UPPER NEWPORT BAY LIVING SHORELINE PROJECT
CSU FULLERTON SUBCONTRACT PROGRESS REPORT
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Introduction

The Upper Newport Bay Living Shoreline Project is a collaborative effort being undertaken by Orange County Coastkeeper and the Coastal Conservancy. The Coastal Conservancy is granting funds to Orange County Coastkeeper. Dr. Danielle Zacherl of CSU Fullerton is a subcontractor under Orange County Coastkeeper. The team is implementing a plan to restore native Olympia oyster (*Ostrea lurida*) and eelgrass (*Zostera marina*) habitat in Upper Newport Bay using natural structures as habitat that will also serve to buffer and protect adjacent shorelines from sea level rise and erosion. The team will conduct baseline monitoring, implement restoration efforts and monitor restoration effectiveness through March 2018.

Dr. Zacherl is assisting Coastkeeper in evaluating the success of the oyster restoration project, measured in terms of native oyster population growth and restoration unit integrity. In support of this effort, Dr. Zacherl has conducted baseline oyster recruitment and biological field investigations during the winter 2015 and the summer recruitment season of 2016 (May – September). Post-restoration monitoring will occur six months and then annually one and two years after project initiation. Zacherl and her students will provide expertise to implement oyster spat recruitment rates, density, size and growth studies collected using settling plates or other appropriate material. Zacherl will collect density data for naturally occurring oysters at the study sites. Zacherl will assist with data analysis and reporting of results for field studies and provide interpretation of project effectiveness.

Project Objectives

The broad goal of the Upper Newport Bay Living Shoreline Project is to create biologically rich native oyster beds in Newport Bay *as part of a complete marsh system that restores an ecological niche that was historically present, is ecologically functional and resilient to changing environmental conditions, and also protects bay tidelands and shoreline.*

The objectives of the CSUF subcontract are to provide field monitoring of native and non-native oyster populations as a result of an oyster restoration project in Newport Bay.

The broad **objectives** for the CSU Fullerton subcontract were to:

1. Conduct baseline and post-restoration monitoring of native and non-native oyster populations, including densities, recruitment, growth and size frequency.
2. Conduct baseline and post-restoration monitoring of integrity of oyster restoration units, including percent shell cover and sedimentation.
3. Provide data analysis, interpretation and reporting of results to OC Coastkeeper for integration into larger project reports submitted by OC Coastkeeper.

SCIENTIFIC STUDY DESIGN AND METHODS

We will be completing a variety of monitoring surveys twice yearly within 4 treatment plots at each of the 4 different field sites in Upper Newport Bay

Site Locations:
Pacific Coast Highway (PCH): 33°37'9.55" N, 117°54'17.41" W to 33°37'12.53" N, 117°54'14.96" W

Permission from land owner: County of Orange, *approved*

Westcliff: 33°37'16.81" N, 117°54'7.29"W to 33°37'17.03" N, 117°54'3.04" W

Permission from land owner: County of Orange, *approved*

DeAnza Peninsula: 33°37'13.18" N, 117°53'54.81" W to 33°37'12.54"N, 117°53'50.65"W

Permission from land owner: County of Orange, *approved*

Shellmaker Island: 33°37'21.60" N, 117°53'32.19" W to 33°37'18.06" N, 117°53'32.81" W

Permission from land owner: CA Department of Fish and Wildlife

Our long-term monitoring surveys will follow the principles of a BACI design (Before-After-Control-Impact) although we will layer a "blocking" effect onto the design such that all treatment plot types will be represented at each of four field sites. We will collect data in winter and spring 2016, and winter 2017, before constructing the Olympia oyster beds. All subsequent sampling will occur after the addition of oyster shell into two of four treatment groups. Importantly, our blocked BACI design will allow for our analysis to take natural variation among field sites into account.

The treatments at each site include: 1) restored oyster bed (20 m by 1.5 m) alone, 2) restored eelgrass bed (20 m by 8 m) alone, 3) restored oyster bed above a restored eelgrass bed, and 4) a control treatment left un-manipulated, with ~ 10 meters separation between each treatment. The intertidal oyster plots will be situated at a tidal elevation at -0.5 ft MLLW. Sara Briley will use GPS coordinates, land-line-ups and wooden stakes to identify the corners of each plot.

General Approach and Generating Random Numbers for Density and % Shell Cover Surveys:
At each treatment plot (n=16), we will survey along a 20 m X 2 m transect parallel to shore at -0.5 MLLW. We provide random X,Y coordinates for each of **10 quadrats.**
Prior to the field work time, research techs will generate random numbers for the X coordinates ranging from 0.0 to 19.5 m. Y coordinates will also be generated ahead of time and will range from 0 (near water) to 1.5 m (land-ward). The Y transect crosses the transect tape at 1 m. Quadrat placement involved laying the upper left corner of the quadrat at the X-Y coordinate. For example, if the X-Y coordinate is 0.0 and 1.5, then the upper left corner of the quadrat will be placed at 0.0 (extending to 0.5 m) along the X and 1.5 (extending to 2 m) along the Y.

Percent Shell Cover Surveys:
We designed the point-contact surveys to assess the amount of hard substrate habitat (and shell cover, once the beds have been constructed) available for oyster settlers.

- Surveyors place a gridded 0.25 m^2 quadrat on the first XY coordinate.

- One surveyor uses a probe to determine the substrate that falls exactly under 49 points in the gridded quadrat (the points are each corner in rows of 7, with 7 total rows).
- If the probe strikes mud with another substrate underneath (e.g. a shell fragment), the surveyor counts that as mud, if the mud is at least 1 cm deep.
- If the mud is less than 1cm deep, then the surveyor calls out the substrate that is under that shallow layer of mud and estimate the depth of the mud in mm.
- *Enteromorpha* and Eelgrass are only counted if we hit an attachment point. The idea here is that when the tide is in, we want to know what substrate the oyster larvae will hit when they go to settle on the substratum. So if we hit a loose tip / piece of eelgrass or *Enteromorpha*, we move it and assess the substrate beneath it.
- In all other cases, we call out the **sessile** substrate that the probe hits first.
- If the probe falls onto a mobile invertebrate, we call out the substratum underneath it.
- At the end of the substrate assessment for the quadrat, the data recorder checks to ensure that there are a total of 49 points.

Density Surveys:
These are designed to assess the number of **LIVING** epibenthic bivalves and other large epibenthic invertebrates in the quadrat. The surveyor replaces the gridded quadrat with a smaller ungridded frame. The critical thing here is making sure that we count EVERY living large epibenthic invertebrate (including *Ostrea lurida, Crassostrea gigas, Musculista* sp., *Mytilus galloprovincialus, Crucibulum spinosum*, a variety of clam species, bubble snails) and identify it properly.

- We first excavate all hard substrata into a bucket down the mudflat surface.
- If an organism/hard substratum is 50% or more than 50% inside the quadrat, we excavate it.
- A looker partly fills the bucket with seawater to wash mud off hard substrata and carefully pulls pieces out searching for living bivalves. We separate each piece of hard substratum into two piles – one that has something living on it and one that does not. For every piece, a second looker examines to confirm numbers and IDs.
- When time runs short in the field, we archive the remaining samples to be sorted and counted into labeled Ziploc bags and freeze them. They get sorted and enumerated back in the lab.

Oyster Bed Area Surveys:
This method involves mapping the boundary of the oyster bed with a differential Geographic Positioning System (GPS) unit. In this way, the bed area can be measured and mapped. This method provides estimates of bed area, and combined with density data, can be used to calculate total abundance of oysters within the bed.

- Determining the boundary of the bed can be subjective as densities on the outside edges (especially in the high intertidal) can become very low. For our purposes, the edge of the reef is defined as a continuous line where the percent coverage of surficial living or non-living shell substrate (or alternate material) is equal to or greater than 25%.
- This method will be implemented once beds are installed

Oyster Settlement and Recruitment Surveys:
Recruitment and growth of oysters is monitored from mid-April through early October 2016-2018 within each plot using ceramic tiles that are attached to a tee made of schedule 80 gray ¾ " PVC (as in Seale and Zacherl 2009). The vertical component of the tee is driven into the mud so that the tile sits approximately 10 cm above the substrate.

- We deploy settlement and recruitment tiles in mid-April– one of each tile type per tee. One tee is deployed in the center of each treatment plot across all four sites, for a total of 16 tees.
- One tile from each tee is swapped out every two weeks until mid-September each year. The second tile on each tee remains in place through the entire study period.
- The tiles collected every two weeks are analyzed in the laboratory to assess the settlement dynamics for both native *Ostrea lurida* and non-native *Crassostrea gigas oysters.* Settler density is assayed under a dissecting microscope.
- The recruitment tiles are collected, photographed, and analyzed at the conclusion of the recruitment season (early October) to measure recruitment and size frequency distributions for each species of oyster. Each visible oyster on the tile is identified to species and measured. Length is measured using Vernier calipers measuring from the umbo to the maximum length, and width is measured perpendicular to the length. These data provide inferred information about survival and growth of oysters over the recruitment season.

Oyster Growth Surveys:
In addition to the growth information inferred from the recruitment tiles, we plan to measure growth and survival of a subset of up to 20 oysters per plot each year.

- In August of 2017, we will locate and tag (using bee tags) up to 20 individual oysters located within a 0.5 meter radius of the tee on each plot.
- These oysters will be mapped and we will attempt to re-locate them every month until they experience mortality or until August 2018, whichever comes first. In this way, we will have growth rate data on two cohorts of oysters – one cohort for two years of growth and one cohort for one year of growth.
- If no oysters are located within the 0.5 m radius of the tee, then we will attempt to locate up to 10 individuals from the bed and transplant them to the tee area.

Oyster Adult Size Frequency Distribution:
In addition to the size frequency data collected from the recruitment tiles in mid-September of each year, we plan to measure the length and width of all oysters excavated from the density surveys at 6, 12, and 24 months after bed construction. This will happen in the field during excavations unless we decide to archive excavated samples to analyze in the lab.

Scientific Study Pre-Restoration Results

Percent Shell Cover:
In both January and May 2016, average percent cover of hard substrata (including rock, gravel, dead shell and living bivalves) was below 6% on all treatment plots at all study locations except at DeAnza peninsula, where the % cover of hard substrata on the future "Oyster" treatment plot was 17.14 ± 3.25% in January 2016 and 15.31 ± 4.67% in May 2016 (Table 1, Figure 1). On that plot, the majority of the hard substratum was composed of dead clam and oyster shell, though living *O. lurida* were present and detectable (average was ~ 2% in both Jan and May) in these quadrats.

Density Surveys:
In January 2016, density of Olympia oysters, *Ostrea lurida*, ranged from 0 to 75 oysters per m^2 across all treatment plots and study locations (Figure 2). Oysters were not detected at Shellmaker or Westcliff, and they were only detected on one treatment plot at DeAnza ("oyster", at 75 per m^2). At PCH, they were present on 3 of 4 treatment plots, ranging from 4.8 - 46.4 oysters per m^2. We detected no *C. gigas* in our surveys, though they were present in low abundances at all study locations, but at much higher tidal elevations than we were surveying. May 2016 patterns were nearly identical to those in January, though small changes in density were detected. Results from ANOVA examining the effects of treatment and date with site as a random blocking factor, plus all two-way interactions, indicated a significant interaction between site and treatment, driven by the pattern at DeAnza where there were significantly more oysters on the plot designated as an (future) "oyster" treatment than on the other treatments, where we found zero oysters (Table 2). Again, we detected no *C. gigas* in our surveys, though they were present in low abundances at all study locations.

Outside of oysters, date mussels, *Musculista senhousia*, and the spiny cup-and-saucer snail, *Crucibulum spinosum*, were by far the most common species detected. Bubble snails*, Bulla gouldii,* bay mussels, *Mytilu*s sp., and a few unidentified clams (tentatively identified as *Venerupis phillipinarum*) were also detected in small numbers.

Oyster Settlement and Recruitment Surveys:
We deployed settlement and recruitment tiles in mid-April 2016 at all study locations on all plots. We have changed out settlement tiles bimonthly since deployment, have enumerated settlement through early August, and have collected and enumerated recruitment tiles on September 16, 2016. With the settlement data through mid-August, we graphed patterns in settlement across time as a function of treatment and (separately) site for visual inspection. We also analyzed the effects of treatment on cumulative settlement with site scored as a random block effect using ANOVA in JMP 12.1.0. Several patterns have emerged. First, we have not detected any *C. gigas* settlers but *O. lurida* has been settling at all sites throughout the census. Next, there was a peak in oyster settlement in late July/August, which was strongest at DeAnza and PCH (Fig. 3). We cannot detect any effect of treatment (blocked ANOVA, Treatment $p>0.05$, Table 3, Fig. 4).

In September we collected and assayed recruitment tiles. We found oysters on all tiles across all sites and treatments, ranging from 1-34 *O. lurida*. We also detected one *C. gigas* recruit on the top side of one tile at PCH. Preliminary 2-way ANOVA analyses for the effect of cumulative settlement and treatment and their interaction found no effect of treatment or interaction, so we treated the four tiles per site as replicates and analyzed whether there was a relationship between average cumulative settlement and average recruitment across sites. We evaluated whether the relationship could be best described as linear or asymptotic (by fitting a line through untransformed and log-transformed data) using linear least squares regression in JMP 12.1.0. The best-fit line was asymptotic, described by $y = -6E\text{-}06x2 + 0.1228x$, $r^2 = 0.65$, $p<0.001$(Fig. 5). This suggests that the relationship between settlement and recruitment is density dependent, with increasing recruitment as settlement pressure increases until cumulative settlement rises higher than about 8,000 spat per m^2, at which point recruitment asymptotes.

Oyster Adult Size Frequency Distribution:
We measured the length and width of all *O. lurida* oysters excavated from the density surveys during our May 2016 surveys. Two study locations (DeAnza and PCH) had a good representation of oysters across a broad range of size classes (Fig. 6), with PCH showing a slightly broader range, with the smallest size class (0-10 mm) and the largest size class (>50 mm) both represented. We did not detect oysters in our excavations at the other two study locations.

Discussion

We have proposed to analyze effects of Living Shorelines restoration using a Before-After-Control-Impact (BACI) design. A BACI design of study is often used to assess environmental impact and the benefit is that one can detect simple temporal shifts in the data that might otherwise be confounded with environmental impact and one is able to detect whether changes at sites are unrelated to environmental impact. A BACI design assumes that if no impact resulted from our restoration activity, then our plots, regardless of treatment, would track one another (i.e., the difference between the control and impact sites, called delta △, would be expected to be the same, Murray et al. 2006). Our prediction is that we expect to see increases in oyster densities across our oyster restoration plots that are significantly greater than changes occurring on control and eel-grass restoration only plots (i.e. a significant change in the difference between the control and impact sites, delta Δ, will indicate an impact, Murray et al. 2006).

All sites and plot locations within sites are dominated by mud, with >80% cover of mud on all treatment plots across all sites. The only treatment plot with <90% mud cover was the (future) “oyster” plot at DeAnza Peninsula, which contained a scattering of shell fragments that also supported the highest density of oysters across all plots at all sites. In fact, DeAnza was the only site that showed a significant difference in oyster density across treatments (Table 2). This might be problematic for detecting an impact of oyster restoration if oysters do not recruit to constructed beds at densities higher than DeAnza currently supports (e.g. the difference between control and impact sites at DeAnza does not change). However, recruitment plates deployed across all treatment plots at DeAnza (Fig. 5) indicate that the site is able to recruit oysters to densities nearly an order of magnitude above current densities, given cumulative settlement pressure experienced in summer 2016.

The timing and magnitude of settlement pulses are also of interest. In summer 2016, the pulse of oyster settlement occurred in late July – early August, and the magnitude of the pulse varied considerably across sites, from ~ 50 oysters/m^2/day at Shellmaker and Westcliff to ~ 225/m^2/day at DeAnza and PCH. Using identical methods to quantify settlement at a restoration site along Castaways beach, situated nearest to our current PCH site, Zacherl et al. (2015) observed differences in the timing and magnitude of settlement across three years. In 2010, the pulse occurred in June, in 2011, no significant pulse was detected, and in 2012, pulses occurred in both May and August. Across all three years, the highest pulse recorded averaged ~ 60 oysters/m2/day, at least three times lower settlement than recorded at PCH during the July pulse in 2016. These data carry significant implications for the timing of bed construction and also predicted oyster response to bed construction. Firstly, it would be most conservative to construct beds prior to May in order to capture potential pulses in May/June. Second, it may take several years to capture cumulative settlement pressure equivalent to that which occurred in summer 2016. Indeed, after two years of settlement pressure significantly lower than our sites experienced in summer 2016, Zacherl et al. (2015) recorded adult densities on beds at ~ 60 oysters/m^2, lower than our recorded averages on the DeAnza "oyster" plot (but note this was 26 times starting density at their restoration site).

Acknowledgements

Thanks to City of Newport Beach and CA Dept. of Fish and Wildlife for permission to access the sites, and a special thanks all of our hardworking Whitcraft lab (CSULB), OC Coastkeeper, and Zacherl lab volunteers, technicians, and students. Funds were provided by CA State Coastal Conservancy, and CSUF Dept. of Biological Science.

Works Cited

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Zacherl DC, Moreno A, Crossen S. 2015. Exploring Restoration Methods for the Olympia Oyster, *Ostrea lurida* Carpenter, 1864: Effects of Shell Bed Thickness and Shell Deployment Methods on Shell Cover, Oyster Recruitment, and Oyster Density. Journal of Shellfish Research 34(3): 819-830.

TABLES AND FIGURES

Table 1. Percent cover of hard substrata (± 1 SE) in January and May 2016 prior to oyster shell additions, determined via point-contact techniques per treatment (n= 10 quadrats per treatment) at four study locations in Upper Newport Bay, CA.

Location	Date	Control	Eelgrass	Oyster	Oyster/eelgrass
DeAnza	Jan-16	0.61 (0.31)	0 (0)	17.14 (3.25)	0.20 (0.20)
	May-16	0.61 (0.31)	0.61 (0.44)	15.31 (4.67)	3.06 (2.84)
Shellmaker	Jan-16	0 (0)	0.61 (0.31)	0.20 (0.20)	0 (0)
	May-16	0 (0)	4.29 (1.38)	0.20 (0.20)	0 (0)
PCH	Jan-16	4.29 (2.50)	1.43 (1.01)	0.41 (0.27)	0.82 (0.33)
	May-16	2.86 (0.87)	5.31 (2.34)	0.41 (0.27)	0.20 (0.20)
Westcliff	Jan-16	1.02 (0.46)	0 (0)	0 (0)	0 (0)
	May-16	2.04 (0.53)	0.20 (0.20)	0.20 (0.20)	0 (0)

Table 2. Results from ANOVA testing for effects of treatment, date, and the blocking effect of site, plus all two-way interactions on density of *O. lurida* per meter squared at four study locations in Upper Newport Bay, CA in January and May 2016.

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Treatment	35548.0	11849.3	3	0.7	0.5718
Date	500.0	500.0	1	0.3	0.6218
Treatment*Date	5192.8	1730.9	3	1.4	0.3036
Site (random)	38063.2	12687.7	3	0.7	0.5552
Site*Treatment (random)	150906.4	16767.4	9	13.6	**0.0003**
Site*Date (random)	4994.4	1664.8	3	1.4	0.3182

Table 3. Results from ANOVA testing for effects of treatment and the blocking effect of site on cumulative settlement of *O. lurida* at four study locations in Upper Newport Bay, CA from April-August 2016. Bold is a significant effect.

Source	**SS**	**MS Num**	**DF Num**	**F Ratio**	**Prob > F**
Treatment	1.81e+7	6032431	3	1.0187	0.4289
Site(random)	3e+8	1e+8	3	16.9011	**0.0005**

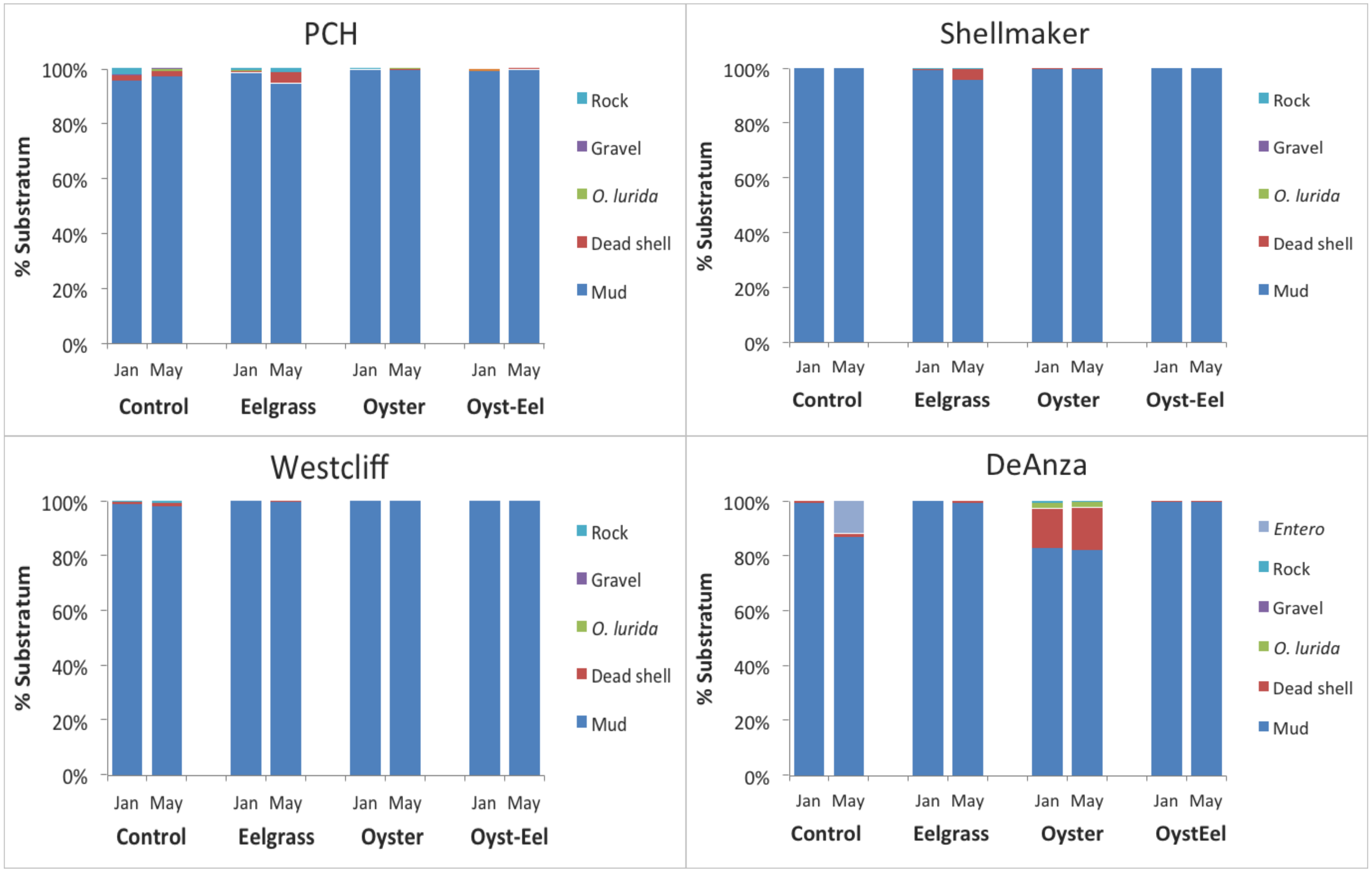

Figure 1. Percentage cover of various substrate types in January and May 2016 prior to oyster shell additions determined via point-contact techniques per treatment (n= 10 quadrats per treatment) at four study locations in Upper Newport Bay, CA.

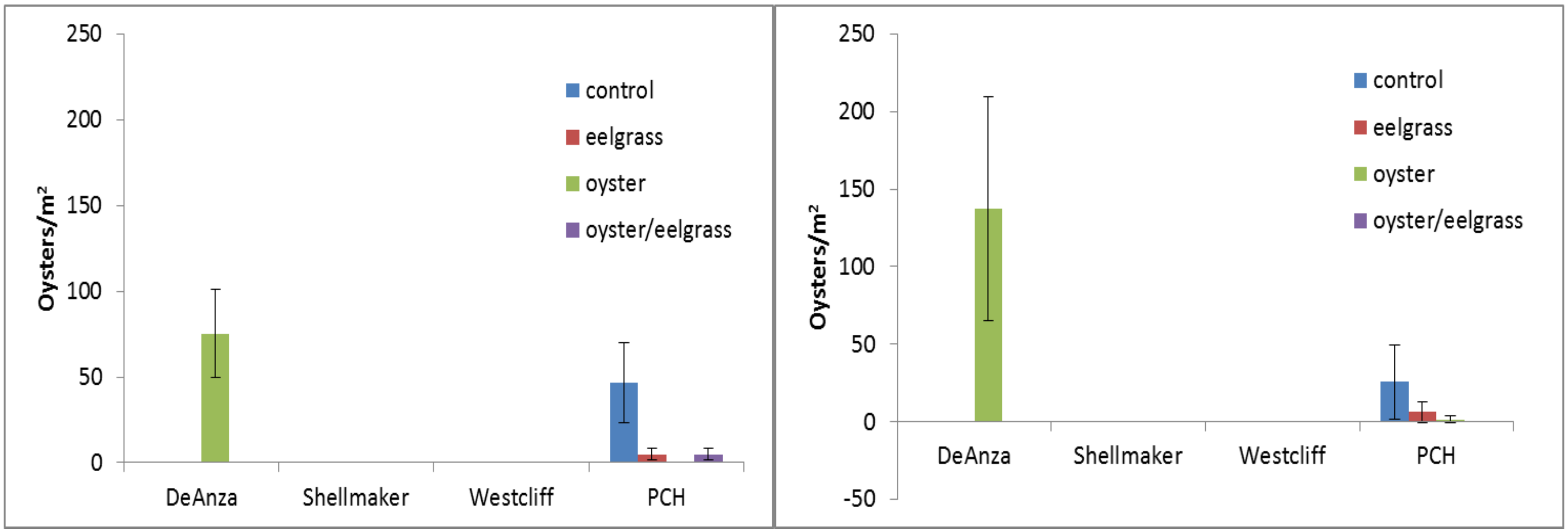

Figure 2. Density (± 1 SE) of oysters (*Ostrea lurida*) per treatment (n= 10 quadrats per treatment) at four study locations in Upper Newport Bay, CA in January 2016 (left panel) and May 2016 (right panel) prior to oyster shell additions.

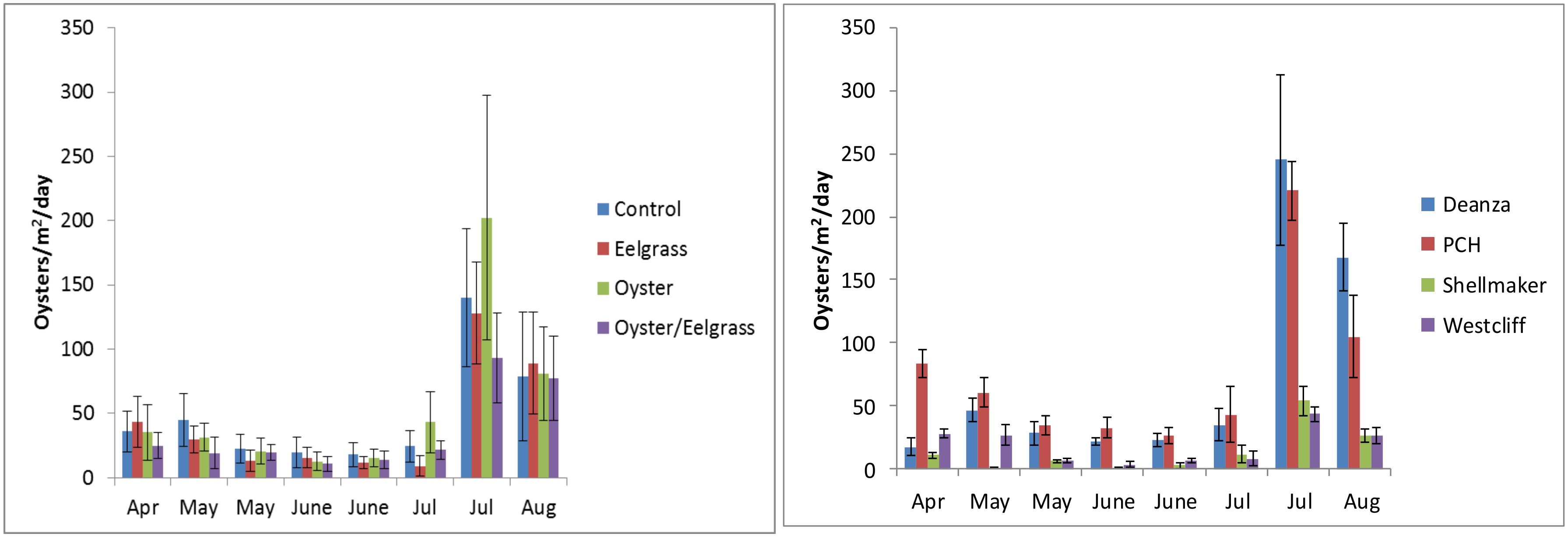

Figure 3. *Ostrea lurida* settlement per m^2 per day (± 1 SE) as a function of treatment (left panel) and site (right panel) in Upper Newport Bay, CA from April-August, 2016 prior to oyster shell additions.

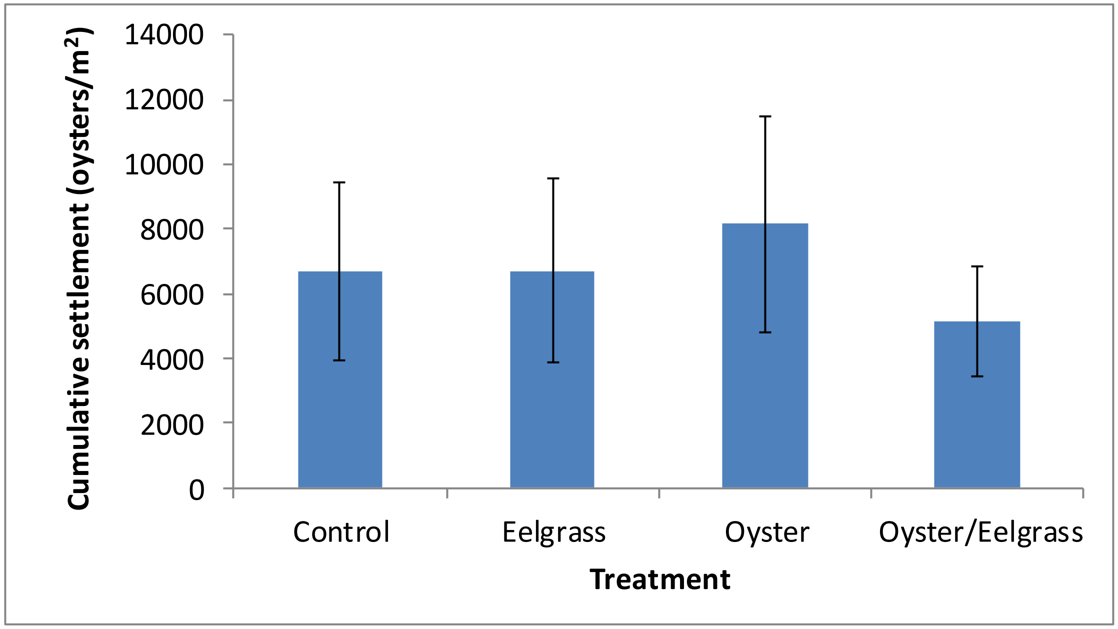

Figure 4. Cumulative *Ostrea lurida* settlement per m^2 (± 1 SE) from April-August, 2016 as a function of treatment in Upper Newport Bay, CA prior to oyster shell additions.

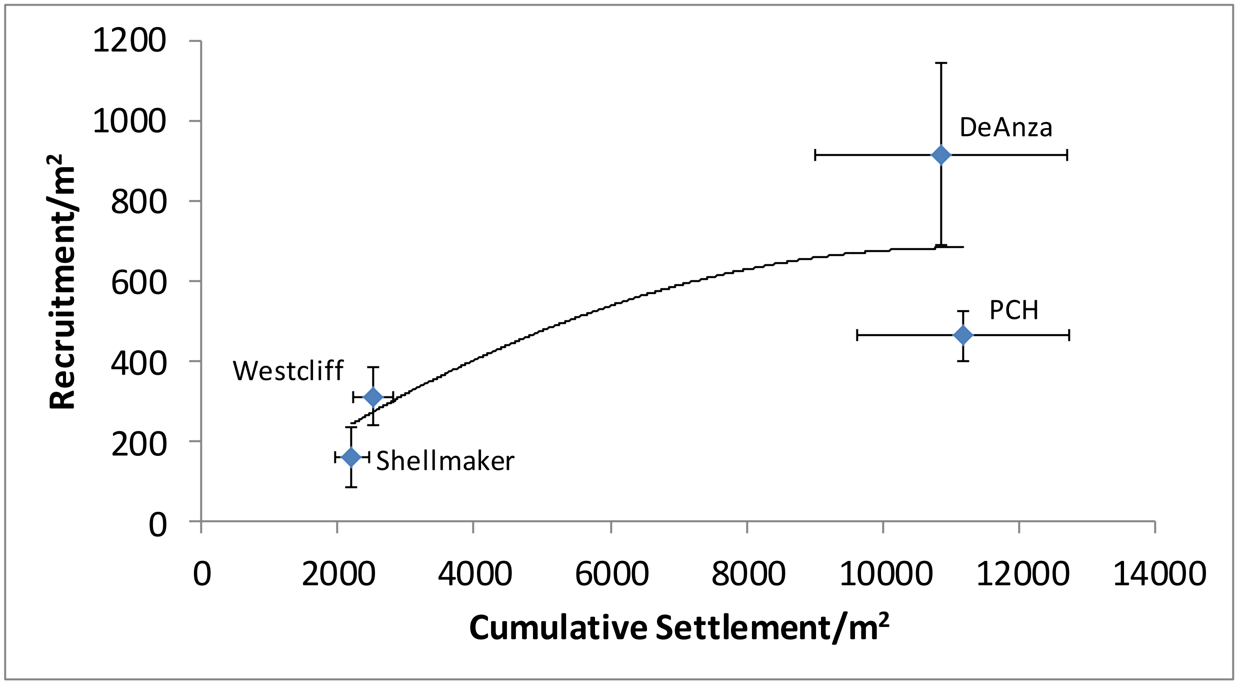

Figure 5. Relationship between cumulative settlement and recruitment of *O. lurida* at Living Shorelines sites in Newport Bay, CA in summer 2016, with best-fit line, $y = -6E\text{-}06X^2 + 0.1228X$, $r^2 = 0.65$, $p<0.001$. Each data point represents a site average (± 1 SE).

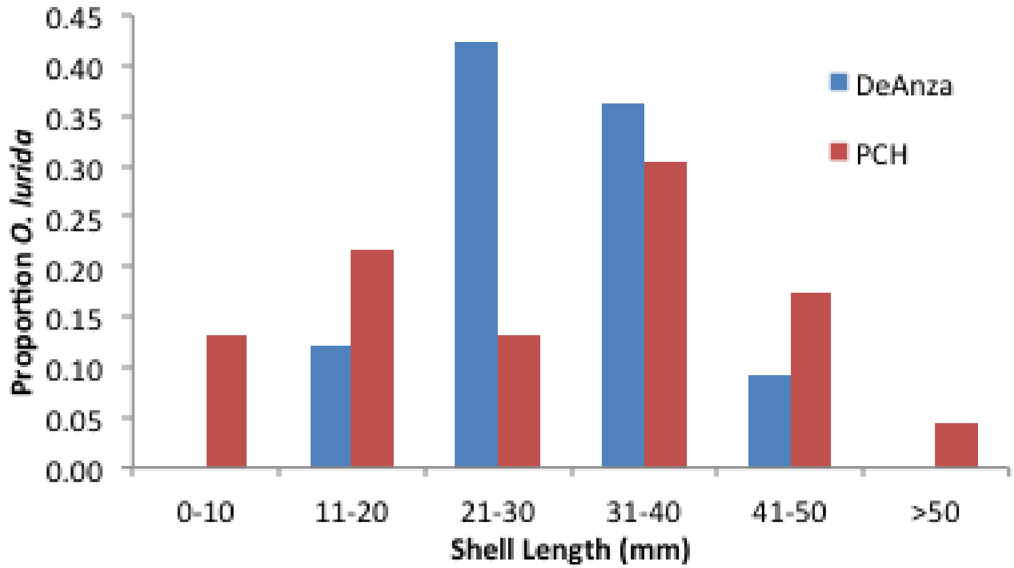

Figure 6. Proportion of oysters (*Ostrea lurida,* n= 66 oysters and 23 oysters at DeAnza PCH, respectively) in 10 mm size class bins based upon shell length at two study locations in Upper Newport Bay, CA in May 2016 prior to oyster shell additions.