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The effects of habitat and competitive/facilitative interactions on reintroduction success of the endangered wetland herb, *Arenaria paludicola*

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Abstract Establishing new populations is essential for preventing the extinction of critically endangered plant species. However, defining the range of environmental conditions suitable for the most severely endangered species is challenging, since few wild populations remain for study. Experimental reintroductions of these species can achieve multiple conservation goals by improving our understanding of habitat and management requirements while simultaneously establishing new populations. We demonstrate this with *Arenaria*

paludicola, a critically endangered wetland plant species now known from a single wild population in coastal California. Before transplanting, we tested salinity tolerance in the greenhouse, and found tolerance of a broader range of soils than expected based on the current distribution. We then transplanted *A. paludicola* in three different habitat types, with and without neighbor removal. Success of *A. paludicola* transplants differed dramatically between the three habitat types, indicating the importance of variation at the habitat and microhabitat level. The best practices for transplant management are context-dependent: neighbor removal may promote the growth of *A. paludicola*, but neighbors may also facilitate transplant establishment in unstable substrates. After one year, *A. paludicola* continued to thrive in habitats dominated by *Oenanthë sarmentosa* with open canopies and moist soil. This habitat differs from that of the remaining wild population. Our discovery of an additional habitat type suitable for *A. paludicola* will allow more effective selection of future transplant sites.

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Introduction

Human disturbance and habitat destruction are putting wild plants at higher risks of extinction than ever before; an estimated 20 % of plant species worldwide are considered in danger of extinction (Pimm et al. 1995; Thuiller 2007; Brummitt and Bachman 2010). Reintroduction, in which a species is placed into an area where it formerly occurred but is now extirpated, is an essential step in the prevention of the extinction of many rare and endangered plants (Maunder 1992; IUCN 1998). Biodiversity recovery increasingly relies on the creation of new, anthropogenic populations; and reintroductions are recommended in the recovery plans of many listed species (Montalvo et al. 1997; Ma et al. 2013). However, reintroduction attempts can be costly and often end in failure, and the best practices for reintroductions still require study (Montalvo et al. 1997; Godefroid et al. 2011). Therefore, reintroductions should be designed not only to achieve biological goals (i.e., create new populations), but also to contribute to our general understanding by testing questions of conservation method or theory (Guerrant and Kaye 2007, Drayton and Primack 2012).

Selecting an appropriate site is crucial for the success of a reintroduction (Godefroid et al. 2011). Site selection decisions must be made at a hierarchy of scales. First, at the regional scale, the choice of reintroduction sites may consider historic collections (Maunder 1992; Allen 1994) but will usually also be strongly constrained by land ownership, legal considerations, and other logistics (Allen 1994; Sarrazin and Barbault 1996). Second, the most suitable habitat must be selected within the larger site. Finally, within a chosen habitat, the choice of particular planting locations—with particular microhabitats—will likely have a strong influence on reintroduction success.

In a review of U.S. Fish and Wildlife service recovery plans for federally listed plant species, Schemske et al. (1994) found that more than half of the species represented were known from five or fewer populations. When considering potential reintroduction sites, special problems are presented by these species that are known from few extant populations. When population extinction is due to habitat loss or alteration, the few remaining localities may not provide an example of ecologically optimal habitat (Roncal et al. 2012; Fowler et al. 2012). Additionally, it is highly likely that the species is capable of living in a broader range of conditions than its

extant locations represent; but how can that range of conditions be defined? Experimental reintroductions, when designed to specifically address this problem, can provide essential information on the habitat breadth of species with extremely limited ranges.

The term “indicator species” can be broadly used to describe taxa that are considered proxies for habitat quality, environmental change, and biodiversity (Larsen et al. 2009; Lindenmayer and Likens 2011). The indicator species concept can also be used when considering reintroduction sites. In this circumstance, an indicator species is one that co-occurs with the focal species and has a similar ecological niche (Baumberger et al. 2012). To identify potential indicator species associated with a focal species, one approach is to review information from historic observations. The presence of frequently co-occurring species can then be used as a starting point for identifying suitable reintroduction sites. Although it is a much simpler and potentially more efficient approach than developing a formal species distribution model (Baumberger et al. 2012), the use of indicator species has rarely been tested formally as a tool to select habitat for rare species (but see Halme et al. 2009; Ren et al. 2010; Baumberger et al. 2012).

Site management is often central to reintroduction success. In an analysis of 47 plant reintroductions, Godefroid et al. (2011) found that both pre-planting and post-planting management (such as fence building and removal of competitors) significantly increased the percentage of plants surviving for 3 years. Competition can be a barrier to the establishment of a focal species, and neighbor removal may be an essential component of a successful reintroduction (Budelsky and Galatowitsch 2000; Buisson et al. 2008). However, in stressful environments, facilitation can be the dominant interaction among plant species (Bertness and Hacker 1994; Ervin 2005). In a wetland, established plants may create a more hospitable soil environment by increasing oxygen availability, provide a good microhabitat with a stable rooting substrate, or provide protection from strong water flow (Ervin 2005; Luo et al. 2010). It is, therefore, possible that removing neighboring vegetation could decrease the likelihood of successful reintroduction in wetland habitats. Since plant–plant interactions may switch from competition to facilitation along a stress gradient (Bertness and Callaway 1994; Luo et al. 2010; Boughton et al. 2011), it may be difficult to

predict when it is appropriate to remove neighbors when implementing a reintroduction.

Arenaria paludicola (marsh sandwort, Caryophyllaceae) is a critically endangered plant species (Service 1993). *A. paludicola* is an herbaceous perennial species with slender green stems; it can root from leaf nodes, making it an easily propagated plant. *A. paludicola* was historically found from Washington State to southern California, with California populations known from San Francisco, Santa Cruz, San Luis Obispo, Los Angeles, and San Bernardino Counties (Service 1998). Historic populations are thought to have been extirpated by development, habitat conversion, and encroachment by invasive trees (United States Fish and Wildlife Service 1993). *A. paludicola* is currently known from only one natural population located in San Luis Obispo County, California. The remaining population grows in a freshwater marsh area in Oso Flaco Lake, with co-occurring vegetation that includes *Mimulus guttatus*, *Sparganium eurycarpum*, *Berula erecta*, *Epilobium ciliatum*, *Hydrocotyle* sp., and *Carex cusickii* (Service 1998). Other dominant species nearby include *Salix* sp. and *Typha domingensis* (Service 1998; M. Elvin, personal observation). In this population, *A. paludicola* is found growing in the peat at the bases of *C. cusickii* tussocks. Herbarium records from extirpated populations nearby report that *Juncus* sp. and *Scirpus* sp. have also co-occurred with *A. paludicola* in San Luis Obispo County (California Academy of Sciences, specimen CAS456473).

Due to the small number of wild individuals and populations and the limited literature available on *A. paludicola*, little is known about the range of conditions under which it could persist. The known historic populations were reported to occur in freshwater marshes from sea level to 450 meters (Service 1998). In Santa Cruz County, *A. paludicola* was last seen in 1976 at Camp Evers near Scotts Valley (San Jose State University Herbarium, specimen SJSU2277), and is reported to have co-occurred with *Oenanthе sarmen-tosa* and *Lupinus polyphyllus* var. *grandifolius* in this area (Rancho Santa Ana Botanic Garden, specimen RSA45354). Based on information about extant and historic populations, the US Fish and Wildlife Service recovery plan suggests that *A. paludicola* would prefer saturated acidic bog soils with high sand content (Service 1998). However, as acidic freshwater bogs in protected areas are not common, an important step in managing this species is exploring whether these plants can grow and reproduce in a broader range of habitats.

We did a preliminary study to investigate habitat breadth of *A. paludicola* in terms of its tolerance of soil salinity. We predicted that *A. paludicola* survival and growth would be restricted to soils with low salinity and low pH levels as suggested by the U.S. Fish and Wildlife Service recovery plan (Service 1998). We carried out a reintroduction experiment to explore factors that may influence the success of establishing populations of *A. paludicola* within its historic range. We investigated the relationship between moisture availability and survival at two scales: across habitats and among the microhabitats of individuals. We also used a neighbor removal experiment to test for either competition or facilitation of *A. paludicola* by other native wetland species.

Methods

In December 2007, USFWS provided plants representing 11 *A. paludicola* genotypes from Oso Flaco Lake (35.02°N, 120.62°W) in San Luis Obispo County to the greenhouse facility at the University of California, Santa Cruz. These 11 genotypes represent nearly the entire germplasm collection for this species.

Study sites

Our study sites included three coastal lagoons near Santa Cruz, California. These lagoons feed into the ocean and drain independent and relatively small sections of coastal land that include both natural and agricultural habitats. Younger Lagoon Reserve (36.95°N, 122.06°W) is on the western edge of Santa Cruz. Wilder Ranch State Park (36.99°N, 122.10°W) and Baldwin Creek (36.96°N, 122.12°W) are located on the coast about 1 km and 7 km west of Santa Cruz, respectively. Originally all three sites were considered for reintroduction of *A. paludicola* populations, but Younger Lagoon was eventually dropped from the field outplants for logistical reasons.

Growth in soils collected across a salinity gradient

To investigate the range of edaphic conditions that *A. paludicola* can tolerate, we did a greenhouse study using soils collected across a salinity gradient. First, we classified the wetland area of Younger Lagoon Reserve into 5 distinct vegetation zones, starting from

the ocean and following the lagoon inward. Each zone was delimited by the dominant plant species: *Salicornia pacifica*, *Atriplex patula*, *Typha latifolia*, *Oenanthе sarmentosa*, and *Salix lasiolepis*. On September 16, 2009; ten 5 cm diameter by 16 cm deep intact soil core samples were collected in each zone. We planted 3 shoot cuttings directly into each of the 50 (5 zones \times 10 replicates) soil cores. Cuttings were not rooted prior to planting, since this species roots from cuttings very readily (>98 % success, $n = 550$). Differences in initial size of the cuttings grown in soil from each vegetation zone were tested using a one-way ANOVA. There was no significant difference in the length ($F_{4,44} = 0.20$, $p = 0.936$) or weight ($F_{4,44} = 0.07$, $p = 0.991$) of the cuttings used in soil from each vegetation zone prior to planting. After planting, samples were randomized and left under a fertilizer-free sprinkler-mist system. The soil cores were collected in transparent sleeves, so visual inspection allowed us to keep the soils moist but not saturated. The bottoms of the soil cores were sealed so no nutrients or ions leached out.

Sixty-five days after planting, we harvested the plants from each sleeve and separated them into above- and below-ground parts, combining all three shoots or roots. We collected only live individuals for biomass assessment. We dried and weighed the plants to obtain above- and below-ground dry biomass per pot and calculated biomass per surviving plant. Above- and below-ground biomasses were highly correlated, and belowground biomass showed the same patterns as aboveground biomass (data not shown). Therefore, we only present results for aboveground biomass.

We oven-dried the soil samples and took the wet and dry weight to calculate the percent saturation. To test soil salinity, we made a 1:1 soil to water extract solution, following the guidelines outlined in Richards (1954). In order to approximate the true field salinity for our soils, we divided the salinity by the percent saturation. To test the soil pH, we made a 1:1 soil to CaCl_2 solution for each sample and measured the pH using a pH meter (Accumet AB15, Fisher Scientific). A 0.01M CaCl_2 solution was used instead of water in order to accurately measure the pH of these saline soils (Kissel and Vendrell 2006). All statistical analyses were performed using R (R Core Development Team 2013). We compared the soil from the five vegetation zones for *A. paludicola* aboveground biomass at harvest, soil salinity, pH, and percent saturation using an ANOVA

with post-hoc multiple comparisons. We compared the soil from the five vegetation zones for the proportion of cuttings per sleeve surviving to harvest using a generalized linear model with a binomial distribution and a logit link function. Post-hoc multiple comparisons of survival were carried out in the R package “agricolae” (Mendiburu 2014).

Reintroduction experiment

In December 2010, we performed an experimental outplant of *A. paludicola* into Wilder Ranch State Park and Baldwin Creek. We selected habitats based on dominant species in order to examine whether indicator species should be used to select future potential outplant locations. In both sites we defined three distinct habitat types, dominated by (1) *Oenanthе sarmentosa*, (2) *Salix lasiolepis*, or (3) *Scirpus microcarpus*. Unlike the greenhouse study above, we included only freshwater habitat types. Although *Scirpus microcarpus* was not investigated in our initial greenhouse study, we chose to include it in the outplant design because of its abundance at our experimental sites. At each site, we placed one 6 m \times 8 m plot within each of the habitat types. At Baldwin Creek, the three plots were within a 6,000 m² area. At Wilder Ranch, the three plots were within a 3,000 m² area. Dominant vegetation was quantitatively characterized in each plot (see supplementary material for methods).

Plants were propagated from cuttings in 115 mL containers containing potting soil and grown for several months. On December 8–10, 2010, we planted a total of 264 *A. paludicola* individuals into the three habitat types at the two sites. We planted 4 replicates of the 11 genotypes in each of the 6 plots ($n = 44$ plants per plot per site), arranged on a grid with 1 m spacing. We randomized genotypes and treatments. Half of the replicates of each genotype ($n = 2$ per plot) were assigned to a cleared treatment and half to a non-cleared (control) treatment. For the cleared treatment, we clipped competing vegetation to ground level in a 30 cm radius around each plant on planting day. Every 2 weeks we removed any vegetation that grew back in this area for the entire length of the study.

Microsite soil moisture and light availability

Soil moisture was measured on March, 3–4; May, 12–13; and September 2–3, 2011. A time domain

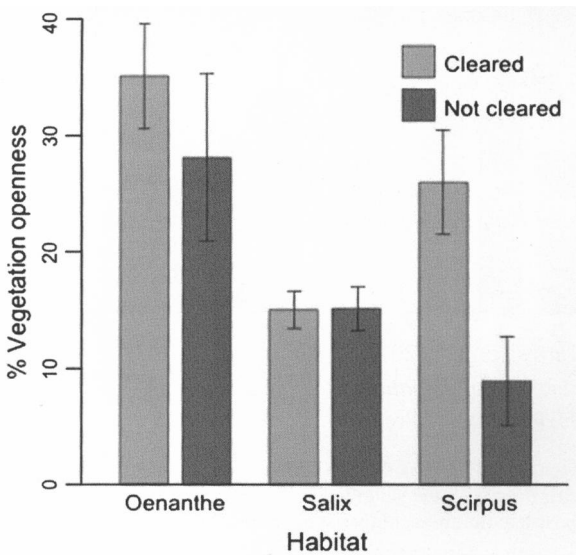


Fig. 1 Vegetation openness at 20 cm above ground level at Wilder Ranch. Light bars represent a “cleared” treatment with neighboring plants removed from within 30 cm; dark bars represent non-cleared controls. Error bars represent 1 SD

reflectometer (Hydrosense TDR Soil Water Measurement System, Campbell Scientific, Inc.) was used to measure soil moisture at each plant location (for details of measurement techniques, see supplementary material). Soil moisture at control plants was compared between sites and habitats at each time point (March, May, and September) using an ANOVA with the site x habitat term used as the error term.

On September 2–3, 2011, we measured canopy openness at 1 m above the ground using a spherical densiometer. Readings for the four cardinal directions were averaged for each of 10 randomly selected points within each plot. The four readings at each point were averaged before statistical analysis. We compared canopy openness between habitats and sites using an ANOVA with the site x habitat term used as the error term.

At Wilder Ranch only, we assessed the light environment at plant height (20 cm above the soil surface) for each individual using a Nikon Coolpix camera with a hemispherical lens (FC-E8, Nikon Corporation). Percent vegetation openness was assessed by analyzing the photos obtained using Gap Light Analyzer V2 software (Frazer et al. 1999). Treatment affected vegetation openness differently in the different habitat types. In the *Salix* plot, clearing did not affect vegetation openness. In the *Oenanthe* plot the clearing treatment increased vegetation openness by 25 %, and in the *Scirpus* plot

clearing increased vegetation openness by 19.2 % (Fig. 1). We tested for an effect of vegetation openness and habitat on final survival at Wilder Ranch using a GLM with a binomial distribution and a logit link function. This analysis was performed using data from control planting locations only.

Survival and growth

We monitored plants for a year following outplanting. Every two weeks, the survival of each plant was assessed and cleared treatments were maintained. We used a Weibull proportional hazards model to examine the effect of site, habitat, neighbor removal, habitat x neighbor removal, and soil moisture at three time points on survival. This analysis was performed using the “survival” package in R (Therneau 2014). This full model was tested against reduced models. The final model included habitat, soil moisture in March, soil moisture in May, and neighbor removal treatment; while site, soil moisture in September, and the interaction between habitat and neighbor removal were dropped. The significance of the habitat term in this model is inflated because individuals are not independent samples of habitats; therefore, we also performed a conservative analysis in which we used a binomial GLM with a logit link function to test the effect of habitat, site, treatment, and habitat x treatment on the proportion of plants that survived in each treatment–habitat–site combination. We used the stepAIC function in the R package “MASS” to determine which factors contributed most to model fit. The best fitting model included habitat only. There was no significant effect of genotype in any of our exploratory data analyses, so it was not incorporated into final models.

Plants were measured on May 26–27, 2011 and February 1, 2012. The length of each green shoot was measured and summed to obtain a total length of green shoots per plant. We used an ANOVA to test for the effects of habitat and treatment on plant size (log-transformed shoot length) in May with the site x habitat term used as the error term. We lacked sufficient degrees of freedom to test for an interaction of habitat and treatment. In February, sufficient numbers of surviving plants remained only in *Oenanthe* habitats; so we used an ANOVA to test effects of treatment on plant size, with the site x treatment term as the error term.

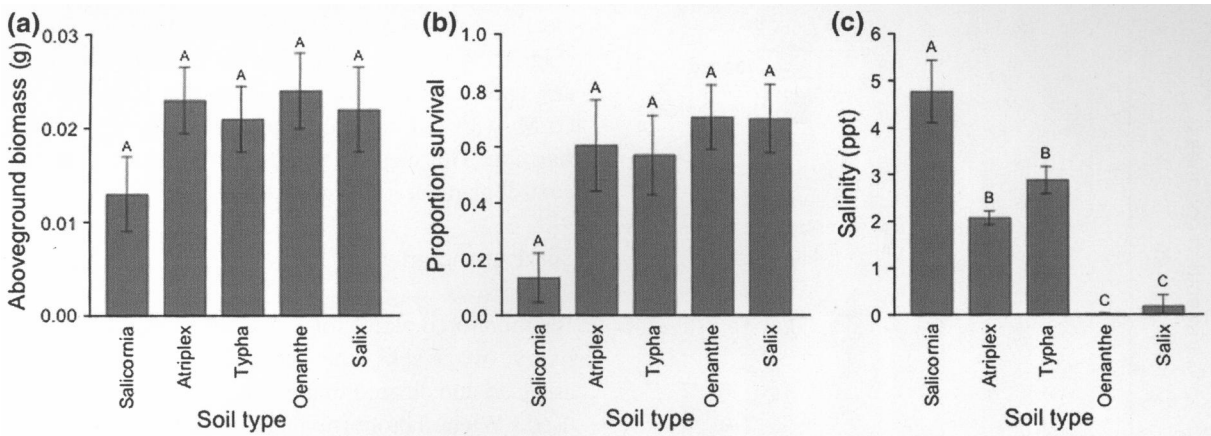


Fig. 2 Final biomass (a) and survival (b) of *Arenaria paludicola* plants grown from cuttings in soils collected from five habitats representing a gradient of coastal influence (*Salicornia*) to freshwater influence (*Salix*). Salinity (ppt) varies

significantly across the five habitats (c). Error bars represent 1 SE. Bars with the same letter are not significantly different by post-hoc multiple comparisons ($p > 0.05$)

Results

Growth in soils collected across a salinity gradient

There was a significant effect of soil collection zone on proportion survival in the greenhouse ($\chi^2_{4,44} = 9.49$, $p = 0.050$); however, individual post-hoc comparisons were not significant (Fig. 2b). Survival was reduced in the *Salicornia* soil, which has the most saline influence. There was no significant effect of soil collection zone on per plant aboveground biomass ($F_{4,26} = 0.55$, $p = 0.699$; Fig. 2a). There was a significant difference in salinity between the soils collected in each zone, with *Salicornia* soil having the highest salinity ($F_{4,44} = 21.03$, $p < 0.0001$; Fig. 2c). Salinity in *Salix* and *Oenanthe* soils was negligible. There was a significant difference in percent soil saturation among the soils collected, with the soils with the most freshwater influence (*Salix* and *Oenanthe*) also being more saturated (Table 1). There was also a significant difference in pH among the soils collected in each zone, although pH did not vary linearly across the gradient of saltwater influence (Table 1).

Outplant site and habitat characteristics: soil moisture and vegetation cover

There were no significant effects of habitat (March, $F_{2,2} = 1.77$, $p = 0.362$; May, $F_{2,2} = 2.09$, $p = 0.323$; and September, $F_{2,2} = 6.97$, $p = 0.125$) or site (March,

$F_{1,2} = 0.39$, $p = 0.594$; May, $F_{1,2} = 0.98$, $p = 0.426$; and September, $F_{1,2} = 1.67$, $p = 0.326$) on soil moisture. The non-significant trend was for *Oenanthe* plots to maintain higher soil moisture levels, especially later in the dry season (Table 2; Fig. 3).

There were marginally significant differences in canopy openness among habitats ($F_{2,2} = 15.07$, $p = 0.062$), and no significant difference between sites ($F_{1,2} = 0.28$, $p = 0.651$). At both sites, *Salix* plots had very heavy canopy cover; while *Oenanthe* plots had mostly open canopies. Canopy openness was intermediate in the *Scirpus* plots (Table 2). The canopy and vegetation openness measures show that although *Salix* plots had the most canopy cover, *Scirpus* plots were highly light-limited at the plant level due to understory vegetation (Table 2).

Effect of habitat, site, treatment, light, and moisture on transplant survival and growth

Our final model predicting survival of outplanted *Arenaria paludicola* included habitat, soil moisture in March, soil moisture in May, and neighbor removal treatment. Habitat had the strongest effect on survival ($\chi^2_{2,258} = 135.49$, $p < 0.0001$). Plants in *Oenanthe* plots survived the longest, followed by those in *Salix* and *Scirpus* plots, respectively (Fig. 4). Neighbor removal had a significant positive effect on survival ($\chi^2_{1,257} = 9.14$, $p = 0.003$). Soil moisture in March and May also significantly affected plant survival (March, $\chi^2_{1,261} = 61.22$, $p < 0.0001$; May, $\chi^2_{1,260} = 6.16$,

Table 1 pH and percent saturation of soil from five vegetation zones used in the greenhouse experiment

Dominant vegetation	<i>Salicornia pacifica</i>	<i>Atriplex patula</i>	<i>Typha latifolia</i>	<i>Oenanthе sarmentosa</i>	<i>Salix lasiolepis</i>	<i>F</i>	<i>df</i>	<i>p</i>
pH	7.24 (0.22)	7.15 (0.35)	6.96 (0.36)	7.53 (0.27)	7.53 (0.26)	6.91	4.44	0.0002
Saturation (%)	73.46 (4.23)	63.26 (17.94)	69.27 (12.40)	79.18 (6.94)	77.02 (3.81)	3.48	4.44	0.01

Soils were collected across a gradient representing a range of coastal versus freshwater influence. SD are in parentheses. The pH and percent saturation of soil collected from habitats dominated by different species are compared with an ANOVA

Table 2 Geographic and environmental characteristics of outplant locations

	Wilder Ranch			Baldwin Creek		
	<i>Oenanthе</i>	<i>Salix</i>	<i>Scirpus</i>	<i>Oenanthе</i>	<i>Salix</i>	<i>Scirpus</i>
Latitude	36.9568°N	36.9568°N	36.9570°N	36.9685°N	36.9684°N	36.9680°N
Longitude	122.0828°W	122.0827°W	122.0831°W	122.1232°W	122.1237°W	122.1230°W
Soil moisture, March (%VWC)	93.7 (9.65)	84.0 (5.49)	93.2 (6.13)	107.1 (1.61)	72.0 (7.02)	72.5 (7.71)
Soil moisture, May (%VWC)	78.0 (7.31)	71.3 (5.92)	64.9 (7.43)	113.1 (5.92)	63.4 (6.68)	74.8 (8.66)
Soil moisture, September (%VWC)	75.1 (16.6)	52.2 (18.9)	37.9 (12.2)	98.2 (8.70)	45.4 (12.2)	58.8 (9.31)
Canopy openness (%)	80.5 (22.3)	5.2 (2.5)	76.7 (22.3)	89.8 (26.2)	6.9 (2.5)	46.6 (22.7)
Vegetation openness (%)	28.1 (7.20)	15.1 (1.90)	8.9 (3.79)			

Plots within sites are described by their dominant cover species: *Oenanthе sarmentosa*, *Salix lasiolepis*, or *Scirpus microcarpus*. Soil moisture and vegetation openness means are calculated from measurements at control planting locations only. Standard deviations are in parentheses. Soil moisture is represented as percent volumetric water content (%VWC)

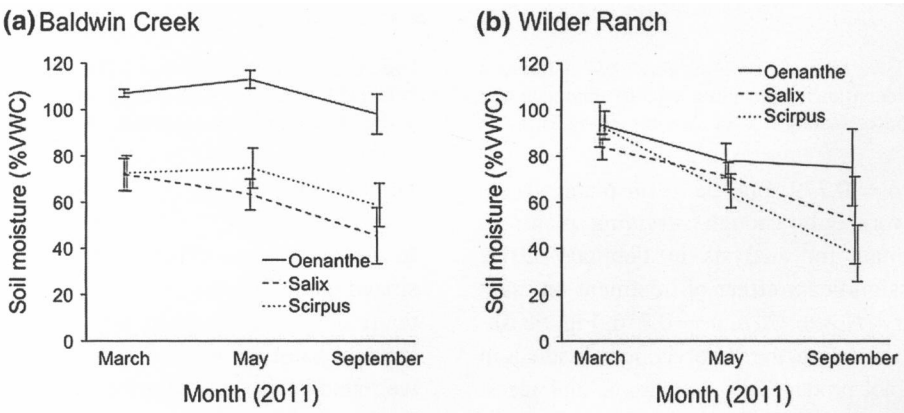


Fig. 3 Soil moisture [represented as percent volumetric water content (%VWC)] in March, May, and September, 2011 at **a** Baldwin Creek and **b** Wilder Ranch, in habitats dominated by *Oenanthе* (x), *Salix* (triangles), or *Scirpus* (circles). Only data from non-cleared controls are represented. Error bars represent 1 SD

$p = 0.013$). However, soil moisture in March and May had conflicting effects: in March, moisture contributed positively to survival [$\exp(\text{coefficient}) = 1.015$]; but in May, soil moisture negatively affected survival [$\exp(\text{coefficient}) = 0.988$]. Our most conservative analysis using the proportion of surviving plants in each site–habitat–treatment combination showed a

marginally significant effect of habitat ($\chi^2_{2,9} = 5.98$, $p = 0.050$). There was a significant positive effect of vegetation openness on survival at Wilder Ranch ($\chi^2_{1,64} = 14.52$, $p = 0.006$), and no effect of habitat ($\chi^2_{2,62} = 0.92$, $p = 0.630$).

There were no significant effects of habitat ($F_{2,2} = 1.02$, $p = 0.496$, Fig. 5a,b) or treatment

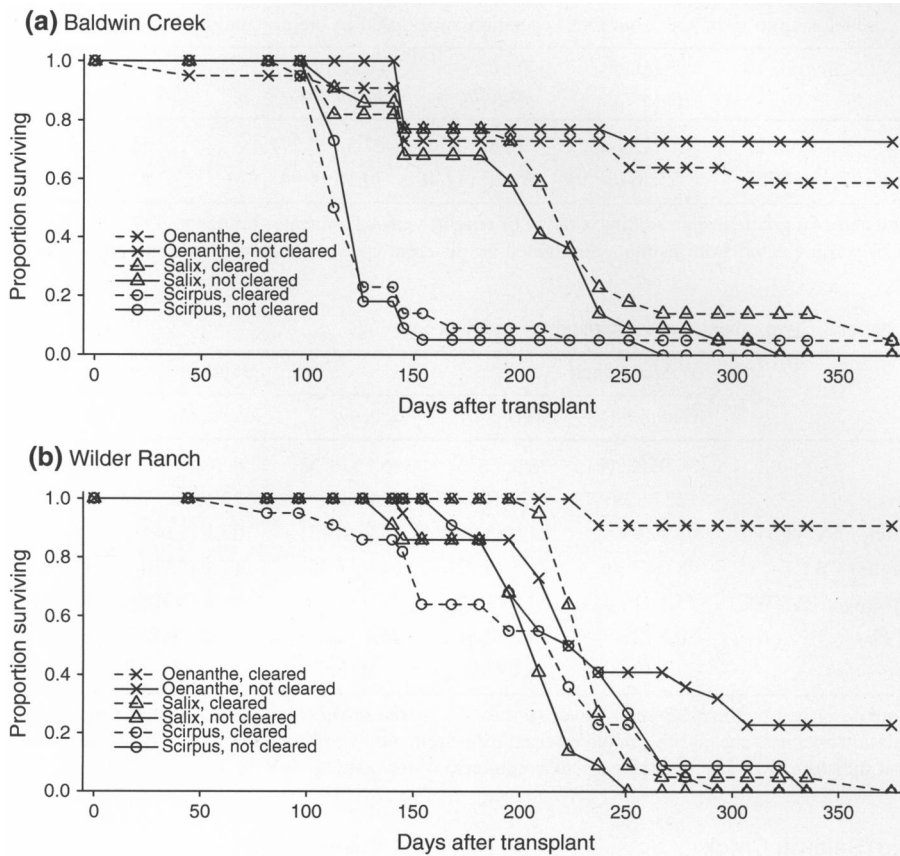


Fig. 4 Survival over time of *Arenaria paludicola* at Baldwin Creek **a** and Wilder Ranch **b**, in three habitats dominated by *Oenanthe* (x), *Salix* (triangles), or *Scirpus* (circles), from

December 2010 to December 2011. Dashed lines represent a “cleared” treatment with neighboring plants removed from within 30 cm; solid lines represent non-cleared controls

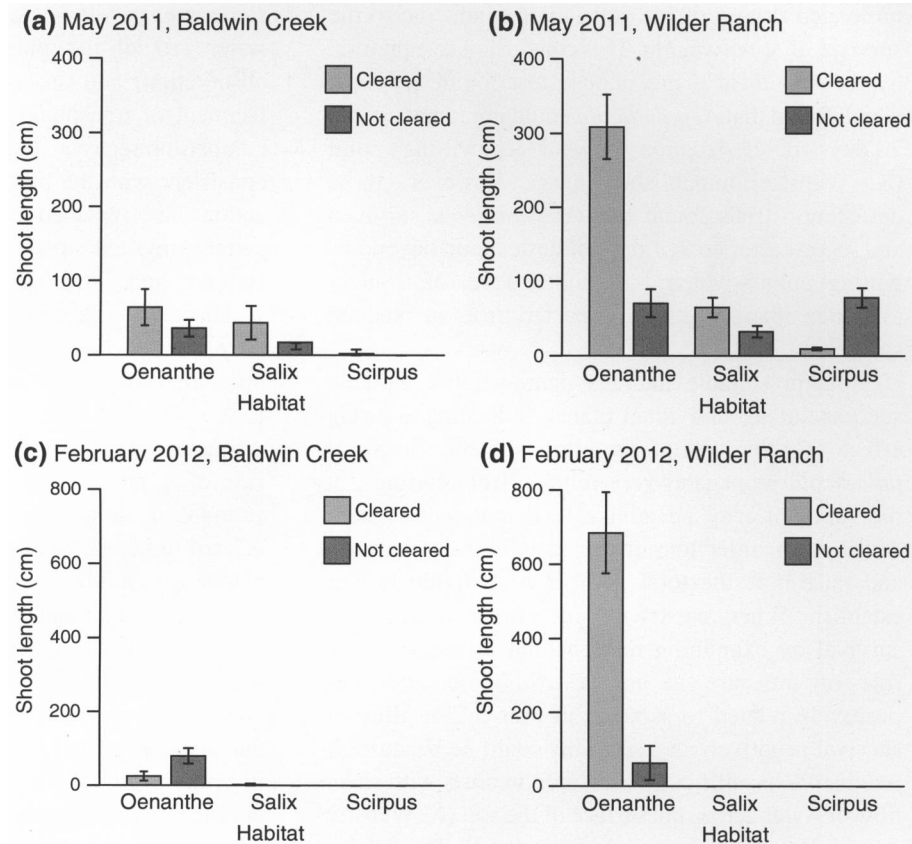
($F_{1,2} = 0.10$, $p = 0.779$, Fig. 5a,b) on plant size in May. There were only enough surviving plants in *Oenanthe* habitats for analysis in February 2012. There was no significant effect of treatment on plant size in February ($F_{1,2} = 0.18$, $p = 0.716$, Fig. 5c,d).

At Wilder Ranch, 17 of the 26 surviving individuals in the *Oenanthe* plot produced flowers, fruits, and seeds. One plant was observed flowering in the *Oenanthe* plot at Baldwin Creek, but no fruits were observed. Seeds collected from the population at Wilder had a germination rate of 83 % ($n = 52$) in the greenhouse, but it should be noted that seeds required 3–6 months to germinate. On 23 August 2012, 20 months after the initial outplanting, the *Oenanthe* plots were revisited and checked for continuing survival. At Baldwin Creek, there was no further mortality and plants were growing larger. At Wilder Ranch, 20 of the 26 plants surveyed in December 2011 were still alive. Some had partially died back, but all had recent growth (M. Bontrager, personal observation).

Discussion

In our greenhouse experiment, *A. paludicola* demonstrated an ability to tolerate a wider range of salinities than expected; although its survival was reduced in soils from the most saline areas (Fig. 2). The threshold trend we found is common in plants, displaying a non-linear decline in plant growth with salinity that includes a pronounced threshold effect (Steppuhn et al. 2005). We were surprised by this result, because historic records of *A. paludicola* in freshwater sites led to the hypothesis that the species would be highly sensitive to soil salinity. However, other closely related species of *Arenaria* and *Eremogone* live in dry desert or disturbed habitats (Baldwin et al. 2012) so mechanisms for osmotic regulation may be common in the group. Our results indicate that potential habitats for reintroduction of this species may not be restricted to the entirely freshwater habitat of the extant natural population.

Fig. 5 Plant size (total combined length of all shoots, cm) in May 2011 at Baldwin Creek (a) and Wilder Ranch (b), and in February 2012 at Baldwin Creek (c) and Wilder Ranch (d). *Light bars* represent a “cleared” treatment with neighboring plants removed within a 30 cm radius; *dark bars* represent non-cleared controls. *Error bars* represent 1 SE



At our reintroduction sites, we saw dramatic differences in establishment success between habitat types. One year after outplanting, more than half of the transplants were alive in *Oenanth* plots at both Baldwin Creek and Wilder Ranch; while no more than two individuals remained in any of the other plots (Fig. 4). This indicates that ecological characteristics varying on the scale of tens of meters are critical to establishment of our species. In the region of our study, *Oenanth sarmentosa* seems to be an indicator of habitats where *A. paludicola* has the potential to thrive and it could provide a starting point when searching for other locations with reintroduction potential. However, *O. sarmentosa* has a broad range, and we do not mean to suggest that every patch of *O. sarmentosa* will be a suitable reintroduction site. The results of our experimental transplants reveal habitat requirements of *A. paludicola* that should be considered along with indicator species occurrence to refine site selection in future transplants. We recommend using *O. sarmentosa* as a tool to identify candidate transplant locations within a site, but only in habitats

with relatively open canopies. The efficacy of this site selection method was supported in a subsequent experiment at Golden Gate National Recreation Area in Marin County, where transplanted *A. paludicola* showed the greatest success in a habitat with a high abundance of *O. sarmentosa* and an open canopy (Acierto et al. 2012).

Although differences in success among the reintroduction plots were striking, it is difficult to pinpoint exactly what features of the *Oenanth* plots made them hospitable to *A. paludicola*. At the plot level, our results indicate that *A. paludicola* success may be related to both light availability and moisture. *A. paludicola* failed to survive in the *Salix* plots, which had dense canopies; and in the *Scirpus* plots, which had understory vegetation that was denser than the *Oenanth* plots (Table 2). At Baldwin Creek, the *Oenanth* plots had higher soil moisture throughout the year than the *Salix* or *Scirpus* plots (Fig. 3a); and at Wilder Ranch, moisture levels in the *Oenanth* plots remained high throughout the year, while the other habitats dried out (Fig. 3b). This suggests that

prolonged drying of the soil is not conducive to the survival of *A. paludicola*. This conclusion is supported by the results of a greenhouse test for desiccation, which found that *A. paludicola* could not recover from 20 days of desiccation beyond its wilting point (K. Webster, unpublished data). However, these desiccation trials found that *A. paludicola* survived and regrew after up to 8 days of desiccation beyond its wilting point—which is a higher level of drought tolerance than might be expected from a wetland species.

Plots in suitable habitats demonstrated variable success among individual plants, indicating a strong effect of microhabitat on establishment. Since *A. paludicola* propagates very reliably from cuttings, in the future it may be advisable to outplant at high densities in order to saturate suitable microhabitats and maximize the total number of individuals that establish. When we try to understand variation in survival by examining microhabitat differences, the role of moisture is unclear. Soil moisture was positively related to survival in March, but affected survival negatively in May. This could be because *A. paludicola* has difficulty surviving in areas with sheet flow of water across the surface of the soil (K. Webster and M. Bontrager, personal observation). This is also consistent with what we have observed at Golden Gate National Recreation Area, where mortality in *A. paludicola* transplants increased in the wettest sites (Acierto et al. 2012). This pattern is unexpected because plants at the one extant site are associated with mats of floating vegetation in a bog (M. Elvin, personal observation). However, based on our experimental results analyzing variation both among plots and among individuals, we conclude that in northern California *A. paludicola* performs best in constantly moist soils but does not require complete soil saturation and it may be negatively affected by sheet flow or puddled water on the soil surface. Our study presents an example of how experimental reintroductions may reveal unexpected ecological patterns in endangered plants that have experienced dramatic range reductions.

Interactions with neighboring plants were generally negative, although in some cases neighbors appeared to facilitate growth. Conflicting effects of clearing may be due to the variety of stresses that *A. paludicola* transplants faced. In areas with sheet flow of water across the soil surface, the neighbor removal treatment

sometimes resulted in loose mud and areas of standing water around the plants (M. Bontrager, personal observation) and this may have impeded the establishment of transplants. The patterns of response to competitor removal that we have observed may be consistent with the prediction that competitive interactions are replaced by facilitative interactions as certain physical stressors intensify, as articulated by Bertness and Callaway (1994).

These patterns lead us to the important insight that the effects of post-planting treatments vary depending upon the context they are utilized in. This means that for a single rare species, there may not be an optimal management regime universal to all habitats. In the case of *A. paludicola*, competitor removal improved survival in some circumstances; but in plots that offered suitable habitat, there was also the potential for plants to establish without treatment. Endangered species management always faces severely limited resources; in the case of *A. paludicola*, our data support allocating effort to additional outplant sites and replication rather than to extensive post-planting management. An effective strategy might be to plant in winter without clearing, and then return to clear around surviving plants in the late spring. This could allow transplants the opportunity to establish in an intact soil substrate, but provide a reprieve from competition to accelerate their growth and reproduction once they have established. Results from our reintroduction at Golden Gate National Recreation Area corroborate this recommendation (Acierto et al. 2012).

The fate of reintroductions can change in the years and decades following transplanting (Drayton and Primack 2012), and continued monitoring will reveal the fate of these newly established populations as time passes. A successful reintroduction can initially be characterized by the survival of the outplanted propagules; but in order to promote the survival of the species, a reintroduction must result in a self-sustaining population (Menges 2008; Guerrant and Kaye 2007). Seed production and recruitment are essential for long-term population viability. The *A. paludicola* populations resulting from our experimental reintroductions flowered and produced viable seeds, indicating potential for recruitment. Our experimental reintroductions have provided specific guidelines for management that we hope will increase the success of future reintroductions for this species, while providing

a model for testing habitat breadth of other critically endangered plants.

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