



# Genetic, elevational and community structuring in the endangered vernal pool plant species Sebastopol meadowfoam (*Limnanthes vincularis*)

Michelle Jensen<sup>1</sup>, Christina Sloop<sup>2</sup>, and Nancy C. Emery<sup>1</sup>

<sup>1</sup>Purdue University, Department of Botany and Plant Pathology; <sup>2</sup>Laguna de Santa Rosa Foundation



## ABSTRACT

Just east of the Laguna de Santa Rosa lies the Santa Rosa Plain, a region that provides unique vernal pool habitat to many federally and state-listed endangered species. In this region and throughout California, vernal pool habitat has been dramatically reduced due to agricultural expansion and urban encroachment. Over the last 20 years, mitigation for impacts to vernal pools and their associated species has often involved the creation of new wetlands. As a result, the movement of soil inoculum has typically occurred without consideration for the spatial genetic structuring of the species or other localized ecological processes that may affect species persistence (e.g. plant community composition or microtopographic position within pools). Utilizing spatial genetic data collected for an endangered vernal pool plant species (Ayres & Sloop 2008), Sebastopol meadowfoam (*Limnanthes vincularis*), we recognize that genetic diversity may be structured within individual pools, instead of solely on a site by site or regional level. Additionally, data on topographic and community gradients have provided insight into the patterns of distribution for *L. vincularis* within and among created pools, natural pools, and natural swales. Through further genetic analyses we anticipate to assess patterns of intraspecific genetic variation, and the degree to which mitigation measures have modified such patterns within and among populations inhabiting both natural and created vernal pools.



Figure 1 – Created vernal pool on the Santa Rosa Plain in Sonoma County, California.



Figure 2 – Volunteers reading relative elevations in a created vernal pool.

## METHODS

### Spatial Genetic Structure in *L. vincularis* populations

Data collected in 2006 by Ayres and Sloop (2008) were used to identify the presence of spatial genetic structure within populations of *L. vincularis* occupying 34 vernal pools and swales. Individual genotypes were assigned using 10 neutral microsatellite loci as described by Ayres and Sloop (2008). Geographic data was collected using Geographic Positioning System (GPS) (NAD83, 10m accuracy). All spatial autocorrelation and Mantel tests were conducted using GenALEX 6.2 (Peakall and Smouse 2006).

### *L. vincularis* distribution, abundance and community structure

Field data were collected during the flowering season for *L. vincularis* from April through May 2009. The sampling design involved placing 0.25m x 0.25m quadrats at random positions off a transect that bisected the maximum length of a vernal pool. Within each quadrat, we estimated the percent cover of *L. vincularis*. Additionally, the most abundant species present in the quadrat aside from *L. vincularis* were documented and categorized according to the vernal pool plant classifications listed in the *California Vernal Pool Assessment Preliminary Report* (Keeler-Wolf et al. 1998). The elevations at each quadrat position were measured using a CST/Berger 24x Automatic Level (Figure 2) and corrected using the mean elevation from the pool edge as the 0.00 meter mark. A total of 16 pools from across the species' range were sampled (6 created pools, 6 natural pools, and 4 natural swales), with an average of 40 samples collected per pool (Figure 3, Calistoga population not shown). All data were statistically analyzed with SAS 9.2 (SAS Institute, Inc. 2008).

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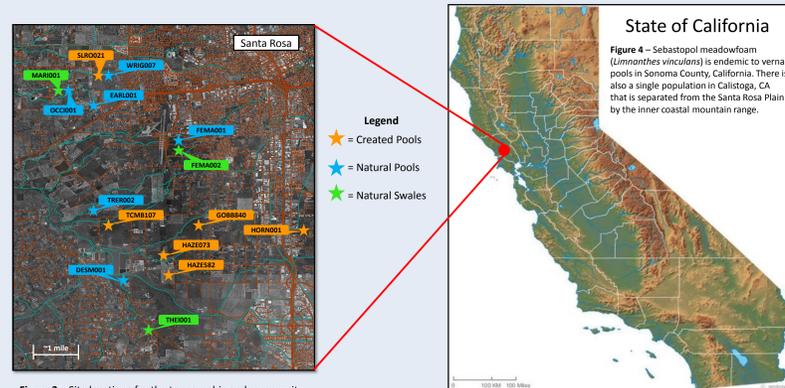


Figure 3 – Site locations for the topographic and community structure analyses within Sonoma County, California. Pool IDs are present in the balloon markers. The marker color refers to the pool type. The single population in Calistoga, CA is not shown.

## RESULTS

### Spatial Genetic Structure

The spatial autocorrelation analysis of the mean geographic coordinates of 34 pools provides evidence that genetic isolation by geographic distance is statistically significant ( $p=0.01$ ), albeit slight ( $r_{max}=0.03$ ,  $r_{min}=-0.007$ ). Pools with a geographic distance between 0 to 1 kilometer and 2 kilometers apart are significantly more genetically similar than by random chance ( $p=0.01$ ). Alternatively, pools that are approximately 3.25 to 10.25 kilometers apart are significantly less related than expected ( $p=0.01$ ) (Figure 5). This indicates that even with extensive movement of seed throughout the species' range from past mitigation events there is a subtle, but statistically significant decrease in the level of gene flow between pools at greater distances apart.

Since natural background levels of seed movement may occur on a more local scale, we looked at the relation between spatial distance and genetic distance within an individual pool at the Shiloh Mitigation Bank. According to the Mantel test, approximately 14.42% of the genetic distance is explained by spatial distance ( $R^2=0.1442$ ,  $p=0.01$ ) (Figure 6). This suggests that spatial genetic structure may develop on a local scale within individual pools compared to larger distances among different pools or sites.

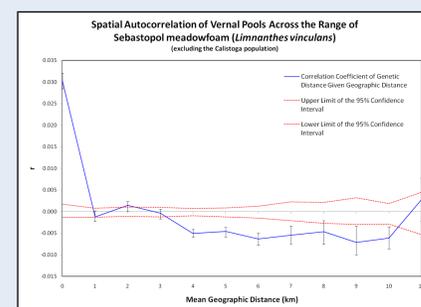


Figure 5 – Spatial autocorrelation analysis of genetic distance explained by mean geographic distance. Correlation coefficient values outside the upper and lower 95% confidence interval lines are statistically significant at the  $\alpha=0.05$  level. Pools that are between ~0.1km apart and 2km apart are more genetically related than expected by chance ( $p=0.01$ ). Pools that are ~3.25-10.25km apart are less related than expected ( $p=0.01$ ).

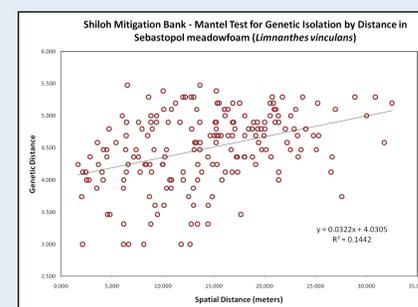


Figure 6 – Analysis of genetic isolation by distance using a Mantel test for a single pool in the Shiloh Mitigation Bank. Approximately 14.42% of the degree of genetic relatedness between individual plants in this pool is due to spatial distance ( $p=0.01$ ).

### Community Structure

We compared the mean number of predominant species across individual pools as well as across pool types. According to our model ( $R^2=0.604$ ,  $F=64.92$ ,  $p<0.0001$ ), the mean number of predominant species co-occurring with *L. vincularis* varied among pools ( $F=40.74$ ,  $df=12$ ,  $p<0.0001$ ) and pool types ( $F=232.42$ ,  $df=2$ ,  $p<0.0001$ ), with created pools having the greatest mean number of species, and natural swales having the least (Figure 9). The mean numbers of species within created pools, natural pools and natural swales are significantly different from each other (Tukey's HSD test,  $\alpha=0.05$ ) (Figure 9). Variation in the pooled number of vernal pool associates and vernal pool indicator species can also be explained by pool type ( $F=305.41$ ,  $df=2$ ,  $p<0.0001$ ) and individual pool ID ( $F=46.32$ ,  $df=12$ ,  $p<0.0001$ ), with all pool types being significantly different from each other (Tukey's HSD test,  $\alpha=0.05$ ). In contrast, the number of generalist species was found to not be significantly different between pool types (Figure 9).

### Relative Elevations and Abundance

The mean elevations where *L. vincularis* was present, relative to the pool edge, were weighted by the percent cover (a proxy for abundance) of *L. vincularis* in the respective quadrat. Relative weighted means ranged from approximately 0.0077 meters above the pool edge at the DESM001 site (which was the only natural convex feature) to -0.2203 meters below the pool edge at the FEMA002 site (Figure 7, Figure 3 – site locations). According to our model ( $R^2=0.479$ ,  $F=38.64$ ,  $p<0.0001$ ), patterns of species distributions and abundances vary among pools ( $F=36.35$ ,  $df=13$ ,  $p<0.0001$ ) as well as among pool types ( $F=74.58$ ,  $df=2$ ,  $p<0.001$ ), with natural swales being significantly different than created and natural pools (Tukey's HSD test,  $\alpha=0.05$ ). Since relative elevations in vernal pools and swales may be associated with the degree of inundation, these results imply that the geomorphology and hydrologic function of natural swales may be different than vernal pools, and perhaps should be considered as a unique case when mitigating impacts to endangered plant species found in this habitat type.

Additionally, we compared the mean percent cover of *L. vincularis* among pool types and individual pools. This model was also highly significant ( $R^2=0.526$ ,  $F=46.02$ ,  $p<0.0001$ ) for both pool type ( $F=75.36$ ,  $df=2$ ,  $p<0.0001$ ) and individual pool ID ( $F=34.63$ ,  $df=12$ ,  $p<0.0001$ ). All pool types had significantly different mean percent cover from each other (Tukey's HSD test,  $\alpha=0.05$ ). Overall, percent cover of *L. vincularis* was lowest in created pools and highest in natural swales (Figure 8). These results correspond to our spring 2008 field observations that individual *L. vincularis* plants in created pools were much smaller and produced fewer flowers than those found in natural swales.

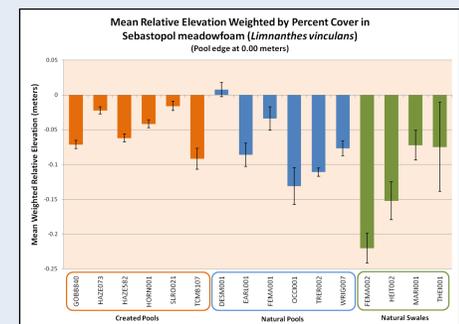


Figure 7 – Mean elevations relative to the pool edge (0.00m) and weighted by percent cover of *L. vincularis*. The pool with a positive relative elevation was the only convex feature sampled (DESM001). Species distribution and abundance varied among individual pools ( $F=36.35$ ,  $df=13$ ,  $p<0.0001$ ) and among pool types ( $F=74.58$ ,  $df=2$ ,  $p<0.001$ ). Natural swales as a pool type were significantly different than created pools and natural pools (Tukey's HSD test,  $\alpha=0.05$ ).

## DISCUSSION AND FUTURE RESEARCH

Even with extensive movement of seed throughout the species' range from past mitigation events there remains a subtle, but significant decrease in the level of gene flow between pools that are further apart compared to pools that are closer together (Figure 5). This effect of genetic isolation by geographic distance was also apparent at a local scale when analyzed within an individual pool (Figure 6). This indicates that spatial genetic structure in *L. vincularis* may be occurring at a local level within individual pools.

The distribution and abundance patterns of *L. vincularis* varies among created pools, natural pools and natural swales (Figures 8 and 9). Furthermore, the composition of the predominant species co-occurring with *L. vincularis* differs between pool types. These patterns indicate that important ecological differences occur between created pools, natural pools and natural swales, and that gene flow among these habitat types could influence the potential for populations to locally adapt to their resident pool. Furthermore, elevation gradients within pools are significant, and could be driving the spatial genetic structure observed at small spatial scales.

In order to expand our understanding of localized structuring in *L. vincularis*, we are currently working to quantify patterns of genetic variation in neutral markers within and among populations of *L. vincularis*, as well as patterns of spatial genetic structure within and among individual pools containing *L. vincularis*. Given our current results, we recommend that mitigation for impacts to *L. vincularis* and other vernal pool species should be considered on a case by case basis with special attention paid to localized structure within individual pools and pool types.

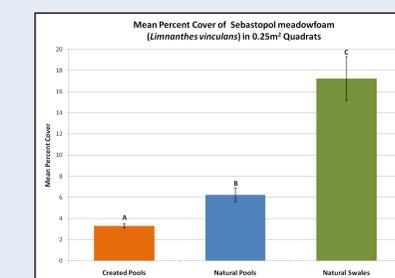


Figure 8 – Mean percent cover of *L. vincularis* in 0.25m<sup>2</sup> quadrats differed among pools ( $F=34.63$ ,  $df=12$ ,  $p<0.0001$ ) and pool types ( $F=75.36$ ,  $df=2$ ,  $p<0.0001$ ). Abundance in all three pool types are significantly different from each other (Tukey's HSD test,  $\alpha=0.05$ ).

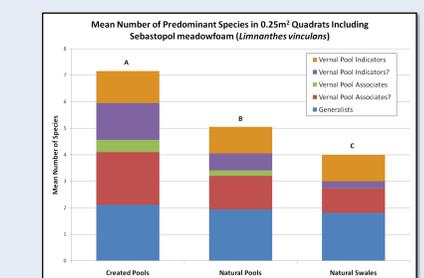


Figure 9 – Mean number of predominant species in 0.25m<sup>2</sup> quadrats with *L. vincularis* broken into subgroups according to Keeler-Wolf et al. (1998). The mean number of species varied between individual pools ( $F=40.74$ ,  $df=12$ ,  $p<0.0001$ ) as well as between pool types ( $F=232.42$ ,  $df=2$ ,  $p<0.0001$ ). All three pool types are significantly different in their community structure (Tukey's HSD test,  $\alpha=0.05$ ).