

EFFECTS OF LONGLINE OYSTER AQUACULTURE ON BENTHIC  
INVERTEBRATE COMMUNITIES IN HUMBOLDT BAY, CALIFORNIA

By

Hannah C. Coe

A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Natural Resources: Fisheries

Committee Membership

Dr. Mark Henderson, Committee Chair

Dr. Andre Buchheister, Committee Member

Dr. Sean Craig, Committee Member

Dr. Rick Zechman, Graduate Coordinator

July 2019

## ABSTRACT

### EFFECTS OF LONGLINE OYSTER AQUACULTURE ON BENTHIC INVERTEBRATE COMMUNITIES IN HUMBOLDT BAY, CALIFORNIA

Hannah C. Coe

Oyster aquaculture has had a commercial presence in Humboldt Bay for nearly 60 years and has experienced changes in scope and methodology as the industry has grown. The traditional method of bottom-culture oyster beds has been phased out, with longline oyster aquaculture becoming the common replacement. However, this transition has preceded much of the research regarding potential impacts to the broader ecosystem. The benthic invertebrate community of Humboldt Bay is a vital food source for many commercially important fishes, as well as for the many shorebirds that utilize Humboldt Bay. The importance of the invertebrate community to the ecosystem highlights the need to investigate how off-bottom culture affects invertebrate community composition. During the summer of 2017 and the winter of 2017/18, I collected benthic and epibenthic invertebrate samples from Humboldt Bay's North Bay. I then used multivariate analyses to compare the invertebrate community composition between eelgrass and mudflat habitats with and without aquaculture. I found that invertebrate communities responded most to the presence of structure and were not

significantly different between aquaculture and eelgrass habitats. Transects conducted to measure eelgrass cover revealed significantly lower eelgrass coverage and shoot count when aquaculture was present. Eelgrass beds are important refuge areas for many juvenile fish species, as well as a vital food source for many migrating waterbirds. This study found that the benthic invertebrate communities were comparable between aquaculture and eelgrass habitats but that eelgrass densities were reduced in aquaculture habitats, which should be considered when managing current and future oyster aquaculture in Humboldt Bay.

## ACKNOWLEDGEMENTS

There are many individuals without whom this work would not have been possible. First, I would like to thank all the intrepid volunteers who contributed their time, effort, comfort, and at moments, their sanity, to help me collect samples from the mudflats of Humboldt Bay! Grace Ghrist, Wesley Hull, Lara Jansen, Chris Loomis, and Laura Solinger sacrificed sleep for the pre-dawn sampling of the summer season. Ian Butler, Maddie Halloran, Emerson Kanawi, and Max Ramos braved the dark, rainy nights of winter sampling, and the lucky Emily Chen got to experience the delights of both as her love for walking the mudflats could not be restricted to only one season! Without Ashley Kay, my sampling rock with perfect handwriting, I would have lost my sanity...thanks for helping to keep it fun! Thank you to Pong Xayavong, Coast Seafoods Farm Manager, who has an astounding ability to navigate the North Bay- thank you for helping to plan the most efficient sampling route...and especially for the boat ladder! Thanks to Anthony Desch for building sampling equipment, Dave Baston and Susan Marshall for guidance while processing sediment samples, and Leslie Harris at the Los Angeles County museum for her help in certifying polychaete IDs.

I would like to thank my committee- Dr. Andre Buchheister and Dr. Sean Craig, for their time and effort in meetings, reading thesis drafts, and advising me through the process. My advisor, Dr. Mark Henderson, for initially accepting me as a student, and then providing support and guidance along the way...and of course for not kicking me out of the lab group because I wasn't studying fish!

Funding for this project was provided by the National Oceanic and Atmospheric Administration's Saltonstall-Kennedy Grant. Thanks to Marlene Meaders and Phil Bloch at Confluence Environmental Company for their support. Many thanks and much appreciation for additional funding from the International Women's Fishing Association, the Rotary Club of Eureka, Granite Bay Flycasters, and the St. Mary's College of Maryland Alumni Foundation.

Finally, thank you to my friends in the Fisheries Cooperative, and those in the Fish Ecology lab! Thanks to Leslie Farrar for administrative support and always having a friendly word and a smile to share. Thank you to my family across the country for the years of support getting me to this point and for understanding when you wouldn't hear from me for weeks. Lastly, to my partner, Chris Diviney, for your unending love and support, for reminding me that there was still a world outside my desk in the fisheries building, and making sure I balanced work with living and loving life.

## TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
LIST OF APPENDICES.....	x
INTRODUCTION.....	1
METHODS.....	6
Study Site.....	6
Sampling Methods.....	7
Sample Processing.....	10
Statistical Analyses.....	13
Eelgrass and sediment metrics.....	13
Benthic invertebrates.....	13
RESULTS.....	20
Eelgrass and Sediment Metrics.....	20
Benthic Macrofauna.....	23
Taxa accumulation curves.....	23
Multivariate analyses.....	25
DISCUSSION.....	33
Benthic Invertebrate Habitat Use.....	33
Seasonality.....	34
Ecosystem Implications.....	36

Management Implications.....	38
LITERATURE CITED.....	40

## LIST OF TABLES

- Table 1. Breakdown of main macrofaunal phyla into the taxonomic groups used for classification. Superscript indicates functional feeding group for that taxa (D= deposit, Su= suspension, Sc= scavenger, P= predator, H= herbivore, O= omnivore, M= mixed.. 12
- Table 2. Seasonal mean environmental characteristics for each habitat type within the three regions. AE= eelgrass with aquaculture, AM= mudflat with aquaculture, NE= eelgrass without aquaculture, NM= mudflat without aquaculture..... 22
- Table 3. Indicator taxa analysis and the associated functional feeding group for the summer and winter sampling seasons for the three sampled regions of Humboldt Bay. Superscript indicates functional feeding group for that taxa (D= deposit, Su= suspension, Sc= scavenger, P= predator, M= mixed..... 29

## LIST OF FIGURES

Figure 1. Cultch-on-longline bed in Humboldt Bay, CA.....	3
Figure 2. Humboldt Bay is located in northern California. This study focused on the North Bay subsection of Humboldt Bay, which was divided into three sampling regions: Bird Island, Mad River, and East Bay, where samples were collected from four habitat types: 1) eelgrass with aquaculture, 2) eelgrass without aquaculture, 3) mudflat with aquaculture, and 4) mudflat without aquaculture. ....	7
Figure 3. Left: Collection of 10cm deep sediment cores for benthic invertebrates and analysis for sediment characteristics. Center: Measuring eelgrass percent cover and shoot count using five 0.5m <sup>2</sup> quadrats along a 50m transect. Right: Collection of epibenthic organisms using a manual bilge pump with a 500micron net attachment. ....	9
Figure 4. Comparison of eelgrass percent cover (left) and shoot count (right) in habitats with and without longline oyster aquaculture. Because both seasons resulted in significantly less eelgrass when longlines were present, the seasons were pooled for simplicity of visual comparison. Error bars indicate standard error of the mean. ....	21
Figure 5. Taxa accumulation curves for each habitat type for both the summer (left) and winter (right) seasons.....	24
Figure 6. Comparison of the mean number of taxa encountered within each habitat type during the summer and winter seasons. All three regions are compared, from left to right: Bird Island, East Bay, Mad River. For each habitat type, there were significantly more taxa encountered during the winter season. Although there were exceptions, generally there were no strong trends or differences between habitat types. Error bars indicate standard error of the mean. ....	24
Figure 7. NMDS ordinations of the invertebrate samples collected from Humboldt Bay's North Bay. Habitat codes within ellipses indicate habitat types: AE= aquaculture and eelgrass, AM= aquaculture and mudflat, NE= eelgrass no aquaculture, and NM= mudflat no aquaculture. Overlapping ellipses indicate similarity in community composition, while clearly separated ellipses represent habitat types with differing invertebrate communities. Arrows indicate the strength and direction of increase of the significantly correlated environmental variables. ....	28
Figure 8. Graphical outputs of GradientForest analysis for the Bird Island, East Bay, and Mad River regions of Humboldt Bay. Plots display taxa which were most impacted by changes along that gradient; top three most impacted taxa are indicated in legend. ....	32

## LIST OF APPENDICES

Appendix A. Summer and winter sampling sites for the Bird Island, East Bay, and Mad River regions of Humboldt Bay's North Bay. ....	46
Appendix B. Top five most abundant taxa per habitat type per region, divided into the summer and winter sampling seasons. ....	48
Appendix C. Results of permutational analysis tests to evaluate equality of dispersion prior to PERMANOVA analysis of regional community composition. Significance values greater than 0.05 indicate failure to reject the null hypothesis of equal dispersion, indicating fulfillment of this assumption. Rejection of the null hypothesis indicates a need to further evaluate equality of dispersion using visual plotting of habitat dispersions. ....	50
Appendix D. Results of permutational analysis of variance (PERMANOVA) for each region for each sampling season. Significant results were evaluated using a post-hoc test, found in FILL. ....	51
Appendix E. Results of post-hoc testing of significant PERMANOVA results for the summer (top) and winter (bottom) seasons. Dashes indicate those habitats were not significantly different for any region. Regional entries indicate those habitats were found to be significantly different within that region. ....	52
Appendix F. Additional axis comparisons of NMDS ordinations for the invertebrate samples collected from Humboldt Bay's North Bay. Habitat codes within ellipses indicate habitat types: AE= eelgrass with aquaculture, AM= mudflat with aquaculture, NE= eelgrass no aquaculture, and NM= mudflat no aquaculture. Arrows indicate the strength and direction of increase of the significantly correlated environmental variables. ....	53
Appendix G. Stress plotted against number of dimensions to determine the correct number of dimensions for ordination plotting. ....	53
Appendix H. R <sup>2</sup> weighted importance of environmental variables based on GradientForest analysis. The top two variables for each season and region combination were used for further analysis of the relationship between individual variables and invertebrate taxa, except in the case of the top two variables being eelgrass shoot cover and count, in which case the next best variable was used, due to the similarity in invertebrate response to the two eelgrass metrics. ....	53
Appendix I. Plots of trends in compositional change of invertebrate communities within the Bird Island, East Bay, and Mad River regions of the North Bay. The x-axis indicates	

the environmental parameters most influential to driving changes in the invertebrate community. The black line indicates the density of regression tree splits at that level of the environmental gradient (how much community sorting occurred at that point in the gradient) and the red line indicates the density of samples taken at various points along the gradient. The blue line displays the ratio of the black line to the red; peaks in the blue line indicate gradient locations where the compositional change of the invertebrate community occurred. .... 53

## INTRODUCTION

Benthic invertebrates (i.e. polychaetes, amphipods, isopods, molluscs, crustacea, etc.) are a vital part of estuarine food webs, and are essential to a sustainable aquatic ecosystem (Beaumont et al. 2007). Invertebrates are characterized by a diversity of feeding methods, from suspension and deposit feeders to active predators, and thereby forge connections that create the base of a healthy foodweb for most ecosystems (Herman et al. 1999). The response of the benthic invertebrate community can be used to gauge the health of an ecosystem, and tracking invertebrate communities through time can disclose whether a system is improving or worsening in its overall health (Pearson and Rosenberg 1978). In Humboldt Bay, an estuary located in northern California, the benthic invertebrate community also serves as a vital food source for many commercially important fishes, including several species of juvenile rockfish and an assortment of clupeids, surfperches, and hexagrammids (Simenstad and Fresh 1995; Pinnix et al. 2005). Also, of the more than thirty species of shorebirds that utilize Humboldt Bay, benthic invertebrates are necessary to the health and sustainability of many (Colwell 1994; Danufsky 2000). Within Humboldt Bay, intertidal habitats are naturally composed of eelgrass and/or open mudflat environments. However, in portions of the Bay, these habitats are modified by the addition of longline oyster aquaculture. The effects of these aquaculture beds, particularly to the benthic invertebrate community, are somewhat uncertain. To ensure a sustainable ecosystem for the fishes and birds of Humboldt Bay, it is essential to understand how benthic invertebrate communities differ between these

various habitats, and thus how any potential changes to the ecosystem (i.e., expansion of aquaculture) may impact the rest of the ecosystem.

The most diverse invertebrate communities have historically been associated with eelgrass beds, and Humboldt Bay contains over 30% of all remaining eelgrass in the state of California (Trianni 1996; Gilkerson and Merkel 2014). Eelgrass beds provide habitat complexity to the otherwise homogenous mud substrate which naturally characterizes much of Humboldt Bay (Skeesick 1963). When compared to bare sand/mud substrate, eelgrass beds have been shown to foster higher abundance and species density of benthic macroinvertebrates (Orth 1973; Stoner 1980; Orth et al. 1984; Bostrom and Bonsdorff 1997). Eelgrass beds increase habitat complexity and food availability, decrease flow rate, and create refuge from predation (Summerson and Peterson 1984; Simenstad and Fresh 1995).

Similar to eelgrass beds, oysters introduced via aquaculture provide areas of refuge and attachment surfaces for invertebrates. The oysters themselves are autogenic ecosystem engineers- the physical structures they create change the environment around them, impacting the biotic and abiotic resources available to the surrounding aquatic community (Jones et al. 1994; Gutierrez et al. 2003). Through increased habitat complexity they also support a population of prey species which can have impacts on the food web of the entire system (Ruesink et al. 2005). In a study by van der Zee et al. (2015), it was found that the addition of an ecosystem engineering mussel resulted in a shift in the species composition of benthic infauna, as well as an increase in the number and diversity of functional feeding groups represented in the intertidal ecosystem.

Although small changes in diversity are sometimes disregarded, the impacts to functional diversity can influence how that ecosystem operates (Tilman 2001; Micheli and Halpern 2005).

Aquaculture-produced shellfish is a growing market worldwide (Ababouch et al. 2016), but production in the United States is hindered by concerns about the uncertain environmental impacts (Costa-Pierce 2002; Dumbauld et al. 2009). Although many predict that aquaculture-produced protein will be vital to feeding the growing world's population, there is also a concern about the terrestrial crops and wild fish food inputs required for aquaculture production of fish species (Troell et al. 2014). Oysters produced within an open, ocean-connected system however, do not require such external inputs and do not add strain to terrestrial crops or wild fish populations. Humboldt Bay, with its connection to the Pacific Ocean, is one such fishery. As the largest producer of aquaculture oysters in the state of California, Humboldt Bay produces over 70% of California's oysters (Coast Seafoods Company 2016) and the oyster aquaculture industry has an economic impact of almost \$20 million to the region's economy (Richmond et al. 2018). The oysters produced in Humboldt Bay, Pacific and Kumamoto (*Crassostrea gigas* and *Crassostrea sikamea*, respectively), are cultivated using the cultch-on-longline aquaculture method, which requires settling oyster spat onto empty oyster shells and braiding them into the longline rope (Figure 1; Cote et al. 2017). Longline oyster culture in Humboldt Bay has replaced the traditional method of



Figure 1. Cultch-on-longline bed in Humboldt Bay, CA.

on-bottom oyster beds harvested via mechanical dredge (Chew 2001). On-bottom culture methods had a substantial direct impact on eelgrass, and the change to off-bottom methods was motivated by a desire to lessen the impacts to the benthic habitat (Gilkerson and Merkel 2014). However, the transition to longline aquaculture has preceded the scientific research to inform best management practices for this culture method; the majority of the oyster aquaculture research that exists today has examined the impacts of on-bottom oyster beds to the ecosystem, resulting in gaps of knowledge regarding the relationship between longline oyster culture and invertebrate communities (Dumbauld et al. 2005).

The introduction of oysters on longline can change the physical environment, with the potential to impact the associated benthic community. The physical structure of longline oyster culture can change the flow regime, while the oysters themselves eject biodeposits (feces and pseudofeces), the products of active suspension feeding (Newell and Landgon 1996; Newell and Koch 2004; Ruesink et al. 2005). These aggregated biodeposits have a faster rate of sinking than do non-aggregated particles in the water column, causing them to be more quickly incorporated into the bottom sediment (Kautsky and Evans 1987). However, the spatial extent of biodeposition from oysters depends upon how quickly those deposits settle to the substrate beneath the lines. Accumulation of feces and pseudofeces can result in over-enrichment and anoxia; but sufficient mixing, driven by hydrodynamic forces, could potentially spread biodeposits throughout the system. A study by Forrest and Creese (2006) found enhanced deposition and slowing of water flow within off-bottom oyster culture beds, and found these areas to

have macrofaunal communities with composition patterns reflecting impact from disturbance. However, other studies have found oyster aquaculture habitats to foster similar benthic invertebrate communities as eelgrass beds (Hosack 2003; Rumrill and Poulton 2004; Hosack et al. 2006; Ferraro and Cole 2007). Due to the potential for changes to the physical habitat as a result of aquaculture, it is important to understand the differences in benthic invertebrate assemblages between eelgrass and mudflat habitats within Humboldt Bay, both with and without longline oyster aquaculture present.

In recent years, the aquaculture industry in Humboldt Bay has sought to expand the footprint of longline beds within the Bay. However, these proposals have been rejected due to the uncertain impact to the ecosystem (Weiner et al. 2017). Understanding how benthic invertebrate communities differ between habitats with and without longline oyster aquaculture will provide insight into how potential expansion of aquaculture practices may influence the ecosystem of Humboldt Bay and similar estuaries. Thus, the primary objectives of my thesis were to:

1. understand how benthic and epibenthic communities are affected by the presence of longline oyster aquaculture in Humboldt Bay.

2. compare seasonal (summer and winter) macroinvertebrate assemblages between four habitat types: 1) eelgrass with aquaculture, 2) eelgrass without aquaculture, 3) mudflat with aquaculture, and 4) mudflat without aquaculture.

## METHODS

### Study Site

Humboldt Bay, located in northern California, is the second largest enclosed bay in the state (Schlosser and Eicher 2012). Oyster aquaculture in Humboldt Bay primarily occurs in the North Bay, the region of the Bay where this study was focused. The North Bay is a shallow area characterized by extensive mudflats and drained by three channel systems converging to flow into the Central Bay and ultimately into the Pacific Ocean. The North Bay has a mean high water (MHW) area of approximately 14.2 square miles and a mean lower low water (MLLW) area of only 6.9 square miles (Skeesick 1963). For this study, aquaculture sites in the North Bay were delineated into three regions- Bird Island, East Bay, and Mad River (Figure 2**Error! Reference source not found.**). These three regions experience differences in flow regimes and turnover rates; physical parameters which can influence other abiotic factors as well as the biological characteristics of a region. To address this potential variability between regions, sampling sites were distributed equally within each region.

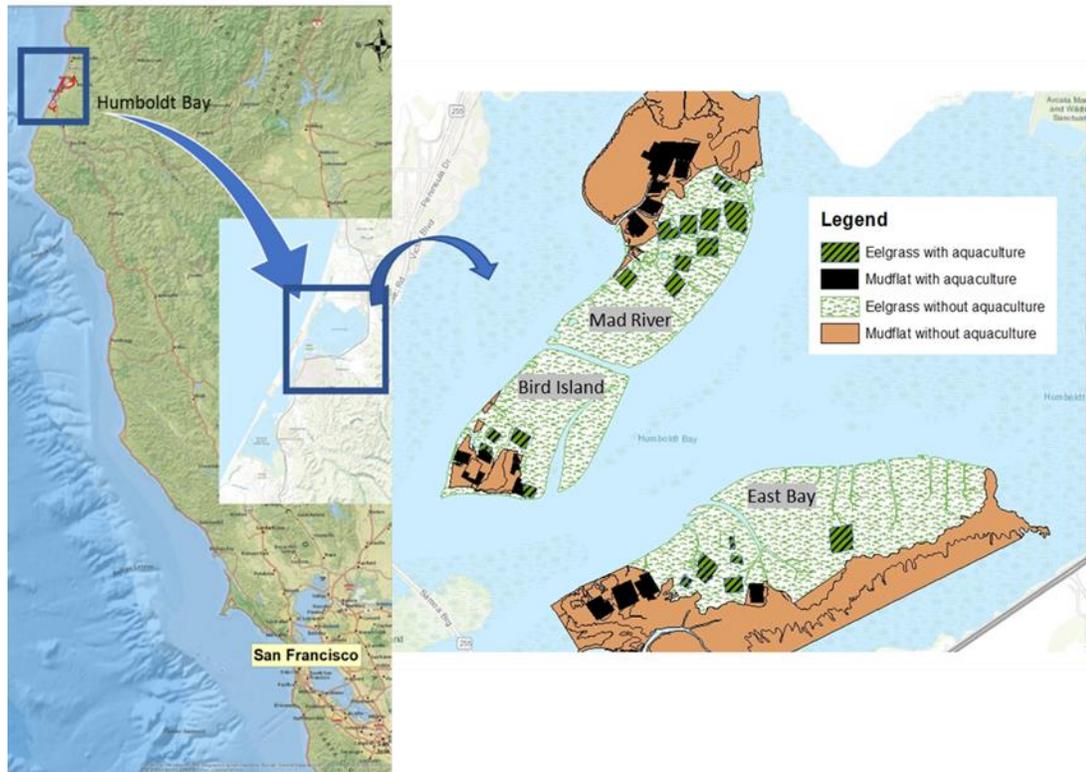


Figure 2. Humboldt Bay is located in northern California. This study focused on the North Bay subsection of Humboldt Bay, which was divided into three sampling regions: Bird Island, Mad River, and East Bay, where samples were collected from four habitat types: 1) eelgrass with aquaculture, 2) eelgrass without aquaculture, 3) mudflat with aquaculture, and 4) mudflat without aquaculture.

### Sampling Methods

Within each of the three regions of the North Bay, I used ArcMaps's random sample tool (ArcMap 10.4.1) to locate sampling sites within each of four habitat types: 1) eelgrass with aquaculture, 2) eelgrass without aquaculture, 3) mudflat with aquaculture, and 4) mudflat without aquaculture. Based on previous studies, I selected at least five sites per habitat type per region to sample each season; this was found to be a sufficient

number of samples to detect benthic macrofaunal community differences due to habitat type (Ferraro and Cole 2004). I conducted separate random sample site draws for the summer and winter sampling seasons (Appendix A).

The primary study goal was to quantify potential differences between benthic invertebrate communities in habitats with and without longline oyster aquaculture. To do this, a 2.5cm by 10cm tall core was collected using a PVC pipe with a vacuum seal when the mudflats were exposed during low tides (Figure 3). The diameter of the cores was selected based on a study by Ferraro and Cole (2004) which showed this volume to be sufficient to capture the diversity and abundance of representative benthic fauna. In addition to the cores, epibenthic organisms were sampled using an epibenthic pump similar to the one used by Toft et al. (2013). With the pump base resting on the sediment surface, the epibenthic pump pulled approximately five liters of water through the 500micron mesh bag attached to the output pipe of a hand bilge pump (Figure 3). For the early morning tides of the summer sampling season, epibenthic pump samples were collected on the incoming tide, following the collection of low tide core samples. During the winter season, low tides occurred in the evenings, so we conducted epibenthic pumps on the outgoing tide, prior to core collection, to avoid high water sampling after dark. For both seasons, samples were collected when the water was between 25-90cm deep.

Several environmental habitat variables were measured at all sampling sites. First, five 0.5m<sup>2</sup> quadrats (Figure 3) were evenly spaced along a 50m transect to measure eelgrass percent cover and eelgrass shoot count. Eelgrass percent cover was estimated by eye while the shoots were individually counted (Tallis et al. 2009). Cores were collected for analysis of carbon content and particle size of the sediment, as these factors have also been correlated with benthic community composition (Bott and Diebel 1982). The elevation of each sampling site was recorded from the ArcMap sampling map.



Figure 3. Left: Collection of 10cm deep sediment cores for benthic invertebrates and analysis for sediment characteristics. Center: Measuring eelgrass percent cover and shoot count using five 0.5m<sup>2</sup> quadrats along a 50m transect. Right: Collection of epibenthic organisms using a manual bilge pump with a 500micron net attachment.

These samples were collected during both the summer and winter seasons to quantify community differences between the highly productive summer months and the less productive winter months. Summer samples were collected between June 22-28, 2017 and July 21-27, 2017 and winter samples were collected between December 2-7,

2017, January 2-5, 2018, and January 28 – February 2, 2018. Sampling dates were chosen to correspond with the lowest set of low tides during those months. During summer sampling, tides ranged from -0.17m to -0.55m, while the tidal range during the winter months was -0.09m to -0.48m.

### Sample Processing

All samples were stored on ice in the field; following transport to Humboldt State University, sediment cores for carbon content and particle size analysis were stored at -80°C and -18°C, respectively. Samples collected for carbon content were analyzed using the loss on ignition protocol (Gavlak et al. 2005) and the equipment of the College of Natural Resources and Sciences Core Research Facility at Humboldt State University. Particle size analysis was performed using the sieve and hydrometer method for percent sand, silt, and clay (Day 1965). All samples collected for invertebrates were stored at 4°C to await sieving to remove invertebrates from the sample. Infauna sediment samples were washed with seawater through a series of stacked sieves (4mm to 2mm to 0.5mm) (Lewis and Stoner 1981), and the organic material left on each sieve was fixed in buffered 10% formalin and stained with Rose Bengal. The benthic pump samples were washed on the 0.5mm sieve and similarly fixed. The fixed samples were examined under a dissecting microscope, invertebrates were removed and placed in 70% ethanol for storage, and individual organisms were later identified to the taxonomic level indicated in Table 1. Forrest and Creese (2006) found similar taxonomic levels to be sufficient to detect spatial differences in soft-bottom invertebrate communities. Copepoda are generally considered

to be meiofauna (organisms which will pass through a 0.5mm mesh) (Watzin 1983), and as such were not counted in any sample, as those encountered were a result of entanglement in detritus within samples and were not targeted in this sampling scheme. As the aquatic invertebrate community was the target of this study, incidental terrestrial organisms were not identified.

Table 1. Breakdown of main macrofaunal phyla into the taxonomic groups used for classification. Superscript indicates functional feeding group for that taxa (D= deposit, Su= suspension, Sc= scavenger, P= predator, H= herbivore, O= omnivore, M= mixed).

Macrofaunal phylum	General groups used
Arthropoda	Class level: Ostracoda <sup>Su</sup> , Order level: Amphipoda <sup>Sc</sup> , Cumacea <sup>Sc</sup> , Isopoda <sup>Sc</sup> , Tanaidacea <sup>Sc</sup> Infraorder: Brachyura <sup>Sc</sup> , Caridea <sup>Sc</sup> Family: Caprellidae <sup>M</sup> , Chironomidae <sup>D</sup>
Mollusca	Class level: Bivalvia <sup>Su</sup> , Gastropoda <sup>H</sup>
Annelida	Subclass: Oligochaeta <sup>D</sup> Family level: Polychaeta: Ampharetidae <sup>D</sup> Capetellidae <sup>D</sup> Cirratulidae <sup>D</sup> Cossuridae <sup>D</sup> Dorevilleidae <sup>P</sup> Eunicidae <sup>Sc</sup> Glyceridae <sup>P</sup> Lumbrineridae <sup>P</sup> Maldanidae <sup>D</sup> Nephytidae <sup>P</sup> Nereididae <sup>O</sup> Opheliidae <sup>D</sup> Orbiniidae <sup>D</sup> Oeniidae <sup>D</sup> Pholoidae <sup>P</sup> Phyllodocidae <sup>P</sup> Spionidae <sup>M</sup> Syllidae <sup>O</sup>
Echinodermata	Phylum level: Echinodermata <sup>P</sup>
Other phyla	Phylum level: Nemertea <sup>P</sup> , Cnidaria <sup>P</sup> , Platyhelminthes <sup>P</sup> , Sipuncula <sup>D</sup>

## Statistical Analyses

### Eelgrass and sediment metrics

For the eelgrass measurements of percent cover and shoot count, differences between seasons, regions, and habitat types were evaluated. Because the mudflat habitats were chosen for their lack of eelgrass, only the two eelgrass habitat types- with and without aquaculture, were used for this analysis. Using R version 3.4.0, two-way analysis of variance (ANOVA) was conducted with factors of aquaculture (presence or absence), season (summer or winter), region (Bird Island, East Bay, and Mad River), and interaction terms. For all ANOVA comparisons, the assumptions of homogeneity of variance and normality were evaluated.

All site characteristics- elevation, sediment metrics (percent carbon, sand, silt, and clay), as well as eelgrass shoot count and percent cover, were summarized as an average per habitat per season.

### Benthic invertebrates

Comparisons of benthic invertebrate communities in habitats with and without aquaculture were conducted to evaluate potential differences in community composition due to the addition of oyster longlines. For all analyses, the invertebrate counts for benthic cores and epibenthic pumps were combined for each site sampled. Taxa accumulation curves were generated for each habitat within each season. These plots show the rate of accumulation of new species with increasing numbers of samples (Ugland et al. 2003), and can be used to determine if adequate sampling has occurred.

No new taxa would be expected with increasing samples if the accumulation curve achieves an asymptote. If, however, the curve does not reach an asymptote, additional samples would likely continue to result in increasing numbers of taxa being encountered, indicating that an insufficient number of samples were collected. Seasonal comparison of the number of taxa encountered within each habitat type (1. eelgrass with aquaculture, 2. eelgrass without aquaculture, 3. mudflat with aquaculture, and 4. mudflat without aquaculture) was conducted using a two-way ANOVA with factors habitat type and season. Within each season, the number of invertebrates within the two eelgrass habitat types (with and without aquaculture) were also compared using one-way ANOVA with factor aquaculture presence or absence.

Patterns in invertebrate community structure within the North Bay were examined using non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA). Due to their spatial locations within the North Bay, the three regions (Bird Island, East Bay, and Mad River) experience differences in flow regime and turnover rates- factors which can influence other abiotic factors. Due to their ability to influence the biotic community, seasons and regions were treated separately for all multivariate analyses. Because sites were placed a priori into habitat groups (eelgrass and mudflat with or without aquaculture), PERMANOVA can be used to determine if there are statistical differences between the habitat types. PERMANOVA partitions the variation in the space of the dissimilarity measure chosen when conducting the NMDS. In this case, the Bray-Curtis dissimilarity measure was used to create a dissimilarity matrix comparing sites based on taxa composition (Bray and Curtis 1957).

The Bray-Curtis distance is commonly used for community composition datasets (Peterson and McCune 2001; McCune et al. 2002). PERMANOVA is suitable for multivariate community data because it does not make distributional assumptions of either the original data or the calculated dissimilarity matrix (Anderson 2017). To address homogeneity of spread, a dispersion test can be conducted to evaluate differences in variability between groups. The null hypothesis of the dispersion test is no difference in spread of the groups being compared (Anderson 2005, 2017). If a significant PERMANOVA result is obtained, a post-hoc test can be used to determine between which groups the differences occur.

Following statistical comparison of the habitat groups, non-metric multidimensional scaling (NMDS) can be used to visualize patterns in community composition. NMDS has several benefits for biotic community data; there is no assumption of multivariate normality and it is accepting of a large number of zero values, which is likely in taxa comparisons (Field et al. 1982; MacNally 1990; Clarke 1993; McCune et al. 2002; Ferraro and Cole 2007). For ecological community data, NMDS uses a multivariate dissimilarity matrix based on the taxa composition dataset to condense the data into a reduced number of dimensions, in which sites are plotted based upon their taxa similarities. For this multivariate analysis, sites were grouped by season and region. To prepare the taxa data, I first used the Hellinger transformation, which is commonly used for ordinations of taxa abundance data, and which gives low weight to rare taxa (Legendre and Gallagher 2001). With these transformed values, I again used the Bray-Curtis dissimilarity measure to create the dissimilarity matrix, as this measure has been

proven to be among the most robust and effective ordination distances for community data when using non-metric multidimensional scaling (Faith et al. 1987).

The number of axes used to plot community similarities is a balance between maintaining the relationship between the similarity of sites in the original data and the similarity of sites in the synthesized and condensed data (McCune et al. 2002). Too many dimensions can result in the information being spread over too many axes, lessening the ability to discern covariation between the taxa composition of sampling sites. Generally, a stress value less than 0.2 would represent a useful ordination with low likelihood of misinterpretation, with lower stress levels indicating that the ordination better represents the actual data. A stress value greater than 0.3 represents an NDMS solution that should not be used, as it is uninformative and little better than a random placement of sampling sites (Field et al. 1982; Clarke and Warwick 2001). The correct number of dimensions was determined by plotting the stress level against different numbers of dimensions and determining at which dimension there was an asymptote (or elbow) in the stress level. This indicates that additional dimensions would not result in a large reduction in stress, and the dimension at which the elbow occurs should be the number of dimensions used for ordination plotting.

NMDS ordination can be used to visualize similarities between groupings of sampling sites. When sample units are grouped a priori to ordination mapping (i.e. by habitat type), an ellipse can be drawn about the centroid of a group, to represent the standard deviation of that grouping of sites (Oksanen et al. 2017). These ellipses can indicate the similarity between groups- ellipses which have no overlap would represent

groups which have dissimilar taxa composition. In addition to the results of PERMANOVA analysis, which statistically analyzes similarities of groups, ordinations can be used to observe trends in group similarities.

To evaluate whether any habitat types were characterized by particular taxa, indicator taxa analysis was performed using the “indicspecies” package in R (De Caceres and Maintainer 2016). Indicator analysis assesses the statistical significance of taxa associations to specified groupings of sampling sites, providing information about the fidelity of taxa to certain habitats. By comparing taxa occurrence within the different habitats, taxa are assigned a strength of association which is compared to a permuted association level likely to occur by chance. If the observed association level is not found to be likely to occur by chance, then that taxa is determined to be significantly associated with a particular habitat (De Caceres and Maintainer 2016). Additionally, invertebrates were categorized into functional feeding groups (predator, herbivore, omnivore, suspension, deposit, and suspension/deposit feeder) (Ferraro and Cole 2007; Macdonald et al. 2010; Barnes and Hamylton 2015; van der Zee et al. 2015) to better understand how community structure is linked to broader ecological function (Fauchald and Jumars 1979; Jumars et al. 2015).

The correlation of habitat variables (sediment characteristics of carbon, sand, silt, and clay, as well as elevation, eelgrass shoot count and percent cover) to the ordination was examined using the Envfit function within the package ‘Vegan’ (Oksanen et al. 2017). Using continuous environmental data, Envfit fits vectors to show the direction of increase for that environmental variable. The strength of the correlation to the ordination

is reflected in the length of the arrow, with a stronger correlation being displayed with a longer arrow (Oksanen et al. 2017). This can help to determine what environmental factors might be driving potential differences in community composition between groups.

To investigate further the relationship between invertebrate community composition and the environmental variables, I used a gradient forest analysis (Ellis et al., 2012; Pitcher et al. 2012; Stephenson et al., 2018). Using taxa abundances and continuous environmental data, a gradient forest analysis evaluates compositional community changes along the environmental gradients of interest, in this case: elevation, eelgrass cover, eelgrass shoot count, sediment carbon content, and sediment size. I conducted this analysis using the R package “GradientForest” (Ellis et al. 2012). This package creates regression trees to group sites based upon the community response to the environmental predictors. Regression trees use the taxa dissimilarity data at each site to split different sites into two groups (or ‘branches’) based upon the community response to an environmental predictor variable. The diverging branches are organized relative to a split value, ‘s’, such that one branch is composed of sites having predictor values less than s, while the other contains sites having predictor values greater than s. In many cases, a set of taxa sensitive to an environmental gradient react to a threshold that sorts taxa composition above and below that threshold level; in these cases, the first split value would be the most informative and would occur at an ‘s’ value close to that of the threshold. The gradient forest method uses an aggregation of many individual regression trees, created using bootstrapped samples. This method evaluates which environmental variables have the strongest overall impact on the invertebrate community, as well as

where along the environmental gradient the response occurs (Ellis et al. 2012; Pitcher et al. 2012).

## RESULTS

### Eelgrass and Sediment Metrics

Comparison of eelgrass shoot count and percent cover revealed similar effects of oyster aquaculture on both eelgrass metrics. Two-way ANOVA comparison using the model: Percent Cover ~ Season \* Region \* Aquaculture (present or absent), resulted in a significant result for the aquaculture comparison only, with less eelgrass occurring when longlines were present ( $p < 0.001$ ;  $F = 31.31$ ; Figure 4); all other comparisons and interactions were found to be non-significant. Shoot counts, using the same model factors as above, were found to be higher in the winter season ( $p = 0.002$ ;  $F = 10.42$ ; Figure 4), although the difference between count averages was less than one eelgrass shoot. There was also significantly lower shoot counts ( $p = 0.012$ ;  $F = 6.76$ ) when oyster longlines were present. Although a significant interaction existed between season and region ( $p = 0.004$ ;  $F = 6.21$ ), for the purpose of comparing the impacts of oyster culture on eelgrass cover and count, it was considered to be of low importance and is not considered further. For both analyses, the assumptions of homogeneity of variance and normality were met.

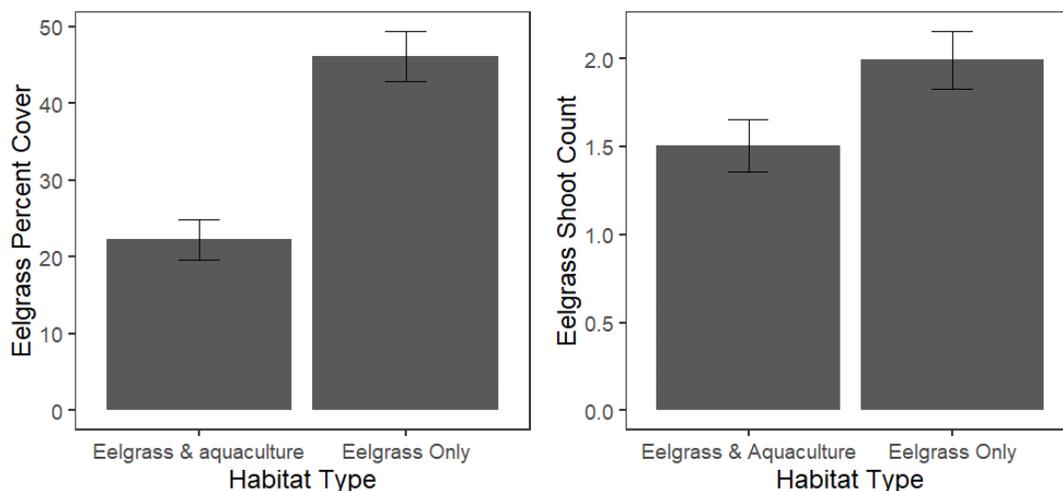


Figure 4. Comparison of eelgrass percent cover (left) and shoot count (right) in habitats with and without longline oyster aquaculture. Because both seasons resulted in significantly less eelgrass when longlines were present, the seasons were pooled for simplicity of visual comparison. Error bars indicate standard error of the mean.

Due to their potential to drive community differences, the other environmental characteristics of site elevation, percent carbon, sand, silt, and clay were summarized by average per habitat type per region for each season (Table 2). Carbon content of the sediment was highest in the summer, with Mad River region having the highest percent carbon in the winter months, and East Bay having the highest carbon content during the summer. Of the three regions, Bird Island sediment had the highest sand content and the lowest silt and clay composition. Eelgrass habitats, both with and without aquaculture were located in the lowest elevations, followed by mudflat with aquaculture, with mudflat only habitats located in the highest elevations.

Table 2. Seasonal mean environmental characteristics for each habitat type within the three regions. AE= eelgrass with aquaculture, AM= mudflat with aquaculture, NE= eelgrass without aquaculture, NM= mudflat without aquaculture.

		Bird Island Region				East Bay Region				Mad River Region			
		AE	AM	NE	NM	AE	AM	NE	NM	AE	AM	NE	NM
Eelgrass Percent Cover	Summer	22.04	0	45.1	0.08	22.44	2.2	51.42	0.04	24.68	0.2	45.92	0.04
	Winter	19.15	0	54.3	0	29.21	0	47.55	0	14.1	0.5	34.16	0
Eelgrass Shoot Count	Summer	1.94	0	2.52	0	2.32	0.1	2.58	0.02	1.68	0.04	1.55	0.02
	Winter	1.89	0	3.02	0	2.09	0	2.8	0	0.95	0.02	1.71	0
Elevation (m to MLLW)	Summer	-0.22	0.13	-0.25	0.27	-0.15	-0.08	-0.24	0.53	-0.28	0.32	-0.12	0.36
	Winter	-0.16	0.15	-0.12	0.36	-0.14	-0.09	-0.27	0.09	-0.21	0.16	-0.21	0.26
Sediment Percent Carbon	Summer	3.66	3.47	3.93	3.35	5.45	5.44	5.11	5.864	6.58	4.68	4.69	5.86
	Winter	3.07	4.1	2.96	3.28	3.35	4.1	3.79	3.25	5.84	3.97	4.73	3.45
Percent Sand	Summer	50.2	46.29	44.2	55.83	28.2	20.2	33.83	22.4	11.83	31	28.67	22.4
	Winter	72.29	35.6	46.4	44	21.83	35.6	36.4	27	15.2	22.8	28.67	24
Percent Silt	Summer	33.4	43.43	44.8	34.33	47	54.8	49.17	52.2	47.83	49.2	48.17	52.2
	Winter	48.2	45.2	39	42.6	50.33	45.2	42.2	47.67	45.6	51.2	47.5	54.2
Percent Clay	Summer	16.4	10.29	11	9.83	24.8	25	17	25.4	40.33	19.8	23.17	25.4
	Winter	18.2	19.2	14.6	13.4	27.83	19.12	21.4	25.33	39.2	26	23.83	21.8

## Benthic Macrofauna

### Taxa accumulation curves

Taxa accumulation curves evaluate the sufficiency of the invertebrate sampling protocol. The taxa accumulation curves for both the summer and winter seasons indicate that community analysis would benefit from additional samples. For either season, although some habitats were close, none completely achieved an asymptote, indicating that a complete census of the invertebrate community did not occur (Figure 6), and additional taxa may have been identified had more samples been collected. A two-way ANOVA of the number of taxa, with main factors Season, Habitat, and an interaction term resulted in significantly different numbers of taxa between seasons ( $F= 57.536$ ;  $P<0.001$ ) and habitat types ( $F=12.017$ ;  $P<0.001$ ). For all habitat types, more taxa were encountered during the winter season (Figure 5). Comparing the abundance of invertebrates in eelgrass habitats with and without aquaculture revealed no difference in abundances, during either season (summer:  $p= 0.40$ ,  $F= 0.708$ ; winter:  $p= 0.60$ ,  $F= 0.263$ ). The top five most abundant taxa for each habitat type within each region were summarized by season (Appendix B).

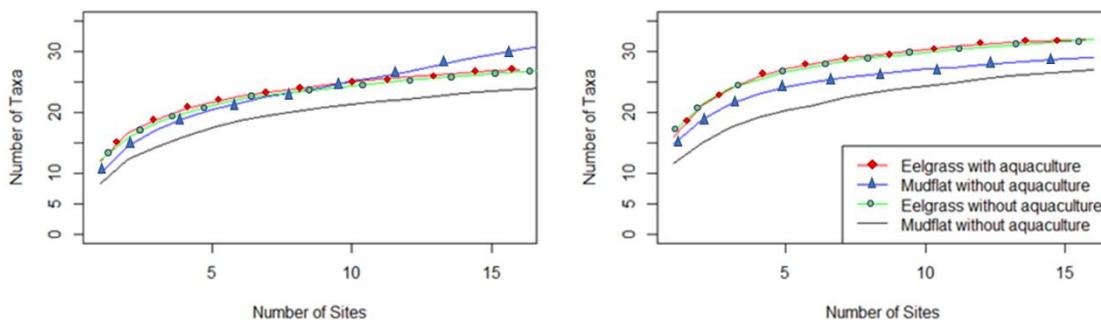


Figure 6. Taxa accumulation curves for each habitat type for both the summer (left) and winter (right) seasons.

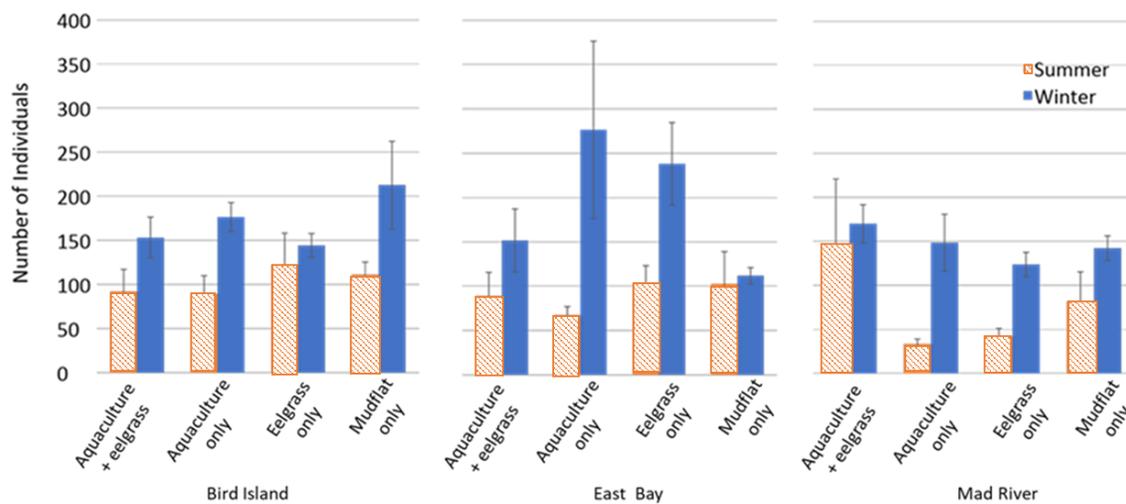


Figure 5. Comparison of the mean number of taxa encountered within each habitat type during the summer and winter seasons. All three regions are compared, from left to right: Bird Island, East Bay, Mad River. For each habitat type, there were significantly more taxa encountered during the winter season. Although there were exceptions, generally there were no strong trends or differences between habitat types. Error bars indicate standard error of the mean.

## Multivariate analyses

### Summer NMDS results

Using NMDS ordination and PERMANOVA analysis, invertebrate communities within the four habitats were statistically and visually compared. For all three regions, permutation tests revealed equal dispersions (Appendix C). Following Hellinger transformation, the Bray-Curtis distance was used to create regional dissimilarity matrices. For the Bird Island region, PERMANOVA and post hoc analyses resulted in differences between community composition of mudflat habitats with and without aquaculture (Appendix D and Appendix E). The ordination for the summer Bird Island sites had a stress level of 0.122 with three dimensions, and Envfit analysis resulted in no environmental variables being significantly correlated to the ordination. PERMANOVA analysis of the East Bay dissimilarity matrix resulted in differences in the community composition between mudflat without aquaculture and both eelgrass habitats (Appendix D and Appendix E). The ordination for East Bay had a stress value of 0.124 with three dimensions, and elevation relative to MLLW was found to be correlated to the ordination. For the Mad River region, PERMANOVA analysis resulted in differences in community composition between mudflat with aquaculture and both eelgrass habitats (with and without aquaculture), as well as differences between eelgrass with aquaculture and mudflat without aquaculture (Appendix D and Appendix E). The three-dimensional ordination for Mad River had a stress value of 0.144, with eelgrass percent cover, shoot count, carbon, clay, and elevation relative to MLLW significantly correlated to the ordination. Each region, regardless of significant differences between habitat types, had

significant indicator taxa (Table 3). For all regions, ordinations for axes 1 and 2 are displayed below (Figure 7), with the additional axis comparisons in Appendix F and stress plots in Appendix G.

#### Winter NMDS results

Community analysis of the regional winter samples revealed differences in community composition between several habitat types. Permutational tests resulted in equal dispersion for Bird Island, East Bay, and Mad River regions (Appendix C). For all regions, taxa abundances were Hellinger transformed, and the Bray-Curtis distance was used to create a dissimilarity matrix for each region. For the Bird Island region, post-hoc testing of a significant PERMANOVA result found differences in the community composition of eelgrass without aquaculture to both mudflat habitats (Appendix D and Appendix E). The three-dimensional ordination had a stress value of 0.150, with eelgrass percent cover, shoot count, and elevation relative to MLLW reported by Envfit to be significantly correlated to the ordination. East Bay winter PERMANOVA results showed differences in the community composition of mudflat without aquaculture and all other habitat types (Appendix D and Appendix E). The East Bay ordination had a stress value of 0.140 with three dimensions, and Envfit analysis found no environmental variables to be significantly correlated to the ordination. PERMANOVA and post-hoc analysis of the Mad River winter sites resulted in a significantly different result between mudflat without aquaculture and both eelgrass habitats (Appendix D and Appendix E). Three-dimensional ordination of this region had a stress value of 0.140, with Envfit finding eelgrass shoot count, percent cover, and elevation relative to MLLW to be significantly correlated to the

ordination. Ordination solutions for axes 1 and 2 are displayed below (Figure 7), with additional axes comparisons in Appendix F and stress plots in Appendix G.

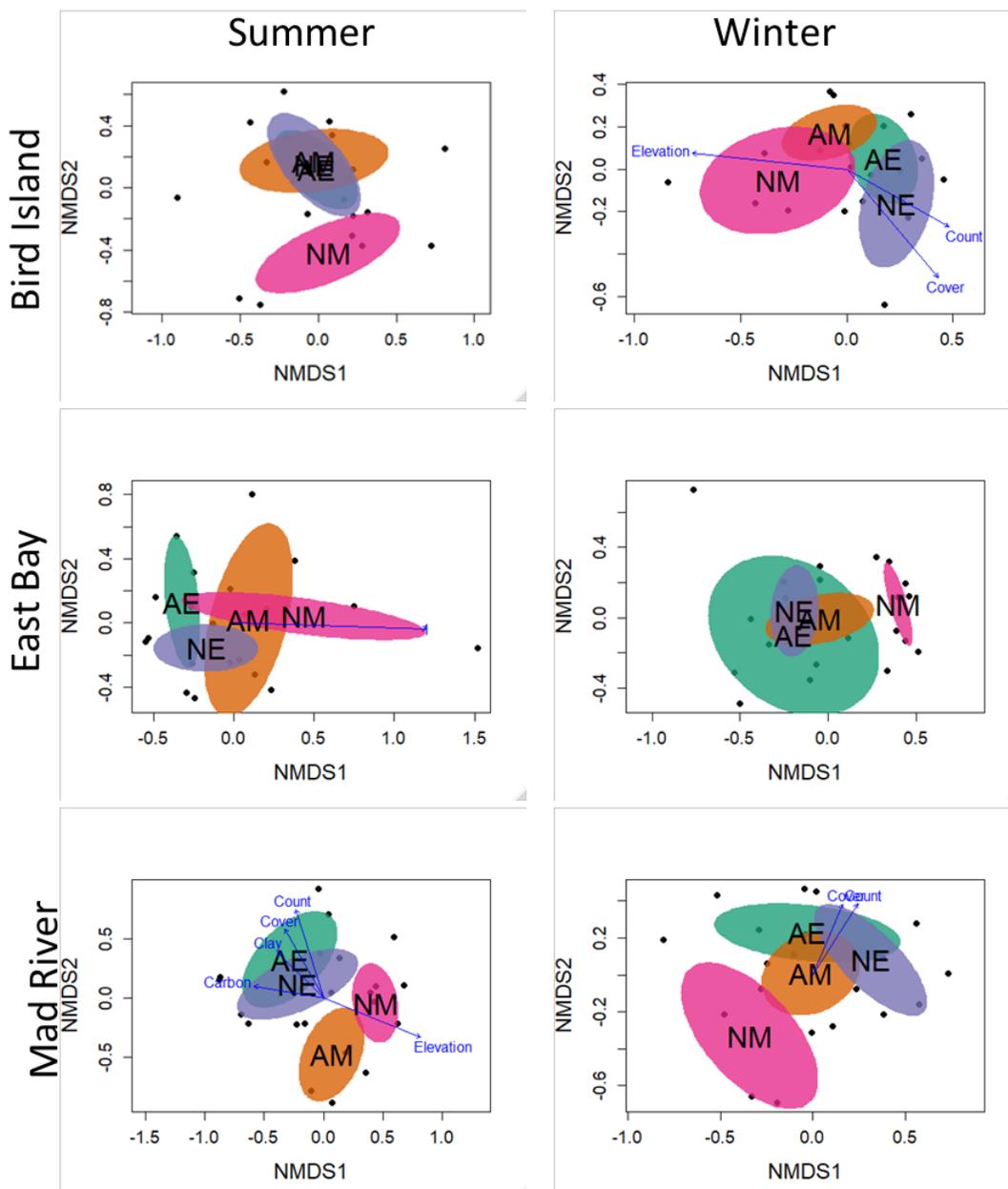


Figure 7. NMDS ordinations of the invertebrate samples collected from Humboldt Bay's North Bay. Habitat codes within ellipses indicate habitat types: AE= aquaculture and eelgrass, AM= aquaculture and mudflat, NE= eelgrass no aquaculture, and NM= mudflat no aquaculture. Overlapping ellipses indicate similarity in community composition, while clearly separated ellipses represent habitat types with differing invertebrate communities. Arrows indicate the strength and direction of increase of the significantly correlated environmental variables.

Table 3. Indicator taxa analysis and the associated functional feeding group for the summer and winter sampling seasons for the three sampled regions of Humboldt Bay. Superscript indicates functional feeding group for that taxa (D= deposit, Su= suspension, Sc= scavenger, P= predator, M= mixed).

Habitat Type	Bird Island		East Bay		Mad River	
	Summer	Winter	Summer	Winter	Summer	Winter
Eelgrass with aquaculture	Caprellidae <sup>M</sup> Oligochaete <sup>D</sup> Oweniidae <sup>D</sup> Phyllodocidae <sup>P</sup>	Ampharetidae <sup>D</sup>	Ostracoda <sup>Su</sup> Oligochaete <sup>D</sup>	Isopoda <sup>Sc</sup>	Ostracoda <sup>Su</sup>	Cirratulidae <sup>D</sup> Bivalvia <sup>Su</sup>
Mudflat with aquaculture	Oligochaete <sup>D</sup> Oweniidae <sup>D</sup> Phyllodocidae <sup>P</sup>	Ampharetidae <sup>D</sup>	-	Oweniidae <sup>D</sup>	-	Cirratulidae <sup>D</sup> Oweniidae <sup>D</sup>
Eelgrass no aquaculture	Caprellidae <sup>M</sup> Oligochaete <sup>D</sup> Oweniidae <sup>D</sup> Phyllodocidae <sup>P</sup>	Ampharetidae <sup>D</sup>	Ostracoda <sup>Su</sup> Oligochaete <sup>D</sup>	Nephytidae <sup>P</sup> Oweniidae <sup>D</sup>	Ostracoda <sup>Su</sup> Bivalvia <sup>Su</sup>	Cirratulidae <sup>D</sup> Bivalvia <sup>Su</sup> Oweniidae <sup>D</sup>
Mudflat no aquaculture	-	Chironomidae <sup>D</sup>	Oligochaete <sup>D</sup>	Oweniidae <sup>D</sup>	Bivalvia <sup>Su</sup>	Bivalvia <sup>Su</sup> Oweniidae <sup>D</sup>

### Gradient Forest results

The gradient forest analysis was used to determine which of the seven environmental variables (elevation relative to MLLW, eelgrass percent cover, eelgrass shoot count, percent sand, silt, clay, and sediment carbon content) had the largest influence on the invertebrate community in each region, and where along the gradient of each of the variables the invertebrate community had the largest response. The overall importance of each of the environmental variables was expressed as  $R^2$  weighted importance (Appendix H). To evaluate the response of the invertebrate community along those environmental gradients, the two most important variables were analyzed further. Because eelgrass shoot count and percent cover revealed similar trends in invertebrate response, in situations where these eelgrass measures were the two most important environmental parameters, the second eelgrass measure was excluded and the next most informative environmental factor was used in its place. The selected habitat factors were plotted to evaluate where along the environmental gradient the split density, and therefore community response, was greatest (Figure 8). These plots delve into the community response, displaying the cumulative importance of an environmental factor on individual taxa, where shallow slopes are indicative of a slow rate of compositional change, and a steep slope indicates a relatively high rate of change in community composition.

For most regions, I found substantial invertebrate compositional change to be related to changes in elevation, while the other factors that influenced invertebrate compositional change varied by region and season. In the Bird Island region, the gradient forest analysis indicated that the primary factor influencing the invertebrate community

was elevation, while silt was the secondary factor, for both summer and winter. During the summer in Bird Island, at an elevation of approximately -0.2 m, and at 25% silt content, there were considerable changes in the importance of Lumbrineridae, a predatory polychaete. During the winter, the composition of bivalvia varied at an elevation of approximately 0.4m and the composition of Pholoidae, another predatory polychaete, varied at approximately 30% silt content. In the East Bay region, the important factors influencing the invertebrate community differed between the summer (percent silt and carbon) and winter (eelgrass cover and elevation). During the summer, the major change was in the composition of the taxa Maldanidae, a deposit feeding polychaete, at carbon content of approximately 4.0%. In the winter, the largest observed changes were also in polychaete taxa. For eelgrass cover, it was a predatory taxa that had the largest change (Nephytidae), whereas for elevation it was an omnivore (Nereididae). Finally, elevation and eelgrass metrics were the factors that had the largest influence on invertebrate community composition in the Mad River region in summer and winter. During the summer, a predatory polychaete (Glyceridae) was the taxa whose composition varied the most for both elevation (at approximately -0.2 m) and eelgrass count (at approximately 1.5 shoots). During the winter, the composition of another predatory polychaete (Pholoidae) varied with cover (at approximately 30%) and the composition of bivalvia varied with elevation (at approximately 0.2 m).

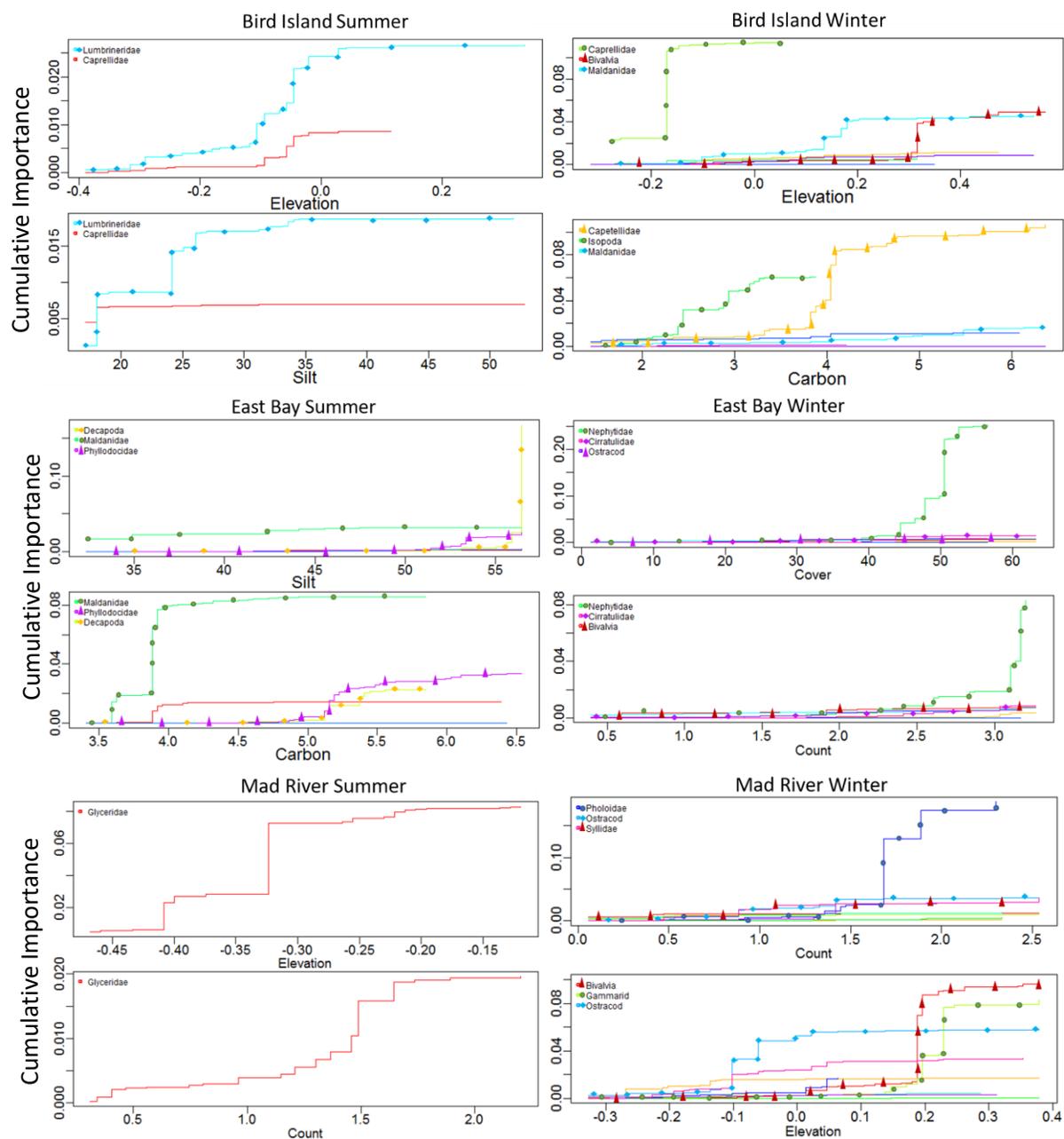


Figure 8. Graphical outputs of GradientForest analysis for the Bird Island, East Bay, and Mad River regions of Humboldt Bay. Plots display taxa which were most impacted by changes along that gradient; top three most impacted taxa are indicated in legend.

## DISCUSSION

### Benthic Invertebrate Habitat Use

Based on the results of this study, benthic invertebrate assemblages are most strongly impacted by the presence of structure. Although the trend was stronger for some regions than others, throughout both seasons the communities of mudflat without aquaculture differed from the invertebrate communities of aquaculture and eelgrass habitats. The slight exception to this trend was found in the Mad River summer and Bird Island winter samples, where there was also a significant difference between mudflat with aquaculture and eelgrass communities. In general, the results from the NMDS ordinations, in which the confidence intervals for the oysters and eelgrass habitats overlap, support the findings of other studies that have also found that invertebrate communities are similar in various types of structures habitats (Dumbauld 2003; Hosack 2003; Dealeris et al. 2004; Rumrill and Poulton 2004; Ferraro and Cole 2007). This is true regardless of whether, or not, that structure was native eelgrass beds or commercial oyster aquaculture.

In the otherwise barren bottom of the intertidal mudflats in Humboldt Bay, oyster longlines contribute broken shell pieces as well as complete, living oysters to the bottom habitat. These fallen oysters add heterogeneity to the bottom environment, providing substratum for boring and attachment and a refuge from predation and tidal currents (Gutierrez et al. 2003). The production of habitat heterogeneity, complexity, and structure

appears to support a similar suite of invertebrates, whether that habitat is eelgrass or aquaculture. However, in addition to creating physical habitat, oysters can also increase deposition to the sediment. Although organic inputs are necessary for a thriving invertebrate community, an overabundance of organics can have considerable impacts to the local area. Soft sediment communities are commonly characterized by relatively large filter feeders, though as organic inputs increase, a shift towards smaller deposit-feeding organisms often occurs (Pearson and Rosenberg 1978; Forrest et al. 2009; Mckindsey et al. 2011). The indicator taxa analysis generally associated deposit feeders with aquaculture habitats (both eelgrass and mudflat), as well as eelgrass without aquaculture. Suspension feeders, however, were associated with habitats lacking aquaculture, as well as eelgrass with aquaculture (Table 3). If a shift towards deposit feeders (which are associated with organically enriched habitats) were occurring, additional field studies would be needed to investigate this further. However, as evidenced by the taxa accumulation curves, this study would have benefitted from additional samples, which might support or contradict this potential trend in functional feeding group shifts.

### Seasonality

Both the summer and winter seasons were characterized by distinctions between habitat types, particularly between habitats with structure and the barren mudflats. Interestingly, there were, regardless of habitat type, more taxa encountered during the winter season than the summer season. This is in contrast to the findings of other local studies, which found either fewer or no difference in taxa in the winter months compared

to the summer season (Rumrill and Poulton 2004; Osborn 2017). Differences in seasonal patterns could have been impacted by the timing of the winter sample collection.

Additionally, pump collection occurred in the early morning during the summer, and in the evening in the winter. This introduced variability with the potential for diurnal movement from mobile epifauna to impact the invertebrate community collected via the pump sampler.

In contrast to the expected seasonal differences, eelgrass percent cover was not found to be significantly different between seasons, and shoot count was actually higher in the winter season. However, it has been well documented that eelgrass undergoes seasonal fluctuations, with the active growing season ranging from May to September, and eelgrass coverage often decreasing in the winter months (Rumrill and Poulton 2004; Gilkerson and Merkel 2014). If the winter of 2017-2018 had a late start, as might be indicated by the lack of eelgrass decline, then perhaps the invertebrate community was still in transition during the winter sampling season. However, despite this slight seasonal inconsistency with some published literature, the overall patterns of community composition between habitat types are consistent with similar studies.

## Ecosystem Implications

This study did not find significant differences in the invertebrate community of habitats with and without longline oyster aquaculture. In the case of foraging shorebirds and wading birds in Humboldt Bay, the invertebrate community within longline beds may be preferred. A study by Connolly and Colwell (2005) found greater abundances of these birds within longline plots than in control areas. Pinnix (2004) likewise found similar numbers of fish species between habitats with and without longline culture, although this study was primarily to investigate the effectiveness of sampling equipment rather than comparison of community assemblages. Because this study was focused on the invertebrate community, the predator-prey connections can only be speculated upon, and additional studies focusing on the foodweb implications would be instrumental in drawing connections between invertebrate community and the Humboldt Bay ecosystem.

I found that longline aquaculture habitats had reduced eelgrass densities, which are an important component of the Humboldt Bay ecosystem. Comparison of eelgrass habitats with and without aquaculture resulted in significantly less eelgrass, both shoot count and cover, when aquaculture was present. This is consistent with previous research investigating the impacts of off-bottom bivalve culture on eelgrass. A recent meta-analysis by Ferriss et al. (2019) reported that longline off-bottom culture methods resulted in a 44% decrease in eelgrass density and a 61% decrease in eelgrass reproduction. Although the presence or absence of aquaculture in eelgrass habitats may not have a strong effect on the number of invertebrates present, there may still be impacts

to the broader ecosystem. Semmens (2008) found smolts of the ecologically and economically important Chinook salmon to exhibit a strong preference for native eelgrass habitats in Willapa Bay. Pacific Herring, an important forage fish, preferentially spawn on the blades of eelgrass (Barnhart 1988). Most herring spawning in Humboldt Bay occurs in the North Bay (Rabin and Barnhart 1986), and although there has been a recent dearth of research into Humboldt Bay herring populations, anecdotal reports of declines through the 1990s may link Herring and eelgrass populations in Humboldt Bay (Watters et al. 2001).

Results from the gradient forest analysis suggest that the factors that influence invertebrate composition varies regionally throughout Humboldt Bay, but is generally related to elevation. In addition to elevation, the factors that had the largest impact on invertebrate concentration were related to the sediment composition or a measure of the eelgrass density (cover or shoot count). The Bird Island region had percent silt concentration as the second most important factor influencing the invertebrate community in both summer and winter. In contrast, the Mad River region in both the summer and winter seasons resulted in at least one eelgrass measure as a primary factor influencing changes in community composition. The East Bay region was a mix, with sediment composition in the summer and eelgrass cover in the winter. Based on the gradient forest analysis, the taxa which were shown to be most strongly impacted in all regions were predators in the polychaete family. Predators have been found to be an important factor in structuring soft-bottom communities (Ambrose 1984; Wilson 1990).

Based on these findings, future research is warranted to examine how invertebrates in the predator functional feeding group respond to aquaculture.

Benthic invertebrates, as well as eelgrass habitats, are also vital to the survival of wintering waterbirds in Humboldt Bay. While invertebrate consumption by waterfowl can be varied, many shorebirds feed primarily upon aquatic invertebrates (Afton et al. 1991; Euliss and Grodhaus 1991; Skagen and Oman 1996) In addition, the dynamics of piscivorous waterbird populations in Tomales Bay, CA, were found to be tied to the availability of herring roe, with pulses of herring spawning leading to increases in the abundance of waterbirds for the next three winters (Kelly et al. 2018). Dabbling waterbirds, including *Branta bernicla nigricans*, the Black Brant goose, are highly dependent upon eelgrass success. Moore et al. (2004) found variability in Brant numbers across flyway sites to be strongly correlated to the presence of high eelgrass abundance. Although I measured that longline aquaculture habitats had reduced eelgrass densities, a resource important to a variety of estuarine species, the impacts appear to be localized to the area encompassed by the longline bed. This indicates that careful management of the oyster aquaculture industry could conserve eelgrass beds for use by dependent members of the broader ecosystem.

### Management Implications

This study of benthic invertebrate habitat use provides insight into one component of the complex ecosystem of Humboldt Bay. The increasing importance of oyster aquaculture to feeding the earth's growing population suggests that this and similar

studies should be applied to developing options for management. As the need for global protein production grows, efforts should be focused on methods which can achieve a balance between production and environmental sustainability.

With the balance between providing resources for sustaining human populations while also conserving the natural environment, the available research should be used to inform best management practices for oyster aquaculture in Humboldt Bay. Rumrill and Poulton (2004) found that small changes to longline culture methods can have large impacts on eelgrass success. For example, they found that increasing line spacings from 2.5 feet (which is standard in Humboldt Bay) to five or ten feet resulted in significantly increased eelgrass cover and shoot count. With the potential for positive impacts to other eelgrass-associated species, and negligible impacts to the benthic invertebrate community (Dumbauld 2003), increased line spacing of longline oyster aquaculture beds within Humboldt Bay could be considered.

When viewing the benthic invertebrate community in isolation, the results of this study are encouraging for the lack of impacts of longline oyster culture. However, I did find that the eelgrass cover was lower in longline oyster culture habitats relative to habitats without oyster culture. These effects can have implications throughout the ecosystem, and these should be considered when planning placement of oyster aquaculture in Humboldt Bay.

## LITERATURE CITED

- Ababouch, L., J. Alder, A. Anganuzzi, U. Barg, D. Bartley, M. Bernal, G. Bianchi, M. Boccia, M. Camilleri, V. Chomo, T. Farmer, N. Franz, C. Fuentesvilla, S. Funge-Smith, L. Garibaldi, J. Gee, M. Hasan, R. Hilborn, N. Hishamunda, G. Laurenti, A. Lem, A. Lovatelli, P. Mannini, R. Metzner, J. Sanders, D. Soto, A. Stankus, P. Suuronen, M. Torrie, J. Turner, S. Vannuccini, Y. Ye, X. Zhou, M. Taconet, S. Tsuji, J. Aguilar-Manjarrez, C. R. Liermann, D. Lymer, E. Fluet-Chouinard, P. McIntyre, D. Bartley, D. Kalikoski, P. Suuronen, S. Siar, N. Franz, U. Barg, F. Martin, M. E. D'Andrea, P. Bueno, D. Soto, G. Marmulla, J. Caffrey, J. Dick, C. Gallagher, F. Lucy, J. Toppe, F. Poulain, R. Metzner, U. Barg, T. Farmer, and S. Vannuccini. 2016. The state of world fisheries and aquaculture. Rome.
- Ambrose, W. G. 1984. Influences of predatory polychaetes and epibenthic predators on the structure of a soft-bottom community in a marine estuary. *Biol. Ecol.*
- Anderson, M. J. 2005. Permutational Multivariate Analysis of Variance (PERMANOVA). Page Wiley StatsRef: Statistics Reference Online.
- Anderson, M. J. 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Page Wiley StatsRef: Statistics Reference Online.
- Afton, A.D., R. H. Hier, and S. L. Paulus. 1991. Lesser scaup diets during migration and winter in the Mississippi flyway. *Canadian Journal of Zoology* 69:328–333.
- Barnes, R. S. K., and S. Hamylton. 2015. Uniform functional structure across spatial scales in an intertidal benthic assemblage. *Marine Environmental Research* 106:82–91. Elsevier.
- Barnhart, R. A. 1988. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Southwest)-- Pacific Herring. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.79). U.S. Army Corps of Engineers, TR EL-82-4.
- Beaumont, N. J., M. C. Austen, J. P. Atkins, D. Burdon, S. Degraer, T. P. Dentinho, S. Deros, P. Holm, T. Horton, E. Van Ierland, A. H. Marboe, D. J. Starkey, M. Townsend, and T. Zarzycki. 2007. Identification, definition and quantification of goods and services provided by marine biodiversity: Implications for the ecosystem approach. *Marine Pollution Bulletin* 54:253–265.
- Bostrom, C., and E. Bonsdorff. 1997. Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L.) beds in the northern Baltic Sea. *Journal of Sea Research* 37:153–166.
- Bott, L., and C. Diebel. 1982. A survey of the benthic invertebrate communities in the channels of central Humboldt Bay, California. Contract No. DACW07-81-C-0010. San Francisco, CA.
- Boyle, M., D. Janiak, and S. Craig. 2004. Succession in a Humboldt Bay Marine Fouling Community: The Role of Exotic Species, Larval Settlement and Winter Storms. Pages 1–20 Humboldt Bay Symposium. Eureka.
- Bray, J. R., and J. T. Curtis. 1957. An Ordination of the Upland Forest Communities of

- Southern Wisconsin. *Ecological Monographs* 27(4):325–349.
- De Caceres, M., and F. J. Maintainer. 2016. Package “indicspecies” Type Package Title Relationship Between Species and Groups of Sites.
- Chew, K. K. 2001. A changing scene for oyster culture in Humboldt Bay, California. *Aquaculture* 27(5):87–91.
- Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117–143.
- Clarke, K. R., and R. M. Warwick. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition, 2nd edition. PRIMER-E, Plymouth.
- Coast Seafoods Company. 2016. Draft environmental impact report: Coast Seafoods Company Humboldt Bay shellfish aquaculture permit renewal and expansion project, Humboldt County, California. Eureka.
- Connolly, L. M. and M. A. Colwell. 2005. Comparative use of longline oyster beds and adjacent tidal flats by waterbirds. *Bird Conservation International* 15:237-255.
- Colwell, M. A. 1994. Shorebirds of Humboldt Bay, California: Abundance estimates and conservation implications. *Western Birds* 25:137–145.
- Costa-Pierce, B. A. 2002. Ecology as the paradigm for the future of aquaculture, in *Ecological Aquaculture: The evolution of the blue revolution*. Page B. A. Costa-Pierce, editor. Blackwell Science, Oxford, UK.
- Cote, J., C. Czesla, H. Hurn, M. Meaders, P. Bloch, M. McDowell, L. Odle, R. Park, and A. Rae. 2017. Coast Seafoods Company, Humboldt Bay Shellfish Aquaculture: Permit Renewal and Expansion Project. Seattle, WA.
- Danufsky, T. 2000. Winter shorebird communities of Humboldt Bay: Species diversity, distributions, and habitat characteristics. Humboldt State University.
- Day, P. R. 1965. Particle Fractionation and particle-size analysis. Pages 545–567 in C. A. Black, editor. *Methods of Soil Analysis, Part 1*. American Society of Agronomy, Madison.
- Dealteris, J. T., B. D. Kilpatrick, and R. B. Rheault. 2004. A comparative evaluation of the habitat value of shellfish aquaculture gear, submerged aquatic vegetation and a non-vegetated seabed. *Journal of Shellfish Research* 23(3):867–874.
- Dumbauld, B. 2003. WRAC Project Termination Report Part I: Summary: The Ecological Role and Potential Impacts of Molluscan Shellfish Culture in the Estuarine Environment. Seattle.
- Dumbauld, B. R., J. L. Ruesink, and S. S. Rumrill. 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries.
- Euliss, N. H., Jr. and G. Grodhaus. 1987. Management of midges and other invertebrates for waterfowl wintering in California. *California Fish and Game* 73:238–243.
- Faith, D. P., P. R. Minchin, and L. Belbin. 1987. Compositional dissimilarity as a robust measure of ecological distance.
- Fauchald, K., and P. A. Jumars. 1979. The diet of worms: A study of polychaete feeding guilds. *Annual Review of Oceanographic Marine Biology* 17:193–284.

- Ferraro, S. P., and F. A. Cole. 2004. Optimal Benthic Macrofaunal Sampling Protocol for Detecting Differences among Four Habitats in Optimal Benthic Macrofaunal Sampling Protocol for Detecting Differences Among Four Habitats in Willapa Bay, Washington, USA. Source: *Estuaries* 27(6):1014–1025.
- Ferraro, S. P., and F. A. Cole. 2007. Benthic macrofauna-habitat associations in Willapa Bay, Washington, USA. *Estuarine, Coastal and Shelf Science* 71:491–507.
- Ferriss, B. E., L. L. Conway-Cranos, B. L. Sanderson, and L. Hoberecht. 2019. Bivalve aquaculture and eelgrass: A global meta-analysis. *Aquaculture* 498:254–262.
- Field, J. G., K. R. Clarke, and R. M. Warwick. 1982. A Practical Strategy for Analysing Multispecies Distribution Patterns. *Marine Ecology Progress Series* 8:37–52.
- Forrest, B. M., and R. G. Creese. 2006. Benthic impacts of intertidal oyster culture, with consideration of taxonomic sufficiency. *Environmental Monitoring and Assessment* 112(1–3):159–176.
- Forrest, B. M., N. B. Keeley, G. A. Hopkins, S. C. Webb, and D. M. Clement. 2009. Bivalve aquaculture in estuaries: Review and synthesis of oyster cultivation effects. *Aquaculture* 298:1–15.
- Gavlak, R., D. Horneck, and R. O. Miller. 2005. Soil, plant, and water reference methods for the western region, 3rd edition.
- Gilkerson, W. 2008. A spatial model of eelgrass (*Zostera marina*) habitat in Humboldt Bay, California. Humboldt State University.
- Gilkerson, W. A., and K. W. Merkel. 2014. Humboldt Bay eelgrass comprehensive plan. Prepared for Humboldt Bay Harbor, Recreation and Conservation District.
- Gutierrez, J. L., C. G. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* 101:79–90.
- Herman, P.M.J, J. J. Middelburg, J. V. D. Koppel, and C. H. R. Heip. 1999. Ecology of estuarine macrobenthos. *Estuaries* 29: 195-240.
- Hosack, G. 2003. Effects of *Zostera marina* and *C. gigas* culture on the intertidal communities of Willapa Bay. University of Washington.
- Hosack, G. R., B. Dumbauld, J. L. Ruesink, and D. A. Armstrong. 2006. Habitat associations of estuarine species: Comparisons of intertidal mudflat, seagrass (*Zostera marina*), and oyster (*Crassostrea gigas*) habitats. *Estuaries & Coasts* 29(6):1150.
- Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as Ecosystem Engineers. *Oikos* 69(3):373–386.
- Jumars, P. A., K. M. Dorgan, and S. M. Lindsay. 2015. Diet of Worms Emended: An Update of Polychaete Feeding Guilds. *Annual Review of Marine Science* 7:497–520.
- Kautsky, N., and S. Evans. 1987. Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem. *Marine Ecology Progress Series* 38:201–212.
- Kelly, J., C. Rothenbach, and W. Weathers. 2018. Echoes of numerical dependence: responses of wintering waterbirds to Pacific herring spawns. *Marine Ecology*

- Progress Series 597:243–257.
- Lacoste, E., and N. Gaertner-Mazouni. 2015. Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. *Reviews in Aquaculture* 7(3):187–196. John Wiley & Sons, Ltd (10.1111).
- Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129:271–280.
- Lewis, F. G., and A. W. Stoner. 1981. An examination of methods for sampling macrobenthos in seagrass meadows. *Bulletin of Marine Science* 31(1):116–124.
- Macdonald, T. A., B. J. Burd, V. I. Macdonald, and A. van Roodselaar. 2010. Taxonomic and feeding guild classification for the marine benthic macroinvertebrates of the Strait of Georgia, British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2874:63.
- MacNally, R. C. 1990. Modelling Distributional Patterns of Woodland Birds along a Continental Gradient. *Ecology* 71(1):360–374.
- Mazouni, N., J. C. Gaertner, and J. M. Deslous-Paoli. 2001. Composition of biofouling communities on suspended oyster cultures: an in situ study of their interactions with the water column. *Marine Ecology Progress Series* 214:93–102.
- McCune, B., J. B. Grace, and D. L. Urban. 2002. *Analysis of ecological communities*. MJM Software Design, Gleneden Beach, OR.
- Mckindsey, C. W., P. Archambault, M. D. Callier, F. Olivier, C. W. Mckindsey, and P. Archambault. 2011. Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review 1. *Can. J. Zool* 89:622–646.
- Moore, J. E., M. A. Colwell, R. L. Mathis, and J. M. Black. 2004. Staging of Pacific flyway brant in relation to eelgrass abundance and site isolation, with special consideration of Humboldt Bay, California. *Biological Conservation* 115:475–486.
- Newell, R. I. E., and E. W. Koch. 2004. Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. *Estuaries* 27(5):793–806.
- Newell, R. I. E., and C. J. Landgon. 1996. Mechanisms and Physiology of Larval and Adult Feeding. Pages 185–269 in V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editors. *The Eastern Oyster: Crassostrea virginica*. Maryland Sea grant College, University of Maryland System, College Park, College Park, MD.
- Oksanen, Jari, F., G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2017. *vegan: Community Ecology Package*. R package version 2.4-4.
- Orth, R. J. 1973. Benthic Infauna of Eelgrass, *Zostera marina*, Beds’. *Chesapeake Science* 14(4):258–269.
- Orth, R. J., K. L. Heck, and J. Van Montfrans. 1984. Coastal and Estuarine Research Federation Faunal Communities in Seagrass Beds: A Review of the Influence of Plant Structure and Prey Characteristics on Predator: Prey Relationships. Source: *Estuaries* 7(4):339–350.
- Osborn, K. 2017. Seasonal fish and invertebrate communities in three northern California

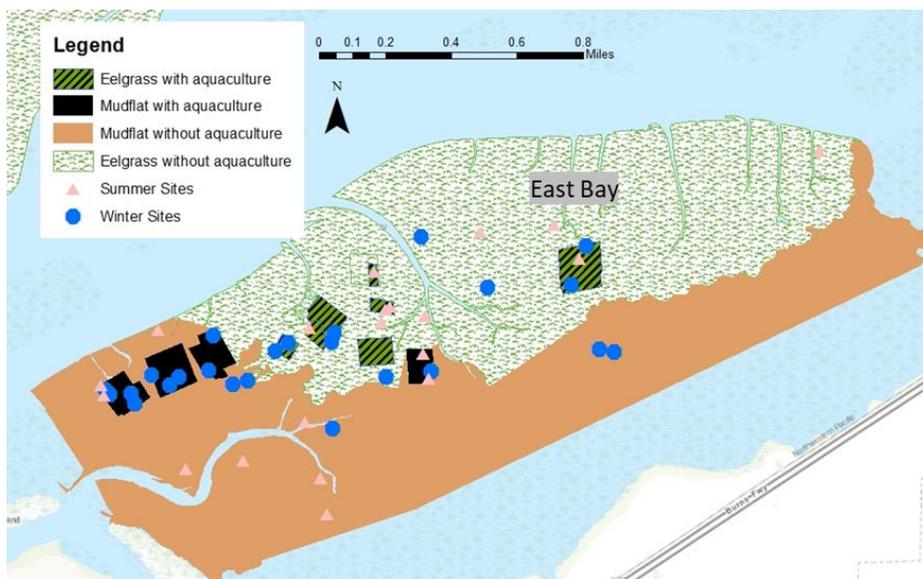
estuaries.

- Pearson, T. H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Review* 16:229–311.
- Peterson, E. B., and B. McCune. 2001. Diversity and succession of epiphytic macrolichen communities in low-elevation managed conifer forests in western Oregon. *Journal of Vegetation Science* 12(4):511–524.
- Pinnix, W. D., T. A. Shaw, K. C. Acker, and N. J. Hetrick. 2005. Fish Communities in Eelgrass, Oyster Culture, and Mudflat Habitats of North Humboldt Bay, California Final Report.
- Rabin, D. J., and R. A. Barnhart. 1986. Fecundity of Pacific herring *Clupea harengus pallasi*, in Humboldt Bay, California. *California Fish and Game* 72(1):4–16.
- Reise, K. 1978. Experiments on epibenthic predation in the Wadden Sea. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 31:55–101.
- Ruesink, J. L., H. S. Lenihan, A. C. Trimble, K. W. Heiman, F. Micheli, J. E. Byers, and M. C. Kay. 2005. Introduction of non-native oysters: Ecosystem Effects and Restoration Implications. *Annu. Rev. Ecol. Evol. Syst* 36:643–89.
- Rumrill, S., and V. Poulton. 2004. Ecological role and potential impacts of molluscan shellfish culture in the estuarine environment of Humboldt Bay, Ca. Oregon Department of State Lands, Final Annual Report to the Western Regional Aquaculture Center.
- Schlösser, S., and A. Eicher. 2012. Humboldt Bay and Eel River Estuary Benthic Habitat Project.
- Semmens, B. X. 2008. Acoustically derived fine-scale behaviors of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) associated with intertidal benthic habitats in an estuary. *Canadian Journal of Fisheries and Aquatic Sciences* 65(9):2053–2062.
- Simenstad, C. A., and K. L. Fresh. 1995. Influence of Intertidal Aquaculture on Benthic Communities in Pacific Northwest Estuaries: Scales of Disturbance. *Estuaries* 18(1):43–70.
- Skagen, S. K. and H. D. Oman. 1996. Dietary flexibility of shorebirds in the Western Hemisphere. *Canadian Field-Naturalist* 110: 419–444.
- Skeesick, D. G. 1963. A study of some physical-chemical characteristics of Humboldt Bay. Humboldt State University.
- Stoner, A. W. 1980. The role of seagrass biomass in the organization of benthic macrofaunal assemblages. *Bulletin of Marine Science* 30(3):537–551.
- Summerson, H. C., and C. H. Peterson. 1984. Role of predation in organizing benthic communities of a temperate-zone seagrass bed. *Marine Ecology Progress Series* 15:63–77.
- Tallis, H. M., J. L. Ruesink, B. Dumbauld, S. Hacker, and L. M. Wisheart. 2009. Oyster and aquaculture practices affect eelgrass density and productivity in a Pacific Northwest estuary. *Journal of Shellfish Research* 28(2):251–261.
- Toft, J. D., A. S. Ogston, S. M. Heerhartz, J. R. Cordell, and E. E. Flemer. 2013. Ecological response and physical stability of habitat enhancements along an urban

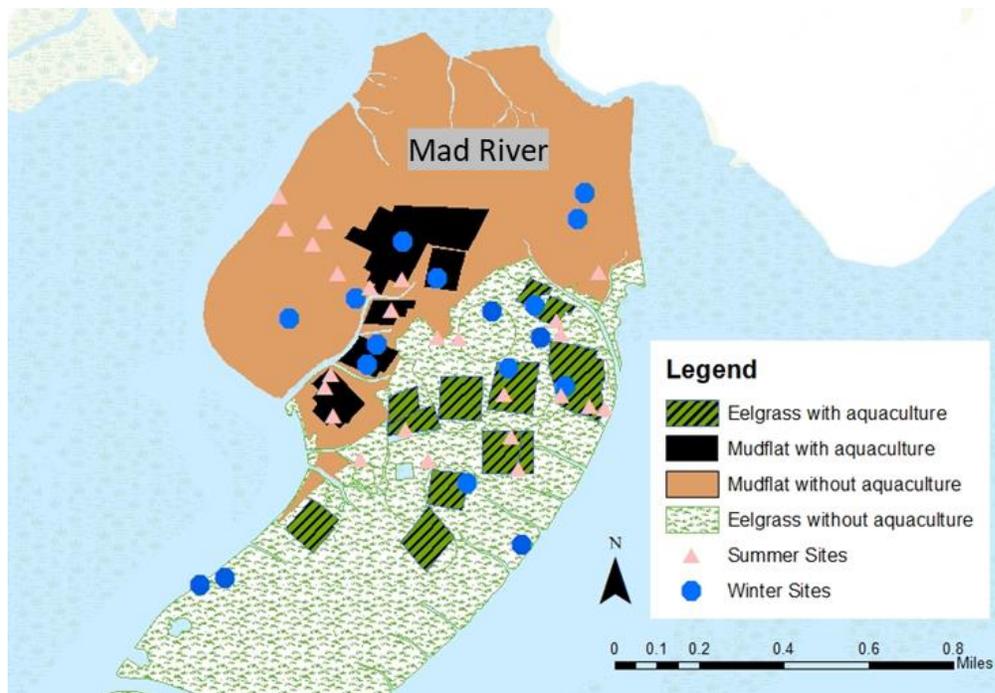
- armored shoreline. *Ecological Engineering* 57:97–108.
- Trianni, M. S. 1996. The influence of commercial oyster culture activities on the benthic infauna of Arcata Bay. Humboldt State University.
- Troell, M., R. L. Naylor, M. Metian, M. Beveridge, P. H. Tyedmers, C. Folke, K. J. Arrow, S. Barrett, A. S. Crepin, P. R. Ehrlich, A. Gren, N. Kautsky, S. A. Levin, K. Nyborg, H. Osterblom, S. Polasky, M. Scheffer, B. H. Walker, T. Xepapadeas, A. deZeeuw. Does aquaculture add resilience to the global food system? *Proceedings of the National Academy of Science of the United States of America*. 111(37): 13257-13263.
- Ugland, K. I., J. S. Gray, and K. E. Ellingsen. 2003. The species-accumulation curve and estimation of species richness. *Journal of Animal Ecology* 72:888–897.
- Watters, D. L., K. T. Oda, and J. Mello. 2001. California's Living Marine Resources: A Status Report Pacific Herring. Eureka.
- Watzin, M. C. 1983. The effects of meiofauna on settling macrofauna: meiofauna may structure macrofaunal communities. *Page Oecologia*.
- Weiner, P., P. Hastings, Billy Plauché, R. Smith, and Bonnie Neely. 2017. Staff report: Regular Calendar: Coast Seafoods Company. San Francisco.
- Wilson, W. H. 1990. Competition and predation in marine soft-sediment communities. *Annual Review of Ecology and Systematics* 21:221–241.
- van der Zee, E. M., E. Tielens, S. Holthuijsen, S. Donadi, B. K. Eriksson, H. W. van der Veer, T. Piersma, H. Olff, and T. van der Heide. 2015. Habitat modification drives benthic trophic diversity in an intertidal soft-bottom ecosystem. *Journal of Experimental Marine Biology and Ecology* 465:41–48.

APPENDIX A

Appendix A. Summer and winter sampling sites for the Bird Island, East Bay, and Mad River regions of Humboldt Bay's North Bay.



Appendix A, continued. Summer and winter sampling sites for the Bird Island, East Bay, and Mad River regions of Humboldt Bay's North Bay.



## APPENDIX B

Appendix B. Top five most abundant taxa per habitat type per region, divided into the summer and winter sampling seasons.

Habitat Type	Bird Island		East Bay		Mad River		
	Taxa	Count	Taxa	Count	Taxa	Count	
Summer	Eelgrass with Aquaculture	Capetellidae	67	Tanaid	87	Tanaid	418
		Cirratulidae	57	Capetellidae	78	Gammarid	127
		Tanaid	55	Syllidae	66	Syllidae	124
		Bivalvia	55	Bivalvia	53	Capetellidae	88
		Cumacea	45	Cirratulidae	40	Spionidae	32
		Gammarid	45				
	Mudflat with Aquaculture	Cumacea	114	Tanaid	70	Tanaid	56
		Capetellidae	92	Syllidae	64	Capetellidae	45
		Tanaid	88	Capetellidae	63	Cirratulidae	30
		Cirratulidae	79	Bivalvia	45	Syllidae	11
		Syllidae	50	Cirratulidae	30	Gammarid	8
	Eelgrass Without Aquaculture	Tanaid	120	Gammarid	120	Tanaid	59
		Syllidae	108	Bivalvia	90	Capetellidae	53
		Cumacea	74	Capetellidae	83	Gammarid	33
		Bivalvia	70	Tanaid	71	Syllidae	21
		Gammarid	51	Ostracod	66	Bivalvia	21
	Mudflat Without Aquaculture	Gammarid	209	Gammarid	230	Tanaid	220
		Tanaid	99	Syllidae	102	Gammarid	100
		Capetellidae	86	Bivalvia	62	Syllidae	70
		Cirratulidae	75	Tanaid	54	Bivalvia	50
Bivalvia		46	Capetellidae	27	Capetellidae	26	

Appendix B, continued. Top five most abundant taxa per habitat type per region, divided into the summer and winter sampling seasons.

	Habitat Type	Bird Island		East Bay		Mad River	
		Taxa	Count	Taxa	Count	Taxa	Count
Winter	Eelgrass with Aquaculture	Syllidae	113	Syllidae	183	Syllidae	293
		Capetellidae	111	Tanaid	162	Tanaid	154
		Cirratulidae	108	Capetellidae	154	Capetellidae	105
		Spionidae	98	Cirratulidae	124	Gammarid	70
		Tanaid	84	Spionidae	65	Ostracod	58
	Mudflat with Aquaculture	Tanaid	186	Tanaid	555	Tanaid	273
		Cirratulidae	148	Syllidae	520	Gammarid	174
		Syllidae	130	Gammarid	171	Syllidae	88
		Spionidae	125	Capetellidae	152	Spionidae	56
		Capetellidae	124	Bivalvia	129	Capetellidae	47
	Eelgrass Without Aquaculture	Capetellidae	142	Capetellidae	200	Capetellidae	155
		Syllidae	126	Tanaid	185	Tanaid	140
		Cumacea	83	Syllidae	170	Syllidae	136
		Cirratulidae	78	Cirratulidae	145	Spionidae	56
		Spionidae	64	Spionidae	119	Cumacea	36
	Mudflat Without Aquaculture	Tanaid	386	Bivalvia	183	Bivalvia	220
		Capetellidae	145	Tanaid	165	Tanaid	219
		Bivalvia	141	Gammarid	96	Gammarid	117
		Spionidae	113	Syllidae	81	Capetellidae	76
		Syllidae	86	Capetellidae	52	Syllidae	37

## APPENDIX C

Appendix C. Results of permutational analysis tests to evaluate equality of dispersion prior to PERMANOVA analysis of regional community composition. Significance values greater than 0.05 indicate failure to reject the null hypothesis of equal dispersion, indicating fulfillment of this assumption. Rejection of the null hypothesis indicates a need to further evaluate equality of dispersion using visual plotting of habitat dispersions.

Season	Region	Degrees of Freedom	F-value	Significance
Summer	Bird Island	3	0.4039	0.7552
	East Bay	3	0.4441	0.7333
	Mad river	3	0.6084	0.6374
Winter	Bird Island	3	1.0827	0.3736
	East Bay	3	1.6335	0.2008
	Mad River	3	0.1101	0.958

## APPENDIX D

Appendix D. Results of permutational analysis of variance (PERMANOVA) for each region for each sampling season. Significant results were evaluated using a post-hoc test, found in Appendix E.

Season	Region	Degrees of Freedom	F-value	Significance
Summer	Bird Island	3	1.5889	0.0170*
	East Bay	3	1.2001	0.1389
	Mad River	3	2.1444	0.0021*
Winter	Bird Island	3	1.7859	0.0051*
	East Bay	3	2.4454	<0.001*
	Mad River	3	2.1552	< 0.001*

## APPENDIX E

Appendix E. Results of post-hoc testing of significant PERMANOVA results for the summer (top) and winter (bottom) seasons. Dashes indicate those habitats were not significantly different for any region. Regional entries indicate those habitats were found to be significantly different within that region.

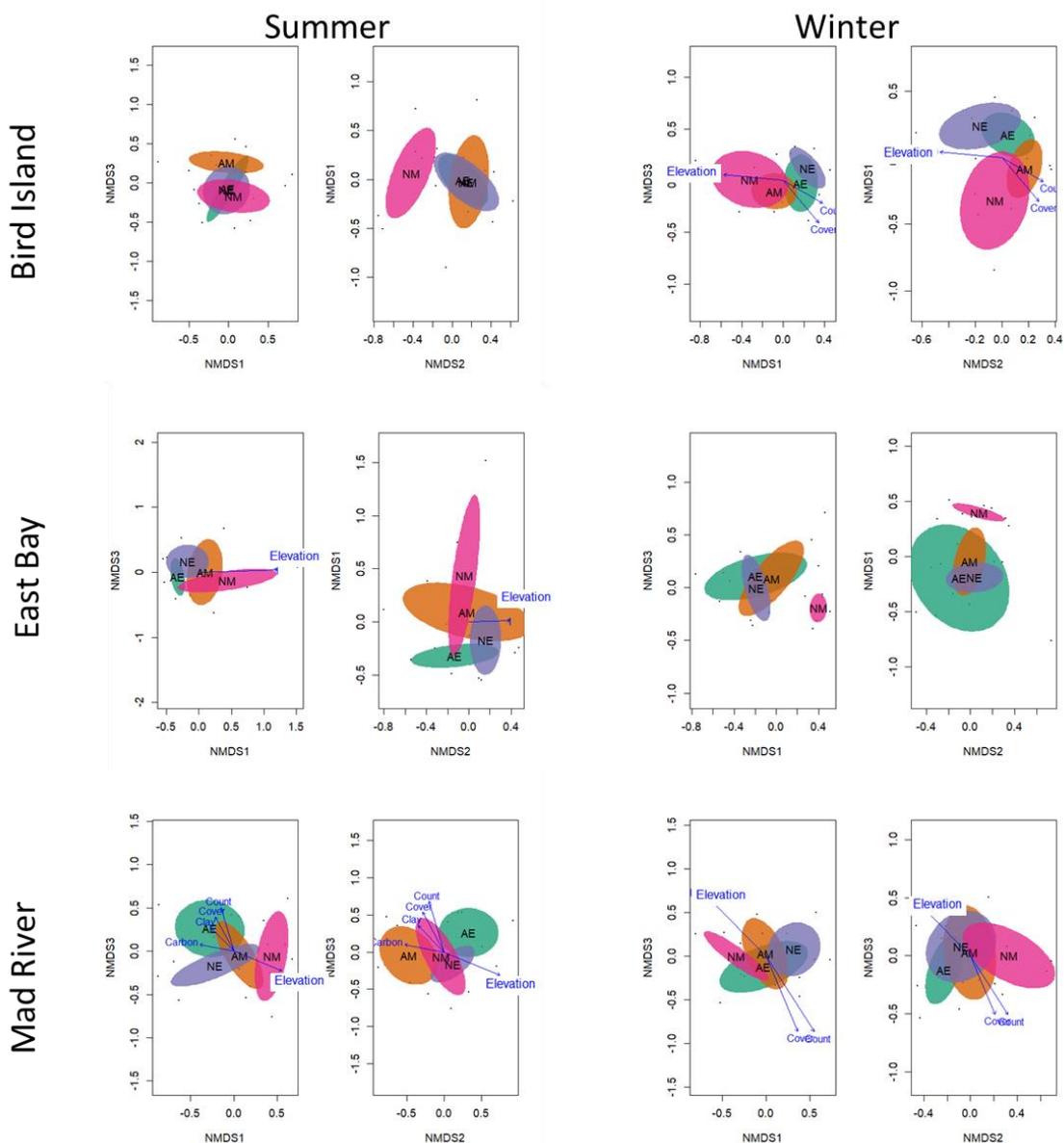
Habitat Type			
Summer season	Eelgrass with aquaculture	Mudflat with aquaculture	Eelgrass no aquaculture
Mudflat with aquaculture	Mad River	-	-
Eelgrass no aquaculture	-	Mad River	-
Mudflat no aquaculture	Mad River	Bird Island	-

Habitat Type			
Winter season	Eelgrass with aquaculture	Mudflat with aquaculture	Eelgrass no aquaculture
Mudflat with aquaculture	-	-	-
Eelgrass no aquaculture	-	Bird Island	-
Mudflat no aquaculture	East Bay Mad River	East Bay	Bird Island East Bay Mad River

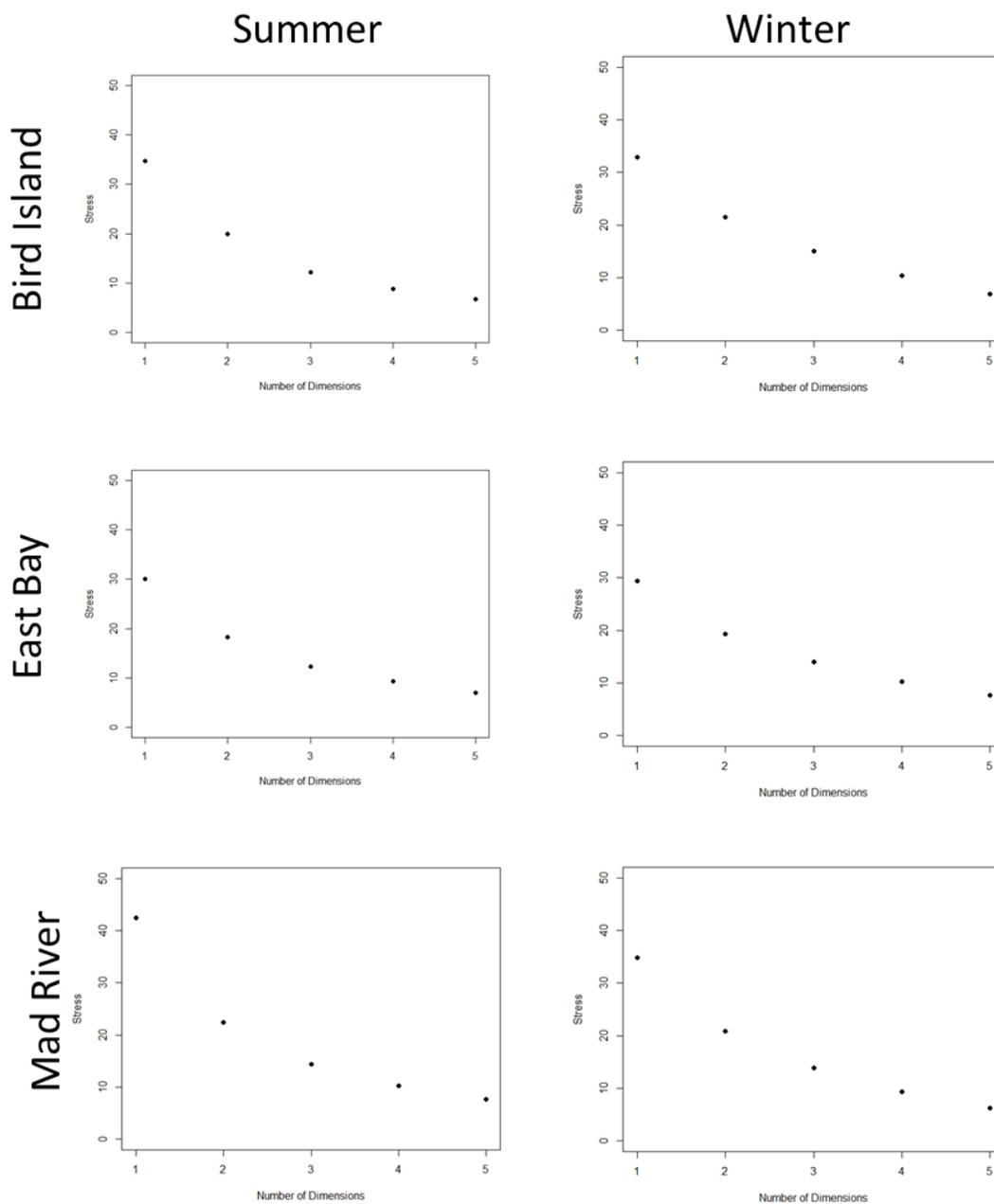
## APPENDIX F

Appendix F. Additional axis comparisons of NMDS ordinations for the invertebrate samples collected from Humboldt Bay's North Bay. Habitat codes within ellipses indicate habitat types: AE= eelgrass with aquaculture, AM= mudflat with aquaculture, NE= eelgrass no aquaculture, and NM= mudflat no aquaculture. Arrows indicate the strength and direction of increase of the significantly correlated environmental variables



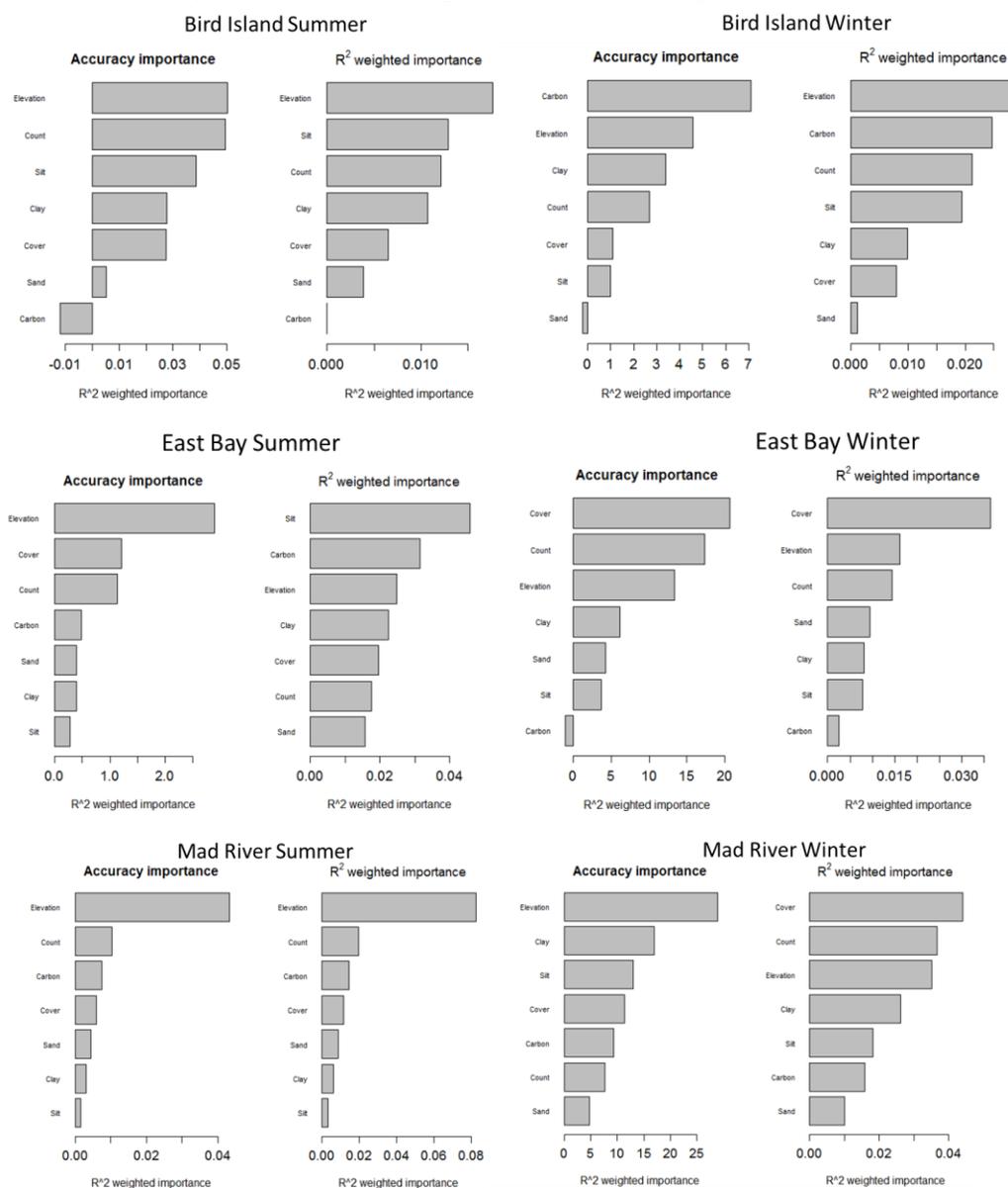
## APPENDIX G

Appendix G. Stress plotted against number of dimensions to determine the correct number of dimensions for ordination plotting



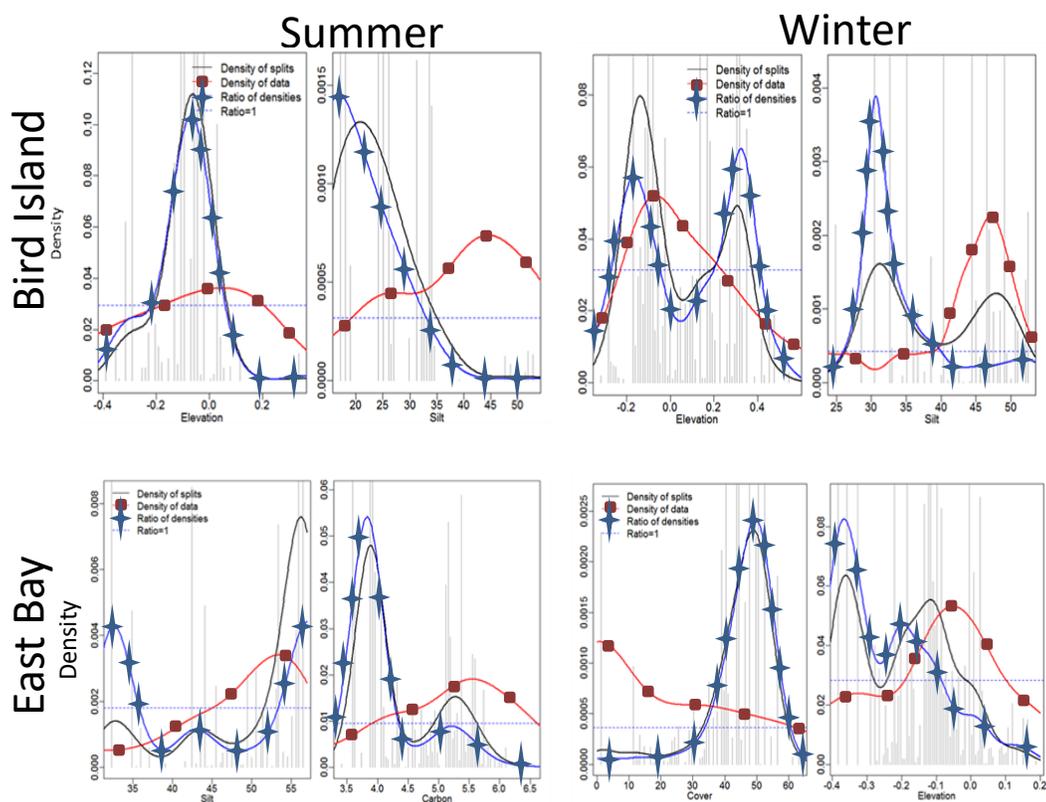
## APPENDIX H

Appendix H.  $R^2$  weighted importance of environmental variables based on GradientForest analysis. The top two variables for each season and region combination were used for further analysis of the relationship between individual variables and invertebrate taxa, except in the case of the top two variables being eelgrass shoot cover and count, in which case the next best variable was used, due to the similarity in invertebrate response to the two eelgrass metrics.



## APPENDIX I

Appendix I. Plots of trends in compositional change of invertebrate communities within the Bird Island, East Bay, and Mad River regions of the North Bay. The x-axis indicates the environmental parameters most influential to driving changes in the invertebrate community. The black line indicates the density of regression tree splits at that level of the environmental gradient (how much community sorting occurred at that point in the gradient) and the red line indicates the density of samples taken at various points along the gradient. The blue line displays the ratio of the black line to the red; peaks in the blue line indicate gradient locations where the compositional change of the invertebrate community occurred.



Appendix I, continued. Plots of trends in compositional change of invertebrate communities within the Bird Island, East Bay, and Mad River regions of the North Bay. The x-axis indicates the environmental parameters most influential to driving changes in the invertebrate community. The black line indicates the density of regression tree splits at that level of the environmental gradient (how much community sorting occurred at that point in the gradient) and the red line indicates the density of samples taken at various points along the gradient. The blue line displays the ratio of the black line to the red; peaks in the blue line indicate gradient locations where the compositional change of the invertebrate community occurred.

