

Developing a Habitat Suitability Index to Compare Off-Bottom Oyster Culture and Eelgrass (*Zostera marina*) Beds in Pacific Northwest Estuaries

Submitted to: Washington Sea Grant

July 30, 2020



By: Pacific Shellfish Institute

Katie Houle, Daniel Cheney, Bobbi Hudson



Table of Contents

List of Figures	3
List of Tables	4
Introduction	5
Methods	6
Refining Parameters	7
Complex Structure	7
Prey Availability	8
Utilization	8
Results	9
Conclusions & Recommendations	20
References	22

List of Figures

Figure 1. Pacific Northwest estuaries selected as study sites for collected data.....	5
Figure 2. Study design in habitat gradient: oyster long-line culture, edge and eelgrass	9
Figure 3. Metrics calculated along 50m benthic transects.....	10
Figure 4. Habitat Suitability Indices generated for three regional bays.....	11
Figure 5. Spring 2017 eelgrass shoot density	13
Figure 6. Habitat Suitability Indices generated for two management scenarios.....	15
Figure 7. Abundances of epibenthic invertebrates sampled in April 2017	16
Figure 8. Habitat Suitability Indices generated for two management scenarios.....	19

List of Tables

Table 1. Two-way ANOVA results from indicator response variables and two factors, habitat (eelgrass, edge, long-line oyster culture) and bay.....	18
Table 2. Mean values and $\pm 1SE$ for indicator response variables tested for analysis of variance (ANOVA).....	19

Introduction

Shellfish aquaculture activities, including new farm sites, expansions, new gear and/or farming methods, are required to obtain numerous federal, state and county permits. Resource managers must assess environmental impact of these activities in complex intertidal habitat that overlaps with native seagrass (*Zostera marina*), which is designated as Essential Fish Habitat (EFH) for multiple Pacific salmon species in Washington State (Pacific Fishery Management Council 2008) and for species within the Pacific Coast Groundfish Fisheries Management Plan. Some jurisdictions' interpretation of seagrass differs, as does the buffer distance between shellfish culture and native seagrass, creating inconsistencies in the process. Furthermore, no-net-loss provisions in federal and state regulations have resulted in a precautionary approach by managers, impeding shellfish production on the west coast. In response to this issue, PSI led a collaborative team of scientists in a comparative habitat analysis of off-bottom oyster culture and seagrass beds from 2016-2018 (Hudson et al. 2018, NOAA grant no. NA15NMF4270318). In an effort to develop a regionally applicable tool for resource managers, PSI developed a biotic index referred herein as the Habitat Suitability Index (HSI), to objectively assess habitat suitability of shellfish aquaculture for critical species of fish and invertebrates.

The HSI is a simplified model using biological indicators of ecosystem functions relevant to Pacific coast species of juvenile salmonids, juvenile English sole and Dungeness crab. By design, the suite of indicators selected for target species are also critical for survival and growth of many other estuarine species. This method of selecting a suite of indicators as opposed to focusing on a single indicator (e.g. eelgrass presence/absence) or metric (e.g. eelgrass shoot density) is an improvement over other established methods to assess habitat suitability for nekton in aquaculture. We believe the HSI, as a management tool, moves us toward a more holistic, ecosystem-based approach to habitat assessment in shellfish aquaculture in our region. Biotic indices have been widely used in freshwater, wetland and terrestrial environments to compare a range of habitat types and system responses to change over time or anthropogenic disturbances (Zhao et al. 2016, Beck and Hatch 2009, Whittier et al. 2007). Globally, biotic indices in coastal, marine and estuarine environments are still developing and have leaned on established indices or approaches from other systems to create tools appropriate for saltwater



Figure 1. Pacific Northwest estuaries selected as study sites for collected data, summer 2016-spring 2017.

habitats (Borja et al. 2009, Borja and Dauer 2008, Diaz et al. 2004, Rosenberg et al. 2004). The HSI we developed borrows conceptually from existing benthic indices and mechanistically from Habitat Evaluation Procedure (HEP) to assess level of suitability for critical species. The HEP method has been in place since the mid-1980's as a simple tool developed by the U.S. Fish and Wildlife Service (USFWS)(USFWS 1980a, USFWS 1980b) and is employed by the U.S. Army Corps of Engineers and other resource developers to allow the integration and comparison of complex environmental variables (Barnes et al 2007, Vincenzi et al. 2006, Entz 2005, Cheney et al. 1994, Brown and Hartwick 1988). This procedure calculates one score that can be used to compare habitat suitability in sampled seagrass, shellfish and unstructured habitats. The synthesis of these approaches led to the construction of the novel HSI for intertidal bivalve aquaculture in the Pacific Northwest. The following summarizes improvements made to the HSI since the culmination of prior work (Hudson et al. 2018), data outputs generated from two management scenarios, discussion of results, and recommendations for next steps in furthering the development and application of this management tool.

Methods

The following equation is used to determine the foundation or benthic (BSI) portion of our Habitat Suitability Index:

$$\text{(Existing habitat increment condition / Optimum habitat increment condition)} \times 100$$

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

NBT - Number of benthic taxa

DHS - Density of total Harpacticoids (#/m²)

DPS - Density of total Peracarids (#/m²)

Epibenthic richness and density of select prey groups are quantified for each strata being compared and then ranked 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:

$$\text{(NBT + DHS + DPS)} \times \text{RVI}$$

To provide examples of possible RVI, the following parameters were included in initial analyses from Hudson *et al.* 2018: 1) structural complexity of native eelgrass (*Z. marina*), 2) epiphyte loads on *Z. marina* blades and 3) macroalgal (typically *Ulva* and *Enteromorpha* species) density. These indicators were selected to trial the mechanics of the index during the project period and did not fully represent all collected data or all possible support functions critical for growth and survival of target species. The core of the work completed during this project period was

refining parameters included in the RVI to assess ecological value of cultured vs. non-cultured habitats for target nekton.

After refining parameters, a final analysis was completed to examine the robustness of parameters included in the HSI. Two-way Analysis of Variance (ANOVA) tests were applied to indicator response variables with two factors, habitat (eelgrass, edge, long-line oyster culture) and bay. Four sampling events from oyster long-line culture sites were included in this analysis: Humboldt Bay, CA, Samish Bay, WA, Tillamook Bay, OR from summer 2016 and Samish Bay, WA from spring 2017. Data included in the analysis met assumptions of ANOVA. Data were either normally distributed or log transformed. Levene's Test was used to confirm all data had equal variance. Tukey's HSD post-hoc tests were used to determine factor interactions with significant p-values.

Refining Parameters

- 1. Complex Structure** – provides juvenile fish and invertebrates refuge from predation while resting, transiting and foraging in nearshore habitats

Indicators: Leaf Area, Macroalgae Cover, Oyster Gear Type

It's well documented that structured habitats within estuaries including marshes, seagrass and other submerged aquatic vegetation (SAV) provide trophic resources and predator refugia, allowing small fish and invertebrates to reach greater density and diversity relative to unstructured habitats such as open mudflat (Plummer et al. 2013, Fresh 2006, Heck et al. 2003, Minello 2003, Deegan et al. 2000, Mattila et al. 1995, Orth et al. 1984). Structure formed by other organisms such as oysters has also been documented to contain diverse and abundant nekton (Ferraro and Cole 2010, Stunz et al. 2010, Coen and Grizzle 2007, Dumbauld et al. 2005, Glancy et al. 2003, Lehnert and Allen 2002, Minello 1999). Novel structures created by intertidal oyster aquaculture have also been documented to create habitat for a diverse array of fish and invertebrate species (Muething 2018, Clarke 2017, Dumbauld et al. 2015, Coen et al. 2011, Dumbauld et al. 2009, Hosack et al. 2006, Dealteris et al. 2004, Coen et al. 1999). Oyster culture methods assessed in this study were long-line and flip-bag systems, both now widely used in U.S. west coast estuaries.

Z. marina leaf area (cm²) as an indicator was calculated as the mean max leaf area = length (cm) x width (cm), from 20 randomly collected shoots along each 50m. benthic transect. We use max leaf area as an indicator of eelgrass health, light regime, inferred canopy cover, and potential surface area for epiphytic algae (McMahon et al. 2013). Macroalgae density was determined in the field using areal percent cover in a 0.0625 m² quad within the same plot as *Z. marina* shoot density was determined. In summer months, macroalgae recruits into shellfish growing areas, especially where eelgrass is sparse. This creates some habitat complexity in the benthic environment often inhabited and foraged on by epibenthic invertebrates and mesograzers (Cheney et al. 1994).

2. **Prey Availability** – foraging potential for juvenile salmonids, juvenile flatfish and crab species

Indicators: Epibenthic Taxa Richness, Harpacticoid Density, Peracarid Density, Epiphyte Load

The foundation of our index focuses on epibenthic invertebrate taxa occurring in intertidal shellfish culture and seagrass habitats in Washington, Oregon and Northern California estuaries. These small invertebrates, also referred to as meiofauna, are the base of the estuarine food web serving as essential prey for shorebirds, benthic macroinvertebrates and many species of fish. They are important indicators of ecosystem health, which is why numerous studies have identified the importance of including these groups of organisms in long-term environmental monitoring efforts (Schratzberger and Ingels 2018, Villnas and Norkko 2011, Coull 2009, Ferraro and Cole 2007, Simenstad and Cordell 2000, Kennedy and Jacoby 1999, Gee 1989, Simenstad et al. 1988). Our index integrates three epibenthic invertebrate metrics: taxa richness, density of harpacticoid copepods and density of peracarids (e.g. isopods, amphipods and cumaceans). These two primary groups of invertebrates are included because they are considered essential prey for juvenile salmonids, chum and Chinook specifically (Simenstad and Cordell 2000, Simenstad et al. 1982), and juvenile English sole (Gunderson et al. 1990, Rogers 1988, Toole 1987, Toole 1980). In addition to epibenthic invertebrates, we include epiphytes growing on *Z. marina* eelgrass blades. Epiphytes are sessile organisms and algae that settle on eelgrass blades and are grazed on by mesograzers including shorecrabs, juvenile Dungeness crabs (Jensen and Asplen 1998) and many of the epibenthic invertebrates we are targeting in these habitat types, that in turn provide food for larger predators (Reynolds et al., 2018, Thomsen et al. 2018, Hovel et al. 2016, Ruesink 2016, Cullen-Unsworth and Unsworth, 2013). Epiphytes are quantified by scraping eelgrass blades, drying the contents, collecting weights and dividing that value by the total dry biomass of the eelgrass blade. The following equation is used to calculate epiphyte load: $Epiphyte\ Load = \frac{dry\ weight\ of\ epiphytes}{dry\ weight\ eelgrass}$. Additional details for processing epibenthic invertebrates, epiphytes and eelgrass are available in Hudson *et al.* 2018.

3. **Utilization** – realized use of habitats by resident fish and crab species serve as indicators of active food web connectivity

Indicators: Fish Abundance, Crab Abundance

Two methods were used to quantify fish and crab use in these habitats. One method included deploying small, 1m x 1m box minnow traps (n=3/habitat type) at low tide that fished for 6 hours and retrieved after the high tide during spring and summer sampling events. Fish and crab were identified, counted and total lengths or carapace widths measured. Crab abundances for the HSI scenarios were extracted from these datasets utilizing this method of capture and quantification. The second method included a series of replicated underwater GoPro video cameras in each habitat (n=3). Cameras were attached to a PVC frame and snorkeled into the location. Cameras recorded video for ~2.5 hours around the high tide while the traps were fishing. Video data went through an initial quality index review. After review, it was determined that the middle hour of video had the best visibility and therefore selected for analysis. Video

data was processed by biologists using the free online software BORIS (<https://boris.readthedocs.io/en/latest/>) to code species presence and behavior categories. Behavioral categories were entered into BORIS as point events. Point events tagged the start time of the activity associated with the observed fish or crab. Common behavior categories included: transit, forage, fight, school, and refuge. The vast majority of fish were seen transiting in the videos followed by foraging, and then limited schooling, refuge and fighting behaviors. Behavior data extracted from this method ended up not being informative enough to serve as a functional indicator or RVI for the HSI. We were however able to export the point events into Excel to develop the fish abundance parameter for the HSI. Smaller crabs (e.g. juvenile Dungeness and shore crabs) caught by minnow traps were not readily visible using the underwater camera methods. Due to our interest in juvenile Dungeness crabs, we selected the trap data to develop the crab abundance parameter versus the video data which only provided information on larger adult crabs. The non-invasive method of underwater video seemed to provide a more realistic view of fish species composition and abundances in the habitats observed versus minnow traps that are limited at a fixed depth, trap opening and bias of attracting certain types of fish more readily, e.g. Pacific staghorn sculpin (Dumbauld, B., *pers. comm.*).

Results

Two management scenarios were constructed using the HSI to model possible application and adaptability of the tool:

$$\text{Scenario \#1} = (\text{BSI} * \text{LA} * \text{EP} * \text{MA}) / \text{MAX} * 100$$

This scenario prioritizes primary production, e.g. *Z. marina* leaf area (LA), epiphytic algae (EP), macroalgae cover (MA) and low-trophic level production of epibenthic invertebrates (BSI).

$$\text{Scenario \#2} = (\text{BSI} * \text{LA} * \text{EP} * \text{FA} * \text{CA}) / \text{MAX} * 100$$

This scenario includes nekton abundance of resident juvenile fish (FA), small/juvenile crab abundances (CA), primary production (LA, EP) and low-trophic level production of epibenthic invertebrates (BSI). It excludes macroalgae cover (MA).

The following results apply and compare the two management scenarios of the HSI across a habitat gradient: off-bottom oyster culture -> edge -> dense eelgrass (Figure 2). Figure 3 provides baseline information on field collected data from benthic surveys conducted at each estuary including the following metrics, a) *Z. marina* shoot density, b) macroalgae % cover, c) epiphyte % cover and d) epiphyte loads on *Z. marina* blades. These datasets helped inform

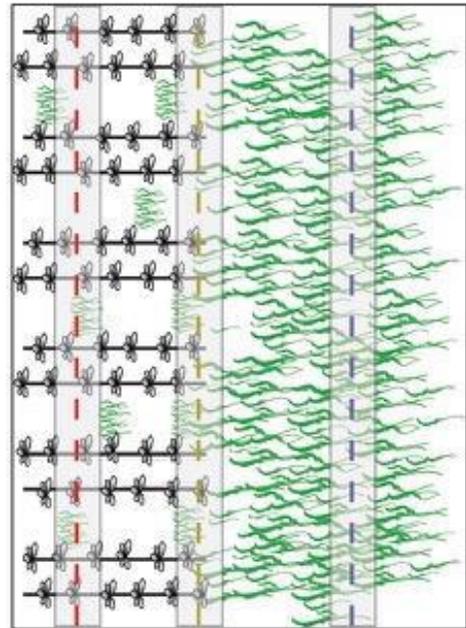


Figure 2. Study design in habitat gradient: oyster long-line culture, edge and eelgrass. Benthic transects were 50m long and at 30m. apart. (Image credit: Dan Sund)

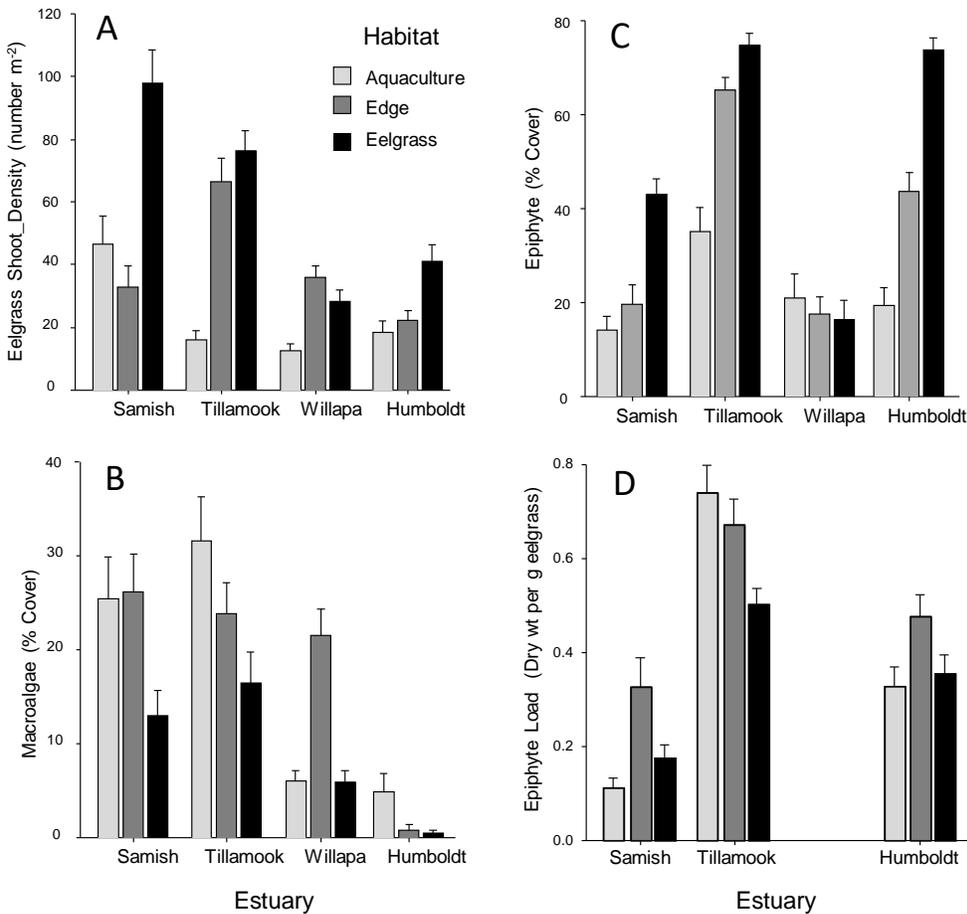


Figure 3. Metrics calculated along 50m benthic transects within each estuary, a) *Z. marina* shoot density, b) macroalgae % cover, c) epiphyte % cover, and d) epiphyte load on *Z. marina* blades (Hudson et al. 2018).

parameter selection for the HSI and interpretation of results.

Figure 4 demonstrates the two management scenarios and calculated Habitat Suitability Indices generated for three regional bays: a) Tillamook Bay, OR, b) Humboldt Bay, CA and c) Samish Bay, WA in oyster long-line culture. Index values for each habitat type are relative to one another within a single sample location and are not compared across bays in this analysis. Absolute values are less relevant than the curves produced along the habitat gradient from eelgrass -> edge -> aquaculture. In management scenario #1 (primary production/low-trophic), we see three different outcomes in each bay along this gradient. In Tillamook, OR (Figure 4a), the edge habitat is nearly twice the projected value of the eelgrass bed and the value is significantly reduced in oyster culture. While the BSI or benthic rating is comparable across the gradient, several metrics differ driving the differences seen here. Eelgrass shoot density in

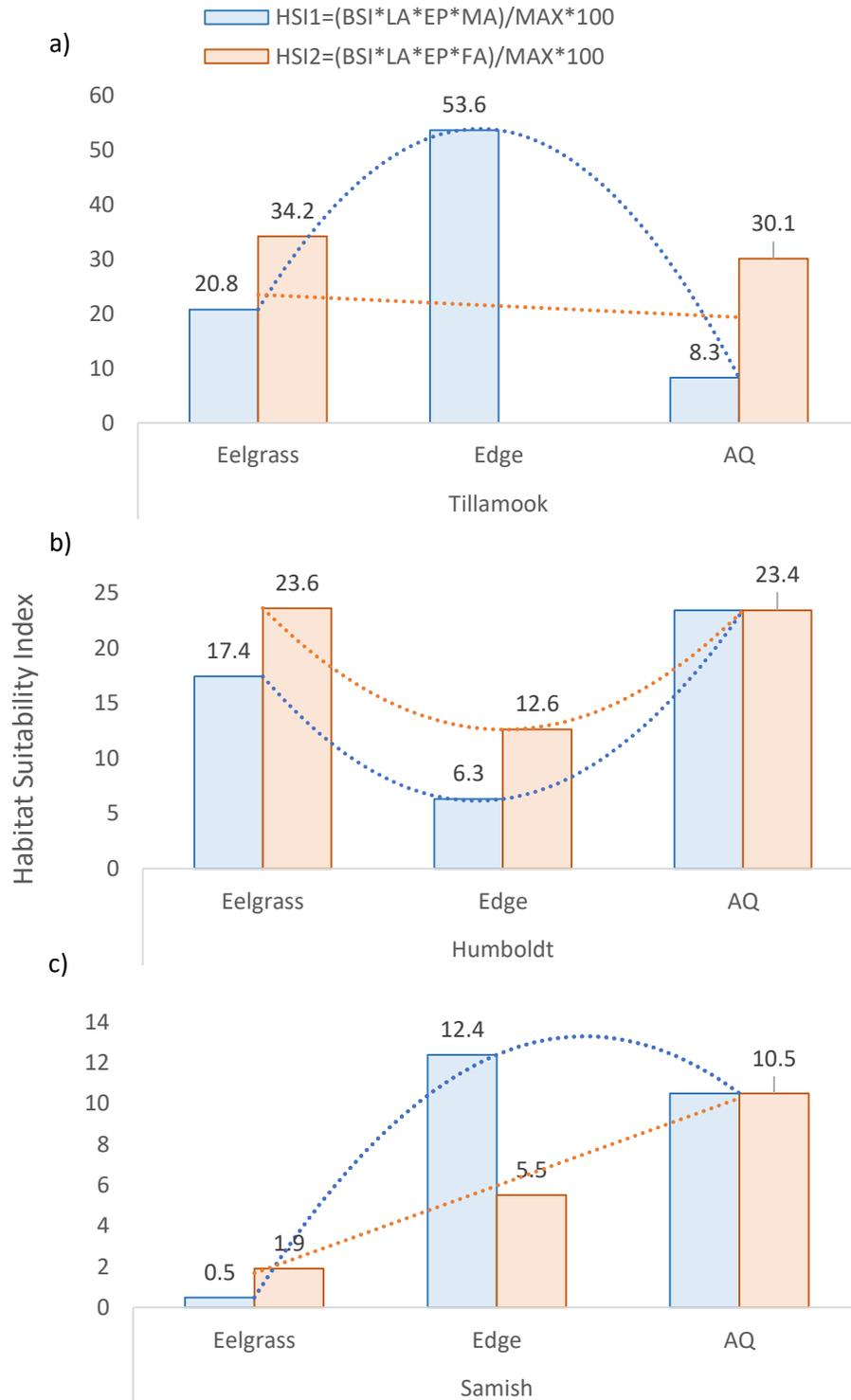


Figure 4. Habitat Suitability Indices generated for three regional bays: a) Tillamook Bay, OR, b) Humboldt Bay, CA and c) Samish Bay, WA. HSI functions represent two management scenarios. Scenario #1 = $(BSI \cdot LA \cdot EP \cdot MA) / MAX \cdot 100$, prioritizes primary production, e.g. *Z. marina* leaf area (LA), epiphytic algae (EP), macroalgae cover (MA) and low-trophic level production of epibenthic invertebrates (BSI). Scenario #2 = $(BSI \cdot LA \cdot EP \cdot FA) / MAX \cdot 100$, includes nekton abundance of resident juvenile fish (FA), primary production (LA, EP) and low-trophic level production of epibenthic invertebrates (BSI).

oyster culture is relatively low ($9.6/m^2$) compared to edge ($30/m^2$) and dense eelgrass ($43/m^2$).

Shoot density is not explicitly included as a metric in the HSI calculation, however max leaf area (cm^2) is and values indicate some shading effects from the long-lines at this location may have reduced leaf size. Interestingly, epiphyte loads (d.w. epiphytes/d.w. eelgrass biomass) however were highest in oyster culture compared to edge or eelgrass. Macroalgae cover was also low in oyster culture (6%) and eelgrass (8%) compared to edge (22%), further depressing the HSI value for oyster culture. When management scenario #2 (primary production, low trophic & nekton) is considered, oyster culture is fairly comparable to eelgrass, namely due to the inclusion of fish abundances, high epiphyte loads and comparable epibenthic invertebrate metrics. No fish data was available for the edge habitat at this location and therefore not included in scenario #2 analysis. This example from Tillamook Bay illustrates the possibility of different outcomes based on parameter selection and prioritization from resource managers. Figure 4b. describes two relatively similar outcomes from both management scenario #1 and scenario #2 for Humboldt Bay, CA. Oyster culture performs slightly better or comparably at this location to eelgrass, and both relatively outperform edge habitat. In Samish Bay, WA (Figure 4c.) management scenario #1 and #2 rank eelgrass comparatively low to both edge and oyster culture with HSI values increasing dramatically from eelgrass -> edge -> in scenario #2 and edge slightly higher than oyster culture in scenario #1. Depressed values in eelgrass at this location are a result of very low macroalgae cover (0.8%), slightly lower epibenthic values and very low fish abundances compared to edge and oyster culture. It's possible that the high shoot density ($128/m^2$) at this site had some effect on the underwater camera methods ability to accurately quantify fish in the eelgrass due to reduced visibility. While minnow traps were deployed at the same time as cameras, they collected very few fish at this location and could therefore not be included in the analysis as a complimentary method to characterize fish use.

In spring 2017, data was collected at two adjacent sites within Samish Bay, WA that had two methods of off-bottom oyster culture: flip bags and long-line culture. Within each of these gear types the same methods to assess benthic environment and nekton were used as summer 2016 sampling efforts. *Z. marina* shoot densities from benthic surveys along the habitat gradient during the sample period are illustrated in Figure 5. Collectively these datasets enabled the production of Habitat Suitability Indices under the two management scenarios with slight modification. In Figure 6, both scenarios do not include macroalgae as it was not present during the spring sample period. In scenario #2, crab abundances are included from minnow trap collections, but fish video data was not available for this time point due to inclement spring weather.

Results from this analysis indicate an increasing trend from eelgrass -> edge -> oyster culture at both flip-bag and long-line sites for scenario #1. Oyster flip-bags ranked the highest for both

scenario #1 and scenario #2. High peracarid invertebrate densities and crab abundances increased the index value of oyster flip-bags. While shoot density and leaf area in flip-bags was lower than eelgrass and edge, epiphyte loads on sampled eelgrass blades in flip-bags ranked highest. Oyster long-lines ranked slightly higher or comparable to eelgrass at both locations. Edge at the oyster long-line location was not analyzed due to

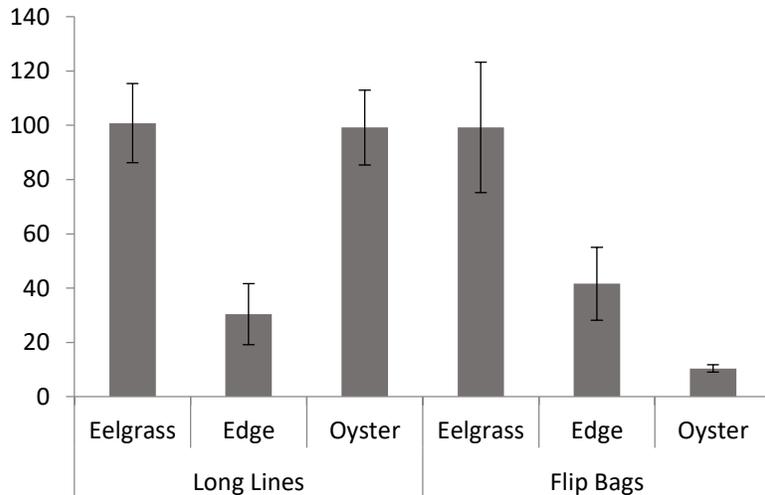


Figure 5. Spring 2017 eelgrass shoot density (#shoots/m²) at oyster long-line and flip-bag farm sites in Samish Bay, WA

epiphyte samples contaminated with sediment. It is notable that the two locations differed significantly in invertebrate species composition (Figure 7a-b), likely due to differences in salinity, elevation or sediment. This makes a true comparative analysis of these gear types challenging for this sample period. More data is necessary to draw any significant conclusions between gear types. For the purposes of this report, Figure 6 should be considered a model for future applications given robust data collection.

Results of the ANOVA are included in Table 1. A total of eight indicators were tested: Epibenthic Taxa Richness, Harpacticoid Density/m², Peracarid Density/m², Macroalgae Cover, Leaf Area, Epiphyte Load, Fish Relative Abundance, and Crab Abundance. Of the eight indicators only one, leaf area had statistically significant differences between habitat types, eelgrass > oyster (p=0.05). Five out of eight indicators had statistically significant differences between bays and there was indication that sample time (spring vs summer) may drive some of these differences, but this aspect would need to be explored further with future data collection in spring verse summer months. Overall, these results indicate that 88% (7/8) of the indicators used in the Habitat Suitability Index do not vary significantly along the gradient of eelgrass -> edge -> oyster long-line culture, but have more distinct differences spatially between bays and perhaps seasonally.

To further explore the relationship of habitat value across the eelgrass -> edge -> oyster gradient with replication (n=4), the same datasets from the ANOVA analysis were used to generate mean values for each indicator. Table 2 includes the means ±1SE and index values generated for the HSI analysis featuring the two management scenarios: #1 (primary production, low trophic) and #2 (primary production, low trophic and nekton – fish and crab) for the three bays sampled (Figure 8). Scenario #1 shows an increasing trend from eelgrass -> edge and then a slight decrease to oyster culture, however HSI value of oyster is almost twice that of eelgrass. Edge ranks highest in this scenario due to high macroalgae cover and epiphyte

loads. As seen in previous scenarios, eelgrass ranks low due to low macroalgae cover. When macroalgae is excluded from the analysis given scenario #1, HSI values along the gradient increase from oyster (20) -> edge (46) -> eelgrass (82). Given the removal of one indicator in this scenario, habitat value of eelgrass is three times that of oyster culture. This example, among others, throughout the generation of HSI scenarios illustrates the need for a system to weigh indicators based on prioritization of management needs. We did not develop such a system during the project period, but see it as a necessary part of any future development or application of the HSI for intertidal aquaculture. Scenario #2 shows an increasing trend along the habitat gradient: oyster (20) -> edge (27) -> eelgrass (30), when nekton (fish and crab abundance) are included and macroalgae is removed from the model. The outcome of scenario #2 seems more balanced than scenario #1 and in contrast, encompasses multiple trophic levels in the model.

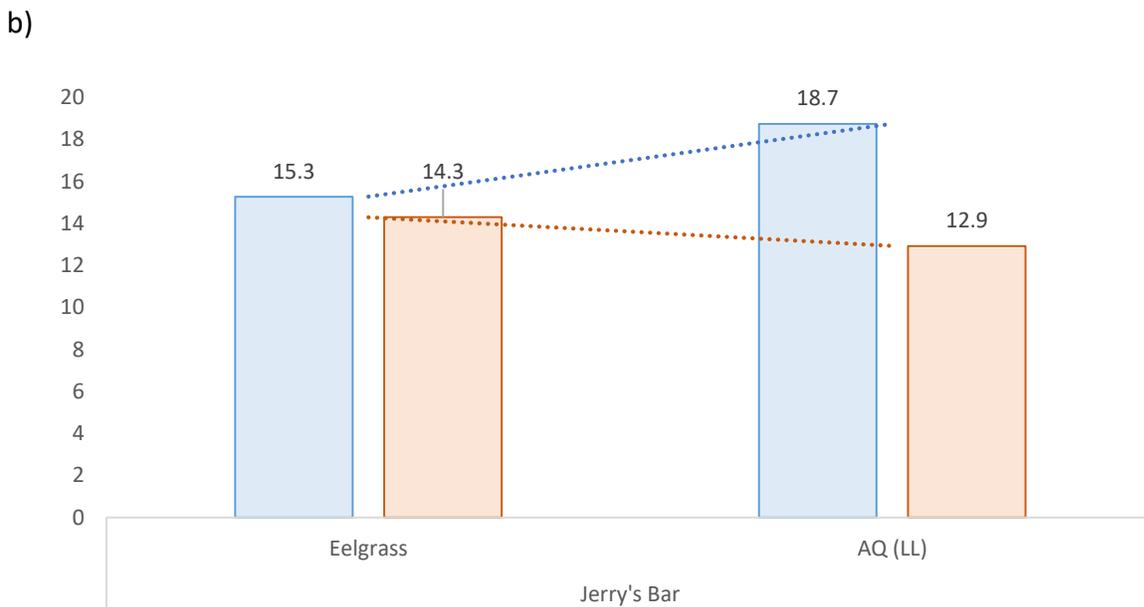
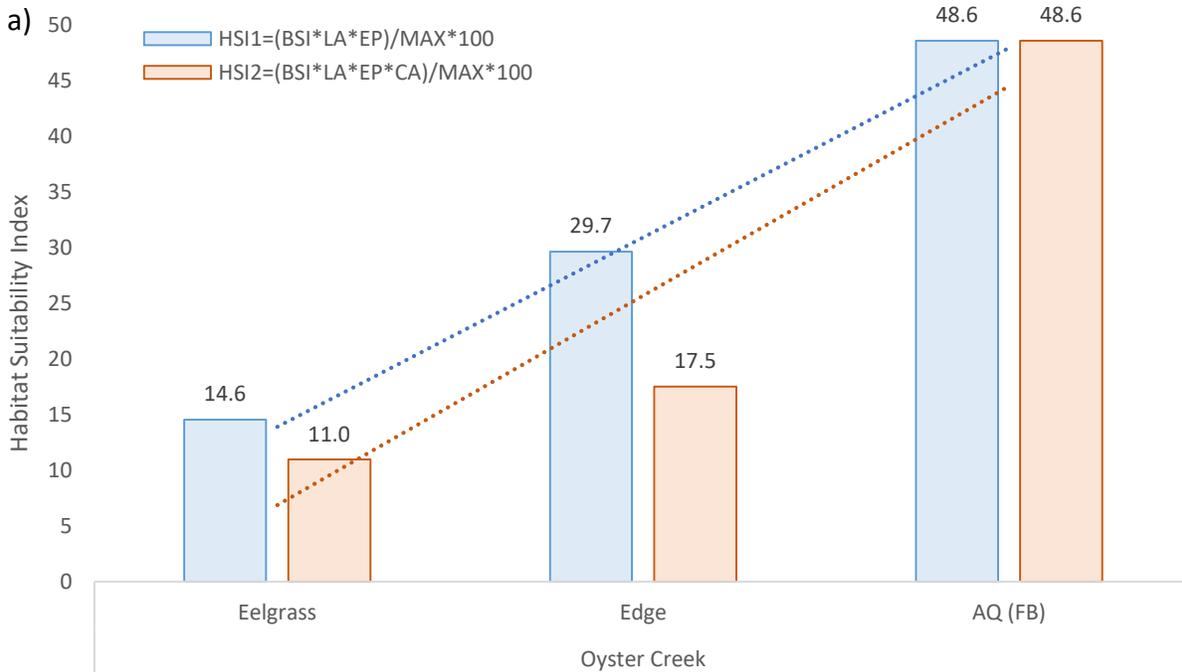


Figure 6. Habitat Suitability Indices generated for two management scenarios for two off-bottom oyster culture methods: a) flip-bags and b) long-lines in Samish Bay, WA. Field data was collected in April 2017 at two neighboring farm plots. Scenario #1 = $(BSI*LA*EP)/MAX*100$, prioritizes primary production, e.g. *Z. marina* leaf area (LA), epiphytic algae (EP) and low-trophic level production of epibenthic invertebrates (BSI). Scenario #2 = $(BSI*LA*EP*CA)/MAX*100$, includes abundance of resident crab species (CA), primary production (LA, EP) and low-trophic level production of epibenthic invertebrates (BSI).

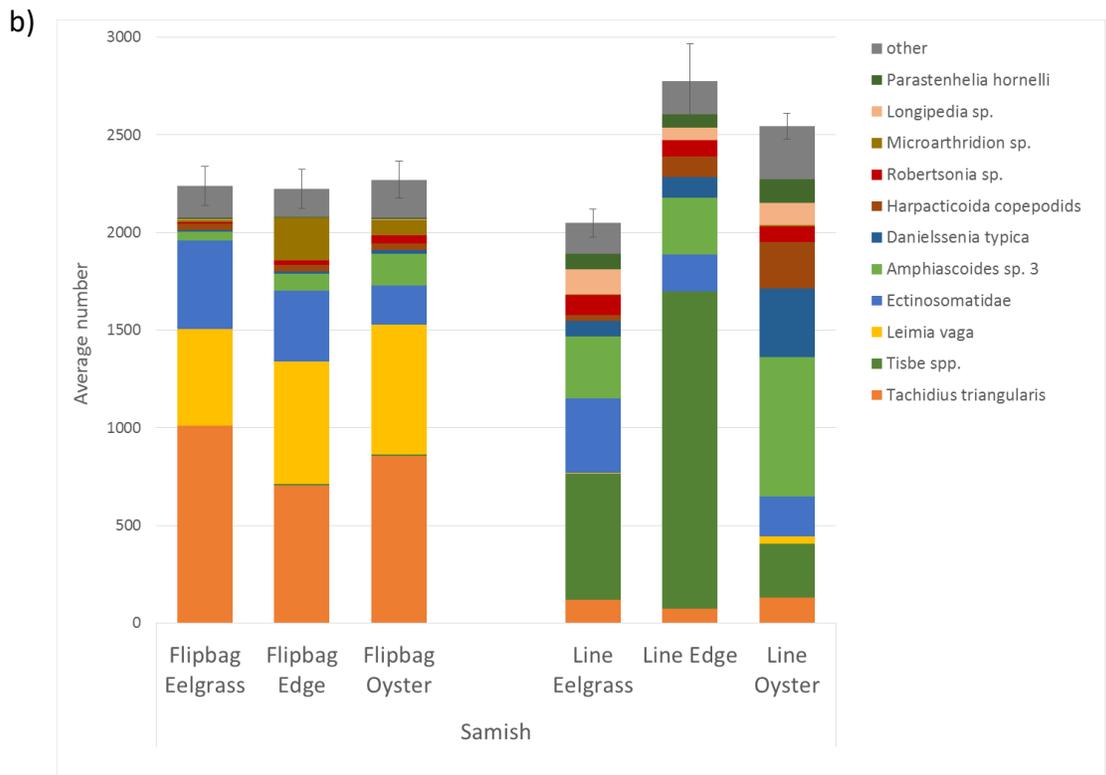
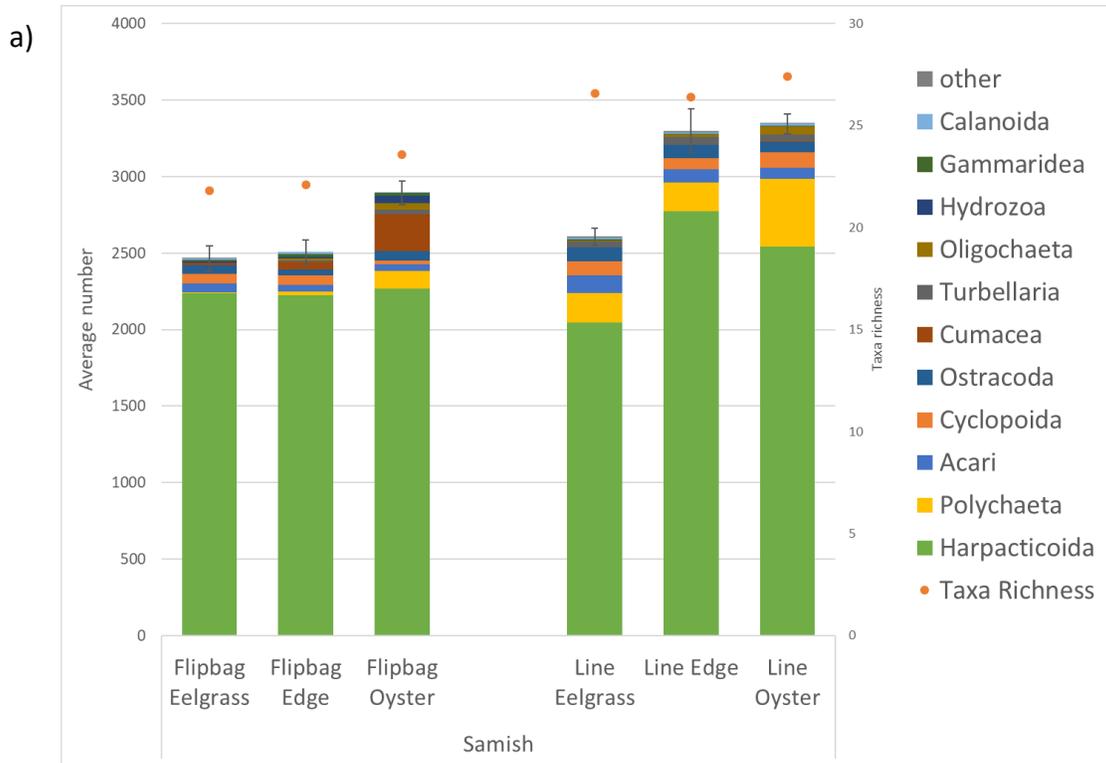


Figure 7. a) Abundances of epibenthic invertebrates sampled in April 2017 in oyster long-line and flip-bag culture sites in Samish Bay, WA. b) Abundances of harpacticoid copepods. Data collection and analysis completed by J. Cordell and J. Toft (UW) (Figures: Hudson *et al.* 2018).

Table 1. Two-way ANOVA results from indicator response variables and two factors , habitat (eelgrass, edge, long-line oyster culture) and bay. Results for Tukey’s HSD post-hoc tests shown for factor interactions with significant p-values.

Factors	Indicator	<i>df</i>	MS	<i>F-value</i>	<i>p</i>
Habitat	Epibenthic Taxa Richness (NBT)	2	0.00146	0.295	0.754
Bay		3	0.04877	9.860	0.009**
SB17-HB16					0.011
	SB17-SB16				0.016
Habitat	Harpacticoid spp. Density/m ² (DHS)	2	0.0121	0.261	0.778
Bay		3	0.6863	14.756	0.003**
SB17-HB16					0.003
TB16-HB16					0.031
	SB17-SB16				0.013
Habitat	Peracarid spp. Density/m ² (DPS)	2	0.01917	0.179	0.841
Bay		3	0.31125	2.903	0.124
Habitat		2	1.838	2.035	0.212
Bay	Macroalgae Cover (%)	3	3.954	4.376	0.059
Habitat		2	1759	4.778	0.057
Bay	Leaf Area (cm ²)	3	3685	10.007	0.009**
SB17-HB16					0.026
TB16-SB17					0.101
Habitat	Epiphyte Load	2	0.01721	1.543	0.301
Bay		3	0.04426	3.969	0.086
Habitat	Fish Relative Abundance	2	0.10453	2.442	0.182
Bay		3	0.00415	0.097	0.958
Habitat		2	0.294	0.596	0.580
Bay	Crab Abundance	3	4.006	8.118	0.015**
SB16-HB16					0.058
TB16-SB16					0.014

Table 2. Mean values and $\pm 1SE$ for indicator response variables tested for analysis of variance (ANOVA). Associated index values generated for the Habitat Suitability Index analysis for four sample periods during 2016-2017, in three bays: Samish Bay, WA, Tillamook, OR, and Humboldt Bay, CA.

Indicator	Habitat	Means $\pm 1SE$	Index Type	Index Value
NBT	Eelgrass	28.4 \pm 1.7	BSI	3
	Edge	27.6 \pm 0.5		2
	Oyster	28.4 \pm 0.9		3
DHS/m2	Eelgrass	78196 \pm 18804	BSI	3
	Edge	76563 \pm 21580		2
	Oyster	70260 \pm 19216		1
DPS/m2	Eelgrass	1138 \pm 172	BSI	3
	Edge	1092 \pm 341		2
	Oyster	1051 \pm 160		1
Leaf Area (cm2)	Eelgrass	87.7 \pm 22.4	RVI	1
	Edge	61.1 \pm 21.5		0.696
	Oyster	46.3 \pm 11.7		0.528
MA Cover (%)	Eelgrass	2.6 \pm 1.8	RVI	0.138
	Edge	14.6 \pm 7.9		0.789
	Oyster	18.6 \pm 13.8		1
Epiphyte Load (g)	Eelgrass	0.368 \pm 0.064	RVI	0.824
	Edge	0.447 \pm 0.064		1
	Oyster	0.306 \pm 0.097		0.684
Fish RA	Eelgrass	0.20 \pm 0.079	RVI	0.386
	Edge	0.31 \pm 0.09		0.605
	Oyster	0.52 \pm 0.13		1
Crab Abundance	Eelgrass	13.3 \pm 8.2	RVI	0.951
	Edge	13.3 \pm 7.5		0.951
	Oyster	14.0 \pm 6.9		1

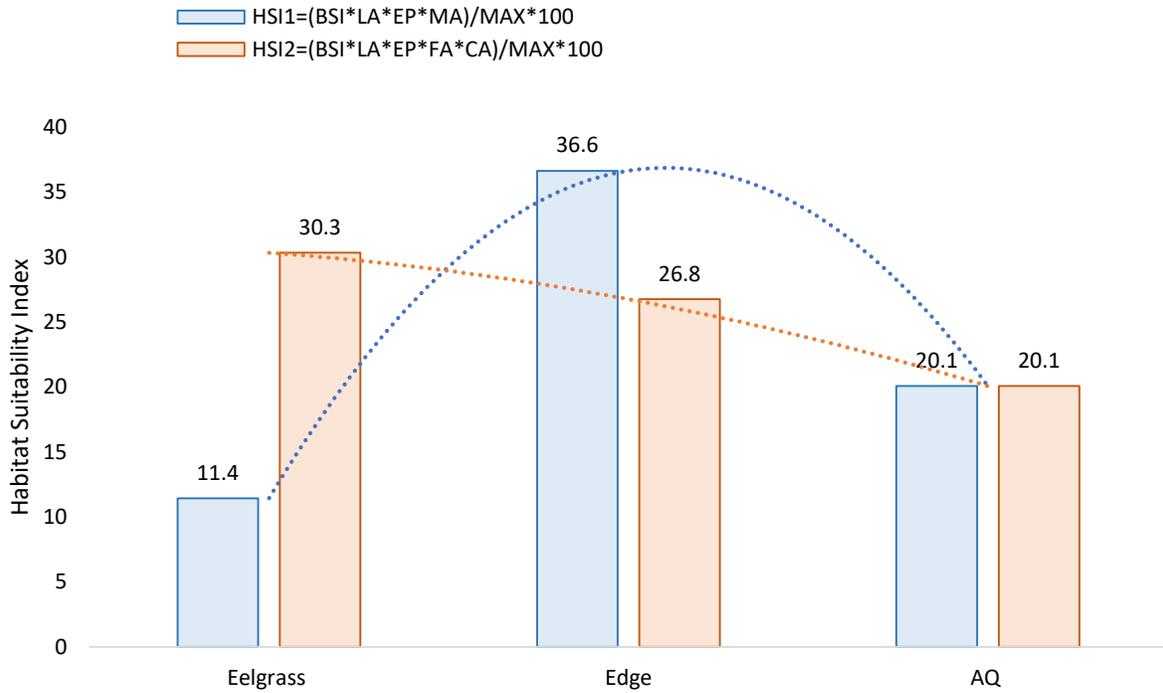


Figure 8. Habitat Suitability Indices generated for two management scenarios. Scenario #1 = $(BSI * LA * EP * MA) / MAX * 100$, prioritizes primary production, e.g. *Z. marina* leaf area (LA), epiphytic algae (EP), macroalgae cover (MA) and low-trophic level production of epibenthic invertebrates (BSI). Scenario #2 = $(BSI * LA * EP * FA * CA) / MAX * 100$, includes nekton abundance of resident juvenile fish (FA) and crab species (CA), primary production (LA, EP) and low-trophic level production of epibenthic invertebrates (BSI).

Conclusions & Recommendations

How to make the Habitat Suitability Index scalable? The initial intention of developing the HSI was to be able to characterize farm plots and surrounding areas in the bay not directly assessed on the ground with survey work. The index values would be translated to “Habitat Units” depending on strata (oyster culture, edge and eelgrass) and overlaid on GIS maps produced for each bay. One challenge of the data collection was the time and cost to speciate the epibenthic invertebrates, which limited the spatial scale and replication of the HSI analysis. Three sites within each regional bay were assessed for all other metrics excluding invertebrates, which could have provided more bay-specific information to scale up the HSI results. To mitigate this issue in any future application of the HSI, the final versions presented in this report include broader categories of invertebrates (e.g. harpacticoid copepod and peracarids) that encompass important prey species for juvenile salmonids and English sole and many other fish species without the need to identify organisms down to the species level. 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 without a robust sampling effort. 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 broader spatial scales (e.g. farm, bay, region) to assess habitat value, it is recommended that multiple locations be measured over several years at important time points (early spring and summer) to determine a mean value. Any level of regional scaling would benefit from the generation of ecologically relevant benchmarks for indicators included in the index. Our analysis simply compared values along the habitat gradient as they occurred at a single time point. The development of regional or bay-specific benchmarks could improve the interpretation and transparency of HSI results.

We acknowledge that the HSI is limited to the data we were able to collect and analyze during the project period and is therefore limited in scope and application. For example, early spring is a critical time for juvenile salmonids and data collected for this analysis largely represents habitat conditions in late spring (May/June) and summer months (July/August) in the Pacific Northwest. Intertidal off-bottom oyster aquaculture and mature dense eelgrass beds of native, *Zostera marina* do not function ecologically in exactly the same way. As evidenced by this analysis however, oyster culture does provide a suite of ecological functions for the estuarine ecosystem, some overlap with existing functions provided by eelgrass, while others are unique and augmented by the presence of cultured oysters and three dimensional structures created from growing methods. Not all ecological functions of eelgrass are provided by oyster aquaculture, specifically detritus from decaying macrophytes that feed the entire system, including cultured bivalves (Conway-Cranos et al. 2015). The HSI should be viewed as a tool for initial scoping and direction for further research and exploration by managers. Values are

represented along a habitat gradient and thus interpreted as a trend and proportional to each other as opposed to absolute values dictating quality of habitat. The methods within 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. During the project period, it was clear that a system to weigh indicators based on specific management needs would be necessary to improve the future application of the HSI in intertidal shellfish aquaculture. Overall, the HSI method of incorporating a suite of ecological indicators representing multiple trophic levels in Pacific Northwest estuaries is an improvement over established methods of habitat suitability for nekton determined by a single indicator or metric. We believe the HSI, as a management tool, moves us toward a more holistic, ecosystem-based approach to habitat assessment in intertidal shellfish aquaculture in our region.

References

- Barnes, T. K., et al. (2007). "A habitat suitability index model for the eastern oyster (*Crassostrea virginica*), a tool for restoration of the Caloosahatchee Estuary, Florida." *Journal of Shellfish Research* 26(4): 949-959.
- Beck, M. W., & Hatch, L. K. (2009). "A review of research on the development of lake indices of biotic integrity." *Environmental Reviews*, 17: 21-44.
- Borja, A., Ranasinghe, A., & Weisberg, S. B. (2009). "Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: challenges for the future." *Marine Pollution Bulletin*, 59(1-3), 1-4.
- Borja, A., Bricker, S. B., Dauer, D. M., Demetriades, N. T., Ferreira, J. G., Forbes, A. T., ... & Zhu, C. (2008). Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. *Marine pollution bulletin*, 56(9), 1519-1537.
- Brown, J. and E. Hartwick (1988). "A habitat suitability index model for suspended tray culture of the Pacific oyster, *Crassostrea gigas* Thunberg." *Aquaculture Research* 19(2): 109-126.
- Cheney, D., et al. (1994). "Creation of rocky intertidal and shallow subtidal habitats to mitigate for the construction of a large marina in Puget Sound, Washington." *Bulletin of Marine Science* 55(2-3): 772-782.
- Clarke, L. M. (2017). "Functional Comparison of Longline Oyster Aquaculture and Eelgrass (*Zostera marina* L.) Habitats Among Pacific Northwest Estuaries, USA, Oregon State University." M.S. Thesis. Oregon State University.
- Coen LD, Dumbauld BR, Judge ML (2011). "Expanding shellfish aquaculture: a review of the ecological services provided by and impacts of native and cultured bivalves in shellfish dominated ecosystems." In: Shumway SE (ed) *Shellfish Aquaculture and the Environment*. Wiley-Blackwell, West Sussex, UK, p 239-296.
- Coen L, Grizzle RE (2007). "The importance of habitat created by molluscan shellfish to managed species along the Atlantic coast of the United States." In: Thomas J, Nygard J (eds) *Habitat Management Series*. Atlantic States Marine Fisheries Commission, Washington, D.C.
- Coen, L. D., M.W. Luckenbach, and D.L. Breitburg (1999). "The role of oyster reefs as essential fish habitat: A review of current knowledge and some new perspectives." *American Fisheries Society Symposium* 22: 438-454.
- Coull, B. C. (2009). "Role of meiofauna in estuarine soft-bottom habitats." *Australian Journal of Ecology* 24(4): 327-343.

- Cullen-Unsworth, L., & Unsworth, R. (2013). "Seagrass meadows, ecosystem services, and sustainability." *Environment: Science and policy for sustainable development*, 55(3), 14-28.
- Dealteris JT, Kilpatrick BD, Rheault RB (2004). "A comparative evaluation of the habitat value of shellfish aquaculture gear, submerged aquatic vegetation and a non-vegetated seabed." *J. Shellfish Res* 23:867-874.
- Deegan LA, Hughes JE, Rountree RA (2000). "Salt marsh ecosystem support of marine transient species. In: Weinstein MP, Kreeger DA (eds) *Concepts and Controversies in Tidal Marsh Ecology*." Kluwer Academic Publishers, Boston, p 333-363.
- Diaz, R. J., Solan, M., & Valente, R. M. (2004). A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of environmental management*, 73(3), 165-181.
- Dumbauld, B. R., Hosack, G. R., & Bosley, K. M. (2015). "Association of Juvenile Salmon and Estuarine Fish with Intertidal Seagrass and Oyster Aquaculture Habitats in a Northeast Pacific Estuary." *Transactions of the American Fisheries Society*, 144(6):1091-1110.
- Dumbauld BR, Ruesink JL, Rumrill SS (2009). "The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries." *Aquaculture* 290:196-223.
- Dumbauld BR, Ruesink JL, Rumrill S (2005). "The ecological role and potential impacts of molluscan shellfish culture in the estuarine environment." In: *Final Report to the Western Regional Aquaculture Center U.S. Dept. Agriculture, ARS, Newport, Oregon*.
- Entz, Ray, "Habitat Evaluation Procedures (HEP) Report; West Beaver Lake Project", 2005 Technical Report, Project No. 199206100, 32 electronic pages, (BPA Report DOE/BP-00004604- 8)
- Ferraro SP and Cole FA (2010). "Ecological periodic tables for nekton usage of four US Pacific Northwest estuarine habitats." *Can J Fish Aquat Sci* 67:1957-1967.
- Ferraro, S. P. and F. A. Cole (2007). "Benthic macrofauna–habitat associations in Willapa Bay, Washington, USA." *Estuarine, Coastal and Shelf Science* 71(3-4): 491-507.
- Fresh KL (2006) "Juvenile salmon in Puget Sound." *Puget Sound Nearshore Partnership Report No. 2006-06*. Seattle District Army Corps of Engineers, Seattle, Wa. 21 p.
- Gee, J. M. (1989). "An ecological and economic review of meiofauna as food for fish." *Zoological Journal of the Linnean Society* 96(3): 243-261.

- Glancy, T. P., et al. (2003). "Comparative Patterns of Occupancy by Decapod Crustaceans in Seagrass, Oyster, and Marsh-edge Habitats in a Northeast Gulf of Mexico Estuary." *Estuaries* 26(5): 1291-1301.
- Gunderson, D. A., D. A., Yung-Bing Shi and McConnaughey, R. A. (1990). "Patterns of Estuarine Use by Juvenile English Sole (*Parophrys vetulus*) and Dungeness Crab (*Cancer magister*)." *Coastal and Estuarine Research Federation* 13(1): 59-71.
- Heck K.L, J. H. G. O. R. J. (2003). "Critical evaluation of the nursery role hypothesis for seagrass meadows." *Marine Ecology Progress Series* 253: 123-136.
- Hosack, G. R., et al. (2006). "Habitat associations of estuarine species: comparisons of intertidal mudflat, seagrass (*Zostera marina*), and oyster (*Crassostrea gigas*) habitats." *Estuaries and Coasts* 29(6): 1150-1160.
- Hovel, K. A., Warneke, A. M., Virtue-Hilborn, S. P., & Sanchez, A. E. (2016). "Mesopredator foraging success in eelgrass (*Zostera marina* L.): relative effects of epiphytes, shoot density, and prey abundance." *Journal of Experimental Marine Biology and Ecology*, 474, 142-147.
- Hudson, B., Cheney, D., Dumbauld, B., Cordell, J., Tomas-Nash, F., Kramer, S., Houle, K., Suhrbier, A., Middleton, M., Toft, J., Kralj, J., Mercer, D., Clarke, L., Meuthing, K., Mack, C., Minch, J. (2018). "Quantification of functional relationships between shellfish culture and seagrass in US west coast estuaries to inform regulatory decisions." Final Report for NOAA Saltonstall-Kennedy Program Award No. NA15NMF4270318. pp. 36.
- Jensen, G. a. A., M. (1998). "Omnivory in the diet of juvenile dungeness crab, *Cancer magister* Dana." *Journal of Experimental Marine Biology and Ecology* 226: 175-182.
- Kennedy, A. D. and C. A. Jacoby (1999). "Biological indicators of marine environmental health: meiofauna—a neglected benthic component?" *Environmental Monitoring and Assessment* 54(1): 47-68.
- Lehnert, R. L. and D. M. Allen (2002). "Nekton use of subtidal oyster shell habitat in a southeastern US estuary." *Estuaries* 25(5): 1015-1024.
- Mattila, J. (1995). "Does habitat complexity give refuge against fish predation? Some evidence from two field experiments." *Biology and Ecology of Shallow Coastal Waters. Proceedings of the 28th European Marine Biological Symposium, Crete, Greece, 23-28 September 1993. International Symposium Series: pp. 261-268.*
- McMahon, K., Collier, C., & Lavery, P. S. (2013). "Identifying robust bioindicators of light stress in seagrasses: a meta-analysis." *Ecological Indicators*, 30, 7-15.
- Minello TJ (1999). "Nekton densities in shallow estuarine habitats of Texas and Louisiana and the identification of essential fish habitat." In: Benaka LR (ed) *Fish Habitat: Essential Fish*

Habitat and Rehabilitation, Book American Fisheries Society, Symposium 22. American Fisheries Society, Bethesda, MD, p 43-75.

- Minello TJ, Able KW, Weinstein MP, Hays CG (2003). "Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis." *Mar Ecol Prog Ser* 246:39-59.
- Muething, K. (2018). "On the edge: Assessing fish habitat use across the boundary between Pacific oyster aquaculture and eelgrass in Willapa Bay, WA" M.S. Thesis. Oregon State University.
- Orth R. J., H., K. L. Jr., and Van Montfrans, J. (1984). "Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predatory-prey relationships." *Estuaries* 7: 339-350.
- Pacific Fishery Management Council (2008) Pacific Coast Groundfish Fishery Management Plan for the California, Oregon, and Washington Groundfish Fishery as Amended Through Amendment 19
- Plummer ML, Harvey CJ, Anderson LE, Guerry AD, Ruckelshaus MH (2013) "The role of eelgrass in marine community interactions and ecosystem services: Results from ecosystem-scale food web models." *Ecosystems* 16:237-251.
- Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., & Dimming, A. (2004). Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine pollution bulletin*, 49(9-10), 728-739.
- Ruesink, J. L. (2016). "Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk." *Journal of Experimental Marine Biology and Ecology*, 481, 1-8.
- Reynolds, L. K., Chan, K. M., Huynh, E., Williams, S. L., & Stachowicz, J. J. (2018). "Plant genotype identity and diversity interact with mesograzers species diversity to influence detrital consumption in eelgrass meadows". *Oikos*, 127(2), 327-336.
- Rogers, C. W. (1988). "UTILIZATION OF A WASHINGTON ESTUARY BY JUVENILE ENGLISH SOLE, *PAROPHRYS VETULUS*." *Fishery Bulletin* 86(4): 823-831.
- Schratzberger, M. and J. Ingels (2018). "Meiofauna matters: The roles of meiofauna in benthic ecosystems." *Journal of Experimental Marine Biology and Ecology* 502: 12-25.
- Simenstad CA, Cordell JR (2000). "Ecological assessment criteria for restoring anadromous salmonid habitat in Pacific Northwest estuaries." *Ecol Eng* 15:283-302

- Simenstad, C. A. and E. O. Salo (1982). "Foraging success as a determinant of estuarine and nearshore carrying capacity of juvenile chum salmon (*Oncorhynchus keta*) in Hood Canal, Washington." PROCEEDINGS OF THE NORTH PACIFIC AQUACULTURE SYMPOSIUM., ALASKA SEA GRANT REP. ALASKA SEA GRANT PROGRAM ALASKA UNIV. (Journal Article): 21-37.
- Simenstad, C., Cordell, J.R., Wissmar, R.C., Fresh, K.L., Schroder, S.L., Carr, M., Sanborn, G., Burg, M.E. (1988). "Assemblage structure, microhabitat distribution, and food web linkages of epibenthic crustaceans in Padilla Bay National Estuarine Research Reserve, WA." NOAA Technical Report Series OCRM/MEMD FRI-UW-8813: 1-69.
- Stunz GW, Minello TJ, Rozas LP (2010). "Relative value of oyster reef as habitat for estuarine nekton in Galveston Bay, Texas." *Mar Ecol Prog Ser* 406:147-159.
- Thomsen, M. S., Altieri, A. H., Angelini, C., Bishop, M. J., Gribben, P. E., Lear, G., ... & Watson, D. M. (2018). "Secondary foundation species enhance biodiversity." *Nature Ecology & Evolution*, 2(4), 634-639.
- Toole, C. (1980). "Intertidal recruitment and feeding in relation to optimal utilization of nursery areas by juvenile English sole (*Parophrys vetulus*: Pleuronectidae)." *Environmental Biology of Fishes* 5(4): 383-390.
- USFWS. U. S. Fish and Wildlife Service. 1980a. Habitat as a basis for environmental assessment. Ecological Services Manual 101. U. S. Fish and Wildlife Service Division of Ecological Services, Washington D.C.
- USFWS. U. S. Fish and Wildlife Service. 1980b. Habitat evaluation procedures. Ecological Services Manual 102. U. S. Fish and Wildlife Service Division of Ecological Services, Washington D.C.
- Villnäs, A., & Norkko, A. (2011). "Benthic diversity gradients and shifting baselines: implications for assessing environmental status." *Ecological applications*, 21(6), 2172-2186.
- Vincenzi, S., et al. (2006). "A GIS-based habitat suitability model for commercial yield estimation of *Tapes philippinarum* in a Mediterranean coastal lagoon (Sacca di Goro, Italy)." *Ecological Modelling* 193(1-2): 90-104.
- Whittier, T. R., et al. (2007). "A structured approach for developing indices of biotic integrity: three examples from streams and rivers in the western USA." *Transactions of the American Fisheries Society* 136(3): 718-735.
- Zhao, Q., Bai, J., Huang, L., Gu, B., Lu, Q., & Gao, Z. (2016). "A review of methodologies and success indicators for coastal wetland restoration." *Ecological Indicators*, 60, 442-452.