RESTORATION OF NATIVE OYSTERS, *OSTREA LURIDA*, IN ALAMITOS BAY, CA

FINAL REPORT

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INTRODUCTION

We implemented a native Olympia oyster, *Ostrea lurida*, restoration and educational outreach effort at the Jack Dunster Marine Reserve (33°45'43.98"N, 118° 7'10.74"W, **Figure 1**) in Alamitos Bay, CA. More than a century ago, this bay supported oyster beds (Gilbert 1889, Bonnot 1935) purported to be 0.25 miles long at one location in the bay, with scatterings of oysters reported elsewhere (Gilbert, 1889).

Alamitos Bay is located within the City of Long Beach, CA and was historically part of the greater Los Cerritos Wetlands. The Los Cerritos Wetlands once encompassed 2400 acres of habitat, but the wetlands have been significantly altered since the land was developed in the late 19th century. The wetland habitats remaining in Alamitos Bay include the 13-acre Colorado Lagoon and the 44-acre Los Cerritos Wetlands, as well as the mitigated wetland of Jack Dunster Marine Reserve on 2.4 acres of land. These small parcels of open space provide necessary refuge for wildlife and native species, and also represent a critical opportunity to engage an otherwise urban populace with the natural history of their neighborhood.

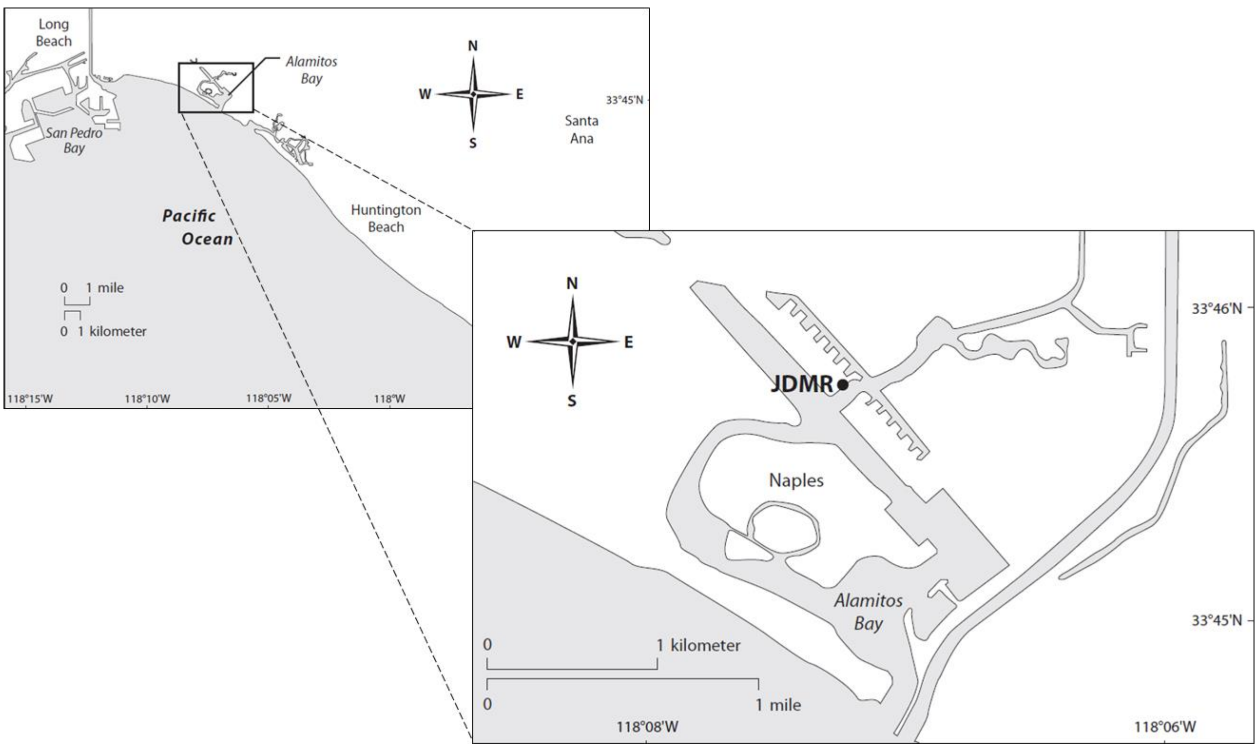

Figure 1. Restoration study site located on an intertidal mudflat at Jack Dunster Marine Reserve (JDMR) in Alamitos Bay, Long Beach, CA.

One particular habitat heavly impacted by development is native Olympia oyster beds. The Olympia oyster is the only native oyster species on the West Coast of the United States. Within the last century it was a widely distributed habitat-forming species in bays and estuaries (Bonnot, 1935; Baker, 1995), including in California, and was exploited as a food resource by California native Americans. In the early 1900s, some combination of over-harvesting (Kirby, 2004), pollution (Hopkins, 1935), and habitat loss/degradation (Dahl et al., 1991; Lotze et al., 2006) led to significant declines throughout this species' range. Oyster beds are now absent in California estuaries, though remnant low-density populations exist (Polson & Zacherl, 2009).

Oysters have been long recognized as an especially important component of a healthy and resilient estuarine ecosystem because of the myriad ecosystem benefits they provide. This includes providing structured habitat in the form of both refuge and hard substrate for a whole community of organisms, stabilizing sediments, and improving water quality and/or clarity, which can facilitate seagrass bed recovery.

Until recently (Polson and Zacherl 2009, Polson et al. 2009), the Olympia oyster has been relatively understudied in Southern California, despite ongoing restoration efforts in Washington, Oregon and in Northern California. Preliminary field surveys of Alamitos Bay in spring and summer 2010 revealed that native oysters were present, however, there were no natural intertidal "beds" of oysters anywhere in Alamitos Bay. Non-native Japanese oysters, *Crassostrea gigas,* were also present throughout Alamitos Bay and other southern California bays. Zacherl's research in nearby Newport Bay indicates that the native oyster is actively reproducing and recruiting to local populations during June - September, with maximal settlement typically in August and in especially high densities (as high as 3,000 to 8,000/m2, Seale and Zacherl, 2009, and unpublished data from 2006-2011). Non-native oysters experience more sporadic recruitment, and at barely detectable densities in Newport and Alamitos Bays, though their recruitment was easily detectable in more recent studies performed in San Diego Bay (Zacherl and Henderson, unpublished data, 2013-2104). A qualitative survey of the size frequency distribution of native oysters in Alamitos Bay provided evidence that they, too, are regularly recruiting. However, suitable settlement habitat in the form of an oyster bed was lacking.

Augmentation of habitat appeared to be the most logical first restoration step since spat were present as local supply but suitable settlement habitat was limiting. The hypothesis that augmentation of habitat will increase local oyster density is supported by findings by Zacherl (unpublished data) from a pilot restoration project completed from 2010-2012 in nearby Newport Bay, CA. Thus, we undertook an oyster restoration project at JDMR in June 2012 by augmenting a stretch of mudflat with oyster shell.

This restoration project, while modest in size of actual habitat restored, offered more than acreage. We undertook a community and educational outreach program, alongside the restoration activity, that was expected to act as a catalyst for further restoration efforts throughout southern California.

PROJECT OBJECTIVES

The broad **objectives** for the project were:

1. Restore an Olympia oyster bed to Alamitos Bay that supports native Olympia oysters at higher density than on adjacent mudflat or reference locations throughout Alamtios bay.
2. Understand how a constructed oyster bed changes community diversity and how it affects adjacent eelgrass communities.
3. Understand how the integrity of a constructed oyster bed changes over time and maintain % shell cover above 70% for the two-year study duration.
4. Increase public awareness about the ecology of filter feeding bivalves.
5. Engage the public in restoration activity.

PERMITTING AND CONTINGENCY PROVISIONS

In order to construct an oyster bed, there were multiple **permits** and **certifications** required, applied for, and obtained:

1. California Coastal Commission Coastal Development Permit Waiver # E-11-006-W
2. Army Corps of Engineers Nationwide Permit No. 27 Aquatic Habitat Restoration, Establishment, and Enhancement Activities, file # SPL-2011-00381-JWM
3. State Water Board General Certification – Notification to Proceed
4. Right of Entry Permit from City of Long Beach to enter and work at Jack Dunster Marine Reserve
5. Certificate of Insurance Coverage – Alliant Insurance Services, Inc. for covered party CSU Fullerton

To complete the permitting process with the California Coastal Commission, we were required to include several **contingency provisions**. We proposed to evaluate the outcomes of our study (this report) 2 years after installation of shell habitat. Project outcomes (this report) will be shared with the following permitting agencies: California Regional Water Board, Army Corps of Engineers, Coastal Commission, and National Marine Fisheries Service. If, in consultation with the agencies named above, it is determined that the outcome of the study is contrary to the intent of the project, then we would apply for, obtain additional permits, and carry out any other activities necessary to remove the augmented habitat. Un-intended outcomes that might necessitate removal of the habitat included:

1. A decrease in native oyster density in the constructed bed relative to the control (indicating that native oysters were adversely affected by the habitat augmentation).
2. A decrease in the biodiversity of the native epifaunal community relative to the control.
3. A decrease in water quality downstream of the oyster bed relative to the control.
4. An increase in the ratio of non-native to native oysters in the community bed relative to the control (indicating that the non-natives are benefitting by the augmentation more than natives are benefitting).
5. Adverse impact to adjacent eelgrass beds by the constructed oyster bed.

We also proposed that if, after year 1 and again after year 2, the oyster bed suffered >30% shell loss due to burial, sinking, or storm activity, we would augment bed with more shell in order to maintain the target bed depth and dimensions.

Scientific Study Questions

Based on the **objectives** for the project and on **contingency provisions required for permitting**, the following **scientific study questions** were developed:

1. What is the rate of shell loss due to burial or other causes on the constructed oyster bed?
2. Do native oysters recruit (settle and survivethrough adulthood) on a constructed oyster bed at higher densities than a control mudflat?
3. Is the ratio of non-native to native oysters lower in the oyster bed relative to the control (indicating that the natives are benefitting by the augmentation more than non-natives are benefitting)?
4. Does the biodiversity of the native epifaunal community increase on the constructed oyster bed relative to the control mudflat?*
5. Does water quality downstream of the osyter bed decline relative to the control?
6. Are eelgrass beds adjacent to the constructed oyster bed adversely impacted?[T]

*Question 4 was partly addressed via a Master's student project at CSU Long Beach by Terrance Champieux (committee chair: Dr. Christine Whitcraft, see **Supporting Materials 1**), so his study design and project results are reported within the Master's thesis, however, a discussion of the findings is included in the Discussion of this final report.

[T]Question 5 was partially addressed and Question 6 was addressed in its entirety via a Master's student project at CSU Fullerton by Sara Briley (committee chair: Dr. Danielle Zacherl, see **Supporting Materials 2**), so the study design and project results are reported within the Master's thesis, however, a discussion of the findings is included in the Discussion of this final report.

Scientific Study Design

Jack Dunster Marine Reserve was selected as the target location because it offers educational opportunities to the public, yet provides mudflat habitat in a protected area with no public access. The public could observe the community bed during low tides via access to a meandering pathway throughout the reserve, as well as gangway access to two floating observation platforms and one floating dock. The site was protected from the potentially erosive currents and boat wakes in the Los Cerritos Channel by a floating breakwater.

Bed construction

We constructed a 2m X 30m bed on the intertidal mudflat centered at a tidal elevation of +0.4m MLLW during June 2012 using loose, dead *Crassostrea gigas* oyster shell applied at ~ 8 cm thickness (**Figure 2**). The oyster shell was provided by Carlsbad Aquafarm and was inspected by California Department of Fish and Wildlife biologists. Prior to inspection, shell was stored in 20 cm x 55 cm x 55 cm stackable plastic lattice trays that were air and sun-exposed and located away from seawater for at least 6 months. Shell material was transported onto the mudflat and deployed by community volunteers and students (see Education and Community Outreach below). To minimize footprint impact on the mudflat habitat during construction, we placed a line of plastic lattice trays extending along the mudflat from the observation dock to the end of the plot where the bed was to be built. In this way, volunteers and students could walk to the end of the line of trays and pass shell along a chain gang, depositing shell onto the footprint left by our trays as we worked our way back to the dock (Figure 2). A control plot (also 2m X 30m) was also established (**Figure 2**).

Shell Percent Cover

Prior to oyster bed construction we recorded shell % cover (e.g., dead shell, *Mytilus* spp., *O. lurida*) using replicate 50 cm x 50 cm gridded point-contact quadrats (n=5) on the established control mudflat as well as on the mudflat where the oyster bed was to be constructed. Surveys were again conducted at 6, 12, 18, and 24 months after bed construction, but with increased replication (n=7).

Figure 2. Left: Community volunteers construct the oyster bed in June 2012. Right: Completed bed, with control plot visible at same tidal elevation in the background.

We did not re-survey the substratum immediately after oyster bed construction because we ensured 100 % shell cover for the constructed oyster bed during construction. The purpose of these % cover surveys was to assay the loss of clean shell available on the surface for spat to attach because when the shells are fouled with sediments, they can no longer serve as adequate “cultch”.

Augmenting the bed

After conducting surveys of shell % cover in June 2013, approximately 12 months after construction of the shell bed (that resulted in 100% oyster shell cover), we observed that shell % cover was reduced to 28% on the bed and remained 0% cover on the control plot (see Results and Fig. 4) Excavations of the bed (see methods in ***Spatfall and Oyster density*** section below) revealed significant deposition of sediment. In our qualitative observations of the bed, we noted the creation of two “drainage” channels that divided our once contiguous bed into three sections. Our adaptive management strategy was to augment the bed after 1-yr surveys were complete, but in attempts to alleviate the sedimentation, we augmented the three separate sections and left the drainage channels in place. We augmented the bed with 4.3 cubic yards of additional shell with the help of community volunteers. Augmentation occurred only on the seaward edge of the bed at approximately +0.2 m MLLW since other Zacherl lab field survey results indicated that native oysters reach their density maximum at a tidal height lower than the originally constructed bed. In subsequent sampling periods (mo 18 and 24), we sampled both the original bed and the "New" bed separately to monitor the progress of the oysters at the tidal elevations most seaward (~0.1 m MLLW on the "New Bed") and most shoreward (~0.5 m MLLW on the original "Bed").

Spatfall and Oyster density

Following initial construction of the bed in June 2012, we monitored spatfall through May 2014 within the bed and within the control plot using replicate (n=4) ceramic tiles that were each attached to tees made of schedule 80 gray ¾" PVC. The vertical component of the tee was driven into the mud so that the tiles sat approximately 10 cm above the substratum. We collected tiles every spring tide (every two weeks) during the reproductive season and every month otherwise, and settler density was assayed under a dissecting microscope. Once oysters were counted per unit area of tile, we calculated the # oysters/m^2. These data were analyzed qualitatively to observe yearly patterns of spatfall and to compare spatfall on the control versus constructed oyster bed.

Every 6 months following construction of the bed we excavated shell from randomly placed replicate 25cm X 25cm quadrats (n=7) within the shell bed and control plot (and after June 2013, we also sampled within the "New Bed"). Shell was brought back to the lab, carefully rinsed of sediments, and then we counted juvenile and adult oysters on the shell. After 1 year, we tested the effects of treatment, time, and their interaction on oyster density using Primer 6 (version 6.1.11) with PERMANOVA+(version 1.0.1) extension software. For the full 2-year dataset, because we included the New Bed treatment in our data analysis, we compared the number of oysters within each treatment only in summer 2014 (when we sampled all three treatments) using t-tests that assume unequal variances with a Bonferroni-adjusted p-value of p=0.05/3=0.017.

Community Biodiversity

In addition to monitoring recovery of oysters, we also examined the effects on biodiversity of the habitat by sampling epifaunal and infaunal invertebrates (including oysters) inside and outside of experimental plots and control plots at 0, 6, 12, 18 and 24 months. Epifauna were sampled from the replicate (n=5) 25cmX25cm quadrats (described above). All sediments were rinsed through a 500 micron and then a 300 micron sieve. In addition, epifaunal organisms attached to the hard substrate were counted. Sieved organisms were preserved in a formalin solution, sorted, identified and counted in the Zacherl lab at CSU Fullerton. Infauna were sampled using replicate (n=5) mud cores (6 cm deep, 4.8 cm diameter). The mud cores were sieved through a 300 micron sieve, then preserved, sorted, and counted in the Whitcraft lab at CSU Long Beach. For the purposes of this final report, we sorted taxa into broad categories including the following: polychaetes, oligochaetes, gastropods, bivalves, copepods, ostracods, amphipods, tanaids, isopods, decapods. Also, here we report on the first-year results only, data analysis for the full two-year data set has been scheduled in late August-early September. Sorting to species-level is also ongoing and is expected to be complete by Dec 2015 for the original bed and control and May 2016 for the "New" bed. With the one-year data set, we tested the effects of treatment, time, and their interaction on oyster density using Primer 6 (version 6.1.11) with PERMANOVA+(version 1.0.1) extension software, then used a multidimensional scaling plots to build an understanding of how communities changed over time. Lastly, we analyzed changes in taxon richness and taxon diversity in JMP 12.0.1using two-way ANOVA testing for the effects of time and treatment. Data met the assumptions of ANOVA.

Water quality

To track effects of the construction project on water quality, during initial construction of the oyster bed in June 2012, we monitored the effects of our activity on the mudflat on downstream turbidity from 20 min- 2.5 hours after the oyster bed was submerged immediately following construction. We took turbidity measurements with a Lamotte 2020 handheld turbidity meter at a control location ~ 10 m upstream from oyster bed, on top of the oyster bed, and ~ 10 m downstream from the oyster bed. At each location, we measured turbidity both on the surface and at a subtidal depth of ~ 0.3 m at 20, 50, 80, and 140 minutes after submergence. We took multiple additional downstream measurements at 170 minutes to ensure that turbidity returned to control levels. To track long-term effects on turbidity and light availability to the adjacent eelgrass bed (see paragraph below), Master's student Sara Briley measured light availability seasonally for a year following bed construction (see Appendix for methods and results).

Eelgrass monitoring

Eelgrass, *Zostera marina*, was known to occur within the low intertidal and shallow subtidal habitat at Jack Dunster Marine Reserve (Rick Ware, Coastal Resources Management, Inc. pers. com. with Danielle Zacherl and Amanda Bird, 10 May, 2011). Other submerged aquatic vegetation (SAV) that were known from the project area included the invasive *Sargassum muticum*, and the green alga *Enteromorpha* spp. The proposed bed construction occurred in the intertidal zone at least 2 m above the "edge" of existing eelgrass. The habitat in Jack Dunster Marine Reserve supported eelgrass beds up to an elevation of +0.5 ft MLLW, based upon surveys completed in 1994 by Rick Ware (President and Senior Biologist, Coastal Resources Management, Inc.). Prior to oyster bed construction, the eelgrass extended to -0.1 ft MLLW at its highest tidal elevation, based upon field surveys conducted May 20, 2011. In collaboration with Rick Ware, who has been restoring eelgrass habitat in southern California bays for more than 20 years, we monitored changes in eelgrass biomass both adjacent to the constructed bed and at a nearby control site as part of our 0, 6, 12, 18, and 24-month community surveys. In addition, we mapped the shallow edge of the eelgrass bed in the vicinity of the oyster restoration project with a differential GPS unit and visually presented this on a map with the location of the oyster restoration beds annually. (For methods, results and discussion of eelgrass studies, see Sara Briley's Master's thesis in Appendices. Discussion of her overall findings is also included in this final report)

SCIENTIFIC STUDY RESULTS

Shell Percent Cover

Approximately 12 months after construction of the shell bed, shell % cover was reduced to 28% on the bed and was 0% cover on the control plot (**Figure 3**). Qualitative observations indicated that the reduction in shell cover was due to sedimentation. After augmentation (building the "New Bed") in June 2013, shell % cover decline was not as severe on the "New Bed", with shell cover at 82% in January 2014 and 79% in June 2014. In fact, we recorded moderate shell % cover gain on the old section of the bed (with % shell cover increasing from 28% in June 2013 to 42% in June 2014).

Spatfall and Oyster density

Spatfall varied wided from year to year, with greatest spatfall occurring in June 2013 (**Figure 4**). In general, spatfall did not differ substantially between Bed and Control, except during a couple of census dates in summer 2013, when more oysters settled onto census tiles deployed on the oyster bed relative to the control plot.

After one year, In June 2013, there were significantly more *O. lurida* oyster juveniles and adults on the constructed bed relative to the control (**Figure 5,** Two-way PERMANOVA, Time $P<0.05$, Treatment $p<0.05$, Time X Treatment $p>0.05$), and adult density (6.9 ± 3.2 oysters/m^2) was comparable to the average population density at reference locations throughout Alamitos Bay (5.3 ± 0.6 oysters/m2, D. Zacherl and N. Tronske, unpublished data). By June 2014, there were

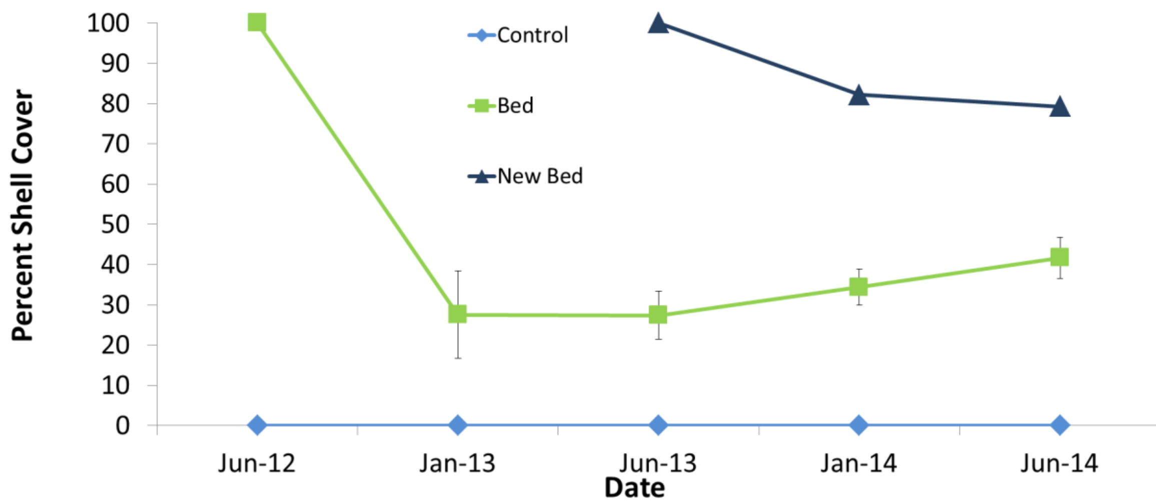

Figure 3. Change in percent shell cover ±1SE on the control plot and constructed beds at JDMR, Alamitos Bay, CA. The control plot ("Control") and original oyster bed ("Bed") were located at a tidal elevation of approximately 0.4 m MLLW. "New Bed" refers to the additional shell placed during summer 2013, and is centered at a tidal elevation of 0.2 m MLLW.

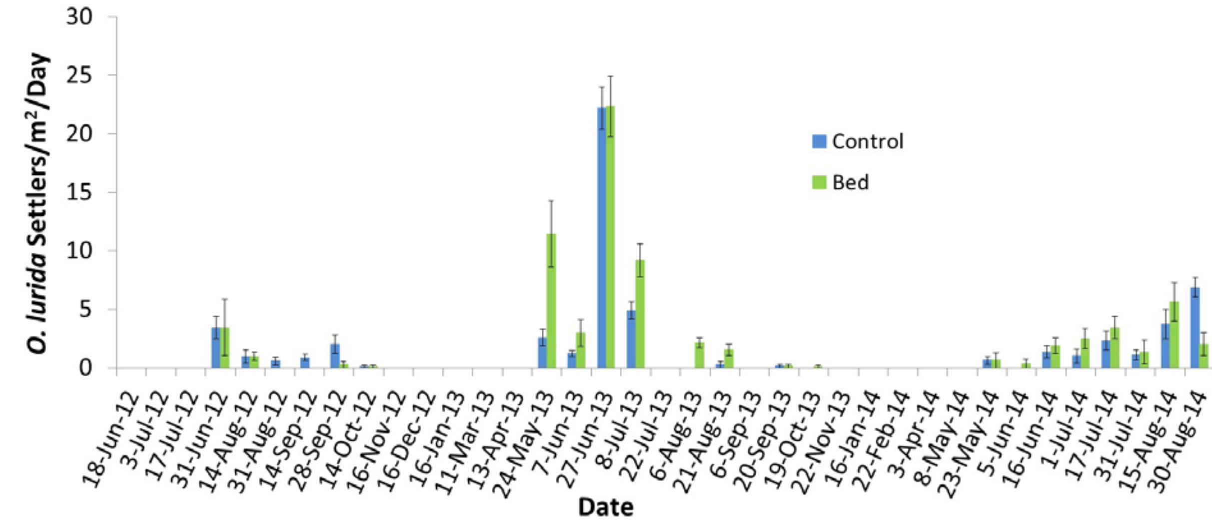

Figure 4. *Ostrea lurida* settlers per meter squared on a control plot and constructed bed at JDMR, Alamitos Bay, CA from June 2012 to August 2014. Error bars = ±1SE.

significantly higher densities of *O. lurida* recruits and adults on the "New Bed" relative to the initial bed (**Figure 5**, t-test, p=5.31E-5) and control (t-test, p=5.62E-5), with an adult density of 358. 86 ± 52.70 oysters/m^2 that was 68 times the average population density at reference locations throughout Alamitos Bay. The initial bed was, however, not significantly greater than the control (t-test, p=0.033) with an adult density of 29.7 ± 14.99 oysters/m^2, but that was 5.6 times the average population density at reference locations throughout Alamitos Bay. There were no adult *O. lurida* oysters on the control plot in June 2014.

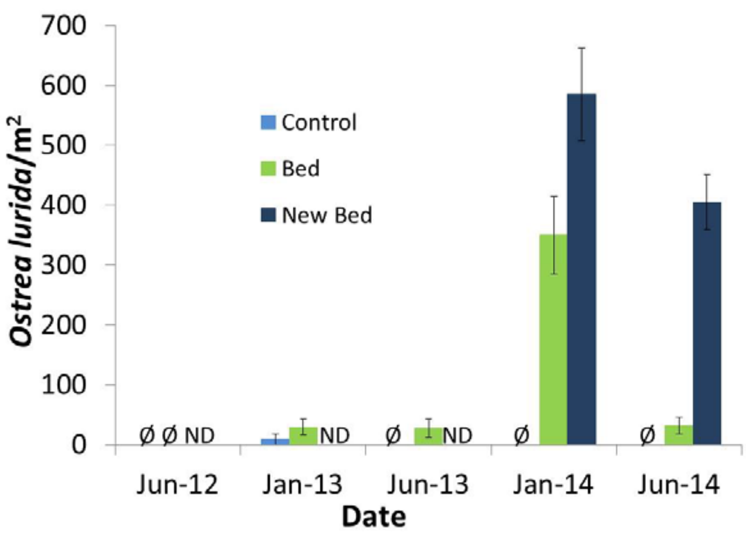

Figure 5. *Ostrea lurida* juveniles and adults per meter squared ± 1SE over time on a control plot and constructed beds at JDMR, Alamitos Bay, CA. ND = no data.

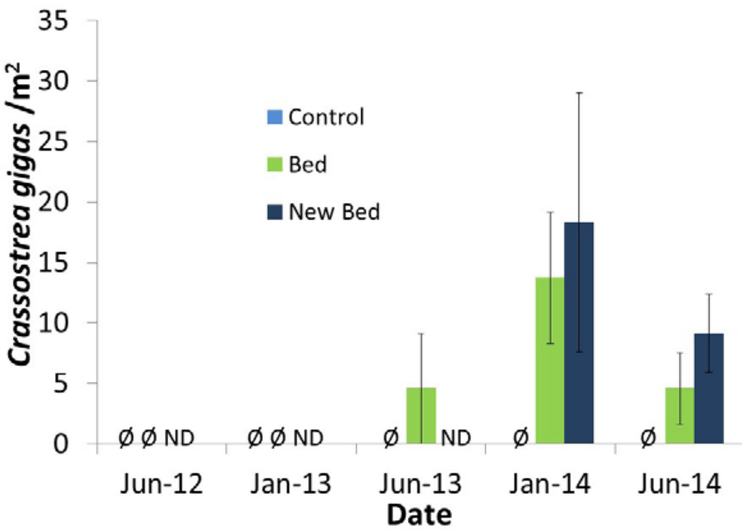

Figure 6. *Crassostrea gigas* adults per meter squared ± 1SE over time on a control plot and constructed beds at JDMR, Alamitos Bay, CA. ND = no data.

Non-native *C. gigas* also recruited to the bed but their density in June 2013 was not significantly different than zero (the control plot, **Figure 6,** Two-way PERMANOVA, Time P>0.05, Treatment p>0.05, Time X Treatment p>0.05). By June 2014, there were significantly higher densities of *C. gigas* adults on the "New Bed" relative to the control (**Figure 6**, t-test, p=0.015) with an adult density of 9.14 ± 3.23 oysters/m^2 that was 6.5 times the average *C. gigas* population density at reference locations throughout Alamitos Bay (at 1.41 ± 0.47 oysters/m^2). The *C. gigas* density on the "new Bed" was not significantly different than the initial bed (which had an adult density of 4.57 ± 2.95 oysters/m^2 (t-test, p=0.158). The *C. gigas* density on the initial bed was not significantly greater than zero (the control plot, t-test, p=0.086).

In June 2014, the ratio of native to non-native oysters on the initial bed (sampled at ~ +0.5 m MLLW) was 7:1, while on the "New Bed" (sampled at ~ +0.1 m MLLW), it was 44:1.

Community Biodiversity

Using broad taxonomic groupings, we found that both taxon richness and diversity increased on the constructed oyster bed after a year, but remained unchanged on a control mudflat (**Figure 7,** Richness: 2-way ANOVA, Time*treatment interaction, p=0.0057; Diversity: 2-way ANOVA, Time, p = 0.0018, Treatment, p<0.0001, Time*treatment interaction, p=0.07). This increase in richness and diversity was not due to the appearance of new taxonomic categories at JDMR, but rather, prior to bed construction (June 2012 surveys), decapods, copepods, ostracods, bivalves, and gastropods were only rarely or occasionally detected on both the bed and control, but after a year (June 2013 surveys), each of these taxonomic groups increased in abundance and were regularly detected among samples collected on the constructed bed. On the other hand, polychaete and oligochaete abundances dropped dramatically on the constructed bed but not on the control, while amphipods declined on both the bed and control. Lastly, tanaids and isopods remained rare on both treatments.

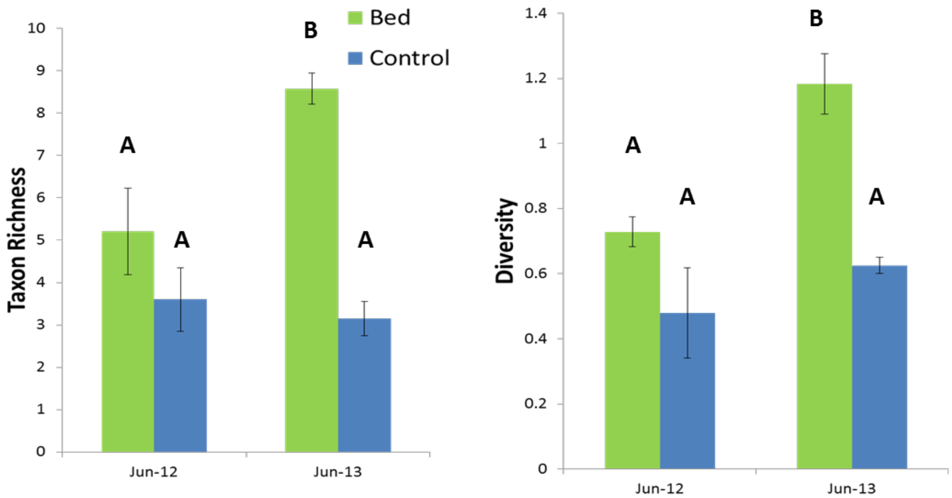

Figure 7. Taxon richness and taxon diversity on a constructed oyster bed relative to a control mudflat at JDMR, Alamitos Bay, CA in summer 2012 and 2013. Letters above bars indicate significant differences among treatment based upon post-hoc Tukey comparisons.

These shifts in community structure are detectable in the PERMANOVA analysis and MDS plot as well (**Figure 8,** PERMANOVA, time*treatment interaction, p=0.001), except PERMANOVA post-hoc analysis indicated that both the bed and control communities shifted significantly from their starting communities over the year, but each shifted in a fundamentally different direction. In the case of the control, a decline in amphipods and an increase in polychaetes were the most signrficant changes, while the constructed bed saw increases in decapods, copepods, ostracods, bivalves, and gastropods (**Figure 8**). The 2D stress of 0.12 indicates a good ordination with no significant chance of a misleading interpretation in 2 dimensions, however it should be noted that a 3-D ordination (not shown) had significantly reduced stress (0.07), and probably did a better job of interpreting changes in community structure.

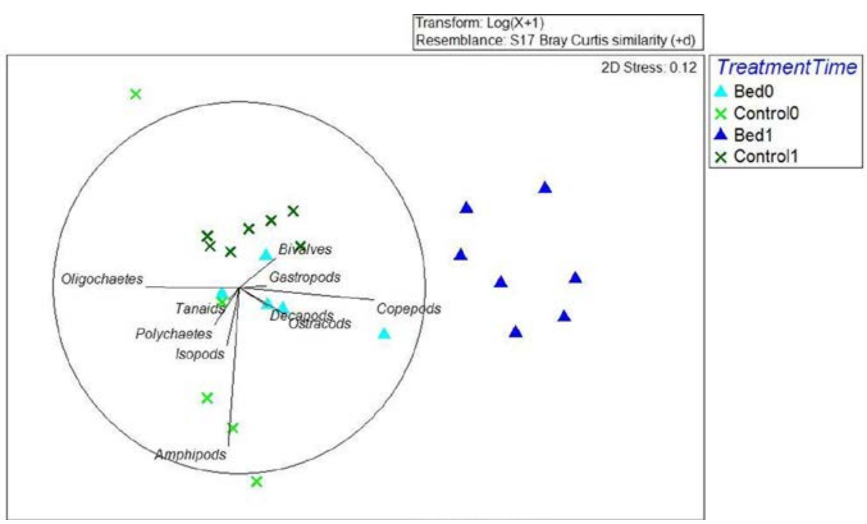

Figure 8. 2-D MDS configuration of community composition on a constructed oyster bed relative to a control mudflat at JDMR, Alamitos Bay, CA in summer 2012 and 2013. Multiple correlation vectors are overlaid.

Turbidity and Light Availability

In the hours immediately following bed construction in June 2012, as the incoming tide submerged the bed, we qualitatively observed a well-defined turbidiy plume of about 2-3 meters width and 0.3 m depth downstream (up bay) of the oyster bed. The plume maintained its integrity for about 10 m upstream before dispersing and for about 2 hours following submergence of the oyster bed. Turbidity measurements indicated that the plume was isolated in its impact (**Table 1**) with the biggest effect isolated to downstream at the surface of the water for about 140 minutes following bed submergence. By 170 minutes, the plume was not detectable quantitatively or qualitatively. Bed effects on light availability to an adjacent eelgrass bed are reported in the Appendices in Sara Briley' Master's thesis (**Supplementary Materials 2**).

Table 1. Turbidity (NTU) measured ~ 10 m upstream ("Control"), on the constructed oyster bed, and ~ 10 m downstream from the oyster bed in the 2.5 hours after bed submergence by the incoming tide on the day of oyster bed construction in June 2015. Numbers in bold exceed the 20% maximum allowable increase in turbidity for the Los Angeles Region.

Treatment	Minutes after submergence				
	20	50	80	140	170
Control	0.76	0.84	2.07	1.14	ND
Control surface	11.36	1.10	5.38	1.51	ND
Oyster bed	**1.40**	4.46	2.43	1.63	ND
Oyster bed surface	1.33	**14.51**	1.72	1.26	ND
Downstream	**2.59**	**1.06**	**2.71**	1.34	ND
Downstream surface	**52.57**	**80.47**	**41.82**	**11.30**	1.6

Eelgrass monitoring

Effects of the constructed oyster bed on an adjacent eelgrass bed are reported in the accompanying Appendices in Sara Briley' Master's thesis.

DISCUSSION

Despite significant loss of shell volume from the constructed oyster bed in 2013, the combined settlement, and juvenile and adult oyster density data gathered during our study provide evidence that oyster densities in Alamitos Bay, CA can be increased significantly by augmenting habitat with oyster shell. Our constructed oyster bed quickly and significantly increased adult *Ostrea lurida* density relative to un-manipulated mudflat. After only two years, average oyster density on the "New" bed was greater than 50 times the average population density at reference locations throughout Alamitos Bay, and the original bed boasted a density 5 times greater. In other locations, depletion of Olympia oyster populations has necessitated the introduction of seed stock to revive locally extinct oyster beds (Dinnel et al. 2009). When restoration can be accomplished via simple habitat augmentation, the risk is lowest for unintended genetic impacts (Camara & Vadopalas 2009). Addition of seed stock does not appear to be necessary to increase the local density of Olympia oysters in Alamitos, CA. Others have similarly found that simply adding oyster shell to mudflat quickly augments eastern oyster (*Crassostrea virginica*) and Olympia oyster densities (O'Beirn et al. 2000, Nestlerode et al. 2007, Dinnel et al. 2009).

Restoration ecologists have underscored the importance of using reference sites in establishing target metrics for restoration goals (Brumbaugh et al. 2006, Baggett 2014). For the Olympia oyster throughout its range, it remains difficult to establish these metrics due to the lack of suitable reference sites and quantitative data on historical species abundance and density. For example, elsewhere in Alamitos Bay, there are no existing Olympia oyster beds against which to compare our adult oyster density data. Although beds of oysters were observed and known to exist throughout southern CA estuaries through the early 1900's (Ingersoll 1881, Gilbert 1889, Bonnot 1935), quantitative data on density of Olympia oysters in beds are nonexistent. However, the average density (358. 86 ± 52.70 oysters/m^2) we recorded after 1 year on our "New" constructed bed are much greater than the maximum density of Olympia oysters (19.2 oysters/m^2) on any other habitat (e.g., cobble, mud, riprap, seawall, pier piles) in nearby Newport Bay recorded by Polson and Zacherl (2009) in their 2005 surveys.

Elsewhere across the U.S. West Coast, only one site in San Francisco, which underwent recent restoration (Point San Quentin), achieved a maximum density somewhat comparable to our average density (146.8 oysters/m2 at Point San Quentin, Polson and Zacherl 2009). However, in British Columbia, Olympia oyster density at multiple sites (at 240-360 oysters/m2) are equivalent that recorded in this study and elsewhere along the range (Gillespie 2009, Jacobsen 2009).

Importantly, we observed non-native Pacific oysters, *Crassostrea gigas*, recruiting onto our constructed beds. Adults of this species are present throughout estuaries in southern California, including Newport Bay in Orange County, Alamitos Bay in Los Angeles County and estuaries throughout San Diego County, especially San Diego Bay (Crooks et al. 2015). The Pacific oyster is now regularly recruiting throughout southern California estuaries (H. Henderson, personal communication, Merkel Consulting, August 2014). *C. gigas* shows zonation patterns with other oyster species (Krassoi et al. 2008) and this is also the case in southern California; *C. gigas* reaches its maximum density well above +0.5 m MLLW, while *O. lurida* achieves highest densities at -0.1 m MLLW and lower (D. Zacherl and T. Parker, unpublished data, CSU Fullerton, July 2015). Importantly, a restoration project in nearby Newport Bay reported the recruitment of only one *C. gigas* individual in their two-year restoration study, but their beds were located at ~ -0.2 m MLLW, where Our constructed beds were located at a tidal elevation (ranging from ~+0.15 to 0.5 m MLLW) that may have favored the recruitment of *C. gigas*. In fact, even across the tidal range of our constructed beds, we saw a shift in the ratio of native to non-native oysters from 44 natives: 1 non-native on the “New” bed to 7 natives: 1 non-native on the original bed. Nonetheless, native oysters clearly benefitted more from the restoration activity, particularly on the “New” bed at the lower tidal elevation.

Mann & Powell (2007) argued that maintaining structured shell cover is a critical element of any restoration effort because without a clear settlement destination for spat, oysters cannot self-perpetuate. We observed significant loss of shell volume in our study in the first year. Qualitative observations of our beds suggested that shells were buried by sediments. Shell volume loss may have stabilized after the secondyear, but that does not ameliorate the finding that shell loss was significant. Clearly, shell loss should be a major consideration for future restoration projects (Coen & Luckenbach 2000, Mann & Powell 2007) and our study findings reinforce the need to monitor this important metric. Our study was designed as a short-term project, but if constructed beds are to be maintained in the long term (i.e. restoration), shell beds will likely require ongoing augmentation.

As far as impacts on the community, we saw increases in richness and diversity of broad taxonomic groups commonly associated with mudflat and intertidal communities in estuaries. In addition, the impact on an adjacent eelgrass bed and its associated community appears to be of little consequence. We found no evidence that the constructed oyster bed impacted water column light intensity above an eelgrass bed, despite a signirficant turbidity plume associated with the initial construction of the bed. This turbidity plume quickly resolved (within two hours of submergence of the newly constructed bed) and thereafter, we gathered no evidence of further impacts on light availability, particularly over the adjacent eelgrass bed. Overall eelgrass bed structure (total above-ground biomass and shoot density) and eelgrass epiphyte load were similarly unaffected by the presence of the constructed oyster bed. We did meaure a decline in eelgrass below-ground biomass, though values were never lower than the control. Individual shoot characteristics also showed evidence of impact in the first year only through increases in leaf width (17 % increase) and shoot biomass (78 % increase), though it is unclear whether this is a positive impact or a temporary adaptive response by the eelgrass to short-term stresses associated with oyster bed construction activities. For a more complete discussion of the impacts of this constructed bed on the adjacent eelgrass, see **Supplementary Materials** 2. In sum, our findings support the coexistence of constructed oyster beds and eelgrass, which is relevant to the design of future restoration efforts for both species.

It is useful to now return to the contingency provisions and provide commentary on each one. Here we list un-intended outcomes that might have necessitated removal of the habitat and then commentary on the observed outcomes of our studies (*in italics following each provision*):

1. A decrease in native oyster density in the constructed bed relative to the control (indicating that native oysters were adversely affected by the habitat augmentation). See discussion above. *No native oysters were observe don the control mudflat after Jan 2013, while native oyster density on the original bed and “New” bed increased significantly.*

2. A decrease in the biodiversity of the native epifaunal community relative to the control. *We observed increases in richness and diversity of the combined infaunal and epifaunal communities on the bed relative to the control mudflat after a year. Because so far the individuals have been enumerated only to broad taxonomic categories, we cannot*

completely address this provision yet. Sorting to species level is anticipated to be completed by Dec. 2015 for the original bed and May 2016 for the "New" bed.

3. A decrease in water quality downstream of the oyster bed relative to the control. *We saw an extrtemely short-term perturbation in turbidity in the 2 hours of submergence of the newly constructed bed, but thereafter, a variety of measures indicated no impact. Importantly, this included no impact on the light availability to an adjacent eelgrass bed.*
4. An increase in the ratio of non-native to native oysters in the community bed relative to the control (indicating that the non-natives are benefitting by the augmentation more than natives are benefitting). *We observed no oysters on the control plot with which to calculate a ratio. However, surveys of native and non-native oysters throughout Alamitos Bay in 2012 recorded antive:non-native ratios ranging from 1:1 to 3:1 on mudflat and riprap, respectively, while ratios on our constructed beds ranged from 7:1 to 44:1. Clearly the native oyster benefitted from the augmentation more than the non-native. Berds constructed at even lower tidal elevations would be expected to tilt the ratio in favor of the Olympia oyster even more conclusively.*
5. Adverse impact to adjacent eelgrass beds by the constructed oyster bed. *We observed no adverse impacts on the adjacent eelgrass beds, except possibly a short-term impact on below-ground biomass that never differed from the control.*

With limited available scientific information on *O. lurida* restoration in many locations throughout its range, the integration of science-based planning and monitoring as part of any future restoration effort is crucial. Monitoring provides valuable data that can drive the direction of future restoration goals and efforts, reveal variation of method efficacy across regions, and bolster success in restoring this ecologically important and recovering species.

EDUCATION AND COMMUNITY OUTREACH

Education and community outreach events consisted of primarily five different activities: volunteer participation in bed construction, community participation in a shell-string deployment endeavor, development and deployment of an outreach education labs for K-12 participants, multiple poster and oral presentations at scientific conferences, and installation of informational signage onsite at the restoration location.

Bed construction

When the original bed was constructed on June 20-21, 2012, 35 student and community volunteers contributed a total of 81 volunteer hours by participating in the bed-building event by helping to unload dead oyster shell from a truck to a spot nearby the restoration site, and laying the shell onto the mudflat at JDMR. In June 2013, 42 community volunteers and interns from OC Coastkeeper participated in a bed-building event, contributing 129 volunteer hours. Nine unpaid project scientists and students from the Zacherl lab and from CSU Fullerton prepped shell, co-hosted and participated in the event with OC Coastkeeper, and provided clean-up, contributing an additional 63 volunteer hours. In total, 86 volunteers contributed a total of 273 volunteer hours constructing oyster beds.

Shell string deployment

We invited the public to participate in “oyster gardening” where community members each hung their own shell strings off of private docks or off public access docks that collected locally produced oyster settlers. Each participating homeowner was provided with multiple (3-10) strings; each string consisted of 10 oyster shells arrayed vertically onto a 12-inch long piece of 16 gauge steel galvanized wire with a loop on the top and attached to polypropylene line for easy deployment off docks. Volunteers “tended” their shell strings for a short period of time before transferring the shells with recruited spat to the growing oyster bed. We hosted 5 half-day workshops over two years at the Sea Base Aquatic Center or the Long Beach Yacht Club, where Zacherl, along with undergraduate research scholars and graduate students trained interested participants in methods for deployment, retrieval, and transfer of shell strings. The training sessions varied in emphasis depending upon need. In July 2012, we hosted the first Shell String Workshop at the Sea Base Aquatic Center. Seven community volunteers attended the first training workshop to learn how to deploy and retrieve shell strings. Example shell string workshop flyers are **Supporting Materials 3**. Two months later (September 2012), we hosted the 2nd Shell String Workshop at the Long Beach Yacht Club. Community volunteers used dissecting microscopes to count oysters that recruited to shell strings deployed from around the bay and then Zacherl lab students deployed some of those shell strings onto the bed. In the following year, we hosted three workshops (April, July and September, 2013) which folled the same format as the above. While participation by the community homeowners remained modest, their enthusiasm was remarkable, and some of the shell strings recruited 100’s of oysters to the restoration bed.

Outreach education

Orange County Coastkeeper developed a curriculum specific to this restoration project that emphasized the role of filter feeders in the environment and introduced middle school and high school students to the wetlands and to oyster ecology. In 2013, OC Coastkeeper hosted seven educational field trips for local schools, bringing 284 students on field trips to JDMR. They collaborated with the Los Cerritos Wetlands Stewards by having two volunteer environmental professionals give a short introduction each field trip day the discussed the history and ecology of JDMR. They also taught 15 in-class presentations to over 500 students, and worked with students to construct over 100 shell strings that were deployed by school groups. Example curricula are **Supporting Materials 4.**

Presentations

The research conducted on this restoration activity provided data that fueled the production of 9 student poster presentations at scientific conferences of local, regional and national significance from Fall 2012 through spring 2015, including student posters presented at Southern California Academy of Sciences, Western Society of Naturalists, Bethic Ecology, National Shellfisheries Association, and on the CSU Fullerton campus at two STEM^2 conferences. The data also were presented in 5 student oral presenrtatiopn at Western Soceity of Naturalists, National Shellfisheries Association, and, most recently, at the Western Society of Malacologists meeting in June 2015 where undergraduate scholar Cristina Fuentes won the Best Student Oral Talk Award. Example posters and talks are **Supporting Materials 5**.

In addition, PI Zacherl delivered 8 oral presentations on data generated by this restoration activity – 3 at scientific conferences, including National Shellfisheries Association and Western Society of Malacologists, 2 to local shell clubs Pacific Conchological Society and the San Diego Shell Club, and 3 public outreach talks to community members sponsored by the Newport Bay Conservancy, CSUF Osher Lifelong Learning Institute (OLLI) and the CSUF Colleagues Colloquium.

Educational signage

Partner Orange County Coastkeeper worked with PI Zacherl to design an interpretive sign to be placed within Jack Dunster Marine Reserve in early September 2015. The 24” x 36” interpretive sign describes the biology, history, and importance of oysters in Alamitos Bay, as well as the role of other filter feeders in healthy bays and estuaries. The sign will be attached to the railing of the observation dock located in the northwest of the Jack Dunster Marine Reserve. The final signage is **Supporting Materials 6**.

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SUPPLEMENTARY MATERIALS