1. STATEMENT OF WORK

INTRODUCTION AND BACKGROUND

Restoration efforts in southern California, where 85% of salt marshes have been lost to human development, have focused on re-establishing populations of the smooth cordgrass, *Spartina foliosa,* and pickleweed, *Salicornia pacifica* (Boyer and Zedler 1996, 1998). These efforts have achieved some success; however, overall outcomes tend to be variable. Cordgrass is a common focus in restoration projects due to its foundational role in structuring southern California salt marsh communities (Boyer and Zedler 1999).

Although the impact of abiotic factors on salt marsh restoration efforts has been well studied (Boyer and Zedler 1996) little attention has been paid to how species interactions impact the growth and survival of transplanted cordgrass. For example, a recent $1.8 million project in Del Mar, CA failed to account for the presence of herbivorous insects when reconstructing salt marsh habitat, likely contributing to their low cordgrass survival. The high herbivore densities characteristic of restored salt marshes suggest that consumers, and the predators that consume them, may profoundly affect restoration efforts. The armored scale insect *Haliaspis spartinae* is a dominant herbivore in southern California salt marshes (Boyer and Zedler 1996), Recent surveys have shown the incidence of heavy scale insect infestations (>1000 individuals per stem) is increasing (Long unpub. data). These densities are sufficient to visibly damage plants (Boyer and Zedler 1996). Previous work in southern California salt marshes has shown that scale insects can reduce leaf growth, above-ground biomass, and the reproductive output of smooth cordgrass (Long unpub. data). Given these emergent threats to cordgrass productivity, it would be beneficial to identify natural predators of scale insects; such predators could be concurrently introduced to restoration sites and may alleviate herbivore stress on transplanted cordgrass.

Beetles in the family Coccinellidae, commonly referred to as ladybird beetles, are major predators of phloem-sucking insects in agricultural systems (Costamagna and Landis, 2011). It has been suggested in that the native ladybird beetle, *Coleomegilla fuscilabris,* is capable of exerting top-down regulation on scale insects within southern California salt marshes. (Johnson 1991, Boyer and Zedler 1996). Until recently no work has been done to empirically test the consumption of scale insects by these lady beetles. Preliminary feeding assays have revealed that lady beetles do consume scale insects when provided no other food source (Rinehart unpub. data, Fig. 1). These results provide the foundation for future studies investigating the role of this predator-prey interaction within southern California salt marshes.

Historically it was assumed that lady beetles hunted through the use of random-walk patterns (Bahlai *et al.* 2008). However, recent studies have shown that lady beetles are capable of tracking prey through the perception of volatile chemicals (Xue et al. 2013), which are commonly released by plants during periods of intense herbivore pressure (Karban et al. 2013). It is likely that cordgrass stems exposed to infestations of scale insects are emitting volatile chemicals, which could be used by lady beetles to detect areas of high scale insect density within salt marshes.

OBJECTIVES AND HYPOTHESES

The aim of this study is to broadly determine the strength of tri-trophic cascade between the salt marsh ladybird beetle, the armored scale insect, and cordgrass.

Objective 1) Compare the productivity of scale insect-infested cordgrass in the presence and absence of ladybeetle predators. **2A)** Determine if lady beetles are capable of perceiving scale insects via tri-trophic signals released from previously scale insect-infested cordgrass tissue in a controlled laboratory experiment. **2B)** Test if lady beetles are capable of perceiving tri-trophic signals from infested and previously infested cordgrass patches under natural field conditions. **Hypothesis 1)** Scale-infested cordgrass will have increased height, leaf count, and leaf surface areas when lady beetles are present. **2A)** Lady beetles will preferentially migrate towards previously scale insect-infested cordgrass tissues. **2B)** Lady beetles will aggregate to infested plots and previously infested plots; however, aggregation to previously-infested plots will diminish with time. Non-infested plots will have low beetle abundance throughout the study.

METHODS

Objective 1: To determine the effects of lady beetles on both scale insects and cordgrass, I will transplant 50 individual cordgrass stems from Sweetwater Marsh National Wildlife Refuge (Chula Vista, CA) to SDSU's Coastal and Marine Institute Laboratory (Point Loma, CA). All plants will be potted and scale insects removed. Adult scale insects will be added to the adaxial leaf surface of half of the transplanted plants by sewing leaf segments with known quantities of pre-established females to the target plants. Since females reproduce asexually, target plants will quickly be colonized by mobile juvenile scale insects. Initial densities of adult female scale insects will be based upon relevant high scale insect densities obtained through survey data. This will be a fully crossed, two factor experiment with scale insects (present, absent) and lady beetles (present, absent). There will be 10 replicates per treatment. Mesh bags will be placed over whole plants to keep beetles on target plants. A cage control will be added as a treatment to ensure mesh bags have no effect on our measured indices of cordgrass and scale insect productivity. All treatments will be placed within outdoor mesocosm with a constant flow of seawater.

Measurements of all plant and scale performance indices will be collected at the start of the experiment and every week thereafter. Plant performance will be determined through measurements of plant height, leaf number, and leaf surface area. Leaf surface area will be measured using weekly images of all leaves on a given stem. Images will be uploaded into Image J software, where total leaf surface area will be calculated. Scale insect performance will be based upon density per plant and growth of individuals through the study. These measurements will be captured through images taken weekly and analyzed using Adobe Photoshop and Image J software. This study will run for 1 month during *S. foliosa*'s *primary* growing season (May-September). Indices of cordgrass and scale insect productivity will be analyzed using a 2-way ANOVA (treatment and cage, presence/absence, as factors) with post-hoc comparisons to compare between treatments. All statistical analysis will be performed using JMP v 10.0 (www.jmp.com).

Objective 2A: I will assess the ability of lady beetles to perceive scale insects through choice experiments within a Y-tube olfactometer. Although there are concerns regarding the realism of olfactometer results, this study will be useful to determine if lady beetles can use chemical cues to find scale-infested cordgrass stems. Within the olfactometer, lady beetles will be provided with the choice of non-infested plants and plants previously infested with scale insects (densities >1000 individuals per stem). Plants used as the previously-infested treatment will have all scale insects removed immediately before the experiment. Treatments will contain either 10 previously scale insect-infested leaves or 10 non-infested leaves, which will provide the volatile source.

A single lady beetle will be placed at the end of the base tube, and will be given 10 minutes to respond to the chemical cues. A positive response will be recorded when beetles cross a 'decision line', which will be defined at 6 cm up the arm leading to the previously scale insect -infested tissue (Xue et al. 2013). The location of the 'decision line' is based upon the size of the insect and previous olfactometer experiments using lady beetles. All responses outside of this will be indicated as no response to previously scale insect-infested tissue. Thirty replicates will be run per trial, trials will be conducted each month during the cordgrass growing season (May-September), so as to capture changes in chemical cues through time. For each month, a Pearson's Chi Square analysis will be used to determine if lady beetles exhibits preference for a specific leaf tissue (previously infested vs. non-infested).

Objective 2B: The aggregation of lady beetles to cordgrass plots with scale insects will be tested in the field at Sweetwater Marsh National Wildlife Refuge (Chula Vista, CA) each month during the *S. foliosa* growing season. We will select two 3m× 4m scale insect-infested plots (averaging ≥1000 individuals per stem), one plot will be randomly selected and all its scale insects removed while the other will remain intact. Scale insects will be removed using a soft bristle toothbrush. A third plot of equal size, with no scale insects present will also be selected. Twelve beetle traps will be deployed in each of our treatment plots (infested, previously infested, and non-infested). Of these 12 traps, half will be tall (145cm) and half will be short (65cm; Hacker and Bertness, 1995). In each plot, traps will be equally spaced (approximately 1m apart). Traps will be made by applying Tangle Trap insect coating to transparency film, which will then be attached to a wooden stake. Traps will be deployed for 2 weeks, and transparencies swapped out every 2-3 days. When transparencies are swapped, I will also remove any new scale insect colonizers to the previously-infested plot and non-infested plot. All insects caught on transparencies will be counted and identified to the lowest possible taxonomic level. Any ladybirds found will be identified to species and measured for maximum length and width. A 2-way ANOVA with post-hoc comparisons will be used to test for differences in *C.fuscilabris* abundance (using treatment and trap height as factors).

2. WORK COMPLETED TO DATE

In fall 2013, feeding assays were performed with lady beetles and scale insects. In each trial, 2 inch cordgrass pieces were cut and all but 9-11 scale insects removed. In half of these trials (n=5) a native lady beetle was added. After four days, final scale insect density was determined for all trials. I was able to determine that, when given no other food source, native lady beetles will consume scale insects (Fig.1; $t_{6.6}$= -5.67, p=0.001; for difference in final scale density of treatments). This is the first quantitative study to confirm this predator-prey interaction.

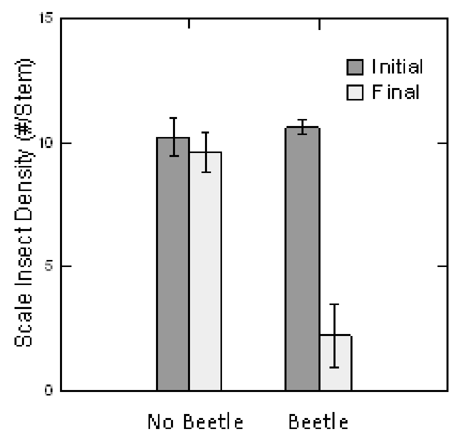

Figure 1: Total consumption of *H.spartinae* by *C.fuscilabris* over a 4 day period. Error bars represent ±1 SEM

3. BENEFITS TO WETLANDS

Previous studies in this system have focused primarily upon the interaction between scale insects and cordgrass (Boyer and Zedler 1998). The study presented here will be the first to quantify the effects of a predatory insect upon the salt marsh scale insect, *H.spartinae,* as well as the ability of lady beetles to perceive prey species. This interaction will provide further insight into the community structure of southern California salt marshes. With more knowledge of marsh systems, we will be able to make better decisions regarding restoration and conservation efforts of southern California salt marshes. Restoration of marshes is particularly important for the conservation of endangered migratory birds such as the Light-footed Clapper Rail, which use cordgrass marshes as primary nesting habitat (Zembal and Hoffman 2012). The success of restored marshes may be improved by a better understanding of the functional role played by ladybird beetles within these marshes.

4. USE OF FUNDS

The funds provided by the Garden Club of America Wetlands Scholarship will be used to purchase essential equipment for general field work (chest waders and insect nets) and field assessments of beetle aggregation (wooden dowels, transparency film, and Tangle Trap Coating). Additionally, funds will be used to cover the costs of transportation to field sites (including gas and tolls). Supplies will also be purchased for mesocosm experiments (plant pots, mesh bag supplies, HOBO temperature loggers and shuttle, a waterproof camera, and Photoshop software) and for laboratory olfactometer experiments (olfactometer, flow meters, and glassware). Lastly, approximately $100 will be used to cover the application fee for a California State Scientific collector's permit.

5. OUTREACH AND BROADER IMPACTS

I plan to produce a music video with the help of students in Dr. Jeremy Long's Chemical Ecology Course. The video will highlight the importance of marsh conservation and how the findings of this project can benefit salt marsh restoration. I will publish my video on the Long Lab webpage, YouTube, and the university's biology department homepage (for examples see www.youtube.com/user/iambient33). I also plan to showcase my work at the Coastal and Marine Institute Laboratory's annual open house event in the form of an informational poster and a 15 minute talk designed for the general public. This event attracts an average of 700 visitors each year, and serves to inform San Diego's citizens of the research being conducted in the area.

Expected products include publications in high-impact, peer-reviewed journals and presentations at multiple national meetings. Additionally, the findings of this study will be shared with local restoration groups (ReCon) and resource managers; in particular, those located at Seal Beach National Wildlife Refuge (Seal Beach, CA) and Sweetwater National Wildlife Refuge (Chula Vista, CA), through annual progress reports and oral presentations to volunteers and staff. With this information, these groups will be able to make more informed decisions regarding salt marsh restoration, which will help to mitigate the effects of harmful scale insect outbreaks.

This project will also serve to train multiple San Diego State University undergraduate students in basic field, laboratory, and data analysis techniques. Students will be contacted through the University's Marine Ecology and Biology Student Association (MEBSA) and Dr. Jeremy Long's Chemical Ecology course.

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