FINAL REPORT Saltonstall-Kennedy Program

Quantification of functional relationships between shellfish culture and seagrass in US west Coast estuaries to inform regulatory decisions

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NA15NMF4270318
6/01/2015 - 5/31/2018
August 2018

Table of Contents

Cover: Pacific oysters (*Crassostrea gigas*) in flip-bag culture in Samish Bay, Washington State.

Executive Summary

This project addressed the twin goals of expanding U.S. shellfish aquaculture opportunities while maintaining and improving environmental conditions for other managed species of estuarine fish and invertebrates. We examined estuaries containing a mix of oyster culture and seagrass habitats at four sites along the U.S. West Coast, all under active management by shellfish aquaculture companies. Research components addressed: 1) measurement of seagrass coverage and growth across shellfish/seagrass boundaries in each of the four estuaries; 2) quantification of fish and invertebrates using minnow traps and underwater video; 3) determination of abundance and taxa of epibenthic invertebrates; 4) tethering experiments to assess predation; 5) analyses for environmental DNA (eDNA); 6) spatial relationships and modeling; and 7) synthesize data and parameterize production functions across habitat types.

A general linear model with estuary and habitat as fixed factors and site treated as a random factor was used to analyze data collected along transects (LMER in R). Results suggest significantly higher eelgrass shoot density occurred in Samish and Tillamook Bays than that found in Willapa and Humboldt Bays, but there was significant interaction between estuary and habitat treatments. Higher shoot density was found in eelgrass habitat, but density of eelgrass along the edge between aquaculture and eelgrass varied amongst estuaries. Similarly, there was higher macroalgal cover in Samish and Tillamook Bays, but generally higher algal density within in the long-line aquaculture, though again significant interaction with habitat due to varying density of macroalgae along the edge amongst estuaries.

Epibenthic analyses focused primarily on samples from long-line culture at all four estuaries in 2016, and at long-line and flipbag culture at Samish Bay in 2017**.** Harpacticoid copepods dominated all sampling strata. This was less pronounced at Willapa and Humboldt Bays where cyclopoid copepods (*Cyclopina* spp.), calanoid copepods (*Eurytemora americana*), and polychaetes accounted for more of the abundance than at the other two bays. Also at Tillamook and Willapa Bays there was a pattern of decreasing overall abundance from eelgrass to edge to oyster culture strata for both total invertebrates and harpacticoid copepods. However, in Samish and Humboldt Bays, there are few differences between oyster culture and edge/eelgrass strata.

Integration of all project results facilitated the development of a Habitat Suitability Index (HSI) as a simplified model to quantify aspects of ecosystem functions in mixed oyster culture and seagrass habitats. Our methods are replicable and the simplified calculations for the index allow for additional parameters to be ranked and included in the model to accommodate site specific variables and organisms/communities of interest. However, if this tool is to be applied at the farm-scale to assess habitat value, it is recommended that multiple locations be measured and assessed for a mean, farm-scale value. We further acknowledge that our index of habitat suitability is limited to the data we were able to collect and analyze during the project period and is therefore limited in scope and application. Our HSI should be viewed as a model, as opposed to absolute values.

Objectives and Goals

This project addresses the twin goals of expanding U.S. shellfish aquaculture opportunities while maintaining and improving environmental conditions for other managed species of estuarine fish and invertebrates. The primary objectives were to:

1) Measure and quantify the effect of shellfish culture on seagrass and its function as habitat for fish and invertebrates.
2) Determine the distribution of, and spatial relationship between, existing shellfish culture and seagrass in several Pacific Northwest estuaries; and use models to predict overlap and conduct change analyses particularly for areas of proposed culture.
3) Synthesize data and parameterize production functions for higher trophic level species of interest (English sole, crab, salmon) across habitat types.

Figure 1. This project's four research sites.

Approach and Results

We examined estuaries containing a mix of oyster culture and seagrass habitats at four sites (Figure 1) along the U.S. West Coast, under active management by the companies noted:

- Samish Bay, WA – Taylor Shellfish
- Willapa Bay, WA – Taylor Shellfish Farms, Pacific Seafoods (Coast), and Ekone Oyster Co.
- Tillamook Bay, OR – Pacific Seafoods
- Humboldt Bay, CA – Pacific Seafoods

Research components addressed: 1) measurement of seagrass coverage and growth across shellfish/seagrass boundaries in each of the four estuaries;
2) quantification of fish and invertebrates using minnow traps and underwater video;
3) determination of abundance and taxa of epibenthic invertebrates; 4) tethering experiments to assess predation; 5) analyses for environmental DNA (eDNA); 6) spatial relationships and modeling; and 7) synthesize data and

parameterize production functions across habitat types. At least 3 sites with longline oyster culture were selected and visited in all four estuaries in July and August 2016. Two sites – one with longline culture and another with flip bag culture were visited in Samish Bay and Willapa Bay and two sites with long-line culture re-visited in Tillamook Bay in April and May 2017.

1. Measurement of seagrass coverage

Measurements were made at 20 random points along a 50m transect (Figure 2) in each habitat type (eelgrass, edge, culture bed) at each site within each bay. *Zostera marina* density was assessed by counting shoots within a 625cm^2 quadrat placed on alternate sides of the transect line at the 20 random points. Percent cover of macroalgae (eg. *Ulva*), inundation of water, and percent cover of epiphytes on blades was also measured and recorded. One shoot was randomly collected from each quadrat and placed into a gallon bag for later analysis of biomass.

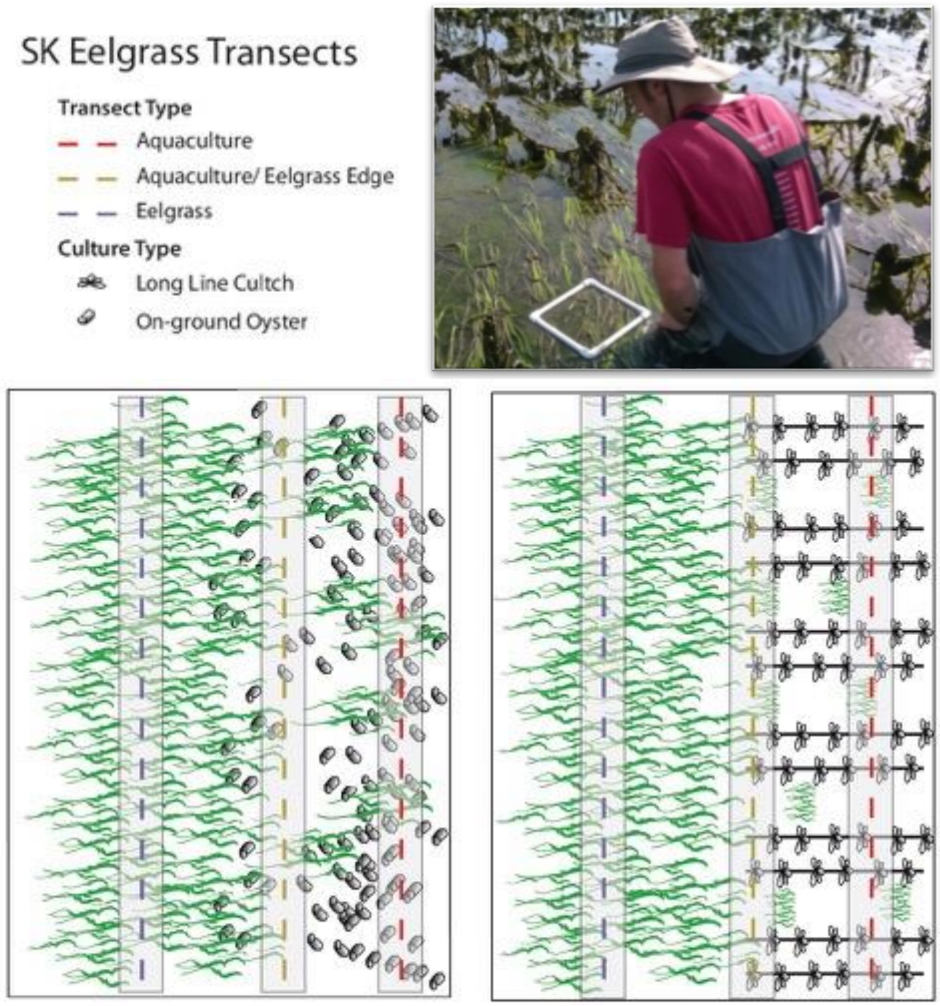


Figure 2. A 50m transect line was used in each habitat type (blue- eelgrass, yellow-edge, and red- longlines). Consistent layouts were attempted but several sites required transect lines to be perpendicular to longlines (right).

Eelgrass shoot samples were processed at Oregon State University. The longest blade of the collected shoot was measured for height and width and then the whole shoot was scraped with the side of a microscope slide to remove all epiphyte cover from each blade. The scraped epiphyte material was placed into a pre-weighed baking tin and dried in an oven at 60^{o} C for 48 hours or until a constant weight was achieved. The shoots were placed into a foil pouch and dried at 60^{o} C for 48 hours. The samples were then weighed and biomass was calculated for each sample. These measurements were then extrapolated to the entire transect area using the shoot density counts taken in the field. Growth measurements were not taken due to logistical challenges (they require repeated site visits).

A general linear model with estuary and habitat as fixed factors and site treated as a random factor was used to analyze data collected along transects (LMER in R). Results suggest significantly higher shoot density occurred in Samish and Tillamook Bays than that found in Willapa and Humboldt Bays, but there was significant interaction between estuary and habitat treatments. Higher shoot density was found in eelgrass habitat, but density of eelgrass along the edge between aquaculture and eelgrass varied amongst estuaries (Figure 3A, Analysis of Deviance, Chi Sq Pr =0.02, <0.001, and <0.001 for estuary, habitat and estuary/habitat interaction respectively). Similarly, there was higher macroalgal cover in Samish and Tillamook Bays, but generally higher algal density within in the long-line aquaculture, though again significant interaction with habitat due to varying density of macroalgae along the edge amongst estuaries (Figure 3B, Analysis of Deviance, Chi Sq Pr =0.02, <0.001, and <0.001 for estuary, habitat, and estuary/habitat interaction respectively). Finally, the epiphyte cover on eelgrass blades assessed visually as cover ranged from 20 to 80%, was highest in Tillamook and Humboldt Bays and lowest in Willapa Bay (Figure 3C). There was again factor interaction but levels were highest in eelgrass and edge habitats in most estuaries. Though we misplaced samples from Willapa Bay, scraped biomass of these epiphytes was also highest per unit eelgrass blade size in both eelgrass and aquaculture habitats in Tillamook Bay (Figure 3D). This may in part be due to surface area of the blades in both this estuary and Humboldt Bay which is larger than that in Samish Bay. Epiphytes have been shown to provide food for mesograzers that in turn provide food for larger predators (Cullen-Unsworth and Unsworth, 2013; Reynolds et al., 2018).

Eelgrass was slightly less dense when measured at some of these sites in April and May 2017 (<100 blades m^2), but mean density was higher in Willapa Bay and there was no apparent difference in the eelgrass treatment between estuaries (Figure 4). Density was clearly lower on aquaculture beds but significant interaction was present due to edge effects differing by estuary.

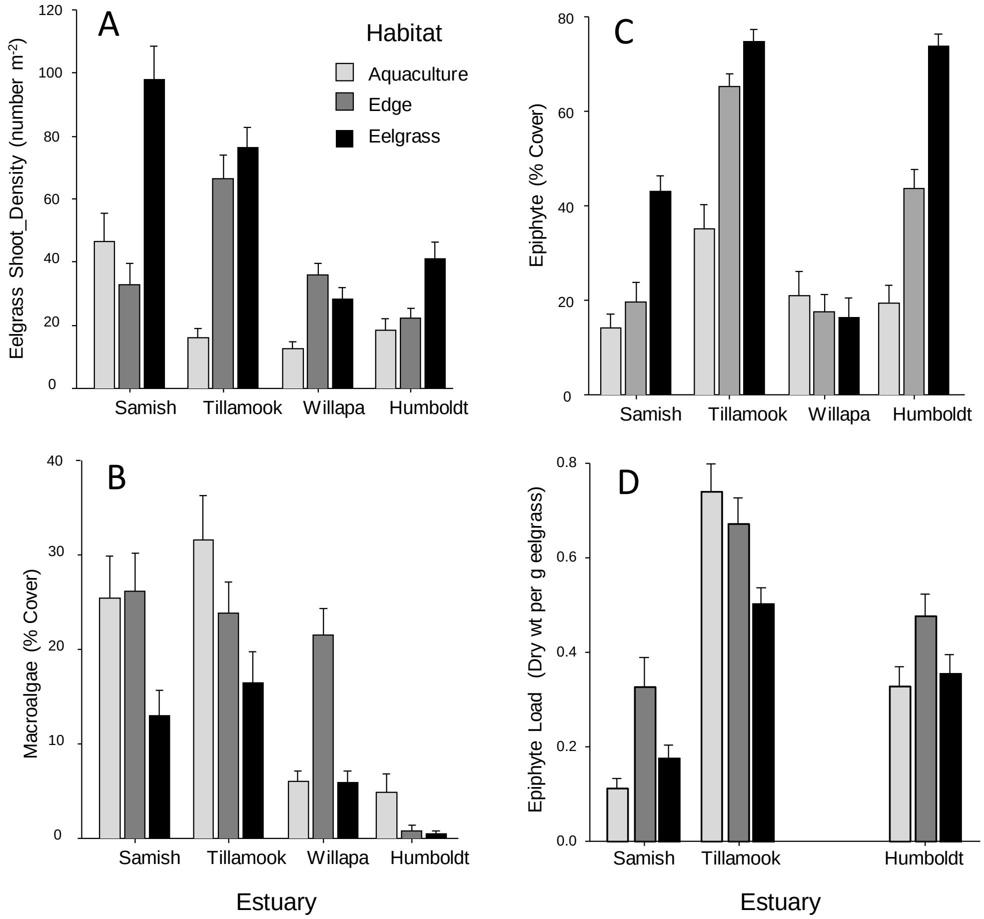


Figure 3. A) Eelgrass Density (shoots m^{-2} , B) macroalgal cover, C) Epiphyte cover and D) Epiphyte load from scrapes and calculated per dry weight of eelgrass blades (g).

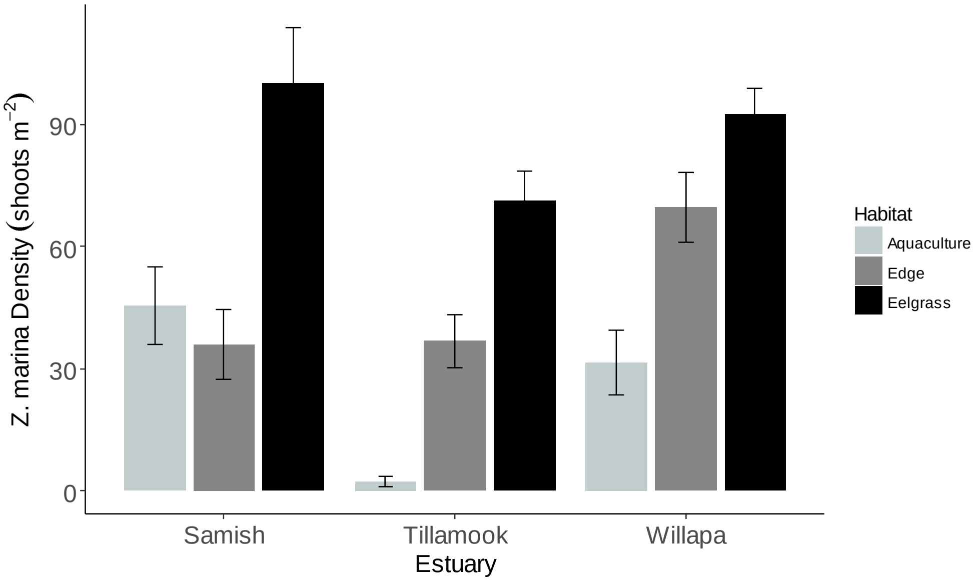


Figure 4. Eelgrass shoot density for early season sampling (spring 2017) by habitat type in three estuaries. Error bars are ±1 SE.

2. Quantification of fish and invertebrates

Minnow Traps

Large (1mx1m) minnow traps with fyke openings on opposite sides (Figure 5) were used to assess fish and invertebrate abundance and verify species seen in underwater video. Three minnow traps were deployed in each habitat at low tide (9 total) at each site and retrieved after high tide at the same time video cameras were retrieved (approximately 4 hours of fishing duration). All fish and invertebrates captured were identified to species if possible, counted and released on site. Most were also measured (fish = total length or fork length, crabs = carapace width using a measuring board and calipers respectfully). Common species captured included Staghorn sculpin (*Leptocottus armatus*, shiner perch (*Cymatogaster aggregata*), juvenile English Sole (*Parophrys vetulis*), juvenile Dungeness crab (*Metacarcinus magister*), shore crabs (*Hemigrapsus oregonensis*), and threespine stickleback (*Gasterosteus aculeatus*). During the 2016 field season, total catches were highest in Humboldt and Samish Bays and there was no apparent habitat effect (Table 1). To compare between seasons, additional sampling occurred in the spring of 2017 at three of the four bays (Table 2). Notably, no shiner perch were caught during the spring sampling (compared to a total of 146 during the summer sampling) but again, there was no obvious habitat effect.

Figure 5. Minnow traps (approximately 1m x 1m) were used to capture fish and invertebrates in each of three habitat types. All captured individuals were identified, measured and released live on site.

Table 1. Total catch of select species of fish and invertebrates captured in minnow traps, by estuary and habitat, during the 2016 field season.

Species	Habitat	Humboldt	Tillamook	Willapa	Samish	Total
English Sole	Aquaculture	2	0	1	0	3
	Edge	0	4	0	0	4
	Eelgrass	0	1	1	0	2
Dungeness Crab	Aquaculture	0	17	0	0	17
	Edge	0	10	2	0	12
	Eelgrass	0	15	3	0	18
Shiner Perch	Aquaculture	17	1	1	35	54
	Edge	10	10	0	19	39
	Eelgrass	23	6	2	22	53
Shore Crabs	Aquaculture	15	1	0	56	72
	Edge	37	5	0	41	83
	Eelgrass	32	1	1	24	58
Stickleback	Aquaculture	1	2	4	4	11
	Edge	2	4	5	5	16
	Eelgrass	3	2	14	4	23
Staghorn Sculpin	Aquaculture	23	23	11	1	58
	Edge	20	15	11	7	53
	Eelgrass	16	14	14	2	46
Total		201	131	70	220	

Table 2. Total catch of select species of fish and invertebrates captured in minnow traps, by estuary and habitat, during spring of 2017 (April-May).

Species	Habitat	Tillamook	Willapa	Samish	Total
English Sole	Aquaculture	2	1	0	3
	Edge	1	0	0	1
	Eelgrass	9	2	2	13
Dungeness Crab	Aquaculture	0	79	0	79
	Edge	0	95	0	95
	Eelgrass	1	97	0	98
Shiner Perch	Aquaculture	0	0	0	0
	Edge	0	0	0	0
	Eelgrass	0	0	0	0
Shore Crabs	Aquaculture	1	0	36	37
	Edge	0	1	17	18
	Eelgrass	1	0	20	21
Stickleback	Aquaculture	0	3	1	4
	Edge	2	12	2	16
	Eelgrass	7	2	1	10
Staghorn Sculpin	Aquaculture	11	12	8	31
	Edge	3	24	7	34
	Eelgrass	2	10	3	15
Total		40	338	97	

Underwater video

Video surveys were conducted using GoPro 4® cameras mounted to PVC pipe mounts with a top camera facing downward and a second camera on the vertical PVC arm looking outward (Figure 6). While we initially proposed using fyke nets to concentrate and observe fish with video, some experimental trials conducted in Yaquina Bay suggested this would be difficult due

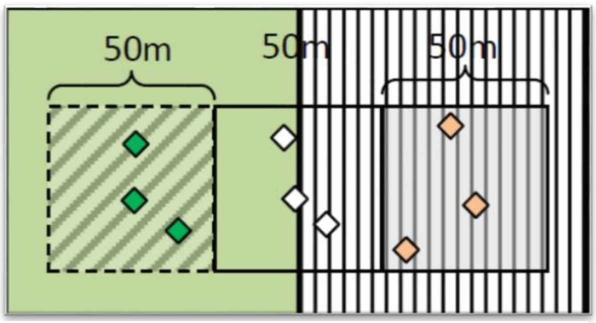


Figure 6. Video deployment on and between eelgrass and shellfish habitats.

to nets blocking field of view and camera entanglement. The additional bias due to fish attraction to the net structures, and permitting issues raised by agencies for some estuaries, caused us to at least temporarily abandon this approach. Snorkelers deployed 3 camera mounts to each habitat type, 9 in total. The cameras were allowed to run for approximately 2 hours during the high tide and were retrieved via boat and gaff hook from the vessel at the end of the 2-hour deployment.

Students and staff from PSI, OSU and USDA-ARS reviewed video from Tillamook, Samish, and Willapa Bays. Humboldt video quality was unfortunately low due to high turbidity levels, so review was less quantitative. After an initial quality index review, the middle 1 hour of video was analyzed for species, abundance, and several basic behavior categories. Species was determined using ID guides and will be marked in the notes when the viewer is uncertain. BORIS, a free software program available online (www.boris.unito.it) was used to code species and behavior categories. Species observed include: English sole, lingcod, shiner perch, threespine stickleback, Pacific herring, tubesnout, surf smelt, Chinook, Coho, starry flounder, Pacific staghorn sculpin, kelp perch, striped sea perch, red rock crab, Dungeness crab (Figure 7).

Behavioral categories were entered in BORIS as point events. Point events tagged the start time of the activity associated with the observed fish or crab. The behavior categories quantified were the following:

1. *Transit*: movement in and out of frame with no other apparent objectives.
2. *Forage*: using predatory or herbivorous tactics to ingest food
3. *Fight*: aggression between one or more species
4. *School*: “an aggregation formed when one fish reacts to one or more other fish by staying near them” (Keenleyside 1955)
5. *Refuge*: using structure to hide from predators or predators seeking vegetated areas where prey are present
6. *Other*

The frame of reference to quantify fish and crab in the x direction was the full camera view and in the y direction is to the edge of the quadrat. This means that an individual that was outside of the quadrat on the side was still counted, however an individual that moved behind the far arm of the quadrat was not. For each count, a key was entered into BORIS that entered both the species and the assumed behavior. Resultant data were saved and exported to R for statistical analyses. Because it was challenging to know whether the same fish or crab repeatedly visited the site, the counts may be artificially inflated. Therefore, “sightings” were used as the response rather than counts (which still may equal the number of counts, but referring to observations as sightings is more true to the data collected).

Figure 7. Screenshot from Tillamook Bay video survey of Dungeness crab utilizing eelgrass habitat.

One of the primary lessons obtained from this research was that underwater video is a very useful technique for assessing fish and invertebrate abundance and potentially behavior in structured estuarine habitats, such as off-bottom shellfish culture and eelgrass where other techniques utilizing nets are untenable. However, sampling issues, primarily with visibility in turbid coastal estuaries (Figure 8) continue to be a challenge and we never obtained useful video footage in Humboldt Bay, nor was video a useful technique during stormy weather in the Spring of 2017. Nonetheless a typical estuarine fish community was observed in the other estuaries, similar to species observed in minnow traps deployed adjacent to cameras, in all four estuaries.

Few directly managed species, except juvenile Dungeness crab and English sole were observed using these methods. Shiner perch were the most sighted fish and there were no statistical differences in abundance of perch among estuaries or habitats sampled (Analysis of Deviance, Chi Sq Pr =0.150, Pr = 0.075, and Pr 0.425 for estuary, habitat, and estuary/habitat interaction respectively, Figure 9). Shiner perch have previously been one of the only fish found in greater abundance in eelgrass compared with open mud or bottom cultured oyster habitat, but we found that they were equally abundant in the three habitats we sampled (eelgrass, long-line oyster culture and habitat along the edge of these beds). This could be significant since all three of these habitats including off-bottom culture had similar vertical structure present which may serve a similar function for these fish. Most perch were observed transiting across the small area we could see in these video observations and few differences in behavior were observed among habitats and estuaries except more perch appeared to be foraging in Samish Bay especially in longline culture habitat.

Figure 8. Underwater video snapshots from a location in Humboldt Bay where visibility was poor (top) and from a location in Tillamook Bay, OR where visibility was good (bottom). Note the perch visible in the bottom picture (arrow).

Abundance of two other commonly observed species, Staghorn sculpin and juvenile Dungeness crab varied among estuaries with fewer observations for both of these species in Samish Bay (Dungeness crab: Analysis of Deviance, Chi Sq Pr =0.056, Pr = 0.033, and Pr 0.221 for estuary, habitat, and estuary/habitat interaction respectively, Figure 9). Results from Humboldt Bay traps suggested that Dungeness crab were mostly absent during the sample period and corresponding video data was too poor to verify presence of this species. No statistically significant differences in habitat use within these estuaries were detected for these two species, however more crab and staghorn sculpin were seen on video and caught in traps in aquaculture and edge habitat than in eelgrass.

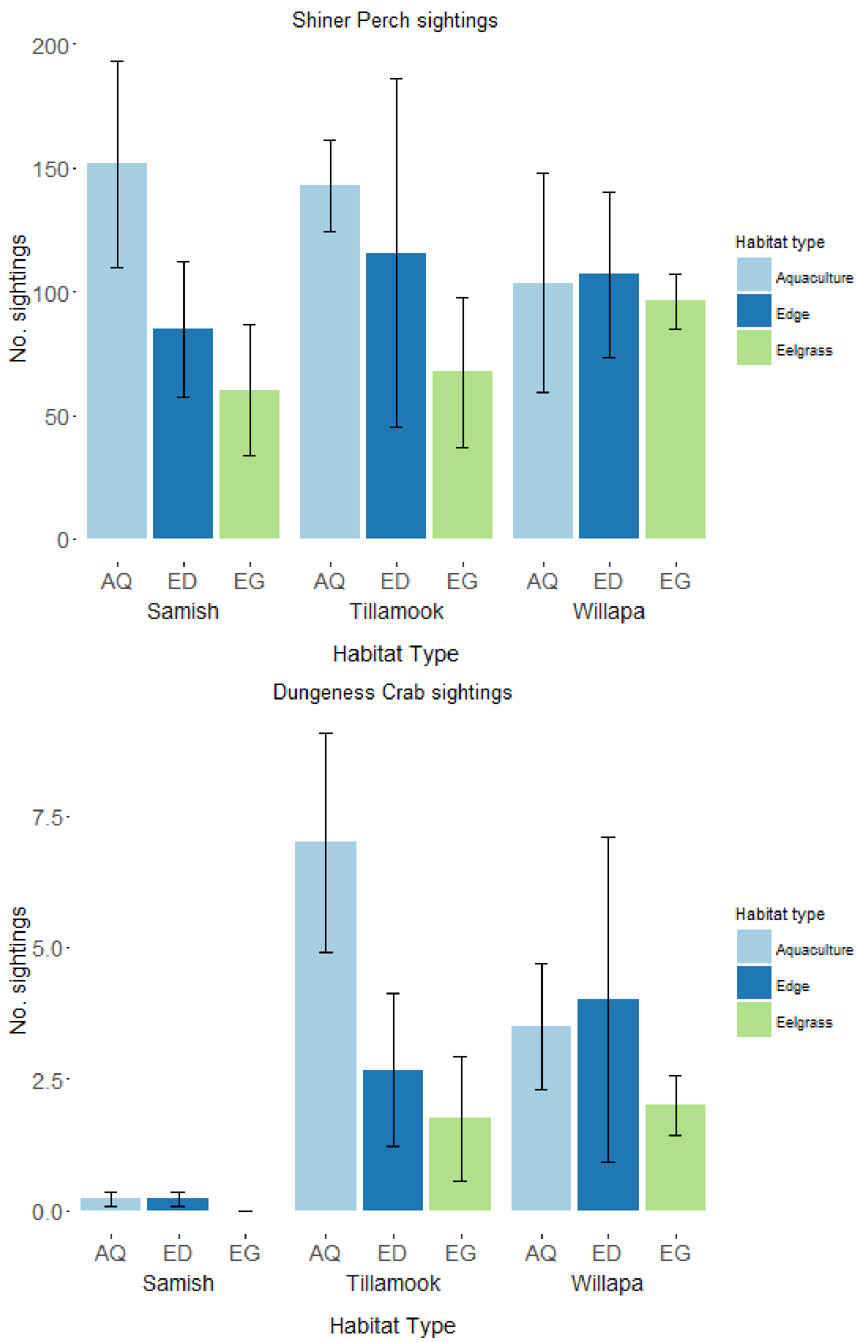


Figure 9. Mean sightings of shiner perch and Dungeness crab in underwater video from three habitats in the three estuaries where video footage was useable. Note no significant difference in habitat use, but significantly fewer Dungeness crab seen in Samish Bay.

3. Determination of abundance and taxa of epibenthic invertebrates

The three strata described above—eelgrass, edge, and oyster line culture—were sampled for epibenthic invertebrates one time during June-July 2016 at each estuary and at one estuary (Samish Bay) in 2017. Either 7 or 10 replicate samples (number determined by the time window allowed by incoming tides) were taken at each stratum. Epibenthic invertebrates were collected by wading using a 2,000 gallon hour^{-1} 12-volt electric bilge pump, housed at the top of a 14.8 cm wide PVC sampling cylinder, open only at the base (Figure 10), which sampled the water ~25 cm above the bottom and encompassed an area of 0.018 m^2 of the benthic substrate. The sampling cylinder was equipped with 0.106 µm mesh screening over replacement water ports, allowing a quantitative sample of the enclosed epibenthos to be obtained without external contamination. For each sample, the cylinder was gently placed on the sediment surface, and water was pumped for 20 seconds, or until benthic sediments were noticed in the pump's clear plastic outflow hose. Material from the pump was collected on a 0.106 µm sieve and the filtrate was fixed in a 5% buffered formaldehyde solution. In the laboratory, invertebrates were identified to the species level for most adult crustaceans (e.g., gammarid amphipods, cumaceans, harpacticoid copepods) and to family or higher taxonomic category for other groups. Strictly planktonic (e.g., most calanoid copepods) or benthic (e.g., nematodes) were not targeted by the sampling methodology, and were not enumerated.

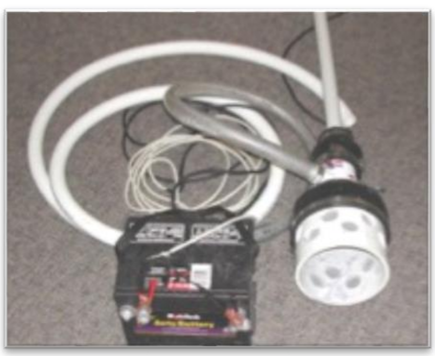


Figure 10. Epibenthic sampling pump (left) and sampling team in Willapa Bay.

Multivariate data describing the epibenthic community was standardized prior to analysis. Species that occurred in less than 3% of samples were excluded, and abundances were log-transformed. Patterns in community composition were visualized by nonmetric multidimensional scaling (NMDS). Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in the overall assemblage compositions. Multivariate tests used the Bray-Curtis similarity, with an unrestricted permutation of the raw data which is recommended for small sample sizes and single-factor tests. P-values <0.05 were considered statistically significant when making comparisons among the three strata. Comparisons of log-transformed densities and taxa richness among the three sampling strata were also analyzed using one-way ANOVA. We then used post-hoc Tukey tests to compare means between pairs of variables when the initial ANOVA showed significant results.

Analyses focused primarily on epibenthic samples from long-line culture at all four estuaries in 2016, and at long-line and flipbag culture at Samish Bay in 2017**.** Harpacticoid copepods dominated all sampling strata (Figure 11). This was less pronounced at Willapa and Humboldt Bays where cyclopoid copepods (*Cyclopina* spp.), calanoid copepods (*Eurytemora americana*), and polychaetes accounted for more of the abundance than at the other two bays. Also at Tillamook and Willapa Bays there was a pattern of decreasing overall abundance from eelgrass to edge to oyster culture strata for both total invertebrates and harpacticoid copepods (Figures 12 and 13). However, in Samish and Humboldt Bays, there are few differences between oyster culture and edge/eelgrass strata (Figure 11). Overall, harpacticoid copepods from Tillamook and Willapa Bays were relatively abundant in eelgrass and edge strata, but reduced in oyster culture strata, whereas they were more evenly distributed among the strata in Samish and Humboldt Bays (Figure 14).

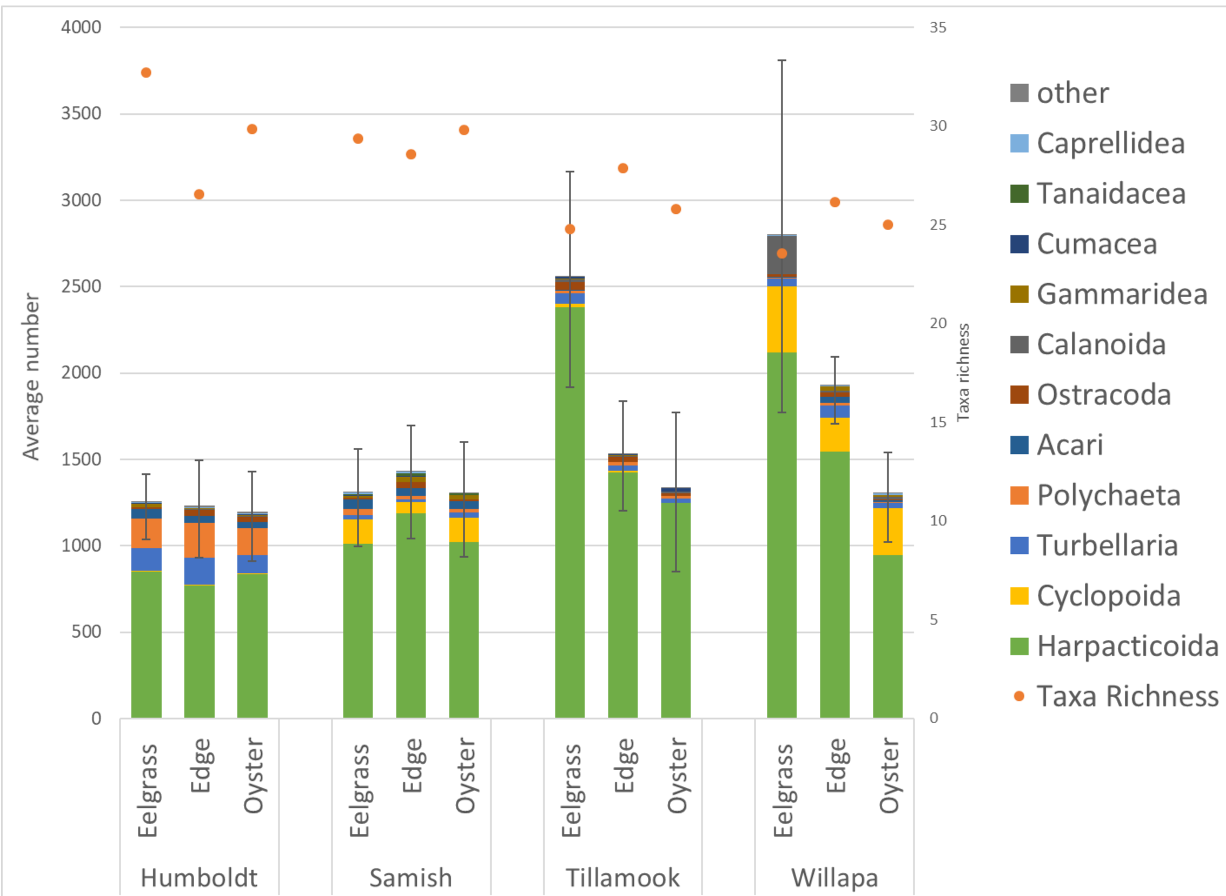


Figure 11. Abundances of major groups of epibenthic animals sampled in 2016 in Humboldt Bay, CA, Tillamook Bay, OR, and Samish and Willapa Bays, WA.

Overall statistical results showed that eelgrass strata had significantly higher total densities and harpacticoid densities than oyster strata. Taxa richness was not significantly different. Multivariate analyses verified that Tillamook and Willapa Bays had the greatest assemblage differences, as PERMANOVA results were significantly different across strata (Figures 15 & 16).

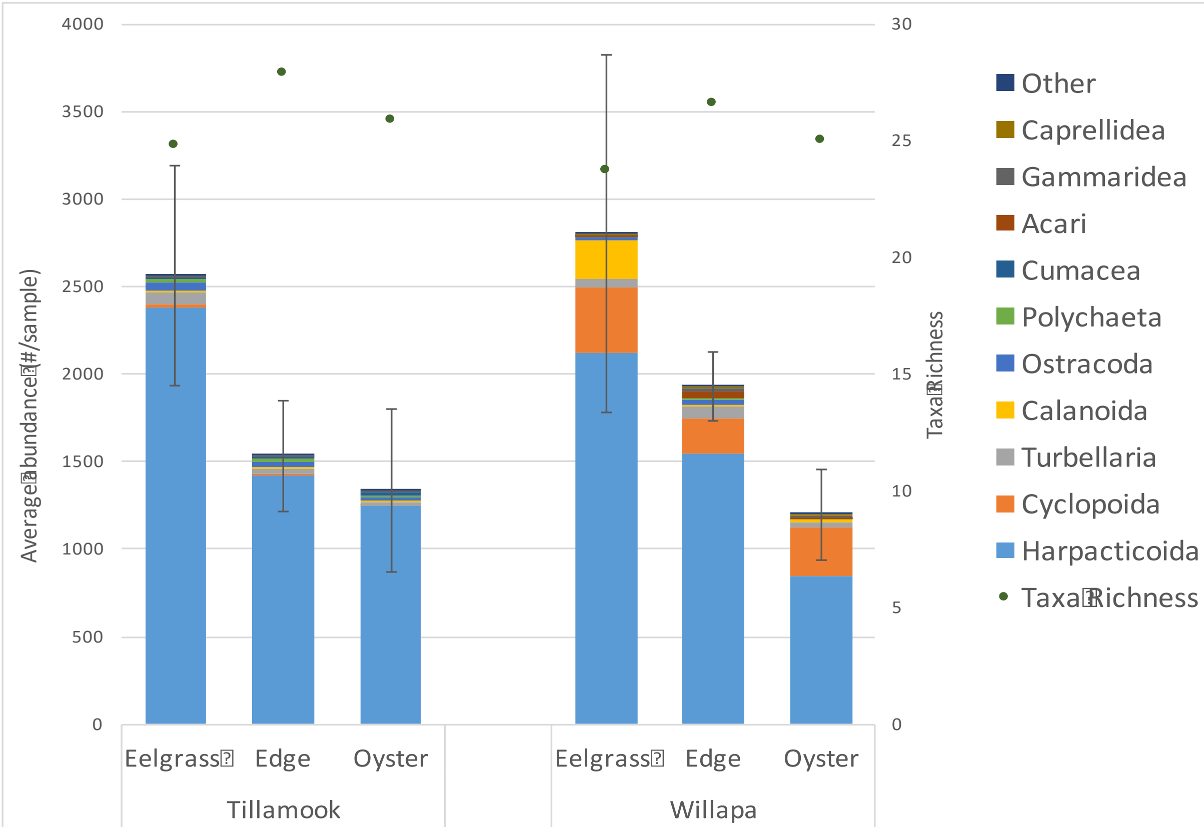


Figure 12. Abundances of major groups of epibenthic organisms.

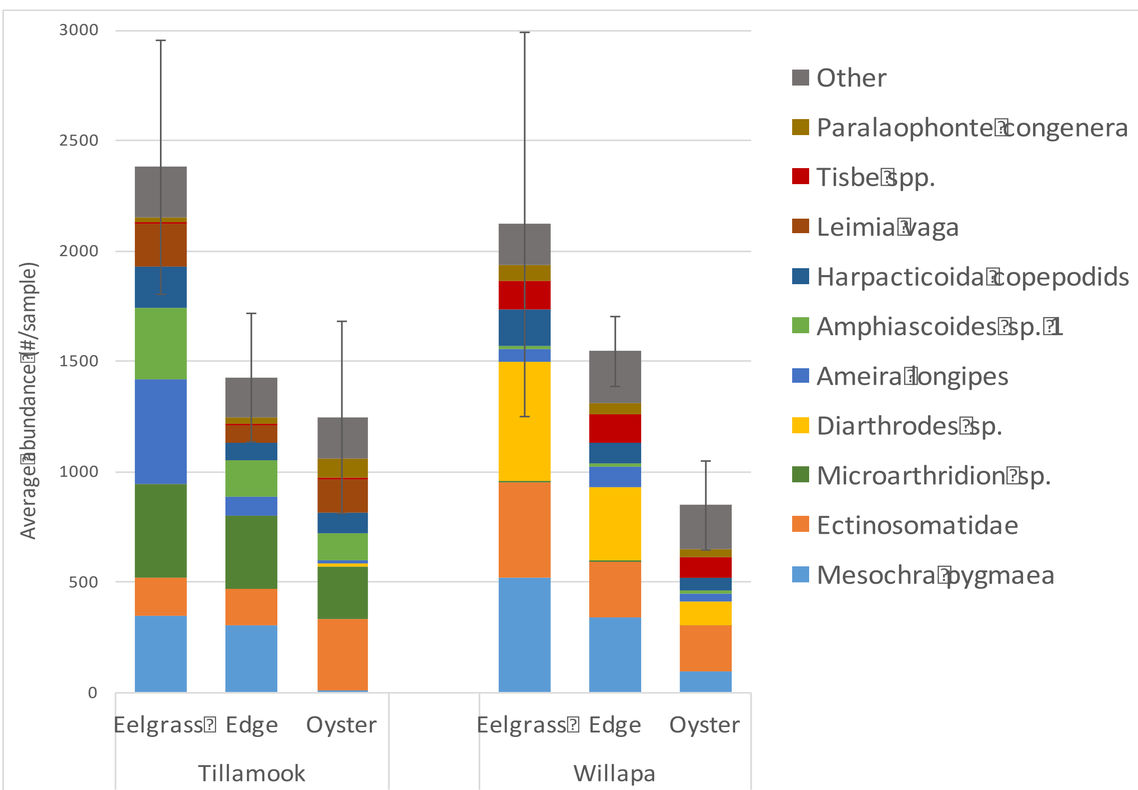


Figure 13. Abundances of harpacticoid copepods.

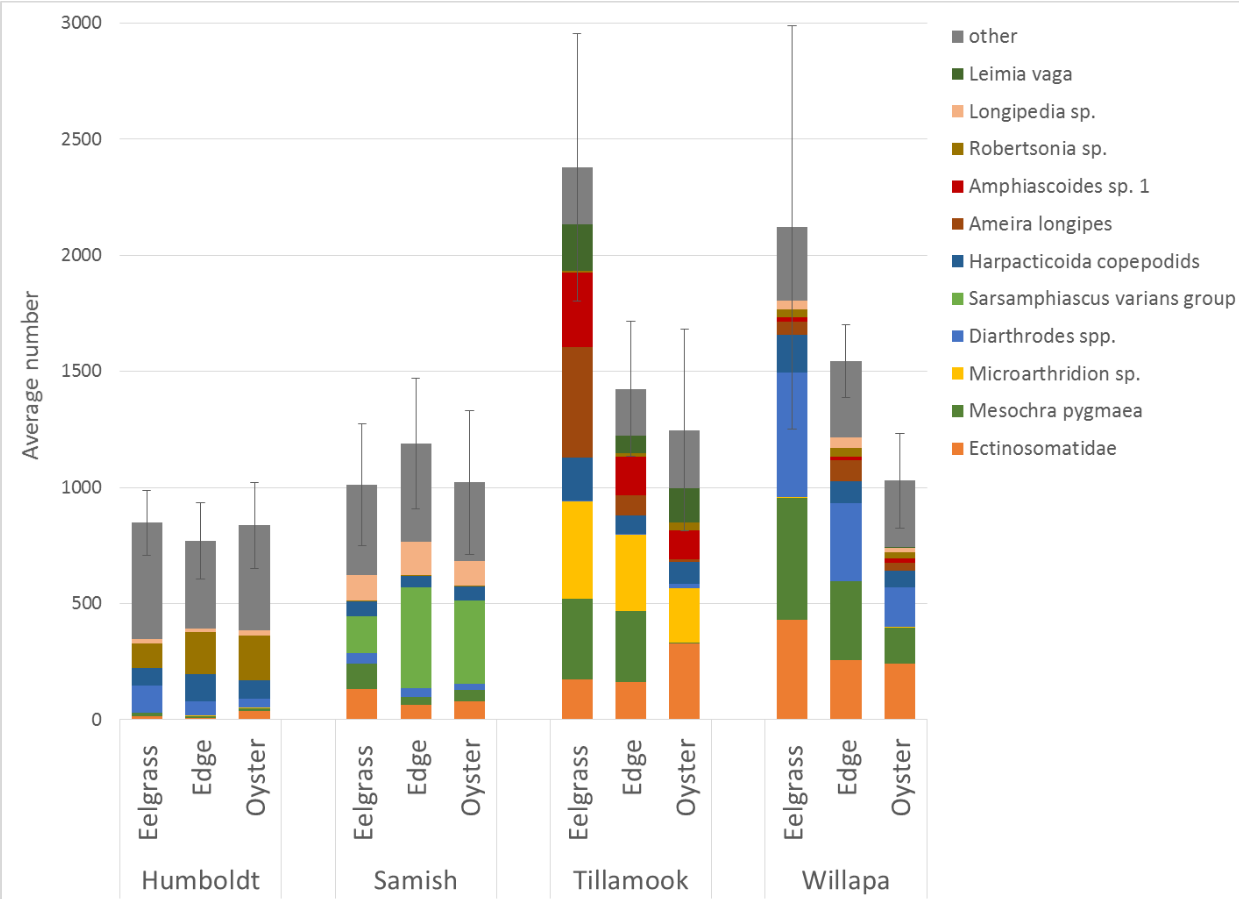


Figure 14. Abundances of major groups of harpacticoid copepods sampled in 2016 in Humboldt Bay, CA, Tillamook Bay, OR, and Samish and Willapa Bays, WA.

For the 2017 results, there were large community differences between the flip bag site and the line culture site for all strata in Samish Bay, especially for harpacticoids (the most abundant group) (Figures 10 and 11). This may have been due to site differences not associated with aquaculture effects (e.g., freshwater input, elevation, sediment)—evidenced by the dominance of more brackish tolerant species at the flipbag culture site (e.g., *Leimia vaga, Tachidius triangularis*). At the line culture site there were more *Tisbe* spp. (a salmonid prey taxon) in eelgrass and edge strata vs. oyster culture. Statistical results confirmed these trends, as assemblages were significantly different between culture types, and taxa richness was significantly higher at line culture. Similarly, assemblages at oyster strata were different than eelgrass and edge at line culture, and the eelgrass assemblage was different from other strata at flipbag culture. Similar to the trends in 2016 sampling at Samish Bay, there were no significant density or taxa richness differences across strata.

PERMANOVA results indicated that assemblages among the three strata at each bay were significantly different (Table 3). These differences are also evident in the NMDS plots in which the three strata cluster separately (Figures 17 and 18). Several taxa were particularly indicative of the eelgrass stratum, including the calanoid copepod *Eurytemora americana* and the harpacticoid copepods *Mesochra pygmaea* and *Ameira longipes*. At Tillamook Bay, the oyster culture stratum was associated with a suite of harpacticoid copepods plus the cumacean *Cumella vulgaris*.

Table 3. Statistical results of epibenthic invertebrates showing p-values, bold if < 0.05.

2016

Linear model

	Strata	Strata post-hoc
Taxa richness	0.96	
Total	**0.025**	**Eelgrass > oyster**
Harpacticoid	**0.027**	**Eelgrass > oyster**

Assemblage (Permanova)

	Strata	Strata post-hoc
Tillamook	**0.0001**	**All strata different**
Willapa Bay	**0.0002**	**All strata different**
Samish	**0.0002**	**Eelgrass different from both, edge not different from oyster**
Humboldt	0.2711	

2017 Samish

Linear model

	Strata	Type (flip, line)
Taxa richness	0.42	**0.00001**
Total	0.36	0.18
Harpacticoid	0.77	0.66

Assemblage (Permanova)

	p-value	post-hoc
Strata	**0.0001**	**Line: oyster different from both; Flipbag: Eelgrass different from both**
Type	**0.0001**	

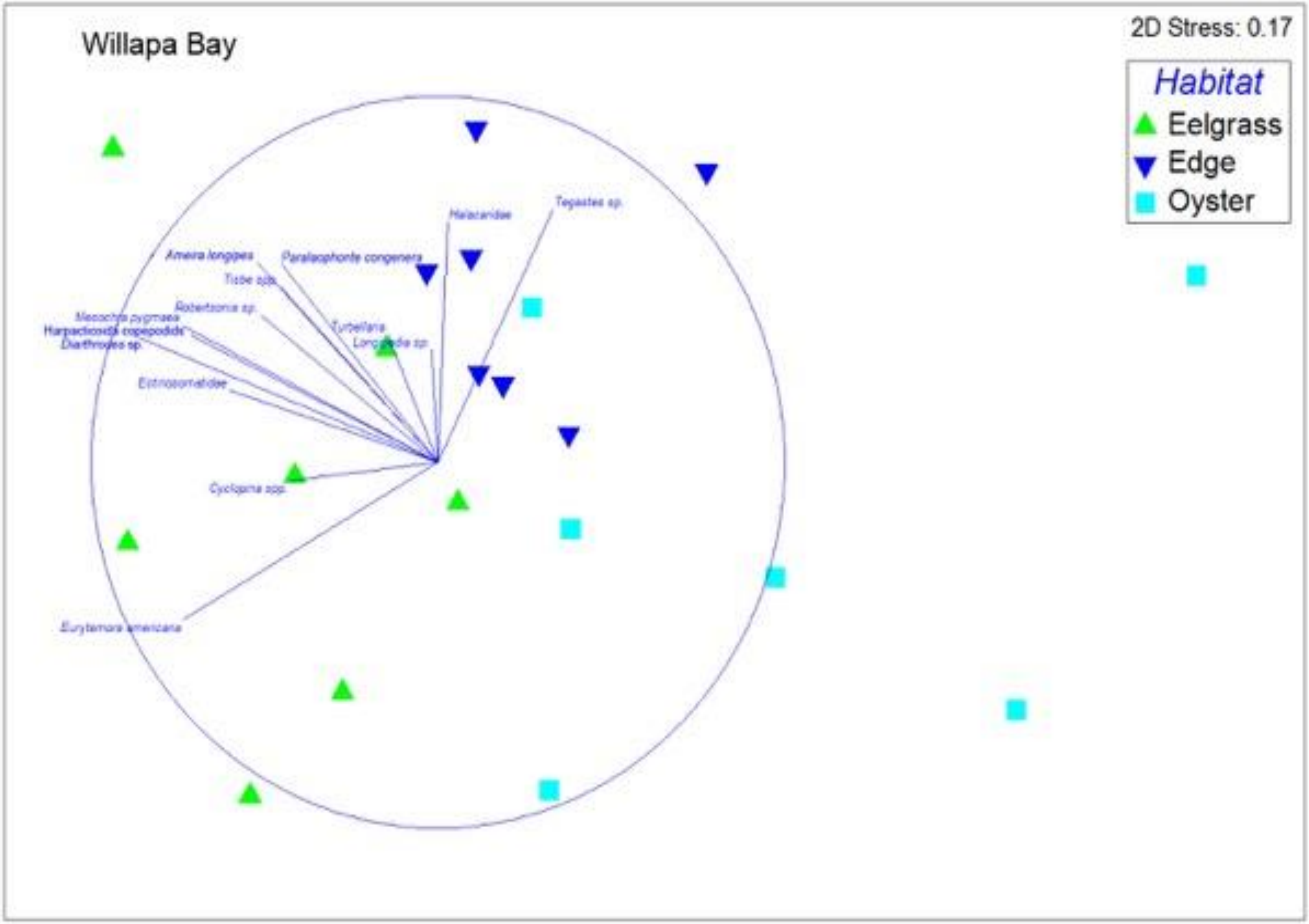


Figure 15. NMDS plots showing epibenthic assemblage structure. Symbols represent replicate samples, and vectors indicate taxa with significant gradients in ordination space based on Pearson's correlation coefficients >0.2.

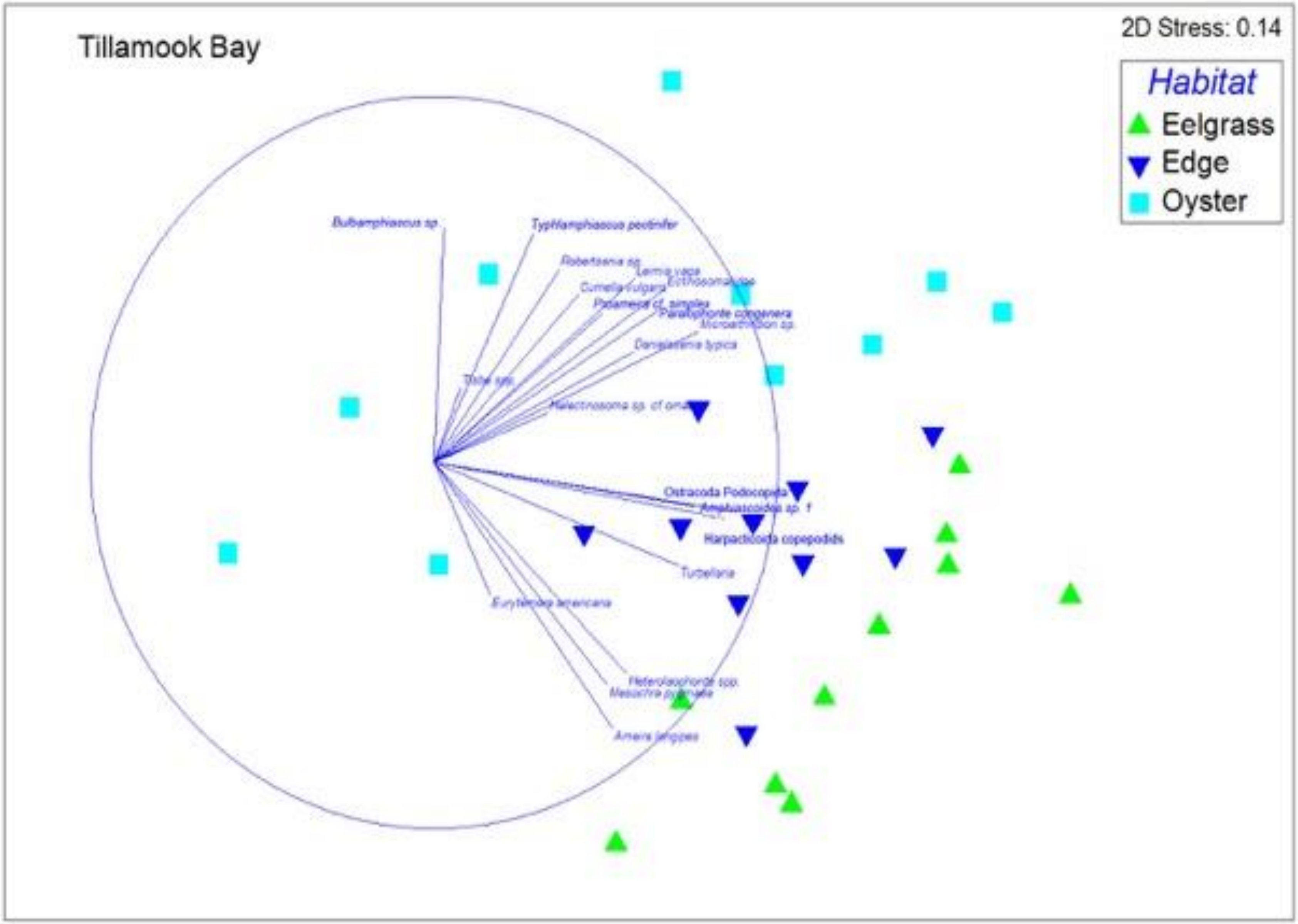


Figure 16. NMDS plots showing epibenthic assemblage structure. Symbols represent replicate samples, and vectors indicate taxa with significant gradients in ordination space based on Pearson's correlation coefficients >0.2.

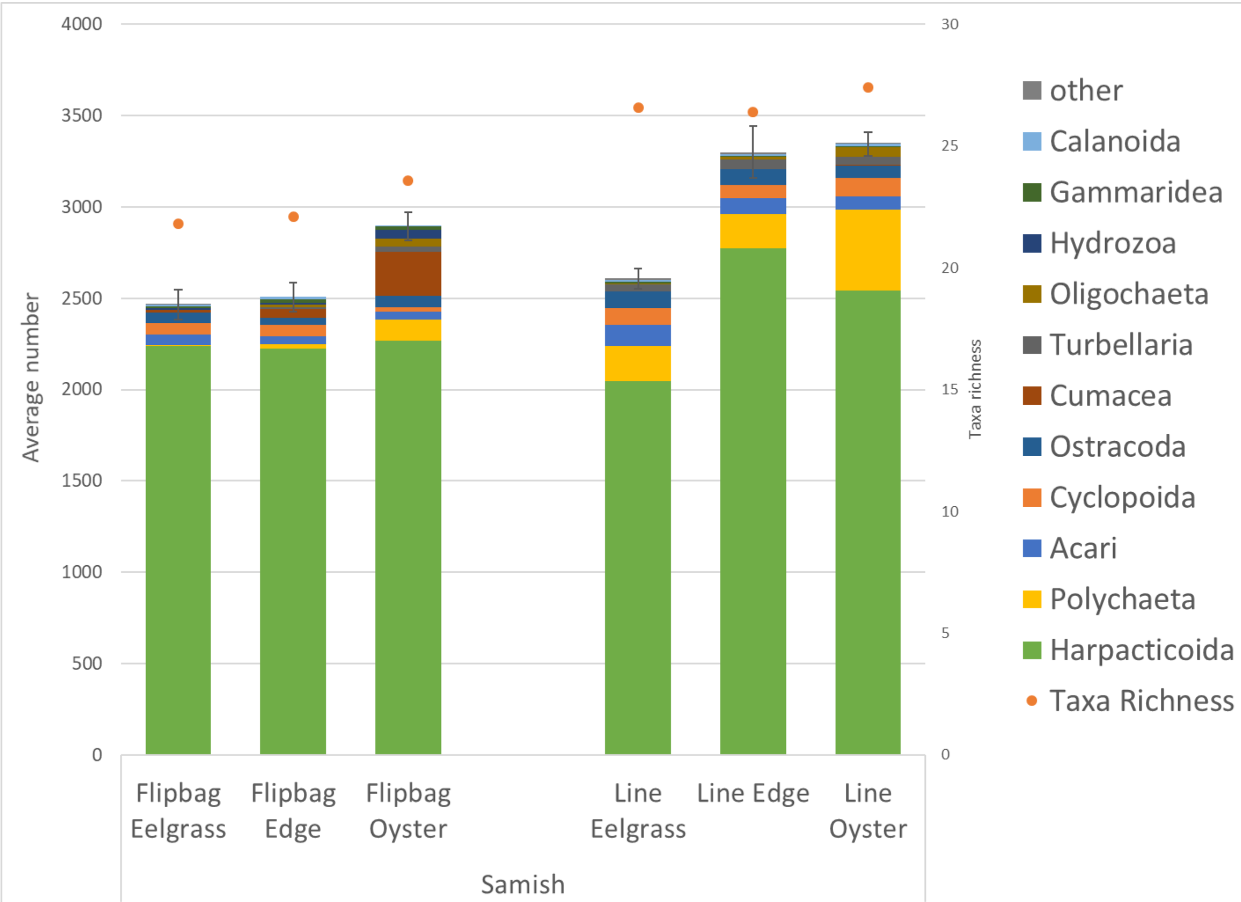


Figure 17. Abundances of epibenthic animals sampled in April 2017 from oyster flip-bag and long-line culture sites in Samish Bay, WA.

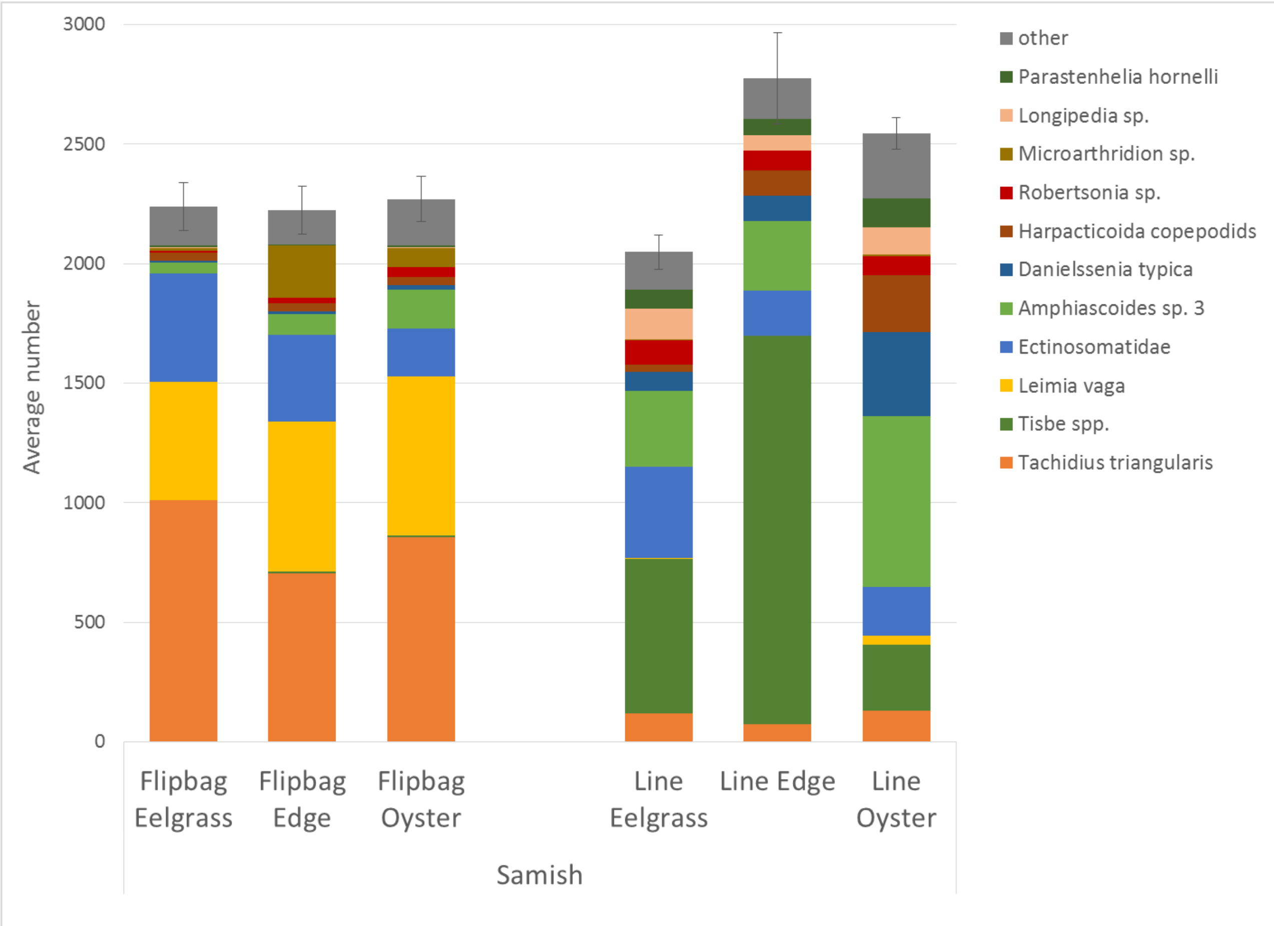
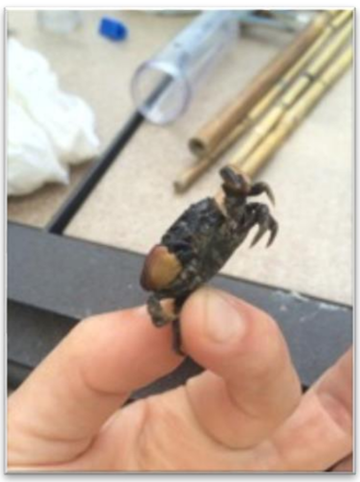


Figure 18. Abundances of harpacticoid copepods sampled in April 2017 from oyster flip-bag and long-line culture sites in Samish Bay, WA.

4. Tethering experiments to assess predation

Predation tethering units (PTU's, Figure 19) were deployed to assess relative predation rate in each habitat type as another way to explain differences in nekton abundance and behavior amongst habitats. A PTU was placed at each of the 20 quadrat locations on the opposite side of the transect from the quadrat as to not disturb the eelgrass sampling. Two to three treatments were placed: a standard sized piece of dried squid that hung 30cm from the sediment on a monofilament line attached to a small stake, a standard squid bait attached to a line so that it sat on the sediment surface, and at one site, a mud or Dungeness crab (10-20 cm CL) also tethered to a stake. Once all PTUs were deployed, they were checked at two intervals (when bait was fully submerged as the tide was flooding and 24 hours post deployment) and squid/crab presence/absence was recorded. In several instances, the PTU was checked and a sculpin or crab were observed eating the squid bait which was also recorded**.**

A generalized linear mixed model (GLMM, using the LME4 package in R, Bates et al., 2012) was used to analyse predation intensity as a function of treatment (high and low), check time (1-2 hours and 24 hours, and habitat type as fixed factors and site within estuary as a random factor. Data was modeled using a binomial distribution with a logit link because it represented presence/absence from a known number of trials. Estuary, treatment, and check time were all significant in the overall model (Analysis of Deviance, Chi Sq Pr < 0.001 for each) and there was no significant habitat-estuary interaction (Chi Sq Pr =0.985). There was little difference among habitats or estuaries and few baits absent at the initial check. At 24 hours post deployment there were no significant habitat differences, but there were significant differences amongst estuaries with lower predation occuring in Samish Bay and lower predation on baits deployed 30 cm off the bottom in Humboldt Bay (Figure 20). Though not quantitative, sculpin and crab were often observed as the predators consuming these baits during the first checks made as the tide flooded these sites. These results agree with those obtained from video and traps presented above that show higher crab (particularly juvenile Dungeness crab) abundance as well as slightly higher staghorn sculpin abundance in the coastal estuaries versus Samish Bay.



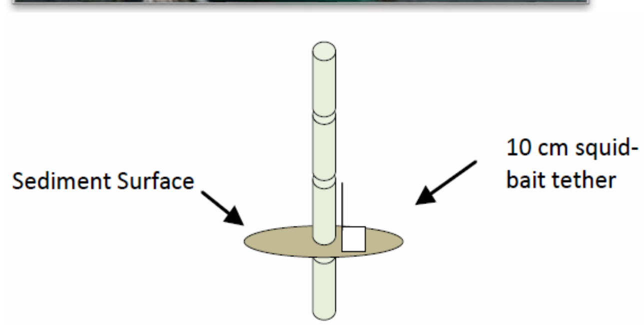
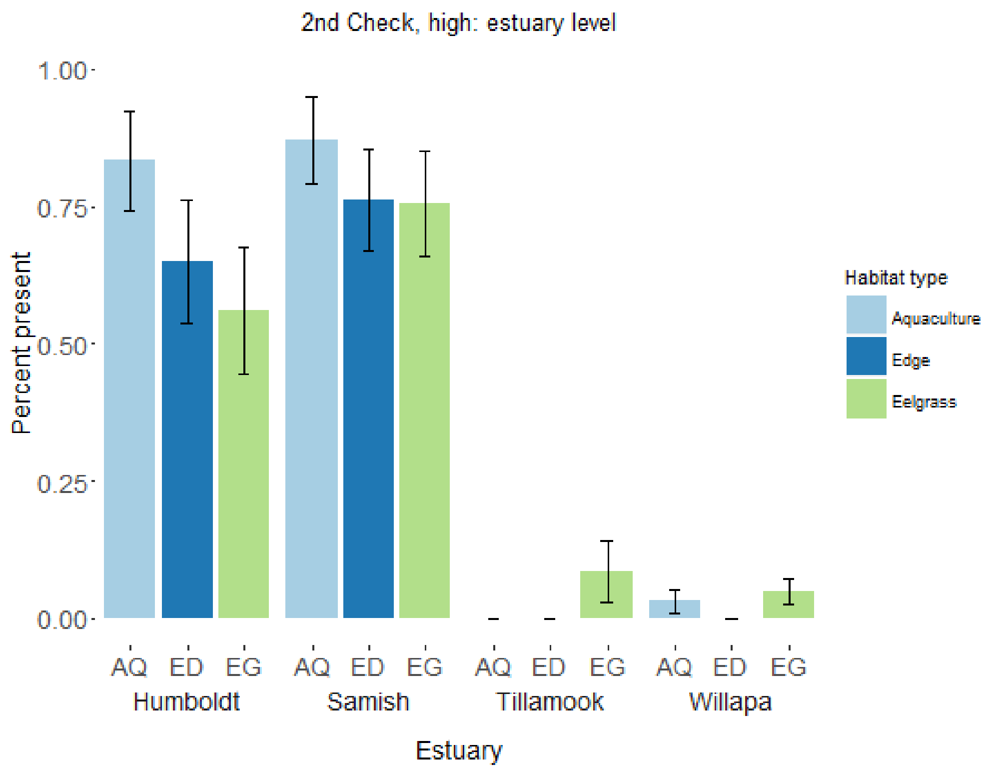


Figure 19. Predation tethering units (PTU's, right) consisted of bamboo poles with small pieces of squid bait or alternatively live tethered crab (like the shore crab depicted above) attached to monofilament line at two distances from the sediment surface.



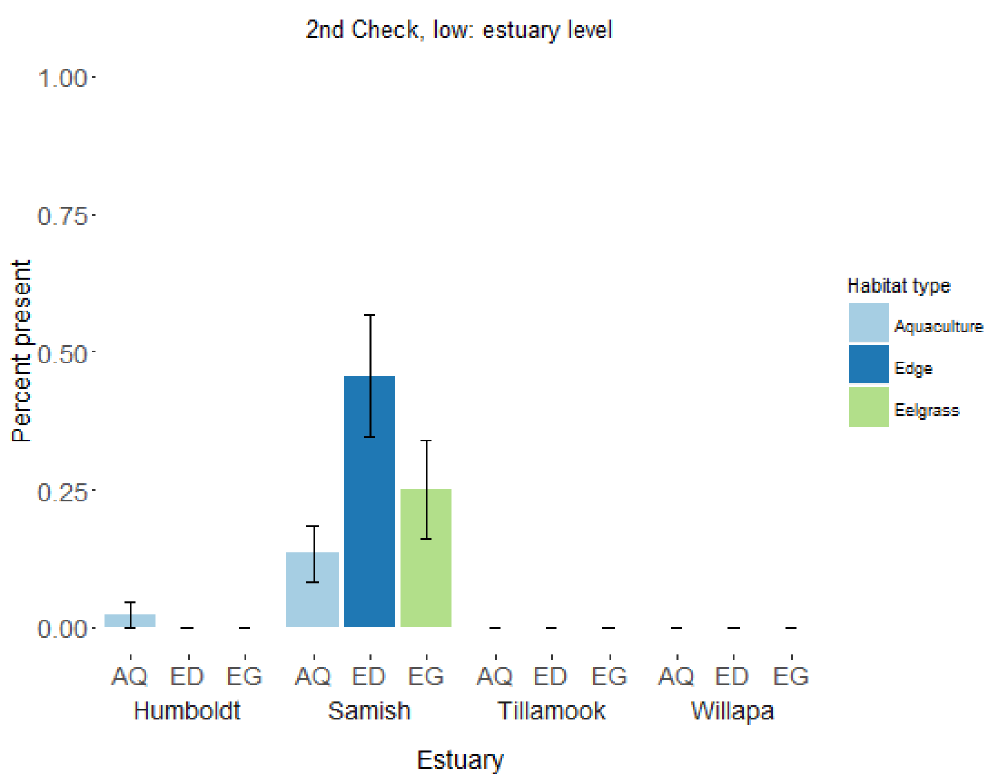


Figure 20. Proportion of squid baits present after a 24 hr deployment on predation tethering units in all four estuaries. Baits were deployed at two heights 20 cm above the sediment surface (top) and directly at the surface (bottom). Baits on the surface were still present in Samish Bay only (bottom) and those at 20 cm in both Humboldt Bay and Samish Bay (top).

5. Environmental DNA

This task was an experimental procedure conducted in association with the invertebrate collections. We collected 15, 1-liter water samples for eDNA analysis at each sampling site (Willapa Bay, Tillamook Bay, Humboldt Bay, and Samish Bay) immediately below the water surface. Five, 1-liter water samples were collected at each of the three strata sampled with the epibenthic pump: eelgrass beds, oyster longline aquaculture plots, and the edge between them. Samples were kept on ice until laboratory processing (within 4-24 hours of collection). We filtered the total volume of the samples (1L) onto cellulose acetate filters (47mm diameter; 0.45um pore size) under vacuum pressure, and preserved the filter at room temperature in buffer. Deionized water (1-liter) served as a negative control for filtering. We extracted total DNA from the filters using a phenol:chloroform:isoamyl alcohol protocol, resuspended the eluate in 200uL water, and used 1uL of diluted DNA extract (1:100) as template for PCR.

We used a 16s mtDNA PCR primer set for eDNA amplicon analysis with the goal of detecting large numbers of taxa present in the sampled environment, in order to compare results to epibenthic samples and fish surveys. Not all primers are expected to amplify all taxa, just as not all nets are expected to catch all fish; our purpose here is to utilize the results of different survey tools (eDNA and manual counts), rather than to ensure survey methods are likely to result in taxonomic overlap. We generated amplicons using a two-step PCR procedure to avoid the taxon-specific amplification bias that results from the use of differentially indexed PCR primers (commonly used to include multiple samples onto the same high-throughput sequencing run to minimize costs).

In the first batch of samples used for preliminary sequencing, we generated 9 PCR replicates from 12 of the 15 1L bottles sampled from Willapa Bay Washington. The goal of this sequencing reaction was to evaluate the impacts of sample pooling during the amplification process. After the first PCR, the 9 replicates of each bottle were pooled such that 3 of the 9 replicates were combined and used as a template for the second PCR. As a comparison, 3 PCR replicates were generated from 4 of the 5 1L bottles collected from the edge habitat in Willapa Bay. Replicates were not pooled and instead each was individually used as a template in the second PCR.

We simultaneously sequenced positive controls (Ostrich, *Struthio camelus* tissue; selected because this taxon does not occur in the Puget Sound region and therefore could not be present in the field samples) and a negative control (de-ionized water). Following library preparation according to manufacturers' protocols (KAPA Biosystems, Wilmington, MA, USA; NEXTflex DNA barcodes, BIOO Scientific, Austin, TX, USA), sequencing was carried out on an Illumina MiSeq nano (250bp, paired-end) platform.

Upon preliminary sequencing, we identified a contamination issue that occurred early in the analysis process, such that PCR replicates did not produce sequences similar to one another. This issue was subsequently resolved, but unfortunately too late to allow trained and available staff to complete the analysis. Nevertheless, our preliminary run produced DNA sequences from a broad array of taxa we expect to see in the sampled environments, including a variety of bivalves (Myidae, Veneridae, Pectinidae, Mytilidae), arthropods (barnacles, amphipods, isopods), fish (Salmonidae, Sebastidae, Embiotocidae), and birds and marine mammals. All samples are now archived and, once personnel are available, will be processed to complete this phase of the research.

6. Spatial relationships and modeling

We assembled data for analyzing the spatial relationship between shellfish culture and seagrass in Tillamook Bay, OR and updated information for Willapa Bay, WA and Humboldt Bay, CA. Where possible, we created shellfish culture vector data and seagrass raster layers at relatively fine spatial resolution (1-5 m) by digitizing farm reports, aerial imagery and other existing data, with some direct ground truthing. These layers were then used as inputs for a generalized linear model predicting eelgrass presence within each bay (Lee II et al. 2014, Dumbauld and McCoy 2015). Predicted values were compared with actual observed values to estimate the impact of aquaculture on eelgrass at an estuary-wide scale.

Tillamook Bay

We interviewed the primary grower in Tillamook Bay to obtain information on bed locations and uses. This information was digitized to produce an aquaculture layer for the bay. A seagrass layer for Tillamook Bay was obtained from Pat Clinton (U.S EPA), which was created using 2005 color infrared aerial imagery. A digital elevation model was obtained from Pat Clinton and Nate Lewis (U.S. EPA), which was produced using LiDAR data from the U.S. Army Corps of Engineers. These three layers were used for spatial analysis of seagrass, aquaculture distribution and overlap, as well as initial modeling of seagrass. Model results predicted lower overall eelgrass cover than was observed, but this could be due to the now 11 year gap in time between observed eelgrass and aquaculture data. Nonetheless, substantially more eelgrass was present in the cultured area than the model predicted (Figure 21 and Table 4). The accuracy of these estimates would be greatly improved by collecting a new eelgrass layer to match the current aquaculture bed information.

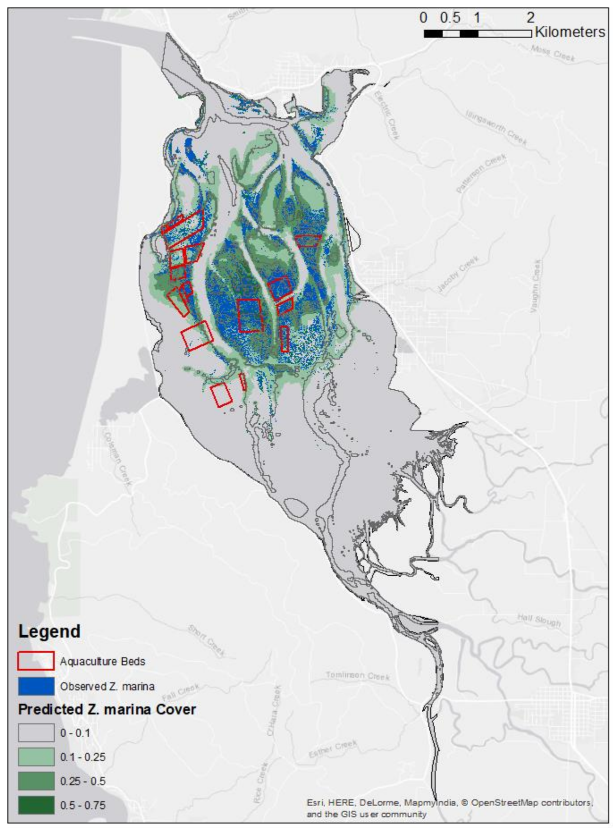


Figure 21. Spatial representation of predicted and observed eelgrass in Tillamook Bay, OR, highlighting locations of existing oyster aquaculture beds.

Table 4. Acreages of Eelgrass (*Zostera marina*) calculated from observed presence/absence values and predicted probabilities of occurrence in Tillamook Bay, OR.

	Observed Values (acres)	Predicted Values (acres)	Observed - Predicted (%Cover)
Total Estuary area	8323.54	NA	NA
Total Aquaculture	387.31	NA	NA
Total Estuary without Aquaculture	7936.23	NA	NA
Total Eelgrass	806.53	840.41	-0.04%
Eelgrass Outside Aquaculture	667.09	738.88	-0.90%
Eelgrass Inside Aquaculture	139.44	101.52	10%

Humboldt Bay

We obtained previous data layers from the 2009 NOAA CSC effort. With this information, we developed a strategy with Whelan Gilkerson (Pacific Watershed Associates) and Greg O'Connell (SHN, Engineers and Geologists) for re-analyzing this data to produce a raster map of seagrass distribution with a finer-scale resolution than the currently available polygonised layer (Figure 22). Efforts to develop a new contemporary layer were hindered by availability of current high-resolution imagery for the entire North Bay portion of the estuary. Another significant issue was lack of credible ground truth information during the 2009 NOAA survey and the now 7-year lag in time to use new data. The point and polygon format of the NOAA data also caused issues. Because of this, eelgrass cover information was instead extracted from 2009 NOAA aerial imagery, using the normalized difference vegetation index (NDVI). Positive values were assumed to correspond to eelgrass, although this is a somewhat basic assumption because these values could also represent macroalgae or other forms of vegetation. High quality layers for both existing and proposed aquaculture were available, in addition to a digital elevation model for the bay, produced and published by the Harbor District. While a more accurate spatial assessment of eelgrass is also planned with more recent aerial imagery and ground truth

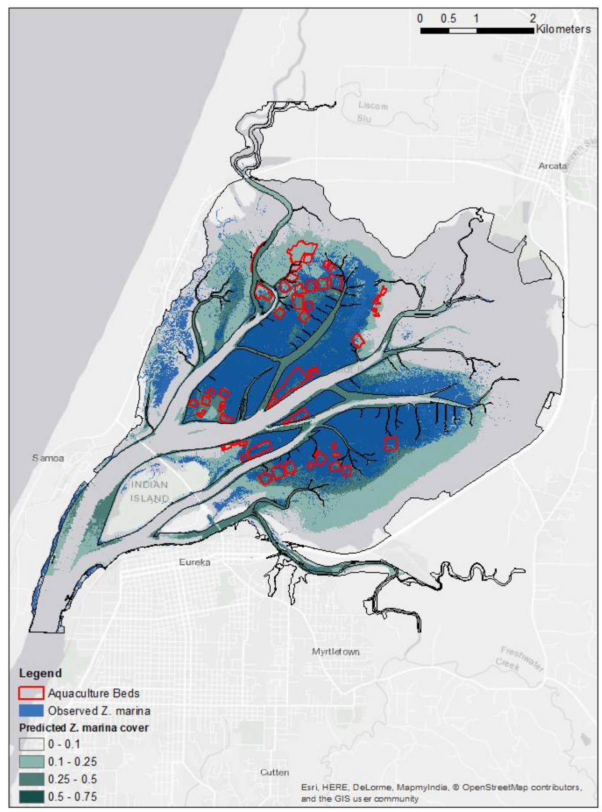


Figure 22. Spatial representation of predicted and observed eelgrass in N. Humboldt Bay, CA, highlighting locations of existing oyster aquaculture beds.

data, project partners deemed this to be a useful first step. As above, these layers were included in a generalized linear model to predict eelgrass presence throughout the bay. This model predicted less eelgrass cover than was observed both inside and outside of aquaculture. Given the above caveats regarding ground truth data for this eelgrass layer, the total eelgrass area calculated here (Table 5) does fall within the range and match that reported by Schlosser and Eicher (2012) using the same imagery. They classified 1,880 acres as continuous eelgrass and 1,697 acres as patchy eelgrass. Thus, it is interesting to note that similar to model results for the Tillamook estuary, substantially more eelgrass was present in the cultured area than the model predicted. While the analysis methods are sound, the eelgrass information that the model is based on and with which it is validated was not adequately ground-truthed and a more current and robust analysis is necessary to accurately capture current eelgrass extent and the effects of aquaculture within N. Humboldt Bay.

Table 5. Acreages of Eelgrass (*Zostera marina*) calculated from observed presence/absence values and predicted probabilities of occurrence in N. Humboldt Bay, CA.

	Observed Values (acres)	Predicted Values (acres)	Observed - Predicted (%Cover)
Total Estuary area	10,449.00	NA	NA
Total Aquaculture	300.77	NA	NA
Total Estuary without Aquaculture	10,147.67	NA	NA
Total Eelgrass	2,297.64	1,520.53	7.44
Eelgrass Outside Aquaculture	2,146.77	1,425.39	7.11
Eelgrass Inside Aquaculture	150.86	95.14	18.53

Willapa Bay

A GIS layer of the density and distribution of *Z. marina* throughout Willapa Bay, WA was created with 2005 aerial photography as baseline, 2006 photography obtained from Washington State Department of Natural Resources (DNR), and a third set of photographs taken in 2009. Therefore, our primary goal was to update the aquaculture layer utilizing current bed information collected during shellfish grower interviews in 2016 and spring 2017. This included digitization of the new information and comparison to the original layer (2005). Following this process, it was determined that there was little change in the spatial extent of active aquaculture beds, making a comparison to the 2005-2009 eelgrass information redundant. We hoped to obtain more recent imagery from partners, but have experienced challenges in securing this data and will continue to pursue this effort once we have more recent eelgrass information.

7. Synthesize data and parameterize production functions across habitat types.

We developed a Habitat Suitability Index (HSI) as a simplified model to quantify aspects of ecosystem functions in mixed oyster culture and seagrass habitats. The HSI has been widely used to normalize ecosystem characteristics across a range of habitat types and to reduce the effects of small-scale variations in species compositions, abundance and diversity within those habitats. This analysis focused on epibenthic taxa occurring in shellfish culture and seagrass habitats in the four primary study sites.

The HSI model applies two general measures: 1) indices of epibenthic taxa richness and densities of epibenthic prey groups targeted by salmonid species and other predators; and 2) index modifiers or Relative Value Indices that characterize environmental conditions that may vary spatially across strata and influence the overall habitat suitability for fish and crab species of interest.

Habitat Suitability Index (HSI)

The following equation is used to determine the epibenthic value of our Habitat Suitability Index determined for each habitat type within each bay sampled:

(Existing habitat increment condition / Optimum habitat increment condition) x 100

Where the existing habitat increment conditions or benthos rating = NBT + DHS + DPS

NBT - Number of benthic taxa

DHS - Density of total Harpacticoids (#/m^2)

DPS - Density of total Peracarids(#/m^2)

Harpacticoida is an order of copepod that includes a number of different species. The taxonomic group Peracarida is a superorder that includes amphipods, isopods and cumaceans. Many species within these groups are preferentially preyed upon by juvenile salmonids and other estuarine fish and crab species of interest and for these reasons are included in our index.

Overall values of epibenthic richness and density of select prey groups are quantified for each strata -- oyster culture, edge and eelgrass -- and then ranked 1-3 relative to one another. The ranked values for each epibenthic parameter (NBT, DHS, DPS) are added to form the existing habitat increment condition. This value is divided by the optimum or max possible habitat increment condition. The total value is multiplied by 100 to acquire the foundation of the HSI.

To integrate environmental conditions that may vary within strata and influence the overall habitat suitability for fish and crab species of interest, we include Relative Value Indices (RVI) as multipliers to the epibenthic “existing habitat increment condition” values:

(NBT + DHS + DPS) x RVI

The following parameters are included as RVI in our analyses: 1) structural complexity of native eelgrass (*Z. marina*), 2) epiphyte loads on *Z. marina* blades and 3) macroalgal (typically *Ulva* and *Enteromorpha* species) density. Structural Complexity = mean shoot density (m^2) x mean leaf surface area (m^2). Three-dimensional, above-ground complexity of eelgrass increases the refuge function of these habitats for juvenile fish, including salmonids. Epiphytes are sessile organisms that settle on eelgrass blades and are grazed on by many of the epibenthic invertebrates we are targeting in these habitat types. Epiphyte Load = dry weight of epiphytes/dry weight eelgrass. Macroalgal density was determined in the field using areal percent cover in a 0.0625 m^2 quad within the same plot as *Z. marina* shoot density was determined.

Relative abundances of resident fish species observed with underwater video within each habitat type and bay accompany final HSI values. Fish observations are described in Section 2 of this report. This additional information is an opportunity to compare resident fish utilization across the relatively ranked habitat types.

One site within each of the four bays was assessed in 2016. Two sites were assessed in 2017 in Samish Bay. All were examined using the Habitat Suitability Index as described above. The findings from these assessments are illustrated in Figures 23 to 26, below.

The resulting Habitat Suitability Indices illustrate the influences of two primary ecosystems services within this system:

a) Structure -- providing refuge from predation; and
b) Enhancing prey availability -- for juvenile salmonids and economically important fish and crab species.

Metrics included in these indices either directly or indirectly support these ecosystem services that ultimately benefit a wide array of organisms at multiple trophic levels beyond select target species that are actively managed.

Samish Bay indices reflect oyster long-line culture as having a significantly higher HSI value than adjacent eelgrass habitat. High macroalgae cover (60%) is the primary variable driving the HSI in aquaculture compared to eelgrass (0.8%). While eelgrass ranked highest for structural complexity (highest shoot density), oyster habitat had eelgrass growing among the rows of long-lines, adding value to the index. Epibenthic values were slightly lower in eelgrass than in aquaculture, diminishing the eelgrass HSI. In contrast to Samish Bay, the location sampled in Tillamook Bay assessed the edge habitat as having the highest habitat suitability value, followed by eelgrass and then oyster culture. In this scenario, the edge of the farm added ecological value to the cultured site and may act as a “seed bank” in the future to augment biological communities within the farm over time. The edge habitat had comparable epibenthic communities, but contained high macroalgal densities and moderate levels of eelgrass shoot density, thus increasing structural complexity and epiphyte values. In Humboldt Bay, eelgrass and aquaculture are fairly comparable, eelgrass had slightly higher epibenthic communities, while aquaculture had higher macroalgal densities, adding to the complexity of the habitat. The edge strata had the lowest HSI due to low epibenthic density, richness and presence of macroalgae. Our spring 2017 data collection at Samish Bay ranked flip-bag oyster culture as having the highest overall HSI value across all strata and sites, followed closely by long-line culture. Both oyster culture habitats had the highest epibenthic densities, with Pericarids driving the high value in oyster flip-bags. Note that this sample period did not include additional RVI metrics and was based exclusively on epibenthic metrics.

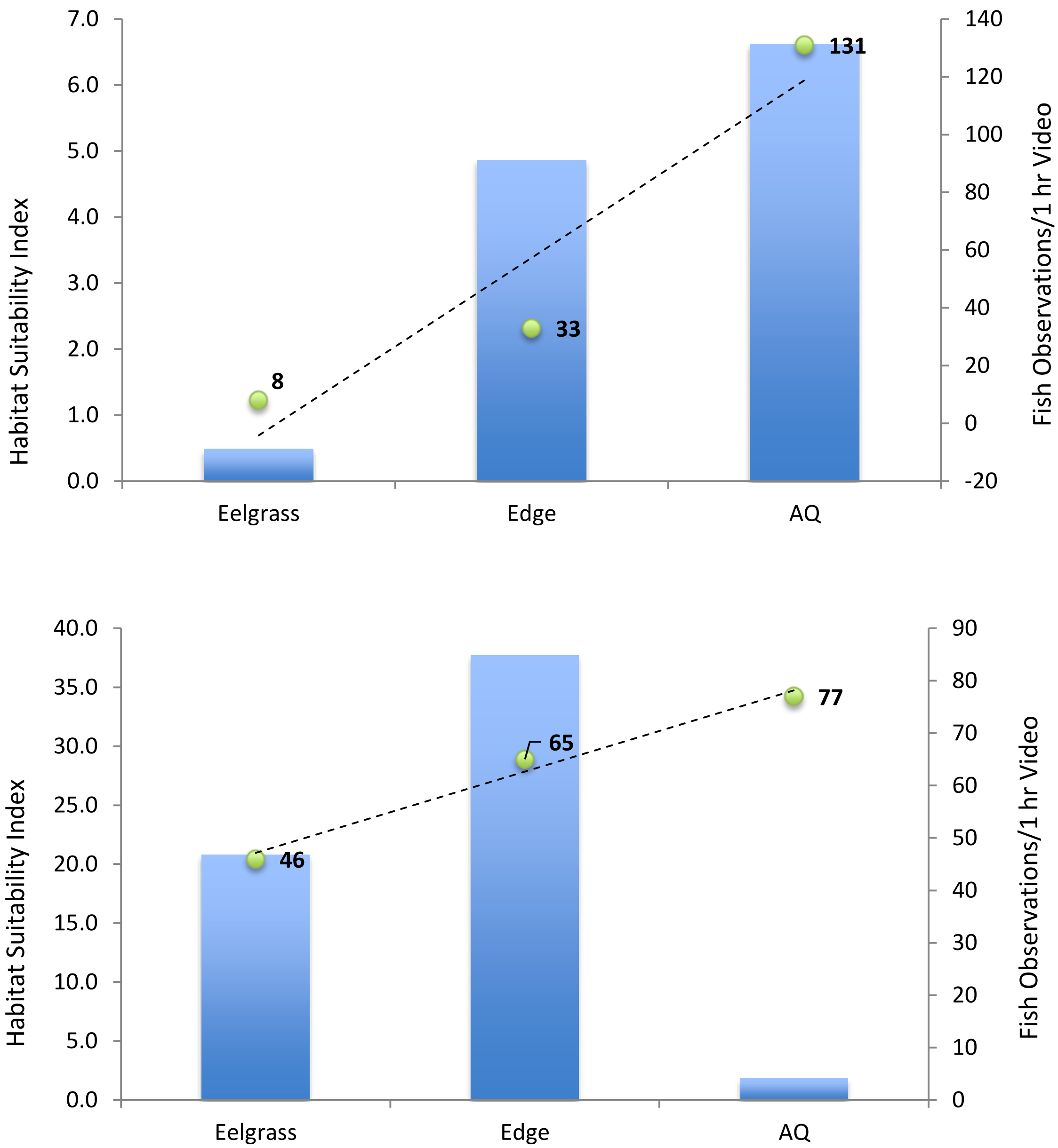


Figure 23. Habitat Suitability Indices for Samish (top) and Tillamook Bays (bottom) estuaries based on synthesized field and lab data collected during the 2016 project period. Indices are paired with the mean # of fish observations/1hr of video. Mean # of fish observations were calculated based on the number of cameras deployed within each habitat type: Samish Bay. EG (n=3), ED (n=3), AQ (n=3) and Willapa Bay. EG (n=3), ED (n=1), AQ (n=3).

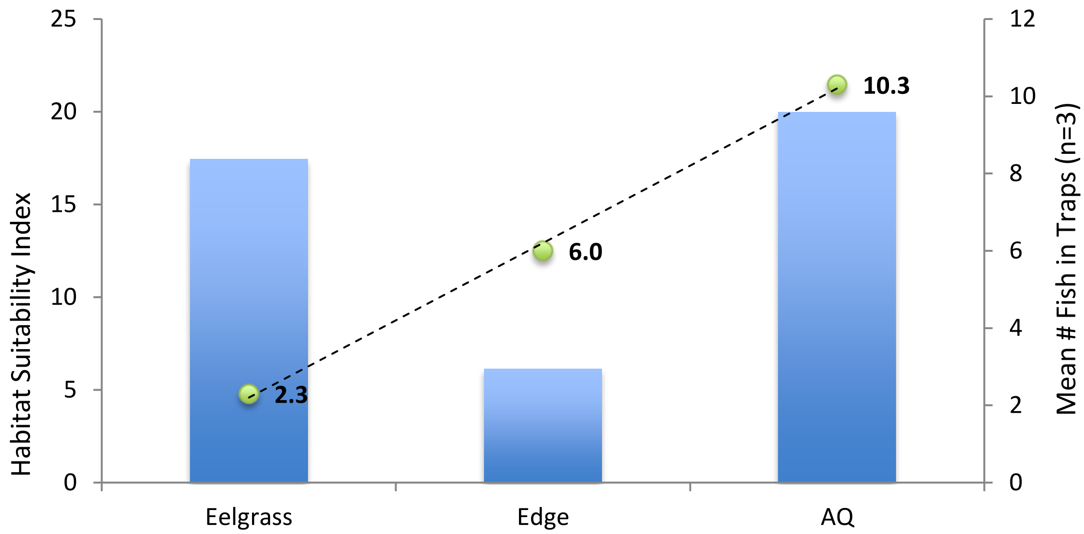


Figure 24. Habitat Suitability Indices for Humboldt Bay based on data collected during the 2016 project period. No video data were available for this site, therefore mean # of fish/minnow trap (n=3 per habitat type) are reported. Note: Low numbers of fish were caught using the trap method across all sample times and bays.

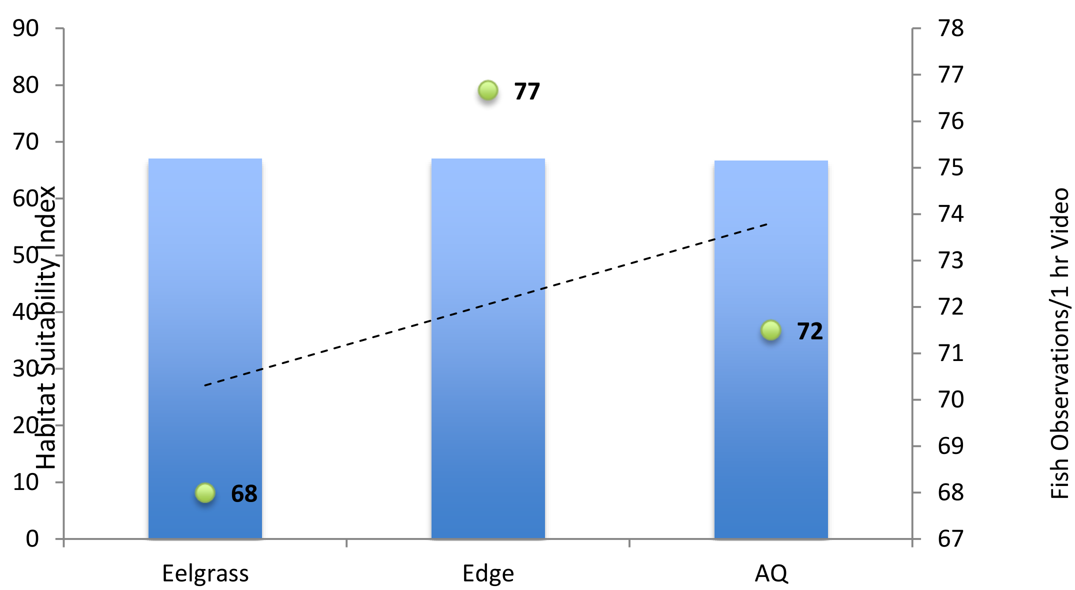


Figure 25. Habitat Suitability Indices for Willapa Bay based on data collected during the 2016 project period. Only epibenthic metrics were used for these calculations as eelgrass data collected to inform the Relative Value Indices were lost. The index values are paired with the mean # of fish observations made from 1hr of video calculated based on the number of cameras deployed within each habitat type: EG (n=3), ED (n=3), AQ (n=2).

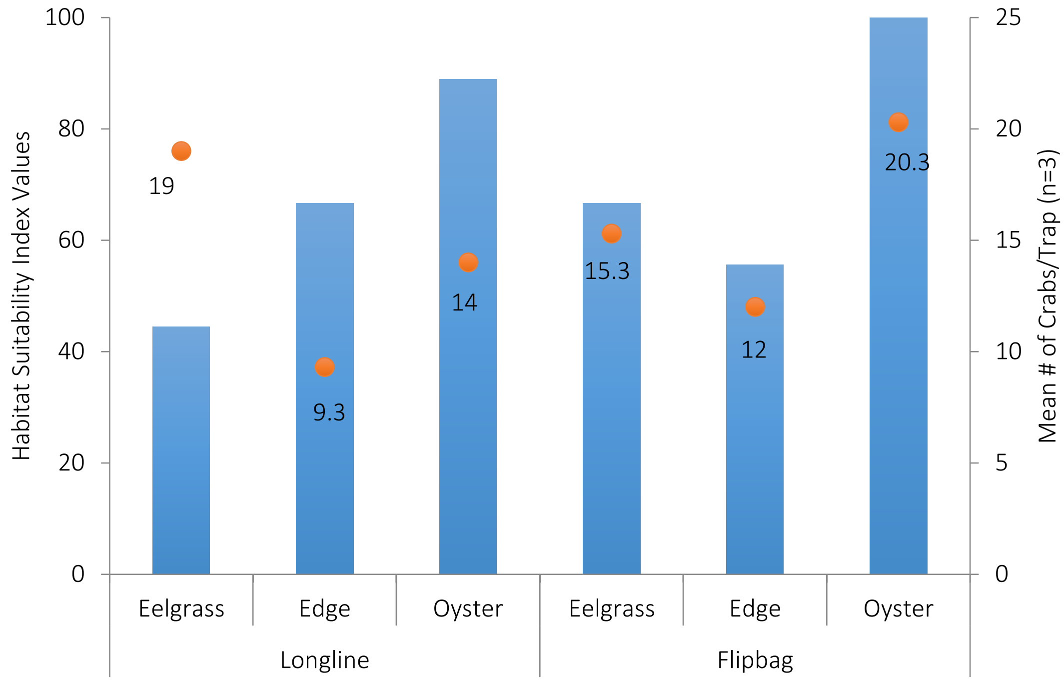


Figure 26. Habitat Suitability Indices for Samish Bay based on data collected during the 2017 project period. Sampling was conducted at two sites: Jerry's Bar (long-line culture) and Oyster Creek (flip-bag culture). Only epibenthic metrics were used to calculate the Habitat Suitability Index for this sample period. Fish observations were very low in video and traps, therefore mean # of crabs (*H. oregonensis* and *Pagurus spp*.) caught in traps (n=3, per habitat) are presented to show mesograzer activity across habitats.

Our comparisons among habitat type reflect one snapshot in time. In general, the distribution and abundance of the species described for this research and previous studies are aligned closely with the morphological and biological complexities and environmental characteristics of the associated habitats. However, certain conditions (e.g. water quality, sediment composition and local predation pressure) vary from site to site and therefore make it difficult to extrapolate index values across a broader spatial scale. Furthermore, these biological communities are driven by seasonal and inter-annual variability that are difficult to capture during one or two sampling events. If this tool is to be applied at the farm-scale to assess habitat value, it is recommended that multiple locations be measured and assessed for a mean, farm-scale value. We further acknowledge that our index of habitat suitability is limited to the data we were able to collect and analyze during the project period and is therefore limited in scope and application. It should be viewed as a model, as opposed to absolute values. Our methods are replicable and the simplified calculations for the index allow for additional parameters to be ranked and included in the model to accommodate site specific variables and organisms/communities of interest.

Problems

Environmental DNA (eDNA)

We were unable to complete a proposed eDNA task due to an unresolved problem in the laboratory analysis. This does not affect the overall outcome of our study and should be resolved at a later date.

Additional Work

An additional study was conducted during the summer of 2017 to compare use of long-line and on-bottom aquaculture habitats in Willapa Bay, WA where these two culture methods are more commonly used side by side than in some of the estuaries reported here (e.g. Humboldt Bay). Transects running perpendicular to the boundary between aquaculture and eelgrass beds were sampled at three sites. The sampling array included a survey of eelgrass density, eelgrass sampling, minnow traps, predation tethering units, and digital video collection. At each site, transects were set up in both long-line aquaculture and on-bottom aquaculture adjacent to eelgrass to allow for a direct habitat comparison between the two culture methods and to adjacent eelgrass. Results from the video data suggest a functional similarity between long-line aquaculture and eelgrass, as assessed by the number of fish observed in video in each habitat. Fish sightings in on-bottom aquaculture were fewer than those made in long-line aquaculture and eelgrass with shiner perch once again dominating the observations. Staghorn sculpin however, were more abundant in both culture areas than in adjacent eelgrass and also appeared to contribute to an edge effect. Eelgrass density data suggest a correlation with available habitat structure, as additional eelgrass present contributed to vertical structure in the long-line aquaculture and there was less present in the on-bottom aquaculture. Predation assessed using predation tethering units, followed similar patterns, with less predation occurring in on-bottom aquaculture than that in long-lines. This study formed the basis of a Master's degree in Marine Resource Management at Oregon State University and a more detailed discussion can be found in the associated thesis (Muething 2018).

Evaluation

All of our goals and objectives were attained. We were able to extend the proposed studies one additional season and complete additional and replicated experiments. We obtained good field data for use in HSI applications and updates of spatial modeling. Finally, we coordinated presentations of the field observations and analyses with the team members, and presented the project findings at meetings for growers, researchers and natural resource agencies, as detailed below. No significant modifications were made to the goals and objectives. We extended the fieldwork for one season to allow additional benthic and epibenthic sampling, and coordination between this project and a follow-up SK study in Humboldt Bay (NA16NMF4270254).

Dissemination of Project Results

On-going project results were presented at:

Cheney, D., Hudson, B., Dumbauld, B., Cordell, J., Toft, J. and F. Nash. 2018. Quantifying Ecosystem Functions in Mixed Oyster Culture and Seagrass/Macroalgae Habitats. 110th Meeting of the National Shellfisheries Association. March 18-22, 2018. Seattle, WA.

Clarke, L., F. Nash, and B. Dumbauld. 2016. An Examination of the Use of Seascape Scale Habitats Including Oyster Aquaculture and *Zostera marina* by Fish and Crab in US Pacific Northwest Estuaries. PCSGA/NSA annual conference, presentation, Lake Chelan, WA.

Clarke, L., F. Nash, and B. Dumbauld. 2016. An Examination of the Use of Seascape Scale Habitats Including Oyster Aquaculture and *Zostera marina* by Fish and Crab in US Pacific Northwest Estuaries. State of The Coast Conference, poster, Lincoln City, OR.

Clarke, L., F. Nash, and B. Dumbauld. 2017. An Examination of the Use of Seascape Scale Habitats Including Oyster Aquaculture and *Zostera marina* by Fish and Crab in US Pacific Northwest Estuaries. NSA annual conference, presentation, Knoxville, TN.

Dumbauld, B., L. Clarke, F. Nash, and B. Hudson. 2017. Do fish recognize shellfish aquaculture and eelgrass as intertidal landscape features and can we tell? PCSGA/NSA-PCS Annual Conference. Welches, Oregon.

Dumbauld, B.R. and S. Rumrill. 2017. Oysters in Oregon. State of The Coast Conference, panel presentation, Florence, Oregon.

Hudson, Bobbi. 2016. Seagrass & Shellfish: Measuring Habitat Use in West Coast Estuaries. PCSGA/NSA annual conference, presentation, Lake Chelan, WA.

Hudson, B. 2017. Aquaculture & Eelgrass, Does Habitat Suitability Differ for West Coast Species of Interest? PCSGA/NSA-PCS Annual Conference. Welches, Oregon.

Muething, K., F. Nash, and B. Dumbauld. 2017. Comparing fish and crab use of the aquaculture/eelgrass (*Zostera marina*) boundary for two different methods of Pacific oyster (*Crassostrea gigas*) aquaculture. State of The Coast Conference, poster presentation, Florence, Oregon.

Muething, Kelly. 2018. On the Edge: Assessing Fish Habitat Use Across the Boundary between Pacific Oyster Aquaculture and Eelgrass in Willapa Bay, Washington. Defense for MSc in Marine Resource Management. Oregon State University. May 23, 2018. Corvallis, OR.

Manuscripts include:

Clarke, L. M. 2017. Functional comparison of longline oyster aquaculture and eelgrass (*Zostera marina L.)* habitats among Pacific Northwest estuaries, USA. MS thesis, Oregon State University 71p.

Muething, K.A. 2018. On the edge: Assessing fish habitat use across the boundary between Pacific oyster aquaculture and eelgrass in Willapa Bay, WA. MS thesis, Oregon State University, 74p.

Additional project publications in preparation include:

QUANTIFICATION OF ECOSYSTEM SERVICES PROVIDED BY EPIFAUNA IN MIXED OFF-BOTTOM OYSTER CULTURE AND SEAGRASS/MACROALGAE HABITATS

A draft manuscript was prepared by Houle, et al. for internal and external peer review with the goal of submission to the Journal of Shellfish Research, Estuaries and Coasts, or Aquaculture Environment Interactions.

NEKTON USE OF SHELLFISH AQUACULTURE AND EELGRASS AS FEATURES OF INTERTIDAL LANDSCAPES IN US WEST COAST ESTUARIES

A draft manuscript was prepared by Clarke, et al. for internal and external peer review with the goal of submission to Estuaries and Coasts or Aquaculture Environment Interactions.

References

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