

NOT SO SHELLFISH AFTER ALL: HOW NATIVE OYSTERS (*OSTREA LURIDA*) MAY AID EELGRASS (*ZOSTERA MARINA*) RESTORATION BY NITROGEN FILTRATION

A Thesis By

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California State University, Fullerton
Summer, 2022

In partial fulfillment of the degree:

Master of Science in Biology

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DOI:

10.5281/zenodo.6955010

Keywords:

Zostera marina, *Ostrea lurida*, foundation species, nitrogen cycle, restoration, spatial statistics

Abstract:

Eelgrass (*Zostera marina*) is a foundation species in coastal waters that provides vital ecosystem services, from habitat provision to trophic support. However, populations have declined globally at alarming rates within the last century. A recent restoration project within Upper Newport Bay (UNB), California, USA implemented a multi-habitat restoration approach using *Z. marina* and *Ostrea lurida*, which may render additional ecosystem services and simultaneously promote more successful eelgrass restoration. Oysters may increase nutrient availability for eelgrass in sediment porewater by mediating nitrogen transfer via filter-feeding and subsequent deposition of nitrogenous waste. In summer 2021, I collected eelgrass shoots, porewater samples, and shoot and oyster densities from three sites within UNB, each restored with eelgrass alone and eelgrass adjacent to oysters. I measured leaf and rhizome growth rates, organic tissue nitrogen, above and below-ground dry weight, and porewater NH_4^+ and NO_3^- concentrations. Since sedentary species may have limited spatial range of impact, spatially explicit co-variogram models, novel to this field of study, related response metrics to distance from and density of associated oysters in restored beds. I found positive associations among oyster density and eelgrass below-ground dry weight and rhizome growth rates that increased in strength inversely to distance between samples. Porewater ammonium and tissue nitrogen also increased with presence, density, and proximity to oyster beds. For above-ground metrics, including dry weight, growth rates, and shoot density, the relationship between oysters and eelgrass was site-dependent, varying from negative to non-significant to positive. The enhanced eelgrass below-ground growth attributable to interaction with oysters suggests that these species can be restored together in a spatial design similar to that used for this study. To capture the full complexity of oyster-eelgrass interactions, future studies should incorporate spatially explicit models and consider a large suite of both above-ground and below-ground eelgrass metrics.

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ACKNOWLEDGMENTS

I would like to thank numerous faculty, students, and friends who made this work possible.

First, I would like to thank Dr. Zacherl for being a guiding light throughout the many challenges faced both developing and conducting this project. Whether it be fieldwork gone wrong or substantial changes to study design, she always found a way to alleviate the stress and help find the way through the weeds. She was a phenomenal mentor and there a few people who put as much effort into each individual student while mentoring so many at once. Next, I want to thank Dr. Walter for his dedication to the fieldwork required for this project. This project would not have been possible without the extensive help of excellent scientific divers like Dr. Walter who trudge through the silty estuary with too much equipment with precision and ease. Dr. Walter not only had an active part in the fieldwork but also acted as a mentor for me by not only providing advice but also inspiring me to continue a career in marine science. I would also like to thank Dr. Schenk for his expertise in plant biology and helping develop this project from the very beginning.

The many variables of this project required a plethora of other experts who helped make this project possible. Firstly, I want to give Dr. Nichols, of the mathematics department at CSUF, a special thanks for working with me to develop our statistical models. His novel models helped us to discover new and significant findings within the field of estuarine ecology. I thank Dr. Kirby and Dr. Carlin, of the Geology department at CSUF, for letting me use Dr. Kirby's lab and Dr. Carlin for taking the time to help process samples. I would like to thank Dr. Der for also letting me use his lab. I thank Dr. Ruesink at University of Washington for sending me old field equipment needed for my project and Dr. Keller at Chapman University for providing expertise on water sampling methods.

Lastly, I would like to thank my funders, students, friends, and family. California Sea Grant, CSU-COAST, the Honda Marine Science Foundation, OC Coastkeeper, and the Coastal Conservancy all made this project possible through funding and resources. A special thanks to Katie Nichols at OC Coastkeeper for helping directly with fieldwork and managing data and Evyan Sloan at the Coastal Conservancy for proving mentorship and guidance thought out the project. I thank Sean

Eckly for not only helping me become a scientific diver, forever changing my career as a marine scientist, but also for taking the time to come help with fieldwork. I cannot thank Valerie Goodwin and Chelsea Bowers, both master students at CSUF, enough for both the direct help on my project and the mental help throughout this program. I can say with confidence that the experiences I had at my time at CSUF would not have been the same without them. Lastly, I want to thank my mom, Jennifer Emery, and my dad, Aaran Emery, for their continued love and support.

CHAPTER 1

INTRODUCTION

Understanding the roles and interactions of foundation species has become increasingly vital as their global decline reveals losses in important ecosystem functions (Ellison et al. 2005; Waycott et al. 2009; Angelini et al. 2011; Youngquist et al. 2017). Foundation species dominate an ecosystem in biomass and provide essential habitat for other species (MacArthur 1964; Dayton 1972; McDevitt-Irwin et al. 2016). Often, ecosystems support multiple foundation species that engage in complex species interactions (Angelini et al. 2011; Gedan et al. 2014). Many of these interactions are fundamentally biogeochemical in nature, facilitating chemical cycles in soils, sediments, and vegetative biomass (Reddy and DeLaune 2008). Estuaries and wetlands are at the forefront of these foundational interactions, possessing large quantities of vegetative marshland and seagrass meadows.

Eelgrass (*Zostera marina*) is an aquatic angiosperm that is distributed widely in the Northern hemisphere and exists in many US West Coast estuaries. Eelgrass is a foundation species that provides vital ecosystem services such as habitat provision and shoreline protection (Beck et al. 2001; Orth et al. 2006; Koch et al. 2006; Canion and Heck 2009). Eelgrass is also presumed to be an ecosystem engineer (Jones et al. 1997) because of its ability to alter abiotic conditions of the local environment through nutrient cycling and pH regulation, in addition to providing trophic support, and enhancing community structure (Van Der Heide et al. 2007). However, despite its importance, populations of eelgrass have declined on a global scale at rates estimated to exceed those of tropical rainforest deforestation (Waycott et al. 2009). Of losses within the US, some California bays have experienced upwards of 95% loss to local extirpation (Ware and Rossmiller 2005; U.S. Department of the Interior 2017; Harenčár et al. 2018). The most likely causes of decline are anthropogenic activities, including coastal development (Orth et al. 2006), nutrient loading from urban runoff (Orth et al. 2006; Waycott et al. 2009; Grech et al. 2012), and recreational use of bays (Waycott et al. 2009; West 2012; Dapuerto et al. 2022). Eutrophication and sediment smothering/burial by urban runoff are

thought to be the most significant causes of eelgrass loss in estuaries (Short et al. 1996; Lee et al. 2004).

Many bivalve species are also important foundation species in estuaries that provide a positive impact on local eelgrass beds in nutrient-loaded environments by filter-feeding nitrate-fixing phytoplankton, algae, detritus, and bacteria (Gosner 1971). Filter-feeding activity mitigates the impacts from eutrophication, and forms bio-deposits of nitrogen from suspended particles (seston) in the water column, which significantly impact sediment nutrients and increases light penetration (Kraeuter 1976; Jordan & Valiela 1982; Gedan et al. 2014; Bilkovic et al. 2017). The bio-deposits are expelled through bivalves' feces and pseudo-feces made of particulate organic matter rich in organic nitrogen (Newell et al. 2005; Booth & Heck 2009). This nitrogenous waste product eventually sinks within the sediments after suspension due the mucous layer that it is packaged with, then undergoes mineralization, ammonification, and nitrification, producing bioavailable forms of nitrogen as ammonium and nitrate for uptake by vegetative roots (Heollien & Zarnoch 2014; Bilkovic et al. 2017). Estuary and saltmarsh vegetation productivity correlates positively with enrichment by nitrogenous nutrients (Tyler 1967; Valiela et al. 1978), and there are direct positive relationships between mussels (*Geukensia demissa*) and cordgrass (*Spartina alterniflora*) (Bertness 1984; Kabala 2020) and oysters (*Crassostrea virginica*) and seagrass (*Halodule wrightii*) (Booth & Heck 2009). This ability to directly facilitate nutrient cycling, however, has been attributed to a range of only several meters; importantly, this observation of limited spatial impact is based upon limited unpublished data (Booth & Heck 2009).

Ammonium is the primary form of nitrogen for absorption by eelgrass, though smaller proportions of nitrate uptake have been observed (Short 1984). Artificial enrichment of ammonium within sediment porewater correlates positively with eelgrass growth metrics and reproduction (Orth 1977; Short et al. 1987; Peralta et al. 2003; Carroll et al. 2008; Jackson et al. 2017); Therefore, by enhancing the sediment nutrients, bivalves may also have this same impact. Oysters not only may directly influence growth of eelgrass but also indirectly by reduction of competitive species. Fast-

growing, nutrient-absorbing species like plankton, algal epiphytes, and *Ulva* sp. have deleterious effects on eelgrass biomass and shoot production (Olyarnik & Stachowicz 2012). Therefore, bivalves that filter nutrients within the water column may reduce competition between eelgrass and planktonic autotrophs and algal epiphytes. (Pederson & Borum 1992).

There is much discussion on the primary mode by which seagrasses absorb nutrients; for *Zostera* species, the roots were thought to be the primary site for nutrient uptake (Short & McRoy 1984); however, others have speculated that the leaves and internal recycling are equally or more important (Lizumi and Hattori 1982; Hemminga et al. 1994; Kraemer and Mazzella 1999; Sandoval-Gil et al. 2016; Pernice et al. 2016). Root absorption is now thought to be the primary source only under limited conditions, such as when water column nitrogen is depleted (Nayer et al. 2018) or when seagrass is outcompeted by other macroalgal species (Vonk et al. 2008).

While much is known about nitrogen dynamics between seagrass and bivalves, few have examined oysters and eelgrass interacting *in situ*. Prior studies have focused on eelgrass metrics relative to oyster presence (Wall et al. 2008; Briley et al. 2021); however, there is a lack of focus on the effects by nitrogen transfer *in situ*. Thus far, studies have used mesocosms and direct manipulation of nitrogen (e.g., Short 1987; Booth & Heck 2009; Sandovil-Gil et al. 2016; Bilkovic et al. 2017; Ferriss et al. 2019; McIver et al. 2019), but few have made *in situ* observations of nitrogen flux between species, therefore questions remain about how bivalves may impact neighboring eelgrass growth.

Oysters, like eelgrass, are also an estuarine foundation species, having an effect on the local community (Gedan et al. 2014) via habitat provision, trophic support, and sediment accretion. Oyster reef restoration may be of critical importance within compromised estuaries due to the oysters' ability to filter seston from the water column to the benthos at significant rates relatively soon after restoration. More established beds may achieve full estuary filtration. However, the oyster native to the US West Coast, the Olympia oyster (*Ostrea lurida*), has been estimated to filter at slower rates than the residence time of water in west coast estuaries (Peter-Contesse & Peabody 2005; Zu

Ermgassen et al. 2013). Nevertheless, the construction of oyster reef habitat also increases the recruitment of other bivalve species (Grabowski et al. 2005), which may further enhance overall filtration rates beyond the scope of oysters alone (Gedan et al. 2014). Importantly, these other bivalve species may also contribute to the nitrogen pool (Newell 2004; Smyth et al. 2017).

Multi-Habitat Restoration

Restoration efforts for both oysters and eelgrass are underway in response to losses in both populations along the coast of California, USA, including within Upper Newport Bay, Newport Beach. A collaborative team from California State University (CSU) Fullerton, CSU Long Beach, and Orange County Coastkeeper has implemented combined eelgrass and native oyster restoration in Upper Newport Bay to explore if multi-habitat restoration yields significantly greater instances of successful restorations via potential reciprocal benefits compared to restoring the eelgrass and oyster habitats in isolation. For example, photoautotrophic species such as eelgrass may improve the carbonate chemistry of the water for calcifiers, like oysters, as well as raise the pH through the uptake of CO₂ for photosynthesis (Bauman and Smith 2017; Wahl et al. 2018). This process progressively reduces the environmental stress on the oysters, allowing for more energy to be put into reproduction rather than focusing on shell growth. Oysters, as discussed above, may provide increased light attenuation by reducing suspended particles via filter feeding, and may provide sediment porewater nitrogen enrichment for the eelgrass rhizosphere (roots and rhizomes) through filtration and excretion. Eutrophication via runoff has contributed to substantial declines of eelgrass (Short et al. 1996) and Newport Bay, likewise, has suffered from nutrient loading from significant amounts of runoff during the wet season from adjacent highly urbanized areas (Taylor 1999; Pednekar et al 2005; Jeong et al. 2005). It is crucial, therefore, that we find natural methods to mitigate this addition of excess nutrients for the benefit of submerged aquatic vegetative species like eelgrass.

Native eelgrass has been co-occurred with the native *Olympia* oyster populations historically in Pleistocene fossil records in northern California (Miller & Morrison, 1988), and both habitats have historically been present throughout southern California estuaries (Dall 1914; Bruff 1946; Ware 1993).

The Living Shorelines project planted beds of eelgrass totaling approximately 1,280 m² in June 2016, and then correspondingly placed beds of oysters totaling around 240 m² in April 2017, with half of the oyster beds adjacent to and up-shore of the eelgrass plots (Wood 2018). This unique design provides an opportunity to observe the impacts oysters may have on eelgrass and their nitrogen resources. The separation of the two species provides a unique opportunity to study the spatial extent of impact by the oysters. In this study, I observed the effect of nitrogen enrichment by oysters on sediment pore-water, eelgrass nitrogen absorption, and a variety of eelgrass growth and productivity metrics. Specifically, four hypotheses were tested in order to assess this relationship.

Hypotheses

H1: Greater sediment porewater nitrogen concentrations will be found near restored oyster beds, increase with oyster density, and decline with distance from the beds.

H2: Percent dry weight nitrogen will be significantly greater in eelgrass root and leaf tissue near restored oyster beds, increase with oyster density, and decline with distance from the beds.

H3: Greater sediment porewater and eelgrass rhizome nitrogen concentrations will correlate with increased eelgrass bed productivity.

H4: Eelgrass beds will show significantly higher densities, growth rates, and biomass when adjacent to restored oyster beds versus in isolation, increase with oyster density, and decline with distance from the beds.

CHAPTER 2

METHODS

I conducted my study at three sites: Westcliff Beach (33°37'16.81" N, 117°54'7.29" W), Pacific Coast Highway (33°37'9.55" N, 117°54'17.41" W, hereafter PCH), and DeAnza (33°37'13.18" N, 117°53'54.81" W) within the Upper Newport Bay MPA (Figure 1). Though originally there were four restoration sites planned for this study, the site Shellmaker lost all eelgrass coverage prior to the study in 2019. Each metric was studied at three sites in each of 2 treatments (Figure 2) -- eelgrass transplanted with oyster beds up-shore and adjacent versus eelgrass transplanted in isolation. Oyster beds were constructed in April 2017 using cured *Crassostrea gigas* shell wrapped up in bags of coir bags laid out in a 20m x 1.5m plot approximately 2 meters up-shore of the eelgrass beds. Eelgrass beds were transplanted in June 2016 using shoots from a nearby donor bed. Shoots were bundled by twine and anchored by a popsicle stick each a meter apart within a 20m x 8m plot.

I measured porewater nitrogen, % nitrogen in eelgrass tissue, above and below-ground dry weight, leaf and rhizome growth rates, and eelgrass and oyster densities in July of 2021.

Nitrogen

To measure porewater nitrogen, I collected sediment cores (2.5 cm diameter) in the top 15-20 mm of the sediment via snorkeling. Cores were then capped to prevent contamination by the water column. The depth of core samples was chosen to emulate the depth of the rhizosphere (Pedersen and Borum 1992). A core was taken every 3 meters at the offshore edge of the oyster bed or mudflat and then every 2 meters going offshore along three transects (n = 20 per bed, Figure 3). This sample design was chosen in order to observe and validate the speculated distance limitation of oyster impact (Booth & Heck 2009). Samples were immediately placed on ice for transport back to the lab. The contents of the cores were then transferred to 50 ml falcon tubes for separation of water and sediment by centrifugation (3000 rpm x 20 min). The water was then extracted by 30 ml syringes and filtered through 0.45-micron filters into 20 ml scintillation vials and frozen. Samples were sent on dry ice to UCSB MSI Analytical Laboratory for ammonium and nitrate analysis.

I conducted a pilot study of ammonium sampling in an intertidal area to determine the accuracy of my coring method. In this study, we used three techniques to sample: piezometers (gifted from the Ruesink Lab at U Wash.), cores, and syringes of overlying water. We considered the piezometers the most accurate since they are sealed off from overlying water. However, this sampling method was not feasible for the subtidal sampling required by this study. I used a pairwise t-test to determine the accuracy of the cores with the piezometers. I did the same with the syringes to justify that the cores were capturing porewater and not overlying water. The coring method proved comparable relative to the piezometer method (p-value: 0.3997) and significantly different from the syringes (p-value: 0.0006).

To measure the organic nitrogen content within eelgrass tissue I collected shoots along with their rhizomes. Shoots were collected via SCUBA along six transects laid out 10 meters going offshore. A shoot was collected either at 1, 2, 6, and 10 meters or 1, 2, 4, and 8 meters, alternating by transect (n = 24, Figure 3). Shoots were placed within a cooler with ambient water and taken back to the lab and frozen. Samples were separated into above and below-ground material before preparation. Each sample was dried (70°C x 29 hours), ground, and placed in a COSTECH elemental analyzer in the Kirby Lab at CSUF to measure the total percent dry weight nitrogen (% mg DW N).

Growth Rates

Growth rates were quantified using the pinhole plastochrone method derived from Short and Duarte (2001). This method involves using a safety pin to prick a hole, as a marker, through the sheath of an eelgrass shoot *in situ* and then returning after the estimated plastochrone interval (the approximate length in time it takes for a new leaf to grow) for collection, which for this study was approximately eleven days. Shoots were marked using flagging tape and landscaping flags in July of 2021 during peak growing season for later retrieval via SCUBA to calculate growth rate and subsequent processing for eelgrass tissue nitrogen. To measure the growth rates of collected shoots I first cleaned the blades of all epiphytes using razor blades for encrusting organisms and cotton swabs for loose algae. I then determined the order of blade age by the position of the hole on each

blade. Due to the differential growth based on age, newer blades had a larger distance between the hole on the blade and the original scar on the sheath. Growth rate was then measured by dividing the mass of the youngest fully mature blade, the 3rd youngest for *Zostera*, by the plastochrone interval. This is calculated by multiplying the number of shoots collected by the number of days passed between marking and collecting, then dividing by the total number of new leaves found in the collected shoots.

Biomass

Above and below-ground biomass per shoot was measured in all the shoot collections. Before weighing, shoots were thawed, rinsed, and removed of epifauna and epiphytes using razor blades and cotton swabs. The shoots were then dried at 70°C for 29 hours in a vacuum oven before weighing. Weights were measured to the thousandth of a gram.

Density

To measure eelgrass density, I used quadrat (0.25 m × 0.5 m) sampling via SCUBA in May of 2021. Six transects, each 3 m apart, were laid out perpendicular to shore at the up-shore edge of the eelgrass beds, and quadrats were placed every 2m along the transects (n = 30) in each bed. Individual shoots were counted within the quadrats by the meristem, and counts were converted to shoots/m².

For oyster density, measurements were also taken via quadrats (0.25 m x 0.25 m) where all hard material within the quadrat was excavated, placed into plastic bags, and transported to the lab and frozen. Ten coordinates were randomly generated at 2-meter intervals along the length of each oyster bed. In the lab, samples were thawed, washed of sediments over a 0.5 cm mesh sieve, and sorted for oysters containing tissue within the valves. Counts were converted to oysters/m².

Statistical Analysis

The first, second, and fourth hypotheses (H1, H2, and H4) were addressed via two-way ANOVAs to determine if relevant response variables for each hypothesis (concentration of nitrogen within the sediment porewater, concentration of dry weight nitrogen, eelgrass density, reproductive

output, growth rates, and biomass) were different depending on whether or not they were adjacent to restored oyster beds with site as a factor. Normality and homoscedasticity, conditions necessary to fulfill assumptions of ANOVA, were assessed using Wilks-Shapiro and Levene tests respectively ($\alpha = 0.05$). Data were transformed accordingly, as needed, prior to ANOVA testing. The appropriate transformation was determined by best fit for Wilks-Shapiro and Levene tests; transformations including $\text{Log}(x+1)$, $\text{Log}(X)$, and square root transformation.

To assess the spatial range of impact of oyster density on the relevant response variables from H1, H2, and H4, a significantly more complex statistical modeling effort was necessary. Dr. Kevin Nichols, a spatial statistician solicited for consulting, helped develop the strategy for assessing spatial impact as a function of distance between response variables and oyster density. First, because of the spatial sparsity of data, kernel regression was used to smooth oyster densities over their respective spatial domains (see Helmstetter et al., 2006 for example). Kernel estimates were constructed using a bivariate Gaussian kernel with bandwidth matrices selected via fivefold cross-validation with a minimum sum of squared residuals loss function. Next, to assess the effective distances of impact, spatial covariograms were constructed between each response variable and smoothed oyster density over the spatial constraints of the restored oyster beds (Cressie 2015). Due to the highly volatile nature of response variables, covariogram estimates were smoothed using a LOESS smoother. While this provides descriptive/graphical evidence of an underlying spatial covariance structure between response variables and oyster density, this fails to address the statistical significance of my findings.

In order to create an ad-hoc graphical statistical test, data were re-simulated under the null hypothesis (that there is no spatial covariance structure underlying respective response variables and oyster density) via a permutation simulation, i.e., where the spatial coordinates of the oyster densities were randomly relocated in their spatial domain. Co-variograms were replicated under the simulated data 10,000 separate times. At a given distance (h), 95% confidence/critical values for the covariograms were estimated empirically using the simulated distributions under the null hypotheses. Interpretation of covariogram results in relation to the hypotheses was determined by observing the

relationship to the confidence intervals and the trend within the 2 – 5 m range (Figure A1). This range was chosen since oyster and eelgrass beds were spaced approximately 2 meters apart, therefore data within the 0-2 m range is highly extrapolated. The proposed limited range of oyster impact to only several meters combined with covariogram results of oyster impact on porewater ammonium (see results and Figure 4 below) support the chosen range of impact (2 - 5 m) for the mechanism of ammonium enrichment being examined in this thesis.

CHAPTER 3

RESULTS

Porewater Nitrogen

Ammonium within the porewater core samples varied from 8.6 μmol to 230.63 μmol with an average of $103.89 \pm 6.6 \mu\text{mol}$ in the control-eelgrass treatments and $110.46 \pm 6.2 \mu\text{mol}$ in the oyster-eelgrass treatments. Ammonium levels varied significantly across the three sites with average ammonium at PCH ($145.31 \pm 6.27 \mu\text{mol}$) nearly double that of Westcliff ($70.69 \pm 6.14 \mu\text{mol}$) and 1.5 $\times$ that of DeAnza at $104.80 \pm 6.00 \mu\text{mol}$ (Figure 5, Table 1, two-way ANOVA, site, $p = 4.96\text{e}^{-14}$). There was no significant difference between treatments nor any interaction effects (Table 1). Covariogram analysis, which relates the compared metrics spatially, revealed significant (95% confidence) associations between elevated oyster density and ammonium concentrations across all three sites (Figure 4). As expected, this effect was greatest at closer distances and reduced as distance increased but stayed significantly positive until approximately 6 meters of distance from the oyster beds.

Nitrate within the porewater core samples varied from 0.035 μmol to 12.99 μmol with an average of $1.61 \pm 0.28 \mu\text{mol}$ in the control-eelgrass treatments and $1.84 \pm 0.3 \mu\text{mol}$ within the oyster-eelgrass treatments. DeAnza, on average, had greater than 4 $\times$ ($2.57 \pm 0.47 \mu\text{mol}$) the nitrate levels compared to PCH ($0.74 \pm 0.08 \mu\text{mol}$). Westcliff also had greater than double the nitrate levels at DeAnza with an average of $1.84 \pm 0.32 \mu\text{mol}$ (Figure 6). Nitrate levels varied significantly across sites but did not show significant differences between treatment nor were there any interaction effects (Table 2, two-way ANOVA, site, $p = 8.88\text{e}^{-4}$). Covariogram analysis of nitrate concentrations varied across sites (Figure 7). Both PCH and DeAnza showed significant reductions in nitrate in relation to the oyster density at the closest ranges, however, DeAnza saw a significant spike in nitrate between the 3 to 6-meter range and PCH nitrate steadily increased within the same range but was still a significant negative association. Westcliff nitrate was significantly positive at the closer ranges and steadily decreased with distance.

Tissue Nitrogen

Leaf dry weight tissue nitrogen varied from 1.25 % of dry weight N to 2.29 % of dry weight N per 0.1 g sample with an average of 1.75 ± 0.03 % dry weight N in the control-eelgrass treatments and 1.85 ± 0.03 % dry weight N in the oyster-eelgrass treatments. DeAnza and PCH had on average greater than 0.1 % of dry weight nitrogen in the oyster-eelgrass treatments compared to their associated control-eelgrass treatments, while WC oyster-eelgrass increased by 0.01% (Figure 8, Table 3, two-way ANOVA, treatment, $p = 0.015$); no site effect was observed. Covariogram analysis revealed significant (95% confidence) associations between elevated oyster density and eelgrass leaf dry weight tissue nitrogen across all three sites (Figure 9), but the positive effect was constrained to closer distances and the effect reduced and became negatively correlated as distance increased.

Rhizome dry weight nitrogen varied from 0.29 % dry weight N to 1.11 % dry weight N per 0.1 g sample with an average of 0.74 ± 0.02 % dry weight N in the control-eelgrass and 0.72 ± 0.03 % dry weight nitrogen in the oyster-eelgrass treatments. Westcliff was the only site to have a greater mean eelgrass Rhizome dry weight nitrogen by about 4 hundredths of a percent, while other sites showed greater nitrogen concentration in the control-eelgrass treatment from a tenth of a percent (DeAnza) to less than a hundredth of a percent (PCH) (Figure 10). No treatment or site effects were observed for eelgrass Rhizome dry weight nitrogen (Table 4). Co-variogram analysis revealed significant (95% confidence) associations between elevated oyster density and eelgrass rhizome dry weight nitrogen at closer distances that steadily reduced with distance at DeAnza and dramatically dropped after 4 meters at Westcliff (Figure 11). PCH showed a reverse trend with a significantly negative association up until 4 meters where it then become significantly positive (Figure 11).

Growth Rates

Eelgrass leaf growth rates varied from 5.48 mg per day to 37.58 mg per day with an average of 16.04 ± 0.83 mg per day in the control-eelgrass treatments and 17.76 ± 0.73 mg per day in the oyster-eelgrass treatments. The oyster-eelgrass and control-eelgrass treatments at DeAnza varied significantly from each other and all other beds where the rest were statistically indistinguishable

(Figure 12, table 5, two-way ANOVA, site $\times$ treatment interaction, $p = 0.041$). Blade growth rates varied among sites, with an approximately 2 $\times$ greater growth rate at PCH and Westcliff (18.92 ± 0.68 & 20.12 ± 1.09 mg per day respectively) compared to DeAnza (11.48 ± 0.53 mg per day). Covariance analysis at DeAnza suggested a weak negative association between oyster density and blade growth rate at distances between 1 to 4 meters and greater than 6 meters (Figure 13). No significant association was found between distances of 4 to 6 meters. Covariance at Westcliff and PCH, however, suggested a positive association up to about 4 meters that then became weakly negative.

Rhizome growth rates varied from 1.11 mg per day to 3.17 mg per day with an average of 4.22 ± 0.3 mg per day in the control-eelgrass treatments and 6.16 ± 0.43 mg per day in the oyster-eelgrass treatments. Eelgrass had greater rhizome growth rates in the oyster-eelgrass treatment compared to control-eelgrass, but this effect could not be differentiated at Westcliff and PCH (Figure 14, Table 6, two-way ANOVA, site $\times$ treatment interaction, $p = 0.049$). At DeAnza the oyster-eelgrass treatment had approximately 2 $\times$ (7.99 ± 1.07 mg per day) the growth rate than the control eelgrass (4.08 ± 0.63 mg per day). Covariance analysis at DeAnza showed a strong association between oyster density and rhizome growth rate at distances between 1 to 5 meters (Figure 15). PCH showed a similar trend to DeAnza with a positive covariance at distances between 1 to 4 meters and 7 to 10 meters (Figure 15), while Westcliff had a highly variable trend with a positive association between 4 to 7 meters. Rhizome growth rate also showed a significant but very weak association with leaf dry weight tissue nitrogen concentration (Figure 16, $p = 0.005$, $r^2 = 0.06$).

Biomass

Eelgrass above-ground dry weights varied from 0.099 g to 0.647 g per shoot with an average of 0.126 ± 0.1 g in the control-eelgrass treatments and 0.118 ± 0.1 g in the oyster-eelgrass treatments. Above-ground dry weight varied greatly between sites but did not show any difference between the treatments (Figure 17). DeAnza (0.19 ± 0.01 g) had the lowest mean above-ground dry weight with PCH (0.37 ± 0.02 g) more than double that and Westcliff (0.25 ± 0.01 g) in the middle. There were no significant differences between treatments at any one site alone, however, statistical

differences were found between certain treatments across sites (Figure 17, Table 7, two-way ANOVA, site $\times$ treatment interaction, $p = 0.044$). Covariance analysis suggested strong negative associations between oyster density and above-ground dry weight at all sites except Westcliff which showed a weak positive association at the closest distances and quickly became non-significant (95% confidence). The weak negative trends were strongest at closer distances between oyster and eelgrass (1-4 meters) and steadily decreased in strength with distance. PCH showed the strongest negative covariance of the sites (Figure 18).

Eelgrass below-ground weights varied from 0.036 g to 0.404 g per shoot with an average of 0.143 ± 0.01 g in the control-eelgrass treatments and 0.171 ± 0.01 g in the oyster-eelgrass treatments. Below-ground dry weight varied significantly across the three sites with the highest average weight at PCH (0.17 ± 0.01 g) followed by Westcliff (0.16 ± 0.01 g), and lastly DeAnza (0.13 ± 0.01 g) (Figure 19, Table 8, two-way ANOVA, site, $p = 0.001$). PCH and DeAnza both showed positive covariance between oyster density and below-ground dry weight (Figure 20). This covariance was strongest with distances of 1 to 4 meters between oysters and eelgrass. Westcliff showed negative covariance up to 4 meters which then turned positive until approximately 7 meters where it returned negative (Figure 20). Eelgrass below-ground weight positively correlated with both above-ground (Figure 21, $p = 2.0e^{-4}$, $r^2 = 0.1$) and below-ground (Figure 22, $p = 0.02$, $r^2 = 0.04$) tissue nitrogen concentrations, but was highly variable for both.

Eelgrass Density

Eelgrass density varied among quadrats from no observed eelgrass to 346 shoots per m^2 with an average of 98.58 ± 6.6 shoots per m^2 in the control-eelgrass treatments and 62.53 ± 5.96 shoots per m^2 in the oyster-eelgrass treatments. DeAnza (142.4 ± 9.43 shoots per m^2) and PCH (113.3 ± 11.4 shoots per m^2) both showed double the eelgrass density in the control-eelgrass treatment while Westcliff showed no treatment effect treatment (Figure 23, Table 9, two-way ANOVA, site $\times$ treatment interaction, $p = 1.26e^{-3}$). The Westcliff control-eelgrass treatment (40 ± 4.64 shoots per m^2) showed the lowest mean density, and the greatest was at DeAnza control-eelgrass (142.4 ± 9.43 shoots per

m²). Treatment covariogram analysis revealed a strong negative covariance between eelgrass and oyster density at PCH and DeAnza until approximately 4-5 meters where it then became positive at DeAnza and non-significant at PCH (Figure 24). Westcliff covariance suggested the opposite trend showing a positive covariance between 3 to 8 meters.

CHAPTER 4

DISCUSSION

Paired restoration of Olympia oysters and eelgrass influences local nitrogenous nutrients and promotes more significant below-ground eelgrass growth; however, it may have the reverse effect on above-ground biomass. This is the first study to observe *in-situ* spatially explicit nitrogen dynamics of up-shore restored *Ostrea lurida* on *Zostera marina* growth and production. To observe the largest potential of this interaction, I conducted the study in the summer during a period when eelgrass growth is considered to be the highest (Orth et al. 2007) while coinciding with high oyster filtration (Ermgassen et al. 2013) and the lowest environmental influence on nitrogen input.

Spatially Explicit Data Analysis

In the field of ecology, the need for geospatial models has become more evident, especially when studying organisms in a spatially explicit arrangement such as in soil science and plant ecology. The classic method of study was to group or classify samples in one-dimensional intervals and run statistical models on the means; however, oftentimes these variables can be continuous and random where spatial gradients are ambiguous (Webster et al. 2005). The relatively low resolution of observing means in ecology has the risk of missing important variables, making multivariable smoothed predictive models like kriging or kernel regression valuable (Webster et al. 2005; Dray et al. 2012; Pommerening et al. 2022). Recent studies in seagrasses have demonstrated the importance of spatially explicit models when studying hydrodynamics, resource allocation, and meadow structure (Morris et al. 2008; Carr et al. 2016).

We suspected that oyster impact on highly variable eelgrass metrics within beds would be spatially limited and, indeed, it was increasingly evident that spatially explicit models returned these patterns that were not revealed via traditional ANOVA frameworks that averaged impact across space. In pilot analyses, even when we incorporated “distance from bed” into ANOVAs, my tests failed to reveal patterns that were subsequently revealed when co-variogram analysis incorporated non-linear spatial data. Each eelgrass bed showed high variability in most metrics among samples

regardless of treatment. This high variability is likely why we could not observe significant patterns when averaging variances within whole beds. This data-shadowing effect by shoots, possibly independent of oyster influence, may be why a refined spatially explicit model is better suited to observe real impact for sedentary species. The use of these models is new relative to studies on submerged aquatic vegetation and invertebrate interactions. Spatially explicit covariogram analysis should be used in further studies involving seagrass given their dynamic nature.

Porewater Nitrogen

Ammonium concentrations in the porewater cores were highly correlated with increasing oyster density and proximity to the oyster beds across all sites. This result supported my hypothesis that oyster feces and pseudofeces would influence local ammonium (Kraeuter 1976; Jordan & Valiela 1982; Gedan et al. 2014; Bilkovic et al. 2017). This effect was not visible, however, across treatments (e.g., eelgrass versus oyster-eelgrass) using a standard ANOVA approach that averaged samples across entire eelgrass beds. The relative homogeneity between treatments despite a significant association with oyster density is discrepant with the observation that these hotspots are undetectable when measured as means of entire beds. This discrepancy again points to the importance of working with spatially explicit models.

Another possible cause of my observations of elevated porewater ammonium concentrations may be the abundance of other bivalves that recruited to the oyster and eelgrass beds (Figure A2). The oyster beds commonly recruited other bivalves such as *Musculista senhousia*, *Modiolus* sp., *Mytilus galloprovincialis*, *Crucibulum spinosum*, and *Argopecten ventricosa*. Many eelgrass samples also contained settled invasive *M. senhousia* on the roots though their abundances were not recorded. Consideration of other bivalves in future studies should be noted because it likely added unexplained variation to my data, and their impact has the potential to overshadow the oyster contribution to the nutrient pool (Newell 2004; Smyth et al. 2017). Importantly, the other bivalves were almost non-existent in the area prior to the construction of the oyster beds (Figure A3), and all are directly associated with the beds, so regardless of which species was elevating porewater

ammonium, the exercise of restoring oyster beds likely directly contributed to the elevated ammonium recorded in the porewater.

Tissue Nitrogen

There are three pathways by which eelgrass obtains inorganic nutrients—via absorption by the roots (Short & McRoy 1984), diffusion through leaves (Hemminga et al. 1994; Pernice et al. 2016), and reclamation from senescing leaves (Sandoval-Gil et al. 2016). The prioritized pathway depends on the environmental conditions and the overall nitrogen budget of the plant. These nitrogenous nutrients are thought to be absorbed primarily through the leaves in the form of ammonium in most cases (Lizumi and Hattori 1982; Hemminga et al. 1994; Kraemer and Mazzella 1999; Pernice et al. 2016). In this study, higher concentrations of nitrogen within the eelgrass leaf tissue were strongly correlated with the presence of oyster beds across all sites (Table 3). Though I observed elevated nitrogen within the leaves, it is difficult to say whether this was the pathway of absorption.

Overall, these results supported my hypothesis that elevated eelgrass nitrogen would correlate with nearby restored oyster beds, and as expected by prior studies, this trend was primarily within the leaves despite elevated nitrogenous nutrients observed in the porewater (Lizumi and Hattori 1982; Vonk et al. 2008; Nayer et al. 2018). Hemminga et al. (1994) found that eelgrass leaves may absorb nutrient-rich porewater and overlying water at low tide when lying in pools. My spatial data may support this finding since the seaward portions of the beds are permanently fully submerged and would not be able to take advantage of this phenomenon while the shoreward portions of the beds closest to the oysters are regularly emersed during low tide. Because eelgrass is able to translocate nutrients between above and below-ground material, we cannot definitively say that the eelgrass is primarily taking up nitrogen through the leaves as opposed to the roots followed by translocation (Lizumi and Hattori 1982; Kraemer and Mazzella 1999; Vonk et al. 2008; Sandoval-Gil et al. 2016; Nayer et al. 2018). Nitrogen is likely to be limiting within the water column in this system during the dry season, typically June, July, and August (Pednekar et al. 2005), due to the terrigenous nature of the local geology and lack of inputs from upstream during storm events. This makes translocation

necessary if more ammonium is obtained through the roots. This may mean that since the oysters are increasing ammonium resources within the porewater it is allowing further nitrogenous nutrient uptake not previously obtainable during either the dry season, or by competition with other autotrophs during eutrophic conditions. The question of the direction and severity of translocation should be assessed in future studies within this system by the use of ^{15}N tracing.

The C:N of the eelgrass samples supports the notion that this system, at the time of study, was nitrogen limited. The average C:N of the above-ground material was 18, slightly higher than ratios found in eelgrass from Tomales Bay, which the authors considered nitrogen-limited (Fourqurean et al. 1997). This limitation may be a regular seasonal pattern because both studies were conducted during the summer when environment inputs of nitrogen are at their lowest. However, it does appear that eelgrass in the oyster treatments are taking advantage of the added ammonium facilitated by the oyster beds, for which we saw lower C:N across all sites (Table A1). The eelgrass leaf tissue in oyster treatments exceeded the 1.8% dw N threshold of nitrogen limitation to primary productivity proposed by Short (1987). All isolated eelgrass treatments' % dw N fell below this threshold for productivity. Although percent carbon was higher in leaf tissue overall than below-ground material, the C:N was lower. This result highlights the importance of C:N ratios in studying nitrogen allocation within seagrasses. The relative stability of C:N across treatments and sites in below-ground material despite changes in the percent nitrogen relating to oyster density and proximity further supports this notion.

Above-ground nitrogen in eelgrass leaf tissue correlated positively with oyster presence, density, and proximity to the oyster beds at all three sites. This result strongly suggests that the eelgrass is obtaining needed nitrogenous nutrients from local restored oyster beds. This trend, however, decreases with distance, which may be due to either the limits of the range of impact by the oysters or the decreased need for nitrogen in the older more established shoots towards the center of the beds.

I found no statistically significant difference in percent nitrogen between the below-ground material treatments. This supports other studies that suggest that the leaves are the primary location

within the plant for storing nitrogen (Iizumi and Hattori 1982; Kraemer and Mazzella 1999; Pernice et al. 2016). However, there was an effect of oyster density and distance on elevated nitrogen content of the below-ground material at two of the three sites, again highlighting the importance of spatially explicit modeling. These results suggest that nutrients may still be absorbed by the roots and translocated to the leaves but the reverse trend is still plausible depending on the plant's needs (Sandoval-Gil et al. 2016; Nayer et al. 2018). Sediment type can also affect the efficiency of below-ground absorption of nutrients (Gleeson 1993; Vogt et al. 1993; Sandoval et al. 2016). Organically rich muddy sediments provide greater nutrient transfer than coarser sandy substrates (Short 1983; Short 1987). Nonempirical assessment of the substrates across the sites revealed potentially significant differences in substrate grain size. Since restoration of the oyster beds, Westcliff appears to have shifted towards smaller grain sizes relative to starting conditions and to the other sites. Results of statistical testing of both the above and below-ground metrics in relation to oysters at Westcliff varied from the other sites (Figure A1).

Biomass, Growth, and Density

Contrary to other studies, I found no association between above-ground biomass and tissue nitrogen (Lee and Durtan; Lee 2004; Sandoval-Gil et al. 2016). Lee et al. (2004) found a negative association between leaf mass and tissue nitrogen in a eutrophic environment, suggesting leaf tissue nitrogen as an indicator of a nutrient-polluted system. Though Newport Bay receives excessive nutrients from urban runoff during the wet season this does not appear to be the case within this study since it was conducted during a dry period and the oysters were shown to have a direct effect on the levels of nitrogen within the eelgrass. My results indicate density and proximity to oysters is negatively correlated to above-ground biomass. Lee and Durtan (2000) found that leaf growth and production were increased when available nitrogen exceeded 100 μmol , which only occurred in the porewater at the PCH and DeAnza oyster/eelgrass treatments. Contrary to these findings, I found a negative trend between above-ground biomass and oyster density and proximity. The ammonium found within these beds still fell far below levels presumed to be toxic (Van Katwijk et al. 1997) and

oyster densities (Figure A4) below that suggesting sulfate toxicity (Booth & Heck 2009). Oyster densities at these sites were also far below previous years like 2019, which had greater than 10× the density of oysters (Figure A5). Because of this, it is likely that some other confounding factor or factors are causing a reduction in above-ground biomass. Though the reduction of above-ground biomass is correlated with oyster density and proximity I speculate that the geomorphology may play a role. Due to the sloping nature of the sites, the farther offshore eelgrass sits in water up to 3 m deep relative to the eelgrass closest to the oysters at 0.3 – 0.6 m, requiring it to grow less to achieve its photosynthetic needs (Krause-Jensen et al. 2000). When accounting for distance within the models it is difficult to differentiate the effect of depth.

Leaf dry weight tissue nitrogen correlated with below-ground biomass and growth rates; however, this trend explained little of the variation. *Zostera* species are clonally reproducing and connected through rhizome networks. Though we cannot say for certain the direction or degree of nitrogen translocation occurring in the samples, I suggest that leaf tissue nitrogen is an indicator of below-ground growth. This study was conducted in the summer when irradiance is high. This could mean that the plants allocate resources to expansion rather than the leaves due to less demand for light (McGlathery et al. 1998). Localized effects of oysters on turbidity were not measured for this study, however, due to the treatment effects observed, the addition of the nitrogen by oysters did appear to facilitate below-ground growth. Since restoration of the eelgrass beds commenced in 2016, we have observed massive lateral expansions of eelgrass, which have led to connections between many of the beds.

I found a positive association between rhizome dry weight nitrogen and below-ground biomass, though this trend was highly variable (Figure 21). This result is contrary to other studies that suggest that below-ground mass increases when nutrients are in short supply in order to increase surface area for more efficient uptake (Short 1987; Lee and Dunton 1999). Below-ground biomass and growth rate also increased with oyster density and proximity at the two sites where porewater ammonium was the highest (i.e., PCH and DeAnza). Because this is purely correlative data, below-

ground growth may be the result of external factors, though oyster presence and their correlative effects on the local nitrogen pool suggest they have some role in the expansion of the rhizome network. Further, my results may be confounding with the sediment type which affects nutrient availability and also may provide differential resistance to growth due to compaction.

Eelgrass density was negatively correlated with oyster presence, density, and distance at two of the sites (i.e., PCH and DeAnza). This result is supported by other studies that observed the effects of aquaculture on local eelgrass beds, though the unique layout of our study makes it difficult to determine whether the same factors are at play (Tallis et al 2009; Sandoval-Gil et al 2016; McIver et al. 2019). Prior studies suggest that eelgrass density is reduced due to spatial competition (Tallis et al. 2009; Wagner et al. 2021), however, this was not a concern with my study since the oyster and eelgrass beds were separated. I hypothesized that density would increase due to the oysters' ability to decrease turbidity through filtration. The differential oyster density across sites may explain why this effect was not observed. The two sites where I saw a negative association (PCH & DA) also had significantly lower oyster densities compared to the site where there was a positive association (WC). This suggests that filtration capacity may depend on the density of oysters. This is consistent with the finding that the highest density of oysters reached within this study correlated with increased eelgrass density. Despite a positive association between eelgrass density and oyster density at closer proximities at Westcliff, this bed had the lowest mean density of eelgrass when observing the bed as a whole (Figure 23). Therefore, the effects of oysters on eelgrass density do appear to be limited to within only several meters. Density dependent effects may have also played a key role in the variability across sites due to differential densities of oysters (Figure A4). Furthermore, when accounting for all filter feeders recruited to the beds DeAnza had the greatest densities, which is also where I observed the greatest overall eelgrass densities. The relationship of densities between eelgrass and the oyster beds, including other filter feeders, may be dependent upon specific filtration capacity of the different species.

Conclusion

Eelgrass and oysters are both critical estuarine ecosystem engineers and foundation species. The reduction in both of these species due to anthropogenic activity has brought focus to their vital roles in these ecosystems. Marine scientists and resource managers have become increasingly curious about the interactions between these species in the interest of promoting shoreline resiliency and more diverse habitats. The protected status of eelgrass has brought much concern to project managers who seek to restore oyster reef or conduct aquaculture practices near eelgrass. In this study, the overall impact of restored oysters adjacent to eelgrass was largely positive with increased below-ground growth and nitrogenous nutrients in a presumed seasonally nutrient-limited system.

I also observed multiple context-dependent negative and non-significant effects, suggesting a complex interaction between these species that warrants further study.

The many site effects suggest that other factors (i.e., depth, turbidity, and sediment type) may affect eelgrass production. However, despite the potential confounding factors, this study revealed for the first time, a spatial framework in which Olympia oysters affect eelgrass growth and production. The significant associations between the below-ground productivity and increased nitrogen when oysters are present and in greater densities shows the importance of this historically coexisting relationship. In the past, a simple metric of restoration success for eelgrass was density, likely due to the relative ease of this type of sampling. However, my results suggest that below-ground growth and lateral expansion are as important to accurately quantify successful restoration. This *in situ* study was correlative and future analysis might focus on tracing nitrogen cycling in similar restoration designs. These findings paired with consideration of the broad array of ecosystem services offered by both habitats implies that paired restoration should be recommended.

APPENDIX A

FIGURES AND TABLES

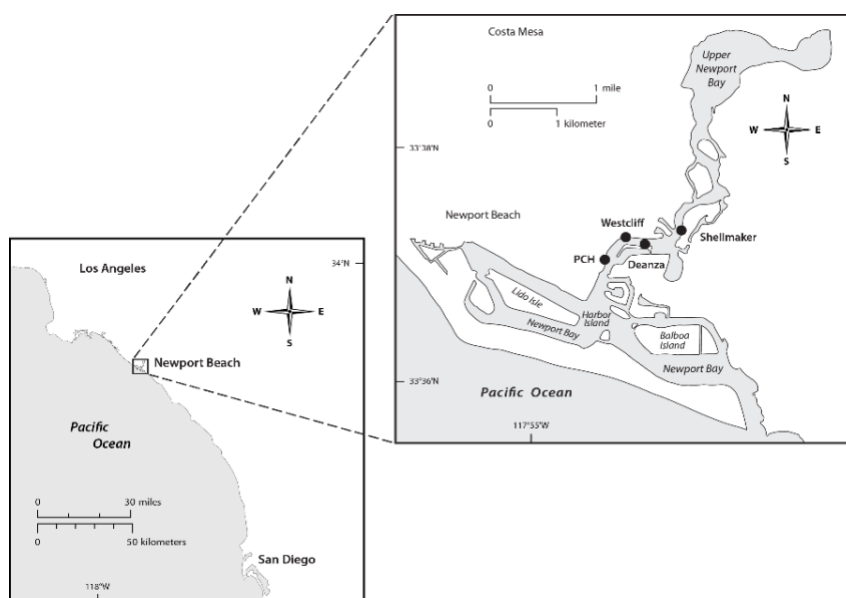


Figure 1. Eelgrass and oyster restoration sites in Upper Newport Bay, Newport Beach, CA: PCH, Westcliff, DeAnza, and Shellmaker.



Figure 2. Orientation and size of the two treatments (oyster/eelgrass and control/eelgrass) studied at each site (PCH, Westcliff, and DeAnza) in Newport Bay, CA in summer of 2021. Treatments were not positioned adjacent to one another, as show in this image, at every site. A full factorial block design including an oyster/control and full control treatment (not shown here) was originally implemented at each site but the other treatments were not part of this study.

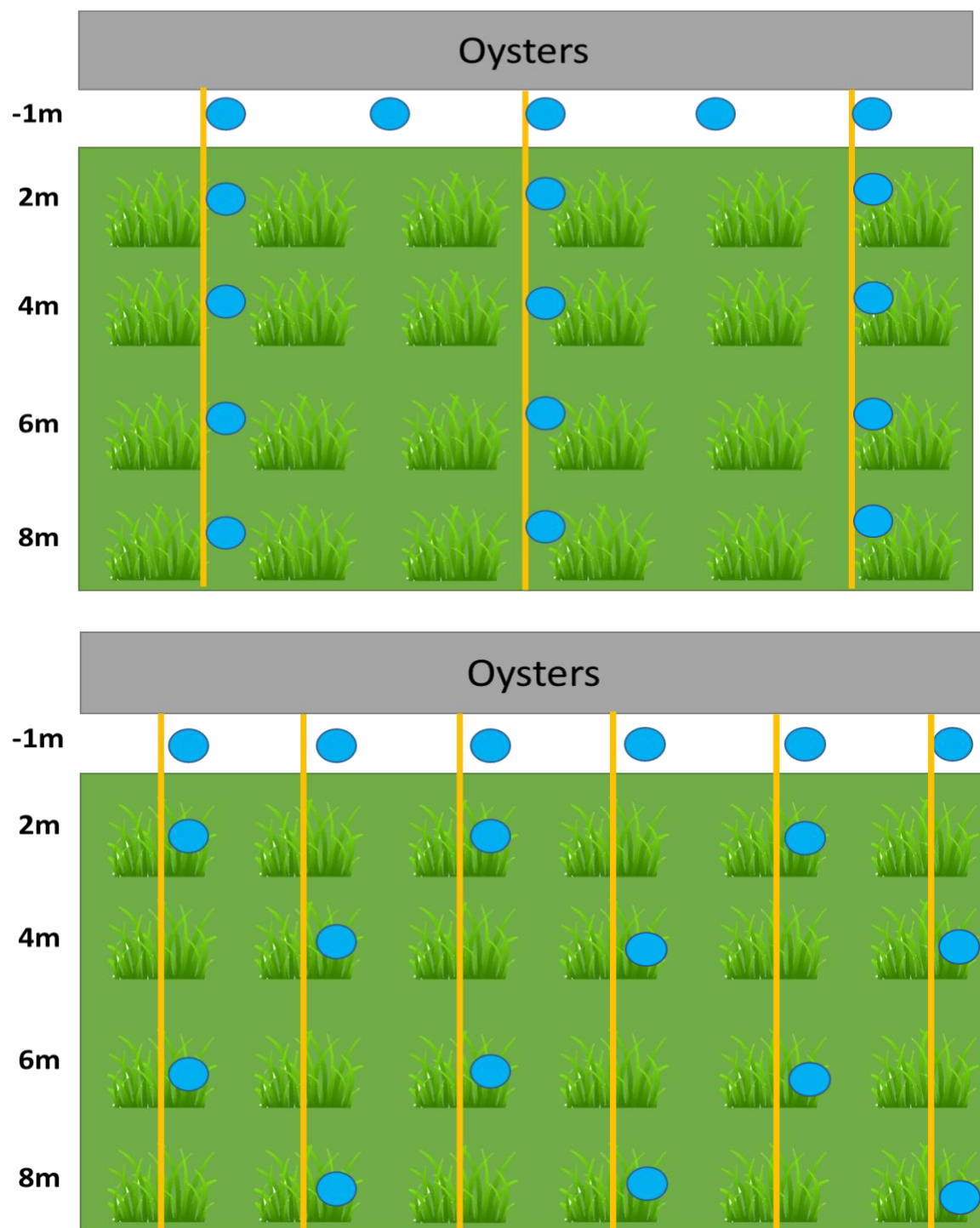


Figure 3. Orientation of the sampling design for porewater cores (above) and shoot collections (below) at each site in Newport Bay, CA in the summer of 2021. Yellow lines represent transects and blue dots represent the sample location. Meter marks represent distance moving away from shore starting a meter above the shoreward edge of the eelgrass bed.

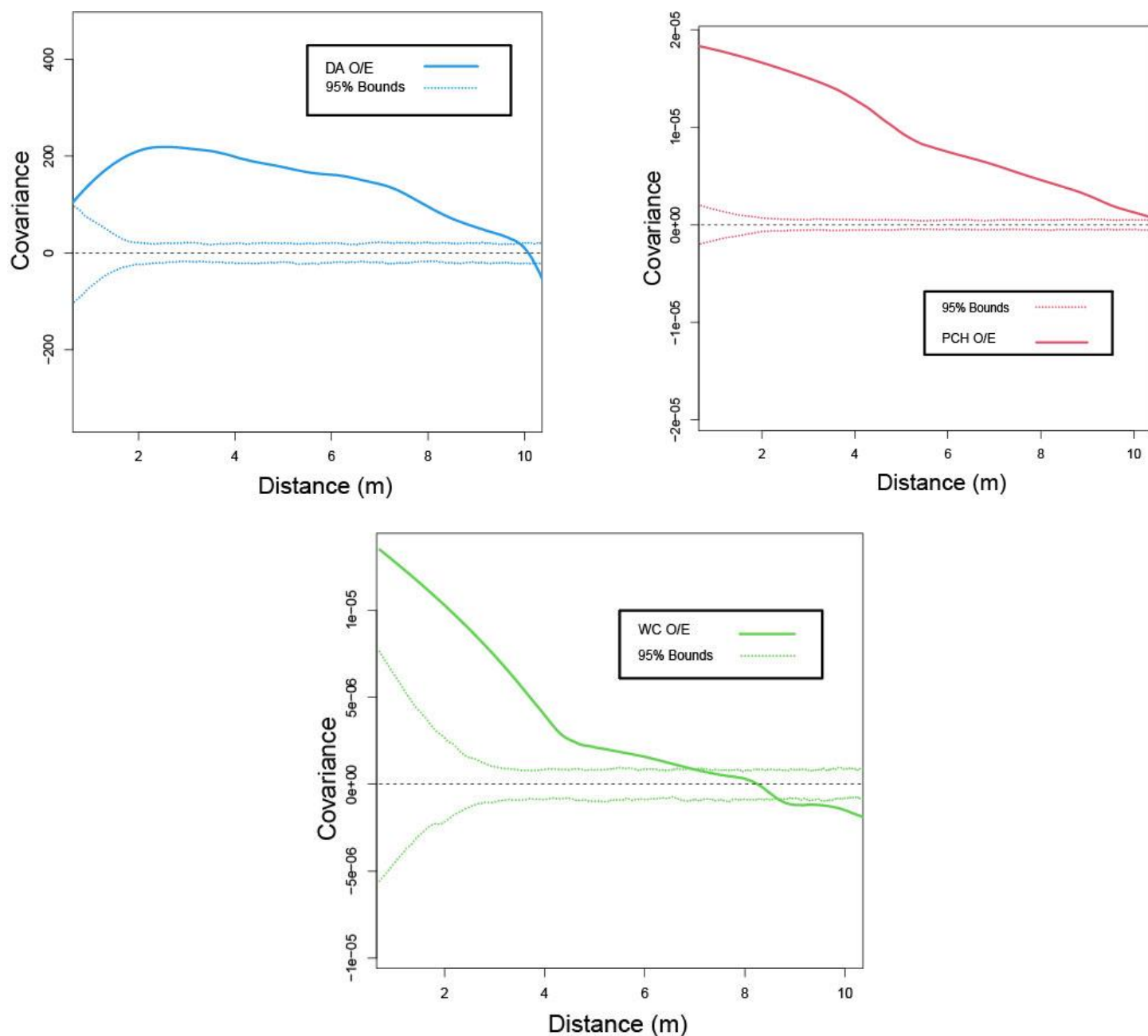


Figure 4. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and ammonium at (A) DeAnza, (B) PCH, and (C) Westcliff in Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

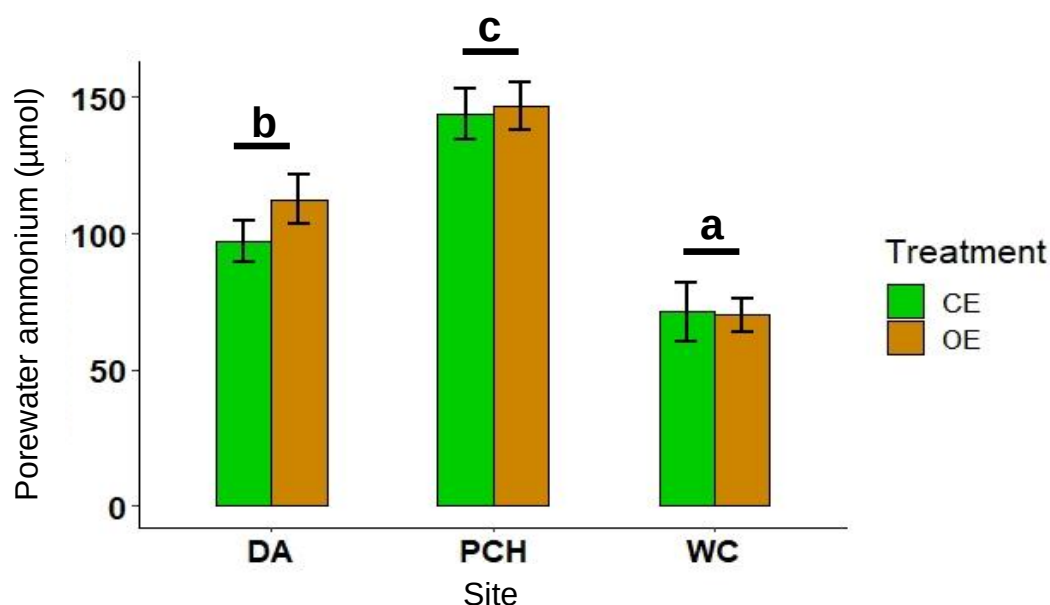


Figure 5. Mean porewater ammonium concentrations across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = CE = control-eelgrass (green) and OE = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons.

Table 1. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OC, CE**) porewater ammonium concentrations during July 2021 in Upper Newport Bay, CA.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Site	2	1.785	0.8925	41.229	4.96E-14	***
Treatment	1	0.0016	0.0016	0.073	0.788	
Treatment × Site	2	0.0219	0.0109	0.506	0.605	
Residuals	108	2.3379	0.0216			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

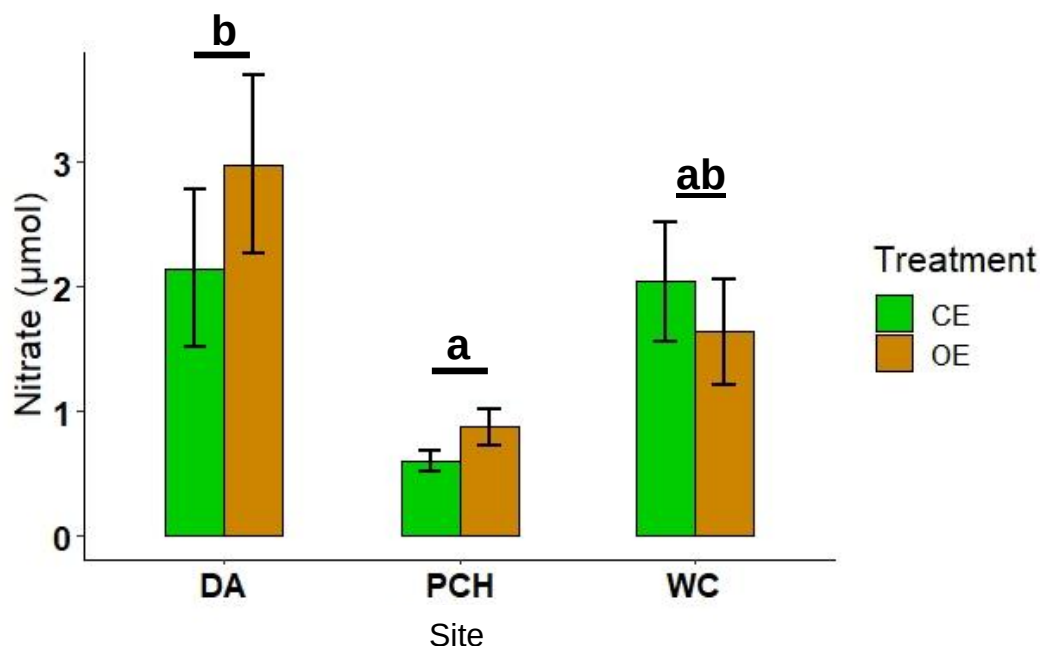


Figure 6. Mean porewater nitrate concentrations across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons

Table 2. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) porewater nitrate concentrations during July 2021 in Upper Newport Bay, CA.

	Df	Sum	Sq	Mean	Sq	F
Site	2	66.6	33.3	7.491	0.000888	***
Treatment	1	1.8	1.76	0.395	0.530742	
Treatment × Site	2	7.5	3.76	0.845	0.432304	
Residuals	111	493.5	4.45			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

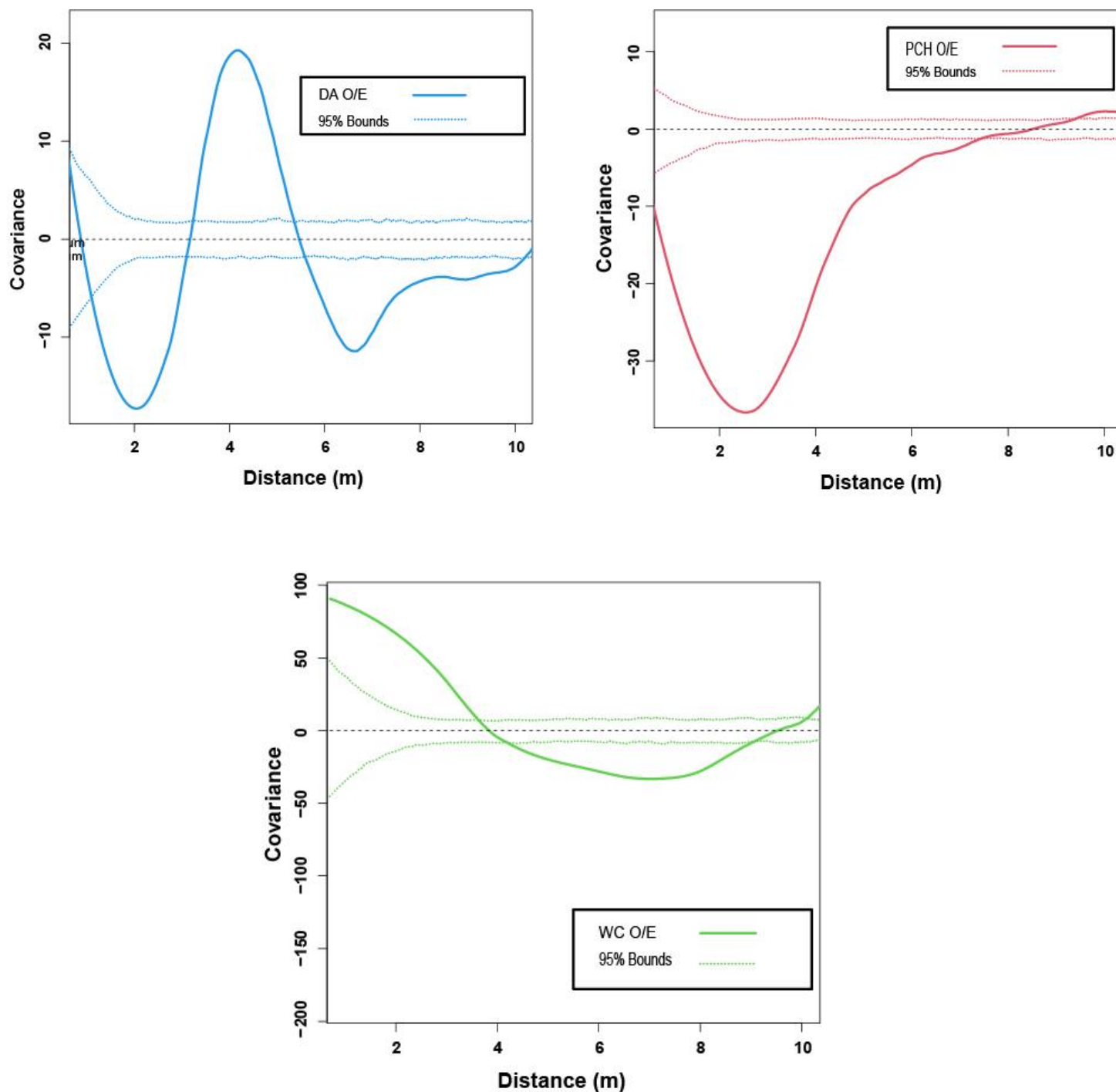


Figure 7. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and nitrate at (A) DeAnza, (B) PCH, and (C) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

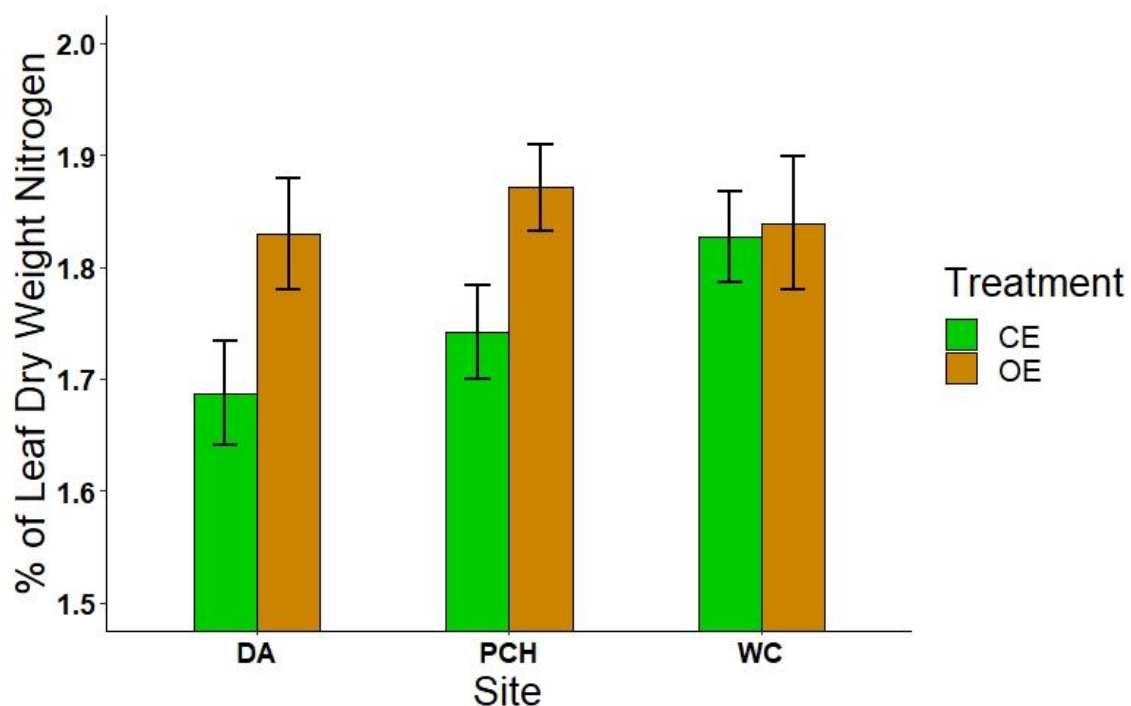


Figure 8. Percent organic nitrogen within above-ground eelgrass tissue across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE.

Table 3. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on percent organic nitrogen within above-ground eelgrass tissue in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Site	2	0.12	0.06003	1.207	0.3025	
Treatment	1	0.302	0.30166	6.067	0.0151	*
Treatment × Site	2	0.111	0.05546	1.115	0.3311	
Residuals	125	6.216	0.04972			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

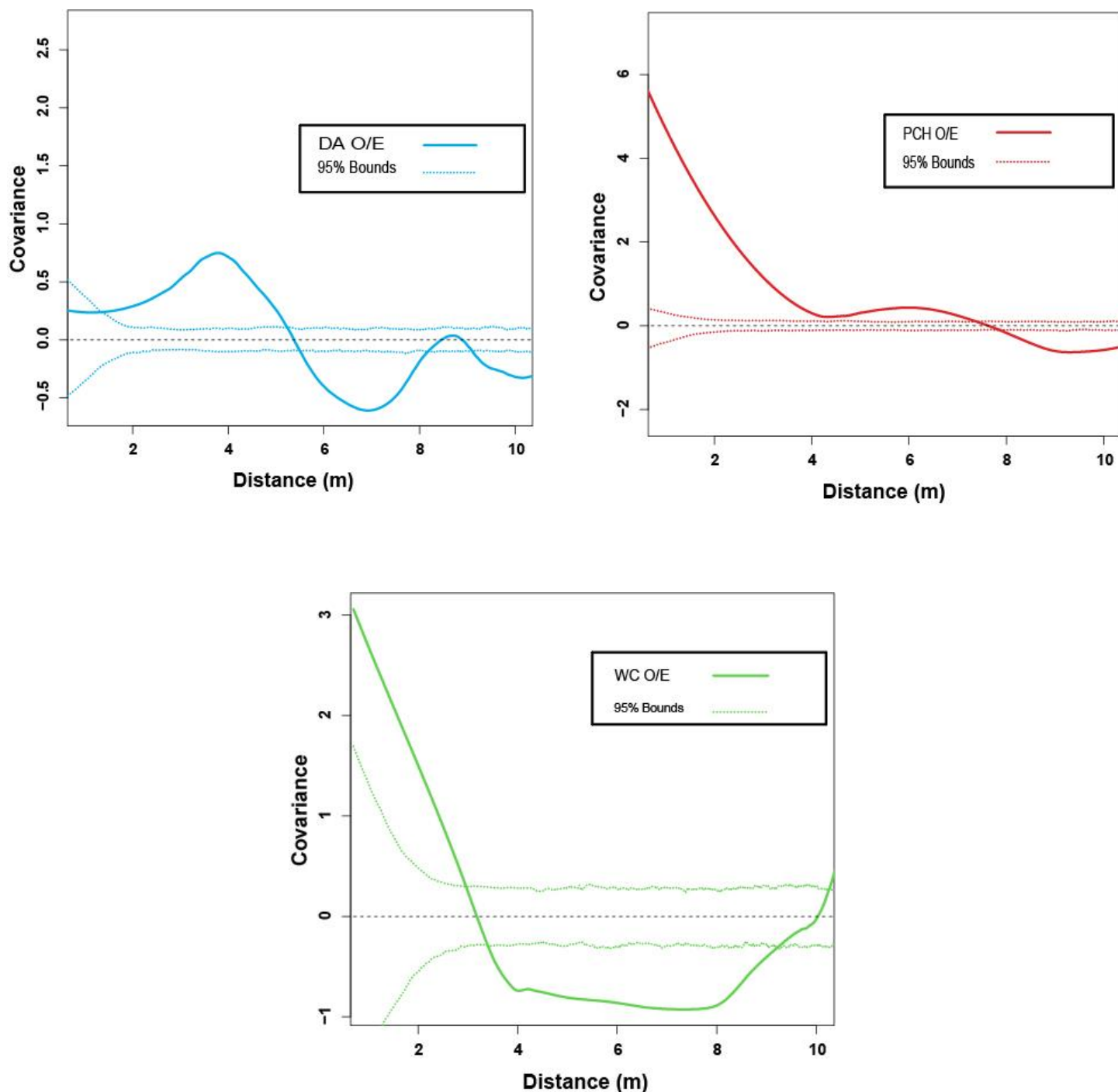


Figure 9. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and percent organic nitrogen within above-ground eelgrass tissue at (A) DeAnza, (B) PCH, and (C) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

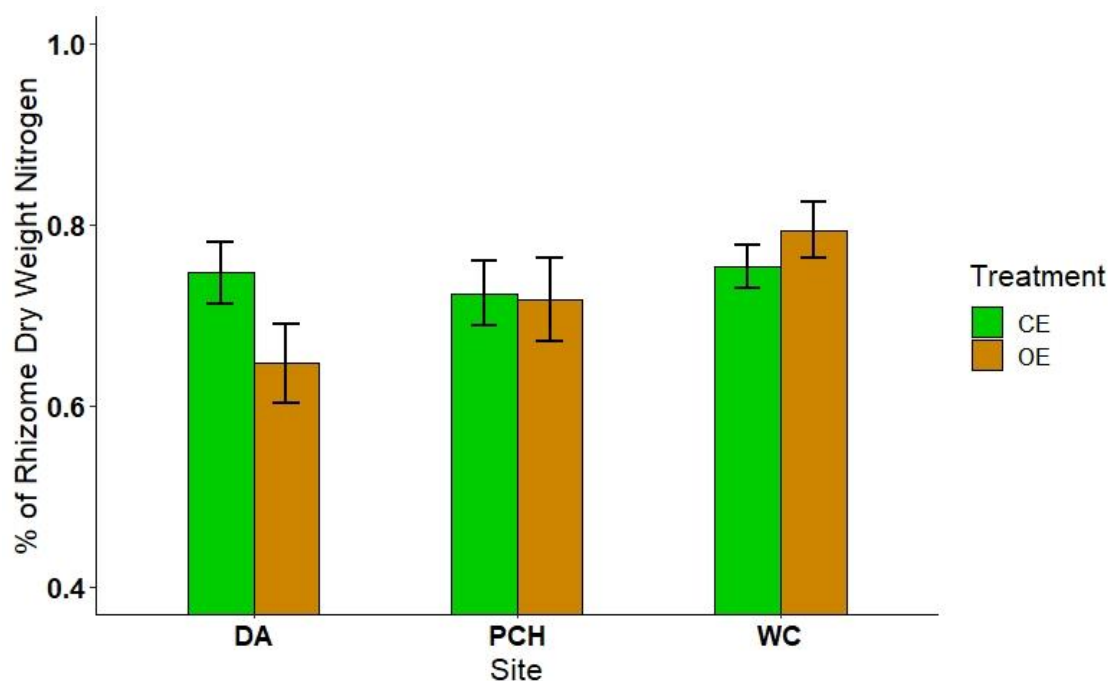


Figure 10. Percent organic nitrogen within below-ground eelgrass tissue across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE.

Table 4. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on percent organic nitrogen within below-ground eelgrass tissue in Upper Newport Bay, CA during July 2021.

	Df	Sum sq	Mean sq	F value	Pr(>F)
Site	2	0.113	0.05645	1.953	0.146
Treatment	1	0.014	0.01428	0.494	0.483
Treatment × Site	2	0.102	0.05119	1.771	0.175
Residuals	120	3.469	0.02891		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

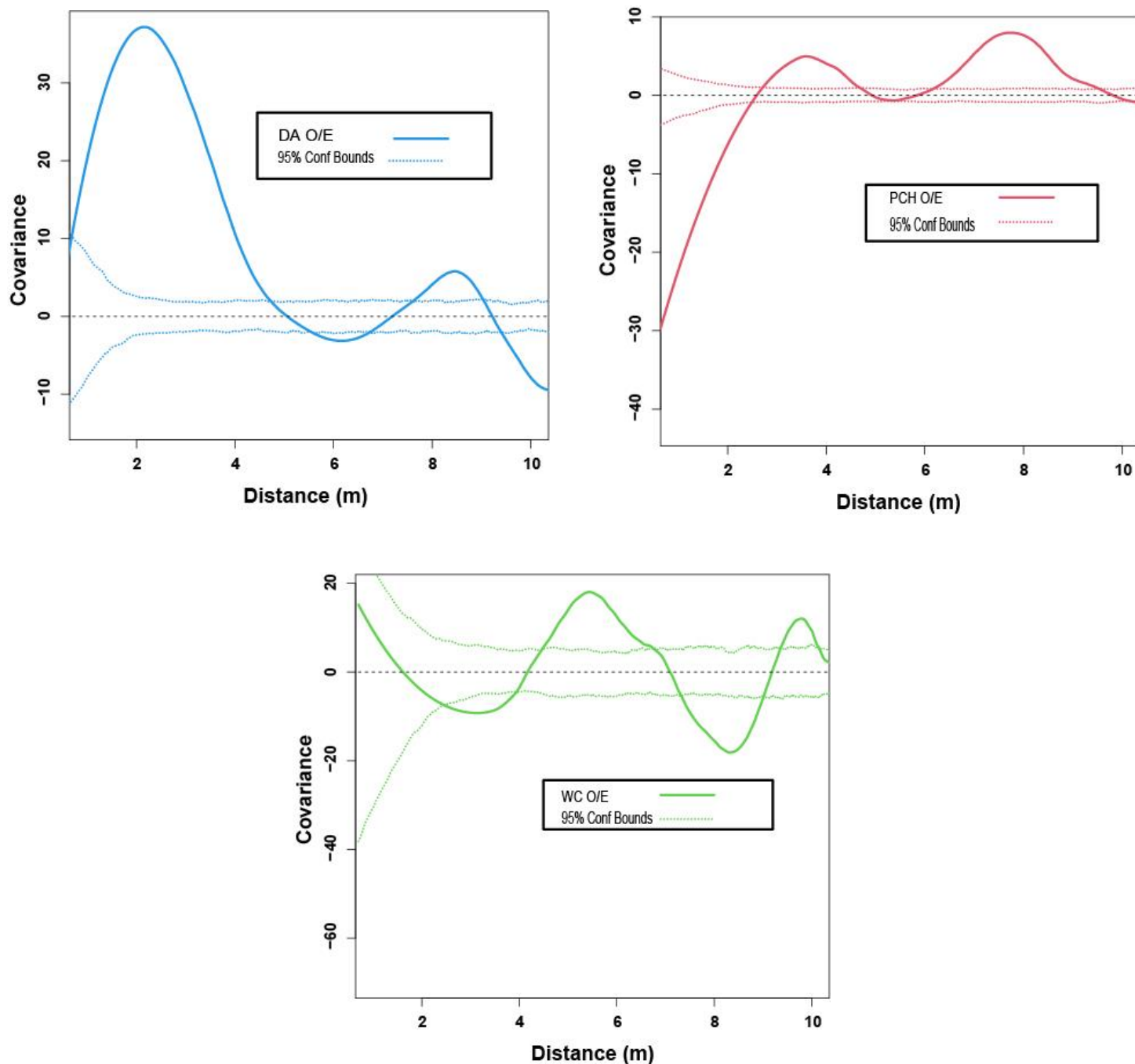


Figure 11. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and percent organic nitrogen within below-ground eelgrass tissue at (**top left**) DeAnza, (**top right**) PCH, and (**bottom**) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

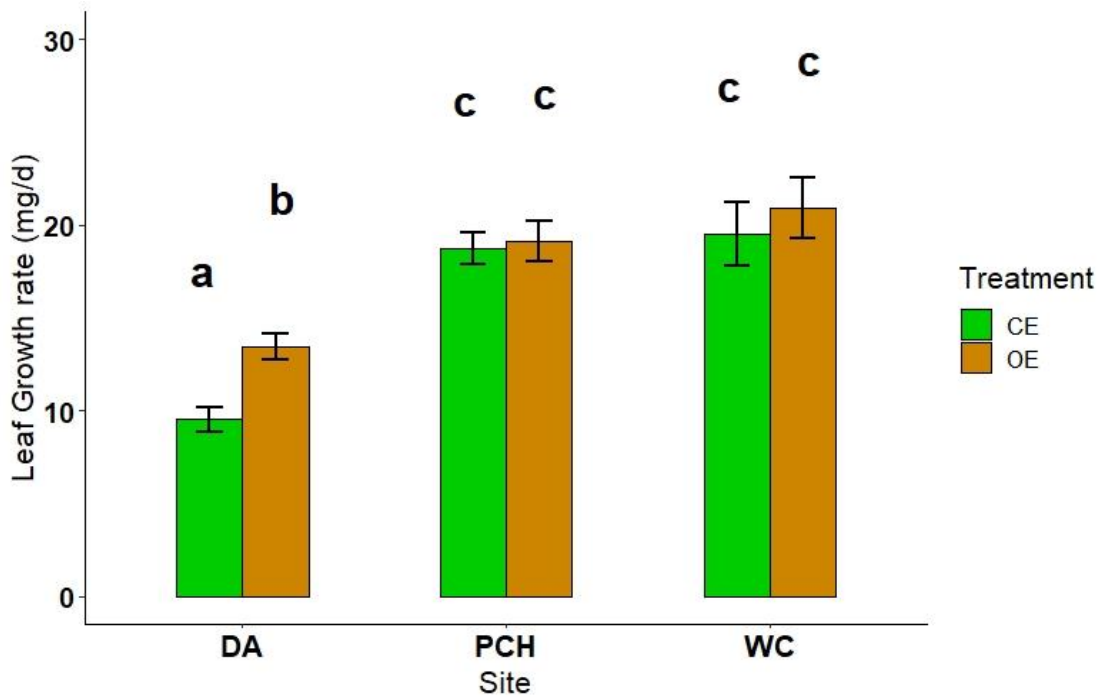


Figure 12. Mean Leaf growth rate across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons.

Table 5. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on eelgrass leaf growth rates eelgrass tissue in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Site	2	1.2807	0.6404	39.63	5.20E-14	***
Treatment	1	0.1143	0.1143	7.077	8.85E-03	**
Treatment × Site	2	0.1059	0.053	3.277	0.04105	*
Residuals	123	1.9875	0.0162			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

B

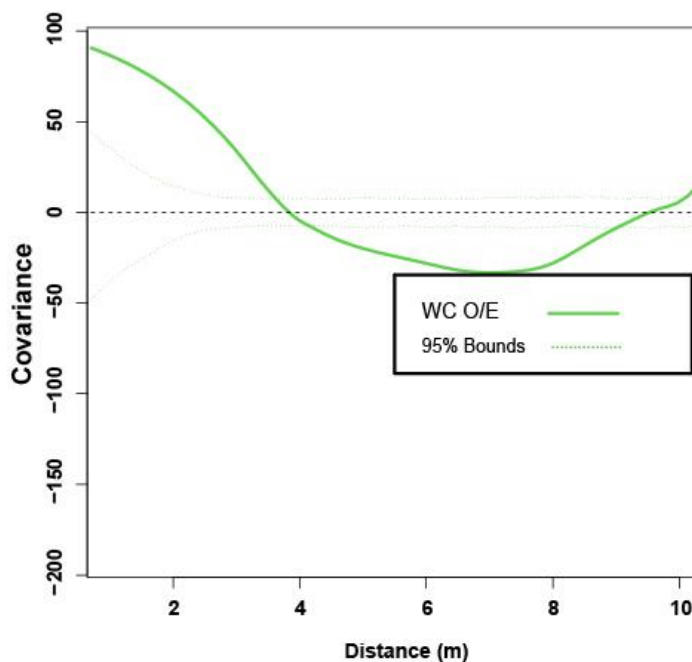
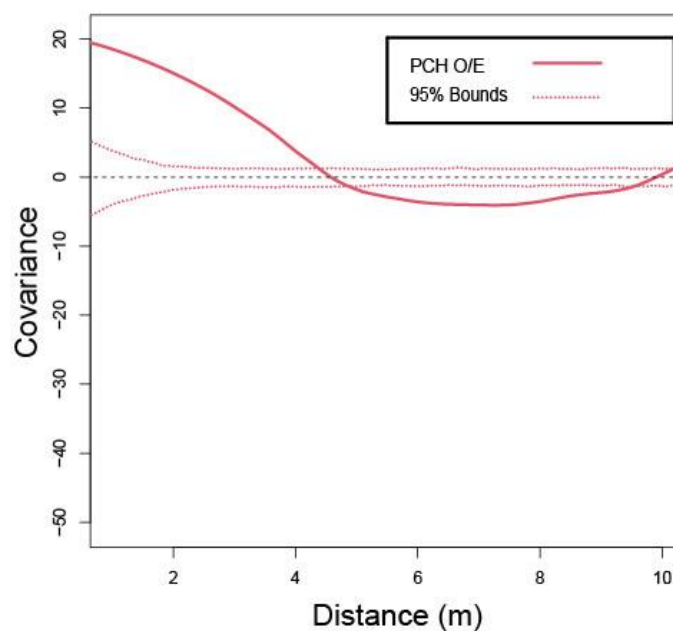
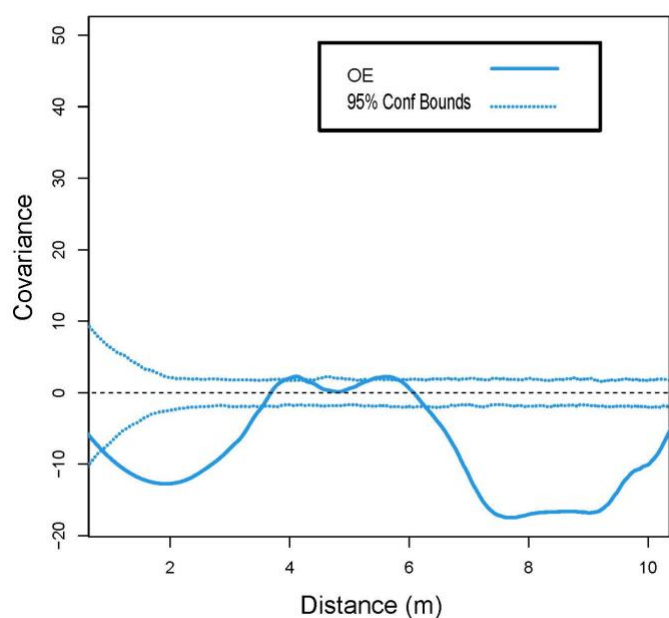


Figure 13. Distance between eelgrass and oysters (**x**) vs. co-variogram estimation (**y**) of oyster density and eelgrass leaf growth rates at (**top left**) DeAnza, (**top right**) PCH, and (**bottom**) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

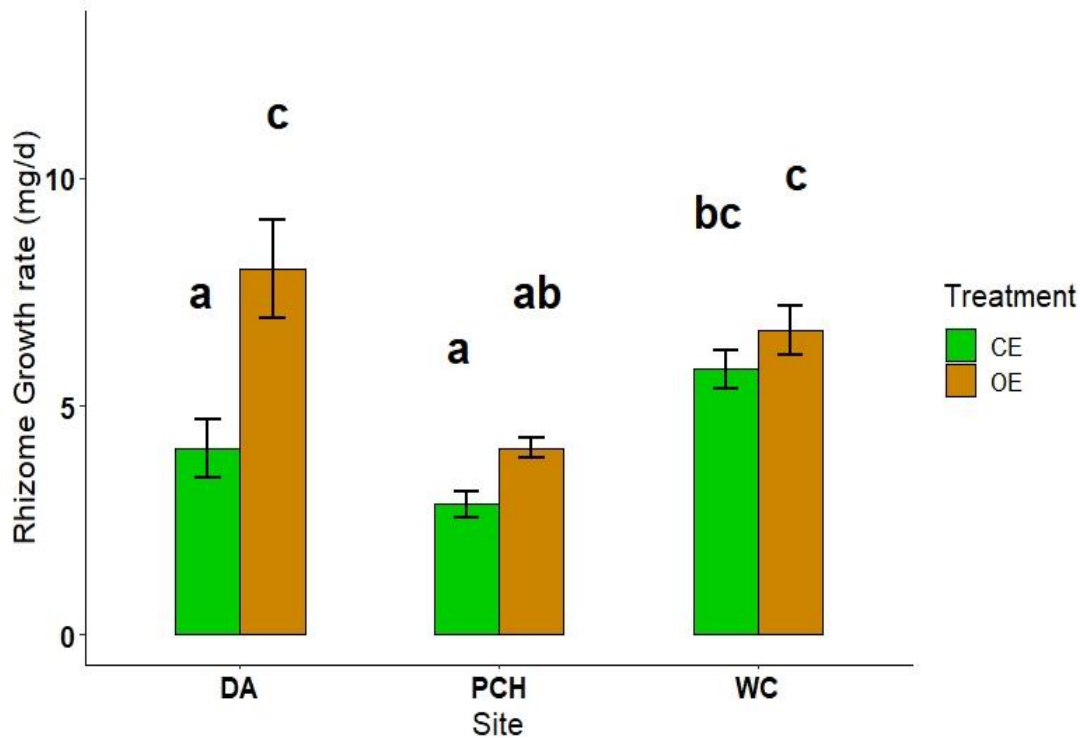


Figure14. Rhizome growth rate across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons.

Table 6. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on eelgrass rhizome growth rates eelgrass tissue in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Treatment	1	1.11	1.1096	23.868	3.07E-06	***
Site	2	1.493	0.7465	16.057	6.14E-07	***
Treatment × Site	2	0.288	0.144	3.097	0.0487	*
Residuals	126	5.858	0.0465			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

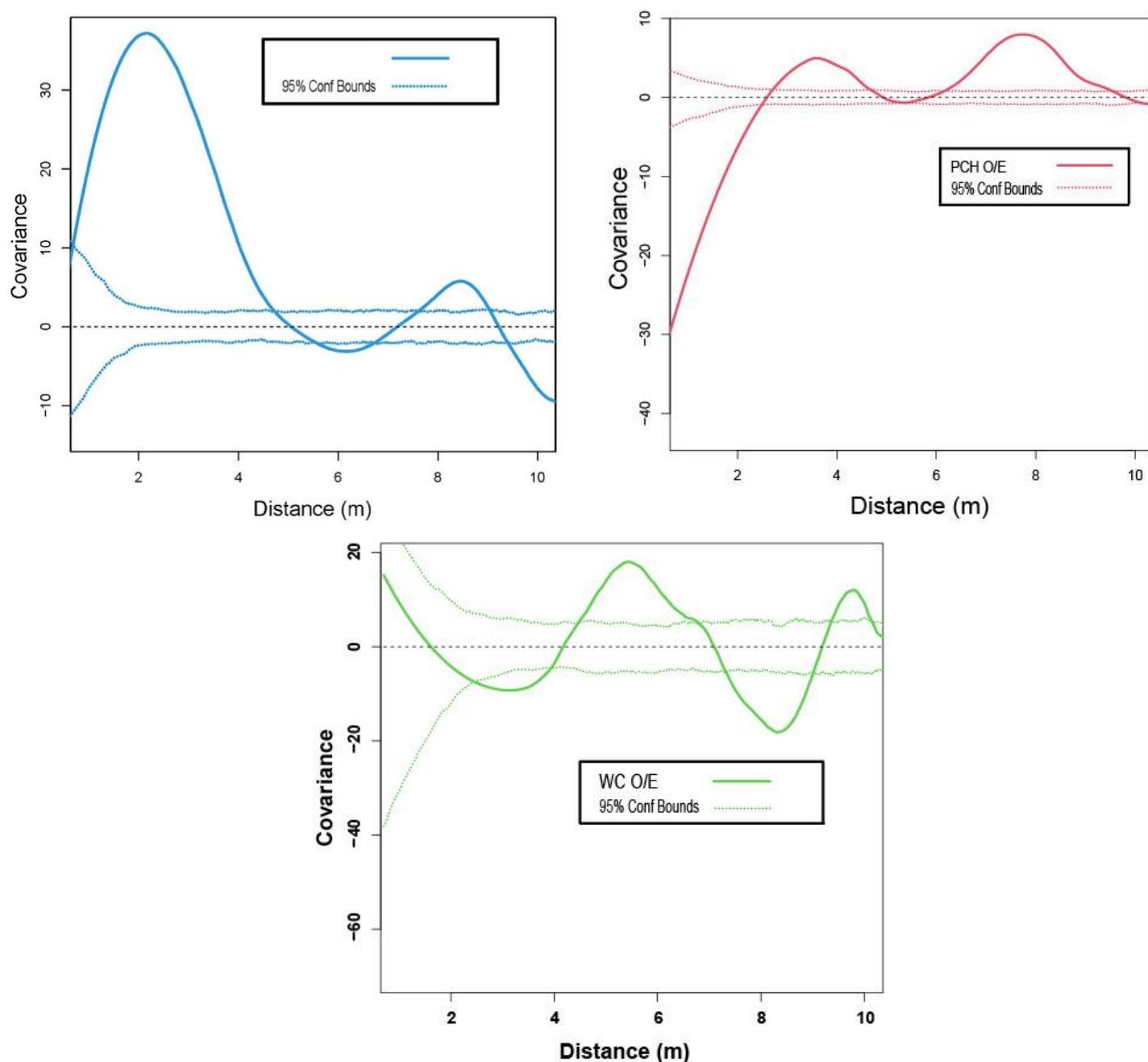


Figure 15. Distance between eelgrass and oysters (**x**) vs. co-variogram estimation (**y**) of oyster density and eelgrass rhizome growth rates at **(top left)** DeAnza, **(top right)** PCH, and **(bottom)** Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

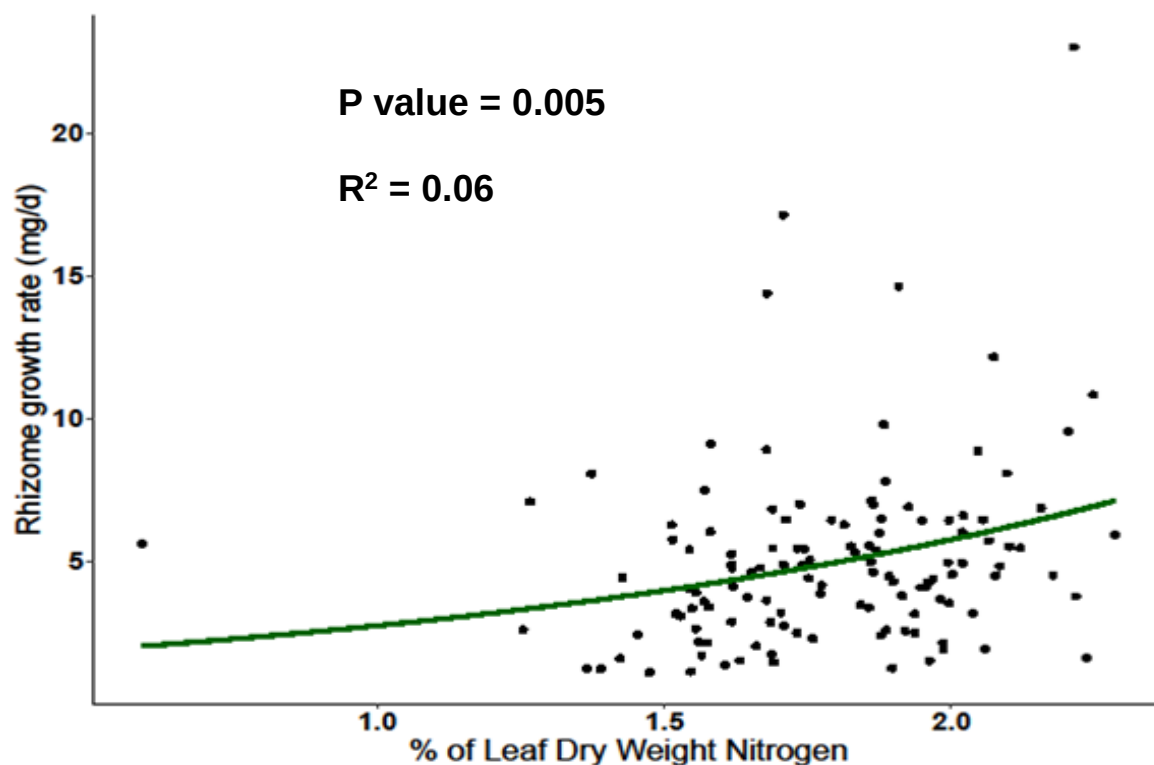


Figure 16. Linear regression of rhizome growth rate vs percent organic leaf nitrogen of eelgrass across all sites and treatments in Upper Newport Bay, CA during July 2021. Green line = log transformed line of best fit.

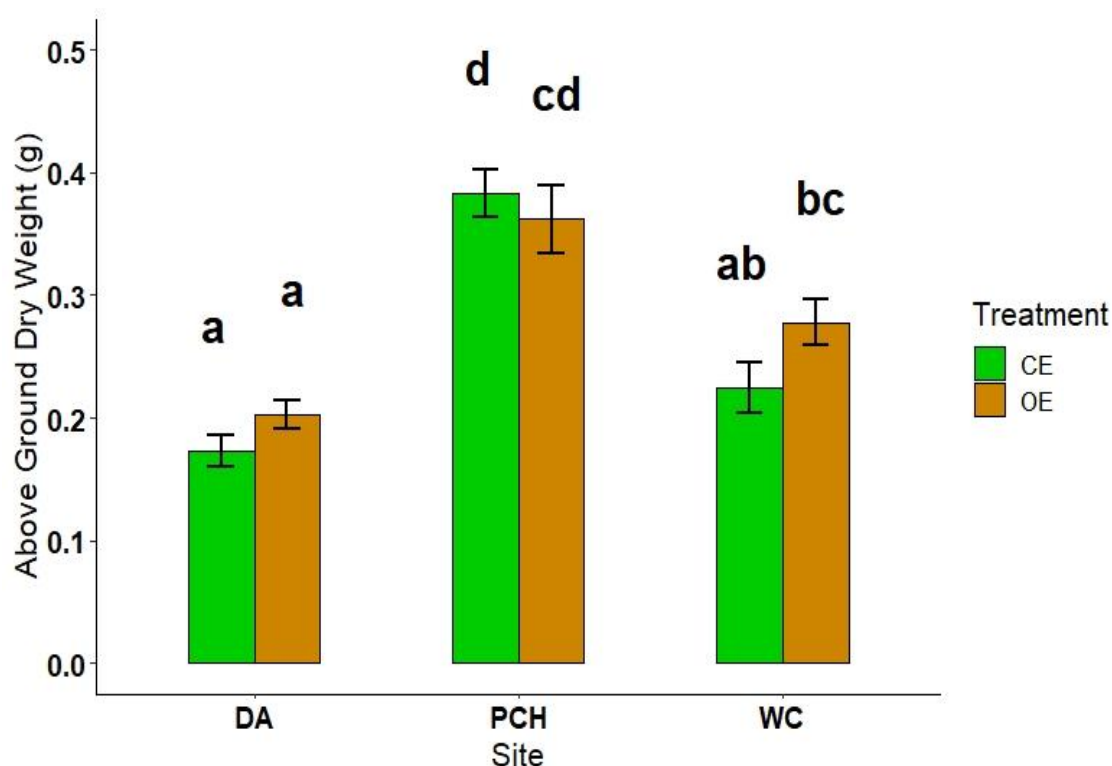


Figure 17. Above-ground dry weight across sites and treatments in Upper Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons.

Table 7. Results of two-way ANOVA testing effects of site (**DA**, **PCH**, **WC**) and treatment (**OE**, **CE**) on eelgrass rhizome growth rates eelgrass tissue in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Site	2	2.1566	1.0783	48.569	<2e-16	***
Treatment	1	0.0819	0.0819	3.689	0.0569	.
Treatment × Site	2	0.1425	0.0712	3.208	0.0435	*
Residuals	134	2.975	0.0222			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

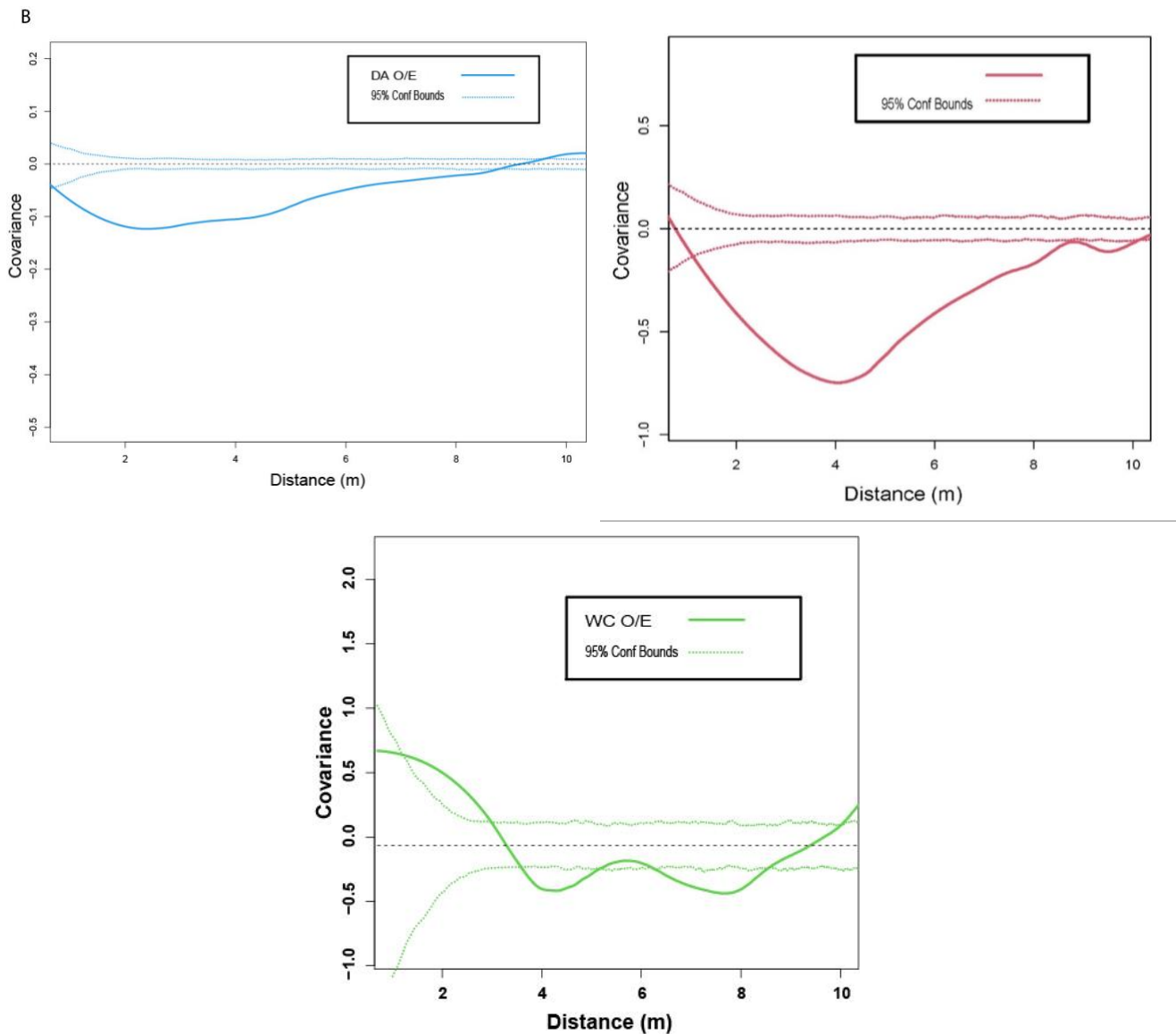


Figure 18. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and eelgrass above-ground dry weight at (**top left**) DeAnza, (**top right**) PCH, and (**bottom**) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95%

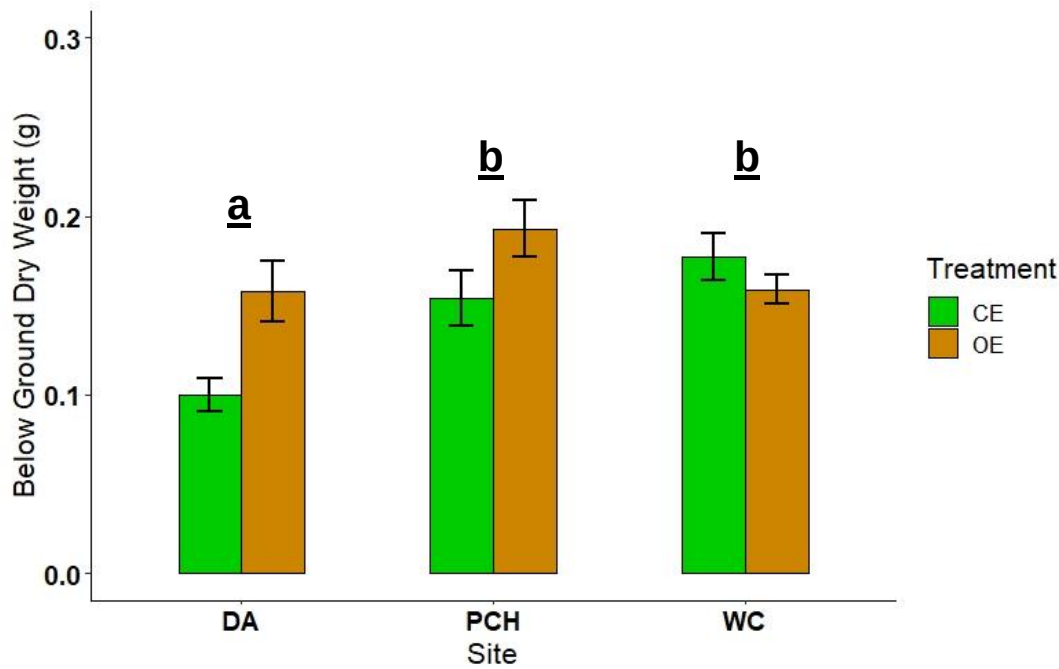


Figure 19. Below-ground dry weight across sites and treatments in Upper Newport Bay, CA during July 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey.

Table 8. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on eelgrass below-ground dry weight in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Treatment	1	0.00076	0.000759	0.925	0.3379	
Site	2	0.01145	0.005724	6.976	0.0013	**
Treatment × Site	2	0.00103	0.000514	0.626	0.5361	
Residuals	138	0.11322	0.00082			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

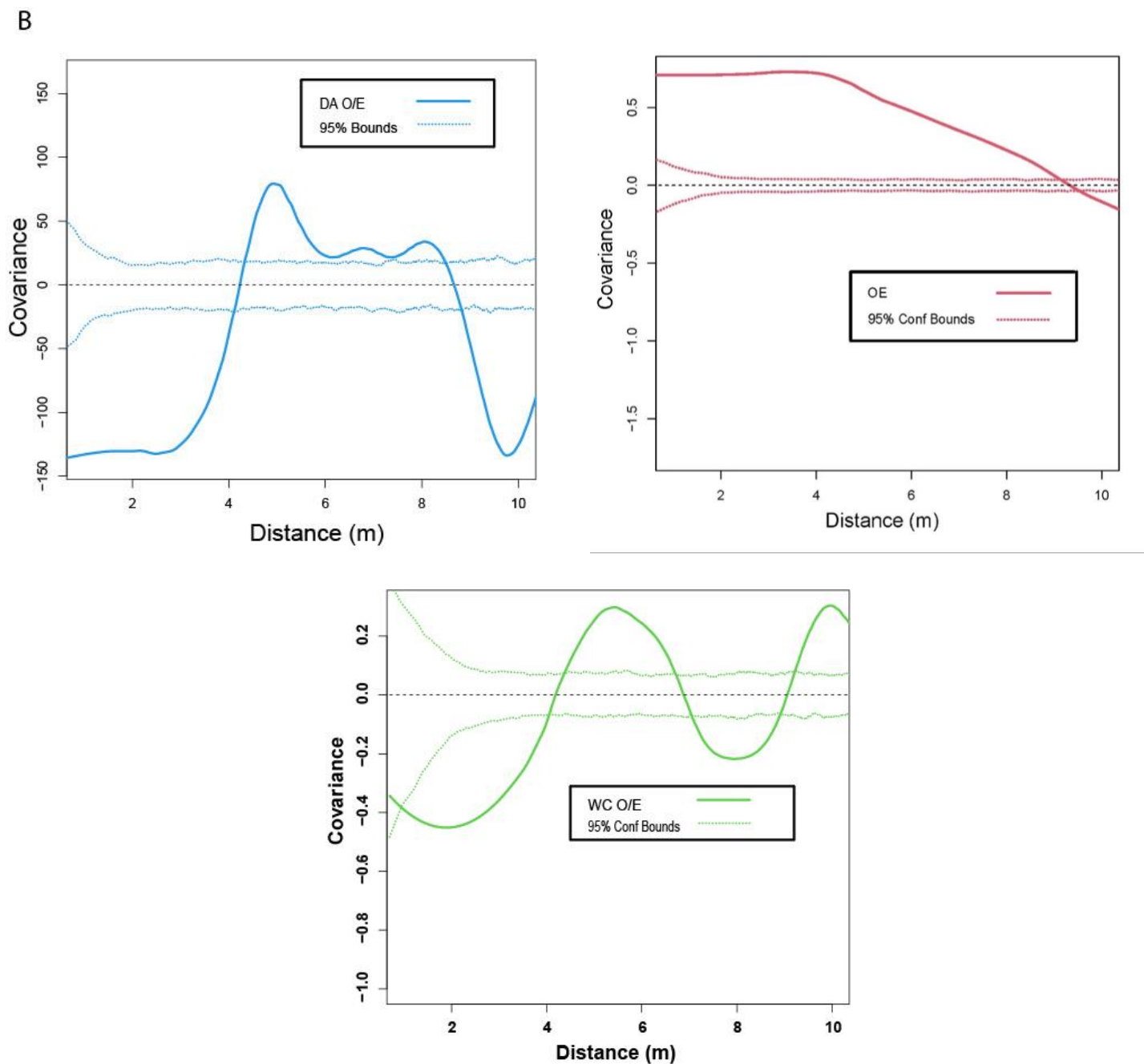


Figure 20. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and eelgrass below-ground dry weight at (top left) DeAnza, (top right) PCH, and (bottom) Westcliff in Upper Newport Bay, CA during July 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

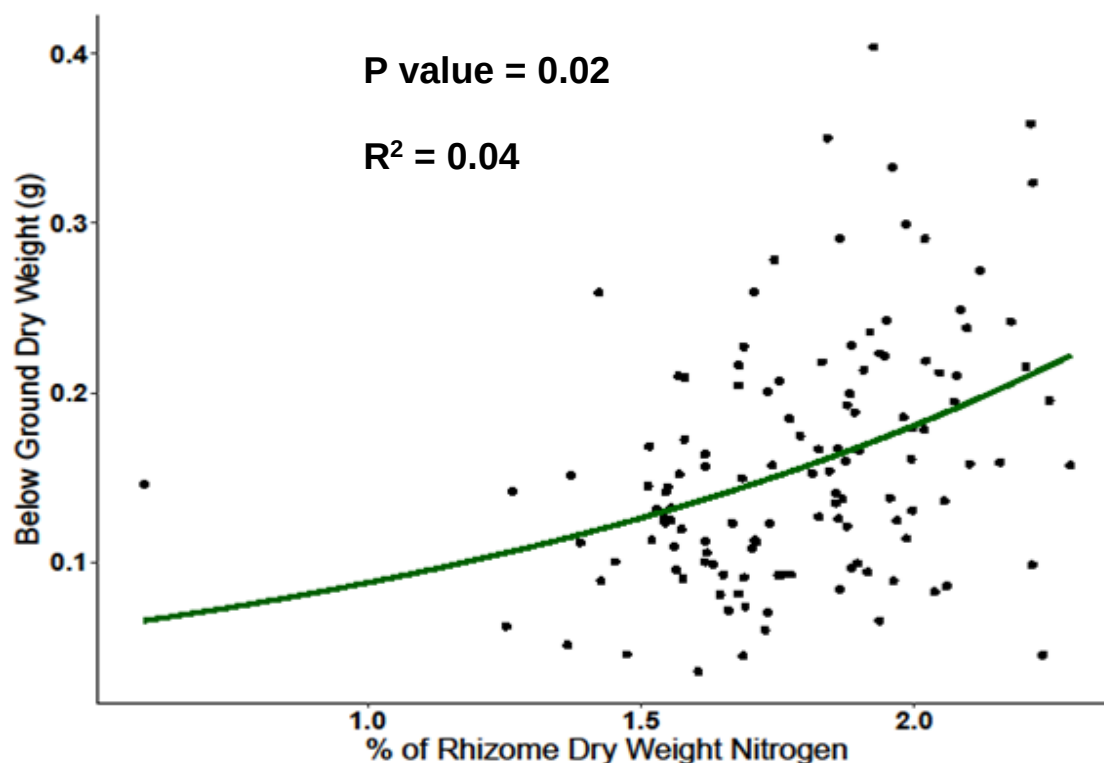


Figure 21. Linear regression of below-ground dry weight vs percent organic leaf nitrogen of eelgrass across all sites and treatments in Upper Newport Bay, CA during July 2021. Green line = line of best fit.

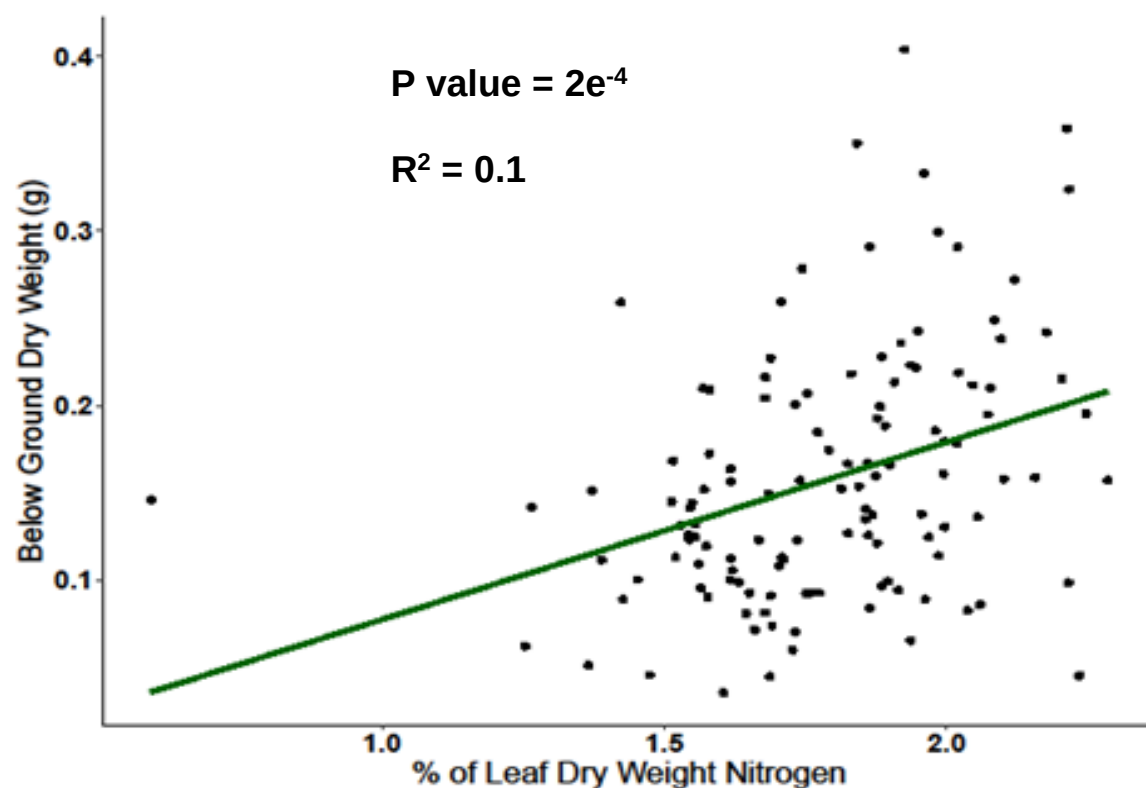


Figure 22. Linear regression of below-ground dry weight vs percent organic rhizome nitrogen of eelgrass across all sites and treatments in Upper Newport Bay, CA during July 2021. Green line = log transformed line of best fit.

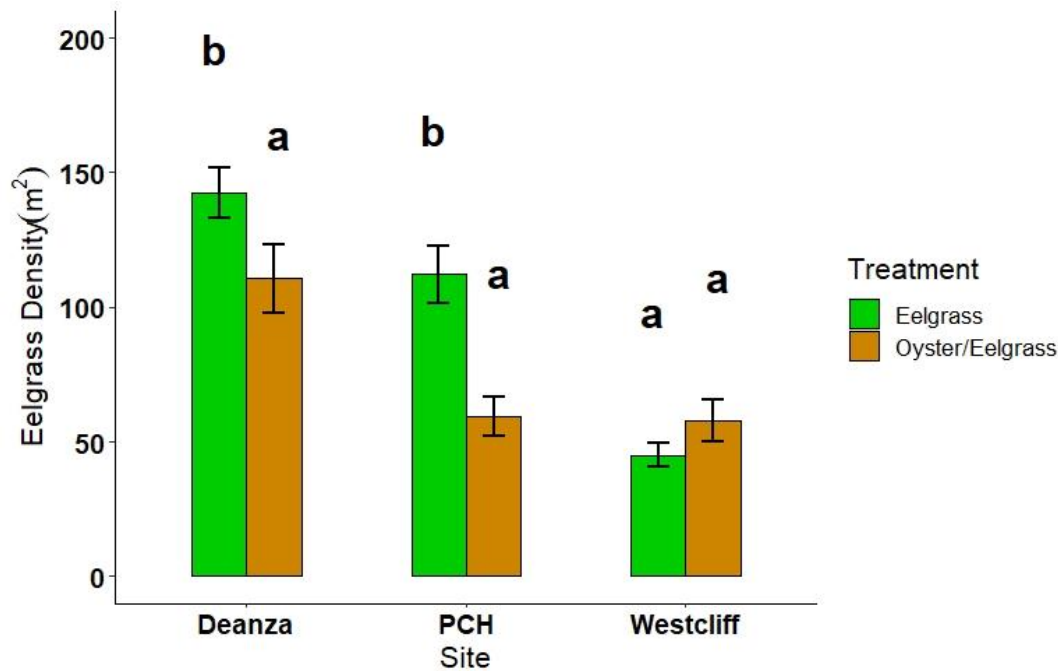


Figure 23. Eelgrass densities across sites and treatments in Upper Newport Bay, CA during May 2021. DA, PCH, WC = **CE** = control-eelgrass (green) and **OE** = oyster-eelgrass (orange). Error bars = 1 SE. Bars not sharing letters indicate significant differences based upon post-hoc Tukey comparisons.

Table 9. Results of two-way ANOVA testing effects of site (**DA, PCH, WC**) and treatment (**OE, CE**) on eelgrass below-ground dry weight in Upper Newport Bay, CA during July 2021.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Treatment	1	118.2	118.21	16.037	9.01E-05	***
Site	2	532.1	266.04	36.09	5.99E-14	***
Treatment × Site	2	102.1	51.05	6.925	1.26E-03	**
Residuals	184	1356.4	7.37			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

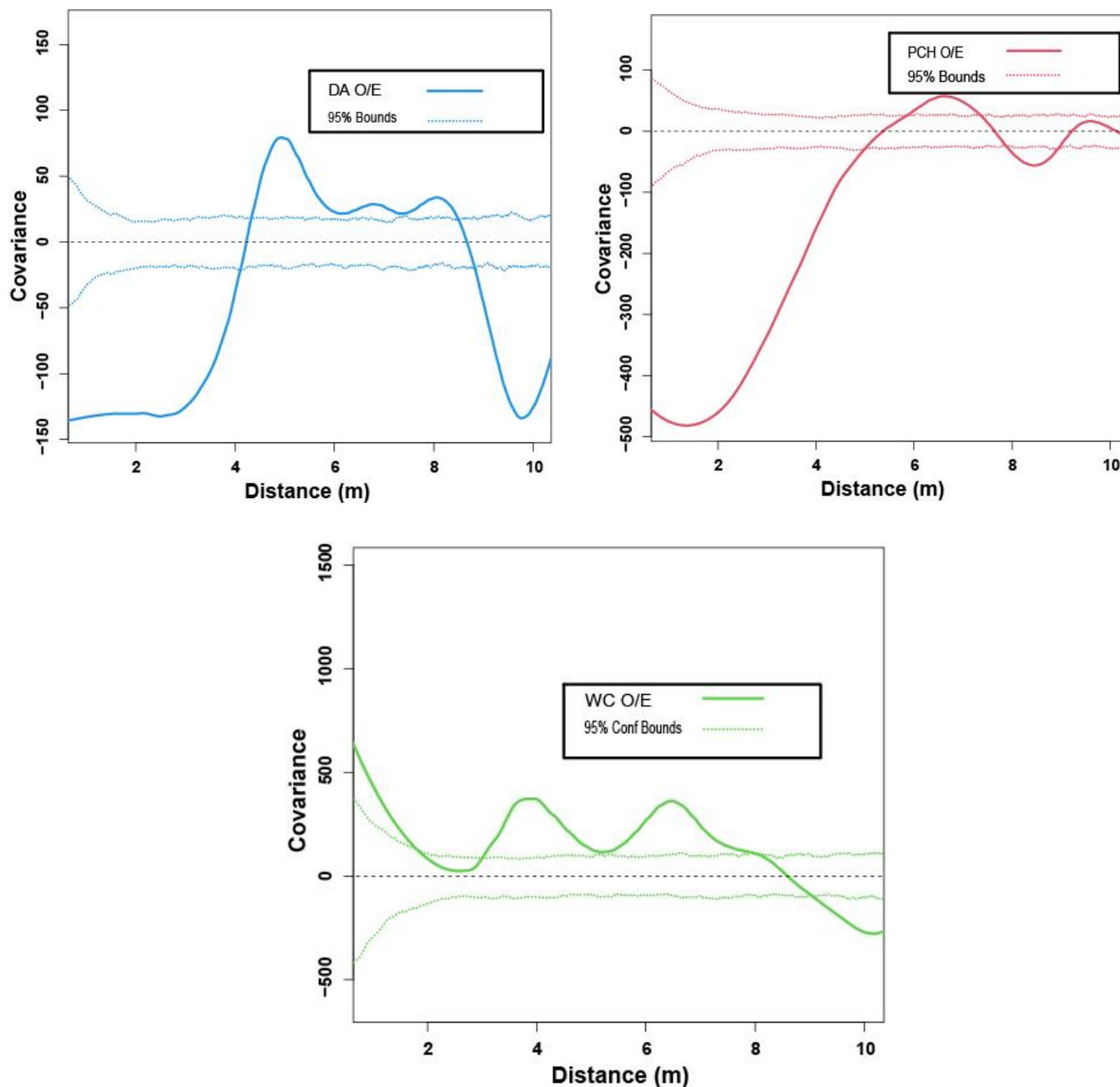


Figure 24. Distance between eelgrass and oysters (x) vs. co-variogram estimation (y) of oyster density and eelgrass density at (top left) DeAnza, (top right) PCH, and (bottom) Westcliff in Upper Newport Bay, CA during May 2021. Positive values represent stronger association at the corresponding distance. Solid line represents association strength. Dashed lines represent 95% confidence bounds based on 10,000 bootstrap simulations.

APPENDIX B

ADDITIONAL FIGURES AND TABLES

Test	ANOVA			Co-variogram			GLM	
Site	WC	DA	PCH	WC	DA	PCH	Leaf N	Rhizome N
Porewater N								
% Rhizome N								
Below ground dry weight								
Rhizome growth rate								
% Leaf N								
Above ground dry weight								
Leaf growth rate								
Eelgrass Density								

	Positive correlation
	No Correlation
	Negative correlation
	No test

Figure B1. Overview table of findings within this study determined by statistical test (ANOVA, co-variogram, and linear regression).

Table B1. Mean C:N across sites (DA = DeZanza, WC = Westcliff, PCH = Pacific Coast Highway) and treatments (OE = oyster eelgrass, CE = Control/eelgrass) in eelgrass leaf samples in Upper Newport Bay, CA 2021.

Average of C:N			
Site	DA	PCH	WC
CE	19.14	18.41	18.96
OE	18.45	17.27	17.69

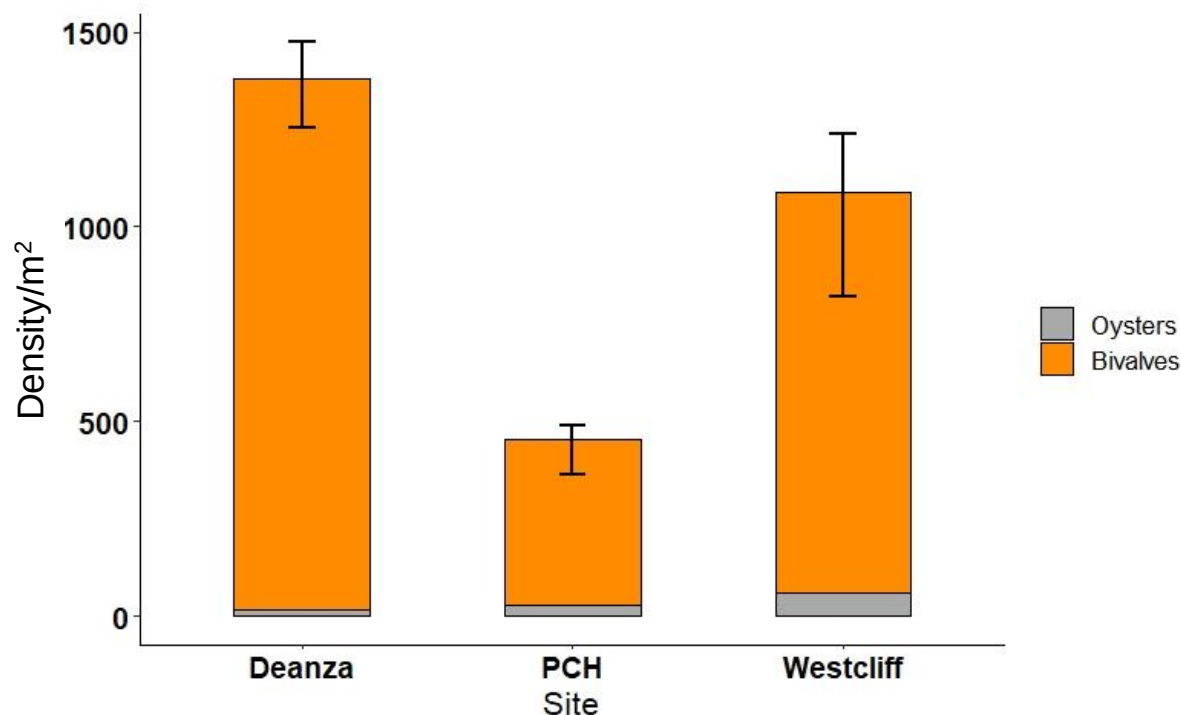


Figure B2. Average density of oysters (gray) and other filter-feeding molluscs (orange) in the oyster-eelgrass treatment beds across sites in Upper Newport Bay in 2021. Error bars = 1 SE. Other filter-feeding mollusc species include *Musculista senhousia*, *Modiolus* sp., *Mytilus galloprovincialis*, *Crucibulum spinosum*, and *Argopecten ventricosus*.

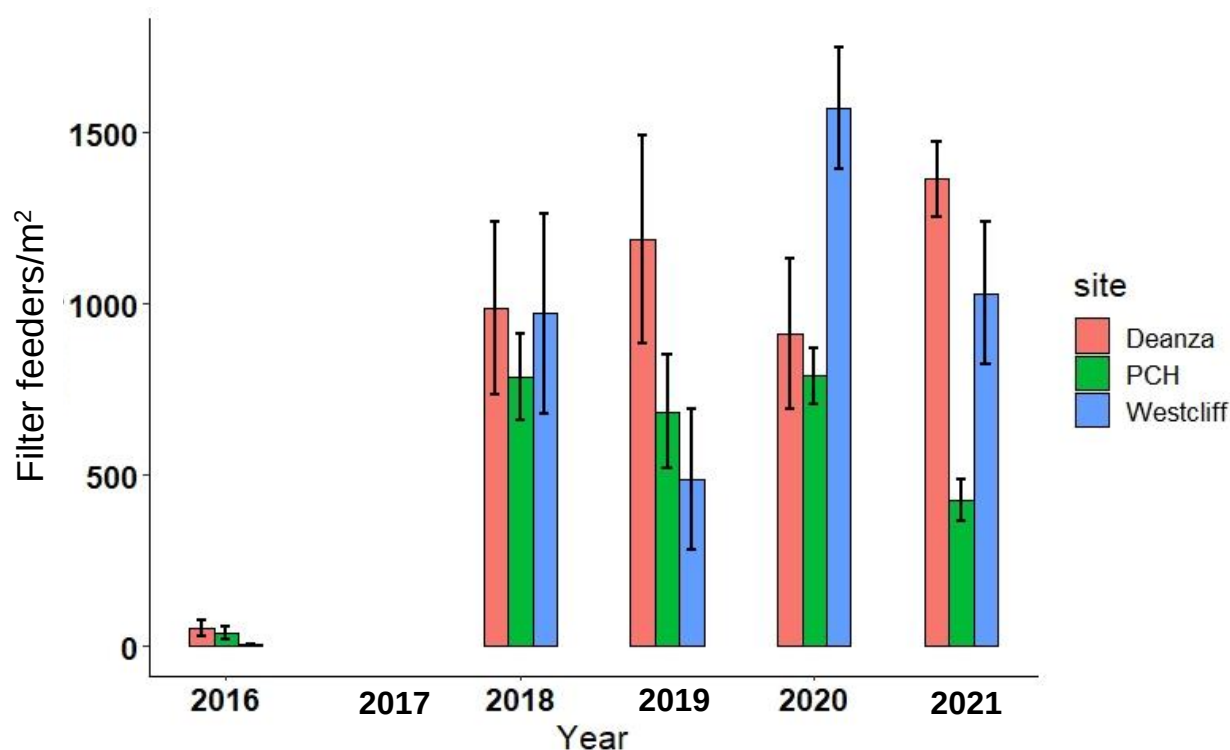


Figure B3. Mean filter-feeding mollusc density in the oyster-eelgrass treatment beds during May each year at each site in Upper Newport Bay, CA. Error bars = 1 SE. Density was not recorded in May 2017. Filter-feeding mollusc species include *Ostrea lurida*, *Musculista senhousia*, *Modiolus* sp., *Mytilus galloprovincialis*, *Crucibulum spinosum*, and *Argopecten ventricosus*.

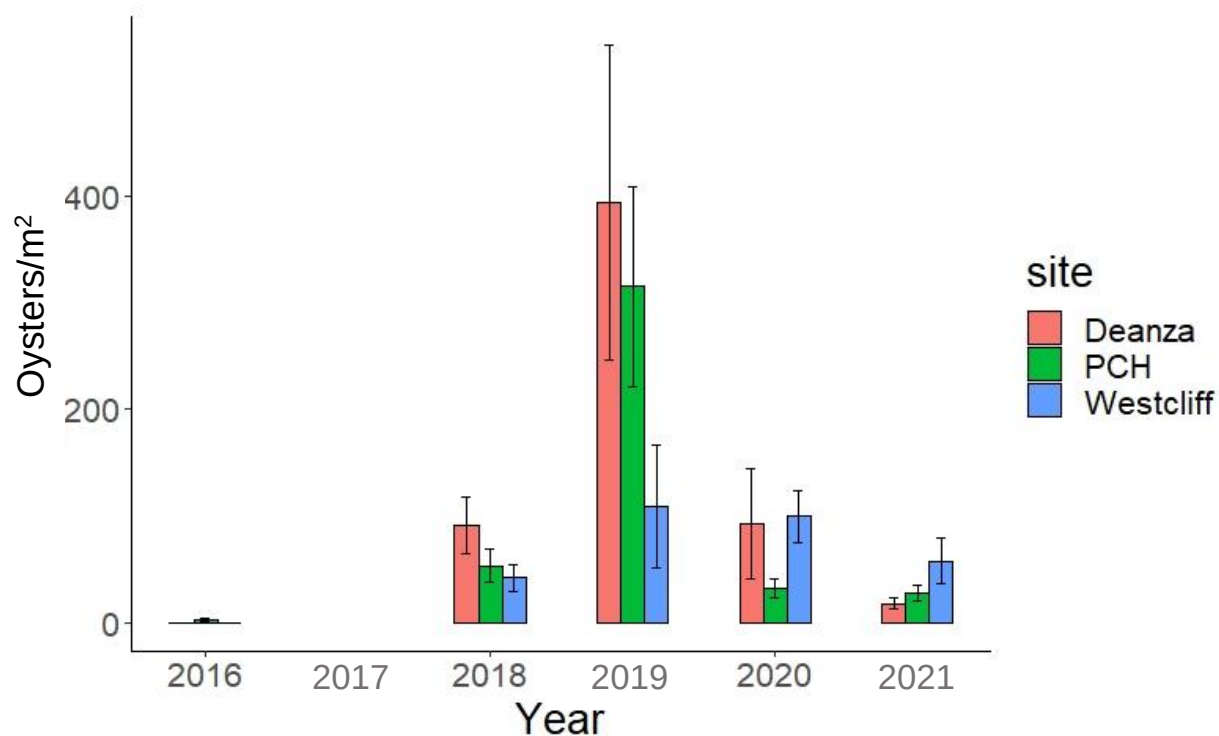


Figure B4. Average density of oysters in the oyster-eelgrass treatment beds across sites in Upper Newport Bay in 2021. Error bars = 1 SE.

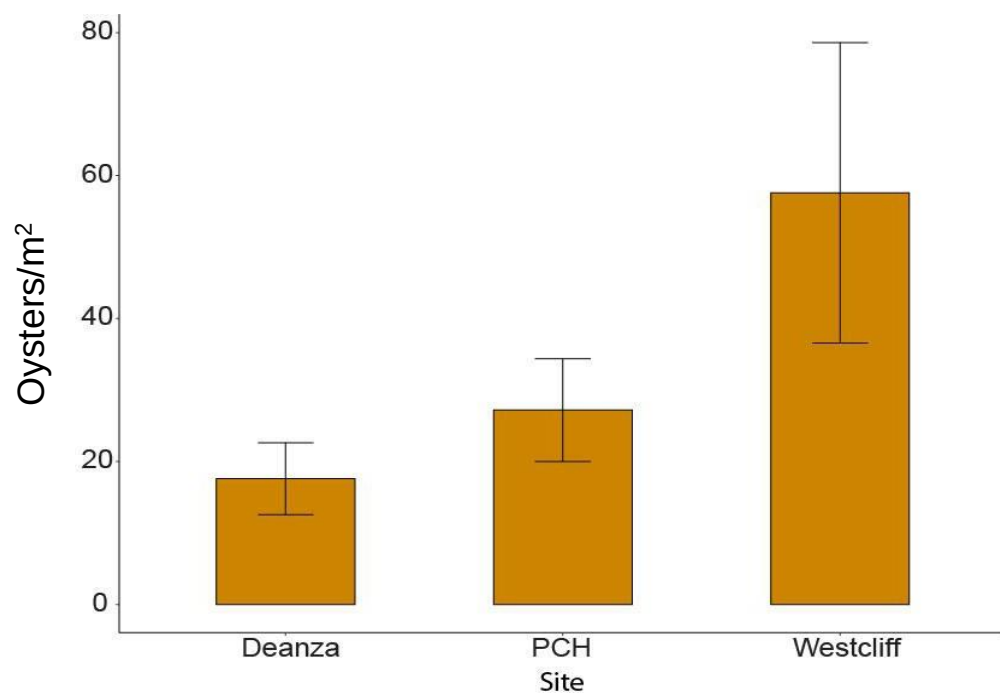


Figure B5. Mean oyster density in the oyster-eelgrass treatment beds by year at each site in Upper Newport Bay, CA. Error bars = 1 SE. 2017 was not recorded.

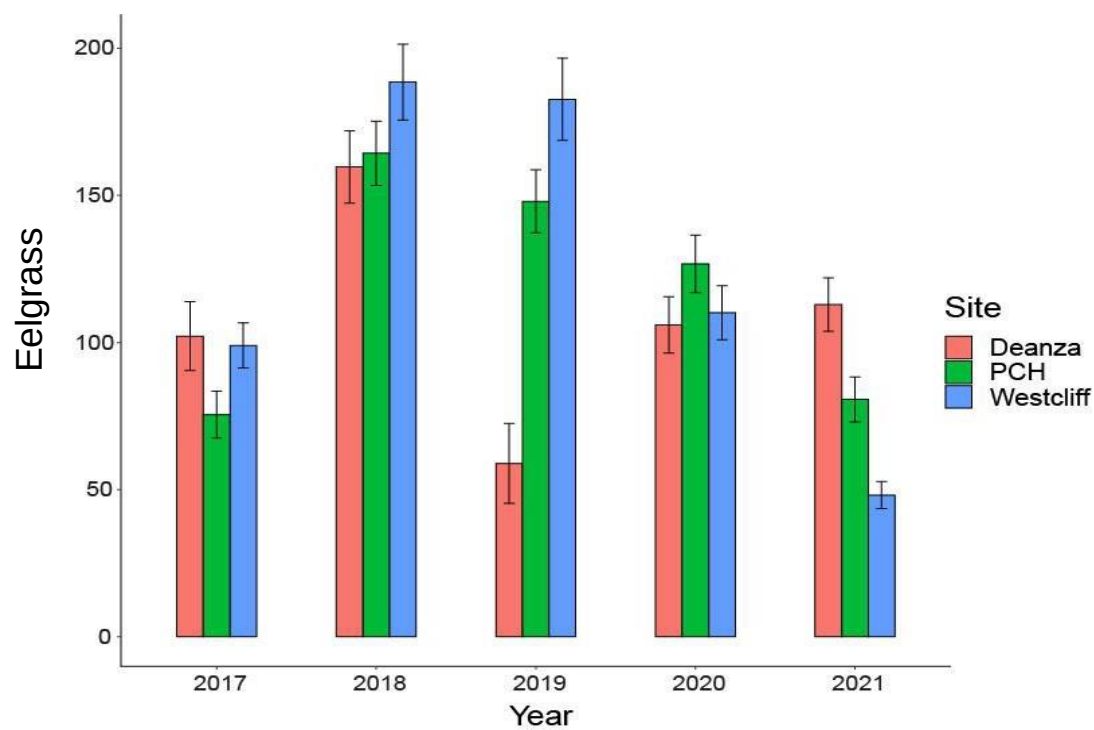


Figure B6. Mean eelgrass shoot density by quadrat sample in the oyster-eelgrass treatment beds by year at each site in Upper Newport Bay. Error bars = 1 SE.

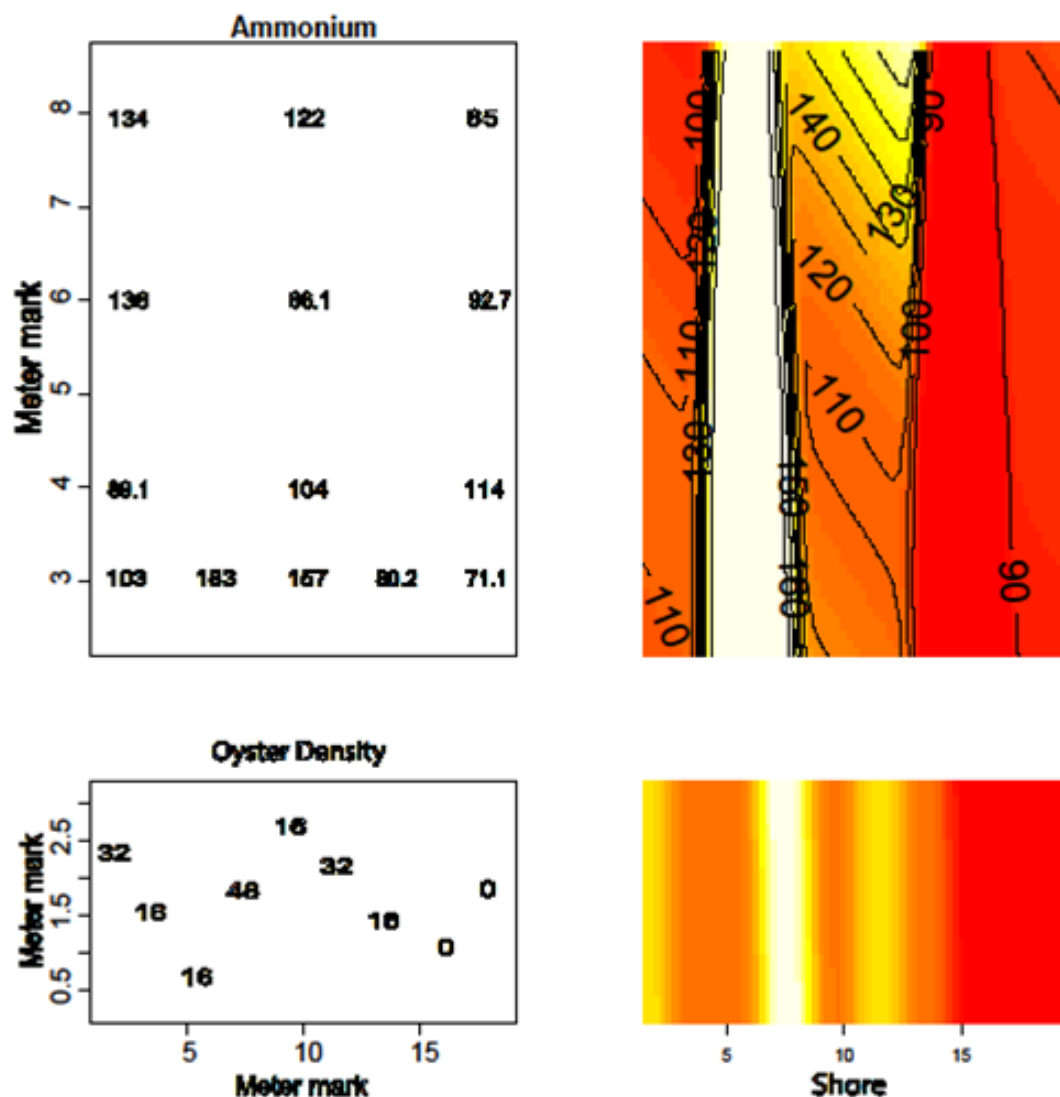


Figure B7. Example of kernel regression smoothing of ammonium and oyster density data from 2021 PCH oyster-eelgrass treatment (right) created from with raw spatial data points (left). Kernel regressions were used for smoothed spatial averaging to create co-variogram analysis. Lighter colors indicate greater values and red indicates lower values. Meter marks represent the position within the bed the data points were collected.

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