Torch Mussel Bed and Fucoid Restoration Project
Final Report

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Project Motivation and Goals:

Rocky intertidal habitats have considerable ecological value and provide a rich environment for education, research, and recreation. The location of intertidal habitats at the coastal interface makes them particularly vulnerable to human disturbances. Oil spills, marine debris, overharvesting, and trampling due to high levels of human visitation have all threatened the health and biodiversity of rocky intertidal ecosystems (Murray et al, 1999; Paine et al., 1996; Smith et al., 2008; Suchanek, 1993). The following project was initiated following the 1997 Torch Platform Irene Oil Spill, which resulted in oiled shorelines, including rocky intertidal habitats, at locations south of Point Arguello. While injury levels greater than 10% were not documented, an effort to address the low level of injury was deemed necessary. We chose to focus restoration and research efforts on two key intertidal species groups: the mussel, *Mytilus californianus*, and fucoid algae, *Fucus gardneri, Pelvetiopsis limitata,* and *Silvetia compressa*.

Mytilus californianus is one of the most abundant primary space holders in rocky intertidal habitats along the west coast of North America. As a foundation species, this mussel forms dense beds that support a diverse array of algal and invertebrate species (Suchanek 1992, Lohse 1993). For this reason, mussels are an excellent target species for restoration efforts. Previous studies have shown that mussel beds in the study region can take 15 years or more to fully recover following a disturbance (Kinnetics Laboratories 1992, Conway-Cranos 2012). This is because mussel recruits are poor settlers of bare rock and typically require complex substrata, such as the byssal threads of adult mussels or turf algae, for post-settlement growth and survival. In this study, we sought to speed up recovery by seeding barren areas with adult mussel transplants. We predicted mussel transplants would result in faster community recovery by facilitating both mussel recruitment and other invertebrate and algal species. Using this approach we hoped to not only benefit areas within the spill region, but also provide valuable information to guide future restoration projects.

In addition to using adult mussel transplants, we also wanted to explore if other invertebrates and algae could be used to increase mussel recruitment. It is well known that mussel recruits settle within complex substrate (Bayne 1964, Seed 1976). Field observations of high mussel recruit densities on particular biological substrates (for example, the turf alga *Endocladia muricata*) suggest that mussel recruits positively associate with these substrates, but this hypothesis has yet to be rigorously tested. Many of the biological substrates that have been identified as potential facilitator species for mussel recruits are abundant within rocky intertidal habitats, raising the alternative hypothesis that high densities of mussel recruits on these substrates may be due to higher encounter rates when settling. To establish that positive associations exist between mussel recruits and a particular settlement substrate, the frequency of mussel recruits observed in association with a substrate must be evaluated relative to the frequency of that substrate in the environment.

With this in mind, the second goal of this project was to survey mussel recruits and available settlement substrate to identify substrates with which recruits positively and negatively associate. Through this work we have identified several species/substrate types that mussel recruits have strong positive associations with, which include fucoid algae. Fucoid algae have not been previously identified as species that mussel recruits associate with and were thought more likely to dislodge recruits through whipping of fronds. These results reflect the general lack of knowledge of factors that promote successful mussel recruitment. With this study we hoped to fill in knowledge gaps surrounding the juvenile life history stage of mussels and aid in the development of future restoration strategies.

Based on the results of the mussel recruit survey, the third goal of this project was to develop and evaluate fucoid restoration techniques. Fucoid algae (*Fucus gardneri*, *Pelvetiopsis limitata*, and *Silvetia compressa*), also known as rockweeds, are fleshy brown algae that are common in the mid intertidal zone. Fucoid species have limited dispersal capability making it difficult for populations to recover following a disturbance event. In this study, we tested two restoration approaches: transplanting of algae propagated in the lab, and the use of seed bags with fertile tips of fucoid fronds. These restoration efforts have the potential to not only benefit fucoid populations following a disturbance, such as an oil spill, but also various invertebrate species, including mussels, that rely on the protection provided by fucoids for growth and survival.

Mussel Transplant Study:

Methods

Study sites:

This study was conducted at two intertidal sites (Pothole and Occulto) located along the coastline of Vandenberg Air Force Base (Fig. 2). These sites were characterized by different abiotic and biotic conditions allowing the effects of mussel transplants on recovery rate to be evaluated in two very different environments. Pothole (N 34.71483, W 120.60725) consists of gently sloping ridges of Monterey shale and experiences moderate to heavy wave action with a southwest coastal orientation (cbsurveys.ucsc.edu). Occulto (N 34.8812, W 120.63594) is composed of highly exposed benches made of conglomerate rock with a west/northwest orientation. Biologically, Pothole is more algal dominated with patchy mussel distribution throughout the middle intertidal zone. In contrast, Occulto is mussel dominated with 60-70% cover in the mid intertidal zone (personal obs.). Experimental plots for this study were established at Pothole and Occulto in December 2009 and April 2010 respectively. Although originally proposed, a third site was not setup due to permitting and logistical constraints.

Transplant methods:

Mussel transplants were used to determine if *M. californianus* adults were able to speed up the recovery of mussel bed communities. It was important to avoid damaging natural mussel beds in the process of collecting transplants. We originally proposed to collect mussel transplants from offshore oil platforms, however, this proved to be logistically challenging. As an alternative, transplanted mussels were collected from the Ellwood Pier in Santa Barbara, CA, which is regularly cleared to prevent fouling. Collected mussels were cleaned of epibionts and notched with a triangular file, which allowed us to measure growth rates. They were then transplanted into plots using a standard protocol for mussel transplantation. To promote attachment, mussels were placed into cleared plots with their byssal organ oriented downward, and then secured with vexar mesh (Fig. 1). Vexar was loosened after one month to allow mussels to reorient, and after two months, the vexar was completely removed. The success of relocated mussels was monitored for one year by counting the number of remaining live and dead mussel transplants in each plot every month.

Experimental design:

Each site contained four replicate blocks of four plot types, for a total of sixteen 50 x 50 cm plots per site. Plots were placed in natural spaces within the mussel bed in the mid intertidal zone. Plot types included a control plot that consisted of natural mussel bed and three treatment plots. Treatments plots were initially cleared of all visible biota using paint chisels and wire brushes. The three treatments included (1) a cleared plot with no mussel transplants (used to simulate a situation where a disturbance occurred and no restoration action was taken), (2) a plot with one large mussel patch containing 100 transplanted mussels, and (3) a plot containing 100 transplanted mussels divided into three small mussel patches. The latter two treatments were used to determine if the spatial configuration (i.e. patchiness) of mussel transplants had any effect on plot recovery (Fig. 3). We hypothesized that increased patchiness would strengthen the positive effects of mussel transplants on recovery by creating more edge space for recruitment and attachment.

Sampling methods:

The percent cover (i.e., community composition) in transplant and cleared plots were compared to the control over time to determine the effect of mussels on recovery rate and trajectory. Sampling at each site occurred monthly throughout the first year of the study. After the first year, sampling was conducted on a quarterly basis. To measure changes in plot community composition, percent cover of species was estimated either in the field or from plot photos using a point contact grid with 100 points. At each point, we identified organisms to the lowest taxonomic level possible. We accounted for habitat complexity by recording both primary substrate and epibionts (if present); therefore, for a given point, multiple species could be recorded. While *M. californianus* may also have positive effects on infauna, this study focused exclusively on epibionts in order to avoid using destructive sampling methods.

In order to measure the amount of mussel recruitment, (i.e. potential new mussel individuals that might be facilitated by the presence of adult mussel transplants) we placed eight recruitment collectors (Tuffy™ dish scrubbers) (Menge 1992) at each site. These collectors are intended to mimic mussel byssal threads, which are known to attract mussel recruits. Two recruitment collectors were associated with each treatment block, one was placed within a mussel patch and one was placed outside of a mussel patch around bare rock. This placement allowed us to determine if there were differences in potential recruitment within the mussel zone due to the presence of conspecifics. Recruitment collectors were retrieved and replaced in the field each month. Collected Tuffy™ dish scrubbers were processed in the laboratory using a standard rinsing protocol (Menge 1992). The resulting content was strained through a 250 μm sieve and preserved in 95% ethanol. The preserved material was then sorted under a dissecting microscope and all bivalves in the Mytilidae family were identified and counted.

To determine if the recruitment of new individuals contributed to any change in mussel cover we needed to be able to account for the growth of transplanted mussels. To do this, we sized transplanted mussels near the end of the study (February 2014) to estimate growth rate. While mussels were originally notched to allow for direct mussel growth measurements, these were no longer visible in the fourth year of the study. As an alternative, random samples of 20 transplanted mussels in each transplant plot were sized. The size range of the transplanted mussels at that point in time was then compared to the size range of mussels when transplanted to estimate an average growth factor.

Data analysis

Mussel cover

Several factors can contribute to changes in mussel cover in treatment plots including loss/death of transplanted individuals, growth of transplants, and recruitment of new individuals into a plot. Through our data collection we were able to estimate transplant growth and count the remaining transplants in each plot. We then estimated the portion of mussel cover that could be attributed to mussel recruitment using the following equation:

$$\text{\% mussel recruitment} = \text{\% mussel cover} - [\text{\# transplants remaining} * 0.3 * \text{growth factor}]$$

For the purpose of this analysis we only included transplant plots where transplantation was successful and no major predation events occurred (six plots at Pothole and three plots at Occulto). The percent mussel cover value was taken from point contact data collected in the field or from photos. Since 100 mussels at the beginning of the study were approximately equivalent to 30% mussel cover, we multiplied the number of transplants remaining in a plot by 0.3 to estimate percent cover of these mussels. We then multiplied this value by the estimated growth factor for each site (calculated using the mussel size data described above). Any mussel cover that was not accounted for by the loss and growth of the original mussel transplants was assumed to be due to new mussel recruits in the plot.

Community composition

Percent cover data were used to analyze overall community composition in plots over time. In processing the data, raw data points where two species were recorded (i.e. mussel and an epibiont) were counted as two points: one for each species present. For this reason, the total number of data points for a plot could be higher than the number of points sampled (100). We also removed all points of barnacle from the data since barnacles were not counted as epibionts and were therefore not counted consistently between cleared plots and plots with mussels. Using these data, we generated Bray-Curtis similarity values for each pairwise comparison of plot communities over time (PRIMER v.6). We were interested in the similarity of treatment plot communities to control plot communities over time so we selected the Bray-Curtis similarity value for every treatment plot (i.e. transplant plots and cleared plots) to each of the four control plots at a site. This resulted in sixteen estimates (four per replicate) of community similarity for each treatment plot type, which were then averaged for each sampling month. We also selected Bray Curtis similarity values for comparisons of the control plots to each other. This allowed us to define the recovery threshold as a point in time when the mean similarity of the treatment plots to the control plots was equal to the similarity of the control plots to each other (Kinnetics Laboratories 1992, Conway-Cranos 2012). The similarity values of treatment plots to control plots were then plotted over time for each block at each site.

We repeated the same analysis but removed all points of mussel from the percent cover data, leaving only points of invertebrates or algae settled on mussel or rock substratum. This allowed us to remove any bias in our data due to inherent experimental effects (i.e., presence or absence of transplants) and to look directly at the effect of mussel presence on the rest of the plot community.

Results

Person-hours for transplant effort:

We tracked hours spent on planning, collection, setup and sampling for both sites and have estimated a total of 162 person-hours/site are necessary for a mussel transplant project of this scale (Table 1). An additional 10 person-hours are needed each time a site is sampled. We believe these estimates provide a reliable starting point for estimating time and cost for future mussel transplant efforts.

Transplant success:

As expected, some mussel transplants were not successful. At Pothole, an average of 60% of original transplants remained after one year (Fig. 4). The only exception was one block where both transplant plots lost nearly all mussel transplants due to a predation event (*Pisaster ochraceus* observed feeding in plots). This appeared to be representative of natural dynamics at the site as a similar event occurred at a later date in a control plot.

The success of transplants at Occulto was considerably more variable (Fig. 4). Five of the eight transplant plots lost at least 50% of original transplants after one year. However, one replicate block of plots retained greater than 70% of transplants during the same time period. Heavy losses of transplants were likely due to site characteristics including increased wave exposure and conglomerate rock type, which is easily broken and sheared off.

Mussel cover:

An examination of mussel cover in plots over time shows that cleared plots gained little to no mussel recruitment at either site during the study period (Figs. 5, 6). This is consistent with past studies of mussel recovery (Conway-Cranos 2010, 2012, Kinnetics Laboratories 1992).

Sharp declines in mussel cover in both block C transplant plots and later in the block B control plot at Pothole where due to the previously mentioned predation events by the seastar *Pisaster ochraceus* (Fig. 5). At Occulto, mussel transplants failed in the majority of transplant plots (blocks B, C and transplant 1 in block D) (Fig. 6). For this reason, trends in mussel cover are clearer at Occulto when plots are separated by success and failure of transplants rather than transplant treatment type (Fig. 7).

Mussel cover in successful transplant plots without predation at both sites decreased initially and then leveled off or increased later in the study period. At both sites, ~2.7% of mussel cover was attributable to mussel recruitment at the end of the study period. A similar increase did not occur in cleared plots.

Recruitment:
We processed and counted mussel recruits for eight of the sample months that were collected for a total of 122 Tuffy™ samples. The recruitment data from Tuffy™ dish scrubbers suggest mussel recruitment levels have been relatively low at both sites during the study period (but typical for this region) with an average of 64 recruits/Tuffy™/month (Fig. 8). However, recruit counts were quite variable and ranged from 2 - 855 recruits/Tuffy™/month. There was no difference in average recruit counts between replicate blocks at either site. There was a significant site x treatment interaction ($F = 8.001$, $df = 115$, $P = 0.006$) such that treatment (i.e. Tuffy™ placement inside or outside the mussel bed) had no effect on mussel recruitment at Pothole but was higher for Tuffy™ dish scrubbers placed within the mussel bed compared to dish scrubbers outside the mussel bed at Occulto (Fig. 9). The difference in average recruit count for the two treatments was largest during late fall and winter months when recruitment pulses are common (Fig. 10).

Community recovery:
Mussel transplants at both Pothole and Occulto increased the similarity of transplant plots to control plots over time relative to cleared plots (Figs. 10, 11). This appears to be driven solely by increased mussel cover in transplant plots. Contrary to our hypothesis, mussel transplants do not appear to increase the recovery of the associated algal and invertebrate community at either site (Figs. 12, 13). Regardless of whether transplants were present, the associated algal and invertebrate community recovered almost immediately at both Occulto (Fig. 13) and Pothole (Fig.12). It should be noted the temporal and spatial variability in this community sets a very low recovery threshold. Also, this community does not include infauna, which would have required destroying mussel bed to accurately sample. However, previous studies have shown that infauna is most abundant and diverse within mussel beds (Borthagaray and Carranza 2007).

Spatial configuration
There was no significant difference between the two transplant treatments with respect to community composition (ANOSIM $p=0.999$). This suggests that the spatial configuration of mussels has no effect on the capability of transplants to speed up recovery. However, spatial configuration may still be an important attribute with respect to infauna, which may benefit more from patchiness than epibionts.

Mussel Recruit Association Study:

Methods:

We conducted surveys of mussel recruit associations along transects within the mussel zone at four rocky intertidal sites in Santa Cruz County, CA. These sites (Terrace Point, Davenport Landing, Waddell Creek, and Greyhound Rock) were selected based on their proximity to UCSC's Long Marine Lab and because historical recruitment data for these sites indicated numerous recruit observations were likely. At each site we sampled along 30 meter transects and recorded two random observations of mussel recruits and their settlement substrate within a 50 x 50 cm quadrat every meter on either side of the transect tape. Recruit observations were made by randomly dropping an object into the plot and searching outward from that object in a circular fashion until the first mussel recruit was encountered. This approach reduced detection bias (e.g., recording most visible recruit present) and ensured observations were independent. For the purposes of this survey, we recorded the substrate to which mussel recruits were directly attached via byssal threads and any overlying canopy (usually algae), as both constituted an association. Therefore, a given recruit observation could be a single substrate or a combination of the primary substrate and the overlying canopy. After recruit observations were made, percent cover of available substrate was estimated for the transect area using uniform point contact methods.

Data Analysis:

For each substrate present at a site, we calculated (1) the number of times the substrate was observed in association with a mussel recruit and (2) the number of times it was found present along each transect. We then used a chi-square test to determine if the number of times mussel recruits were observed in association with a substrate was significantly different from what would be expected based on natural percent cover of that substrate. We then calculated a standardized percent difference for each substrate using the following equation: $\frac{R-A}{R+A}$, where R= % recruits found with substrate X and A= % available substrate composed of substrate X. The standardized percent difference value allowed us to determine the direction of recruit-substrate associations (positive or negative).

Results:

At all four sites, settlement substrate of mussel recruits was significantly different from what one would expect based on available substrate cover ($p < 0.002$). This indicates that mussel recruits exhibit strong positive and negative associations with available substrate. Standardized percent difference values identify several positive associations with biological substrata. At all four sites, mussel recruits had strong positive associations (> 0.5 standardized percent difference values) with the fucoid algal canopies (including species *Pelvetiopsis limitata*, *Silvetia compressa*, and *Fucus gardneri*). Strong positive associations were also seen between mussel recruits and articulated coralline algae and with acorn barnacle tests when in combination with fleshy algal canopies (Fig. 14). Interestingly, mussel recruits showed a slightly negative association with acorn barnacles when an algal canopy was not present. Recruits also had strong negative associations with bare rock and non-coralline crust (Fig. 14).

Fucoid Restoration Study:

As shown above, fucoids may aid in mussel recruitment and mussels are important habitat forming species. Fucoids have also been shown to decline following oil spills. Thus, two pilot techniques were carried out to test the success of restoring fucoids (with the additional goal of avoiding depletion of adult source populations via transplantation). The first method involved collecting reproductive tips of fucoid individuals (which does not harm the individuals) and making these tips release gametes, form zygotes, and settle on small pebbles in the lab for later out-planting (Fig. 15). The second technique required collecting more reproductive tips, putting these in small mesh baggies, anchoring these in the intertidal zone for a short period of time (Fig. 16), and monitoring subsequent fucoid settlement and growth in the surrounding vicinity.

Both pilot techniques were carried out close to the University of California, Santa Cruz using *Fucus gardneri*. *Fucus* recruits were settled similar to Pollock (1970) by collecting tips in the field, returning them to the lab, and placing them over rocks in a dark refrigerator with a covering of paper towels that were moistened with seawater. After approximately 24 hours in the refrigerator, *Fucus* tips were dipped in fresh water and then immersed in seawater over the top of these same rocks. This technique repeatedly and successfully released *Fucus* gametes which then formed zygotes and settled on the rocks below. On average, five *Fucus* tips gave rise to 17,544.4 ± 2,750.7 (SE) recruits. However, literature reports that out of ~5,395 zygotes, only 1-2 survive in the wild (Wright et al., 2004).

It was originally intended for *Fucus* recruits to remain in the lab until small macroscopic thalli formed. After 4 months, recruits were still microscopic. Thus, recruits were out-planted (despite their small size) after being in the lab for 1 month, 2 months, and 3 months. The 3 month and 1 month cohorts had 0% survival after being in the field for less than 1 week. The 2 month cohort showed 20% survival after 2 weeks, 10% after 6 weeks, and 0% after 10 weeks. Initial survival exhibited by the 2 month cohort may have been due to localized site conditions rather than time spent in the lab.

Several outplanting technique treatments were tested in the field; some recruits were placed in the open, some were under nearby algal canopies, and some (both under canopies and in the open) were encircled with copper paint to deter herbivores. The copper paint did not seem to have an effect on recruit survival, however, recruits under algal canopies showed greater survival than recruits in the open. This signifies that desiccation and high light levels have adverse effects on *Fucus* recruit survival. This is congruent with findings from van Tamelen et al. (1997). Concomitantly, *Fucus* recruits on porous pebbles seemed to exhibit greater survival; this could be attributed to more favorable conditions inside micro-habitats created by pits on the surface of the rocks (such as higher moisture retention). It is hypothesized that the overall low survival exhibited by out-planted recruits could have been a manifestation of the unrealistic/protected conditions present in the lab prior to out-planting. Transplantation to rougher surroundings may explain the high mortality observed in *Fucus* recruits accustomed to more placid surroundings.

Consequently, recruits were later settled on pebbles in outdoor tanks to facilitate acclimatization to ambient light levels. Low tides were also simulated so that recruits could become accustomed to exposure and desiccation; however, variable water motion was not accounted for. These outdoor recruits were out-planted at much shorter time intervals (1 day, 1 week, and 2 weeks) so they would be immersed in natural conditions at an early age and hopefully adjust to this environment.

Recruits out-planted at 1 day and 1 week exhibited 0% survival after 4 weeks. Recruits out-planted after 2 weeks showed 20% survival after 5 weeks in the field. It is hypothesized that the 1 day old recruits were too young to show successful survival in the wild. The 1 week old recruits were hypothesized to have shown greater survival but experienced an extreme desiccation event directly before out-planting that likely contributed to their mortality.

It has been difficult to assess the success of mesh baggie experiments in the field because many other organisms settle and grow in the direct vicinity of where baggies are deployed. Thus, it is not recommended that this technique be utilized. It takes at least 4 hours for recruits to attach to the substratum (Brawley and Johnson, 1991; Brawley et al. 1999) and zygotes in the wild may be swept away by the incoming tide before having a chance to settle. This baggie technique has also been attempted using reproductive tips of *Silvetia* which did not produce positive results (pers. comm., Stephen Whitaker).

Conclusions:

We were able to achieve all three goals of this project and gained valuable knowledge that will help guide future rocky intertidal restoration work. In our mussel transplant study, transplants had variable success. Both high wave exposure and the conglomerate rock type at Occulto decreased the success of transplants. We recommend avoiding areas with these characteristics for future mussel transplant projects. When this is not possible, leaving vexar mesh attached longer to allow mussel transplants a longer period to firmly attach and/or using alternative strategies for attaching vexar that avoid drilling multiple holes in the surrounding rock (which may make rock more prone to shearing off), may help increase transplant success.

Despite some transplant failure, mussel transplants that survived sped up recovery of mussel bed communities. It appears that this was accomplished solely by increasing mussel cover through the presence of mussel transplants. It is difficult to assess the degree to which mussel transplants facilitated mussel recruitment because recruitment levels in this area were so low during the study period. We did detect a small increase in mussel cover that we attributed to mussel recruitment in transplant plots that we did not see in cleared plots. However, a more significant recruitment event is necessary to determine if mussel transplants speed up recovery by facilitating mussel recruits. Even though recruitment in the study area was low, we were able to detect some evidence that the presence of adult mussels facilitates recruitment in our mussel recruit samples. Tuffy dish scrubbers at Occulto placed within mussel beds had higher recruitment than dish scrubbers placed outside mussel beds. This effect was most noticeable during recruitment pulse in the late fall and winter. A similar effect was not observed at Pothole; however, this may be due to the sites lower recruitment rates and suggest that there may be a recruitment threshold that must be reached in order to observe an effect.

Although not measured, we also assume that infaunal species were more abundant in mussel transplant plots than cleared plots (Borthagaray and Carranza 2007). The presence of mussel transplants did not speed up the recovery of epifauna, which was almost immediately recovered in both transplant and cleared plots. This is partly due to the temporal and spatial variability of this community, which set a very low recovery threshold.

Overall, the results from the mussel transplant study emphasize the importance of mussels to the recovery of some portions of the mussel bed community and suggest that restoration efforts may benefit from using mussels as target species. However, because transplanting mussels is time and cost intensive, we do not recommend this as a viable restoration approach. These costs would likely outweigh any benefits this strategy might produce, especially on a scale appropriate for a restoration effort. We believe a more beneficial approach would be to develop strategies to facilitate mussel recruitment in ways that do not require adult mussel transplants.

The mussel recruit study conducted in Santa Cruz County demonstrated positive associations do occur between mussels and biological substrata. Many of the positive and negative associations identified in these surveys were unexpected. We did not think mussel recruits would positively associate with fucoid algae because the large fronds of these algae were thought to dislodge mussel recruits via whiplash. However, mussel recruits exhibited one of the strongest positive associations with the fucoid algal canopies. While the current scientific consensus is that mussel recruits require byssal-like substrate to facilitate recruit growth and survival, these results suggest that the presence of a robust algal canopy is more important. We will repeat this study at Vandenberg Air Force base sites to determine if similar patterns are observed. These positive species associations might be utilized in future restoration strategies by using living specimens or materials that mimic these substrates to facilitate mussel bed recovery as an alternative to transplanting mussels.

The fucoid restoration techniques that were tested were largely unsuccessful. The most success was achieved when algae were propagated in outdoor tanks prior to outplanting. This is most likely because propagules in indoor tanks experience a more drastic change in environmental conditions when outplanted compared to propagules in outdoor tanks. We believe outplanting is a preferable technique to using mesh seed-bags to propogate algae in the field. Outplanting allows algae to settle onto substrate in the lab where settlement time can be guaranteed, along with little to no water motion, which has been shown to increase zygote attachment (Pearson and Brawley, 1996).

We planned to use the techniques tested in our pilot study at the two Torch sites on Vandenberg Air Force Base using *Silvetia compressa.* This fucoid is more common in the Vandenberg region than the more robust species of rockweed, *Fucus gardneri,* used in the pilot study. However, because of the very low survival rates exhibited by *Fucus* in the pilot study, this technique will not likely be any more successful with *Silvetia*. Therefore, it is also not recommended that this method be implemented as it would likely not result in high levels of fucoid restoration, nor large amounts of fucoid individuals with which to facilitate mussel settlement and recovery.

Future Studies and recommendations with respect to restoration of mussel communities following a disturbance

1) *Finding*: Transplantation of mussels as a method to restore mussel communities was successful but labor intensive.
    a. Importantly, this approach to restoration, in part, relies on a donor population. The key to such populations is that they be sufficiently local, large and dense to be able to accommodate the scale of removal necessary for transplantation. Such intervention may not be possible: (1) The disturbance may be wide spread, thereby reducing the potential for extant donor populations. (2) The spatial genetic structure of the species may contraindicate transplantation over the necessary spatial scales. (3) Local policy may prelude removal and transplantation.
    b. The utility of this approach is likely to be linked to local recruitment intensity of mussels. While transplantation works, we cannot recommend it as a single strategy for restoration. When transplantation also leads to focused recruitment of larval mussels to the transplant sites (larvae induced to settle by the presence of adult mussels) intervention by transplantation can be successful. The degree of success will be a function of recruitment intensity. The targeted area near Point Conception is an area of remarkably low mussel recruitment and there was very little replenishment via recruitment throughout the experimental period.

   Recommendation:
    a. The transplantation approach should be carried out in areas from high to low (control areas) mussel recruitment to assess the efficacy of the approach. For example based on our earlier work, Mendocino, Santa Cruz and Santa Barbara counties represent high, intermediate and low recruitment areas. We predict that restoration potential of mussel transplantation would vary with these levels of recruitment.
    b. One alternative approach, would be to culture juvenile mussels in lab for use as transplants. Small scale versions of such culturing have been done for consumable mussels (e.g. *Myilus edulis*), and this is the method of choice for terrestrial and some estuarine restorations. We propose to test the idea that the cost-effective culturing to a transplantable size (2-3 cm) can be done in standard seawater systems at (for example) UC Santa Cruz.

2) *Finding*: Mussels disproportionately use fucoid algae as a settlement substrate
   a. We found that recruitment of new individuals of mussels was positively associate with fucoid (and other species) algae. This could lead to an intervention approach that does not require a donor mussel population. Because of the efforts described above we did not envision an approach that required a donor patch for fucoids. Instead we utilized the unusual life history of fucoid algae in the development of a restoration approach for mussels. Fucoids, unlike mussels, have extraordinarily low dispersal of progeny – usually in the meters rather than kilometers scale. Hence, local replenishment of Fucoid populations can be facilitated directly by reproduction by local adults. Our strategy was to enhance local populations of fucoids on a small scale (with very minor use of donor individuals) and let those resultant populations subsequently self –replenish. The result would then act to enhance recruitment of mussels. To that end we used two approaches:
      i. Transplantation of reproductive portions of male and female stipes, to facilitate local recruitment. This required no removal of individuals from donor patches (only non-lethal movement of reproductive tissue)
      ii. Culturing of juvenile fucoids onto cobbles in the lab, with subsequent transplantation to the field.

Recommendation:

a. Subsequent work has indicated that transplantation of cobbles with adult fucoids may act to facilitate replenishment of local fucoid populations. We are currently running a large experiment in San Francisco Bay to determine optimal transplantation design. While this experiment will allow us to refine our design with respect to fucoid replenishment, there is essentially no *Mytilus californianus* in SF bay so we will not be able to assess the ancillary benefit to mussels. Given that mussel recovery may take decades, the two step process of fucoid/mussel recovery may be of great restoration benefit and we propose an experiment similar to the one in SF Bay on the outer coast of Santa Cruz/San Mateo counties, which is described as an area of intermediate mussel recruitment.

Expense allocations

Phase 1 was for the initial mussel transplantation experimental work. Phase 2 was the fucoid/mussel assessment and field trials. The values below are from the last accounting (November 2014), which was prior to all costs being debited.

BUDGET CATEGORY	ALLOCATED BUDGET (IN FIS)	ALLOCATION ADJUSTMENT	TOTAL BUDGET	EXPENDITURES THROUGH 11/16/14	OBLIGATIONS KNOWN AS OF 11/17/14	Phase 1	Phase 2	TOTAL EXPENDITURES & OBLIGATIONS
SALARY & WAGES	108,708.00	0.00	108,708.00	104,198.85	0.00	55,294.10	48,904.75	104,198.85
FRINGE BENEFITS	16,349.00	0.00	16,349.00	31,160.77	0.00	27,473.77	3,687.00	31,160.77
SUPPLIES	8,000.00	0.00	8,000.00	7,280.08	0.00	3,640.04	3,640.04	7,280.08
EQUIPMENT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DOMESTIC TRAVEL	24,000.00	0.00	24,000.00	3,919.48	0.00	2,123.05	1,796.43	3,919.48
FOREIGN TRAVEL	0.00	0.00	0.00	0.00	0.00			0.00
FEE OFFSET & GSHIP	24,170.00	0.00	24,170.00	30,025.04	0.00	0.00	30,025.04	30,025.04
Graduate student								
SUBCONTRACTS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PARTICIPANT SUPPOR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
INDIRECT COST ADJ.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DIRECT COSTS	181,227.00	0.00	181,227.00	176,584.22	0.00	88,530.97	88,053.25	176,584.22
REV. INDIRECT COST ADJ.		0.00	0.00					
INDIRECT COSTS	31,714.00	0.00	31,714.00	30,902.24	0.00	15,492.92	15,409.32	30,902.24
TOTA L AWARD	212,941.00	0.00	212,941.00	207,486.46	0.00			207,486.46

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Table 1: Number of person-hours required for mussel transplant effort for one study site. Eight hundred adult mussels were transplanted per site, an amount that covered ~0.6 m^2

Activity	Hours	Persons	Person-Hours
Planning/Site scouting	27	1	27
Building	12	1	12
Mussel collection/notching	16	3	48
Transplanting/Site setup	11	5	55
Doming vexar	5	2	10
Removing vexar	5	2	10
Sampling site	5	2	10
* Drive time to sites not included			

Figure 1: Mussel transplants secured with vexar mesh.

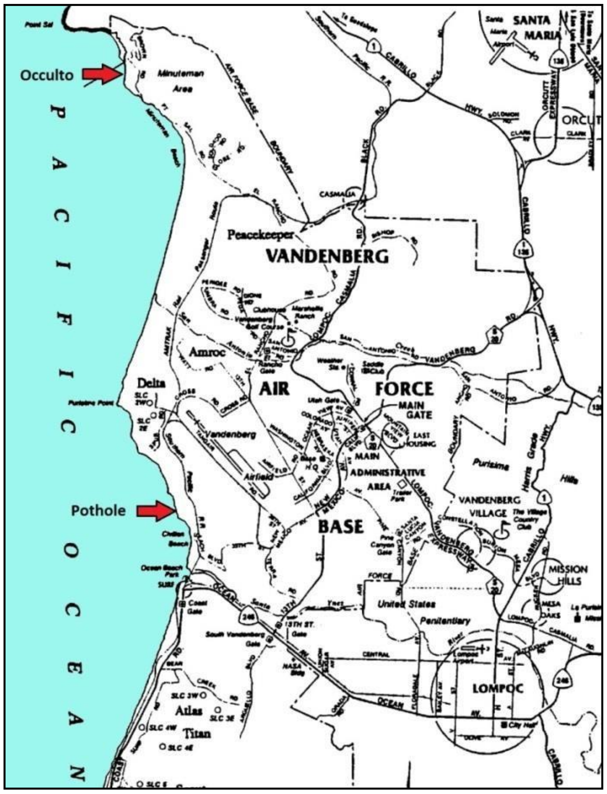

Figure 2: Site locations along Vandenberg Air Force Base coastline

Figure 3: Transplant treatment types: transplant 1 with 100 mussels in one patch (left) and transplant 3 with 100 mussels in three patches (right).

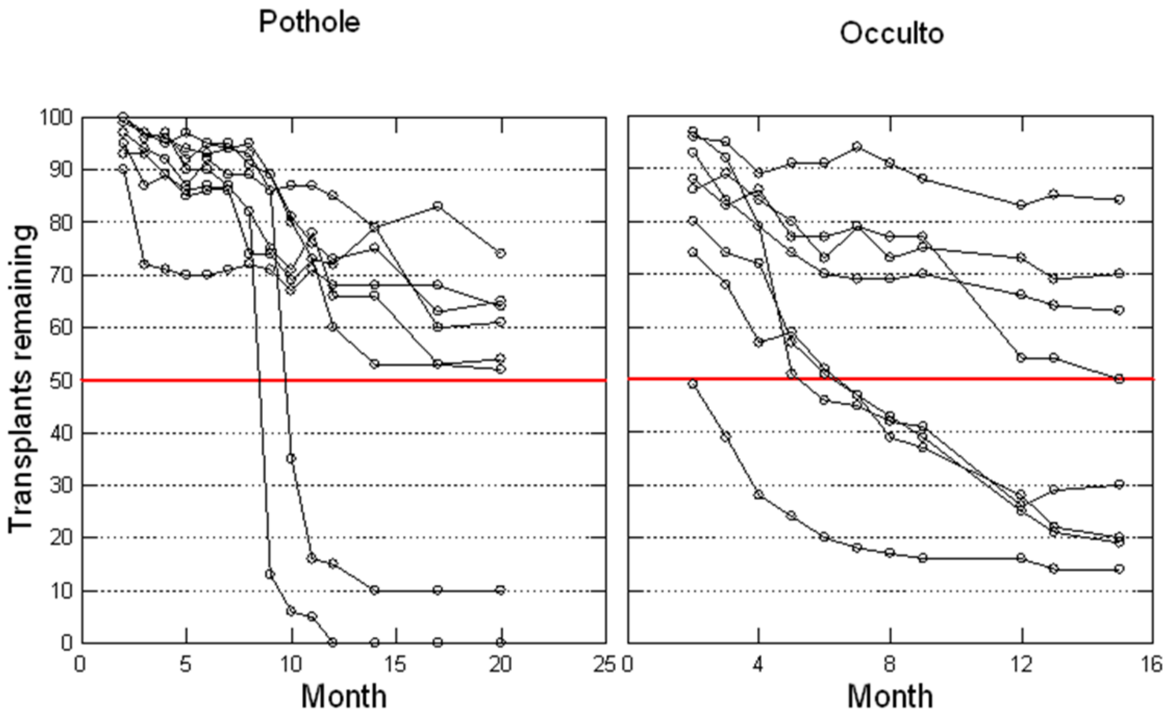

Figure 4: Number of mussel transplants remaining out of the original 100 over time at Pothole (left) and Occulto (right).

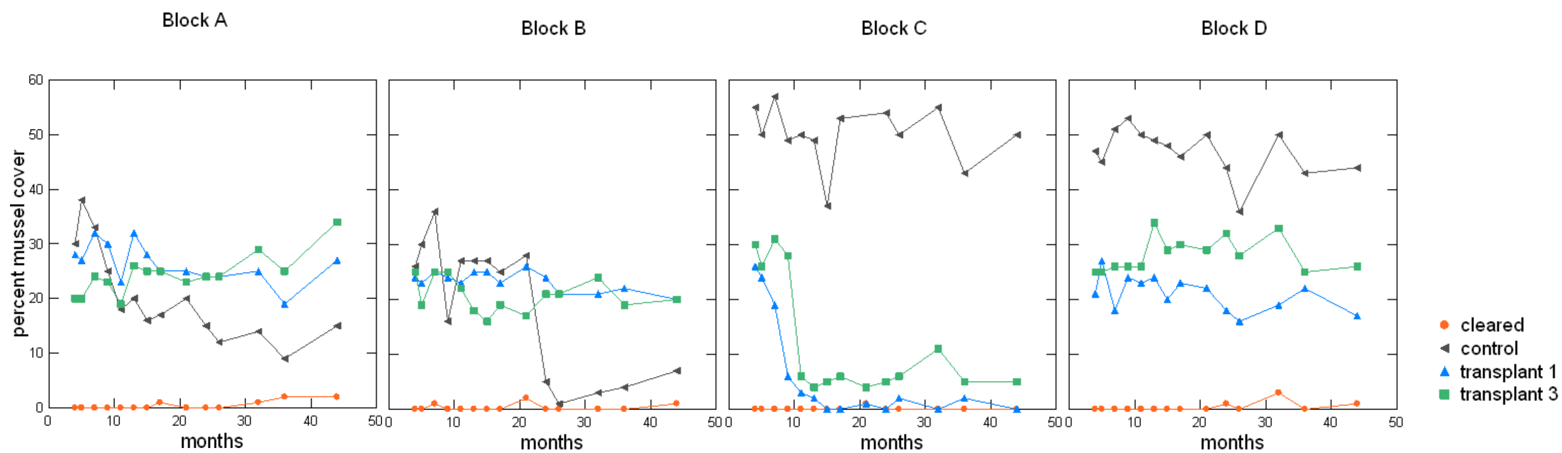

Figure 5: Percent mussel cover at Pothole for each treatment plot and control plot over the study period.

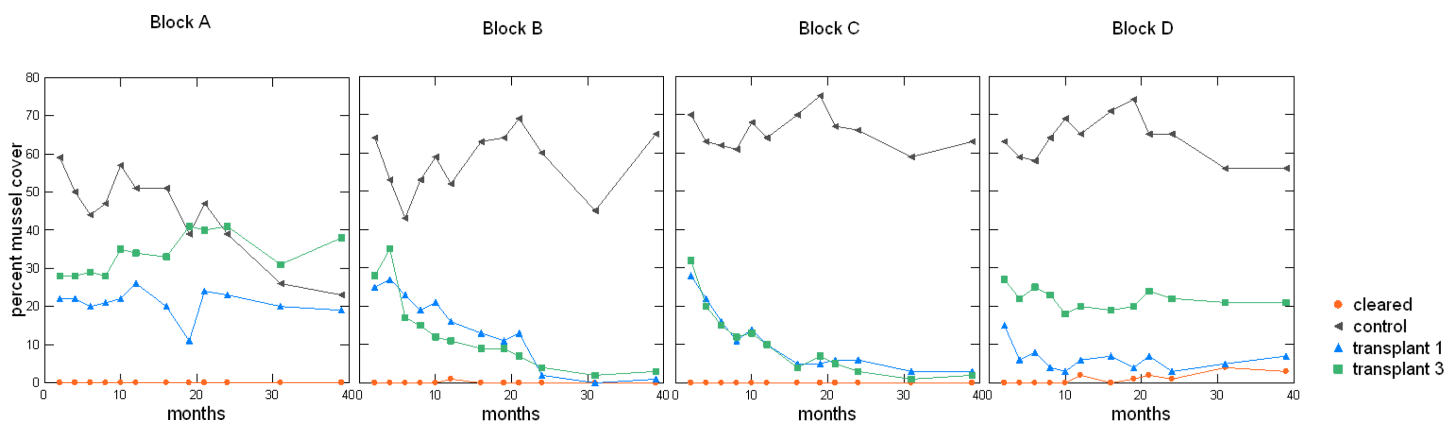

Figure 6: Percent mussel cover at Occulto for each treatment plot and control plot over the study period.

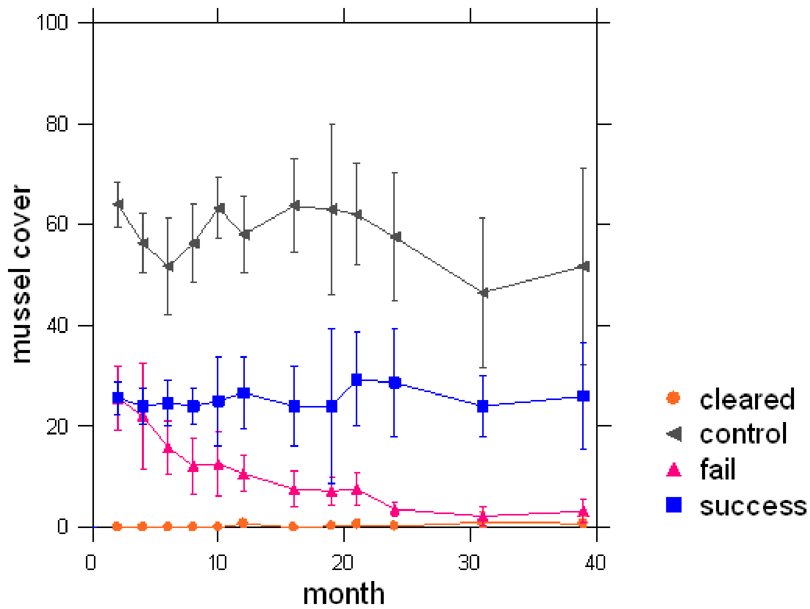

Figure 7: Mussel cover (%) at Occulto in cleared plots, control plots, transplant plots that failed, and transplant plots with successful transplants.

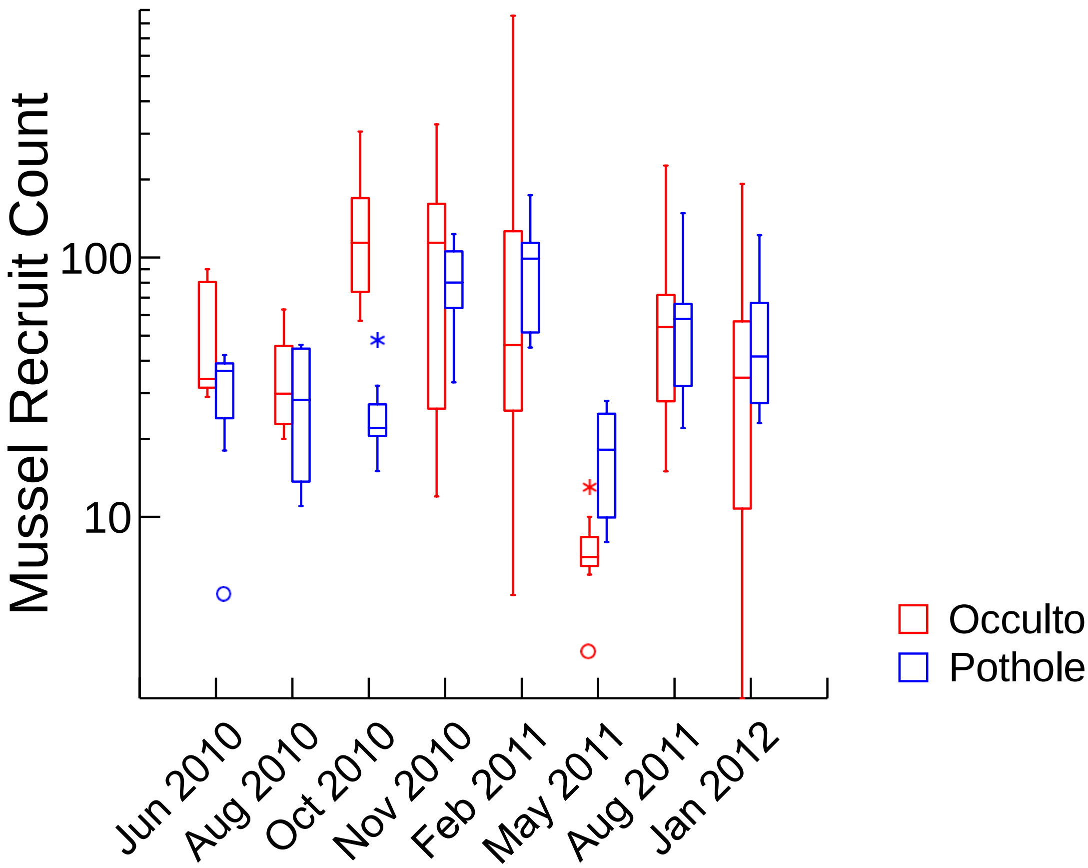

Figure 8: Box plots of mussel recruit abundance for monthly Tuffy™ samples (eight per month)) at Pothole and Occulto**.**

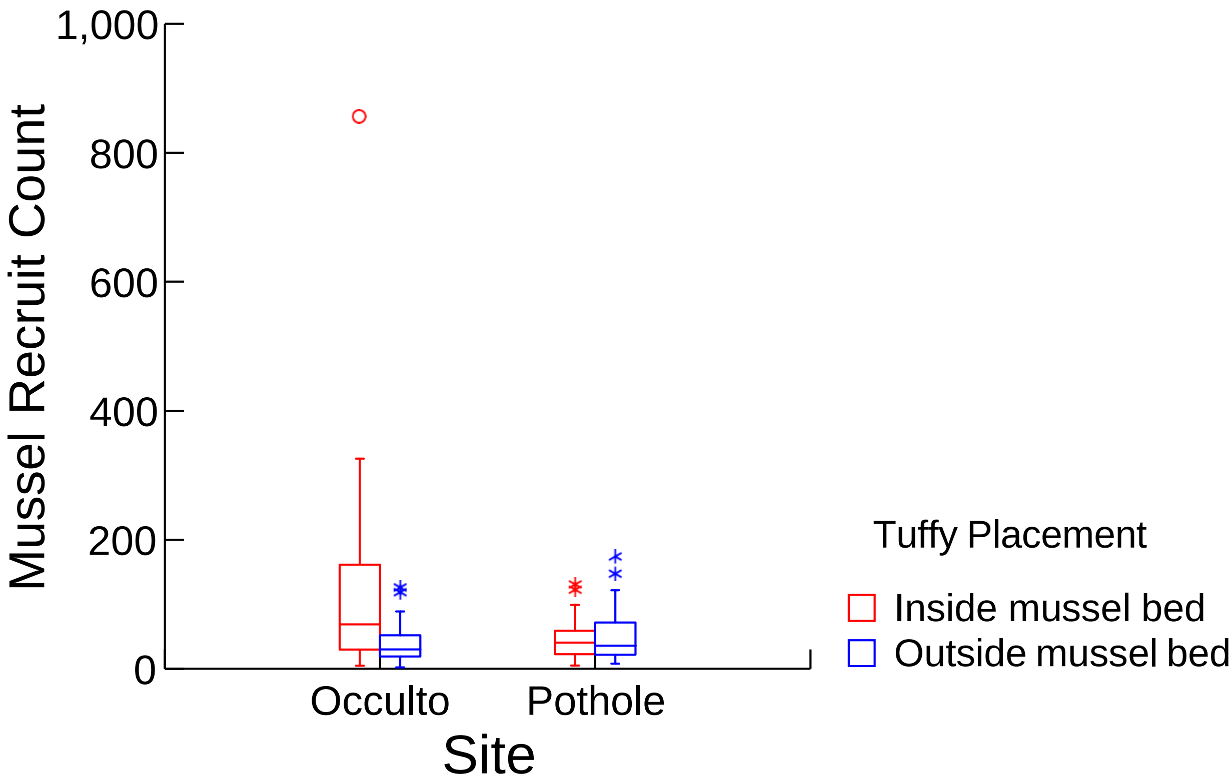

Figure 9: Comparison of mussel recruit count for the two Tuffy™ sample treatments (placement within or outside mussel bed) at Occulto and Pothole.

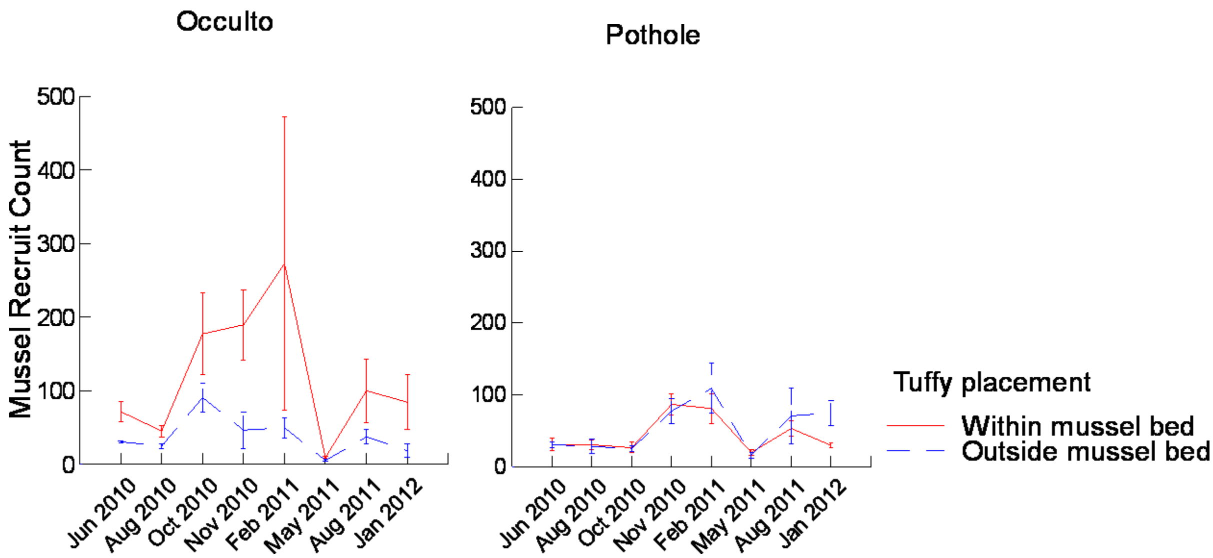

Figure 10: Average mussel recruit counts over time grouped by treatment (i.e. Tuffy™ placement) at Occulto and Pothole

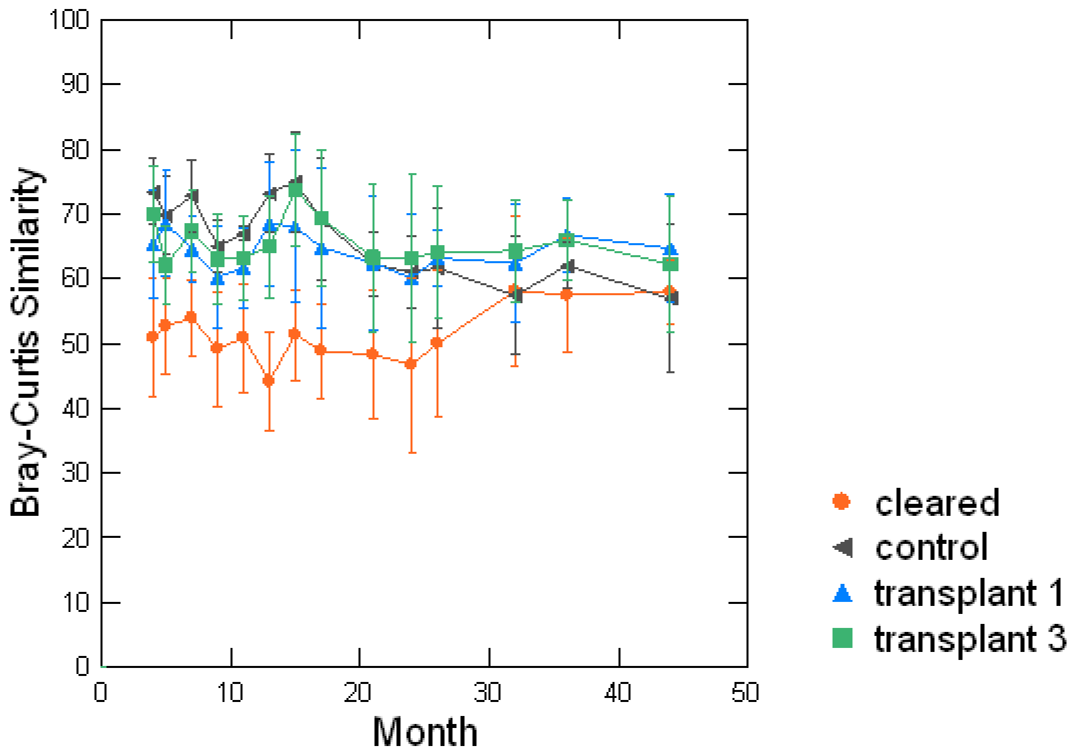

Figure 10: Mean (+/- SD) Bray-Curtis similarity for cleared and transplant plots to each control plot and of the control plots to each other at Pothole. Data include all points of mussel and but exclude plots with major predation events.

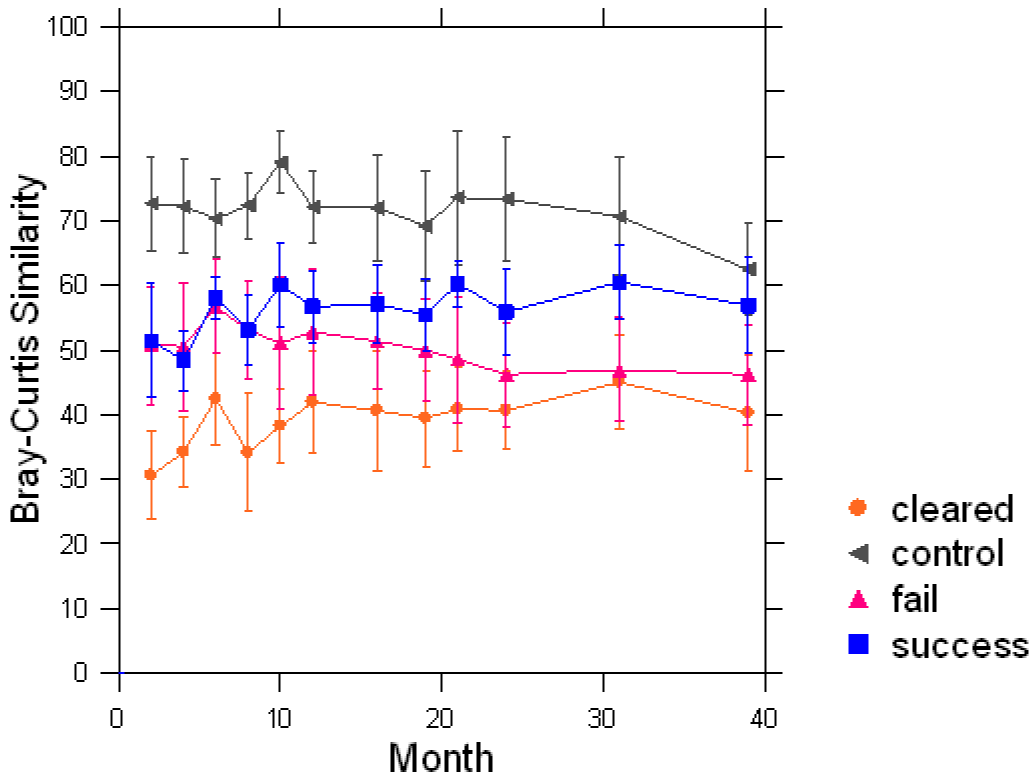

Figure 11: Mean (+/- SD) Bray-Curtis similarity for cleared plots, successful transplant plots, and failed transplant plots to each control plot and of the control plots to each other at Occulto. Data include all points of mussel.

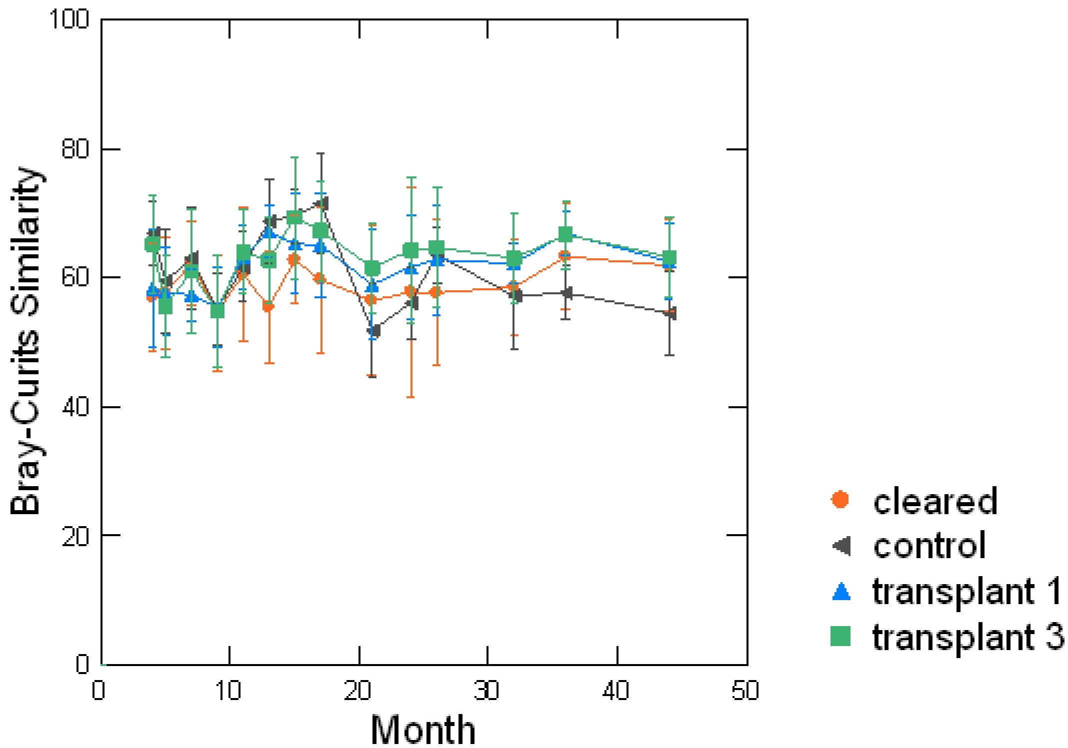

Figure 12: Mean (+/- SD) Bray-Curtis similarity for cleared and transplant plots to each control plot and of the control plots to each other at Pothole. All points of mussel have been removed from the data, therefore, represent only the invertebrate and algal community remaining. These data also exclude plots with major predation events.

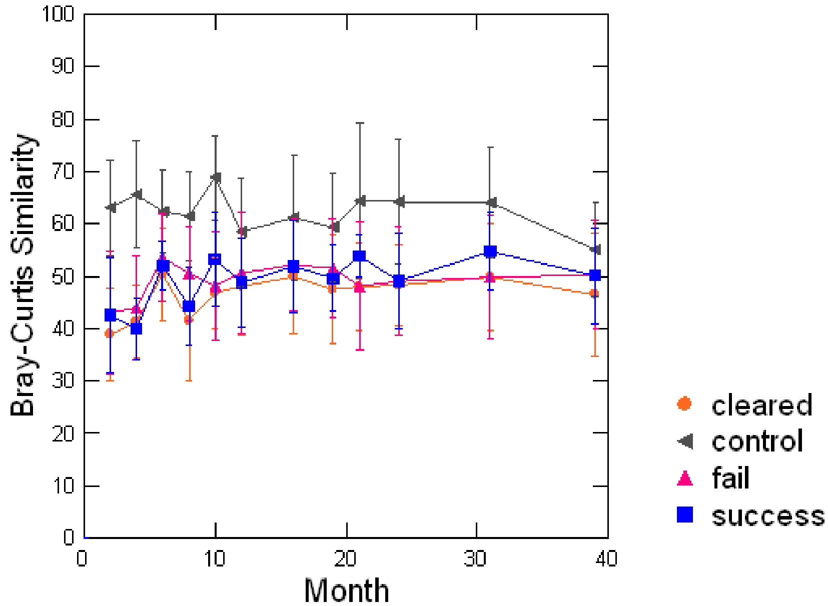

Figure 13: Mean (+/- SD) Bray-Curtis similarity for cleared plots, successful transplant plots, and failed transplant plots to each control plot and of the control plots to each other at Occulto. All points of mussel have been removed from the data, therefore, represent only the invertebrate and algal community remaining.

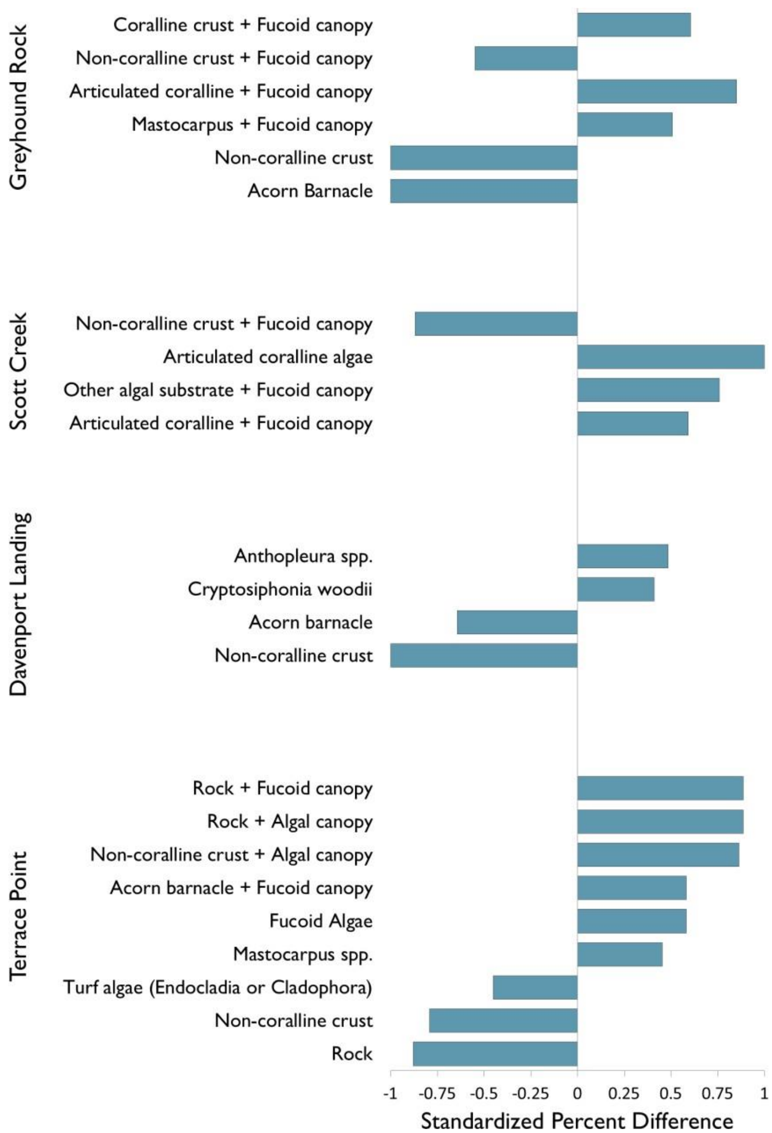

Figure 14: Specific settlement substrates at all four study sites with which mussel recruits had strong positive (> 0.5 standarized percent difference) and strong negative associations (< -0.5 standardized percent difference).

Figure 15: Rock pebble with settled *Fucus* zygotes outplanted in the intertidal.

Figure 16: Mesh seed-bag filled with fertile *Fucus* frond tips, anchored to the intertidal.