

# Comparative Habitat Use of Estuarine Habitats with and without Oyster Aquaculture—Appendices

Prepared for:

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# Appendix A Field Sampling Effort

# Appendix A FIELD SAMPLING EFFORT

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# 1.0 INTRODUCTION

This appendix describes the 2017-2018 field sampling effort that supported the "Comparative Habitat Use of Estuarine Habitats with and without Oyster Aquaculture Project" (the Comparative Habitat Project). The goal of the Comparative Habitat Project was to determine whether oyster aquaculture alters invertebrate and fish assemblages or productivity of habitats where oysters are grown commercially in Humboldt Bay. The research was set up to compare biological communities within two habitat pairs: (1) native eelgrass with and without oyster aquaculture, and (2) unvegetated mudflats with and without oyster aquaculture.

Three research objectives were identified in the Saltonstall-Kennedy Competitive Research Program Grant proposal (SK Grant Number NA16NMF4270254):

- Does oyster culture alter invertebrate communities (prey resources) in Humboldt Bay?
- Does oyster culture alter fish and/or macroinvertebrate communities in Humboldt Bay?
- Does oyster culture alter the food web in Humboldt Bay?

The 2017-2018 field sampling effort addressed the first two objectives (Table A-1). Note that this appendix is divided into the three field sampling categories identified in the table. The last objective, related to the food web in Humboldt Bay, is addressed in Appendix B (Ecosystem Modeling Workshop).

		Field Sampling Category Addressed			
Approach	Performance Measure	Physical and Chemical Habitat Structure	Invertebrate Communities	Fish and Macroinvert. Communities	
Objective: Does oyster culture	re alter invertebrate communities (prey re	sources) in Humbold	lt Bay?		
Collect quadrat samples within each habitat pair	Successful collection of samples and measurement of eelgrass, macroalgae, or shell material within habitat pairs	•			
Collect core samples within the top 10 cm of sediment within each habitat pair	Statistical comparison of all benthic invertebrate species (or functional feeding group) and measurement of total organic carbon and grain size	•	•		
Collect epibenthic samples along the sediment surface within each habitat pair	Statistical comparison of all epibenthic invertebrate species (or functional feeding group) within habitat pairs		•		
Objective: Does oyster cultu	re alter fish and/or mobile macroinvertebr	ate communities in H	lumboldt Bay?		
Collect and enumerate species within rapidly deployed enclosures	Statistical comparisons of all fish and macroinvertebrate within habitat pairs			•	
Identify other fish or macroinvertebrates that use Humboldt Bay	Observations of other fish or macroinvertebrates using direct and indirect sampling methods			•	
• = field sampling category address	essed by the sampling approach and performance	measure.			

Table A-1.	Objectives, Approa	ch, and Performand	ce Measures for the	2017-2018 Field Sampling



# 2.0 METHODS

Physical and chemical habitat structure, invertebrate communities, and fish communities were sampled within two habitat pairs in Humboldt Bay. Specifically, the North Bay (or Arcata Bay) region of Humboldt Bay was sampled, which is where oyster aquaculture is located. The type of oyster aquaculture that was studied during the 2017-2018 field sampling effort was cultch-on-longlines spaced approximately 0.8 meters apart. Some of the culture plots (i.e., groups of longlines together) included 1.5-meter gaps between longlines. The data collected, and other available data in Humboldt Bay, were assessed for sufficiency to include in an Ecopath with Ecosim (EwE) model to understand energy flow within the Humboldt Bay food web (see Appendix B).

## 2.1 Study Location

The 2017-2018 field sampling effort for the Comparative Habitat Project was located within intertidal habitat of North Bay (Figure A-1). The tidal range in North Bay is approximately -0.6 meters to +2.6 meters mean lower low water (MLLW). Intertidal areas in North Bay have substrates that are comprised mainly of silty mud with some sand. The total surface area of North Bay is approximately 3,432 hectares (456 hectares subtidal and 2,976 hectaries intertidal) at mean higher water (NOAA 2012).

There are three general geographical regions of North Bay – intertidal areas near Mad River, on Bird Island, and in East Bay – that were sampled for various target components of the Comparative Habitat Project (Table A-2).

Sampling Method	Target Component	Mad River	Bird Island	East Bay
Physical and Chemical Habitat S	tructure			
Core samples	Total organic carbon and grain size	•	•	•
Quadrat samples	Eelgrass, macroalgae, or shell material	•	•	•
Invertebrate Communities				
Core samples	Benthic invertebrates	•	•	•
Epibenthic pumps	Epibenthic invertebrates	•	•	•
Fish and Mobile Macroinvertebra	te Communities			
Enclosure nets	Fish			•
Fyke nets	Fish			•
Minnow traps	Fish and mobile macroinvertebrates	•	•	•
Underwater video <sup>1</sup>	Fish	•	•	•
Predation tethering units (PTUs) <sup>1</sup>	Predation potential	•	•	•
• = sampling method used in the geog	graphical region of North Bay.	1	1	

Table $\Lambda_2$	Sampling Methods	used for the Com	parative Habitat Project
I ADIE A-Z.	Sampling Methods (	used for the com	

<sup>1</sup> While these sampling methods were used by Hudson et al. 2018, they did not occur as part of the current project.

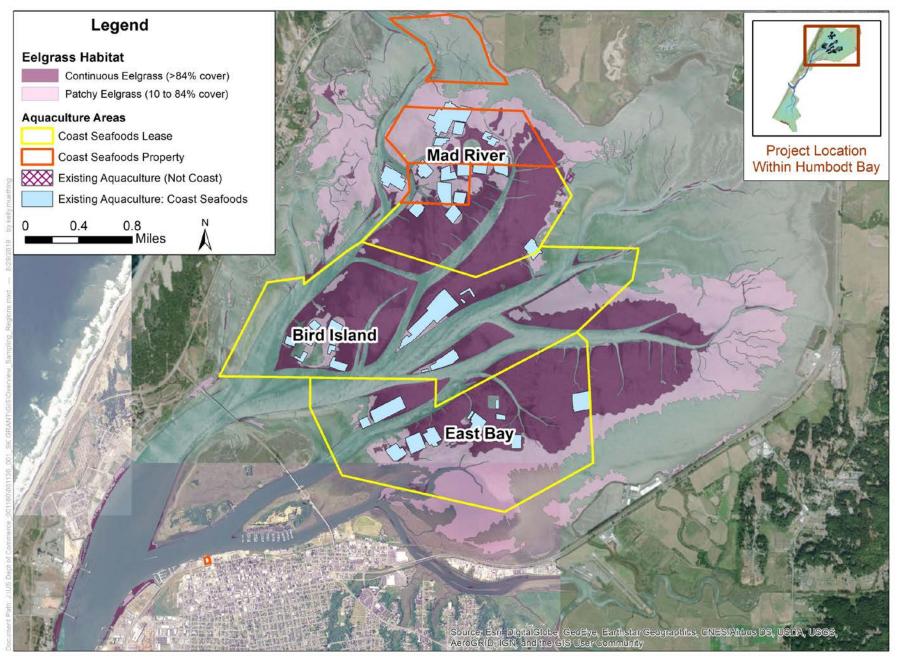


Figure A-1. General Geographical Regions used for Sampling within North Bay



# 2.2 Sampling

This discussion of sampling methods are broken down by the three main categories used for the 2017-2018 field sampling effort: (1) physical and chemical habitat structure, (2) invertebrate communities, and (3) fish and mobile macroinvertebrate communities. Note that the predation tethering units (PTUs) were not originally identified in the SK Gant Number NA16NMF4270254 proposed work, but were added to the fish and mobile macroinvertebrate communities category because it provides information on benthic predators using invertebrate prey species as bait. A discussion of each sampling method, and the target components sampled, is discussed below.

#### 2.2.1 Physical and Chemical Habitat Structure

The physical and chemical habitat structure was sampled with core and quadrat samples (Figure A-2). Both sampling methods were used in the three general geographical regions of North Bay within the two habitat pairs described above. Sampling occurred during winter and summer months, allowing for a seasonal comparison. Core samples provided information on both the physical and chemical structure of the sediment, while quadrat samples provided detail about the physical structure creating habitat above the sediment. Physical and chemical habitat structure was a key component of understanding the invertebrate communities (or community structure). According to Dethier and Schoch (2005), "patterns of benthic community structure are functionally linked to estuarine processes and physical characteristics of the benthos."



# **Figure A-2.** Representative Photographs of Sampling Gear Photo A: Collection of 10 cm deep sediment cores. Photo B: Measuring eelgrass percent cover and shoot count using 5-0.25 m<sup>2</sup> quadrats along a 50 m transect. Photo C: Epibenthic pump with a 500 micron (µm) mesh bag attachment.



The following information is a summary of the detailed methods provided in Coe (2019), one of the project partners for the Comparative Habitat Project.

#### **Core Samples**

Sediment samples using a 2.5 centimeter (cm) diameter by 10 cm tall core were collected when the sample plot was exposed during low tides. These samples were used for percent total organic carbon (%TOC) and particle size analysis. All samples were stored on ice in the field and then transferred to appropriate storage at Humboldt State University depending on the intended analysis: -80°C for %TOC and -18°C storage for particle size.

Specific sampling dates were chosen based on the lowest tide series<sup>2</sup>. Sampling locations were chosen using a random sample tool (ArcMap 10.4.1) within the three general geographical regions of North Bay (Figure A-3). At least five sites per habitat type per region were sampled each season for a total of 120 core samples (Table A-3).

Socon	Low Tide Series*	Habitat Type (Sample Number)			
Season Date	(meter MLLW)	Eelgrass with Oyster Culture	Eelgrass without Oyster Culture	Mudflat with Oyster Culture	Mudflat without Oyster Culture
Mad River					
Summer					
Jun 22-28, 2017	-0.52	3	3	3	4
Jul 21-27, 2017	-0.38	3	3	2	2
Winter					
Dec 2-7, 2017	-0.46	2	2	3	3
Jan 2-5, 2018	-0.47	2 0	0	0	0
Jan 28-Feb 2, 2018	-0.39	2	2	0	2
Bird Island				•	
Summer					
Jun 22-28, 2017	-0.52	3	3	3	3
Jul 21-27, 2017	-0.38	2	2	4	3
Winter					
Dec 2-7, 2017	-0.46	3	1	3	2
Jan 2-5, 2018	-0.47	0	0	0	0
Jan 28-Feb 2, 2018	-0.39	2	2	3	2
East Bay					
Summer					
Jun 22-28, 2017	-0.52	3	3	3	3
Jul 21-27, 2017	-0.38	2	3	2	2
Winter					
Dec 2-7, 2017	-0.46	3	3	2	3
Jan 2-5, 2018	-0.47	2	4	2	3
Jan 28-Feb 2, 2018	-0.39	1	2	1	1
MLLW = mean lower low water *Low tide series is a 4- to 8-day		ts. Values represent n	neans of predicted lower	r low tides for the spec	ified date range

#### Table A-3. Sampling Dates for Core Samples

(NOAA 2019)

<sup>2</sup> Low tide series = a 4- to 8-day series of extreme low tide events.

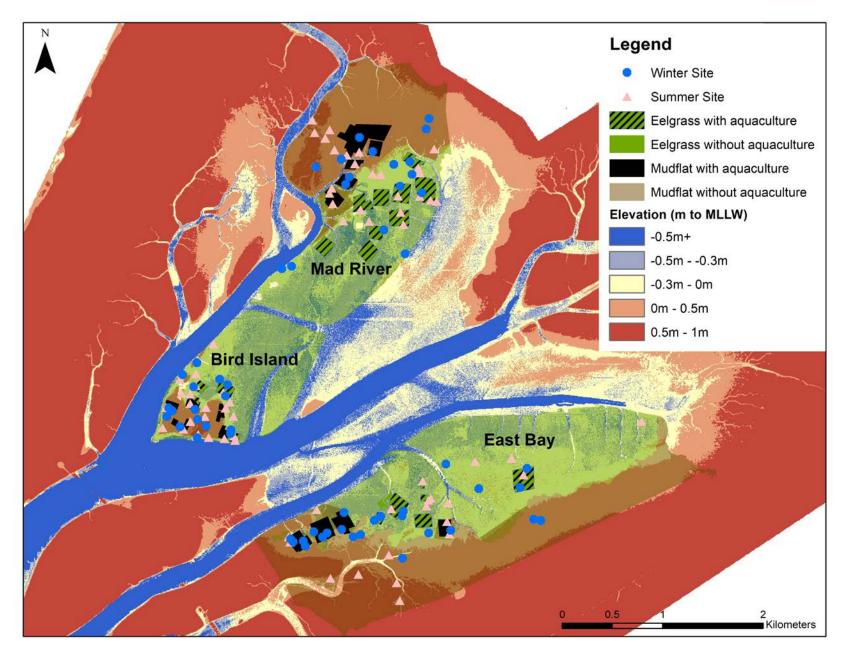


Figure A-3. Core and Quadrat Sampling Locations within the Geographical Regions of North Bay



#### Quadrat Samples

Eelgrass habitat variables were measured at all sample sites using a 0.25 square meter (m<sup>2</sup>) quadrat. Five measurements were taken along a 50 meter (m) transect, and each transect was 10 m apart. Eelgrass percent cover and shoot<sup>3</sup> counts were recorded within each quadrat. Percent cover was estimated visually and all shoots within the quadrat were counted, similar to the methods described by Tallis et al. (2009). The same sampling locations and dates used for the core samples (refer to Table A-3) were also used for the quadrat samples.

#### 2.2.2 Invertebrate Communities

Invertebrate were sampled using cores and epibenthic pumps (refer to Figure A-2). Cores sample invertebrates within the substrate while epibenthic pumps collect fauna along the top of the substrate. Samples were taken at the same locations that were used for the physical and chemical habitat structure samples (refer to Section 2.2.1 and Figure A-3). More information on these methods can be found in Coe (2019). Pitfall traps and drop samplers were also proposed as part of the invertebrate sampling regime, but initial results indicated that the methods were inappropriate for sampling the target taxa and also were time intensive. A "lessons learned" section on gear used during the 2017-2018 field sampling effort is provided in Appendix C.

#### **Core Samples**

Core samples were collected using a 2.5 cm diameter by 10 cm tall core when the tideflats were exposed during low tide. This size core results in a volume of approximately 50 cubic cm (cm<sup>3</sup>). Ferraro and Cole (2004) showed that this volume was sufficient to capture the diversity and abundance of benthic infauna. Samples were stored on ice in the field and then transferred to 4°C storage prior to sieving to remove invertebrates from the sample.

#### **Epibenthic Pumps**

Epibenthic organisms were sampled using an epibenthic pump similar to the one used by Toft et al. (2013), with a 500 micron ( $\mu$ m) mesh bag attached to the output pipe of a hand bilge pump. 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 epibenthic pumps were conducted on the outgoing tide to avoid high water sampling after dark. For both seasons, samples were collected when the water was between 25 cm and 90 cm deep.

<sup>3</sup> Shoot (or turion) = an individual eelgrass leaf. Eelgrass can produce asexually through rhizome growth and creation of new buds. Each bud forms a leaf that is considered a shoot or turion.



#### 2.2.3 Fish and Mobile Macroinvertebrate Communities

The fish and mobile macroinvertebrates were sampled directly using enclosure nets, fyke nets, and minnow traps. The enclosure nets sampled eelgrass and mudflat habitats in East Bay, fyke nets sampled eelgrass habitat in East Bay, and the minnow traps sampled eelgrass and mudflat habitats in Mad River, Bird Island, and East Bay (Figure A-4). Each sampling method comes with its own inherent bias, which was compared against the other methods in terms of suitability for sampling the fish communities in North Bay. As noted above, Appendix C has a lessons learned section on gear used during the 2017-2018 field sampling effort.

Habitat use by the fish community was measured indirectly by using predation tethering units (PTUs). PTUs provide an estimate of the predation intensity within the different habitats by measuring the time it takes for a piece of bait to be consumed. Underwater video sampling was also attempted but did not prove to be an effective sampling method in Humboldt Bay (see Appendix C for details).

#### **Enclosure Nets**

Enclosures nets were located within 9.1-meter by 9.1-meter (83.6 square meter) plots with netting rapidly deployed around the perimeter of the enclosure to isolate the fish or mobile macroinvertebrates within the sample plots. The target habitat area for the enclosure nets was a sample plot that drained into a single tidal channel. The nets had a live box attached to a tunnel that extended from the corner of the net to the adjacent tidal channel. The live box was positioned within the adjacent tidal channel for the live capture of fish and macroinvertebrates (Figure A-5). The enclosure net was held up by a total of 12 steel posts that were 3 meters tall. Each side of the enclosure net had 3 posts, creating a square enclosure. The enclosure net was weighted at the bottom and collected fish from the entire water column.

The enclosure nets were set up typically the day before sampling during the higher of the low tides in North Bay (approximately +0.6 meters MLLW) and left with the sides of the nets tied up during the flood tide. Enclosure nets were rapidly deployed at water depths of approximately 1.2 meters above the sediment surface during the next ebb tide. Fish caught in enclosure nets and within the enclosures were monitored every 30 to 60 minutes to limit injury to fish in nets and facilitate rapid release of organisms back to Humboldt Bay. All fish captured within the enclosure nets were collected, identified to species (or lowest taxonomic level possible), measured to total length and fork length (as applicable), counted, and released. Observations of mobile macroinvertebrates in the enclosure nets were recorded but were not enumerated or measured.

A field test of the gear was performed in April and May 2017 to test the timing, challenges, and effectiveness of sampling using the enclosure net system. Following the field test, sampling within enclosure plots occurred during 4 different events during spring, summer, and winter



(Table A-4). Sampling for habitat types occurred during the same tidal series and sampling each habitat pair occurred on the same day.

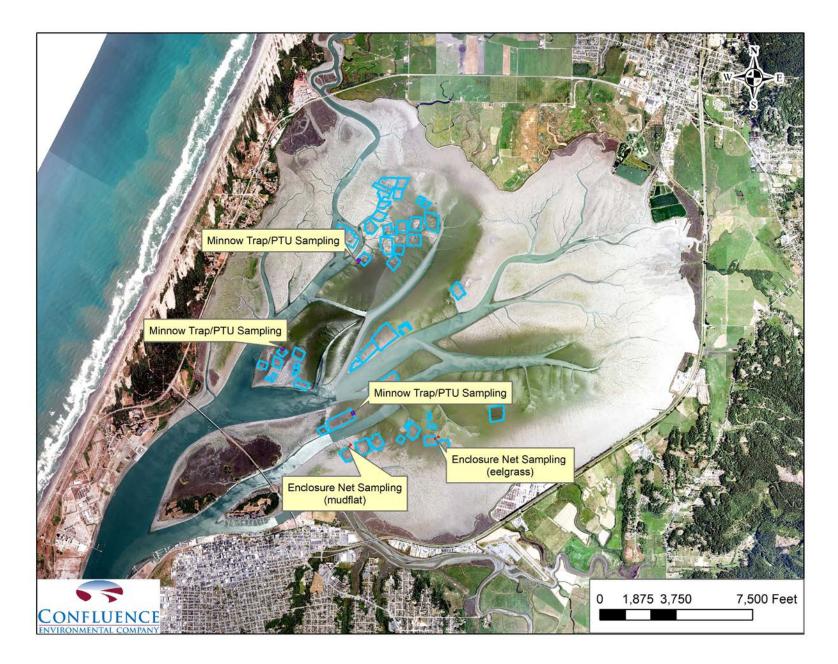


Figure A-4. Fish and Macroinvertebrate Sampling Locations within the Geographical Regions of North Bay





#### **Figure A-5.** Example of Enclosure Net Set Up for the Comparative Habitat Project Photo A = Set up for the paired enclosure nets; Photo B = example of setting posts for the nets; Photo C = Fully set up net ready for deployment.

Low Tide	Habitat Type				
Elevation* (meter MLLW)	Eelgrass with Oyster Culture	Eelgrass without Oyster Culture	Mudflat with Oyster Culture	Mudflat without Oyster Culture	
-0.46	•	•			
-0.28			•	•	
-0.64	•	•			
-0.67			•	•	
-0.33	•	•			
-0.27			•	•	
-0.34	•	•			
-0.50			•	•	
	Elevation* (meter MLLW) -0.46 -0.28 -0.64 -0.67 -0.33 -0.27 -0.27 -0.34	Elevation* (meter MLLW)         Eelgrass with Oyster Culture           -0.46         •           -0.28         •           -0.64         •           -0.67         •           -0.33         •           -0.27         •           -0.34         •	Elevation* (meter MLLW)Eelgrass with Oyster CultureEelgrass without Oyster Culture-0.46••-0.28••-0.64••-0.67••-0.67••-0.33••-0.27••-0.34••	Elevation* (meter MLLW)Eelgrass with Oyster CultureMudflat with Oyster Culture-0.46••-0.28••-0.64••-0.67••-0.67••-0.33••-0.27••-0.34••	

Table A-4.	Sampling	Dates for	r Enclosure	Net Samples
------------	----------	-----------	-------------	-------------

\*Lowest tidal elevation possible during the sampling of the enclosure net.

#### Fyke Nets

The methods and nets used for the fyke net sampling was similar as used by Pinnix et al. (2005) with the exception that the nets were deployed during the receding tide to match the enclosure net deployments. In comparison, Pinnix et al. (2005) deployed for a total of 4 hours after low tide, which resulted in a much longer sampling period. The fyke net used was a 1.22 m Maine fyke net with 30.48 m leads and 6.35 mm mesh. The wings were deployed along the edges of the habitat (Figure A-6). The target habitat area for the fyke nets was a broad sample plot that drained into a single tidal channel. The fyke net had a live box that was attached to a tunnel in the middle of the net. The tunnel extended from the intertidal habitat out to the tidal channel where the live box was positioned. The same 3-meter posts described above were used to set up the fyke net, which included a post for each side of the tunnel draining into the live box, one post to hold up the live box, one post along the middle of each wing wall. The lead line and net tension on the posts kept the net sampling the midwater column when deployed. In comparison, the enclosure net had a finer mesh size and sampled the entire water column.

The live box for the fyke nets was attached at water depths of approximately 1 meter above the sediment surface. Fish collected were processed using the same methods described above for the enclosure nets.



Fyke nets were set up in paired eelgrass habitat. Sampling using the fyke nets occurred on August 22, 2017, and December 4, 2017 (2 events). Sampling for the paired fyke nets occurred during the same tidal cycle.



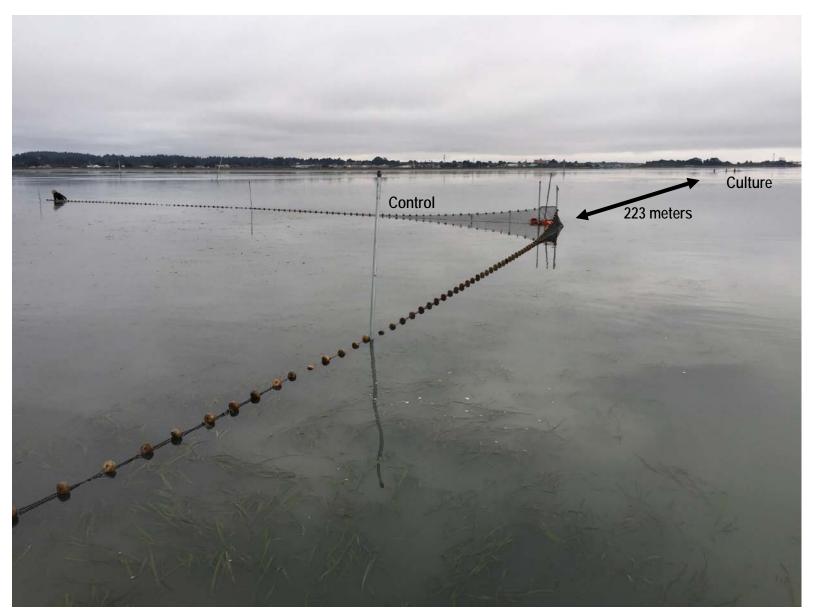


Figure A-6. Example of Fyke Net Set Up for the Comparative Habitat Project



#### Minnow Traps

Five minnow traps (approximately 60 cm x 60 cm x 46 cm, with a ~1.2 cm opening) were evenlyspaced (15 m apart) along a 60 m transect running from an oyster aquaculture bed into an adjacent eelgrass bed (Figure A-7a). The transect was centered at the edge of the aquaculture bed, with traps placed at the edge, 15 m, and 30 m from the edge in each direction. Traps were considered to represent five different parts of the habitat matrix along each transect (Figure A-7b):

- 1. Aquaculture interior (Location A 30 m from edge),
- 2. Aquaculture intermediate (Location B 15 m from edge),
- 3. Edge (Location C),
- 4. Eelgrass intermediate (Location D 15 m from edge), and
- 5. Eelgrass interior (Location E 30 m from edge).

Traps (un-baited) were deployed at low tide and retrieved approximately one hour after the local high tide. Captured fish and mobile macroinvertebrates were identified, counted, and then returned to the water. Sampling using minnow traps occurred at three sites within the bay during two separate tidal cycles: December 2 to 4, 2017 and June 14 to 16, 2018 (2 events).

To allow for a more straightforward comparison between this data and other data collected within the 2017-2018 field sampling effort, the traps within aquaculture (Locations A and B) and traps within eelgrass (Locations D and E) were respectively pooled. Catch from oyster aquaculture and eelgrass habitats could then be compared.





Figure A-7a. Example of Minnow Trap.



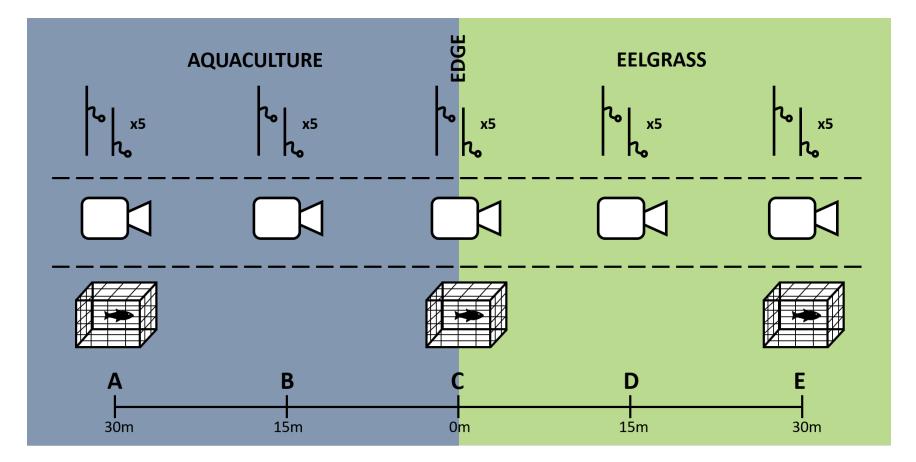


Figure A-7b. Example of Minnow Trap Set Up for the Comparative Habitat Project (Note: predation tethering units (PTUs) and underwater video were not part of the current study).



## 2.3 Sample Processing

Sampling processing following typical methods for each category sampled.

#### 2.3.1 Physical and Chemical Habitat Structure

Core samples collected for %TOC were analyzed using the loss on ignition protocol with equipment of the College of Natural Resources and Sciences Core Research Facility at Humboldt State University (Gavlak et al. 2005). Particle size analysis was performed using the sieve and hydrometer method for percent sand, silt, and clay (Day 1965). These methods allowed for characterization of the sediment profile at the sampling locations. %TOC and particle size were then correlated with the benthic invertebrate communities (Bott and Diebel 1982, Ferraro and Cole 2004, Dethier and Schoch 2005).

#### 2.3.2 Invertebrate Communities

Core samples for benthic invertebrates were washed with seawater through a series of sieves (from 4 mm to 2 mm to 0.5 mm), and the organic material left on each sieve was fixed in buffered 10% formalin and stained with Rose Bengal (Lewis and Stoner 1981). The epibenthic pump samples were washed on a 0.5 mm sieve and similarly fixed. The fixed samples were examined under a dissecting microscope, invertebrates were removed and placed in 70% ethanol for storage and were later identified to the taxonomic level indicated in Table A-5. Forrest and Creese (2006) found similar taxonomic levels to be sufficient to detect spatial patterns of impact to soft-bottom invertebrate communities.

Phylum	General Group Used
	Class level: Malacostraca, Ostracoda
Arthropoda (subphylum Crustacea)	Order level: Amphipoda, Cumacea, Isopoda, Decapoda, Tanaidacea
	Family level (examples): Caprellidae, Gammaridae
Mollusca	Class level: Bivalvia, Gastropoda
Appelide	Class level: Oligochaeta, Polychaeta
Annelida	Family level (examples): Capitellidae, Nephtyidae, Oweniidae, Phyllodocidae
Echinodermata	Phylum level: Echinodermata
Other phyla	Phylum level: Nemertea

Table A-5	Taxonomic Grou	ps in Classifications	on Sampler	Invertebrates
Table A-3.		ps in Glassifications	s un Sampleu	

#### 2.3.3 Fish and Mobile Macroinvertebrate Communities

As indicated in Section 2.2.3 above, fish that were captured in the enclosure nets, fyke nets, and minnow traps were identified to species (or lowest taxonomic level possible), measured, enumerated, and released. Fish were monitored for health prior to release, and any fish that perished during sampling were identified in the field notes. Additional conservations measures for fish processing followed the protocols established under the California Department of Fish and Wildlife (CDFW) memorandum of understanding, CDFW scientific collecting permit (SC-



13657), additional amendments under the same scientific collecting permit, and the NMFS Permit (Permit 20622):

- All species collected were handled with extreme care and kept in cool aerated water to the maximum extent possible during sampling and processing. Adequate circulation and clean water in holding units were used. If Endangered Species Act (ESA) listed fish species, sensitive species, or other protected fish species, were collected, then their handling included additional precautions.
- Fish were released underwater while the vessel(s) used during field sampling was stationary. Releasing fish from a vessel underway shall only be conducted when trying to avoid predation on listed species by piscivorous birds and/or marine mammals.
- There was no intentional lethal take of ESA listed fish species, sensitive species, or other protected fish. Additional measures were taken for true smelt (family Osmeridae) collected while in the field.
- No ESA listed fish or true smelt (family Osmeridae) were encountered during this field study.

Mobile macroinvertebrates (e.g., crabs) observed within the nets or minnow traps were recorded, but no collection method was suitable for collecting and enumerating crabs. Indirect methods for mobile macroinvertebrates (e.g., underwater video, PTUs) were primarily used to understand presence or absence in a habitat type.

### 2.4 Data Analysis

Several visual and statistical tools were used to analyze the data in each of the three main categories used in the 2017-2018 field sampling effort (Table A-6). Similar to previous work by Dethier and Schoch (2005), the invertebrate samples were pooled. The data were not analyzed for variability within each sampling location or by sampling method because the goal was to quantify the community structure and not the inherent variability of sample units. In a similar manner, fish samples were pooled for the different sampling events and with other background literature.



#### Table A-6. Visual and Statistical Tools used for Data Analysis

Tool	Goal	Physical and Chemical Habitat Structure	Invertebrate Communities	Fish and Macroinvert. Communities
Visualization				
Bar Charts	To visualize averages by habitat pair for eelgrass metrics and sediment grain size.	•		
Univariate Analyses	-			-
One-Way Analysis of Similarities (ANOSIM)	To test for habitat differences in the fish communities			•
Two-Way Analysis of Variance (ANOVA)	To test the interaction between community structure, habitat type, and season. The assumptions of homogeneity of variance and normality were evaluated.		•	•
Tukey's honest significant difference (HSD) test	To complete post-hoc analysis on the ANOVA results. This analysis can show which factors drive a significant ANOVA result.			•
Taxa Accumulation Curves	To determine if adequate sampling occurred.		•	
Multivariate Community	Analysis			
Bray-Curtis Dissimilarity	To create a dissimilarity matrix comparing sites based on taxa composition.		•	
Non-Metric Multidimensional Scaling (NMDS)	To identify the similarities and differences in the data represented by distances between NMDS plots.		•	
Permutational Multivariate Analysis of Variance (PERMANOVA)	To determine if there are statistical differences between the habitat pairs following NMDS analysis. If a significant PERMANOVA result is obtained, a post-hoc test can be used to determine between which groups the differences occur.		•	•
Indicator Taxa Analysis	To evaluate whether particular taxa were significantly associated with habitat types analyzed in the NMDS.		•	
Functional Feeding Groups	To understand how community structure is linked to broader ecological function.		•	
Envfit within the Vegan Package	To determine what environmental factors might be driving potential differences in community composition between groups.	•	•	
Taxa = lowest level of ident	ification for each organism			

• = sampling method used in the geographical region of North Bay.

Sources: Bray and Curtis 1957, Clarke and Warwick 2001, Ugland et al. 2003, Dethier and Shoch 2004, Ferraro and Cole 2007, De Caceres and Maintainer 2016, R Core Team 2016, Oksanen et al. 2017, Partridge et al. 2018

# 3.0 RESULTS

The following sections include the results of the 2017-2018 field sampling effort that supported the Comparative Habitat Project broken down by the three main categories: (1) physical and chemical habitat structure, (2) invertebrate communities, and (4) fish and mobile macroinvertebrate communities. The results for the first two categories are summaries of the work provided in Coe (2019). Please refer to that document for more detailed information.



# 3.1 Physical and Chemical Habitat Structure

Core and quadrat samples for physical and chemical habitat structure were collected within a tidal range of -0.28 m to -0.12 m MLLW (Table A-7). There were also seasonal and regional difference in where samples were collected. Overall, there were a total of 131 core samples (67 summer and 64 winter) for %TOC and sediment grain size, and a total of 660 quadrat samples collected at 132 sites (67 summer and 65 winter) for eelgrass shoot count and percent cover throughout North Bay.

Liekitet Deire	Tidal Elevatio	on (m MLLW)
Habitat Pairs	Summer	Winter
Bird Island		
Eelgrass with Aquaculture	-0.22	-0.16
Eelgrass without Aquaculture	-0.25	-0.12
Change in Elevation	-0.03	+0.04
Mudflat with Aquaculture	0.13	0.15
Mudflat without Aquaculture	0.27	0.36
Change in Elevation	+0.14	+0.21
East Bay		
Eelgrass with Aquaculture	-0.15	-0.14
Eelgrass without Aquaculture	-0.24	-0.27
Change in Elevation	-0.09	-0.13
Mudflat with Aquaculture	-0.08	-0.09
Mudflat without Aquaculture	0.53	0.09
Change in Elevation	+0.61	+0.18
Mad River		
Eelgrass with Aquaculture	-0.28	-0.21
Eelgrass without Aquaculture	-0.12	-0.21
Change in Elevation	+0.16	0.00
Mudflat with Aquaculture	0.32	0.16
Mudflat without Aquaculture	0.36	0.26
Change in Elevation	+0.04	+0.10
MLLW = mean lower low water		

#### Table A-7. Elevation for Core and Quadrat Sample Locations

### 3.1.1 Eelgrass Metrics

Comparison of eelgrass shoot count and percent cover revealed similar effects of oyster aquaculture on both eelgrass metrics. Two-way ANOVA comparison resulted in no difference in eelgrass percent cover between the summer and winter seasons for all quadrat samples collected (p= 0.592; F= 0.291). Shoot counts were found to be higher in the winter season for the pooled data (p= 0.006; F= 8.164), although the difference between count averages was less than one eelgrass shoot, indicating that eelgrass resources were stable during both winter and summer seasons. There were significantly lower shoot counts (p= 0.0311; F= 4.864) and lower percent cover (p= 0.0005; F= 15.21) when oyster longlines were present. For both analyses, the assumptions of homogeneity of variance and normality were met.



While the overall pattern of eelgrass with and without aquaculture was consistent between the three geographical regions of North Bay, there were differences that confounded the sampling results. There were larger differences in shoot counts between paired habitats on Bird Island and in the East Bay region, and fewer differences in Mad River. In addition, although not significant, mean shoot counts were greater in the summer within Mad River, while the relationship was switched in the other two regions. Both of these differences may be a result of microtopography within sampling locations. As indicated in Table A-5, the sampling locations were not uniform in terms of tidal elevation. The largest elevation differences in where samples were collected included sampling without aquaculture were +0.16 m higher in the summer in the Mad River and -0.09 to -0.13 m lower in East Bay. This is relevant because eelgrass typically does better (i.e., is more dense) at lower elevations.

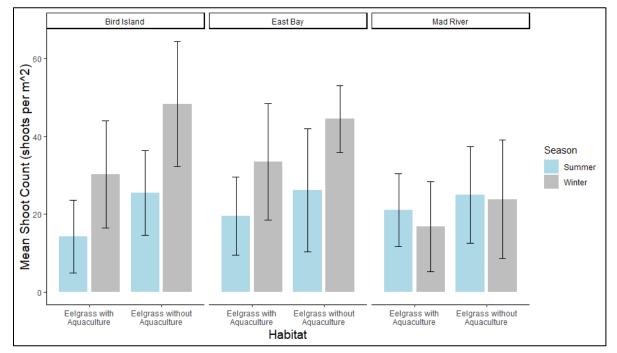


Figure A-8. Eelgrass Shoot Counts in North Bay by Habitat Pair



Due to their potential to drive community structure differences, %TOC and sediment grain size were summarized by habitat type, region, and season. %TOC is a measure of organic carbon accumulation rates or sediment with higher silt and clay content that binds to the organic carbon (Ferraro and Cole 2004). Higher carbon content indicates a higher amount of decaying material that contributes to detritus (e.g., eelgrass decay). Bird Island consistently had lower %TOC than other regions, and summer consistently had higher %TOC for all regions with few exceptions (Table A-8). In eelgrass habitat, in the summer, %TOC ranged from 3.66 to 6.58% with aquaculture and 3.93 to 5.11% without aquaculture. In mudflat habitat, in the summer, %TOC ranged from 3.47 to 5.44% with aquaculture and 3.35 to 5.86% without aquaculture. This indicates almost identical ranges of %TOC by habitat type and habitat pairs.

Liekitet Deire		Summer		Winter
Habitat Pairs	Carbon (%)	Elevation (m MLLW)	Carbon (%)	Elevation (m MLLW)
Bird Island				
Eelgrass with Aquaculture	3.66	-0.22	3.07	-0.16
Eelgrass without Aquaculture	3.93	-0.25	2.96	-0.12
Mudflat with Aquaculture	3.47	0.13	4.10	0.15
Mudflat without Aquaculture	3.35	0.27	3.28	0.36
East Bay				
Eelgrass with Aquaculture	5.45	-0.15	3.35	-0.14
Eelgrass without Aquaculture	5.11	-0.24	3.79	-0.27
Mudflat with Aquaculture	5.44	-0.08	4.10	-0.09
Mudflat without Aquaculture	5.86	0.53	3.25	0.09
Mad River				
Eelgrass with Aquaculture	6.58	-0.28	5.84	-0.21
Eelgrass without Aquaculture	4.69	-0.12	4.73	-0.21
Mudflat with Aquaculture	4.68	0.32	3.97	0.16
Mudflat without Aquaculture	5.86	0.36	3.45	0.26
MLLW = mean lower low water				

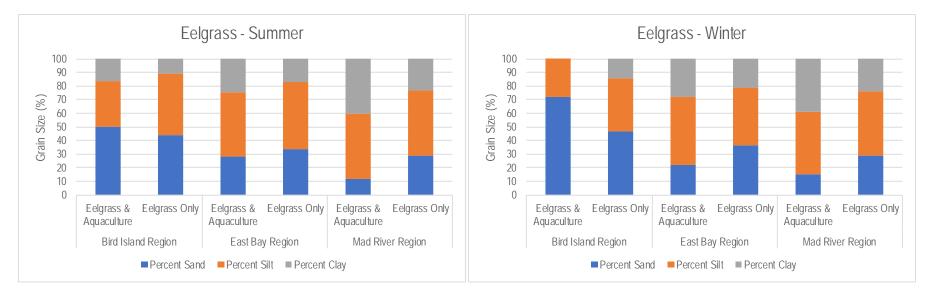
#### Table A-8. Percent Carbon by Geographical Region and Habitat Pair

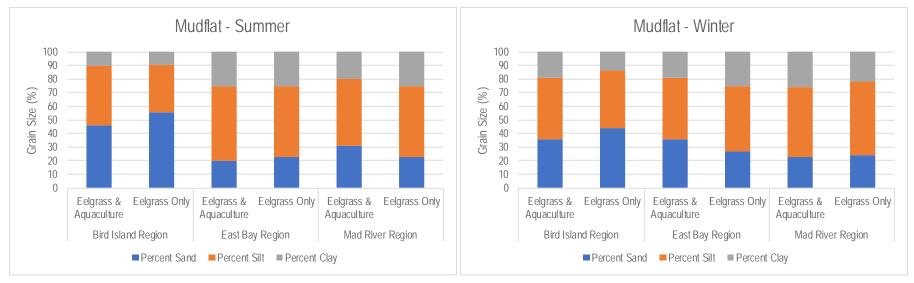
Sediment grain size is a measure of the percent composition of different size classes. Grain size helps to determine the amount of organic carbon that can bind to the sediment, and also drives the community structure that develops within an area. Partridge et al. (2018) specifically identifies sediment grain size as a limiting factor for feeding strategies (e.g., suspension feeders, deposit feeders, carnivores, scavengers, and herbivores), tube-building, and other aspects of benthic invertebrate community structure. For example, lake of sand in the sediment will limit the number of tube-dwelling polychaetes or lack of organic material that binds to the sediment will limit the number of deposit feeders.

Overall, the habitat pairs used to sample invertebrate communities were similar to each other. Using the summer sampling as an example, sand was the dominant grain size on Bird Island, while silt dominated both the East Bay and Mad River regions (Figure A-9). The largest difference between habitat pairs was the amount of clay within the eelgrass habitat with aquaculture compared to areas without aquaculture.

#### APPENDIX A - FIELD SAMPLING EFFORT







#### Figure A-9. Sediment Grain Size by Habitat Pairs and Season

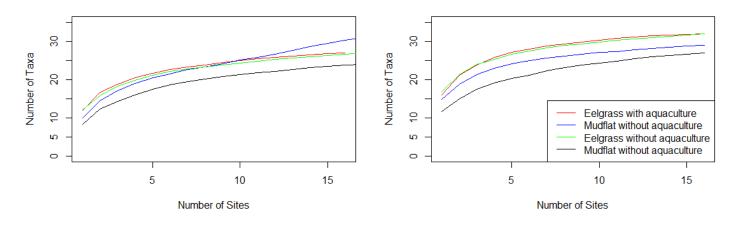


# 3.2 Invertebrate Communities

Core and epibenthic pump samples for invertebrates were collected within the same tidal range described above for core samples (refer to Section 3.1, Table A-7). Overall, there were a total of 131 core samples (67 summer and 64 winter) for benthic invertebrates and a total of 67 epibenthic pump samples (29 summer and 38 winter) for epibenthic invertebrates. Taxa were classified into groups, according to Table A-5 above, and resulted in a total of 17,230 individuals in 35 taxa identified within 7 functional feeding groups (Coe 2019). The resulting communities were analyzed as described below.

#### 3.2.1 Taxa Accumulation Curves

Taxa accumulation curves evaluate the sufficiency of the invertebrate sampling protocol. No new taxa would be expected with increasing samples if the accumulation curve achieves an asymptote. 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 (Figure A-10). This indicates that a complete census of the invertebrate community did not occur, and additional taxa may have been identified had more samples been collected.





#### 3.2.2 Taxa Encountered

The mean number of taxa were analyzed by habitat pair and season. A two-way ANOVA of the number of taxa, with main factors of 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 on average, although this was not always statistically significant (Figure A-11). Comparing information by habitat pair, there were not significant differences in mean number of taxa, with and without aquaculture for eelgrass habitat. In the winter, there was slightly higher total taxa



in areas without aquaculture, but this relationship was not significant. Compared to eelgrass habitat, there were larger differences in mean number of taxa within habitat pairs for mudflat habitat, with higher numbers of taxa sampled from areas with aquaculture compared to areas without aquaculture.

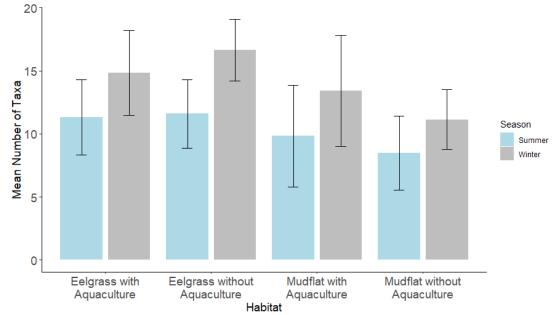


Figure A-11. Mean Number of Taxa Encountered with Each Habitat Pair by Season

Taxa were also analyzed by the specific groups that dominated the different habitats using the indicator taxa analysis and categorizing invertebrates into functional feeding groups. This analysis provides an understanding of the statistical significance of taxa associations. Functional feeding groups (e.g., predator, suspension feeder, deposit feeder) is used to understand the trophic structure of a community (Dethier and Schoch 2005, Macdonald et al. 2010, Partridge et al. 2018). According to Partridge et al. (2018), if there is a balanced trophic structure, then "the functions that the organisms serve in the community are preserved, unless something happens to unbalance the community."

Based on the indicator taxa analysis, the taxa identified within each habitat pair was similar with and without aquaculture (Table A-9). Within eelgrass habitat pairs, the main difference was in East Bay in the winter where isopods (scavengers) defined the invertebrate community structure in areas with aquaculture, compared to two different types of polychaetes – Nephtyidae (predators) and Oweniidae (deposit feeders) – that defined the community structure for areas without aquaculture. Overwhelmingly, the functional feeding groups that defined the invertebrate communities were provide regardless of whether oyster longline were present. Larger differences were observed within the community structure when comparing between eelgrass and mudflats or aquaculture and mudflats. For example, in East Bay during



the summer, the indicator taxa structure included suspension feeders and deposit feeders in eelgrass, but only included deposit feeders in mudflats.

<b>•</b> • • •		Sun	nmer			Wi	nter	
Geographic Region	w/ Aquacul	ture	w/o Aquacu	lture	w/ Aquacul	ture	w/o Aquacu	lture
Region	Таха	FFG	Таха	FFG	Таха	FFG	Таха	FFG
Eelgrass Habit	at Pairs							
	Caprellidae	D	Caprellidae	D	Ampharetidae	D	Ampharetidae	D
Bird Island	Oligochaeta	D	Oligochaete	D				
BILU ISIAIIU	Oweniidae	D	Oweniidae	D				
	Phyllodocidae	Р	Phyllodocidae	Р				
Feet Dev	Ostracoda	Su	Ostracoda	Su	Isopoda	Sc	Nephtyidae	Р
East Bay	Oligochaeta	D	Oligochaeta	D			Oweniidae	D
	Ostracoda	Su	Ostracoda	Su	Cirratulidae	D	Cirratulidae	D
Mad River			Bivalvia	Su	Bivalvia	Su	Bivalvia	Su
							Oweniidae	D
Mudflat Habita	t Pairs							
	Oligochaeta	D			Ampharetidae	D	Chironomidae	D
Bird Island	Oweniidae	D						
	Phyllodocidae	Р						
East Bay			Oligochaeta	D	Oweniidae	D	Oweniidae	D
Mod Diver			Bivalvia	Su	Cirratulidae	D	Bivalvia	Su
Mad River					Oweniidae	D	Oweniidae	D
FFG = Functional	I Feeding Group; D	= deposit f	eeder; P = predator;	Sc = scav	enger; Su = suspens	ion (filter) f	eeder;	

Table A-9. Indicator Taxa Analysis and Associated Functional Feeding Group
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#### 3.2.3 Invertebrate Community Analyses

Invertebrate communities were analyzed using NMDS ordination, Bray-Curtis dissimilarity, and PERMANOVA analysis. NMDS ordination provides an understanding of whether the community structure is similar or different between habitats. Visually, when the ordination plots (or plot of a community) overlaps, then they are similar. Statistically, these plots can be compared using the Bray-Curtis method to quantify the differences in communities by region, and using the PERMANOVA and post hoc analysis to look at differences in communities by composition. For all regions, taxa abundances were Hellinger transformed.

NMDS ordination plots of invertebrate communities by region and season are provided in Figure A-12. The plots are coded by habitat:

- Green (AE) = eelgrass with aquaculture
- Purple (NE) = eelgrass without aquaculture
- Orange (AM) = mudflats with aquaculture
- Pink (NM) = mudflats without aquaculture



The following discussion is how the invertebrate communities within these habitats are similar or dissimilar to each other.



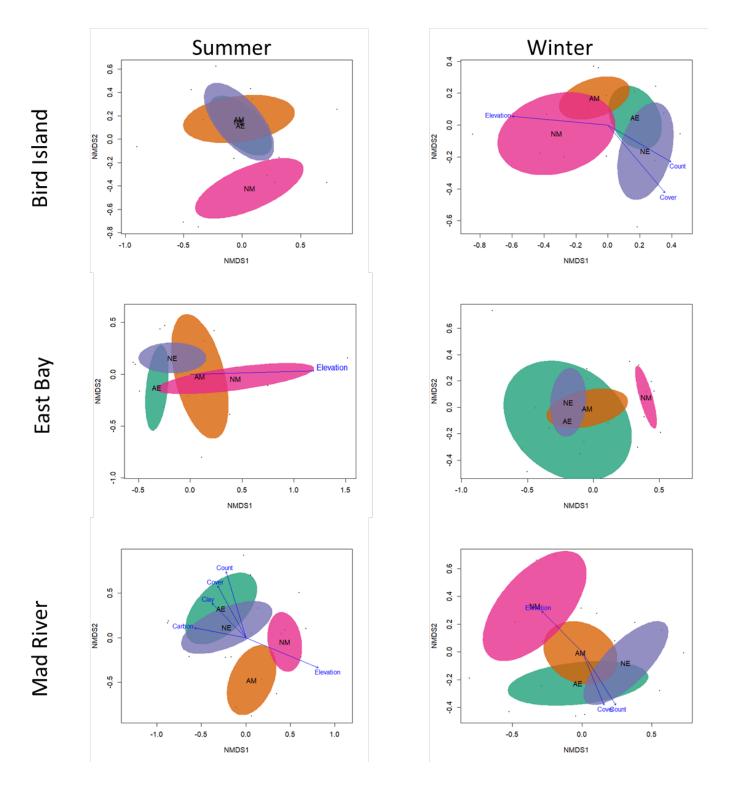


Figure A-12. NMDS Ordination Plots of Invertebrate Community Structure from North Bay Note: AE= eelgrass with aquaculture, NE= eelgrass without aquaculture, AM= mudflat with aquaculture, and NM= mudflat without aquaculture. Source: Coe (2019)



#### Summer NMDS Results

In every summer NMDS ordination plot, there is overlap between eelgrass habitat pairs. This means that the invertebrate communities that develop in eelgrass with and without aquaculture are similar to each other. Comparatively, the communities in mudflat habitats do not always overlap. For example, on Bird Island, there is no overlap in the mudflat habitat types. Based on the NMDS plot, mudflat habitat with aquaculture on Bird Island was more similar to eelgrass habitat with and without aquaculture. The ordination for the summer Bird Island sites had a stress level of 0.122 with three dimensions, and no environmental variables being significantly correlated to the ordination. This indicates that, while the stress value represents useful ordinations (i.e., is below 0.2), there is no factor that drives the community structure.

In East Bay, the summer NMDS plots were more dispersed, but included some amount of overlap between the various habitat types. While mudflat habitat without aquaculture was most dissimilar to the other habitat types, elevation was a main driving force in determining the invertebrate communities. 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.

In the Mad River region, there was substantial overlap between the invertebrate communities found within eelgrass with and without aquaculture, but no overlap with mudflat habitats. In addition, mudflat habitats with aquaculture were not similar to mudflat habitats without aquaculture. The three-dimensional ordination for Mad River had a stress value of 0.144, with eelgrass percent cover, eelgrass shoot count, %TOC, clay content, and elevation relative to MLLW significantly correlated to the ordination.

#### Winter NMDS Results

Similar to the summer NMDS plots, winter NMDS plots showed substantial overlap in eelgrass both with and without aquaculture, but mudflat habitat without aquaculture was typically distinct from the other habitat types. The plots for Bird Island showed some overlap between all habitat types and eelgrass with aquaculture, but the communities that were most similar included eelgrass with and without aquaculture. The three-dimensional ordination had a stress value of 0.150, with eelgrass percent cover, eelgrass shoot count, and elevation relative to MLLW significantly correlated to the ordination.

In East Bay, the winter NMDS plots all overlapped with each other except for mudflat habitat without aquaculture. 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. The East Bay ordination had a stress value of 0.140 with three dimensions, and no environmental variables to be significantly correlated to the ordination.

In the Mad River region, the pattern was similar to that reported for other areas. Eelgrass with and without aquaculture was the most similar to each other, mudflat habitat with aquaculture



was also similar, but mudflat habitat without aquaculture was dissimilar to the other habitat types. Three-dimensional ordination of this region had a stress value of 0.140, with eelgrass shoot count, eelgrass percent cover, and elevation relative to MLLW significantly correlated to the ordination.

#### 3.2.4 Gradient Forest Analysis

The final statistical analysis used for the invertebrate communities was the gradient forest analysis. This provides information on the community response to physical and chemical habitat structure (e.g., eelgrass shoot density, sediment grain size, %TOC). The details of this analysis are provided in Coe (2019), but the end result was similar to the information presented above. Presence of eelgrass and elevation was, by far, the most substantial drivers for invertebrate community composition. Other studies (e.g., Gilkerson 2008) have also reported that elevation is a consistent predictor of eelgrass presence in Humboldt Bay. An example of how this results in an invertebrate community shift was identified through the gradient forest analysis. For example, Caprellidae is an indicator taxa on Bird Island, and is also an eelgrass-associated species. There is a population shift at around -0.2 m MLLW where eelgrass becomes less abundant at higher elevations and Caprellidae also become less abundant.

Overall, the invertebrate communities responded strongly to presence of eelgrass and elevation. All other environmental factors (e.g., %TOC, sediment grain size) were either also driven by eelgrass and elevation, or the communities only responded mildly to these factors.

#### 3.3 Fish and Mobile Macroinvertebrate Communities

Field data collection under this project includes 16 enclosure net deployments, 4 fyke net deployments and 12 unbaited minnow trap deployments. This data was combined with 42 prior fyke deployments described by Pinnix et al (2005) to evaluate the fish populations associations by habitat type (eelgrass vs. mudflat and oyster aquaculture vs. no oyster aquaculture). These 74 fish sampling events include 26 samples in eelgrass without aquaculture, 15 samples in eelgrass with aquaculture, 18 samples in mudflat without aquaculture and 15 samples in mudflat with aquaculture.

Across the research efforts to characterize fish populations in mudflat and eelgrass habitats with and without eelgrass in Humboldt Bay, a total of 5231 fish representing 21 species were identified. A total of 6 species (whitebait smelt, Pacific sardine, speckled sanddab, California halibut, and three-spined stickleback) were only detected in eelgrass habitats while all 15 species identified in mudflat habitats also occurred in eelgrass habitats (Table A-10).



Table A-10.	<b>Fish Species</b>	Collected by	Sampling	Method
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Fish S	pecies	Enclosure		e Net		now
Scientific Name	Common Name	Net	SK Grant	Pinnix		aps
Eelgrass Habitat		w/ w/o	w/ w/o	w/ w/o	w/	w/o
Atherinops affinis	Topsmelt	• •	• •	• •		
Hypomesus pretiosus	Surf smelt			•		•
Allosmerus elongatus	Whitebait smelt			• •		
Porichthys notartus	Plainfin midshipman	• •				
Clupea pallasii	Pacific herring	• •		•		
Sardinops sagax	Pacific sardine			•		
Engraulis mordax	Northern anchovy	• •	• •	• •		
Leptocottus armoratus	Staghorn sculpin	• •	•	• •	•	•
Cymatogaster aggregata	Shiner surfperch	• •	• •	• •	•	•
Hyperprosopon argenteum	Walleye surfperch	• •	• •	• •		
Hyperprosopon ellipticum	Silver surfperch	• •				•
Phanerodon furcatus	White surfperch	•	•	• •		
Rhacochilus vacca	Pile surfperch	•		• •		
Lepidogobius lepidus	Bay goby	• •	•			
Pholis ornate	Saddleback gunnel	• •			•	
Syngnathus leptorhynchus	Bay pipefish		•	• •	•	•
Pleuronectes vetulus	English sole	• •			•	•
Citharichthys stigmaeus	Speckled sanddab			•	-	
Sebastes caurinus	Copper rockfish					
Paralichthys californicus	California halibut	•				
Gasterosteus aculeatus	Three-spined stickleback			• •		
Mudflat Habitat		<u>i</u>	1			1
Atherinops affinis	Topsmelt	• •				
Hypomesus pretiosus	Surf smelt					•
Porichthys notartus	Plainfin midshipman	•				
Clupea pallasii	Pacific herring	•				
Engraulis mordax	Northern anchovy	•				
Leptocottus armoratus	Staghorn sculpin	• •			•	•
Cymatogaster aggregata	Shiner surfperch	• •				
Hyperprosopon argenteum	Walleye surfperch	• •		by SK Grant;		
Hyperprosopon ellipticum	Silver surfperch	• •	no compa	rison made		
Phanerodon furcatus	White surfperch	•				
Lepidogobius lepidus	Bay goby	• •				•
Pholis ornate	Saddleback gunnel	• •			•	
Syngnathus leptorhynchus	Bay pipefish	•			•	•
Pleuronectes vetulus	English sole	• •			•	
Sebastes caurinus	Copper rockfish	•				
w/ = with oyster culture; w/o = without		<b>I</b>				<u>.</u>



Each fish sampling method has limitations that affect the enumeration of species. By combining the observations from multiple sampling methods, the limitations of each individual method can be identified and improve the overall understanding of fish populations. For example, both minnow traps and enclosure nets detected English sole, while fyke nets did not. Plainfin midshipman and bay goby were detected only by enclosure nets. Characteristics of each of these species likely explain why they were detected by some sampling methods and not others.

#### 3.3.1 Taxa Abundance by Habitat Type

Fish captures were highly variable between sampling efforts with catches ranging from 0 in some fyke net captures to 836 with an average catch of 70.7 fish per sample. A total of eleven sampling events resulted in no fish captures. These were primarily fyke net deployments (10), and 1 winter deployment of minnow traps. On three occasions all three methods were deployed simultaneously. During these deployments the enclosure catches slightly exceeded and were similar to the catch for the fyke net, while minnow traps appear to capture slightly fewer individuals.

Sampling by habitat type suggests that catches in eelgrass habitats exceed mudflat habitats, and that the presence of aquaculture gear is associated with greater catches of fish (Figure A-13). Although pairwise comparisons of averages and standard errors suggest that the presence of aquaculture may be associated with higher catch, a one-way ANOVA for these sampling results does not suggest significant differences. However, a two-sample t-test comparing habitats with and without oyster culture suggests that the presence of oyster culture is associated with significantly more fish (p<0.01).



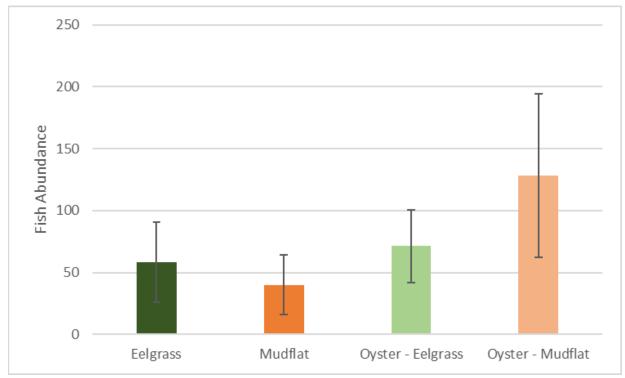


Figure A-13. Fish Abundance for all sampling efforts across all habitat types.



Evaluating catch by functional feeding group shows differences in the composition of catches by habitat type with catches in eelgrass areas being dominated by forage fish – smelt, anchovy and herring (Figure A-14).

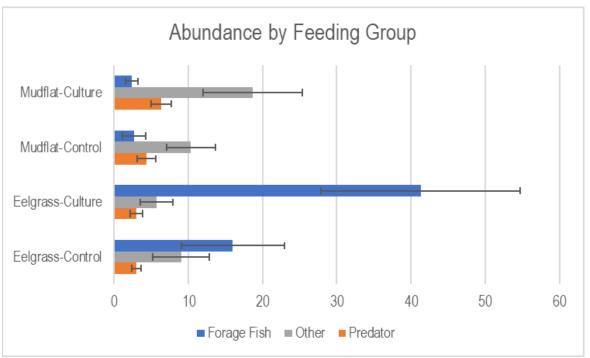


Figure A-14. Fish Abundance by Functional Feeding Group (Enclosure Nets).

The most abundant fish species in each habitat category are shown in table A-11. While Pacific herring and topsmelt are the most abundant species detected in eelgrass habitats, bottom associated species such as English sole, bay goby and staghorn sculpin are most abundant in mudflat areas. Surfperch were detected at similar abundances in both eelgrass and mudflat habitats. Differences in species associations with and without culture are not significant.



Estandar	Summer				
Estuarine Habitat	w/ Aquaculture		w/o Aquacu	ılture	
Habitat	Таха	Count	Таха	Avg. Count	
Eelgrass	Pacific herring ( <i>Clupea pallasii</i> )	30	Pacific herring ( <i>Clupea pallasii</i> )	13	
	Topsmelt (Atherinops affinis)	10	Silver surfperch (Hyperprosopon ellipticum)	4	
	Silver surfperch (Hyperprosopon ellipticum)	7	Walleye surfperch (Hyperprosopon argenteum)	3	
	Shiner surfperch ( <i>Cymatogaster</i> <i>aggregata</i> )	8	Shiner surfperch (Cymatogaster aggregata)	5	
	SIlver surfperch (Hyperprosopon ellipticum)	5	English sole (Pleuronectes vetulus)	5	
	Bay goby (Lepidogobius Lepidus)	4	Bay goby		
	Staghorn sculpin (Leptocottus armoratus)	4	<ul> <li>(Lepidogobius Lepidus)</li> </ul>	4	

Table A-11. Mos	st abundant taxa	identified in	n each habitat
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#### 3.3.2 Fish Abundance by Season

The 2017 and 2018 sampling includes sampling in 3 seasonal periods – Spring, Summer and Winter. Including earlier sampling effort, fish abundance was assessed in March, April, May, June, August and December. As described in Section 3.3.1, forage fish comprise a significant portion of the overall catches in Humboldt Bay. Forage fish captured were primarily larval stage fish. Catches of forage fish appear to vary seasonally, with individual catches comprising large fractions of the total catch for some species. This is illustrated in Pinnix et al. (2005) catch data where a single large catch of topsmelt accounts for nearly all the catch of that species. The 2017/18 field research found more consistent catches of topsmelt and Pacific herring. However, the 2017/18 field data captured far fewer northern anchovy than the earlier field effort.

In evaluating catch rates, it is evident that winter catches were much lower than summer catches. Pairwise t-tests comparing June and December catch rates show this difference is significant (P<0.01) (Figure A-15).



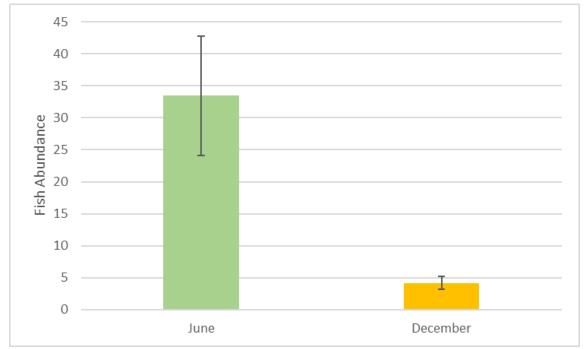


Figure A-15. Fish Abundance by sampling date (2017 and 2018 sampling)

#### 4.0 DISCUSSION

The goal of the Comparative Habitat Project was to determine whether oyster aquaculture alters invertebrate and fish assemblages or productivity of habitats where oysters are grown commercially in Humboldt Bay. The type of oyster aquaculture that was studied during the 2017-2018 field sampling effort was cultch-on-longlines spaced approximately 0.8 meters apart. Some of the culture plots included 1.5 meter gaps between longlines. The field sampling was set up to compare biological communities within two habitat pairs: (1) native eelgrass with and without oyster aquaculture, and (2) unvegetated mudflats with and without oyster aquaculture.

Two research questions related to the response of the biological communities in Humboldt Bay to oyster aquaculture were addressed during the 2017-2018 field sampling effort. Overall, field data indicated that presence of eelgrass and tidal elevation are the two most important factors in defining biological communities. Eelgrass provides structured habitat and is itself controlled by elevation in Humboldt Bay. Oyster aquaculture longlines also provide structured habitat, but in a different way compared to eelgrass. The following discussion compares the data collected during the 2017-2018 field sampling effort with existing data from Humboldt Bay and the West Coast. One of the main themes discussed throughout the literature is the role that habitat complexity and habitat type play in defining biological communities.



# 4.1 Does oyster culture alter invertebrate communities (prey resources) in Humboldt Bay?

Invertebrate community structure responds to structured habitat and complexity. Longline oyster aquaculture is considered a type of structured habitat, and the communities that form within this habitat are similar to eelgrass habitat. The two main lines of evidence to illustrate this are from the indicator taxa analysis and NMDS ordination plots:

- Indicator Taxa Analysis:
  - Eelgrass with and without aquaculture contained nearly the same taxa that defined the community structure, regardless of season or geographical region.
  - Mudflats with and without aquaculture did not contain the same taxa in the summer, but was more closely aligned in the winter.
  - Mudflats with aquaculture were more similar to eelgrass habitats (both with and without aquaculture) compared to mudflats without aquaculture.
- NMDS Ordination Plots:
  - Eelgrass with and without aquaculture consistently overlapped with each other (i.e., were similar in terms of invertebrate community structure) both within the two seasons compared and the geographical regions of North Bay.
  - Mudflats with and without aquaculture did not overlap consistently with each other, and often mudflats without aquaculture did not overlap with any other habitat type.
  - Mudflats with aquaculture were more similar to eelgrass habitats (both with and without aquaculture) compared to mudflats without aquaculture.

These patterns are consistent with the existing scientific literature related to aquaculture and invertebrate populations. Hosack et al. (2006) reported that benthic invertebrates were strongly associated with habitat type, and structured habitats (oyster beds and eelgrass) had higher species abundance than other habitat types. Earlier work by Hosack (2003) reported that important fish prey organisms, such as harpacticoid copepods, exhibited higher densities in both eelgrass and oyster habitats compared to sand or mudflats. Hudson et al. (2018) reported that harpacticoid copepods were a dominant part of the epibenthic community in Humboldt Bay, but their abundance did not drive invertebrate community patterns as it did in other West Coast estuaries (e.g., Willapa Bay [WA], Tillamook Bay [OR]). The Hudson et al. (2018) study reported few differences between oyster aquaculture and eelgrass habitats for epibenthic invertebrates in Humboldt Bay. The data appears to support a conclusion that oyster longlines in eelgrass do not substantially change the invertebrate communities, but oyster longlines in mudflats alter the communities to be more similar to eelgrass habitat.

One of the most comparable work to the 2017-2018 field study conducted in Humboldt Bay was the work by Rumrill and Poulton (2004). Results of the Rumrill and Poulton (2004) study showed that invertebrate biomass was highest in the oyster longline plots and lowest in some of the eelgrass reference sites. However, the composition of the invertebrate communities was not



significantly different between oyster longlines and eelgrass control plot. This is in agreement with the results of the 2017-2018 field sampling effort, namely that oyster longline aquaculture in eelgrass habitat does not significantly change the invertebrate communities compared to eelgrass habitat. The Rumrill and Poulton (2004) did not compare invertebrate communities within mudflat habitats.

While the literature agrees with the result from the 2017-2018 field sampling that oyster aquaculture and eelgrass result in similar invertebrate communities, a recent presentation by Rumrill (2017) indicated that there may be tradeoffs because of the structure of the habitat. Oysters are a secondary producer, compared to eelgrass, which is a primary producer. While nutrients and organic material is created both by oysters (e.g., biodeposition<sup>4</sup>) and eelgrass (e.g., decomposition of shoots) in the sediment, an overabundance of organic material can create impacts within a local area. Indicator taxa and functional feeding groups can be used to determine if there are community shifts due to the presence of oyster aquaculture. In general, deposit feeders (e.g., Caprellidae, Ampharetidae, Oligochaeta) were the main functional feeding group identified throughout North Bay. This is likely due to the abundance of organic material available. However, there was no indicator taxa shifts or specific taxa that was strongly correlated with %TOC. While suspension feeders (e.g., bivalves, ostracods) were common in eelgrass (both with and without aquaculture) and in mudflats without aquaculture, they were not as dominant within mudflats with aquaculture. This could be a result of competition with the cultured species (a bivalve) or a lack of structured habitat that provided no refuge from predators within open mudflat habitat.

Overall, habitat complexity appears to support a similar suite of invertebrates when eelgrass is present, and increases diversity and structure-associated taxa when oyster longlines are present. While this is an alteration of the invertebrate community for at least the mudflat habitat, there is also the potential that additional prey resources are provided with this added structure.

# 4.2 Does oyster culture alter fish and/or macroinvertebrate communities in Humboldt Bay?

Sampling suggests the potential for increased overall abundance of fish in aquaculture areas compared to like estuarine habitats without aquaculture gear present. This difference is likely primarily associated with larval stages of several species of forage fish which appear to be associated with aquaculture gear. There are multiple potential causes for this association including:

• Fish may interact with aquaculture gear in-water habitat

<sup>4</sup> Biodeposition = The process of transferring organic matter containing materials from the water column to the substrate as wastes from bivalve consumption.



- Aquaculture gear may create flow refuge by reducing currents thereby allowing larval stage fish to take refuge in areas where gear is present
- Aquaculture gear may provide in-water forage opportunities

Other species did not appear to have a positive or negative association with aquaculture gear suggesting that effects of aquaculture gear are likely limited to certain groups or guilds of fish species.

These findings add to the tentative evidence provided by Pinnix et al (2005) that aquaculture gear in a longline or basket configuration may have limited or not effect on fish abundance. Hudson et al. (2018) had similar findings for transects between aquaculture and adjacent habitats. However, results presented in Hudson et al. (2018), suggest that some fish and macroinvertebrate abundance and distribution patterns may differ between coastal estuaries complicating efforts to generalize observed patterns in one area to others.

The following criteria were used to evaluate the Project:

Criteria #1: Comparison of the exclusion fyke net combination to data from standard deployment of a fyke net (e.g., Pinnix et al. 2005).

Sampling indicates that a similar suite of species was detected with the fyke sampling in 2017 compared to the 2004 and 2005 sampling by Pinnix et al. (2005). The number of individuals collected in 2017 sampling events is also comparable to the 2004/05 sampling events. However, total numbers of fish captured per sampling event are not evenly distributed, particularly in Pinnix et al. sample effort. The 2017/18 sampling events are all paired events and sampling shows similar catch rates at both sample pairs, whereas Pinnix et al. (2005) sampling shows very high catch rates during a series of sampling events between August 1 and 3, 2005 that comprise 97% of the catch in just 35% of the sample effort. This illustrates both the importance of sample timing and potential for patchy distribution of lack of sample pairing to affect measurement of fish abundance. Sampling with the enclosure and minnow traps show that winter abundances are much lower in Humboldt Bay compared to spring or summer abundances. This appears to be the only study that has attempted to sample winter fish abundances in Humboldt Bay.

Criteria #2: Comparison of previous data conducted in Humboldt Bay or other West Coast estuaries.

Patterns found in the current study are consistent with the observations presented in Hudson et al. 2018 and Pinnix et al. 2005 which also found tentative support for the hypothesis that fish abundance is either unaffected by the presence of aquaculture gear or has a positive relationship in Humboldt Bay. This study is consistent with the findings of Gross et al (2019) that nekton abundance appears to respond to in-water structure.



Criteria #3: Identification of the role that habitat complexity and type plays in trophic interactions and food web productivity.

This study reinforces the importance of habitat structure provided by either eelgrass or oyster aquaculture in influencing the abundance of fish. However, the underlying habitat type – either mudflat or eelgrass – appears to play an important role in structuring invertebrate and fish populations. Aquaculture does not appear to significantly affect the diversity or abundance of invertebrates.

This study does identify potentially important seasonal components to ecosystem structure, species richness and abundance. While invertebrate and fishery abundance declines in winter months, invertebrate species richness appears to increase.

#### 5.0 RECOMMENDATIONS

The current study provides support for the hypothesis that the presence of aquaculture gear in estuarine habitats has no effect on invertebrate abundances. In addition, it provides tentative support for the hypothesis that aquaculture gear is associated with increases in fish abundances when habitat type is held constant. These research findings address concerns enunciated by the public and regulators regarding the impacts of aquaculture activities on ecosystem function in Humboldt Bay.

However, field observations suggest that eelgrass densities, a frequent proxy for biomass, are currently lower in aquaculture areas in Humboldt Bay compared to adjacent areas. Findings regarding eelgrass do not demonstrate causation because of difficulties isolating the effect of current aquaculture activities from the history of aquaculture development in Humboldt Bay. Historical habitat alterations, including placement of fill and dredge harvesting, likely contribute to differences in eelgrass densities, and ongoing research associated with Coast Seafoods activities is designed to address questions about effects to eelgrass using a before-after control impact (BACI) research design (Merkel & Associates 2018).

Future research to better understand the potential relationship between aquaculture and forage fish is needed due to the relatively short sampling opportunities when larval forage fish are present in Humboldt Bay

The following section includes recommendations for management and future work.

#### 5.1 Management

The current research project was developed as a partial response to scientific uncertainty regarding the potential response of invertebrate and fishery populations to the presence of aquaculture gear in Humboldt Bay. This study illustrates that these resources appear to primarily respond to the underlying estuarine habitat and tidal elevation with limited, if any,



response to the presence of aquaculture gear where those underlying habitats are maintained. This study is narrow in its application as it is specific to the habitats and aquaculture methods in Humboldt Bay.

#### 5.2 Future Work

Interactions of aquaculture gear with Pacific herring spawning has been identified as a topic of interest by CDFW, however this study indicates that larval forage fish of multiple species may be positively associated with aquaculture gear. Further evaluation of this relationship would require intensive sampling during periods when these stages of forage fish are present in Humboldt Bay.

Further evaluation of fishery use of aquaculture habitats may be focused on understanding how these habitats are used including evaluations of prey consumption, sources of prey items, and movement patterns of fish.

Ongoing research-based monitoring being led by Merkel & Associates (2018) as part of permit requirements for Coast Seafoods will enhance understanding of the effect of aquaculture development to eelgrass in Humboldt Bay. Additional research focused on use of eelgrass and habitat areas by brant is being led by H.T. Harvey Associates (2018) and will enhance the understanding of ecosystem interactions with aquaculture.



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# Appendix B Ecosystem Modeling Workshop

### Appendix B HUMBOLDT BAY FOOD WEB WORKSHOP – SUMMARY REPORT

Prepared for:

National Marine Fisheries Service Saltonstall-Kennedy Program 1315 East-West Highway, Room13358 Silver Spring, MD 20910 Attn: Daniel Namur

Authored by:

Confluence Environmental Company

November 2019



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#### 1.0 INTRODUCTION

This appendix describes the Humboldt Bay food web workshop that supported the "Comparative Habitat Use of Estuarine Habitats with and without Oyster Aquaculture Project" (the Comparative Habitat Project). The goal of the Comparative Habitat Project was to determine whether oyster aquaculture alters invertebrate and fish assemblages or productivity of habitats where oysters are grown commercially in Humboldt Bay. The research was set up to compare biological communities within two habitat pairs: (1) native eelgrass (*Zostera marina*) with and without oyster aquaculture, and (2) unvegetated mudflats with and without oyster aquaculture.

Three research objectives were identified in the Saltonstall-Kennedy Competitive Research Program Grant proposal (SK Grant Number NA16NMF4270254):

- Does oyster culture alter invertebrate communities (prey resources) in Humboldt Bay?
- Does oyster culture alter fish and/or macroinvertebrate communities in Humboldt Bay?
- Does oyster culture alter the food web in Humboldt Bay?

The workshop addressed the third research objective of the SK Grant proposal. The stated goal of the workshop itself was to explore the interest in and data available for a Humboldt Bay ecosystem model. This involved engaging a wide array of stakeholders and educating participants on the Ecopath with Ecosim (EwE) framework. While the goal of the project was initially to develop a preliminary EwE model to be applied to Humboldt Bay, workshop leaders determined that there was insufficient data prior to the workshop to support a meaningful model. However, there is potential to organize and/or collect sufficient data to support a model, which is what led to the workshop approach.

Notes from the workshop are attached at the end of this appendix.

#### 2.0 WORKSHOP PARTICIPANTS

The workshop brought together a diverse array of stakeholders and experts within the Arcata and Eureka area to understand the state of data available for Humboldt Bay that could contribute toward an EwE model (Table B-1). Unfortunately, the workshop occurred during a hiatus in federal funding, which prevented several federal employees from the National Oceanic and Atmospheric Administration (NOAA) and U.S. Fish and Wildlife Service (USFWS) from being able to participate. A few federal employees were able to participate if budget was already approved for their attendance.

Participant	Entity	Presenter?
Adam Canter	Wiyot Tribe	
Andre Buchheister	Humboldt State University (HSU)	Y
Bobbi Hudson	Pacific Shellfish Institute	Y
Brendan Leigh	U.S. Fish and Wildlife Service (USFWS)	
Brett R. Dumbauld	U.S. Department of Agriculture	
Catalina Cuellar-Gempeler	HSU	
Catherine Peterson	Self	
Diane Ashton	Self, National Oceanic and Atmospheric Administration (NOAA), retired	
Eddie Koch	Wiyot Tribe	
Eric P Bjorkstedt	NOAA	
Frank Shaughnessy	HSU	
Gary Fleener	Hog Island Oyster Company	
Greg Dale	Coast Seafoods Company/Pacific Seafood	
Gretchen O'Brien	SHN Environmental	
Hannah Coe	HSU	Y
Jeff Smith	Harvey Ecological Associates	
Jennifer Kalt	Humboldt Baykeeper Association	
Jeremy Svehla	GHD Civil Engineering	
Joe Tyburczy	California Sea Grant	
Juan Avellaneda	Hog Island Oyster Company	
Kasey Sirkin	U.S. Army Corps of Engineers	
Kelly Muething	Confluence Environmental Company	Y
Kirsten Ramey	California Department of Fish and Wildlife	
LeAnne Sprague	Self	
Lisa Savage	City of Eureka	
Lucas Sawyer	Hog Island Oyster Company	
Mark Henderson	HSU	
Matt Goldsworthy	NOAA	
Mike Wilson	Humboldt County Commissioner	
Phil Bloch	Confluence Environmental Company	Y
Rafael Cuevas Uribe	HSU	
Scott Sterner	North Bay Shellfish	
Stephanie Schneider	H.T. Harvey & Associates	
Su Corbaley	California State Coastal Conservancy	
Ted Romo	Self	
Thomas Gast	Thomas Gast and Associates	
Tim Nelson	Wiyot Tribe	
Vanessa Blodgett	Plan West Partners	
Whelan Gilkerson	Merkel and Associates	
William Pinnix	USFWS	



#### 3.0 AGENDA

An agenda was distributed in advance of the meeting and used to guide discussions and presentation throughout the workshop (Table B-2).

Table B-2	Humboldt Bay	Food Web	Workshop	chaon A
I able D-Z.	Humboldt Bay	у гоой web	workshop	Ayenua

	Time/Topic	Discussion Points	Presenter
9:00	Introduction to Workshop	Objectives of workshop & expected outcomes	Phil Bloch
9:30	Workshop attendee introductions	Name, organization, interest	Phil Bloch
10:00	Ecosystem Modelling using Ecopath with	Justification/motivation of approach	Phil Bloch
	Ecosim (EwE)	Motivation behind Ecosystem modeling Modeling framework Overview of past model applications South Puget Sound Case Study	Dr. Andre Buchheister Bobbi
11:30	Recent Field Studies	Habitat Use of Habitats with and Without Oyster Culture	Hudson Hannah Coe
		Quantification of relationship between shellfish culture and seagrass	Bobbi Hudson and Kelly Muething
1:00	Using Ecosystem Models to Evaluate Potential Uses and Pressures	Discussion Break-out groups Uses/Pressures Management Concern Future Scenario Ecological/Food Web Linkage	Andre Buchheister and Phil Bloch
2:00	Ecopath with Ecosim data needs	Model needs	Andre Buchheister
2:30	Existing and desired data to inform model	Available data Primary Production Fisheries Birds and Marine Mammals Invertebrates	Phil Bloch Breakout Groups
3:30	Conclusion / Wrap-up		All

#### 4.0 OVERVIEW OF ECOSYSTEM MODELING

Dr. Andre Buchheister, assistant professor in the Department of Fisheries Biology at HSU, acted as the subject expert and provided information on the basics of ecosystem modeling. Dr. Buchheister specializes in research integrating ecological modeling, advanced statistics, field research, and laboratory methods to address applied fisheries science questions. A major research focus area is creating scientific model tools to support ecosystem-based fisheries management by developing ecosystem modeling tools that characterize the structure, function, and drivers of fish communities and marine ecosystems. Dr. Buchheister received his graduate training in Marine Science at the College of William & Mary and led development of an EwE model to assist in management of Atlantic menhaden (*Brevoortia tyrannus*).



Dr. Buchheister's presentation covered the general goals of ecosystem modeling and explained that an ecosystem model is a quantitative representation and simplification of an ecosystem. Ecosystem modeling can help to inform natural resource management by highlighting the connections between different resources rather than approaching management from the perspective of individual resources. He underscored that ecosystem models focus on how biomass flows among biotic groups and harvest. Thus, the emphasis is on the biological and ecological metrics of the system, not the physical parameters.

#### 4.1 Motivations for Ecosystem Modeling

Ecosystem models have a wide range of applications, depending on the interests of the developers and end users. Generally, they can help evaluate trade-offs, identify knowledge and data gaps, and develop indicators of system success or failure. Ecosystem models are a tool to view the complexities of an ecosystem and should not be seen as the ultimate answer to management questions.

#### 4.2 EwE Basics

EwE is a publicly available, open-source software that is commonly used in the development of ecosystem models. There are three primary components of EwE: Ecopath, Ecosim, and Ecospace. In this workshop, the focus was on the first two components. Ecopath is the biomass accounting tool for the system. Production and consumption equations define relationships between trophic groups within the ecosystem. Ecosim is the time component of the model, allowing the system, and the inherent trophic relationships, to change through time. This component is especially useful for policy and management exploration because it allows for different scenarios to be evaluated into the future.

Outputs of the Ecopath component of an EwE model include biomass, production, mortality, and consumption values that help to identify "keystoneness" (a quantitative metric for "keystone" species), connectivity, and mixed trophic impacts, among other metrics. Possible outputs of Ecosim include reference points for maximum sustainable yields, fishing rate simulations, or exploration of the effects of uncertainty within the model. EwE models have many benefits, including the ability to model entire ecosystems and integrate a large amount of data, but they can be time intensive and can be data limited for specific groups. Overall, these models should be viewed as tools to inform management decisions, and not as definitive answers to ecological questions.

#### 4.3 Case Studies and Examples

Examples of EwE applications were provided at various scales, starting with the largest scale and narrowing to a small-scale example, with relevance to the proposed application in Humboldt Bay. The Alaska CLIMate Project is a broad, collaborative project that seeks to



integrate and synthesize a variety of models, including an EwE model. Summaries were presented for each model implementation to illustrate the scale and range of analytical applications, including:

- Alaska CLIMate Project (Dr. Andre Buchheister)
- Atlantic Menhaden EwE model (Dr. Andre Buchheister)
- Role of eelgrass in central Puget Sound (Dr. Andre Buchheister)
- Shellfish aquaculture in south Puget Sound (Bobbi Hudson)

#### 4.4 Recent Research in Humboldt Bay

A description of the sampling effort associated with the Comparative Habitat Project was provided, including methods and preliminary results (see main document for more information). Other research that has been occurring in the bay that was described included black brant (*Branta bernicla nigricans*) and eelgrass monitoring associated with Coast Seafoods permitting efforts and eelgrass carbonate chemistry work at HSU. Brant monitoring began in 2018 and is done using trail cameras. A stratified random sample of pictures was selected out and used to compare abundance between control and aquaculture plots. Initial results suggest that black brant move away from aquaculture gear when it is mostly exposed, but are more abundant in aquaculture gear when there is water covering the area. Eelgrass monitoring has been completed using drone photography and subsequent spectral based analysis to identify eelgrass presence. Additional ground-truthing has occurred in order to work towards a relationship between pixel value and turion density. Finally, research by Dr. Frank Shaunessey and Dr. Joe Tyburczy has focused on the potential for eelgrass to change the carbonate chemistry in the bay and the effect that it could have on calcifying organisms.

#### 4.5 Discussion: Management Concerns and Scenarios

Ecosystem models are developed to address specific management interests or concerns. The project team worked with breakout groups of workshop participants to identify management concerns of interest, future scenarios that the participants had an interest in modeling, and ecological outputs or metrics that they would like to see identified (Table B-3).



Table B-3. Humboldt Bay Management Concerns, Scenarios, and Ecological Metrics Identified by Workshop Participants

Management Concern	Scenario(s)	Ecological Metric(s)
Species of special concern – longfin	Increase or decrease in habitat area	Zooplankton
smelt	Reduction in bycatch (especially pink	Predator fish
	shrimp)	Birds
Ocean Acidification (OA)	Increase or decrease in OA levels	Primary Productivity (Plant communities
	Potential to mitigate with seaweed culture	Changes in demoic acid
	Status quo modeled pH projections	Change in organisms that calcify
		Reduced settlement of calcifiers
		Change in primary producers/production
		Phytoplankton composition
		Eelgrass effects
	Feely acidification estimates	Top trophic level effects
		Dungeness Crab abundance
		Juvenile Rockfish abundance
		Oystercatcher abundance
		Recreational shellfishing effort
Olympia Oysters	Change in substrate availability	Habitat value
Brant Population	Changes in forage availability and quality	Change in eelgrass area
	Hunter pressure	Other waterbird abundance
	· · · · · · · · · · · · · · · · · · ·	(competition)
	Aquaculture area	Competitive interactions with other
	Boat Disturbance	waterfowl
Storrmwater runoff/Water Contamination	Watershed development	Change in sedimentation rates
Climate Change – Temperature		Disease
increase (also increased fluctuations	Projected temperature change scenarios	Effects to eelgrass decomposition
and variability)		Changes to nutrient cycling
Recreation Activities (Kayaking, fishing,	Change in recreation activity	Mudflat/benthic disturbances
hunting)	Increased California halibut fishing	Pollution
	increased california halibut fishing	Change in primary production
Oyster production		Potential 'carrying capacity' for oyster
		production in Humboldt Bay (change in
		phytoplankton)
		Change in fish diversity
	Change in oyster lease locations/footprint	Change in eelgrass area or biomass
		Change in fish abundance
		Change in bird use
		Change in nutrient deposition
		Change in invertebrate communities
Introduced non-native species	Introduced non-native species	Introduced non-native species
Sea Level Rise	Changes to land use footprints	Change in habitat types and areas

Several workgroups identified common management concerns for Humboldt Bay, including:

- Ocean acidification
- Climate change/sea level rise
- Development/dredging/shipping
- Oyster culture



Scenarios of interest focused on using predicted ranges of climate change, ocean acidification, or sea level rise to evaluate outcomes. There were also interests in evaluating a range of development scenarios for both oyster aquaculture in the bay and commercial or residential development along Humboldt Bay.

Most groups identified potential effects to primary production, both eelgrass and phytoplankton, as ecological metrics of interest that would affect higher trophic levels. Ocean acidification was recognized as a mechanism that could affect numerous trophic levels and a diverse array of ecological measures.

#### 5.0 DATA AVAILABILITY AND NEEDS

Ecosystem models are data intensive, and the outputs are dependent on the quality of the input data. Therefore, there is an interest in developing a sufficient base of ecological relevant data before attempting to implement an ecosystem model to avoid creating misleading outputs.

#### 5.1 EwE Data Needs

In order to satisfy the needs of the master equations governing the Ecopath part of the EwE model, information about the production and consumption of biological groups is needed. Primarily, the goal is to define different trophic groups within an ecosystem and understand how they relate quantitatively. Information from stock assessments, surveys, and empirical methods can be used to determine the core data needs. These include biomass, production to biomass ratio, consumption to biomass ratio, and ecotrophic efficiency. This last parameter is the proportion of production used within the system and is typically estimated. Assumptions about average individual biomass are often required to convert available count data (e.g., stock assessment results) to biomass of the group or species. Additional data about diet composition and annual catch can be obtained from the literature or landings records, respectively. Physical data (e.g., water chemistry, sediment flux, etc.) are not included directly in this model, but are indirectly represented through responses by biological entities.

#### 5.2 Discussion: Available Data

The following is a summary of the break-out groups (i.e., primary production, fisheries, invertebrates, birds and marine mammals, and water quality) during the session on "existing and desired data to inform model." The participants included several researchers and academic advisors that have overseen or participated in data collection efforts within Humboldt Bay. It was identified that, while there is count data for several species and at several time periods, there is limited information regarding biomass in Humboldt Bay.

The result of these discussions culminated into developing a spreadsheet that provided a description of the various datasets and links to the data. This is a living document that is located



at the following link: <u>https://docs.google.com/spreadsheets/d/1-</u> <u>TfFqvtNUnKsr\_ZP0sECoQ8YVmrZ0RORR1V7Vu3O\_yA/edit#gid=1192032262</u>

#### 5.3 Discussion: Data Gaps

Several data gaps were identified by the workshop participants. The following is a brief list of the data gaps by break-out groups:

- Primary Production: Primary production by eelgrass and submerged aquatic vegetation (SAV) tends to focus on two metrics – cover and for eelgrass plant density. This creates the following challenges or data gaps:
  - It is challenging to convert shoot density to biomass.
  - There is a need for rates of primary production, residence time, and *in situ* primary production.
  - There is a lack of benthic macroalgae data for most habitats in Humboldt Bay.
  - There is a lack of understand below ground biomass for eelgrass.
- **Fisheries:** In general, there is a desire to have more information on biomass for species common within the bay, and how much each species is relying different food resources (e.g., diet data). This creates the following challenges or data gaps:
  - There is a lack of rates of consumption for various food resources by fish, although this could be inferred from the literature.
  - It would be beneficial to have multiple seasons of very frequent, continuous monitoring to detect and quantify habitat usage. Spring and summer are probably the most important.
- Birds & Mammals: In general, there is a lack of understanding migration patterns of birds, number of individuals (aside from snapshots), and food habits. This creates the following challenges or data gaps:
  - There is a lack of understanding migration patterns that are influenced by wind, and general use by nonmigratory birds.
  - There are only snapshots of number of individuals moving through the area.
  - There is a lack of understanding how long birds stay and how healthy they are.
  - There is a lack of data for food habits of birds, aside from some work by Colwell.
  - There needs to be more data associated with black brant use of eelgrass and on culture plots.
- Invertebrates: There is an overall need for more information on biomass of species (e.g., clams, crabs, mussels, scallops, moon snails, nudibranchs, fouling communities, etc.).
- Water Quality and Physical Data: There is an overall need to obtain chemistry and physics of climate change, sea level rise and ocean acidification, more certain projections of ocean acidification, rate of SLR, rate of temp increase (atmosphere, upper and lower ocean), change in upwelling seasonality and strength (jet stream fluctuations, increasing



wind strength vs. deepening thermocline), and change in fog occurrence. This creates the following challenges or data gaps:

- This data may for sea level rise may result in changes to habitat for species. For example, increasing depth may benefit oysters, harm eelgrass and other changes may harm these species.
- A more realistic approach is probably to consult current research and explore high/mid/low estimates for each of these water quality and physical data needs.

#### 6.0 FUTURE DIRECTIONS

Workshop participants recognized that existing data resources to support ecological modeling are spread across multiple resource managers with little effort to create a synthesis to date. Participants recognized that synthesizing this information may provide value and support better decision-making by resource managers and a more thorough understanding of the implications of management decisions.

The workshop participants agreed that the existing data is unlikely to produce ecological model outputs that are useful for decision-makers and, therefore, encouraged that effort be invested in data development and synthesis at this stage rather than model development.

# Appendix C Stakeholder Outreach

### Appendix C OUTREACH AND EDUCATION SUMMARY

Prepared for:

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Table C-1.	Outreach and Education C	Opportunities4	ŀ



#### 1.0 INTRODUCTION

This appendix describes the outreach and education effort that supported the "Comparative Habitat Use of Estuarine Habitats with and without Oyster Aquaculture Project" (the Comparative Habitat Project). The goal of the Comparative Habitat Project was to determine whether oyster aquaculture alters invertebrate and fish assemblages or productivity of habitats where oysters are grown commercially in Humboldt Bay. The research was set up to compare biological communities within two habitat pairs: (1) native eelgrass with and without oyster aquaculture, and (2) unvegetated mudflats with and without oyster aquaculture.

Three research objectives were identified in the Saltonstall-Kennedy Competitive Research Program Grant proposal (SK Grant Number NA16NMF4270254):

- Does oyster culture alter invertebrate communities (prey resources) in Humboldt Bay?
- Does oyster culture alter fish and/or macroinvertebrate communities in Humboldt Bay?
- Does oyster culture alter the food web in Humboldt Bay?

Outreach and education focused on engaging relevant stakeholders and sharing sampling results, including formal and informal efforts in the Arcata and Eureka area in concert with Coast Seafoods/Pacific Seafood, the Humboldt Bay Harbor, Recreation, and Conservation District (the Harbor District), the Wiyot Tribe, and Humboldt State University (HSU). Representatives from each entity participated throughout the project. The Comparative Habitat Project also engaged other project partners during the outreach and education phase, including the U.S. Fish and Wildlife Service (USFWS), Pacific Shellfish Institute (PSI), and Oregon State University (OSU).

The milestones established for the outreach and education portion of the Comparative Habitat Project in the SK Grant Number NA16NMF4270254 proposal included:

- Posting a project report on a website with links from USFWS and the Harbor District.
  - **Milestone Achieved:** A project description with links to project data and reports is publicly accessible at:

https://www.confenv.com/comparative-habitat-use-with-and-without-aquaculture/

The Humboldt Bay Harbor District is providing a description of the project and link to the project website on their "Conservation Program" website and will promote availability of the report in Winter 2019/20.

- Presenting results at appropriate conferences.
  - Milestone Achieved: A total of 7 presentations and workshops at various environmental conferences or locally within the Arcata/Eureka area were provided from 2016 through 2019 (see Section 2.0). An abstract is in preparation for the



upcoming National Shellfish Association Conference which will occur March 29-April 2, 2020.

- Pursuing publication of results in an appropriate peer review journal.
  - **Milestone In-Progress:** A manuscript will be prepared in the winter 2019/20 for submission to the Journal of Shellfish Research.

In addition to the milestones above, the Comparative Habitat Project also had goal of providing research and cross-training on the environmental impacts of shellfish aquaculture by furthering the understanding of how fish and invertebrate communities are affected by the presence of cultch-on-longline oyster aquaculture. This involved providing and understanding of the effects that both fisheries and aquaculture operations have on each other and their relationship to sustainable fisheries practices to both Coast Seafoods/Pacific Seafood and the public. By providing research and cross-training on shellfish aquaculture operations, the Comparative Habitat Project addressed Priority #1 – Aquaculture – of the SK Grant Program:

Demonstrate aquaculture technologies in pilot commercial scale projects that will create jobs in fishing communities, produce healthful local seafood, revitalize working waterfronts and support traditional fishing communities. Provide training for fishermen and others in coastal fishing communities in aquaculture production methods. Document and assess socioeconomic impacts of marine aquaculture operations. Provide research on environmental impacts of aquaculture facilities.

This goal of research and cross-training was achieved in the following ways throughout the Comparative Habitat Project:

- Participation and support by Coast Seafoods/Pacific Seafood employees throughout the field effort, including vessel support, gear cleaning/moving, navigation training, and advice during the experiment setup.
- Creation of a pamphlet on the main project results to be distributed by the Harbor District, Wiyot Tribe, and Coast Seafoods/Pacific Seafood.
- Participation in an Ecopath with Ecosim (EwE) workshop to explore the interest in and data available for a Humboldt Bay ecosystem model (see Appendix B).



#### 2.0 OUTREACH AND EDUCATION OPPORTUNITIES

There were a series of both formal and information outreach and education opportunities that were provided by the Comparative Habitat Project from 2016 through 2019 (Table C-1).

Table C-1.	Outreach and	Education	Opportunities
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Presentation/Workshop Title	Primary Presenter(s)	Date	Notes				
Humboldt State University (HSU)							
General Training on Fish Identification	Marlene Meaders	April 2016	Participants: Wiyot Tribe and Confluence Goal: to cover identification strategies for common species within Humboldt Bay, and provide accuracy.				
Humboldt Bay Food Web Workshop	Phil Bloch Dr. Andre Buchheister Bobbi Hudson	January 11, 2019	Participants: HSU, Coast Seafoods/Pacific Seafood, USFWS, Wiyot Tribe, PSI, the Harbor District, CDFW, USDA, Hog Island Oyster Company, interested parties, and Confluence Goal: to explore the interest in and data available for a Humboldt Bay ecosystem model. See Appendix B for more details.				
Effects of longline oyster aquaculture on benthic invertebrate communities in Humboldt Bay, CA	Hannah Coe	May 2019	Participants: HSU Goal: to describe findings from invertebrate sampling describing associations of invertebrates by habitat type.				
Pacific Coast Shellfish Growers Associatio	Pacific Coast Shellfish Growers Association (PCSGA) Annual Meeting						
Comparative Habitat Use of Estuarine Habitats With and Without Oyster Aquaculture: Challenges, Partnerships, and Initial Lessons	Marlene Meaders	September 2017	Participants: Confluence Goal: to provide lessons learned from the field studies and initial results.				
Habitat Use of Estuarine Habitats With and Without Oyster Aquaculture: Challenges, Partnerships, and Lessons Learned	Phil Bloch	September 2018	Participants: Confluence Goal: to provide an update on the study results.				
National Shellfisheries Association (NSA)	Annual Meeting						
Comparative Habitat Use of Estuarine Habitats With and Without Oyster Aquaculture: Challenges, Partnerships, and Initial Lessons	Marlene Meaders	March 2018	Participants: Confluence Goal: to provide an update on the study results.				
Growing Oysters in the Context of Ecosystem Restoration: Challenges and Opportunities	Phil Bloch	March 2019	Participants: Confluence Goal: to use the study results as an example in how the interactions between oyster aquaculture and ecosystem restoration are affecting both commercial operations, landowners, restoration efforts, and other stakeholders.				

#### 3.0 LESSONS LEARNED

The fish sampling enclosure studies required a large amount of staff to deploy and is a viable sampling method during a limited sampling period which limits the potential to collect data. In addition, this sampling method creates temporary damage to the sampled area which precludes re-sampling the same area until the seabed recovers. The sampling effort is necessarily



constrained by tidal elevations and therefore fish captures may not be representative of fish distribution during all tidal stages. While this inquiry was extremely valuable, limited sample counts preclude statistical findings in some cases where trends are indicated.

Other efforts to sample within oyster culture have recently explored underwater videography as a method to capture interactions between oyster culture and fish. However, associated studies in Humboldt Bay (Hudson et al. 2018) demonstrated that underwater videography is of limited value in Humboldt Bay due to the high turbidity in the intertidal areas during tidal exchanges. This effectively precludes the ability to use underwater video 'traps' to sample fish use.

Inquiries into the food web and data summaries associated demonstrated that these studies are currently disparate and collections for individual species, habitats or associations are often linked to student thesis research. These studies are therefore somewhat sporadic, with bursts of intensive research on selected topics, but limited effort to compile or maintain the data and integrate across studies. The food web workshop explored the potential to generate food web models for the bay, which show promise, however additional investments are needed to compile, integrate and evaluate the existing studies and significant data gaps exist that limit the utility of creating such a model.

The project evaluated and attempted some forms of data collection that ultimately are not included in project reporting due to methodology or data reliability concerns. For example, sediment oxidation data was collected as part of the fieldwork for studying invertebrates. However, the measurement probe took a long time to stabilize readings in the field and appeared to give variable readings. Furthermore, some sites ponded slightly compromising data collection for sediment oxidation. Therefore, results from these measurements were not included in analysis and reporting. Underwater video was considered as a potential method for supplementing field data collection. Researchers attempted to deploy and review underwater video in Humboldt at the onset of the project (Hudson 2018), and while these methods are effective in other West Coast estuaries, poor visibility in Humboldt Bay intertidal areas precludes this as an effective survey method. Even very short distances could not be consistently viewed using underwater video precluding the use of underwater video to capture of behavior or occurrence of fish.

The project experienced some schedule difficulties associated with permitting sampling efforts. Specifically, federal permitting for take of listed species in scientific sampling efforts are processed once or twice per year on the West Coast. Recent communications from NOAA suggest that the challenges of this process for authorizations under section 10(a)(1)(A) of the Endangered Species Act is receiving administrative improvements to reduce uncertainty for field researchers. Improved transparency for this process is improving certainty for field researchers.



Similarly, the study areas fall along the boundary of managed airspace for the Bowerman Field Airfield. This commercial airfield operates an irregular schedule of commercial and recreational flights and regulations surrounding operations of drones adjacent to this airfield and communications with this airfield for aerial surveys was ambiguous at the time of the project. Drone flights were used or attempted to provide aerial views of the habitat complexes surrounding the study areas. Initial efforts at these flights were prevented due to a lack of transparency regarding how to pursue flight authorization for drone operation in areas within 1 mile of the airfield.

#### 4.0 CONCLUSION

The project used multiple outreach venues to reach an array of audiences. Oyster growers and research scientists were engaged through professional conferences where they learned about the study effort and findings. Local individuals, government staff, and scientists contributed to the overall understanding of the role of oysters in the Humboldt food web. The project also integrated staff from a diverse collection of organizations to maximize learning across those organizations as staff from the Wiyot Tribe, USFWS, USDA, HSU, Confluence and Coast Seafoods all contributed to field planning, data collection, analysis and interpretation.

#### 5.0 REFERENCES

Hudson, B., D. Cheney, B. Dumbauld, J. Cordell, F. Tomas Nash, S. Kramer. 2018. Quantification of functional relationships between shellfish culture and seagrass in US west Coast estuaries to inform regulatory decisions. Pacific Shellfish Institute, Olympia, WA. August 2018.

