A description of terrestrial invertebrate assemblages and a comparison of sampling methods in pre and post-restoration Humboldt Bay salt marsh: A pilot study

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ABSTRACT

Literature pertaining to the effects of *Spartina densiflora* invasion on terrestrial invertebrate communities in Humboldt Bay, California is virtually non-existent. Consequently, efforts to restore native salt marsh vegetation, although widely expected to improve biodiversity and system function, have gone largely uninformed regarding invasion and restoration impacts on trophic arrangements of lower organisms. It is suspected that *S. densiflora* has the potential to shift functional group representations by altering various qualities of the salt marsh habitat.

The goal of the study was to determine whether invertebrate abundance and diversity differ significantly at the Order level between *S. densiflora* invaded and *S. densiflora* removed-and-restored sites and to compare sampling methods. A vacuum sampling technique was tested, proved capable of capturing diverse arrays of organisms at both sites, and was suitable as a stand-alone vehicle for diversity and abundance comparisons at the Order level using our methods. The restored site was shown to have more than twice the diversity and abundance than the invaded site using this method, both variables differing significantly ($p<.05$). The Shannon Diversity Index demonstrated higher diversity at the restored site. Sticky traps were deemed ineffective for use in salt marsh habitats using the described methods, while sweep netting was found to be suitable for the detection of flying invertebrates and vegetation top-dwellers. Notable among the sweep net findings was the observed volume of Culicidae (mosquitoes), comprising the bulk of Dipterans observed at the invaded site. Biomass sampling was identified as likely crucial in obtaining adequate representation of the stem-dweller component within *S. densiflora* habitats. At the invaded site this method detected the Order Acari (including mites), which was missed by all other techniques. Our findings imply that *S. densiflora* invasion reduces invertebrate diversity and abundance and casually demonstrates an association with family Culicidae. In addition, vacuum sampling should be used in conjunction with other methods to maximize terrestrial invertebrate representation. Researchers should take into consideration the natural

history of particular species and/or vegetation types to be sampled, as this study demonstrates the concept that no single sampling method addresses vegetative structural complexities in totality.

I. Introduction and Objectives

Humboldt Bay and its wetland habitats have been drastically altered by the introduction of the local railroad and agriculture. Both of these activities brought about major structural changes to the salt marsh. The building of dikes and levees has disrupted tidal prism exchanges, and since their installment in the early 1900s salt marsh habitats have been reduced by 90%, from 3,642 ha (9,000 ac) to 364 ha (900 ac) (Fig. 1).

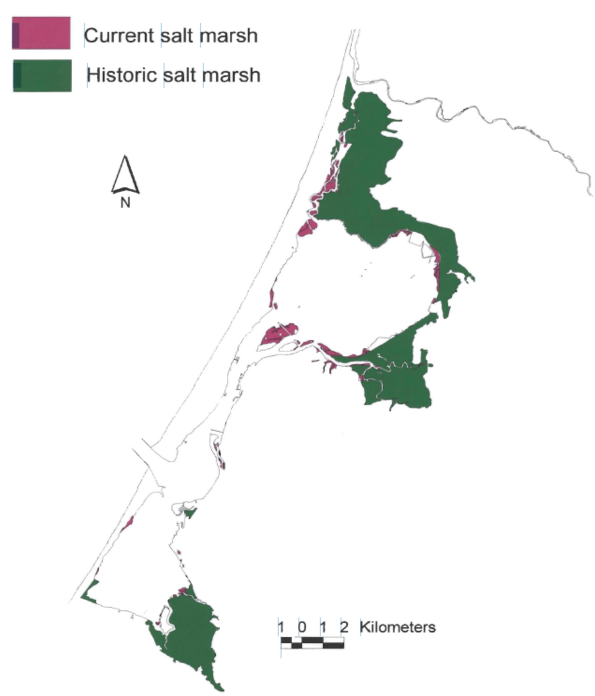

Figure 1. Former and current extent of salt marsh in Humboldt Bay (from Pickart, 2001).

These diked areas are still functioning wetlands, but have been converted from estuarine to palustrine, seasonally-flooded marsh, and most are used as pasture for grazing (Pickart, 2001). Efforts in 1998 and 1999 were made by the U.S. Fish and Wildlife Service to document the distribution of remaining native vegetation assemblages in Humboldt Bay. Since then, active removal of *Spartina densiflora* has taken place using mechanical methods within Humboldt Bay National Wildlife Refuge (Pickart, 2005, 2008).

The Invader

S. densiflora is a tall grass typically occurring in monocultures of tightly packed clumps. Recent observations indicate that it is naturally distributed from Sao Paulo State, Brazil, to Rio Gallegos, Argentina (Bortolus, 2006). It is an aggressive invader of salt marsh habitats and has consequently out-competed many native plants in Humboldt Bay. The physical characteristics of *S. densiflora*-invaded marsh are drastically different than those of a native or restored marsh. Common saltmarsh species such as *Salicornia virginca* (pickleweed) and *Distichlis spicata* (saltgrass) are comparatively shorter, and the native communities are more structurally complex due to the higher diversity of plant species. The relatively lower height of native salt marsh species leads to almost complete immersion at high tide while *S. densiflora's* height keeps the majority of the plant out of water, providing refuge for invertebrates. In addition, the shift to a comparatively structurally homogenous habitat likely favors less and/or alternative species.

S. densiflora is also thought to alter invertebrate assemblages through “bottom up” trophic dynamics (Levin et al. 2006).

Objectives

1) To compare terrestrial invertebrate abundance and diversity at the Order level within *Spartina*-dominated salt marsh with that of native restored salt marsh.

2) To compare the relative effectiveness of a vacuum sampling method in the two habitat types.

3) To determine if biomass, sticky traps, and sweep netting significantly contribute to capture rates when compared to vacuum sampling alone.

4) To compare total capture rates of vacuum, sticky trap, biomass and sweep net invertebrate sampling methods.

Site Description

Two study sites were selected, both located along the Mad River Slough, which flows into the main northern body of Humboldt Bay near Arcata, CA. Site adjacency was a primary factor in deciding where to locate plots in addition to their adequate representation of the desired plant communities. The sites are approximately 0.8 km (.5 mi) apart, with appropriately similar climatic conditions, soils, and topographic exposure. Both sites were at similar tidal elevations, as evidenced by the current or historic presence of dense *S. densiflora*.

The first site, referred to as "Ma-le'l," is within the Ma-le'l Dunes Unit of Humboldt Bay Wildlife Refuge, situated between the Pacific Ocean and the Mad River Slough at Humboldt Bay. The Ma-le'l site is located along the west bank of Mad River Slough in a salt marsh that underwent restoration through the removal of *S. densiflora* between 2008 and 2010 (Pickart 2005). In the two years since the removal/restoration began, native species have re-colonized the site. Within the sampling grid, the dominant species overall were saltgrass (*Distichlis spicata)* and pickleweed (*Salicornia virginica),* each contributing about 50% of total cover of the site overall. Other species were sporadically represented, including Point Reyes Birds Beak (*Cordylanthus maritimus* ssp. *palustris*), marsh rosemary (*Limonium californica*), and arrow-grass (*Triglochin* spp.)*.* The parasitic dodder *Cuscuta salina* was present on several *Salicornia virginica* individuals.

The second site, referred to as "Mad River Slough Outlet" is south and slightly downstream about 0.8 km (0.5 mi) closer to the main body of Humboldt Bay, on the west side of the slough. This site can be characterized as a near monoculture of *S. densiflora* with very minute patches of *Salicornia virginica* interspersed. The site is owned by California Department of Fish and Game, and located within its Mad River Slough Wildlife Area. No restoration has taken place at the site save for one or two small-scale experimental removal plots.

II. Methods

Vacuum, Biomass and Sticky Trap Sampling Grid Setup

One master plot was established in each of the two study areas. The areas selected for master plot establishment were determined by visual estimation of dominant cover, with only consistent patches of *S. densiflora* and native marsh cover considered. The lower left corner of a 9m x 9m grid was initially established by a person standing at the upland/marsh edge, facing directly away from the desired habitat, and throwing an item over their shoulder, with its landing point acting as the lower left grid corner. A random compass bearing then determined the direction of the line forming the left side of the grid. From this initial angle, 90° bearings were taken successively to form the sides of the grid until returning to the original point. The four corners of the grid were established with wooden posts and a pin flag. The bottom row of sample points was numbered vertically starting with the lower left corner as #1 and the top as #10. The top row of points was subsequently numbered 10, 20, 30, up to 100, while the bottom was 1, 11, 21, 31, and up to 90. If any portion of the grid fell outside the low marsh into adjacent vegetation or any standing or flowing water source, a new lower left corner would have been randomly selected to avoid introducing confounding variables. Each coordinate acted as the center of a sample point. A total of two sets of 25 randomly selected sample points were chosen from a possible 100 points at each site and marked with a pin flag. The two sets of numbers were produced using a widely available random number generator.

Vacuum Sampling

Vacuum sampling was conducted on September 26, 2010 during the hours of 1000 to 1050 at Ma-le'l and from 1150 to 1300 at Mad River Slough Outlet.

Terrestrial invertebrates were sampled using vacuum methods similar to those described by Stewart & Wright (1995), Hossain et al. (1999) and Southwood (1978), adapted to suit the conditions described below. A modified Ryobi blower/vac with a 23 cc engine, a nozzle area of 81 cm^2, and a nozzle velocity of 322 km/hr (200 mi/hr) was employed for vacuum collection. This velocity is significantly higher than the minimum threshold effectiveness of 145 km/hr (90 mi/hr) recommended by Southwood (1978). A fine mesh nylon bag was inserted into the vacuum tube as a collector, and a clamp was tightened around it and the tube to prevent it from being sucked into the machine. A 121-l (32-gal) garbage can with a volume of 0.12 m^3, height of 66 cm and surface sampling area of 1,641 cm^2 was used as a sampling enclosure, with a screen mesh over the top to prevent flying insect escape. At each sample point the enclosure was placed with the pin flag at the center. The enclosure had a slit in the screen through which the vacuum sampler was inserted, with the vacuum initially run for 30 seconds. The vacuum was applied to vegetative matter within the enclosure from a vertical position as evenly and thoroughly as possible. With the vacuum still running to prevent insect escape, the enclosure screen was then removed and a visual assessment conducted, followed by a second round of vacuuming for 30 seconds down to the soil surface to maximize catch rates.

Sticky Traps

During an approximate 48-hr period between two successive high tides from September 24 to September 26, 2010, a total of 25 white index cards coated with “Tanglefoot” insect adhesive (The Tanglefoot Company, Grand Rapids, MI) were deployed at the center of the sample points described above. The angle of each card was chosen randomly by stopwatch method, multiplying 360° by the 1/100th second of a random start/stop interval. Cards were collected after approximately 48 hours during vacuum sampling and stored in individually labeled plastic bags.

Sweep Netting

Sweep netting was conducted on November 3, 2010, between the hours of 1115 to 1200 at Mad River Slough Outlet and 1315 to 1400 at the Ma-le'l site. Two surveyors participated, with one taking a single sample at each site and the second taking an additional Ma-le'l sample for comparison purposes. One sweep net sample was standardized to 100 sweeps (once back and forth) of a net with a 30-cm diameter opening. One step was taken forward after each full back-and-forth sweep. Sweeping was conducted haphazardly at each site, following no predetermined path other than staying within the desired vegetation. The Mad River Slough Outlet was sampled first using appropriately labeled plastic bags and a Kestrel 3000™ (Nielsen-Kellerman Company, Chester, PA) to record the weather specs. At each step taken a sweep was made in front of the sampler at the highest area of desired vegetation, in this case the tops of *S. densiflora*. After 100 sweeps, the recorder prepared a bag for the contents of the net to be shaken into. The net was then inverted into the sampler's plastic bag and the contents rigorously shaken out. After this the bag was immediately sealed and labeled. An alternate surveyor collected an additional sample of 100 sweeps at the Ma-le'l site to determine if catches differed between surveyors. For sweep net comparisons between sites only the original was used as only one sample was collected at the Mad River Slough Outlet site.

Biomass

The bulk of the upper *S. densiflora* biomass was removed with clippers after each vacuum sample and stored for further examination in the lab. This method is intended to compensate for inherent vacuum sampler inefficiencies in tall vegetation identified by Hossain et al. (1999).

Laboratory Procedures

Vacuum and biomass samples were frozen for over 24 hrs and sorted. Each sample was removed from its bag and rinsed into 3 layers of sieve trays, starting with a large #5 mesh on top, followed by #10 mesh and finally into # 60 mesh. At each stage of mesh, heavy vegetation was rinsed thoroughly into the next screen, placed in a white tray with water and thoroughly searched. After discarding the vegetation, the water in the tray was rinsed into the screens. After the smallest material had been concentrated into the fine mesh, the material was rinsed into the bottom and flushed into petri dishes with a pipette. The concentrates were then examined under a 30x dissecting microscope, with specimens separated from debris and placed into labeled vials containing 75% ethyl alcohol. Invertebrates were sorted to order within each vacuum sample and tallied on data sheets.

Abiotic Variables

Weather variables for each site were recorded on all occasions of trap deployment and data collection with a Kestrel™ portable weather meter (Nielsen-Kellerman Company, Chester, PA). Variables recorded include wind speed (error

+/-3%), ambient air temperature (error +/- 1ºC), humidity (error +/- 3%), and dew point (error +/- 2ºC). Salinity was measured with an Ecosense EC300™ meter (YSI Environmental, Yellow Springs, OH), and a pHTestr™ (Eutech Instruments) was used for pH.

Statistical Analysis

Data were analyzed for normality and t-tests or a non-parametric equivalent were used to determine whether invertebrate variables differed between restored and non-restored sites. Accumulation curves were constructed for each sampling method, and sample means for diversity and abundance within sample populations at each site were analyzed by ANOVA. T-tests were also used to determine if the addition of sweep, biomass, and sticky trap samples contributed significantly to the vacuum detection of invertebrates. Analysis of data was conducted using Excel 2007 data analysis package.

III. Results

Vegetation Height

Mean vegetative height among samples at the Ma-le'l site (18.26 cm) was significantly higher than at the Mad River Slough Outlet (53 cm, $p<.001$).

Sample Size (Vacuum Method)

Abundance values were highly variable (Ma-le'l: mean= 73.0, SD= 44.6; Mad River Slough Outlet: mean= 33.3, SD= 35.4). Variation in diversity indices was lower at Ma-le'l than Mad River (Ma-le'l: mean= 7.2, SD= 0.9; Mad River Slough Outlet: mean= 3.8, SD= 1.4). Diversity accumulation curves were also highly variable until apparently reaching a plateau at around 20 samples (Figures 1 and 2) implying sample size was sufficient to overcome variability for our purposes.

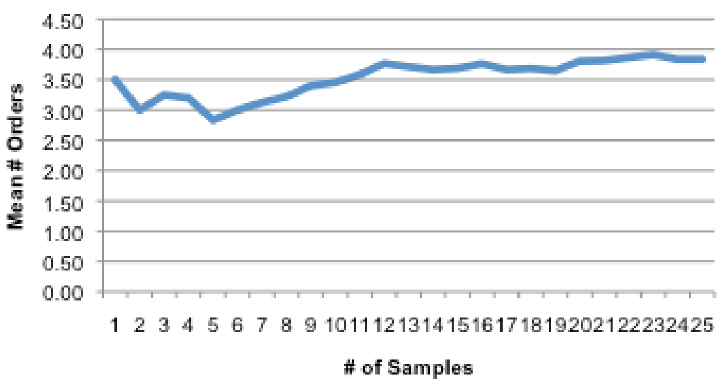

Figure 1. Mad River Slough Outlet Diversity Accumulation

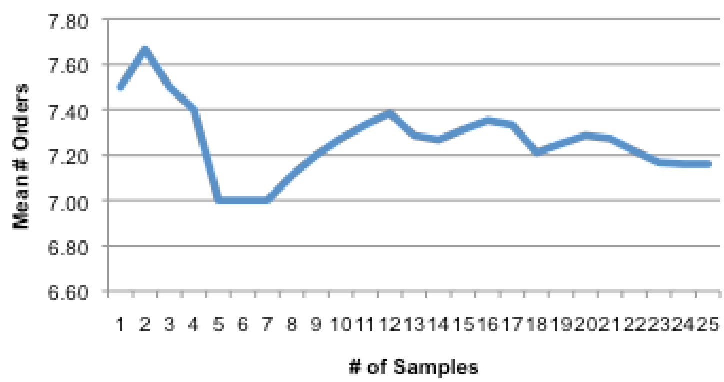

Figure 2. Ma-le'l Diversity Accumulation

Total Catch Diversity and Abundance Between Sites- Vacuum

Mean observed invertebrate abundance for vacuum sampling at Ma-le'l was more than double the amount at Mad River (Fig. 3). Mean diversity was also more than double (Fig. 4).

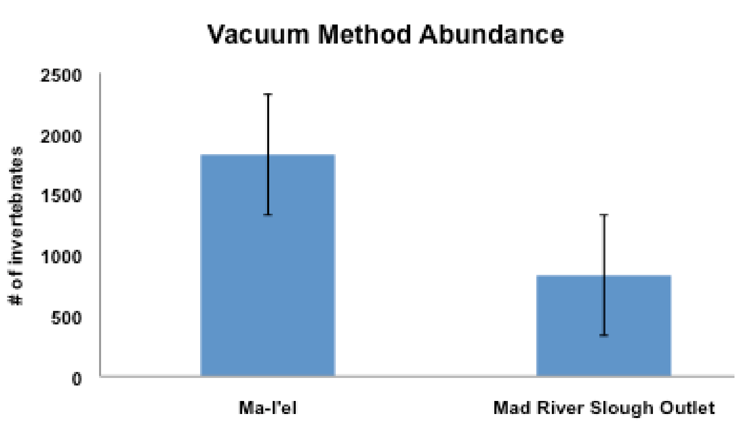

Figure 3. Vacuum method abundance (+/- 1 SE).

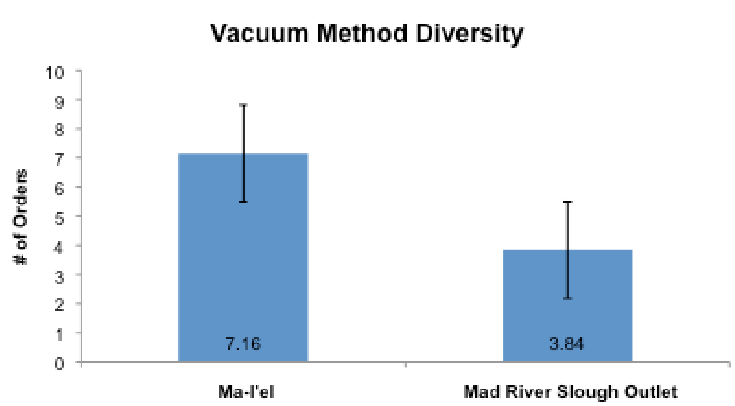

Figure 4. Vacuum method diversity (+/- 1 SE).

An F-test determined variance of both diversity and abundance for the two sites to be equal (diversity p=.056, df=24; abundance p=.267, df=24). Two-tailed, independent, two sample t-tests for equal variance identified significant differences in both diversity (p<.001, df=48) and cumulative abundance by Order between restored and un-restored sites using the vacuum method (p<.001, df=48) (Fig. 5). Shannon Diversity Index results indicate higher diversity at the restored site (Ma-le'l: H1= 2.55, Mad River Slough Outlet: H1= 1.72).

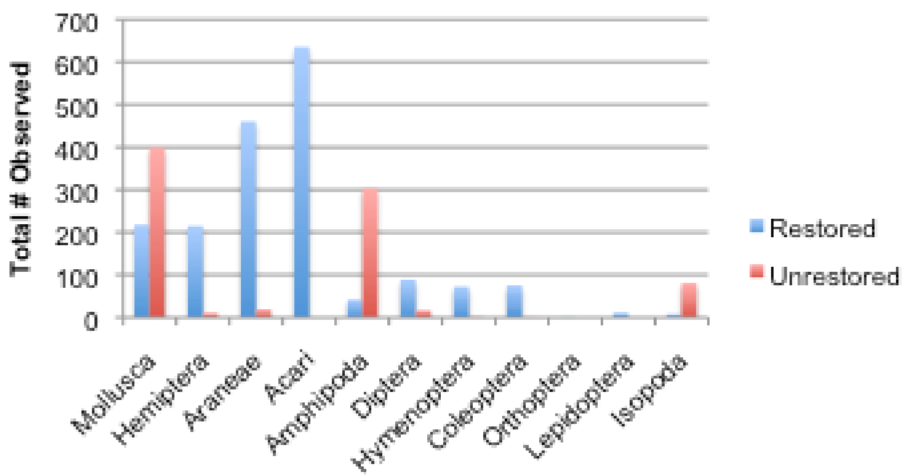

Figure 5. Abundance by Order and location (Ma-le'l = Restored, Mad River Slough Outlet = Unrestored).

Abundance by Order Between Sites- Vacuum

A two-tailed, independent t-test for unequal variances identified significant differences in total abundance for Amphipoda (p<.001., df=27), Acari (p<.001, df=24), Araneae (p<.01, df=24), Hemiptera (p<.001, df =25), Coleoptera (p<.001, df=24), Diptera (p<.001, df=24), Hymenoptera (p<.001, df=24), Lepidoptera (p<.01, df=24), and Isopoda (p.05, df=24). Differences for Mollusca and Orthoptera were not significant (p>.05). The most abundant organisms observed at the restored site were predators and herbivores (Fig. 6). Comparatively, the invaded site showed a significantly higher abundance of detritus feeders, with more abundant gammaridean amphipods and mollusks (Fig. 7).

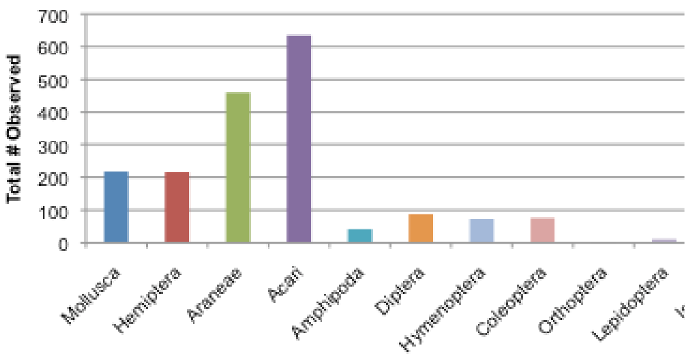

Figure 6. Invertebrate abundance by Order at Ma-le'l site (restored).

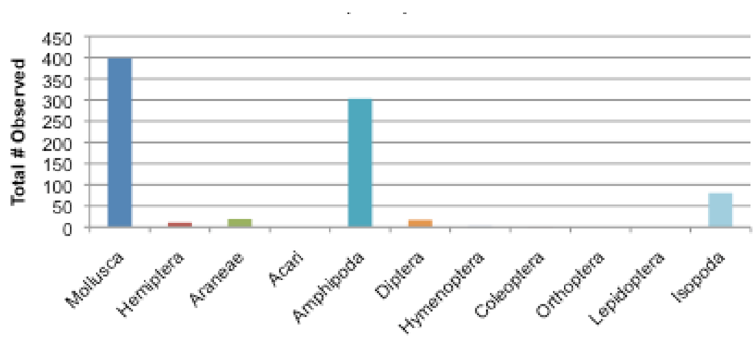

Figure 7. Invertebrate abundance by Order at Mad River Slough Outlet (invaded)

Biomass

A total of five Orders were identified in biomass samples. The dominant invertebrates were Acari (Fig. 8), an order that went undetected by all other methods at the Mad River Slough Outlet site. T-tests between vacuum and vacuum + biomass abundances detected no significant differences between Orders except Acari (P<.05) (Figure 9).

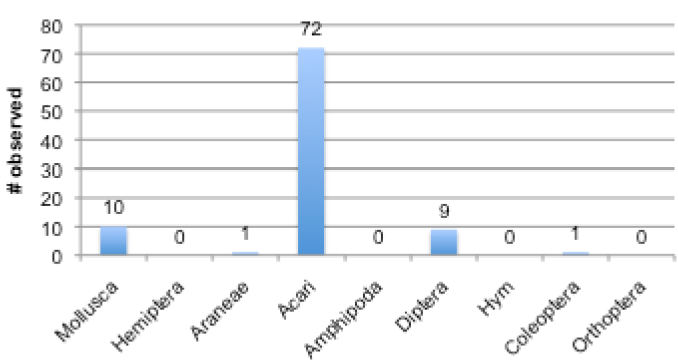

Figure 8. Invertebrate abundance by Order detected through biomass sampling at Mad River Slough Outlet

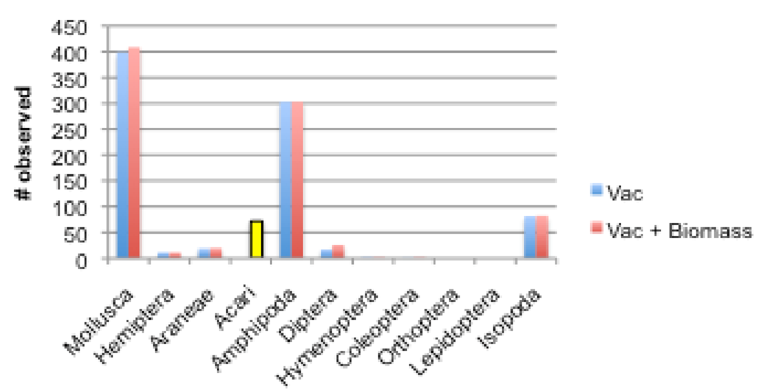

Figure 9. Vacuum and vacuum + biomass comparison

Sticky Traps

Capture rates differed significantly for Diptera between the two sites ($P<.05$) with more captures occurring at the Ma-le'l site. Hemiptera were detected at the Mad River Slough Outlet site using this method while none were captured at Ma-le'l (Figure 10).

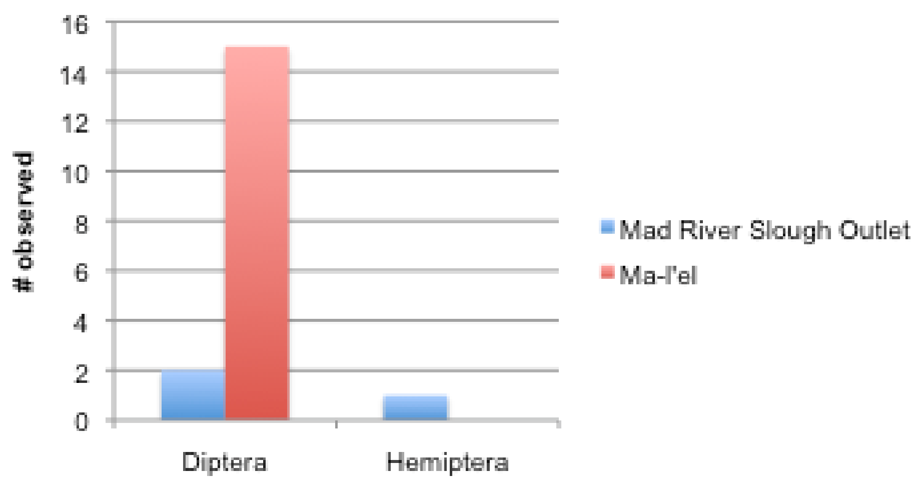

Figure 10. Abundance by Order and site detected by Sticky Traps.

T-tests between vacuum and vacuum + sticky trap abundances determined that there was no significant difference in total catch between individual Orders at either site ($p>.05$).

Sweep Netting

Diptera were the dominant Order within the sweep net sample at the Ma-le'l site, followed by Hemiptera, Araneae, and Acari. Capture rates did not differ significantly between surveyors at the Ma-le'l site ($p>.05$). Represented Orders between surveyors was identical at the restored site with the exception of a single Orthoptera found by Surveyor 2 (Figure 11).

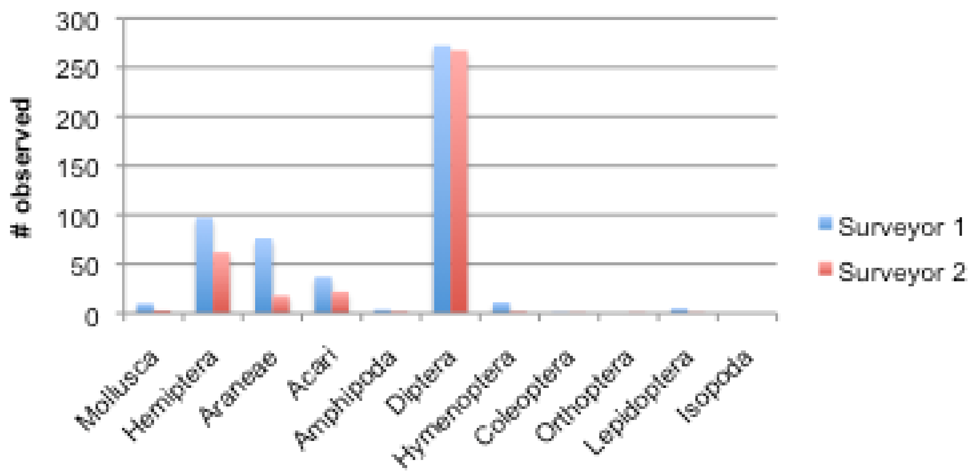

Figure 11. Sweep net Abundance by Surveyor, Ma-le'l site.

The Dipteran group was also the dominant Order at the Mad River Slough Outlet, and was 32x more abundant than the second most abundant, Araneae. A total of eight Orders were collected using the sweep method at Mad River Slough Outlet (Figure 12).

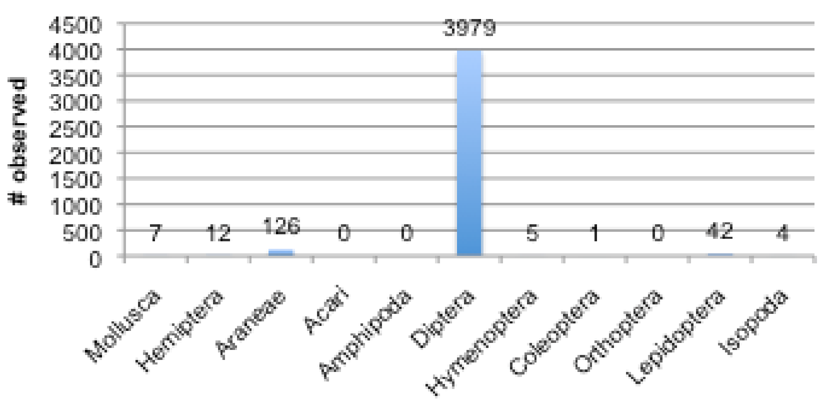

Figure 12. Abundance by Order for sweep method at Mad River Slough Outlet

Diversity and Abundance Between Sites- Sweep Netting

Sweep net contributions to vacuum sampling were significant for Diptera at both sites ($p<.05$). Sweep net samples when compared on their own between sites illustrates the high incidence of Diptera, consisting primarily of Culicidae (Figure 13).

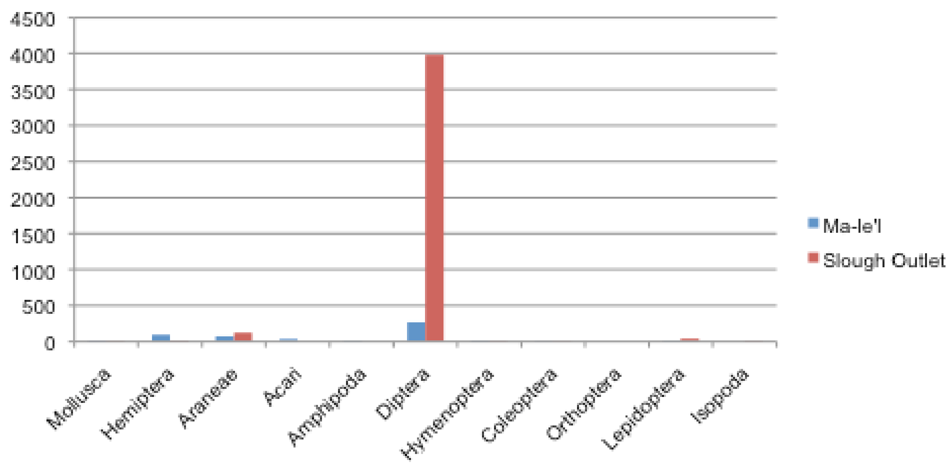

Figure 13. Abundance by Order for sweep method between restored and unrestored sites.

IV. Discussion

Evaluation of Methods

The vacuum method was determined to be successful as a sampling method for salt marsh invertebrate comparisons based upon the array of organisms caught. Although the study supports previous findings that vacuum method is inefficient in taller vegetation such as *S. densiflora*, utilizing additional measures can compensate for this. The vacuum was an excellent method for sampling in the low vegetation of the restored marsh.

Biomass sampling was the only method able to detect Order Acari at the invaded site, confirming the inefficiencies of the vacuum method in tall vegetation. As the *Spartina* stems sampled in this study were disturbed before collection, it is expected that clipping biomass before vacuum sampling has the potential to improve on observed catch rates.

Although the sticky trap method was demonstrated to be ineffective using the described methods, a protocol could likely be altered to increase effectiveness. Height of the trap, color, and length of deployment are all likely variables contributing to catch rates in this type of habitat and warrant further investigation.

The sweep netting method captured several orders and high abundances of both flying and epiphytic invertebrates, although it was generally less effective than the vacuum method alone. The fact that samples obtained between the two surveyors were significantly similar demonstrated our standardization as reasonably replicable. It was recognized that the Dipteran Order consisted primarily of the family Culicidae, apparently in support of similar findings by Caceras and Ruiz (2004). As concerns about the spread of West Nile Virus are increasing, our findings support removal invasive of *S. densiflora* as possibly beneficial to mosquito control efforts.

Ecological Implications

Although absolute species representation for this study is undoubtedly incomplete, the trophic group representation observed at the Mad River Slough Outlet appear to reflect and support the consequences of a *Spartina* invasion as described by the Neira et al. (2006) and Levin et al. (2006) studies. As depicted in Fig. 6, many of the vacuum samples contained higher observed incidences of herbivores, plant piercers and active predators, supporting the notion that non-indigenous plants lack the associated diversity of herbivores present in their ecosystem of origin (Strong et al., 1984). The presence of specialized feeders and a higher invertebrate diversity at the Ma-le'l site is therefore likely reflective of the higher complexity of plant resource availability compared to the monoculture of the *S. densiflora* invaded site.

These results indicate that the *S. densiflora*- invaded salt marsh has a reduced diversity and abundance of terrestrial invertebrates and significantly altered functional group representation compared to the restored site. Previous studies supporting the concept of "bottom up" trophic dynamics have shown that alteration from native to invasive salt marsh vegetation can impact invertebrate assemblages through biodiversity loss and changes to functional groups (Harvey et al. 2009), and that removal of the invader can rapidly restore invertebrate communities to their un-invaded state (Gratton & Denno, 2005). Research by Neira et al. (2006) suggests several factors as being possible culprits behind the apparent biotic re-arrangement observed at the invaded Mad River Slough Outlet site. In the study, the combined effects of decreased water velocities (leading to less organism recruitment), lack of food availability (through a decrease in algae and increase in detritus), and higher sediment deposition (leading to increased soil organic matter content) were identified as leading to significant declines in both diversity and density of macrofauna in hybrid *Spartina* marsh in the San Francisco Bay. Levin et al. (2006)

further examined the role of increased detritus resulting from *Spartina* invasion, attributing changes in faunal arrangements to a shift from algal-based to detritus based primary consumption.

V. Conclusions

This study supports the general consensus that non-native plant invasions can significantly alter the taxonomic array of invertebrate assemblages. Shifts in primary sources of productivity leading to reduced diversity and abundance in invertebrates likely has implications for higher-up organisms such as fish, birds, and mammals. If the primary resources supporting a particular array of organisms in a system are reduced, eliminated, or replaced, the new resource base inevitably will not support an identical array of organisms, thus altering the "links" (functional groups) in the chain. The findings in this study warrant further investigation into the effects of *S. densiflora* invasion in Humboldt Bay, particularly on higher organisms dependent on salt marsh invertebrates.

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