Shellfish aquaculture farms as foraging habitat for nearshore fishes and crabs in Puget Sound

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A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington
2023

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Program Authorized to Offer Degree:
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Abstract

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Oyster reefs across North America have declined precipitously over the past 140 years. In Washington State, Olympia oyster *Ostrea lurida* reefs historically provided water filtration and nearshore structural habitat for fishes and invertebrates but are now functionally extinct across their historical range. In place of these naturally occurring reefs, shellfish farms consisting mainly of non-native Pacific oysters *Magallana gigas* now occupy patches of nearshore habitat across Washington. These farms modify intertidal substrate by adding structural habitat via suspended oyster grow bags, predator exclusion nets, loose oyster beds, and other shellfish grow-out gear. As interest and investment in shellfish aquaculture has expanded both locally and globally, so has interest in how these farms modify intertidal habitat, and whether the complex structure created by the shellfish and shellfish growing gear provides ecosystem services that are comparable to unfarmed areas such as mudflats and eelgrass meadows. In this study, we sought to quantify how shellfish farms are used as foraging habitat for several common nearshore species of fishes and crabs in Puget Sound, Washington. In the first part of this study, we used direct observations of species-specific behaviors from underwater video to model how habitat type affected observed foraging rates. We obtained a total of 393 crab observations, 431 demersal fish observations, and 1,856 pelagic fish observations across seven farm sites. Several common species of pelagic fishes such as shiner perch Embiotocidae used aquaculture growing gear more frequently than unfarmed areas as foraging habitat, but *Metacarcinus* crabs displayed higher foraging frequency in unfarmed mudflats. Species groups such as sculpin Cottidae and small flatfish Pleuronectidae clearly used specific aquaculture growing gear and mudflats in roughly equal proportion. In the second part of the study, we used stable isotope mixing models to estimate, for several species of nearshore fish and crab in two areas of North Puget Sound, Washington, the percent diet originating from eelgrass meadows, pelagic planktonic sources, and oyster farm habitats. Our results indicate that several species of nearshore fish derive a significant proportion of their diets from farm areas, while others derive most of their diets from eelgrass habitat or planktonic sources. Shellfish farms within a larger nearshore habitat mosaic of eelgrass meadows, mudflats, bivalve aquaculture gear, and edge habitat can provide foraging habitat for several species of nearshore fish.

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Acknowledgements

This research was conducted in collaboration with the generous support of shellfish aquaculture farms and Padilla Bay National Estuarine Reserve in Washington, USA. Thank you to Chelsea Wood, Beth Sanderson, Peter Kiffney, and Bridget Ferriss for their insight, advice, and help. Thank you to Jessica Diallo for the use of her mass spectrometer data processing code; and Gordon Holtgrieve for the use of the Holtgrieve lab mass spectrometer. Reference to trade names does not imply endorsement by the University of Washington.

Chapter 1: Shellfish aquaculture farms as foraging habitat for nearshore fishes and crabs

Introduction

Oyster reefs are important habitats for many species of fishes and invertebrates because they provide foraging opportunities (Shervette and Gelwick 2008) and predator refuge (Hixon and Beets 1993). Oysters provide extensive ecosystem services through habitat formation of oyster reefs and water filtration in estuaries (Coen et al., 2007). The physical structure created by the mass of shells in reefs creates habitat complexity (Humphries et al. 2011) and provides refuge habitat for small organisms such as juvenile Dungeness crabs *Metacarcinus* (Holsman et al. 2006), which are a valuable commercial species. Areas such as Chesapeake Bay have undertaken widespread oyster reef restoration efforts to protect and restore the important ecosystem services provided by oyster reefs (Beck et al. 2001; Coen et al. 2007a).

Unfortunately, over the past 140 years, oyster reef habitat has declined about 64% in the United States, with native Olympia oysters in Washington State becoming functionally extinct across most of their historical range (Zu Ermgassen et al. 2012). Some Olympia oyster reefs still exist within limited areas in Washington's Puget Sound, with core historical populations having been reduced by approximately 96% (Horowitz and Hoberecht 2016).

In place of historically occurring oyster reefs, shellfish farms located on tide flats along the coast of Washington State are growing enterprises that confer both economic opportunities and high quality, locally grown food (Horowitz and Hoberecht 2016). Shellfish aquaculture of all farmed bivalves contributes approximately $184 million USD annually to the state's economy (Horowitz and Hoberecht 2016). These farms either grow oysters directly on the tide flat in a method that is analogous to a naturally occurring oyster reef, or on a variety of gear types attached to the tide flat substrate. Increasingly, the preferred gear type for growing oysters in Puget Sound are oyster flipbags, which consist of buoyant, oyster filled mesh bags hanging from wires directly above the tide flat (Figure 1).

A major consideration when permitting and implementing shellfish aquaculture is its impact on nearshore habitats either through effects from the physical addition of structure, or pulse disturbance from activities such as harvesting or gear installation (Dumbauld et al. 2009). Many studies show that certain shoreline modifications can impair habitat function and structure (Griggs 2005; Munsch et al. 2017). However, the introduction of structured artificial habitats via placement of bivalve aquaculture growing gear on tide flats may enhance predator feeding opportunities by providing increased prey availability (Hosack et al. 2006) or predator refuge (Wechsler 1996) for nearshore species that mitigates risks of feeding behavior. Nevertheless, these benefits are probably species and location specific, with different species showing different preferences for structure and habitat (Dumbauld et al. 2009).

There is some evidence that bivalve aquaculture confers some habitat functions (Alleway et al. 2019; Gentry et al. 2020), but how habitat functions in shellfish aquaculture sites compare to natural areas is still not well understood (Dumbauld et al. 2011). Thus, an improved understanding of whether aquaculture sites provide foraging and refuge opportunities for commercially, recreationally, and culturally important fish and crab species, and the ecosystem value of aquaculture sites relative to natural areas, will help inform decisions about the ecological implications of an expanding shellfish aquaculture industry.

In this study, we analyzed underwater video to compare feeding of nearshore fish and crab species among five different farmed and unfarmed habitat types in Puget Sound, Washington State. We sought to understand:

1) how habitat type influenced observed feeding behavior;
2) how substrate cover (i.e. algae vs. eelgrass vs. bare) influenced observed feeding behavior; and
3) whether structure affiliated species used structurally complex habitats for foraging more than structurally simpler habitats.

Methods

Study area

Puget Sound is a complex and highly productive inlet within the Salish Sea ecosystem of Washington State, USA, and British Columbia, Canada, consisting of several large, environmentally distinct sub-basins (Ruckelshaus and McClure 2007). Extensive tidal flushing combined with significant freshwater inputs from rivers create a complex estuarine ecosystem within the various sub-basins (Moore et al. 2008), with salinity levels lower than in the open ocean. Shellfish aquaculture occurs across most of the major basins of Puget Sound, with the largest concentration of farms in South Puget Sound, North Puget Sound, and Hood Canal. The shellfish produced are primarily Pacific oysters and Manila clams *Ruditapes philippinarum* by weight (Horowitz and Hoberecht 2016). Farming occurs on tide flats which depending on location are naturally composed of a combination of eelgrass meadows, mud flats, and naturally recruiting non-native Pacific oyster beds.

Data collection

Using cameras to quantify species and behavior is an increasingly common technique that has been used in similar studies (Gross et al. 2018; Muething et al. 2020; Mercaldo-Allen et al. 2021; Shinn et al. 2021; Ferriss et al. 2021) and which allowed us to capture more observations than would have been possible using dive or snorkel surveys. We used GoPro video cameras to record several thousand hours of underwater video of shellfish aquaculture and adjacent eelgrass and mudflat habitats (Ferriss et al. 2021). A subset of these data was analyzed to identify the species present in these habitats and to classify observed behavior.

We collected data in the spring and summer of 2017 and 2018. GoPro cameras were placed at seven shellfish aquaculture sites across North Puget Sound, South Puget Sound, and Hood Canal (Figure 1). We selected sites based on the shellfish species being farmed, the grow gear present, and the accessibility of the site. Our focus was on the three most predominant grow-out gears used in Puget Sound: oyster flipbags (also known as tumble bags), clam nets, and loose oyster bottom culture, which is roughly comparable to a natural oyster reef. We selected reference sites of similar tidal elevation to the farmed areas, which consisted of unfarmed eelgrass meadows and mudflats that were located 30 to 60 meters away from the edge of the shellfish farm. We chose this distance from the farms to minimize potential environmental influences from the shellfish farms, while maintaining similarity in environmental conditions between the farmed and unfarmed reference sites.

We deployed cameras at low tide inside GoPro dive housing cases, with attached timelapse timers which triggered video collection during high tide on the following day. At each location, we placed GoPro Hero 3+ and Hero 4 cameras in pairs at the centers of several different aquaculture gear types as well as unfarmed reference areas. Cameras were deployed in pairs so that we would have a backup camera if one camera failed to record properly.

We mounted the cameras on individual pieces of PVC pipe approximately 30 cm above the benthos, facing down at a 20-degree angle. Two small PVC stakes were placed in front of each pair of cameras to mark a one-meter square of visibility starting from the bottom edge of the camera frame of view. In the summer of 2017, four of our sites were sampled once and three sites were not sampled at all. In the summer of 2018, we sampled all seven sites twice. Not every habitat type was present at every site. For example, our two study sites in South Puget Sound did not have any eelgrass present. Eelgrass reference sites were present at five of seven sites, and mudflat reference sites were present at six of seven sites. Of the three aquaculture gear types sampled, oyster flipbags were present at six of seven sites, clam nets were present at five of seven sites, and oyster on bottom culture was present at two of seven sites. Each site had a maximum of two reference types: eelgrass and mudflats, and a maximum of three aquaculture gear types: oyster flipbags, clam nets, and oyster on bottom culture.

Preliminary results indicated that visibility was best at or near slack tide. Videos were collected for 2 minutes every 10 minutes for 1.5 hours on either side of high tide when visibility was generally highest. If available, we used morning high tides for analysis due to improved visibility in morning versus afternoon tides. During tidal cycles where daylight morning high tides were not available, we used afternoon high tides for analysis instead.

Data analysis

After camera retrieval, we selected 10 video segments for analysis from each habitat type. Five videos were from timelapse that were recorded over the course of an hour prior to high tide, and five videos were from timelapse recorded over the course of an hour directly after high tide, for a total of 20 minutes of video analyzed from each pair of cameras in each habitat type recorded over a two-hour time span. In all, we collected several thousand hours of video, of which approximately 75 hours were analyzed for species and behavioral observations. For more information on how we collected this data, see Ferriss et al. (2021).

Using the video analysis software BORIS (Friard and Gamba 2016), we counted all fishes and crabs observed within a one-meter square area from each video. Each observation was also assigned a behavioral classification (“feeding” or “not feeding”). Feeding was defined as an individual observed to be eating or attempting to eat something at any point in the video, for any length of time. If there were no feeding attempts observed by an individual during the two-minute video, then they were classified as “not feeding”. Videos were subjectively ranked as either low, medium, or high visibility to account for potential observer bias resulting from varying water quality conditions across the study sites, which could have obscured observations or behaviors in lower visibility videos (Ferriss et al. 2021).

We summed observations from each 2-minute video to calculate the sum of each species and behavior combination observed per video segment. In several videos we observed large schools of fast-moving fish, which were primarily forage fish. We chose to remove these large schools from the dataset, as this would cause a few observations to skew the results. Species with fewer than three observations were also removed along with species that either were not in our three vertical functional groups (e.g., jellyfish medusae, harbor seals *Phoca vitulina*, diving birds Aves), appeared to be attracted to the camera (e.g., dogfish sharks *Squalus suckleyi*), or that were not identifiable. After the data were subset, we obtained a total of 393 crab observations, 431 demersal fish observations, and 1,856 pelagic fish observations across all seven farm sites (Table 1) (Veggerby et al. 2023a). We defined pelagic fish as fusiform style fish primarily associated with the open water column and demersal fish as fish primarily associated with the benthos or near the benthos. We did not separate crabs into species or other groupings for the analysis due to the difficulty of consistently identifying crabs at the species level using video. The likely species of crabs most commonly observed were shore crabs *Hemigrapsus* spp., and Dungeness and graceful crabs *Metacarcinus* spp.

We used a generalized linear mixed effects model approach to analyze the probability of foraging activity among species groups and habitat types using the ‘MuMIn’ package (Barton 2022), the ‘lme4 package (Bates et al. 2022) and the ‘arm’ package (Gelman et al. 2022). The species observations were separated as either pelagic, demersal, or benthic functional groups (Table 1), and analyzed as a group. Our goal was to separate species that were clearly morphologically and functionally different into different groups for analysis, while also maintaining a sufficient sample size to effectively perform analyses. Species within each vertical functional group were modeled together, so the more abundant species drove the observed trends. For example, surf perch (Embiotocidae) dominated our pelagic fish observations, and thus also dominated the pelagic fish model results.

Within the models for each group, habitat type, visibility, and cover type (eelgrass, bare mud, or algae) were included as fixed effects, and site was included as a random effect. Including cover type was important, as there was substantial variability within habitat type based on algae cover. Some mudflats and bivalve farming areas had substantial algae and/or biofouling whereas other areas were bare or mostly bare. We did not include the specific subbasin within Puget Sound as a fixed effect (i.e., North versus South Puget Sound) because differences in behavior across the region were captured by the individual farms spread across the Puget Sound. For each vertical functional group *k*, we modeled the probability of foraging at site *j*, whereby for individual *i* where *P* refers to probability:

$$feeding_{i,j,k} \sim Bernoulli\left(P_{i,j,k}\right)$$

$$\log\left[\frac{P_{i,j,k}}{1-P_{i,j,k}}\right] = \alpha_{k[j]} + \beta_0 + \beta_1\left(habitat_{j,k}\right) + \beta_2\left(substrate_{j,k}\right) + \beta_3\left(visibility_{j,k}\right)$$

$$\alpha_{k[j]} \sim N(\mu_{j,k}, \sigma_{j,k})$$

We first fit a saturated model that included all the covariates with the random effect of site and compared it to a model with no random effects. After determining the appropriate random effect structure via AICc values, we fit all combinations of model that included habitat type and compared model parsimony using AICc values calculated with the ‘MuMIn’ package (Barton 2022). We did not include model combinations without habitat type as a predictor because we were specifically interested in the effect of habitat type. Models within 2 AICc units of each other were considered comparable (Burnham and Anderson 2004). Comparable top models with fewer predictor variables were considered preferential to models with more predictor variables. For models containing both fixed and random effects, we calculated Nakagawa conditional and marginal R^2 metrics (Nakagawa and Schielzeth 2013). The conditional R^2 takes both fixed and random effects into account, while the marginal R^2 takes only the fixed effects into account. For models without random effects, we calculated a single Cox and Snell pseudo R^2 metric (Cox and Snell 1989). Both Nakagawa and Cox and Snell pseudo R^2 metrics are generally comparable to traditional R^2 metrics. However, values that would be considered good are much lower than what would generally be expected from traditional R^2 metrics. For example, a pseudo R^2 of 0.2 would be considered a good fit.

All statistical analyses were conducted in R version 4.2.2 (R Core Team 2022) and RStudio version 2022.12.0 (RStudio 2022). The ‘performance’ (Lüdecke et al. 2021), ‘here’(Müller and Bryan 2022), ‘tidyverse’(Wickham 2023) and ‘RColorBrewer’ (Neuwirth 2022) packages were also used in this project. All data and code necessary to reproduce our analyses and results are available on GitHub at https://github.com/veggerk/Veggerby_2023_fish-behavior.

Results

Of the 393 crabs, 431 demersal fish, and 1,856 pelagic fish seen on video, 11% of crabs, 24% of demersal fish, and 36% of pelagic fish were observed feeding. There were very large overlaps in feeding probability standard errors across habitats for both pelagic fish and crabs, while demersal fish clearly fed at higher rates in mudflats and oyster flipbags compared to the other habitat types. The probability of pelagic fish feeding was highest in clam nets (Figure 2, Table 2). However, the large overlap in standard errors meant that foraging probabilities were overall similar between habitats. When comparing different cover types, the probability of fish feeding was highest in algae-covered substrate. This type of substrate cover was most commonly attached to flipbags and clam nets, particularly in South Puget Sound. The cover type standard errors were much smaller than habitat type standard errors. Algae covered substrate was clearly used by pelagic fish, dominated numerically by surf perch.

The probability of pelagic fish feeding was explained equally well by two models that were within 2.0 AICc of one another. One model contained fixed effects of habitat type, cover type, visibility, and a random effect of site. The second contained fixed effects of habitat type, cover type, and a random effect of site (Table 3). Since these models were considered comparable during AICc ranking, the most parsimonious model was chosen as the top model for generating estimates of feeding probability across habitats. The most parsimonious model had a Nakagawa conditional R^2 of 0.12 and a Nakagawa marginal R^2 of 0.05 (Nakagawa and Schielzeth 2013).

The probability of demersal fish feeding was highest in mud flats and oyster flipbags (Figure 3, Table 2). Unlike pelagic fish, demersal fish had a much higher probability of feeding in these two habitat types compared to clam nets, oysters on bottom, and eelgrass. Standard errors of feeding probability in those two habitat types had little or no overlap with standard errors for the three other habitat types, indicating a clearly higher feeding probability.

The model that best explained probability of demersal fish feeding contained a fixed effect of habitat type (Table 3) and had a Cox and Snell pseudo R^2 of 0.09 (Cox and Snell 1989). There were no other competing models for the demersal fish functional group, so this model was used to generate estimates of feeding for demersal fish.

The probability of crab feeding was highest in mud flats (Figure 4, Table 2). Crabs also had a higher probability of feeding in either bare or algae covered substrate. Feeding probability was higher when visibility was better, indicating that water quality had an impact on whether crabs were observed feeding. The model that best explained the probability of crab feeding contained fixed effects of habitat type, cover type, and visibility (Table 3) and had a Cox and Snell pseudo R^2 of 0.07. There were no other competing models for the crab functional group, so this model was used to generate estimates of feeding probability across habitats for crabs.

Discussion

Habitat played an obvious role in the probability of aquatic organisms observed feeding, yet these patterns were influenced both by organism functional groupings as well as substrate characteristics. There were large overlaps in feeding probability across habitats for both pelagic fish and crabs, indicating more generalist habitat usage, compared to demersal fish which clearly fed in mud flats and flipbags at higher rates. These two habitat types had the least direct benthic structure present, since flipbags are suspended in the water above the substrate. Small flatfish have previously been found to prefer open, unstructured benthic habitat such as mudflats (Williams 1994; Able et al. 2005).

The lack of clearly defined differences in foraging probability between cultivated and uncultivated habitat supports the idea that shellfish aquaculture provides equivalent foraging opportunities as uncultivated reference areas. Demersal fish were much more likely to feed in oyster flipbag and mudflat habitat, but those habitat types encompassed both cultivated and uncultivated areas. Pelagic fish and crab feeding probability differed by habitat, but the differences were seemingly insignificant when factoring in the standard errors. It might be that species abundance differs due to various species preferences, but where these fish and crabs are found, they have equal propensity to forage in shellfish farms relative to eelgrass/mudflat.

Structurally complex habitats, such as eelgrass meadows and oyster reefs, harbor higher densities of epibenthic meiofauna (Bell et al. 1984; Attrill et al. 2000; Jenkins et al. 2002; Hosack et al. 2006). As such, shellfish aquaculture grow gear may provide increased feeding opportunities for structure-associated species by facilitating the attachment and growth of aquatic epiphytes, which similarly may harbor increased invertebrate prey. We observed higher feeding probabilities of surf perch directly above clam nets in South Puget Sound, which were covered in thick filamentous algae. These nets likely provided attachment points for algae to adhere and grow, which in turn created beneficial habitat for preferred prey items such as amphipods (Caine 1991). Algae, as a benthic cover type, was the top foraging cover type for pelagic fish, but not for demersal fish or crabs (Table 2). The relationship between epiphyte covered clam nets as foraging habitat may be location dependent. South Puget Sound has little or no eelgrass (Washington State Department of Natural Resources 2022), so thick algae mats adhered to natural or artificial structure may play a similar role as eelgrass meadows do in other Puget Sound regions with more abundant eelgrass meadows that support invertebrate prey species.

Individual species preferences for the presence or absence of structure likely drove the trends we observed. Responses to the addition of habitat structure are likely species and/or life stage specific. For example, small crabs have been previously documented to use eelgrass and oyster beds as refuge before moving into open mudflats to forage (Fernandez et al. 1993; Holsman et al. 2006). We documented a similar phenomenon here, with mudflats being the habitat with the highest foraging probability for crabs. However, the large overlap in standard errors indicates that foraging habitat usage was highly variable.

Oyster-on-bottom was not a top feeding habitat for crabs, but Ferriss et al. (2021) found that the density of small crabs was generally very high in oyster beds, indicating that this is important habitat for crabs such as young-of-the-year Dungeness crabs (Fernandez et al. 1993). Small crabs, such as shore crabs, were common in video recordings, but were generally not labeled to species level due to difficulty in a definitive identification. Behavior from these individuals was challenging to quantify due to their small size, as well as physical blockage of the video frame by the scattered oysters they were in. Thus, feeding probability of crabs was likely under-estimated in oyster beds specifically. Oyster beds may also provide other habitat functions for small crabs, such as nursery habitat or predator refuge, which has been previously documented (Holsman et al. 2006).

Our results are driven by a few numerically dominant species groups, primarily surf perch, sculpin, flatfish, and stickleback (Table 1). Species that are less common or simply cryptic and difficult to study via video are likely underrepresented. For example, bay pipefish and gunnels are extremely abundant in eelgrass meadows, but due to their camouflaged and cryptic nature we identified very few of them on video, so their contributions to the dataset was too small to draw confident conclusions. Our study also focused only on adults and larger juveniles; small juveniles or larval stages of fish and crab were excluded, as we could only quantify individuals large enough to be seen on video.

Our study adds to the growing body of knowledge on the ecosystem role that bivalve farms play in intertidal and nearshore habitats. We found that structure-associated species such as surf perch (Hosack et al. 2006) foraged in and around bivalve gear types as well as naturally occurring eelgrass meadows and mudflats. Species not associated with structure, such as small flatfish (Williams 1994; Able et al. 2005), foraged in approximately equal proportion in unstructured mudflats and oyster flipbags, indicating that flipbags did not impede feeding for several demersal species. Crabs primarily fed in un-farmed mudflats, however very small crabs such as shore crabs inhabited oyster on bottom style farm plots in high densities (Ferriss et al. 2021), and may have utilized these oyster beds in ways we were not able to accurately quantify on video.

Our results indicate that oyster and clam aquaculture habitat provide foraging opportunities for several common species of nearshore fish, particularly surf perch, sculpins, and small flatfish. The large standard errors of feeding probability for pelagic fish and crabs indicated that feeding in these species' groups were similar across habitats, i.e., they appeared to be generalists. Demersal organisms dominated by sculpin, stickleback, and small flatfish were much more likely to forage in open mudflats and oyster flipbags. Structurally complex habitats usually harbor higher diversity and abundance of nearshore fauna (Orth et al. 1984; Jenkins et al. 1997; Heck et al. 2003). Vertically elevated habitat created by oyster farm gear or eelgrass meadows harbor higher densities of organisms compared to unstructured and unvegetated habitat such as mudflats (Castel et al. 1989; Pinnix et al. 2005; Hosack et al. 2006; Ferraro and Cole 2007). Habitat structure created by the addition of oyster or clam growing gear also provided attachment points for aquatic epiphytes, which may further enhance feeding by creating ideal habitat for invertebrate prey items such as amphipods. Although some species were foraging more in shellfish aquaculture-associated habitat, we do not know if foraging in these habitats leads to higher growth and survival relative to unfarmed natural areas. Shellfish farms within a larger nearshore habitat mosaic of eelgrass meadows, mudflats, bivalve aquaculture gear, and edge habitat can provide foraging habitat for several species of adult and subadult nearshore fish.

Data availability

All data used in this study, and all the R code used for the analysis and figures is available online at zenodo.org: https://doi.org/10.5281/zenodo.7742785

Table 1. Numbers of fishes and crabs observed in underwater videos organized by vertical functional groups.

Vertical distribution	Species grouping	count	% observed feeding
pelagic	Surf perch (Embiotocidae)	1824	36%
pelagic	Forage fish (*Clupea pallasi*, *Hypomesus pretiosus*, *Ammodytes hexapterus*)	23	57%
pelagic	Salmonid (Salmonidae)	9	0%
demersal	Stickleback (*Gasterosteus aculeatus*)	184	27%
demersal	Sculpin (Cottoidea)	141	16%
demersal	Flatfish (Pleuronectidae)	82	37%
demersal	Gunnel (Pholidae)	12	8%
demersal	Snake prickleback (*Lumpenus sagitta*)	5	40%
demersal	Bay pipefish (*Syngnathus leptorhynchus*)	4	0%
demersal	Greenling (Hexagrammidae)	3	0%
benthic	Crabs (*Hemigrapsus*, *Metacarcinus*, *Pugettia*)	393	11%

Table 2. Coefficients and standard errors of parameters in each top foraging behavior model. The model for each vertical functional group with the fewest parameters within delta AICc of 2.0 or less was considered the best model.

		pelagic		**demersal**		**benthic**	
		estimate	2 standard errors	estimate	2 standard errors	estimate	2 standard errors
habitat type	**eelgrass**	0.36	0.14 – 0.65	0.09	0.04 – 0.21	0.10	0.006 – 0.65
	mudflat	0.31	0.15 – 0.54	0.33	0.22 – 0.48	0.59	0.13 – 0.93
	oyster flipbags	0.41	0.23 – 0.60	0.33	0.23 – 0.43	0.14	0.01 – 0.67
	clam net	0.49	0.26 – 0.72	0.09	0.03 – 0.22	0.37	0.04 – 0.89
	oyster on bottom	0.33	0.13 – 0.63	0.09	0.03 – 0.25	0.16	0.01 – 0.75
cover type	**eelgrass**	0.40	0.18 – 0.67	NA	NA	0.91	0.50 – 0.99
	algae	0.49	0.26 – 0.72	NA	NA	0.37	0.04 – 0.89
	bare/algae	0.19	0.07 – 0.45	NA	NA	0.07	0.005 – 0.54
	bare	0.33	0.18 – 0.52	NA	NA	0.27	0.14 – 0.44
visibility	**high**	NA	NA	NA	NA	0.37	0.04 – 0.89
	medium	NA	NA	NA	NA	0.20	0.03 – 0.67
	low	NA	NA	NA	NA	0.05	0.003 – 0.45

Table 3. Top models for each functional group based on a delta AICc of 2 units within the best model. Habitat type, cover type, and visibility were fixed effects, and site was a random effect in the GLMM models. The model with the fewest parameters within delta AICc of 2.0 or less was considered the best model within a functional group. There were no competing models for either the demersal fish or the crab vertical functional groups.

Functional group	Model covariates	Delta AICc	AIC weight
pelagic fish	habitat type + cover type + site	0	0.494
pelagic fish	habitat type + cover type + visibility + site	0.28	0.431
demersal fish	habitat type	0	0.642
crabs	habitat type + cover type + visibility	0	0.977

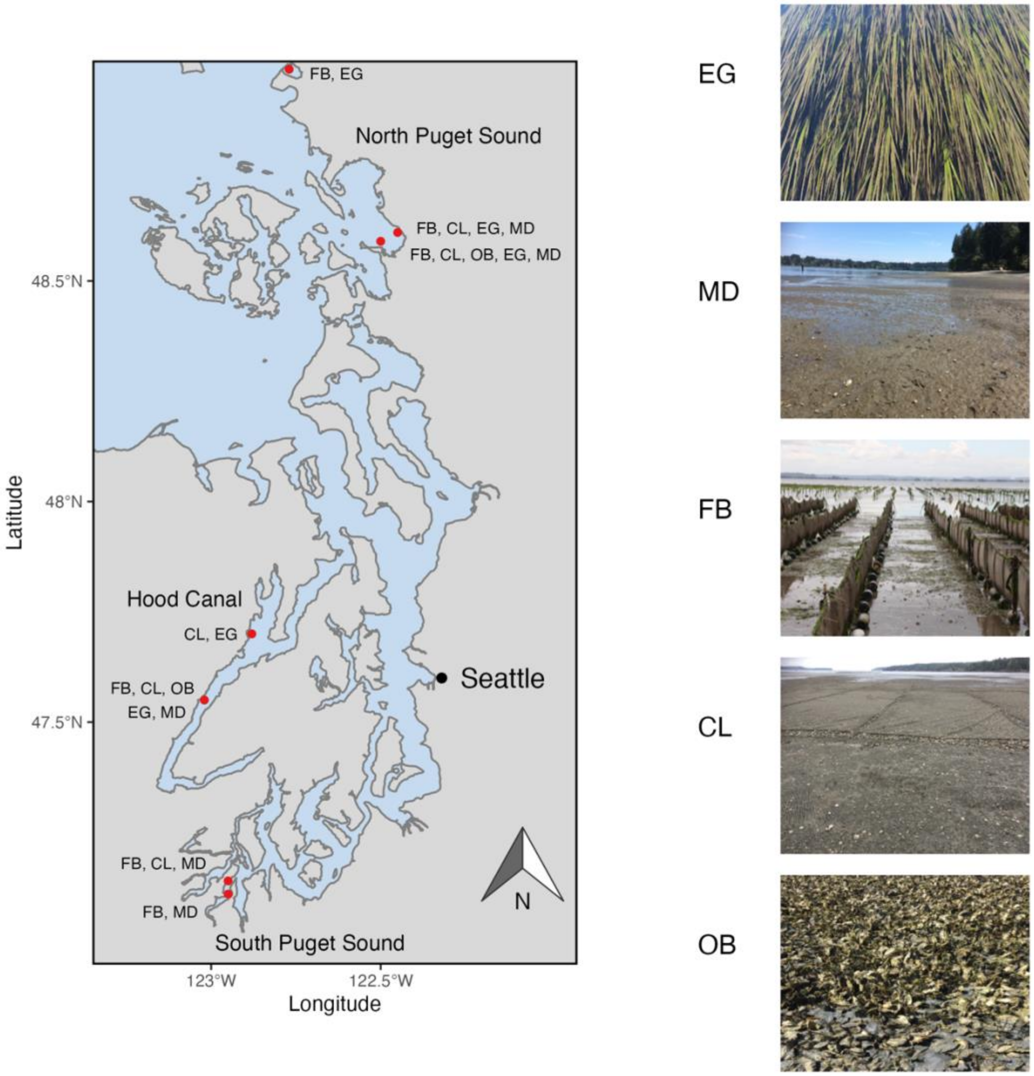

Figure 1. Map of the Puget Sound and the locations of the seven study sites. The five different habitat types used in the study are shown on the right. Eelgrass meadows (EG), mudflats (MD), oyster flipbags (FB), clam nets (CL), and oysters on bottom (OB).

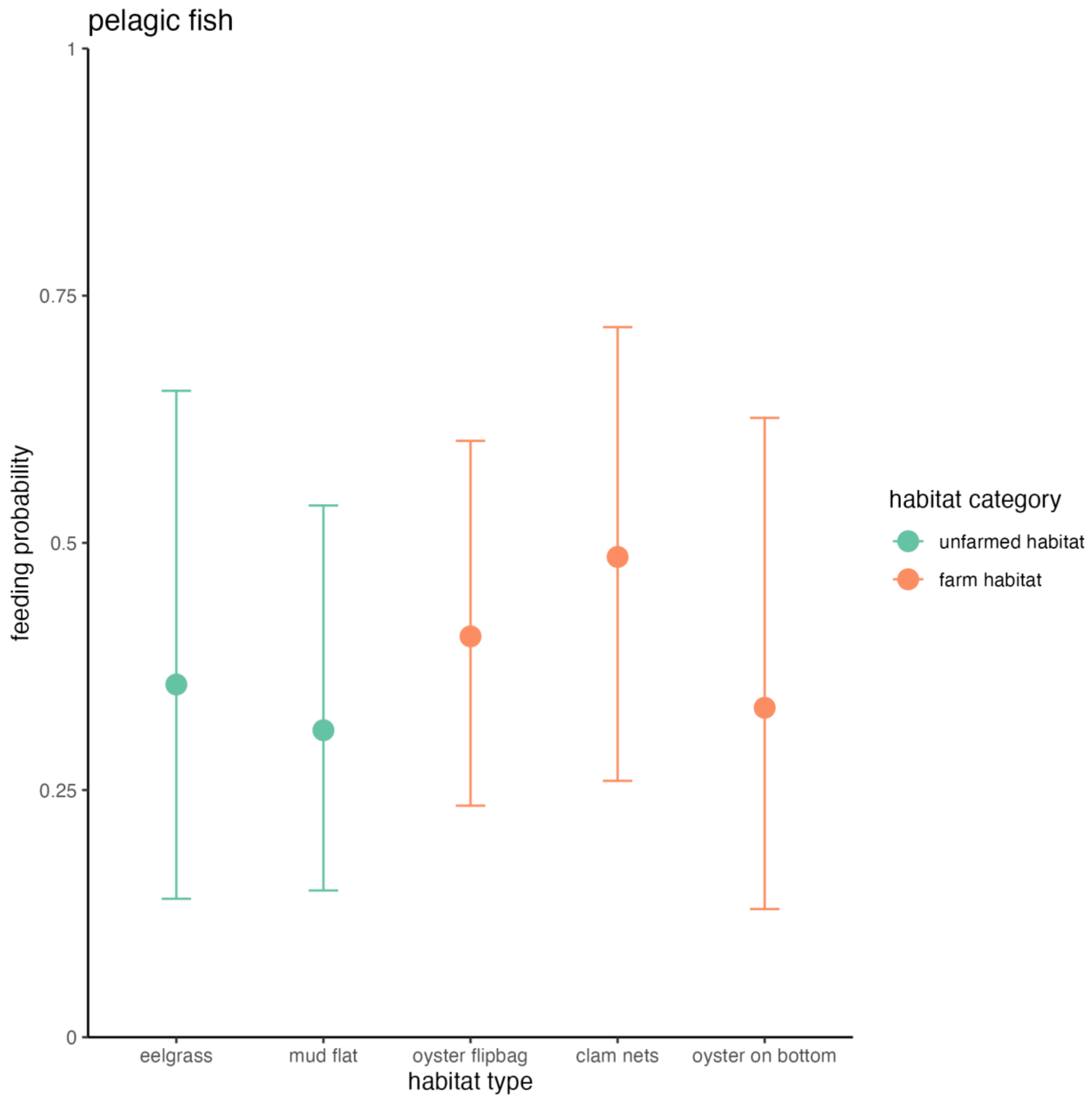

Figure 2. Estimated feeding probability of pelagic fish across the five habitat types estimated from the most parsimonious model ranked within delta AICc of 2.0 or less. Lines denote two standard errors above and below each estimate. Light green color denotes unfarmed habitat types, light orange color denotes farmed habitat types.

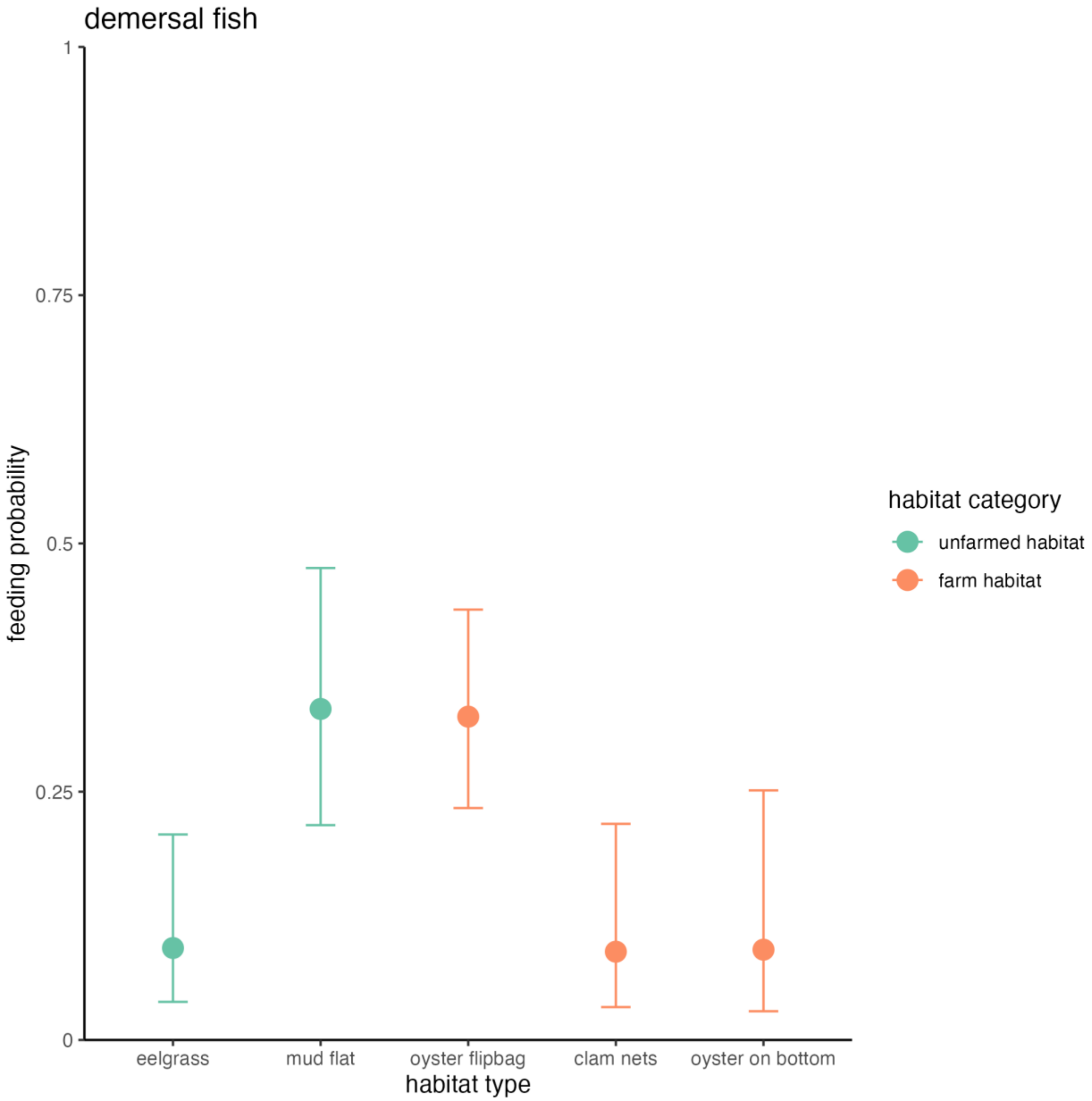

Figure 3. Estimated feeding probability of demersal fish across the five habitat types estimated from the top AICc ranked model. Lines denote two standard errors above and below each estimate. Light green color denotes unfarmed habitat types, light orange color denotes farmed habitat types.

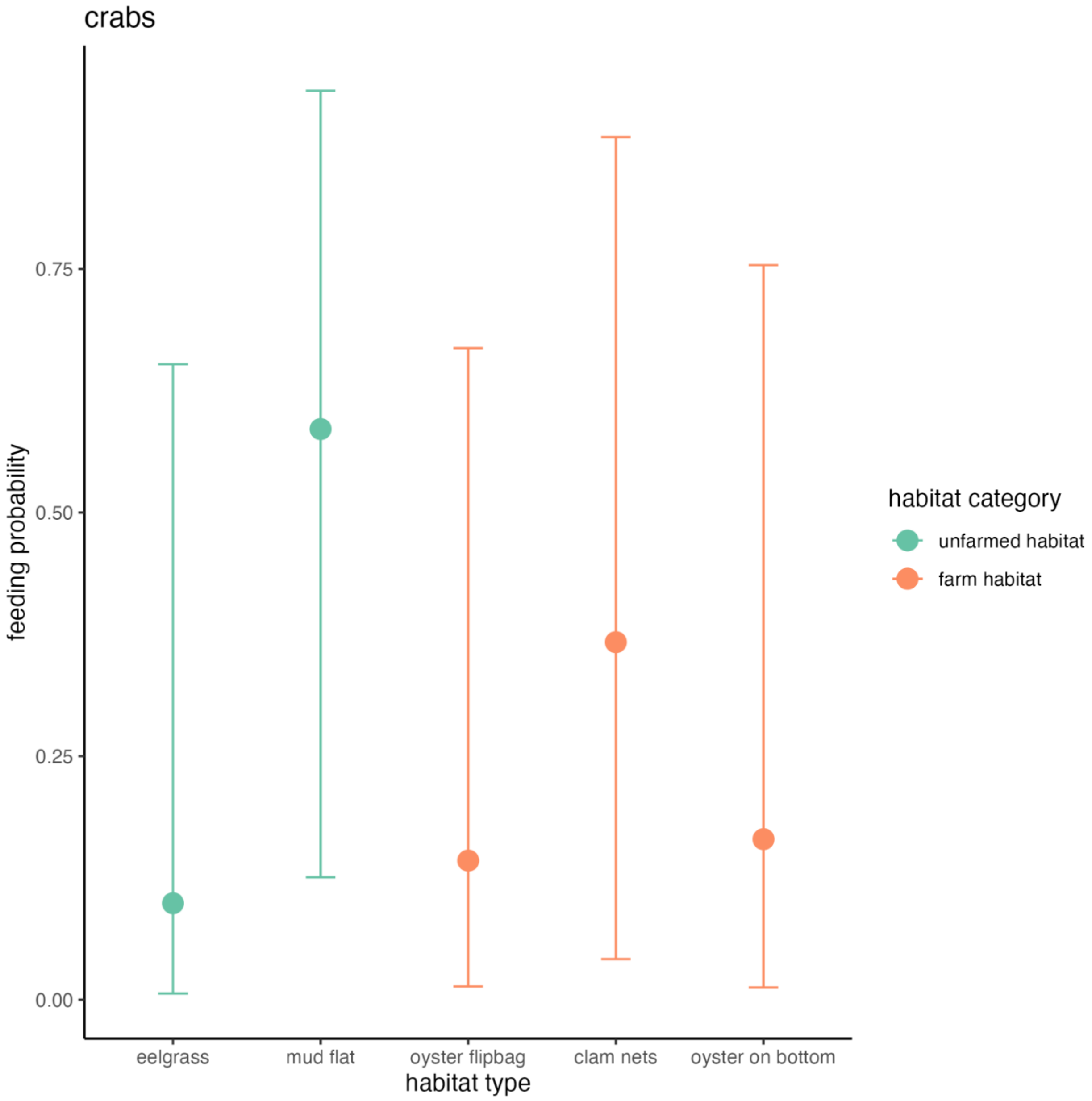

Figure 4. Estimated feeding probability of crabs across the five habitat types estimated from the top AICc ranked model. Lines denote two standard errors above and below each estimate. Light green color denotes unfarmed habitat types, light orange color denotes farmed habitat types.

Chapter 2: Stable isotopes reveal intertidal fish and crabs use shellfish farms as foraging habitat in Puget Sound, Washington

Introduction

Eelgrass meadows are highly productive nearshore ecosystems that have declined globally over the past several centuries (Lotze et al. 2006; Waycott et al. 2009). These protected intertidal meadows serve as nursery habitat for many nearshore species of fish and crab, including commercially and culturally important salmon species (Rubin et al. 2018) and Dungeness crab (Holsman et al. 2006). Eelgrass meadows are also highly productive ecosystems, fixing around one kilogram of carbon per square meter per year (McRoy, 1974), and providing about $1.9 trillion dollars globally in ecosystem services via nutrient cycling alone (Waycott et al. 2009). Eelgrass meadows also sequester carbon, though the native eelgrass species found in Washington *Zostera marina* sequesters lower levels of carbon compared to other seagrass systems elsewhere in the world (Poppe and Rybczyk 2018; Postlethwaite et al. 2018).

In many areas of Washington State, eelgrass meadows overlap with bivalve farming activities (Washington State Department of Natural Resources 2022), some of which have existed for thousands of years (Deur et al. 2015; Lepofsky et al. 2021). Indigenous peoples across the Pacific coast of North America historically modified intertidal areas using clam garden systems (Deur et al. 2015; Lepofsky et al. 2021), and continue to farm and harvest wild oysters, clams, and other bivalves in many areas. Most of the current oyster farming in Washington State is done either by growing oysters loosely on the bottom, or by using mesh bags or lines suspended above the benthic substrate to secure the oysters just above the substrate.

There has been increasing focus on how ecosystem services provided by eelgrass meadows compare to other habitats such as bare mudflats, oyster reefs, and various types of artificial habitat created by the addition of bivalve aquaculture gear on tide flats. Bivalve-growing gear such as flipbags, clam nets, and loose oyster bottom culture modify tide flats by introducing complex vertical structure, while potentially reducing eelgrass cover through increased localized disturbances related to gear movement, crop out-planting, and harvesting etc. (Dumbauld et al. 2009; Ferriss et al. 2021). However, the complex vertical structure added to the tide flats creates attachment points for filamentous algae, aquatic macrophytes, and habitat for epibenthic meiofauna such as amphipods. Epibenthic meiofauna are important prey items for small nearshore fish (Alheit and Scheibel 1982; Gee 1989; Caine 1991), and occur in higher densities around oyster flipbags and eelgrass meadows than in open, unstructured mudflats (Hosack et al. 2006). The vertical structure can be used both as predator refuge and foraging habitat for intertidal fishes and crabs.

Epiphyte density may also play a role in habitat function and usage, as surf perch Embiotocidae have a higher probability of foraging in areas containing high epiphyte cover compared to areas with no epiphyte cover (Veggerby et al. 2023c). Thus, bivalve aquaculture gear that has accumulated epiphyte cover may provide more habitat function than clean gear that has spent less time on the tide flat. For example, Ferraro and Cole (2007) found that the diversity of benthic macrofauna was equal between oyster farm habitat and eelgrass meadows after the oysters were left to grow for 2 - 3 years without disturbance. Nevertheless, the degree to which aquaculture habitats are used for foraging, and how those habitats compare to unfarmed mudflats or eelgrass meadows, is not well (but see Dumbauld et al., 2015; Muething et al., 2020; Ferriss et al., 2021). Understanding the ecological role of habitat that has been modified and created by bivalve aquaculture in the intertidal food web is important for effective management of nearshore systems and the many ecological functions and services they provide.

Stable isotopes are a useful tool for studying food webs and habitat usage (Peterson and Fry 1987; Fry 2006). Stable isotope ratios such as carbon^{12}/carbon^{13} and nitrogen^{14}/nitrogen^{15} can be used to estimate diet sources when both the predator (i.e., consumer) and the potential prey (i.e., source) isotopic values are known (Fry 2006). Nitrogen isotope ratios are commonly used to estimate trophic position, and carbon isotope ratios are commonly used to estimate nutrient flow through an ecosystem. When used together, carbon and nitrogen isotopes can be used to estimate the contribution of different prey or habitat types towards the overall diet of a species of interest. As computational power has increased, more complex and computationally intensive Bayesian methods for modeling predators and prey using stable isotopes have become more accessible to researchers (Stock et al. 2018).

In this study we estimated the proportion of species' diets derived from eelgrass meadows, pelagic planktonic sources, and oyster farm habitat types using a Bayesian stable isotope mixing model. We did not attempt to quantify feeding behavior as in Veggerby et al., (2023), but instead focused on habitat sources of carbon and nitrogen. Specifically, we sought to answer the following questions:

1. How much of each species diet came from eelgrass meadows versus pelagic planktonic sources versus oyster farm habitat?
2. Were diet sources consistent across multiple farmed sites?

Methods

Study area:

The Puget Sound is a structurally complex estuary located in Washington State, USA (Ruckelshaus and McClure 2007). It forms the southern extent of the Salish Sea, which is a marginal sea located along the northwest coast of North America spanning the United States and

Canada. Various types of bivalve aquaculture are present along the outer coast of Washington as well as within Puget Sound. Oysters are often grown either directly on the benthic substrate, or in buoyant mesh bags suspended just above the benthic substrate (Figure 1).

Within the North Puget Sound region, Samish Bay and Drayton Harbor are used for growing oysters and other bivalves. In Samish Bay, a large amount of its tide flat area is dedicated to bivalve farming. Multiple shellfish companies grow oysters, clams, geoducks, and mussels on tide flats across the bay. Drayton Harbor has comparatively much less aquaculture, with only one oyster farm using less than two acres of tide flat for active farm operations. Both areas encompass extensive eelgrass meadows adjacent to farmed areas, and eelgrass, macroalgae, or bare mudflat substrates within the active farm area. Other areas within North Puget Sound such as Padilla Bay National Estuarine Reserve serve as protected estuarine reserves with no bivalve farming activity. Padilla Bay has the second largest eelgrass meadow on North America's Pacific coast with over 8,000 acres of eelgrass (Shull and Bulthuis 2002).

Sample collection:

We conducted sampling in Padilla Bay, Samish Bay, and Drayton Harbor during July and August of 2020, 2021, and 2022, with most of the sampling conducted in the summer of 2022. Padilla Bay was used as a reference site in contrast to bays containing low levels of aquaculture (Drayton Harbor) and bays with higher levels of aquaculture (Samish Bay). We collected species of nearshore fish and crabs that are common in the intertidal area: shiner perch (*Cymatogaster aggregate*), threespine stickleback (*Gasterosteus aculeatus*), Pacific staghorn sculpin (*Leptocottus armatus*), Dungeness crab (*Metacarcinus magister*), shore crab (*Hemigrapsus nudus* and *H. oregonensis*), and small juvenile flatfish comprising either English sole or starry flounder (*Parophrys vetulus* and *Platichthys stellatus* respectively). We chose these species because of their consistent abundance across these study sites, and because these species were the dominant species observed in underwater video collected in the same sites during a previous study (Ferriss et al. 2021).

We targeted nearshore species within or near oyster flipbag farmed areas with a 25-meter beach seine with 1.5 mm mesh, along with opportunistic hand collection of crabs when possible. We deployed crab pots concurrently with beach seining to try to trap additional crabs while we were at each site. At Padilla Bay we sampled an eelgrass meadow for sampling that had similar tidal elevation to the farmed areas in the other bays. We timed seining with the tides so that sites had approximately two to four feet of water present. We counted fish and crabs caught in the seines, immediately released non-target species, and euthanized target species with tricaine mesylate (MS-222) and immediately placed them on ice. We transferred samples to a laboratory freezer for storage within a few hours of collection.

We used two species of snails as proxies for benthic isotopic baselines in lieu of direct benthic primary productivity measurements (Post 2002). Japanese mud snails (*Batillaria attramentaria*) and Japanese bubble snails (*Haminoea japonica*) were ideal species for this use because they were abundant and densely spread across all three of our sites. We collected 15 - 20 individuals of each snail species from the center of flipbag farmed areas near where we seined, as well as from adjacent eelgrass meadows approximately 30 meters away from the edge of the farmed site. We chose 30 meters distance to reduce potential environmental influences from the farmed areas, while maintaining similarity in environmental conditions between the farmed and unfarmed reference sites.

Pelagic sources of primary productivity were measured using Pacific oyster (*Magallana gigas*) gutballs. Oysters are filter feeders, so by dissecting their gutballs, we could quantify the recent planktonic isotopic signature in an area. Oysters and snails were immediately placed on ice after collection. We transferred samples to a laboratory freezer for storage within a few hours of collection. We collected a total of 145 shiner perch, 116 stickleback, 154 staghorn sculpin, 92 juvenile flatfish, 65 Dungeness crab, and 25 shore crab across the three sites. To quantify primary productivity, we collected a total of 67 Japanese mud snails, 73 bubble snails, and 35 oyster gutballs across the three sites.

Sample processing:

We extracted tissue samples from each individual for isotope analysis in the lab. Samples were allowed to partially thaw to make dissections easier. We cut dorsal muscle plugs from each fish, and we cut claw muscle plugs from each crab. We dissected oyster gut balls from each oyster and removed the whole body of snails from their shells. All samples were placed in 20 mL glass scintillation vials, labeled, and freeze dried for 24 hours to remove all moisture. Once samples were freeze dried, we pulverized them, weighed them into 5 mm by 9 mm tin capsules, and analyzed them on a Thermo-Fisher Delta V mass spectrometer at the Holtgrieve Ecosystem Ecology Lab at the University of Washington.

Stable isotope data went through the lab’s QAQC process: sample blanks were run periodically to check for incomplete combustion, and L-Glutamic acid and salmon standards run with the sample sets were used to estimate the precision and accuracy of the isotope ratio measurements. L-Glutamic acid and salmon standards were also used to check for drift over the course of sample set runs and correct for differences in sample weight. Average nitrogen precision and accuracy was 0.12 ‰ and 0.05 ‰, respectively, across all sample sets run. Average carbon precision and accuracy was 0.06 ‰ and 0.04 ‰, respectively, across all sample sets run. We merged stable isotope data with all the collected metadata and combined it into a single large dataset for analysis (Veggerby et al. 2023b).

Statistical Analysis:

To analyze diet sources for each species of fish and crab, we used the MixSIAR package in R to conduct Bayesian stable isotope mixing model analysis (Stock and Semmens 2018; Stock et al. 2022). We used data from Post (2002) to select the necessary trophic enrichment factor. We used 0.4 +/- 1.3 for $\delta\ ^{13}C$ and 3.4 +/- 1.0 for $\delta\ ^{15}N$. Using a trophic enrichment factor corrects for isotopic fractionation in consumer tissue that results from preferential uptake of lighter or heavier isotopes when tissue from prey is digested and incorporated into the predator's tissue. There are very few data on the percentage of diet that originates from eelgrass versus oyster farms for the species included in our study, so we used an uninformative prior in the mixing model. We used the best practices of stable isotope data analysis and mixing model outlined in Phillips et al. (2014).

We modeled each consumer species independently with a base model with no added effects, as well as a model with aquaculture site as a fixed effect. Samples from Padilla Bay were modeled separately with no added effects. We classified diet sources as derived from eelgrass, flipbag farm habitat, or planktonic sources. There were not enough shore crabs collected at Drayton Harbor for site comparisons, so we only ran a base model with no fixed effects for shore crabs. There were not enough staghorn sculpin or flatfish collected at Padilla Bay, so we did not run a Padilla Bay model for those two species.

We used several model diagnostics to ensure that the mixing models had converged properly. Before model runs, we examined isospace plots to ensure that source and consumer isotopic signatures overlapped in isospace. Consumer isotopic signatures that are outside of the source isospace polygon would yield nonsensical model results (Stock et al. 2018). For every mixing model we used three Markov chain Monte Carlo (MCMC) chains with a total chain length of 1,000,000, a burn-in of 500,000, and a thinning rate of 500, for a final chain length of 1,000 steps. The thinning rates are set by default by MixSIAR and not based on the measures of autocorrelation within any of the specific chains.

We calculated Gelman-Rubin statistics for each Markov chain Monte Carlo chain. Gelman-Rubin values should be around 1.0, with values above 1.1 indicating that the chains did not converge (Gelman et al. 1995). We also visually examined MCMC trace plots to confirm that the chains had converged. Proper convergence was determined based on guidance from Gelman et al., (1995).

We conducted all statistical analyses in R version 4.2.2 (R Core Team 2022). We also used the ‘here’ version 1.0.1 (Müller and Bryan 2022), ‘tidyverse’ version 2.0.0 (Wickham 2023) and ‘RColorBrewer’ version 1.1-3 (Neuwirth 2022) packages in this project. All data, code, and model diagnostics necessary to reproduce our analyses and results are available on GitHub at: https://github.com/veggerk/veggerby_2023_stable-iso_habitat.

Results

Several species of nearshore fish collected in the vicinity of bivalve farms derived a substantial portion of their diets from farmed areas. Shiner perch and staghorn sculpin collected in the vicinity of farms obtained about 80% of their diets from farmed areas (Figures 2 and 3), while juvenile English sole and starry flounder derived about 40% of their diets from farmed areas (Figure 4). Stickleback derived most of their diets from pelagic sources (Figure 5), and

Dungeness crabs and shore crabs obtained most of their diets from eelgrass meadow habitat (Figures 6 and 7, Table 1).

Estimates of diet source between the two farm sites were consistent for shiner perch, stickleback, and juvenile flatfish. Staghorn sculpin and Dungeness crab diet source estimates were different between Samish Bay and Drayton Harbor, with variation being driven by the percent diet derived from pelagic versus eelgrass sources. The percent diet derived from flipbag habitat was consistent across the two sites. In Samish Bay where eelgrass cover was lower than Drayton Harbor, we estimated that Dungeness crabs derived about 70% of their diets from eelgrass meadows compared to about 37% in Drayton Harbor (Table 2).

Estimates of diet source derived from pelagic or combined benthic sources between the farm sites and Padilla Bay eelgrass meadows were very similar for the three species where this comparison was possible. In Padilla Bay combined benthic sources were simply eelgrass meadows, whereas in Samish Bay and Drayton Harbor, combined benthic sources consisted of both farm and eelgrass habitat. Shiner perch, stickleback, and Dungeness crab fed on benthic versus pelagic derived nutrients in consistent proportions across sites with different characteristics and eelgrass abundances. In farmed sites where eelgrass cover was broken up by farmed plots, Dungeness crabs on average still fed on about 75% benthic-derived nutrients (Table 3).

Model diagnostics for all models indicated proper convergence. Gelman-Rubin values for all models except the shiner perch location effect model were below 1.1. The shiner perch location effect model had one out of 133 Gelman-Rubin values above 1.1. The other 132 Gelman-Rubin values were below 1.1, indicating that most of the chain steps after the burn-in period remained in a satisfactory stationary distribution. Trace plots for the one Gelman-Rubin value above 1.1 showed that the first chain momentarily left the convergence area for a few steps before returning for the remainder of the chain. Trace plot inspection for all other models further indicated that MCMC chains had converged properly. The satisfactory model diagnostics indicated that we could confidently proceed with interpreting the model outputs.

Discussion

Our analysis provides unique insights into the functional role of different nearshore habitat types for key nearshore consumers. All three potential habitat diet sources were estimated to be important for at least one species, which highlights the benefits of having a diverse array of habitat types within a nearshore area. Different species groups appeared to derive their nutrients from different habitats. Both Dungeness crabs and shore crabs were estimated to derive most of their diet from eelgrass type habitat. Whereas shiner perch and staghorn sculpin were estimated to derive most of their diets from farm type habitat. Stickleback diets were predominantly derived from pelagic planktonic sources, and small flatfish appeared to have diets reflective of habitat generalists, with both eelgrass and farm type habitat both providing similar proportions of their diets. For species such as shiner perch, staghorn sculpin, and juvenile flatfish, bivalve type habitat appeared to provide valuable nutrients. This finding is in line with previous research, indicating that bivalve farm habitat provides foraging opportunities for certain species of nearshore fish, particularly surf perch, juvenile flatfish, and sculpin (Veggerby et al. 2023c).

Eelgrass meadows are highly productive nearshore habitats (McRoy, 1974) that can export nutrients to adjacent mudflats, particularly in areas with high tidal and wave energy (Johannessen and Macdonald, 2016). In previous research, large cancer crabs including Dungeness crabs were observed foraging in mudflats at higher rates than bivalve farm habitat type (Veggerby et al. 2023c). However, bivalve farm habitat that did contain eelgrass supported high crab foraging (Veggerby et al. 2023c). Eelgrass also serves as nursery habitat for small Dungeness crabs (Williams 1994; Holsman et al. 2006), as well as foraging and nursery habitat for a wide range of nearshore species (McDevitt-Irwin et al. 2016; Kennedy et al. 2018). Our findings indicate that eelgrass may continue to provide valuable nutrients for Dungeness crabs even when they move into open mudflats and deeper water to forage as they mature (Veggerby et al. 2023c). This nutrient transfer may be from detrital inputs to the benthos that then supports prey for crabs, or other production that end up in nearby habitats.

Filamentous algae, other aquatic macrophytes, and biofouling anchored to natural or artificial structure such as oyster flipbags or clam-growing nets may harbor high numbers of preferred prey items such as amphipods (Caine 1991) for shiner perch and other structure-affiliated fish (Dumbauld et al. 2015). Shiner perch have previously been observed foraging in both farmed and unfarmed habitat types, particularly over heavily bio-fouled bivalve growing gear (Veggerby et al. 2023c). Most of the shiner perch diet was estimated to be derived from flipbag or similar habitat, indicating that although they do exhibit habitat flexibility, foraging is often associated with complex, vertically structured areas such as oyster flipbags, which provide predator refuge as well as abundant preferred prey items. A previous isotopic study in the same geographic area (Conway-Cranos et al. 2015), found that oysters derived most of their organic matter from autochthonous estuarine production of macrophytes such as algae and eelgrass, which highlights the importance of aquatic macrophytes as producers of energy not just for fish and crabs but for the farmed oysters themselves.

Demersal fish including staghorn sculpin and small flatfish have previously been observed to feed primarily in flipbag and unstructured mudflat habitats (Able et al. 2005). Our results dovetail with this observation, as staghorn sculpin derived a high percentage of their diets from the flipbag type habitat. In contrast, small flatfish had estimated diet sources that were more variable, with each of the three potential habitat sources contributing approximately equally to their diets. The benthic area under flipbags is similar to bare unfarmed mudflats. But the overhead structure in the form of large mesh bags may serve as protection from predators for species or groups like sculpin or small flatfish.

The structure created in bivalve farms may also be used for refuge by species like stickleback, which are abundant in both flipbag habitat and eelgrass meadows (Ferriss et al. 2021) but were estimated to derive most of their nutrition from pelagic sources. In this sense, the flipbag or eelgrass habitats may not be directly generating nutrients for stickleback but rather serve as foraging habitat for planktonic prey items that originate elsewhere while also providing cover from predators. Nutrient cycling is common between local-scale nearshore habitats, with strong linkages between adjacent bays, saltmarshes, and pelagic planktonic sources contributing to a mosaic of interconnected habitats (Conway-Cranos et al. 2015).

Bivalve farms within a larger intertidal habitat mosaic appear to provide ecosystem function in the form of foraging habitat for nearshore fish. In general, eelgrass is often considered key nursery habitat for some earlier life stages of these species (Holsman et al. 2006; Rubin et al. 2018). As subadults or adults, these species of nearshore fish derived a substantial proportion of their diets from bivalve farm habitat types. Other species such as Dungeness crabs and shore crabs appeared to derive most of their diets from unfarmed eelgrass meadows, either through directly foraging within eelgrass, or by consuming nutrients that originated from eelgrass meadows but were exported to adjacent farmed areas or mudflats.

Oyster flipbags modify habitat through the addition of benthic structure and disturbance which can reduce eelgrass density within actively farmed areas (Dumbauld et al. 2009). This leads to the creation of a habitat patchwork of eelgrass meadows and either bare mudflats or sparsely vegetated farm plots with a variety of artificial structure types. These habitats may, in turn, alter the species composition within a localized area to favor species that prefer the habitat characteristics created by a combination of artificial structure and disturbance from harvesting, crop out-planting, and other farm activities. Having a diverse set of habitat types (including bivalve aquaculture) within a nearshore area provides foraging and refuge habitat to a wide range of species. Our results indicate that bivalve farm habitats within a larger nearshore ecosystem function as foraging habitat by generating organic matter that is important for several species of nearshore fish, while Dungeness and shore crabs primarily rely on eelgrass meadows for their nutrient needs. Future studies should incorporate stomach content analysis to supplement stable isotope diet analysis.

Data availability

All data used in this study is available online at Zenodo.org:

https://doi.org/10.5281/zenodo.8118102

Table 1. Model estimates of percent diet coming from each habitat type from Samish Bay and Drayton Harbor sites combined. Flatfish consisted of juvenile English sole and starry flounder.

consumer	source	mean % diet	standard deviation	95% credible interval
shiner perch	eelgrass habitat	2.1%	2.1%	0 - 6.1%
	farm habitat	77.8%	4.3%	71.2% - 84.4%
	pelagic	20.1%	3.4%	14.8% - 25.4%
stickleback	eelgrass habitat	4.4%	3.6%	0 - 11.3%
	farm habitat	7.8%	6.5%	0 - 20.4%
	pelagic	87.8%	6.5%	79.4% - 96.2%
staghorn sculpin	eelgrass habitat	2.9%	2.7%	0 - 8.5%
	farm habitat	86.7%	4.7%	79.6% - 93.8%
	pelagic	10.4%	3.3%	5.2% - 15.6%
flatfish	eelgrass habitat	24.1%	10.8%	5.8% - 42.4%
	farm habitat	42.8%	13.5%	20.7% - 64.9%
	pelagic	33.1%	3.6%	27.0% - 39.2%
Dungeness crab	eelgrass habitat	63.3%	8.6%	51.5% - 75.1%
	farm habitat	13.1%	9.7%	0 - 31.3%
	pelagic	23.7%	4.0%	17.3% - 30.1%
shore crab	eelgrass habitat	56.7%	13.4%	39.2% - 74.2%
	farm habitat	21.4%	16.9%	0 - 54.0%
	pelagic	21.8%	6.6%	11.5% - 32.1%

Table 2. Model estimates of the difference in percent diet between organisms from Samish Bay and Drayton Harbor sites. Flatfish consisted of juvenile English sole and starry flounder. * Not enough shore crabs were collected at Drayton Harbor for site comparisons.

consumer	source	mean % diet Drayton Harbor	mean % diet Samish Bay
shiner perch	eelgrass habitat	3.3%	1.4%
	farm habitat	73.3%	85.3%
	pelagic	23.4%	13.3%
stickleback	eelgrass habitat	5.0%	2.6%
	farm habitat	9.8%	4.5%
	pelagic	85.2%	92.9%
staghorn sculpin	eelgrass habitat	24.1%	10.0%
	farm habitat	46.4%	81.0%
	pelagic	29.5%	9.0%
flatfish	eelgrass habitat	19.8%	34.1%
	farm habitat	45.4%	31.7%
	pelagic	34.8%	34.2%
Dungeness crab	eelgrass habitat	36.5%	70.3%
	farm habitat	11.6%	11.1%
	pelagic	51.9%	18.7%

Table 3. Model estimates of the percent diet coming from pelagic and combined benthic sources between organisms from Samish Bay and Drayton Harbor, and the Padilla Bay reference site. Eelgrass and flipbag sources were combined from farmed sites for comparison of benthic versus pelagic sources. Flatfish consisted of juvenile English sole and starry flounder. *Not enough staghorn sculpin or flatfish collected at Padilla Bay for site comparisons. **Not enough shore crab collected at either Padilla Bay or Drayton Harbor for site comparisons.

consumer	source	mean % diet farmed sites	mean % diet Padilla Bay
shiner perch	combined benthic habitat	79.9%	73.9%
	pelagic	20.1%	26.1%
stickleback	combined benthic habitat	12.2%	19.1%
	pelagic	87.8%	80.9%
staghorn sculpin*	combined benthic habitat	89.6%	NA
	pelagic	10.4%	NA
flatfish*	combined benthic habitat	66.9%	NA
	pelagic	33.1%	NA
Dungeness crab	combined benthic habitat	76.4%	77.1%
	pelagic	23.7%	22.9%
shore crab**	combined benthic habitat	78.1%	NA
	pelagic	21.8%	NA

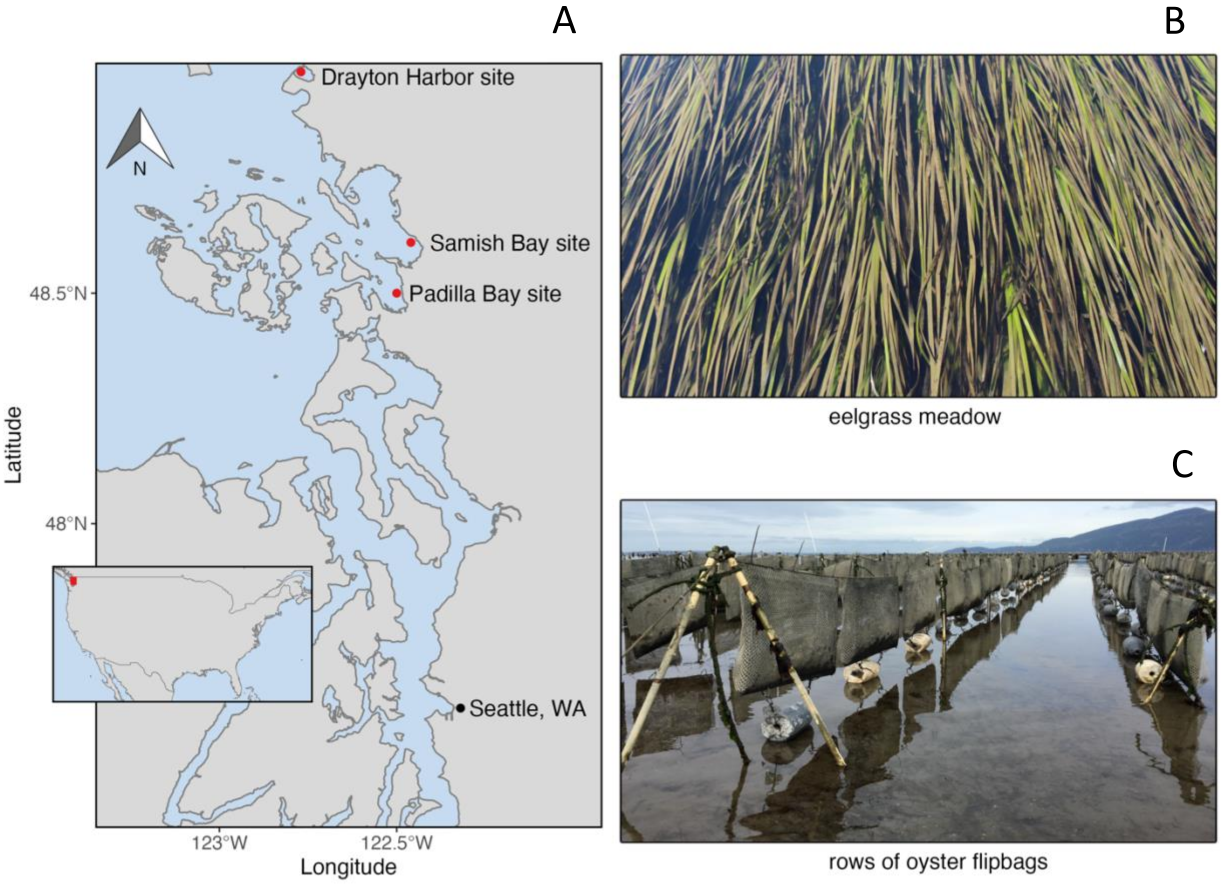

Figure 1. (A) Map of the North/central Puget Sound with locations of the two farm sites (Drayton Harbor and Samish Bay) and one reference site (Padilla Bay). The two different benthic habitat types compared in the study are shown on the right: Eelgrass meadows (B) and oyster flipbags (C).

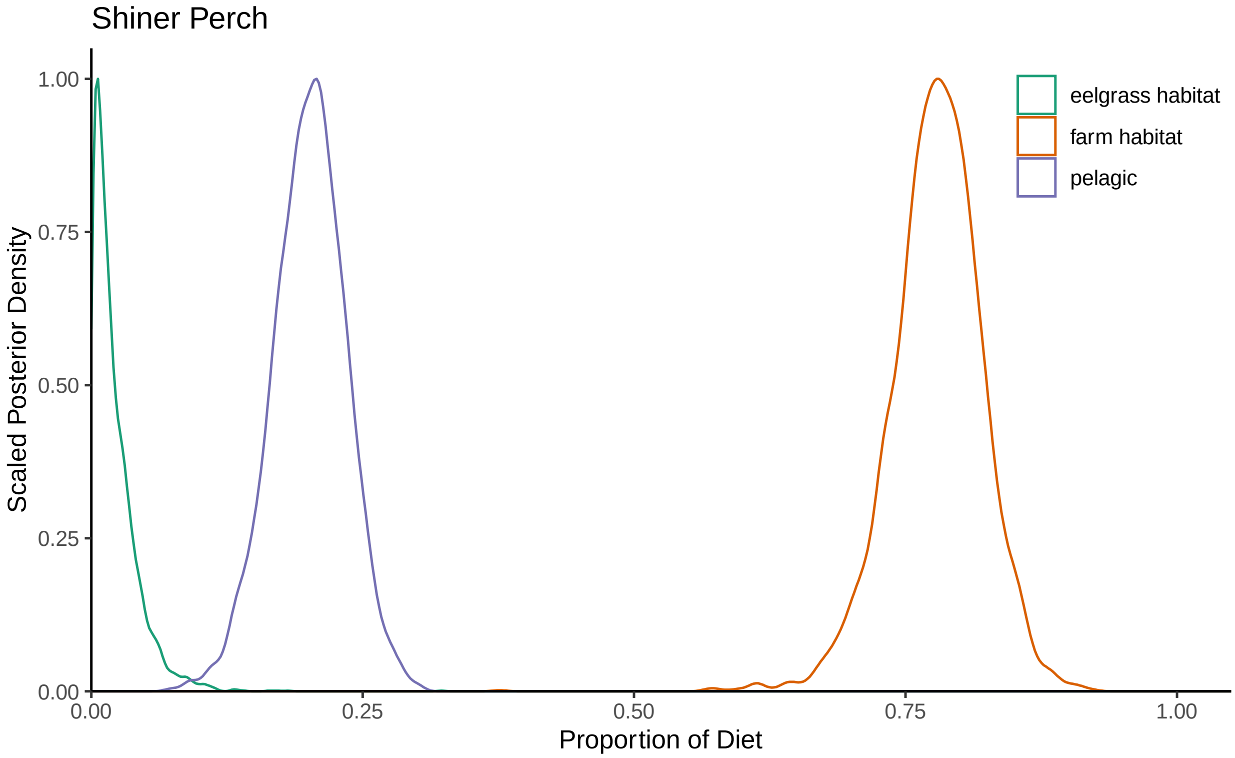

Figure 2. Posterior probability densities of estimated shiner perch diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source.

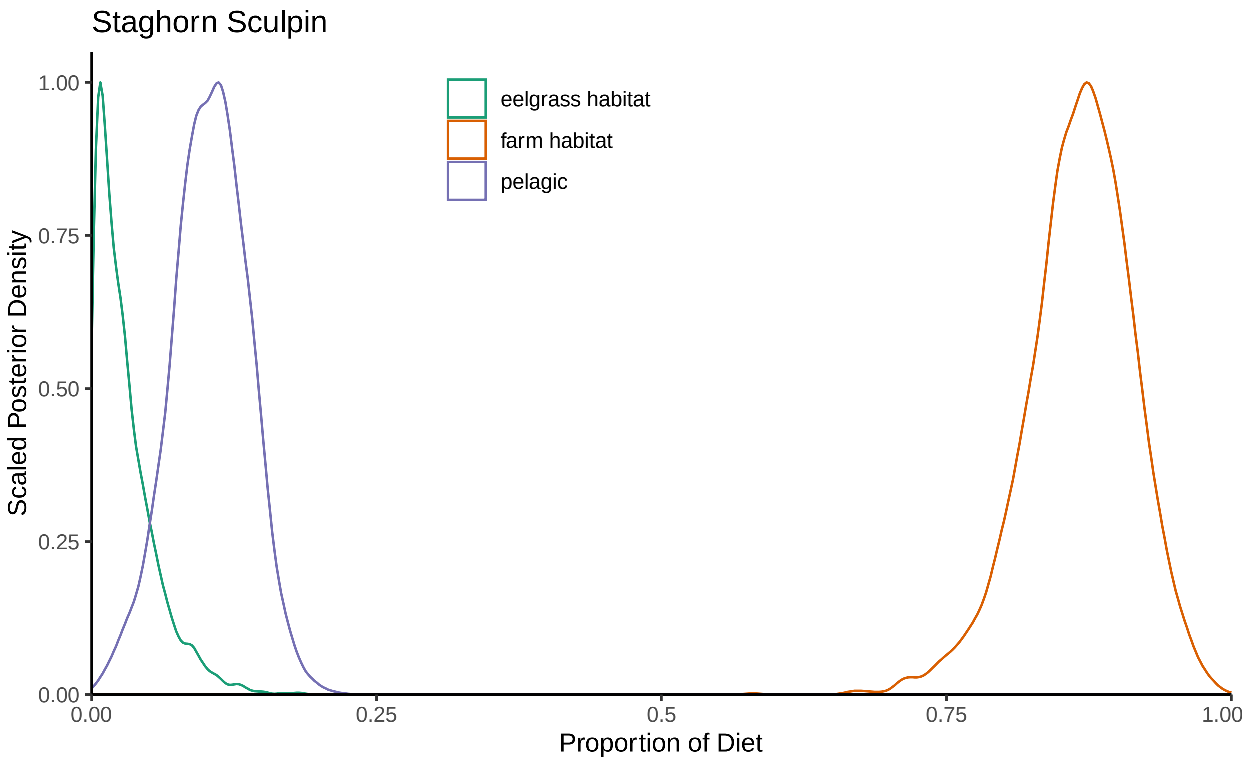

Figure 3. Posterior density plot of estimated staghorn sculpin diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source.

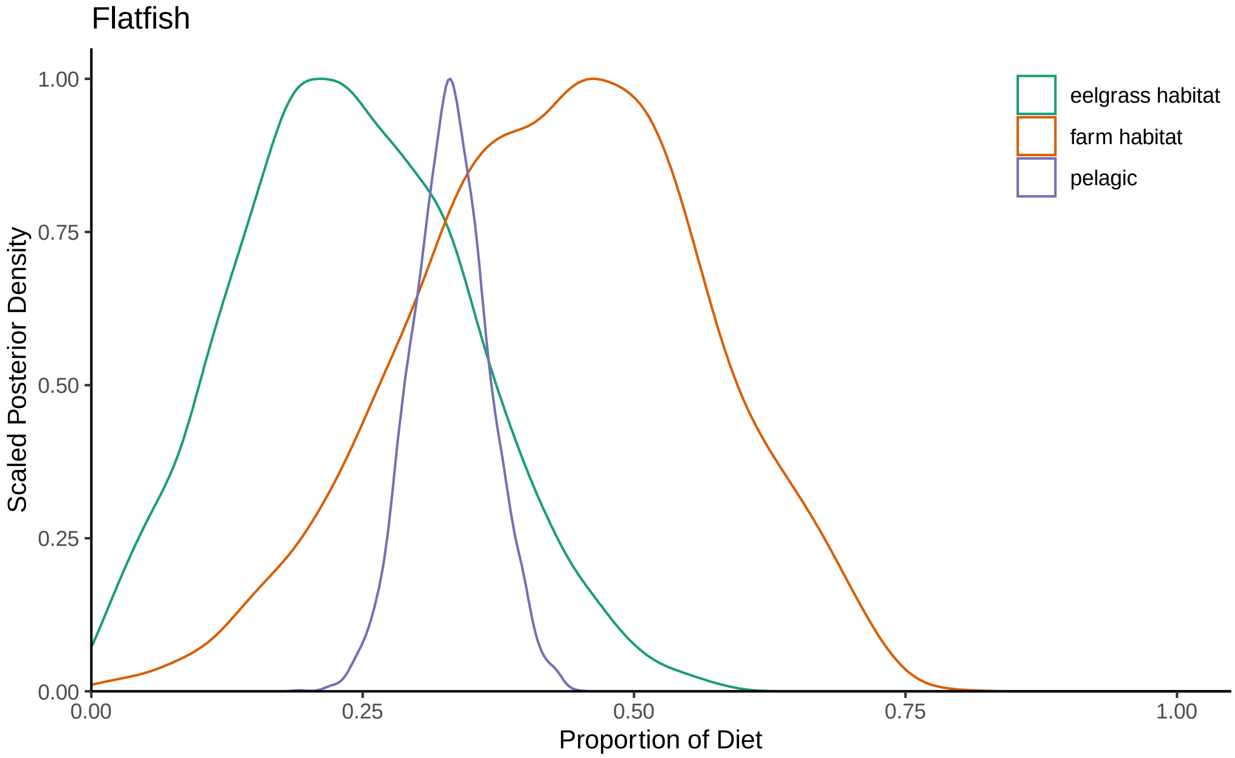

Figure 4. Posterior density plot of estimated flatfish diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source. Flatfish consisted of juvenile English sole and starry flounder.

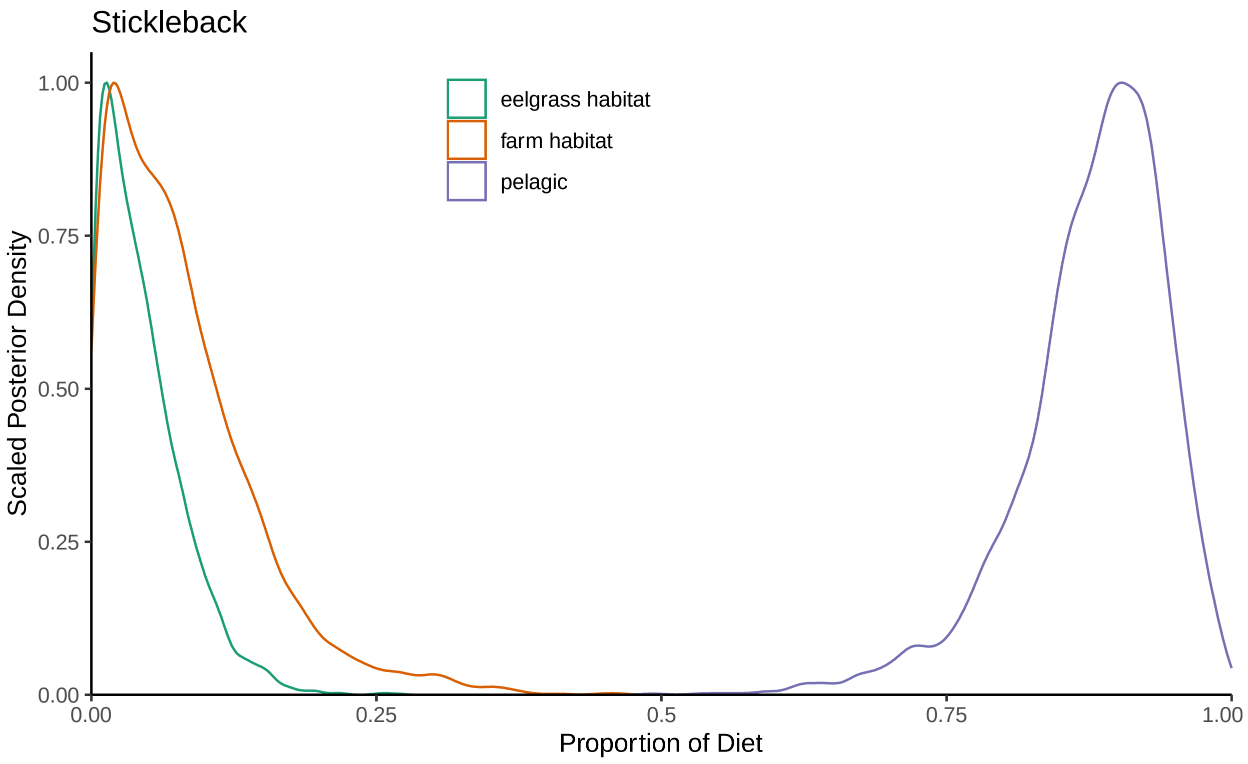

Figure 5. Posterior density plot of estimated stickleback diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source.

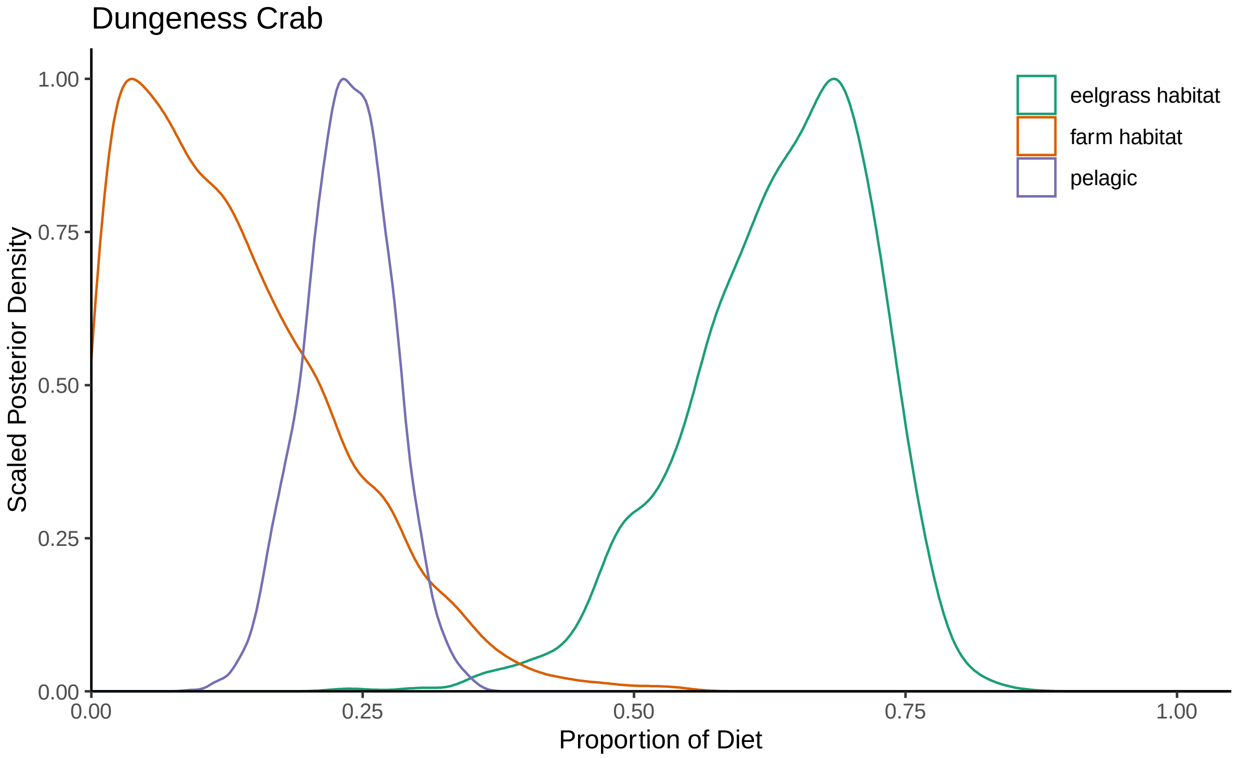

Figure 6. Posterior density plot of estimated Dungeness crab diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source.

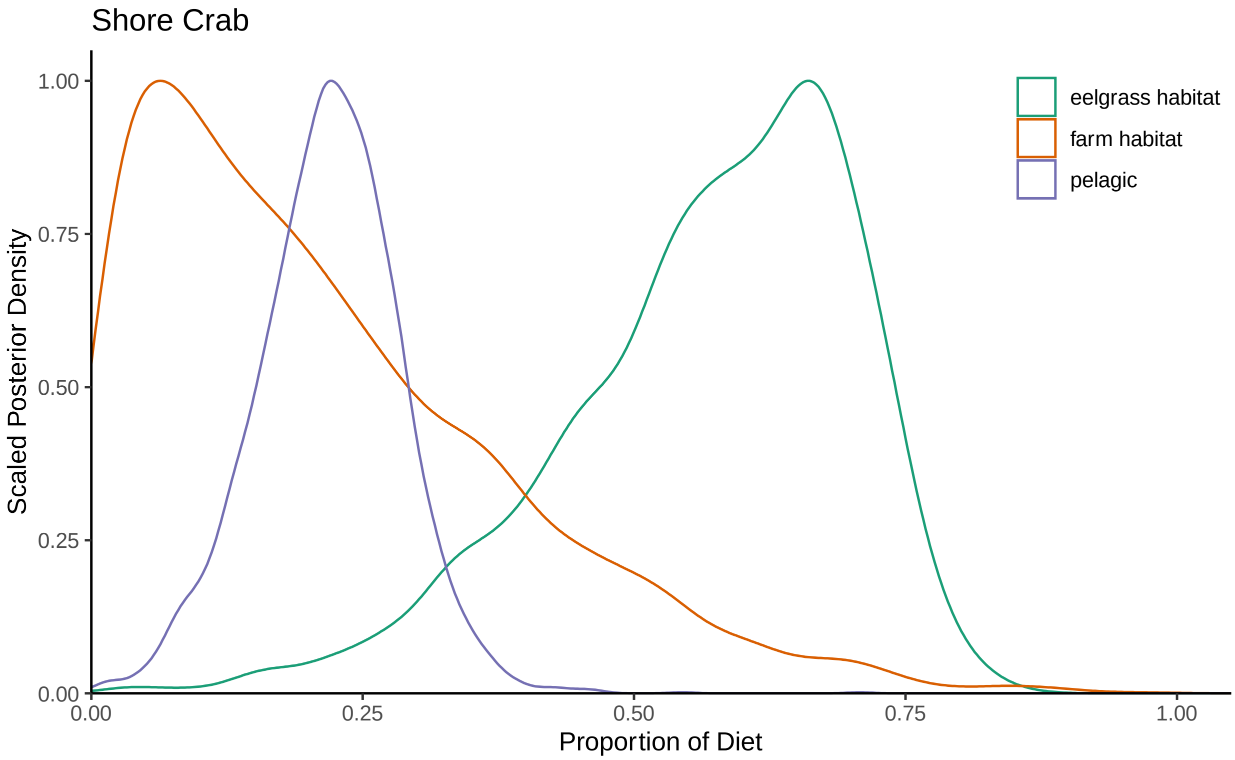

Figure 7. Posterior density plot of estimated shore crab diet sources. The peaks for each diet source represent the highest probability diet percentage coming from that source.

Literature Cited

Able, K. W., M. J. Neuman, and H. Wennhage. 2005. Ecology of juvenile and adult stages of flatfishes: Distribution and dynamics of habitat associations. Pages 164–184 *in* Robin N. Gibson, editor. Flatfishes: Biology and Exploitation. Blackwell Science Ltd.

Alheit, J., and W. Scheibel. 1982. Benthic harpacticoids as a food source for fish. Marine Biology 70(2):141–147.

Alleway, H. K., C. L. Gillies, M. J. Bishop, R. R. Gentry, S. J. Theuerkauf, and R. Jones. 2019. The ecosystem services of marine aquaculture: Valuing benefits to people and nature. BioScience 69(1):59–68.

Attrill, M. J., J. A. Strong, and A. A. Rowden. 2000. Are macroinvertebrate communities influenced by seagrass structural complexity? Ecography 23(1):114–121.

Barton, K. 2022. Package 'MuMIn.'

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, F. Scheipl, G. Grothendieck, P. Green, J. Fox, A. Bauer, and P. Krivitsky. 2022. Package "lme4" Linear Mixed-Effects Models using "Eigen" and S4 (version 1.1-31).

Beck, M. W., K. L. Heck, K. W. Able, D. L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51(8):633–641.

Bell, S. S., K. Walters, and J. C. Kern. 1984. Meiofauna from seagrass habitats: A review and prospectus for future research. Estuaries 7(4A):331–338.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference - understanding AIC and BIC in model selection. Sociological Methods & Research 33(2):261–304.

Caine, E. A. 1991. Caprellid amphipods: Fast food for the reproductively active. Journal of Experimental Marine Biology and Ecology 148:27–33.

Castel, J., P.-J. Labourg, V. Escaravage, I. Auby, and M. E. Garcia. 1989. Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio-and macrobenthos in tidal flats. Estuarine, Coastal and Shelf Science 28:71–85.

Coen, L. D., R. D. Brumbaugh, D. Bushek, R. Grizzle, M. W. Luckenbach, M. H. Posey, S. P. Powers, and S. G. Tolley. 2007a. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341:303–307.

Coen, L. D., R. E. Grizzle, J. L. Lowery, K. T. Paynter, J. Thomas, and J. Nygard. 2007b. The importance of habitat created by molluscan shellfish to managed species along the Atlantic Coast of the United States.

Conway-Cranos, L., P. Kiffney, N. Banas, M. Plummer, S. Naman, P. MacCready, J. Bucci, and M. Ruckelshaus. 2015. Stable isotopes and oceanographic modeling reveal spatial and trophic connectivity among terrestrial, estuarine, and marine environments. Marine Ecology Progress Series 533:15–28.

Cox, D. R., and E. J. Snell. 1989. Analysis of Binary Data2nd Edition. Chapman and Hall/CRC, London.

Deur, D., A. Dick, K. Recalma-Clutesi, and N. J. Turner. 2015. Kwakwaka'wakw "clam gardens": Motive and agency in traditional Northwest coast mariculture. Human Ecology 43:201–212.

Dumbauld, B. R., G. R. Hosack, and K. M. Bosley. 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, B. R., B. E. Kauffman, A. C. Trimble, and J. L. Ruesink. 2011. The Willapa Bay oyster reserves in Washington state: Fishery collapse, creating a sustainable replacement, and the potential for habitat conservation and restoration. Journal of Shellfish Research 30(1):71–83.

Dumbauld, B. R., J. L. Ruesink, and S. S. Rumrill. 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture 290(3–4):196–223.

Fernandez, M., O. Iribarne, and D. Armstrong. 1993. Habitat selection by young-of-the-year Dungeness crab *Cancer magister* and predation risk in intertidal habitats. Marine Ecology Progress Series 92(1–2):171–177.

Ferraro, S. P., and F. A. Cole. 2007. Benthic macrofauna-habitat associations in Willapa Bay, Washington, USA. Estuarine, Coastal and Shelf Science 71:491–507.

Ferriss, B., K. Veggerby, M. Bogeberg, L. Conway-Cranos, L. Hoberecht, P. Kiffney, K. Litle, J. Toft, and B. Sanderson. 2021. Characterizing the habitat function of bivalve aquaculture using underwater video. Aquaculture Environment Interactions 13:439–454.

Friard, O., and M. Gamba. 2016. BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution 7(11):1325–1330.

Fry, B. 2006. Stable Isotope Ecology. Springer US, New York, NY.

Gee, J. M. 1989. An ecological and economic review of meiofauna as food for fish. Zoological Journal of the Linnean Society 96:243–261.

Gelman, A., J. B. Carlin, H. S. Stern, D. B. Dunson, A. Vehtari, and D. B. Rubin. 1995. Bayesian Data Analysis Third edition (with errors fixed as of 15 February 2021).

Gelman, A., Y.-S. Su, M. Yajima, J. Hill, M. G. Pittau, J. Kerman, T. Zheng, and V. Dorie. 2022. Package 'arm' Data analysis using regression and multilevel/hierarchical models.

Gentry, R. R., H. K. Alleway, M. J. Bishop, C. L. Gillies, T. Waters, and R. Jones. 2020. Exploring the potential for marine aquaculture to contribute to ecosystem services. Reviews in Aquaculture 12(2):499–512.

Griggs, G. B. 2005. The impacts of coastal armoring. Shore & Beach 73(1):13–22.

Gross, C., C. Donoghue, C. Pruitt, and J. L. Ruesink. 2018. Habitat use patterns and edge effects across a seagrass-unvegetated ecotone depend on species-specific behaviors and sampling methods. Marine Ecology Progress Series 598:21–33.

Heck, K. L. Jr., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253:123–136.

Hixon, M. A., and J. P. Beets. 1993. Predation, prey refuges, and the structure of coral-reef fish assemblages. Ecological Monographs 63(1):77–101.

Holsman, K. K., S. P. McDonald, and D. A. Armstrong. 2006. Intertidal migration and habitat use by subadult Dungeness crab *Cancer magister* in a NE Pacific estuary. Marine Ecology Progress Series 308:183–195.

Horowitz, J., and L. Hoberecht. 2016. Washington: A Shellfish State.

Hosack, G. R., B. R. Dumbauld, J. L. Ruesink, and D. A. Armstrong. 2006. Habitat associations of estuarine species: Comparisons of intertidal mudflat, seagrass (*Zostera marina*), and oyster (*Crassostrea gigas*) habitats. Estuaries and Coasts 29(6B):1150–1160.

Humphries, A. T., M. K. La Peyre, M. E. Kimball, and L. P. Rozas. 2011. Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. Journal of Experimental Marine Biology and Ecology 409:172–179.

Jenkins, G. P., H. M. A. May, M. J. Wheatley, and M. G. Holloway. 1997. Comparison of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. Estuarine, Coastal and Shelf Science 44:569–588.

Jenkins, G. P., G. K. Walker-Smith, and P. A. Hamer. 2002. Elements of habitat complexity that influence *harpacticoid* copepods associated with seagrass beds in a temperate bay. Oecologia 131(4):598–605.

Johannessen, S. C., and R. W. Macdonald. 2016. Geoengineering with seagrasses: Is credit due where credit is given? Environmental Research Letters 11(11).

Kennedy, L. A., F. Juanes, and R. El-Sabaawi. 2018. Eelgrass as valuable nearshore foraging habitat for juvenile Pacific Salmon in the early marine period. Marine and Coastal Fisheries 10:190–203.

Lepofsky, D., G. Toniello, J. Earnshaw, C. Roberts, L. Wilson, K. Rowell, and K. Holmes. 2021. Ancient anthropogenic clam gardens of the Northwest coast expand clam habitat. Ecosystems 24:248–260.

Lotze, H. K., H. S. Lenihan, B. J. Bourque, R. H. Bradbury, R. G. Cooke, M. C. Kay, S. M. Kidwell, M. X. Kirby, C. H. Peterson, and J. B. C. Jackson. 2006. Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. Science 312:1806–1809.

Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. performance: An R package for assessment, comparison and testing of statistical models. Journal of Open Source Software 6(60):3139.

McDevitt-Irwin, J. M., J. C. Iacarella, and J. K. Baum. 2016. Reassessing the nursery role of seagrass habitats from temperate to tropical regions: A meta-analysis. Marine Ecology Progress Series 557:133–143.

McRoy, C. P. 1974. Seagrass productivity: Carbon uptake experiments in eelgrass, Zostera marina. Aquaculture 4:131–137.

Mercaldo-Allen, R., P. Clark, Y. Liu, G. Phillips, D. Redman, P. J. Auster, E. Estela, L. Milke, A. Verkade, and J. M. Rose. 2021. Exploring video and eDNA metabarcoding methods to assess oyster aquaculture cages as fish habitat. Aquaculture Environment Interactions 13:277–294.

Moore, S. K., N. J. Mantua, J. A. Newton, M. Kawase, M. J. Warner, and J. P. Kellogg. 2008. A descriptive analysis of temporal and spatial patterns of variability in Puget Sound oceanographic properties. Estuarine, Coastal and Shelf Science 80:545–554.

Muething, K. A., F. Tomas, G. Waldbusser, and B. R. Dumbauld. 2020. On the edge: Assessing fish habitat use across the boundary between Pacific oyster aquaculture and eelgrass in Willapa Bay, Washington, USA. Aquaculture Environment Interactions 12:541–557.

Müller, K., and J. Bryan. 2022. Package 'here.'

Munsch, S. H., J. R. Cordell, and J. D. Toft. 2017. Effects of shoreline armouring and overwater structures on coastal and estuarine fish: Opportunities for habitat improvement. Journal of Applied Ecology 54(5):1373–1384.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4(2):133–142.

Neuwirth, E. 2022. Package ‘RColorBrewer.’

Orth, R. J., K. L. Heck, and J. Van Montfrans. 1984. Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7(4A):339–350.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823–835.

Pinnix, W. D., T. A. Shaw, K. C. Acker, and N. J. Hetrick. 2005. Fish communities in eelgrass, oyster culture, and mudflat habitats of North Humboldt Bay, California final report. Arcata, CA.

Poppe, K. L., and J. M. Rybczyk. 2018. Carbon Sequestration in a Pacific Northwest Eelgrass (*Zostera marina*) Meadow. Northwest Science 92(2):80 – 91.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83(3):703–718.

Postlethwaite, V. R., A. E. McGowan, K. E. Kohfeld, C. L. K. Robinson, and M. G. Pellatt. 2018. Low blue carbon storage in eelgrass (*Zostera marina*) meadows on the Pacific Coast of Canada. PLoS ONE 13(6).

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RStudio, T. 2022. RStudio: Integrated Development Environment for R. Boston, MA.

Rubin, S. P., M. C. Hayes, and E. E. Grossman. 2018. Juvenile Chinook salmon and forage fish use of eelgrass habitats in a diked and channelized Puget Sound river delta. Marine and Coastal Fisheries 10:435–451.

Ruckelshaus, M. H., and M. M. McClure. 2007. Sound science: Synthesizing ecological and socioeconomic information about the Puget Sound ecosystem.

Shervette, V. R., and F. Gelwick. 2008. Seasonal and spatial variations in fish and macroinvertebrate communities of oyster and adjacent habitats in a Mississippi estuary. Estuaries and Coasts 31(3):584–596.

Shinn, J. P., D. M. Munroe, and J. M. Rose. 2021. A fish’s-eye-view: Accessible tools to document shellfish farms as marine habitat in New Jersey, USA. Aquaculture Environment Interactions 13:295–300.

Shull, S., and D. Bulthuis. 2002. A methodology for mapping current and historical coverage of estuarine vegetation with aerial photography and ArcView. Mount Vernon, Washington.

Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips, and B. X. Semmens. 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ 2018(6).

Stock, B., and B. Semmens. 2018. MixSIAR GUI user manual v3.1. San Diego.

Stock, B., B. Semmens, E. Ward, A. Parnell, A. Jackson, and D. Phillips. 2022. Package 'MixSIAR.'

Veggerby, K., Mark D. Scheuerell, B. Sanderson, P. Kiffney, and B. Ferriss. 2023a. Shellfish aquaculture farms as foraging habitat for nearshore fishes and crabs [Data set]. Zenodo https://doi.org/10.5281/zenodo.7742785.

Veggerby, K., M. D. Scheuerell, B. Sanderson, and P. Kiffney. 2023b. Stable isotopes reveal intertidal fish and crabs use shellfish farms as foraging habitat in Puget Sound, Washington [Data set].

Veggerby, K., M. D. Scheuerell, B. L. Sanderson, P. Kiffney, and B. Ferriss. 2023c. Shellfish aquaculture farms as foraging habitat for nearshore fishes and crabs. Unpublished.

Washington State Department of Natural Resources. 2022. Seagrass Monitoring in Puget Sound (2000-2020): What we have learned.

Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. PNAS 106(30):12377–12381.

Wechsler, J. F. 1996. Assessing the relationship between the ichthyofauna and oyster mariculture in a shallow coastal embayment, Drakes Estero, Point Reyes National Seashore. University of California, Davis.

Wickham, H. 2023. Package 'tidyverse.'

Williams, G. D. 1994. Effects of habitat modification on distribution and diets of intertidal fishes in Grays Harbor Estuary, Washington.

Zu Ermgassen, P. S. E., M. D. Spalding, B. Blake, L. D. Coen, B. Dumbauld, S. Geiger, J. H. Grabowski, R. Grizzle, M. Luckenbach, K. McGraw, W. Rodney, J. L. Ruesink, S. P. Powers, and R. Brumbaugh. 2012. Historical ecology with real numbers: Past and present extent and biomass of an imperilled estuarine habitat. Proceedings of the Royal Society B: Biological Sciences 279(1742):3393–3400.