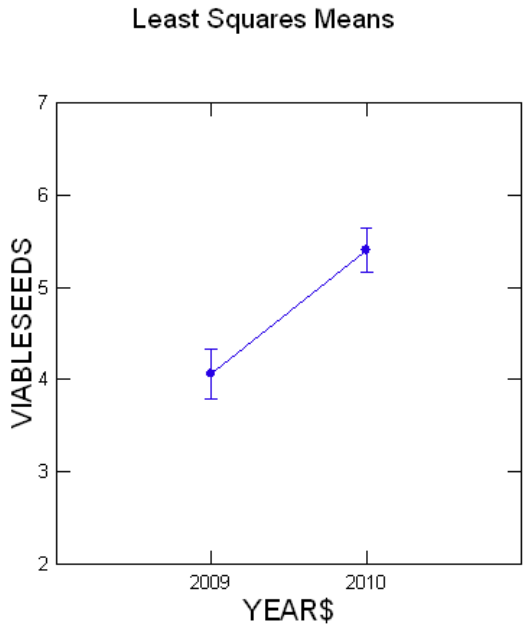


Figure 1.3. Average seed set of *B. bakeri* across sampling years (p = 0.0001)

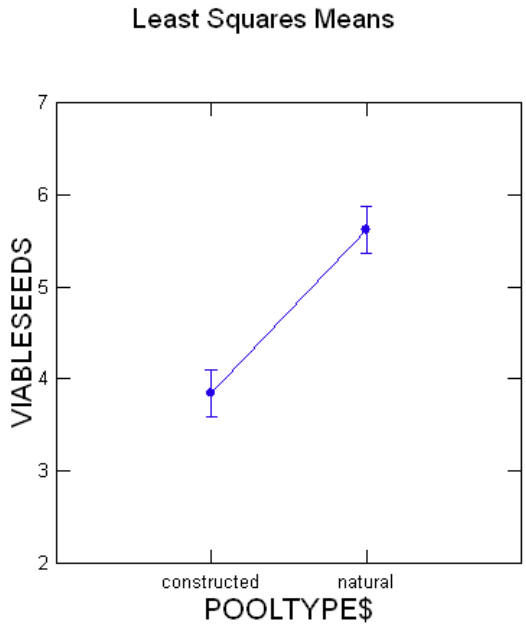


Figure 1.4: Average viable seed set of *B. bakeri* in 2009 and 2010 samples in natural versus constructed pools (p = 0.0001).

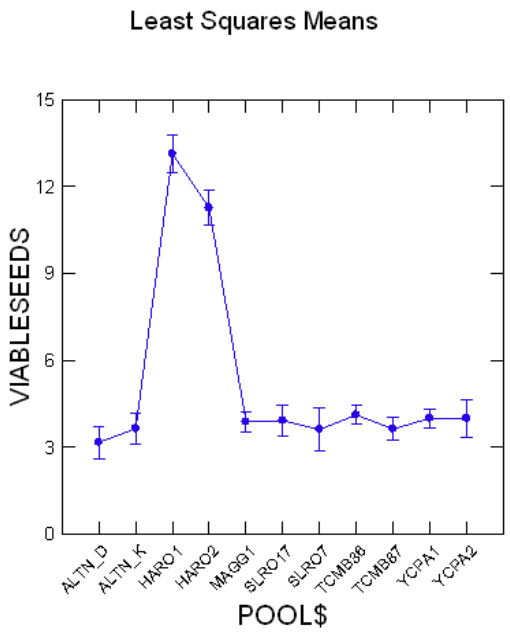


Figure 1.5*:* Viable seed set of *B. bakeri* per pool averaged from 2009 & 2010 data (p < 0.0001)

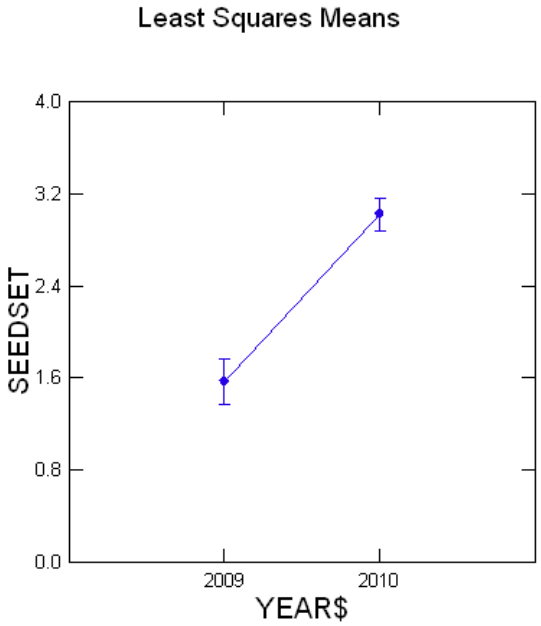


Figure 1.6*:* Average *L. vinculans* seed set across years (p < 0.0001, alpha 0.05)

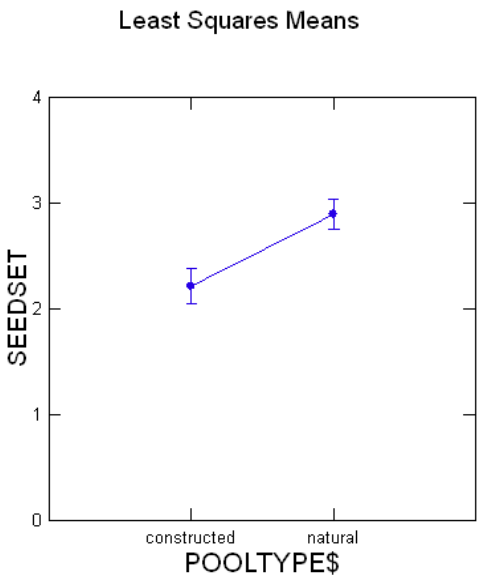


Figure 1.7: Average *L. vinculans* viable seed set of 2009 and 2010 samples in natural versus constructed pools (p = 0.001).

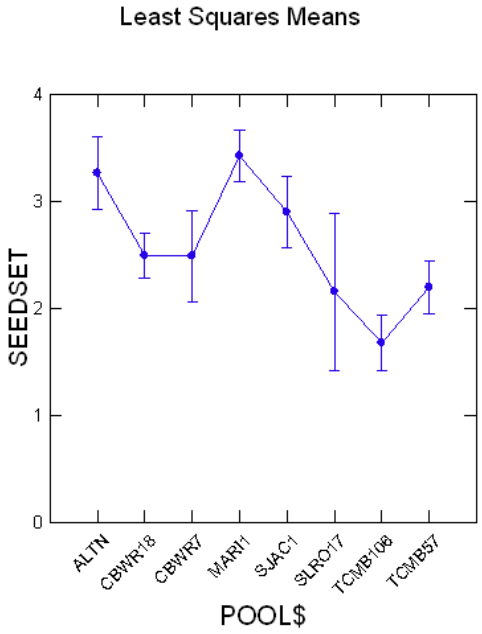


Figure 1.8: Viable *L. vinculans* seed set (bars = s.e.) per pool averaged from 2009 & 2010 data (p < 0.0001)

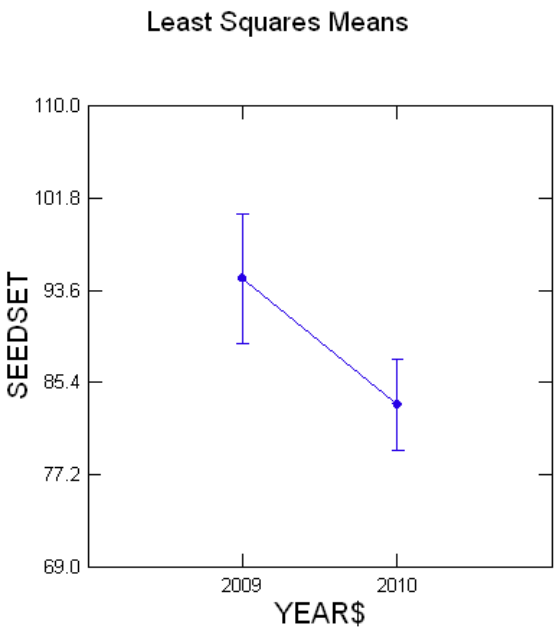


Figure 1.9: Average *L. burkei* seed set across years (p = 0.001)

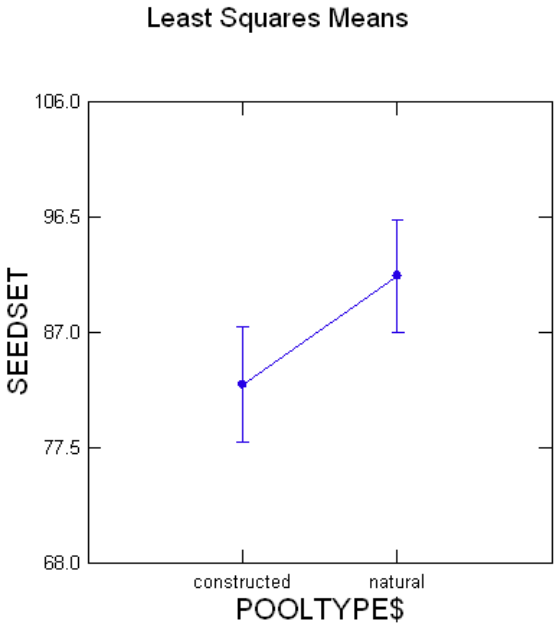


Figure 1.10: Average *L. burkei* viable seed set of 2009 and 2010 samples in natural versus constructed pools (p = 0.117).

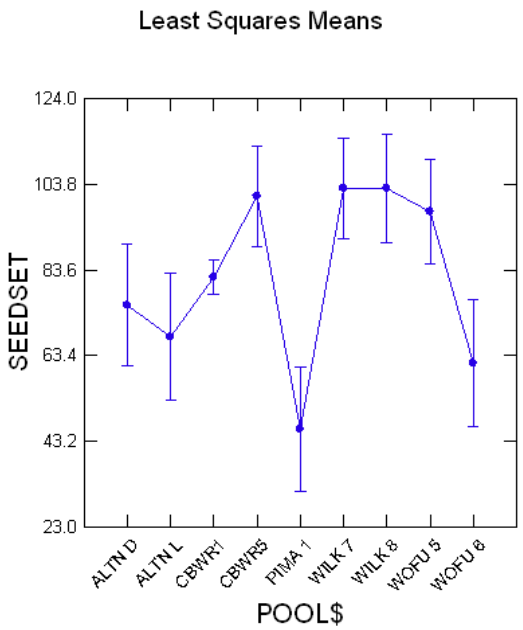


Figure 1.11*:* Viable *L. burkei* seed set per pool averaged (bars = s.e.) from 2009 & 2010 data ($p < 0.0001$)

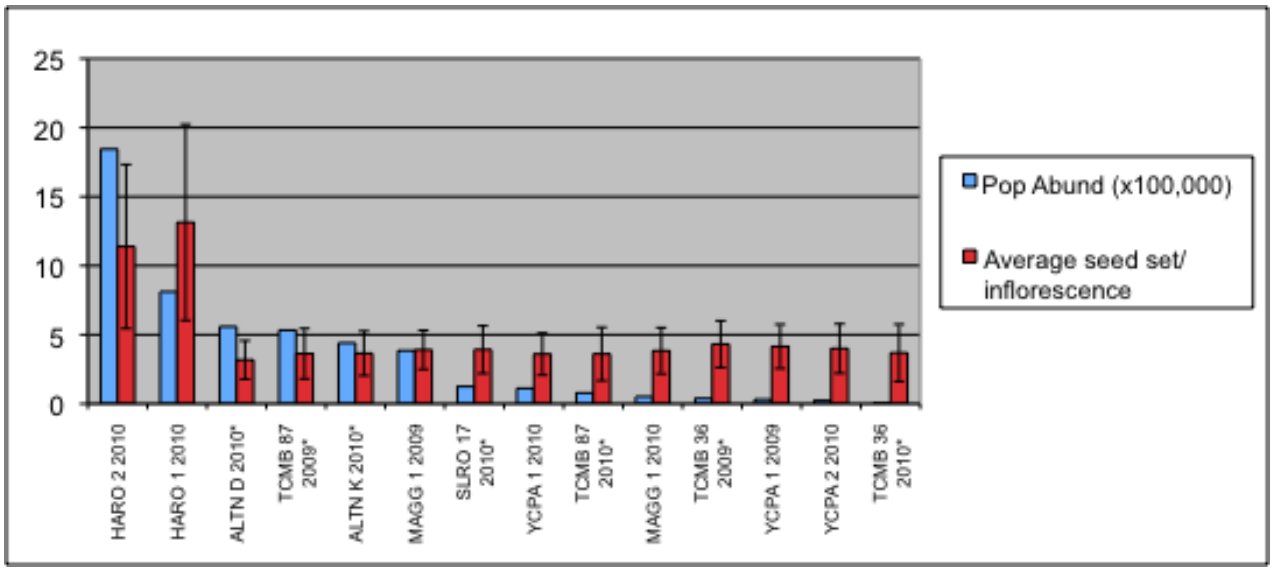


Figure 1.12: Population abundance estimates of *B. bakeri* and average seed set per inflorescence (± standard deviation) per sampled pool. [* constructed pools]

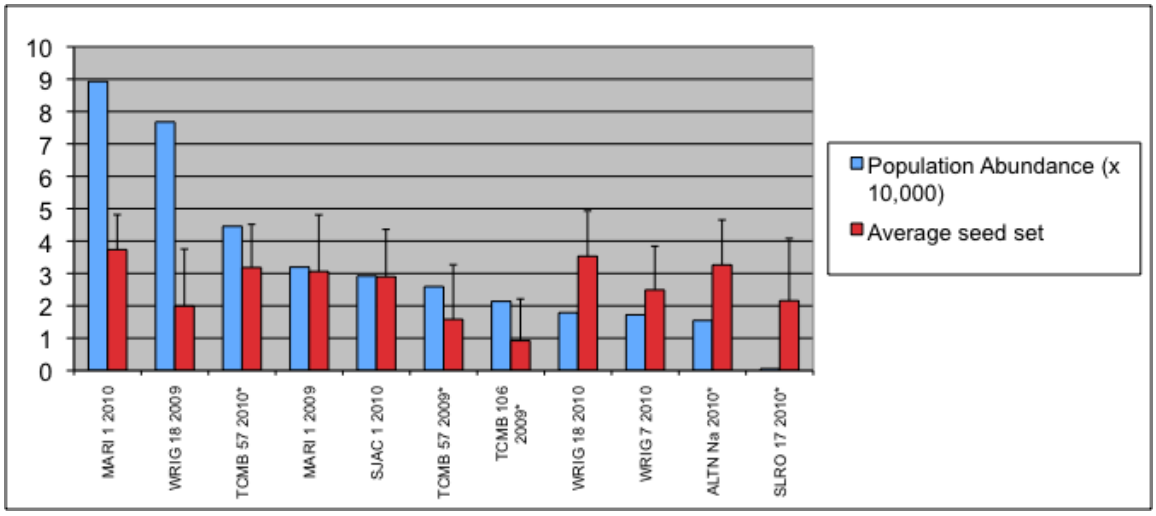


Figure 1.13: Population abundance estimates of *L. vinculans* and average seed set per flower (± standard deviation) per sampled pool. [* constructed pools]

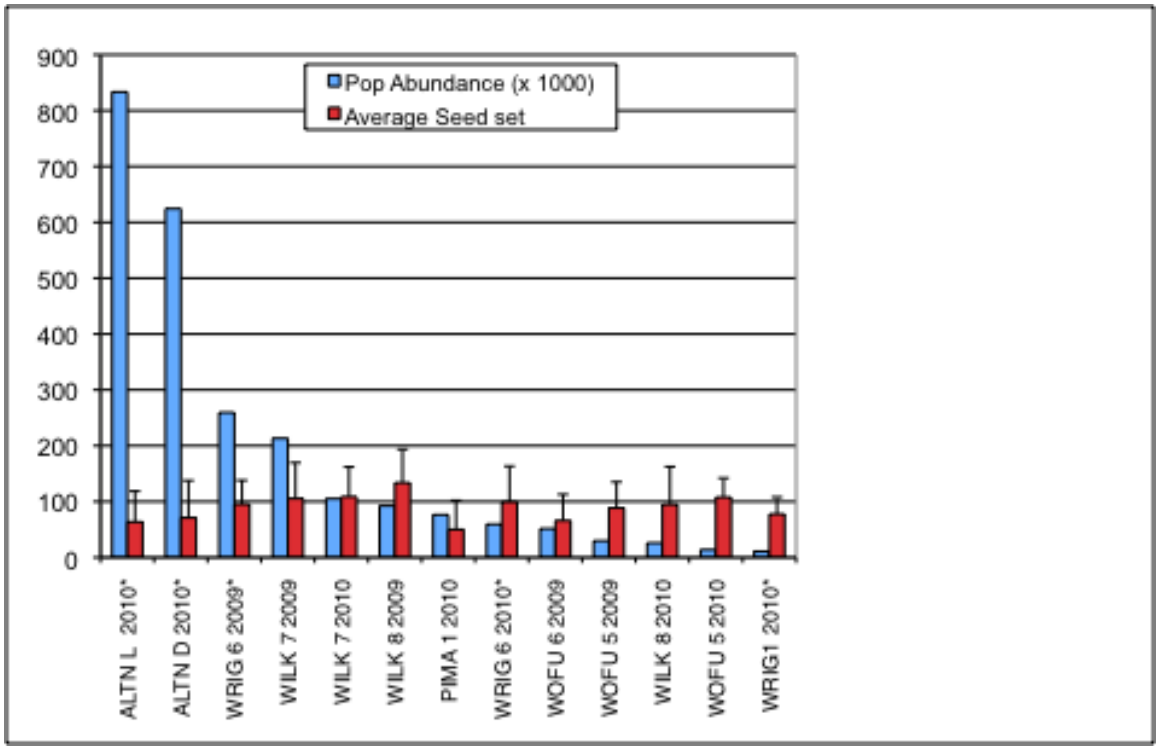


Figure 1.14: Population abundance estimates of *L. burkei* and average seed set per inflorescence (± standard deviation) per sampled pool. [* constructed pools]

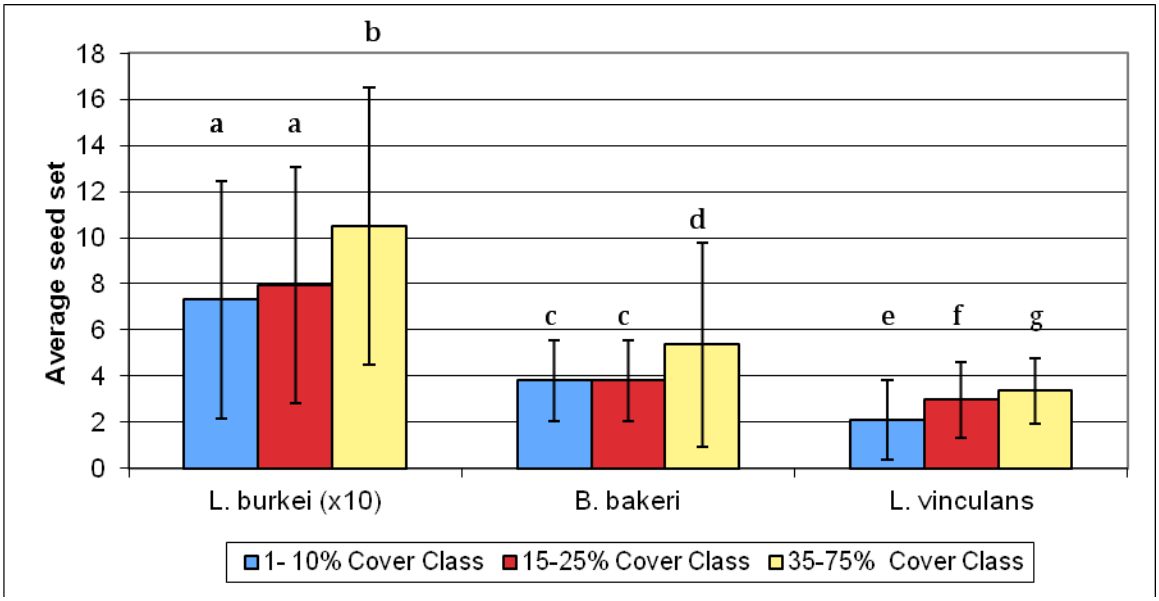


Figure 1.15: Average seed set by cover class group (error bars = standard deviation) . Letters indicate statistically significant differences (within each species): b, d, e, f ($P < 0.001$), and g ($P < 0.01$).

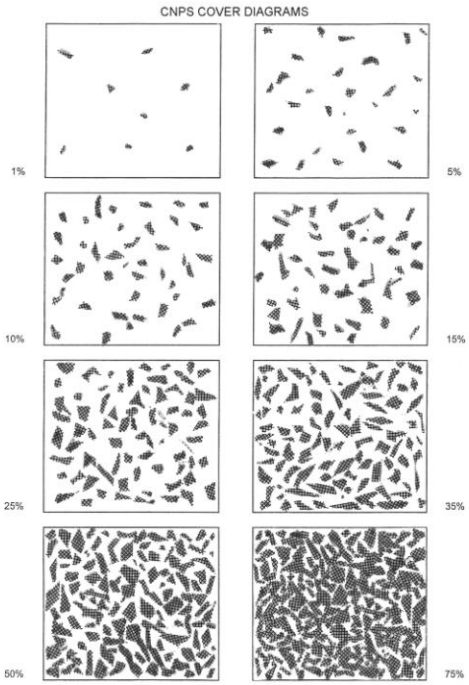
Appendix 1.1 Geographic coordinates of sampling sites.

SITE_CODE	X_COORD	Y_COORD
WILK	-122.81332999999900	38.42668580000000
MARI	-122.79396900000000	38.42823840000000
WOFU	-122.77174500000000	38.48335800000000
SJAC	-122.76739700000000	38.41900220000000
SLRO	-122.77999300000000	38.43445480000000
WRIG	-122.77661700000000	38.43201320000000
PIMA	-122.75386800000000	38.46647250000000
YCPA	-122.77571300000000	38.46370310000000
ALTN	-122.77699000000000	38.47245740000000
HARO	-122.71944400000000	38.37469920000000
MAGG	-122.78135200000000	38.48602650000000
TCMB	-122.77845200000000	38.38411390000000

***The coordinates were recorded in the following map projection:

NAD_1983_StatePlane_California_II_FIPS_0402 _Feet

Appendix 1.2. California Native Plant Society Cover Classes



II. Insights into the Pollination Ecology and Pollinator Communities of *Blennosperma bakeri*, *Limnanthes vinculans* and *Lasthenia burkei*

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Introduction

A presumed keystone component for long-term reproductive success in annual plants is the ecological relationship with their associated pollinators (Davis 1998). Many specialized and rare vernal pool plants have co-evolved with specialized bee pollinators (Thorp & Leong 1998). To date, only information inferred from related congeners was available regarding the specific pollinator relationship(s) and reproductive ecology of *Blennosperma bakeri, Limnanthes vinculans* and *Lasthenia burkei*. Other *Lasthenia, Limnanthes and Blennosperma* species rely on pollination from specialized bee pollinators. Until now, there had been few or no distinct records for the pollinators on the Santa Rosa Plain, the main habitat range of the three target species examined here. Thorp (1969) described two species of solitary bees *Andrena* (*Diandrena*) *submoesta,* and *A. (D.) puthua* as collecting pollen only from flowers of the genus *Lasthenia.* Predominant pollination for the genus *Limnanthes* is carried out by the solitary bee *Andrena* (*Hesperandrena*) *pulverea* (= *A. limnanthis* in older literature, specialist pollinator (R. Thorp, pers. com.). Known pollinators of *Blennosperma bakeri* are also in the genus *Andrena* with *Andrena blennospermatis* as the specialist pollinator (Dr. Robbin Thorp, pers com.).

It is imperative to quantify the role these flower visitors play in successful reproduction of these endangered plants, as the question remains whether these solitary bee populations may be locally waning if their main pollen source plants are in decline. If so, an extinction vortex may ensue, taking with it both plant and pollinator (Gilpin and Soulé 1986). Because specialist bees have only one generation per year and a short flight season to gather nest provisions for the following generation, access to host plants is critical for the bees' persistence (Thorp and Leong, 1998). These specialist pollinators can therefore be particularly vulnerable to habitat loss and fragmentation.

Since *B. bakeri*, *L. vinculans* and *L. burkei* are predominately out-crossing species (see report section I), we asked whether the annual plants in remaining natural and in constructed habitats are receiving pollination services, and in turn provide forage for their specialist pollinators. Prior to our study, it was unknown whether specialist andrenid bees were still extant at remaining natural populations of our endangered target plants. Moreover, it was unclear whether and at what density specialist pollinators had also colonized 'newer' populations of endangered plants established within constructed vernal pools, as opposed to generalist pollinators visiting and pollinating them. To assess ecological relationships between our target plants and their pollinators, it was therefore

important to study plant-pollinator interactions in both natural and constructed vernal pool habitats. The main objective of this investigation was to identify main pollinator(s) and pollinator communities of *B. bakeri*, *L. vinculans* and *L. burkei* relative to pool type (natural or constructed), and if possible, to determine the location of their upland nest sites.

Methods

During 2010, we conducted timed observations, net collections, and pan trapping at six natural and six constructed pools for *B. bakeri*, and at five natural and five constructed pools for both *L. vinculans* and *L. burkei*. In 2011, we conducted timed observations and net collections at three natural and four constructed sites with *L. burkei,* and set out pan traps at sites which we did not sample in 2010 (Appendix 2.1).

Observations

We observed patches of flowering *B. bakeri*, *L. vinculans*, and *L. burkei* within a 0.5 m^2 quadrat for 10 minutes. During the observations, we counted the number of times each type of insect made contact with the reproductive parts of a flower. Insect visitors were classified by category (e.g. solitary bee, honey bee, syrphid fly, beetle).

In 2010 we conducted at least 10 observation sessions per pool. In 2011 our efforts were focused on *L. burkei* and we conducted at least 12 observations per pool sampled, and sampled eight pools at constructed sites and four pools at natural sites. In order to capture peak levels of insect activity, observations took place between the hours of 9:30 a.m. and 3:30 p.m. and only when the temperature exceeded 65^{o}F and wind speed averaged less than 5 miles per hour.

Specimen Collections

We collected insects visiting the *B. bakeri*, *L. vinculans*, and *L. burkei* flowers with net sweeps during a timed 30-minute period at each pool. In order to control for effects of inclement weather on insect activity, collections were only conducted when the temperature is above 65^{o}F and wind speed less than 5 mph. Captured specimens were killed with ethyl acetate vapor or freezing, then pinned for identification and labeled with locality information.

We also set out pan trap arrays at each site in 2010 and at the new sites in 2011. The pan trap arrays consisted of a 3x3 Latin square pattern of yellow, blue, and white plastic bowls halfway filled with a soapy water solution. Soap was added to break the surface tension of the water, so that insects entering the bowl would not be able to climb out. The arrays were set out on dry ground directly adjacent to each sampling site. At several sites with cattle grazing, the traps were knocked over and we were not able to sample this way. We collected contents of traps after 24 hours and stored specimens in alcohol for later processing where they were cleaned, dried, pinned, and labeled. Tiny beetles and flies were stored in alcohol. The specimens are kept in the research collections at the

Sonoma State University museum. Dr. Robbin Thorp at UC Davis made final species determinations of the bee specimens, and Dr. Martin Hauser at the California Department of Food and Agriculture made final species determinations of the syrphid and bombyliid flies.

Results

Observations

Counts of insect visits during timed observations were averaged for each pool, and $\log_{10}$ transformed to meet assumptions of normality. Results from an ANOVA comparing natural and constructed vernal pools are shown in Figures 2.1-2.3. There is a strong indication that solitary bees are more abundant in natural vernal pools for *B. bakeri* and *L. vinculans*. While there is a statistically significant difference in solitary bee numbers for *L. burkei*, the counts are very low. *L. burkei* was visited most often by Bombyliid flies in the genus *Conophorus.* The frequency of *Conophorus* visits was highly variable between sites, as shown by the large error bars in the graph, even after transformation.

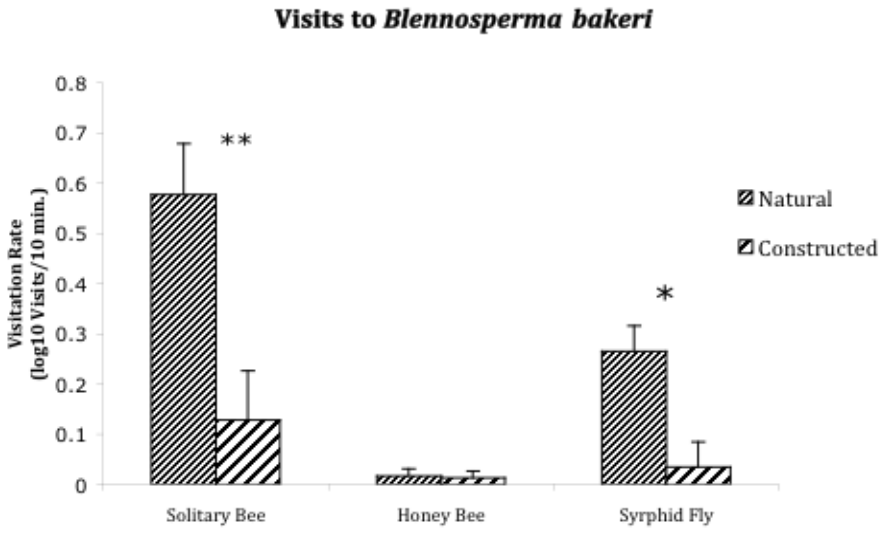

Figure 2.1. Least-squared means (+ 1 standard error) from transformed visitation rates across 7 natural and 6 constructed vernal pools with populations of *Blennosperma bakeri*. Solitary bee and syrphid fly visits differed significantly across pool types. *= significant at the $p = 0.05$ level; ** = significant at the $p < 0.001$ level

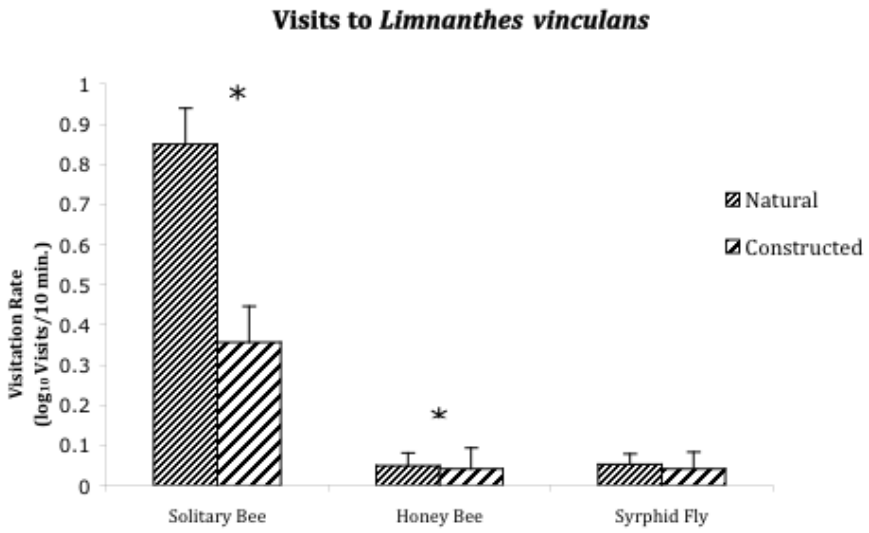

Figure 2.2. Least-squared means from transformed visitation rates (+ 1 standard error) across 5 natural and 4 constructed vernal pools with populations of *Limnanthes vinculans*. Solitary bee and honey bee visits differed significantly across pool types. * = significant at the p = 0.05 level

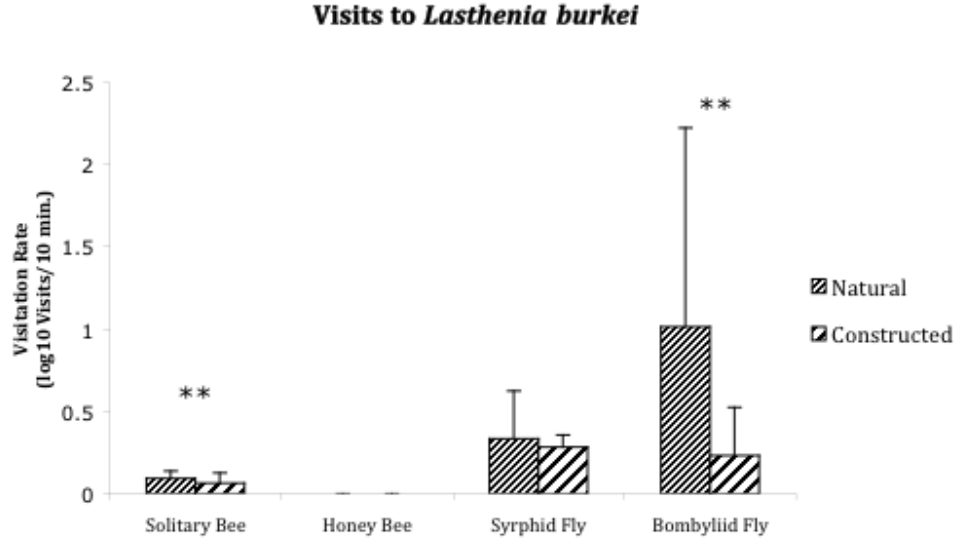

Figure 2.3. Least-squared means (+ 1 standard error) from transformed visitation rates across 5 natural and 5 constructed vernal pools with populations of *Lasthenia burkei* in 2010. Solitary bee and bombyliid fly visits differed significantly across pool types. *= significant at the p = 0.05 level; ** = significant at the p < 0.001 level

Again in 2011, the bombyliid fly *Conophorus cristatus* was the dominant visitor to *L. burkei*, and they were more abundant at natural vernal pool sites than created sites (See Figure 2.4.) Solitary bees and syrphid flies were present at low levels at both types of sites.

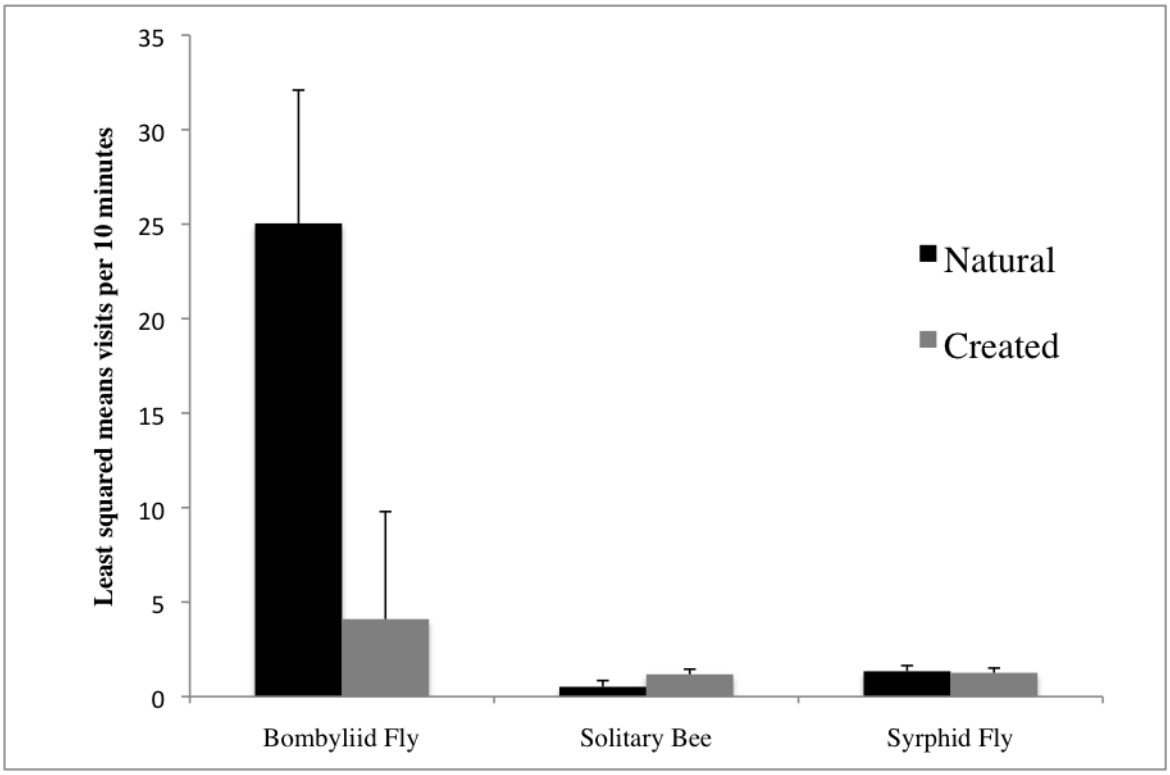

Figure 2.4: Least squared means of visitation rates to *Lasthenia burkei* (+1 standard error) in 2011. There was a significant difference in Bombyliid fly visits between natural and created vernal pools (p= 0.03). Solitary bee and syrphid fly visits did not differ significantly between pool types.

Insect Specimens

Insect observation results show a significant difference between natural and created sites in visits by "solitary bees" as a category (Figures 21. – 2.3). During field obesrvations it is difficult to distinguish which specific species are visiting the plants, and so we used the specimens we collected via net sweeps and pan traps to help with flower visitor identifications. Each of our three target plant species has an associated pollen-specialist solitary bee taxon that gathers pollen exclusively from plants within the genus. *Andrena blennospermatis* is a specialist on *Blennosperma spp., Andrena pulverea* is a specialist on *Limnanthes spp.,* and *Andrena submoesta* is a specialist on *Lasthenia spp*. (Thorp and Leong, 1996.) In addition to the pollen specialists visiting our three focal plant species, there were four generalist bee species that have a similar shape and body size: *Andrena pensilis, Andrena angustitarsata*, *Lassioglossum titusi*, and *Halictus tripartitus*.

Table 2.1 shows the means and standard errors for the number of specimens collected from each of the three plant species. Net collecting was the best method to detect the specialist bees of *B. bakeri* and *L. burkei*, while pan trapping collected many more *Limnanthes* specialists than net collecting. There are no strong patterns for generalist native bees and syrphid flies between natural and created vernal pool sites. Generalist native bees were found in greater numbers at *B. bakeri* sites and play a relatively larger role in the pollinator community for *B. bakeri* than for the other two plant species.

Andrena pulverea, the specialist on *Limnanthes*, is very abundant at both natural and created sites and is the dominant pollinator of *L. vinculans*. It seemed to competitively exclude the other generalist native bee pollinators, since, on average, zero generalists were net collected from the flowers. *Apis mellifera,* the European honeybee, was seen foraging on *L. vinculans* and was higher in abundance in created sites. One explanation for why *A. pulverea* is so abundant on the Santa Rosa Plain may be that the commonly occurring plant *Limnanthes douglasii* (Common meadowfoam) provides additional forage for these populations, boosting their numbers.

The specimens collected from *L. burkei* closely mirror the observation results discussed above; the bombyliid fly *Conophorus cristatus* is the dominant flower visitor, and may be its main pollinator. *Conophorus cristatus* individuals were more abundant in natural pools than created pools, and net collecting is the best way to sample for them.

Tables 2.2-2.4 show the abundance and locations where pollen specialist bees were collected, and are listed in site order from north to south. One interesting observation is that the greatest number of *A. submoesta* (the specialist on *Lasthenia*) was found at a created site, Woodbridge Mitigation Bank. Perhaps the numbers were highest there because of the large number of pools with *L. burkei*, which would provide plenty of forage for the bees, thus supporting a larger population of specialists. Tables 2.5 through 2.8 list the numbers of each species found, broken down by year and insect order.

Discussion

While our pollinator exclusion results (see section I) showed that each of the three target plant species can produce some seed without aid from pollinators, insect pollinators, specifically specialist pollinantors, play the leading role in the reproductive ecology of these plants. Our results from this study show that rate of pollinator visitation is lower in created vernal pools than natural ones for all three target plant species (Figs. 2.1-2.4). These results are consistent with an earlier study of insect visitors to *B. bakeri* between natural and constructed vernal pools at Alton Lane in 1996 (Leong, 2000)

We also found differences in the pollinator community for each plant species. Solitary bees played the largest role for *B. bakeri* and *L. vinculans* while bombyliid and syrphid flies are more prominent for *L. burkei.* Furthermore, specialist solitary bees were far more abundant on *L. vinculans* than on *B. bakeri*, which might mean that *L. vinculans* is the most dependent on its specialist pollinator, or that the specialist is competitively

excluding other pollinator taxa (Table 2.1). Conversely, generalist native bees were found in higher numbers on *B. bakeri* than *L. vinculans*, which indicates the *B. bakeri* system may have more resiliency to the loss of one pollinator species.

Since *B. bakeri* has the most diverse pollinator community, it seems that other pollinators besides the specialist may be able to make up the difference at locations where the specialist is rare. However, at the constructed sites where we observed *B. bakeri* patches, solitary bees as a group and syrphid flies were also less abundant compared to natural sites (Figure 2.1, Table 2.1). The pollinator community of *L. vinculans* appears to be dominated by its specialist pollinator *A. pulverea*. While visitation rates and captured *A. pulverea* specimens were lower at constructed pools, the species was still the most abundant pollinator in our sample. (Table 2.5)

Burke's goldfields' specialist bee, *Andrena submoesta*, was found less frequently and at fewer sites. We also did not observe bees visiting flowers of *L. burkei* very often relative to the amount of bombyliid fly visits. This could mean that the bombyliid fly *Conophorus cristatus* is acting as the main pollinator for this plant species and while the specialist bee may rely on its host plant for food, the plant may not rely on it for pollination. Although *C. cristatus* is less abundant in created pools, it is still the most numerous pollinator in the system.

While it may only be a matter of time for constructed pool ecosystems to "mature" and pollinators to become established at these new sites, without persistent annual seed set, local plant extinctions may occur, once the long-lived seed bank (in many cases brought from former natural sites to inoculate constructed sites) is depleted. If endangered annual plant reproductive success declines due to lack of pollinators, floral displays will also grow smaller and become less attractive to foraging bees, resulting in Allee effects (see section I) and a negative feedback loop of rapid population decline.

The number of specialist bees collected are listed in Tables 2.2 through 2.4 and are depicted from north to south. There is not a strong latitudinal pattern for *A. blennospermatis* or *A. submoesta*, but there appears to be a hotspot for *A. pulverea* in the center of the range sampled. The Mariposa population had the highest number of *A. pulverea* for a single sampling site and may be a good candidate for a source population if bee relocation is deemed necessary.

Management Recommendations

Annual seed set assessments and visual monitoring for pollinators should be considered part of the management strategy for endangered plant species recovery. We noted that constructed sites with the highest numbers of specialist bees were situated in the vicinity of natural sites, which may have facilitated pollinator movement (Tables 2.2-2.4). For example, Woodbridge (c = constructed) is close to Wood Fulton (n = natural), Todd Carinalli Mitigation Bank (c) is across the road from Todd Road Ecological Reserve (n), and Alton Lane has natural pools with *B. bakeri* on site in addition to constructed and restored pools. While it is yet untested, and it may be difficult to move bees when creating constructed vernal pools, it seems likely that building constructed pools near

existing plant populations will increase the chances of their colonization by specialist bees and other pollinators. Moreover, an intermediate level of grazing in vernal pool habitats is important to reduce invasive annual grass growth and create spaces of open soil where native bees can build nests.

Management of pollinator communities should be done on a plant-by-plant basis; as we have seen *L. vinculans* has a strong relationship with its specialist *A. pulverea*. Pollinators of *B. bakeri* and *L. burkei* include several insect species, which should be taken into account when planning pollinator restoration or management projects for these species. Knowing more about the nesting biology and habitat requirements of these pollinator species would be very useful in this regard.

A note on nest searches: During each of the field seasons, we looked for signs of bees nesting in the ground in the upland surrounding the vernal pools. Finding nests would have given us information about the type of substrates these bees prefer and how far of a foraging distance their nests are to the nearest flowering patches. Unfortunately, throughout the three seasons, we were unsuccessful at finding solitary bee nests. Nest appearance is described as small mounds of excavated dirt surrounding a hole ¼ to ½ inch in diameter (Dr. Robbin Thorp, pers. com.). Most of the vernal pool uplands were covered by annual grass growth, and at some sites, with previous year's thatch. Due to the time intensive nature of performing floral visitor observations and net sampling, we prioritized those tasks over more intensive nest searching. More directed investigations into where nests are located would however be very useful, especially at sites where we found a large number of specialist bees (see Tables 2.2-2.4).

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Tables

Table 2.1: Specimens of the major pollinator taxa collected in created and natural pools

	Created				Natural			
	Net		Pan		Net		Pan	
Blennosperma bakeri	*n* = 5		*n* = 7		*n* = 6		*n* = 6	
	Avg	s.e.	Avg	s.e.	Avg	s.e.	Avg	s.e.
Andrena blennospermatis	1.4	± 0.6	1.0	± 0.5	1.7	± 0.5	0.3	± 0.5
Apis mellifera	0.6	± 0.6	0.9	± 0.5	--	± 0.5	0.8	± 0.5
Generalist native bees*	0.5	± 0.3	1.3	± 0.2	0.3	± 0.2	1.8	± 0.3
Syrphid flies	0.2	± 0.3	0.2	± 0.2	0.3	± 0.2	0.4	± 0.2
Limnanthes vinculans	*n* = 5		*n* = 5		*n* = 5		*n* = 5	
Andrena pulverea	7.3	± 3.5	57.3	± 3.0	14.4	± 2.7	69.0	± 2.7
Apis mellifera	2.0	± 3.5	1.3	± 3.0	0.4	± 2.7	1.5	± 3.0
Generalist native bees*	--	± 2.5	0.4	± 2.2	--	± 1.9	0.8	± 1.9
Syrphid flies	0.1	± 1.7	0.3	± 2.6	0.2	± 1.6	0.1	± 2.0
Lasthenia burkei	*n* = 9		*n* = 9		*n* = 4		*n* = 5	
Andrena submoesta	0.9	± 0.6	0.3	± 0.2	0.7	± 0.6	0.6	± 0.9
Apis mellifera	--	± 0.4	0.6	± 0.4	--	± 0.5	0.4	± 0.6
Conophorus cristatus	8.2	± 0.4	0.2	± 0.4	12.8	± 0.4	1.2	± 0.6
Generalist native bees*	0.4	± 0.2	0.4	± 0.2	--	± 0.6	0.3	± 0.3
Syrphid flies	0.1	± 1.2	0.2	± 0.2	0.4	± 0.3	0.2	± 0.3

*Generalist native bees are *Andrena pensilis, Andrena angustitarsata*, *Lassioglossum titusi*, and *Halictus tripartitus*

Table 2.2: Location and numbers of the pollen specialist bee, *Andrena blennospermatis*

Site	Pools Sampled	Site Type	N Collected
Alton Lane (ALTN)	4	Created	13
Youth Community Park (YCPA)	2	Natural	5
Todd Carinalli Mitigation Bank (TCMB)	2	Created	1
Haroutounian (HARO)	2	Natural	9

Table 2.3: Location and numbers of the pollen specialist bee, *Andrena pulverea*

Site	Pools Sampled	Site Type	N Collected
Alton Lane (ALTN)	1	Created	57
Slippery Rock Mitigation Bank (SLRO)	1	Created	46
Wright Ecological Preserve (WRIG)	3	Natural	214
Mariposa (MARI)	1	Natural	175
Sam Jones Air Center (SJAC)	1	Natural	28
Todd Carinalli Mitigation Bank (TCMB)	2	Created	160
Hazel Mitigation Bank (HAZE)	1	Created	27

Table 2.4: Location and numbers of the pollen specialist bee, *Andrena submoesta*

Year	Site	Pools Sampled	Site Type	N Collected
2010	Piner-Marlow (PIMA)	2	Natural	2
	Wilkinson (WILK)	2	Natural	2
2011	Wood-Fulton (WOFU)	1	Natural	2
	Woodbridge (WOOD, #20)	2	Created	11
	Piner-Marlow (PIMA)	1	Natural	2

Table 2.5: List of Bee Species collected in 2010

Family Andrenidae	N
Andrena pulverea	758
Andrena cuneilabris	43
Andrena blennospermatis	28
Andrena chalybaea	23
Andrena torulosa	20
Andrena pencilis	10
Andrena suavis	5
Andrena subchalybea	5
Andrena submoesta	4
Andrena angustitarsata	3
Andrena caerulea	3
Andrena hypoleuca	3
Panurginus nigrellus	3
Andrena candida	3
Andrena cercocarpi	2
Andrena orthocarpi	2
Andrena osmioides	2
Panurginus n. sp.	2
Andrena sp (large black)	2
Andrena miserabilis	1
Andrena (Thysandrena) sp. 1	1
Andrena (Thysandrena) sp. 2	1
Andrena [cymatilis]?	1
Family Halictidae	
Agapostemon texanus	8
Halictus farinosus	2
Halictus ligatus	9
Halictus rubicundus	1
Halictus tripartitus	50
Lasioglossum (Dialictus) shiny sp	12
Lasioglossum (Dialictus) sp	1
Lasioglossum (Dialictus) sp D	39
Lasioglossum (Evylaeus) sp E	22
Lasioglossum (Evylaeus) sp I	7
Lasioglossum (Evylaus)	5
Lasioglossum (Evylaus) kincaidii	1
Lasioglossum (Evylaus) med. sp.	4
Lasioglossum (Evylaus) small sp.	6
Lasioglossum (L., #5) pacifica	8

Family Halictidae, cont.	N
Lasioglossum incompletum	33
*Lasioglossum olym*piae	7
Lasioglossum tegulariforme	2
Lasioglossum titusi	59
Sphecodes sp.	1
Family Apidae	
Apis mellifera	40
Ceratina nanula	5
Eucera edwardsii	4
Nomada sp.	4
Bombus californicus	1
Bombus melanopygus	1
Eucera actuosa	1
Family Colletidae	
Hylaeus conspicuus	2
Family Megachilidae	
Osmia nemoris	62
Osmia regulina	5
Osmia nr trevoris	3
Osmia med. sp.	3
Osmia albolateralis	2
Osmia trevoris	2
Osmia [californica]?	2
Osmia atrocyanea	1
Osmia sp. KG M-1	1
Osmia sp. KG-1	1
Osmia (Chenosmia)	1
Osmia large blue sp.	1

Table 2.6: List of flies found in 2010

Family Bombyliidae	N
Conophorus cristatus	90
Family Syrphidae	
Eristalis arbustorum	2
Eristalis hirta	2
Eupeodes volucris	2
Helophilus fasciatus	4
Lejops polygrammus	5
Parhelophilus sp.	1
Platycheirus stegnus	6
Sphaerophoria sulphuripes	2
Toxomerus marginatus	44
Toxomerus occidentalis	7

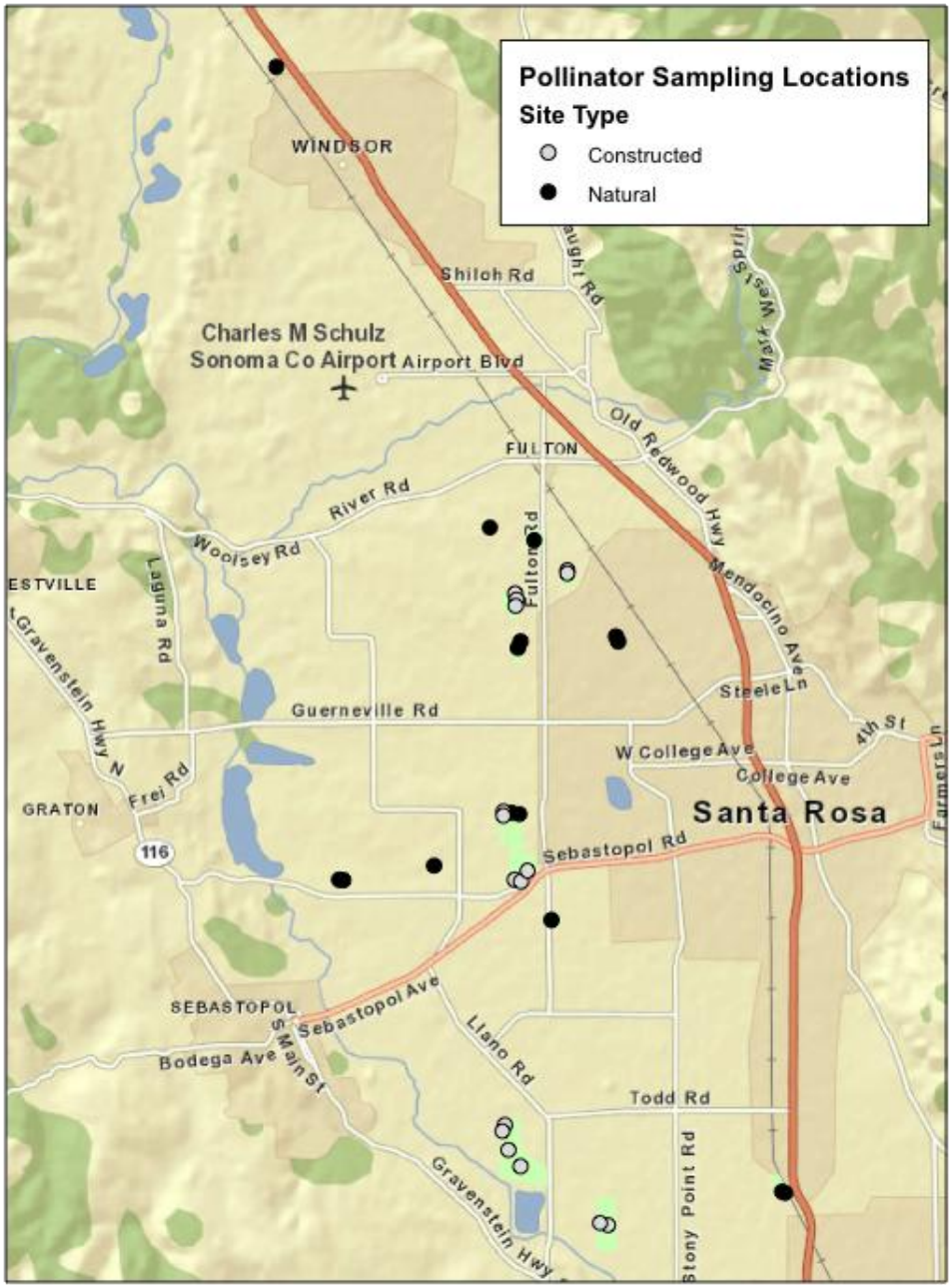
Table 2.7: List of bees found in 2011

Family Andrenidae:	N
Andrena angustitarsata	3
Andrena osmiodes	2
Andrena pensilis	6
Andrena pulverea	1
Andrena subchalybea	1
Andrena sublayiae	1
Andrena submoesta	15
Panurginus morphospecies 1	1
Panurginus morphospecies 2	2
Panurginus sp. (undescribed new species)	3
Family Halictidae:	
Halictus ligatus	3
Halictus rubicundus	1
Halictus tripartitus	1
Lasioglossum (Dialictus) morphospecies 1	6
Lasioglossum (Dialictus) morphospecies D	1
Lasioglossum (Dialictus) morphospecies 'KG-1'	2
Lasioglossum (Evylaeus) kincaidii	8
Lasioglossum incompletum	1
Lasioglossum titusi	16
Family Megachilidae:	
Osmia nemoris	1
Osmia trevoris	1

Table 2.8: List of Flies Found in 2011

Family Bombyliidae:	N
Conophorus cristatus	123
Family Syrphidae:	
Eristalis arbustorum	8
Platycheirus stegnus	2
Sphaerophoria sulphuripes	2
Toxomerus marginatus	12

Appendix 2.1: Pollinator sampling locations.



Map by Kandis Gilmore, 2/28/2012
DeLorme World Base Layer from ArcGIS Online

III. Investigation of seed bank size through soil cores of *Blennosperma bakeri* and *Limnanthes vinculans* populations

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Introduction

Population viability of endangered vernal pool annual plants is ultimately dependent on 1) the potential of a population to set viable seeds each growing season, being closely linked to density of flower patches to attract pollinators, 2) pollinator availability and efficiency, and on 3) long-term seed bank viability. While the annual growth and density of flowers or inflorescences in vernal pool annual plants will vary within and between pools in any given year and depends on environmental factors, the long-lived seed bank of many vernal pool annuals represents a reserve and functional buffer through periods where environmental factors are unfavorable. In some cases, natural variation may be extreme, with thousands of individuals present in one year, and only few or none present in another. The bounty years, when an extremely large amount of seeds are set, buffer those years with low or no seed contribution to the seed bank, ensuring population viability through bad times. The seed bank therefore allows vernal pools to persist in their extreme wet and dry growing regime, enabling population viability not only through the dry summer months, but also for several (1-20) years (Ranch Santa Ana Botanic Gardens). Determination of whether or not an annual vernal pool population is in decline therefore depends on evaluating long-term annual abundance trends and seed set, rate of germination, as well as seed bank size and seed longevity.

Here, we report on methods on how to sample the long-lived seed bank of two of our target species, and discuss the potential for assessing population viability by combining various demographic parameters, including a persistent seed bank into a density-structured model to project plant population viability and dynamics into the future (Freckleton et al. 2011). The main objectives of this investigation were to create and test a seed bank sampling protocol and establish initial seed bank size estimates for *B. bakeri* and *L. vinculans* populations.

Methods

Primary methods for soil seed bank analysis are (1) germination-type studies involving enumeration of emergent seedlings directly from whole soil samples and (2) soil separation-type studies involving some method of sieving or flotation to remove soil followed by enumeration of seeds (Bernhart et al. 2008, Mesgarian et al. 2007, Ambrosio et al. 2004, Gross 1990). We chose a soil-separation technique and tested both serial

sieving through metal screens and cloth bag techniques (Fay and Olsen 1978, Mesgarian et al 2007). Both techniques required lengthy agitated washing of samples with water to separate soil from seed. Cloth bags, commonly used in the wine, beer, and cheese industries were inexpensive relative to metal sieves, however, they proved difficult to clean between samples and are not available in standardized pore sizes. We chose to proceed with the metal sieving technique, which allowed the additional option of passing samples through a series of screens with pores of decreasing sizes. Sieving in series aided the removal of large organic material and coarse stones that would otherwise sort to the seed fraction.

We developed a protocol for soil seed bank analysis including a sampling regime (i.e., number and distribution of samples per site, depth of sample), and seed enumerating methodology. We tested several soil sampling methods, including open bucket and closed bucket augers, and intact core slide hammer samplers to determine the most appropriate equipment for collecting soil from vernal pools. Using an open bucket auger (Figure 3.1), we initially collected soil samples from four different depths in the soil profile (0-2", 2-4", 4-6", and the surface organic layer) in 2008 to determine the greatest depth of seed occurrence and so limit the depth of future sampling to just below the deepest seed occurrence.

We developed a soil sampling protocol using a serial screening technique wherein soil samples are passed through screens of decreasing pore size. Samples were gently agitated in water through 5 mesh (4000 um pore size) and 35 mesh (500um) metal screens. The fraction passing through the 5 mesh and trapped by the 35 mesh was saved, dried, and searched for seed. Seeds were identified by eye and verified under 20x magnification.

In September 2009, we assessed the quantity of *L. vinculans* seeds at one site in thirty-six randomly stratified soil cores within areas of dense *L. vinculans* growth. We subsequently performed additional sampling of 36 soil cores in low-density growth areas of *L. vinculans* at the same site. In 2010, we assessed the quantity of *B. bakeri* seeds at one site, collecting 36 soil cores within areas of both high-density growth (18 randomly stratified cores) and low-density growth (18 randomly stratified cores) of *B. bakeri*. Each core was divided into a surface litter fraction (surface) and the first three inches of mineral soil (sub-surface). Each fraction was sorted by serial sieving and *L. vinculans* seed was identified by eye and verified under 20x magnification. Due to the extremely small size of *L. burkei* seeds, we were not able to apply our soil sampling techniques to this species, and did therefore not sample any *L. burkei* sites.

We germinated seeds from soil samples to verify the correct identification of seeds, yet we did not quantify rate of germination as it was outside the study scope. We took a subset of germinated seeds, transplanted them to soil, and grew them in our greenhouse to verify not only plant identification, but also that seeds were viable.

Each soil core represented a soil volume of 154.4 cm^3. We estimated sampling areas of *L. vinculans* using a hand-held Trimble 500 Geographic Positioning System (GPS) data logger, and assessing polygon areas by cover class for each species using Geographic

Information System (GIS) analytic tools. We then calculated sampling volume from sampling area and soil depth and determined number of possible soil cores. Soil core numbers were then multiplied with the average number of seeds by density class (high or low) to estimate seed bank size (Tables 3.1 & 3.2).

Figure 3.1: Open bucket auger used to extract soil samples.

Results

From our preliminary *B. bakeri* sampling in 2008 we determined that stratified depth sampling of 1) the soil surface and 2) a 0-3 inch depth captured the deepest seed occurrence for this species, and we verified this for *L. vinculans* the following season. In 2009, we found both visibly intact *L. vinculans* seeds and nonviable seed coats that may have 1) already germinated, 2) lost the seed due to insect predation or 3) been damaged in the sieving process.

Limnanthes vinculans
While the number of both intact seeds and nonviable seed coats was highly variable across individual cores, all soil cores from high-density growth areas contained *L. vinculans* seeds. No *L. vinculans* seeds, nor seed coats, were found in 47%, or 17 of 36 soil cores from low-density growth areas. Seven of these cores were taken from the surface, while 10 were obtained at 0-3 inch depth. In high-density (> 15% cover class) floral patches, surface soil cores contained on average 16.25 ± 15.46 (s.d.) seeds and 21.50 ± 21.18 (s.d.) seed coats, while sampling cores from 0-3 inch depth included 4.00 ± 4.55 (s.d.) seeds, and 3.55 ± 2.62 (s.d.) seed coats (Table 3.1). In low-density (< 15% cover class) floral patches, surface soil cores contained on average 1.50 ± 2.55 (s.d.) seeds and 2.17 ± 2.68 (s.d.) seed coats, while sampling cores from 0-3 inch depth included 1.39 ± 2.52 (s.d.) seeds, and 1.78 ± 4.45 (s.d.) seed coats (Table 3.1). We found on average 19.14 ± 16.94 (s.d.) seeds per soil core from high-density, and 4.52 ± 5.78 (s.d.) from low-density areas. In both low- and high-density growth areas, more intact

seeds were found in surface vs. subsurface fractions (P = 0.0001), suggesting that *L. vinculans* seed does not incorporate deeply into the soil. Our seed bank size estimate for *L. vinculans* at the Balletto site was ~ 6 million seeds (Table 3.2).

Blennosperma bakeri

In a total of 36 surface soil cores for *B. bakeri* we found a total of 80 seeds and 14 seed coats, as compared to 17 seeds and 4 seed coats at 0-3 inch depths (p = 0.001). On average, we found 3.28 ± 3.59 (s.d.) seeds, and 0.22 ± .0.65 (s.d.) seed coats at the surface, and 0.50 ± 0.86 (s.d.) seeds and 0.00 ± 0.00 (s.d.) seed coats deeper in the soil in high-density samples (Table 3.1). Low density areas contained on average, 1.17 ± 2.31 (s.d.) seeds, and 0.56 ± 1.04 (s.d.) seed coats at the surface, and 0.44 ± 0.62 (s.d.) seeds and 0.22 ± 0.73 (s.d.) seed coats deeper in the soil (Table 3.1). Approximately 1.3 times the amount of seeds on average was found in high-density growth areas (3.56 ± 3.76 (s.d.)) of *B. bakeri*, as compared to low-density growth areas (2.61 ± 3.18 (s.d.); p = 0.04), suggesting that most *B. bakeri* seed are found in the surface detritus, and that they incorporate to a slightly lesser degree up to 3 inches into the soil surface layer. Our seed bank size estimate *B. bakeri* at Youth Community Park pool 1 was ~165,000 (Table 3.2).

Discussion

Our investigation of how to measure and estimate the seed bank of endangered vernal pool annual plants was targeted to provide methodology and a simple seed bank size estimation framework that could give a sense of the conservation status of individual populations. Annual abundance surveys are crucial in informing long-term fluctuations and potential population declines, yet only with additional consideration of the status of the seed bank will we be able to determine which populations are in serious decline, and which are stable or on route to recovery. While seed bank status assessment is labor intensive, its implementation may only be necessary periodically to verify plant viability model forecasts, making it thus a crucial component of adaptive conservation management.

Examining the results for *L. vinculans* we might assume that the more substantial seed bank estimate of ~6 million seeds (Table 3.2) for *L. vinculans* would forecast long-term population persistence and stability, yet abundance surveys for this species showed a substantial decline from 16,078 plants in 2008 to only two plants in 2009 and 2010 to zero plants in 2011 (Figure 3.1). This type of decline should trigger immediate management actions at this site, aimed at thatch removal, for example, to maximize future potential of seed germination, or other relevant management measures to benefit population persistence.

In contrast, results for *B. bakeri* showed in comparison a much smaller estimated seed bank of 164,548 seeds (Table 3.2), yet population abundance estimates fluctuated from 31,163 in 2009 to 110,072 in 2010 to 24,249 plants (Figure 3.2). For this population, it may be warranted to keep a close eye on annual abundance levels, while identifying potential management actions, such as the removal of non-native competitors, or other

factors, i.e. pollinator availability, that might diminish successful seed set, thus maximizing future contributions to the seed bank to build it up to a more stable size. In each case, it is important to implement a monitoring component into management so that expected outcomes can be tested and management effects can be evaluated.

Assessing whether yearly seed set estimates suggest a substantial addition to the seed bank, or indicate a continual draw down from the seed bank without significant replenishment of seeds each year at natural and constructed sites is possible within a density-structured population dynamics model framework that allows to project plant population viability and related recovery dynamics into the future (Freckleton et al. 2011). Target species focused density-structured model development will allow evaluation of various seed gain or loss scenarios over short and long time frames, crucial for long-term recovery management of extant populations of these endangered annual plant species. Such intricate model development, however, was larger than the scope of this investigation. We strongly recommend the development of such models for these and other endangered annual plant species, however, since we believe they will help in the long-term assessment of population viability at distinct sites, allowing ever diminishing management resources to be focused on those populations with the largest potential for recovery.

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Tables

Table 3.1: Average number of *L. vinculans* and *B. bakeri* seeds and empty seed coats found per soil core in surface detritus and at 0-3 inch depth. Average percent seed "loss" is calculated under the assumption that empty seed coats are the result of seed predation, rather than prior germination.

		Ave # seeds (s.d.)		**Ave # seed coats (s.d.)**		**Ave % Seed "Loss"**	
Species	**Stratum**	**High-density**	**Low-density**	**High-density**	**Low-density**	**High-density**	**Low-density**
L. vinculans	Surface detritus	16.25 (15.46)	1.50 (2.55)	21.50 (21.18)	2.17 (2.68)	0.57	0.59
	0-3 in depth	4.00 (4.55)	1.39 (2.52)	6.19 (5.83)	1.78 (4.45)	0.61	0.56
	Total core	**19.14 (16.94)**	**4.52 (5.78)**	**12.72 (9.29)**	**3.56 (5.92)**	**0.40**	**0.44**
B. bakeri	Surface detritus	3.28 (3.59)	1.17 (2.31)	0.22 (0.65)	0.56 (1.04)	0.06	0.32
	0-3 in depth	0.50 (0.86)	0.44 (0.62)	0.00 (0.00)	0.22 (0.73)	0.00	0.33
	Total core	**3.56 (3.76)**	**2.61 (3.18)**	**0.44 (0.86)**	**1.22 (1.31)**	**0.11**	**0.32**

Table 3.2: Seed bank size estimate calculations

Species	Floral density sampled	Average seeds/ core *A*	Sampling area (m2) *B*	Sampling volume (m3) *C = B* (3 in *0.0254)*	Core volume (m3) *D*	Number of cores/sample volume *E = C/D*	Seed bank size estimate *F = A*E*	High estimate (ave seeds/core *F = (A+ s.d.)*E*	Low estimate *F = (A- s.d.)*E*
L. vinculans	Low (<35% cover class)	4.52	1,536	117	0.0001544	758,052	3,426,394	7,807,934	0
	High (>35% cover class)	19.14	297	23	0.0001544	146,576	2,805,473	5,328,053	0
	TOTAL		**1,833**	**544,518**			**6,231,876**	**13,135,987**	**362,044**
B. bakeri	Low (<35% cover class)	2.61	57.5	4.3815	0.0001544	28,378	74,066	164,306	0
	High (>35% cover class)	3.56	51.5	3.9243	0.0001544	25,416	90,483	186,048	0
	TOTAL		**109**	**8.31**			**164,548**	**350,355**	**0**

Figures

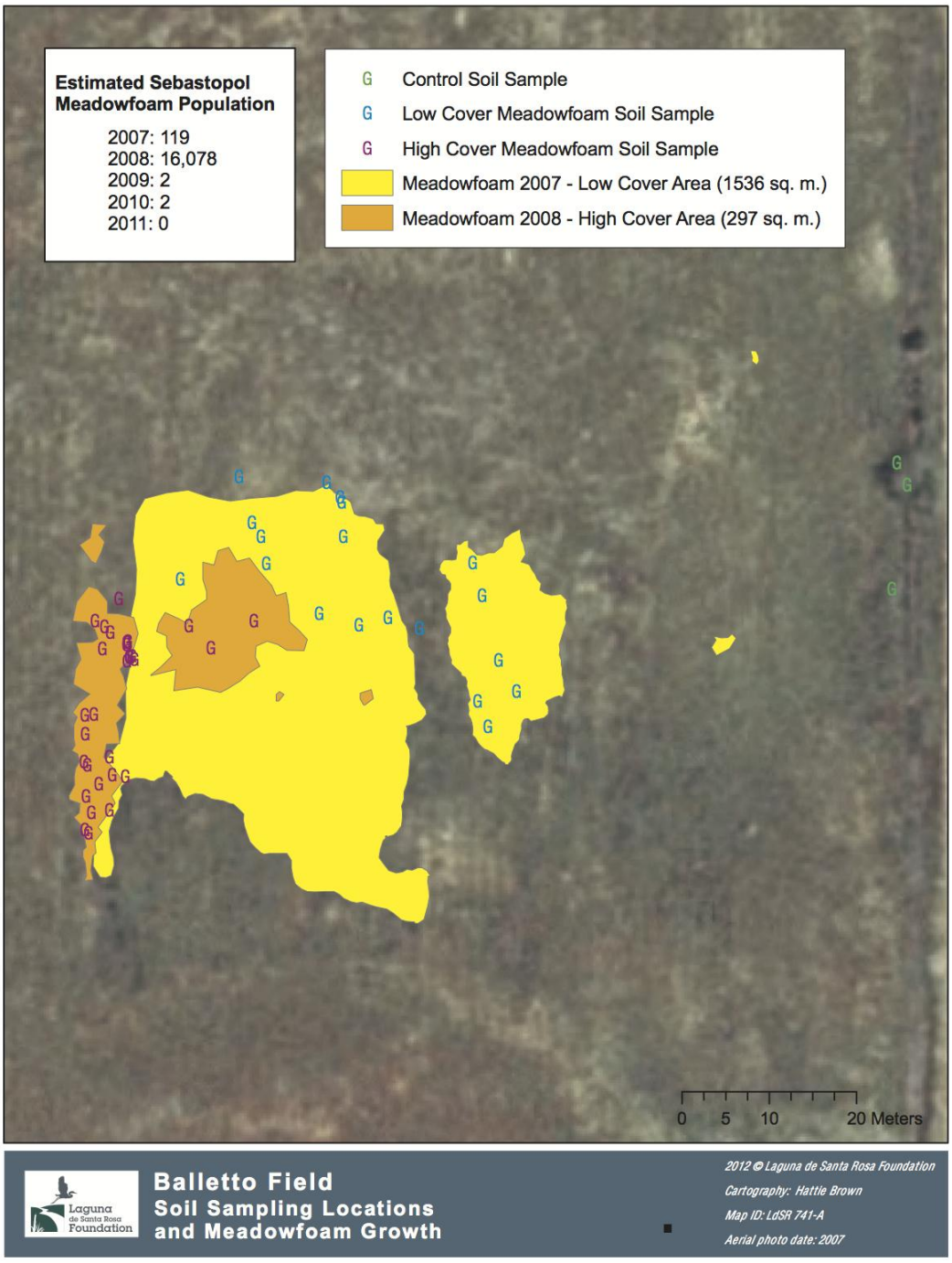

Figure 3.1: Soil core sampling locations by floral density for *L. vinculans* at Balletto Field.

Figure 3.2: Soil core sampling locations by floral density for *B. bakeri* at Youth Community Park pool 1.