Habitat Function of Shellfish Aquaculture Ecosystems: Fish Behavior and Diets

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Introduction

A major consideration when permitting and implementing shellfish aquaculture is its impact on nearshore habitats. Many studies show that modifying shorelines can impair habitat functions. However, most research along the west coast has focused on effects of habitat destruction and we know little about the introduction of structured artificial habitats. Such habitats may provide prey or predator refuge that mitigates risk of feeding behavior, both of which may enhance feeding. There is evidence that bivalve aquaculture can confer some habitat functions (Alleway et al. 2019; Gentry et al. 2020), but whether shellfish aquaculture sites provide habitat function comparable to natural areas is still not well understood (Dumbauld et al. 2011). Thus, understanding whether aquaculture sites provide foraging opportunities for commercially, recreationally, and culturally important fish and crab species, and the value of aquaculture sites relative to natural areas, will help inform decisions about the ecological implications of an expanding shellfish aquaculture industry. To address this high priority knowledge gap, my master's research aims to compare the feeding behavior of consumers at shellfish aquaculture habitats and nearby natural habitats.

Complex nearshore waters are important habitats for many species because they support functions such as 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). However, over the past 100 years, oyster reef habitat has declined about 64% in the United States, with Olympia Oysters on the west coast becoming functionally extinct in most of their historical range (Zu Ermgassen et al. 2012). Olympia Oyster reefs still exist in Puget Sound in limited areas, but have been greatly reduced. Only about 4% of historical Olympia Oyster reefs remain in Washington State (Horowitz and Hoberecht 2016). In response to the loss of native oysters, shellfish aquaculture is a growing enterprise that confers to people both economic opportunities and food (Horowitz and Hoberecht 2016). Although shellfish aquaculture offers direct benefits to people, our understanding of its impacts on nearshore ecosystems and associated habitats is incomplete.

Ecological functions of nearshore habitats, including shellfish aquaculture habitats, are challenging to quantify. Underwater video can describe what species are present in habitats and how they use these habitats. Using underwater cameras to collect data has become common, and has been used in numerous other studies over the past few years (Gross et al. 2018; Muething et al. 2020; Mercaldo-Allen et al. 2021; Shinn et al. 2021). Over the past several years, I have worked with colleagues at NOAA to deploy underwater video cameras to record fish and crab species occupying shellfish aquaculture and adjacent eelgrass and sediment habitats (Ferriss et al. 2021). We have collected thousands of hours of video, a subset of which I analyzed to identify the species present in these habitat types (Ferriss et al. 2021). However, to understand the ecological functions of these habitats more completely, and not just what species are present, we need more information about how those species use the habitat for foraging.

Advances in stable isotope technology have enabled researchers to use naturally occurring elements as tracers of feeding relationships (Fry 2006). Stable isotopes are commonly used in ecology to quantify the proportional importance of prey items in a predator's diet (Peterson and Fry 1987; Benstead et al. 2006; Fry 2006); the stable isotope ratios in a consumer's tissues reflects the proportional contributions of its prey's isotope ratios. Over time, an individual's isotopic signature will shift according to its consumption patterns, thus providing clues to dietary changes (Sakano et al. 2005). Two common isotopes are nitrogen 15 ($\delta 15N$), used to indicate dietary inputs and trophic position within a food web and marine derived nutrient sources; and carbon 13 ($\delta 13C$) which indicates potential sources of energy or dietary inputs (Fry 2006). Anticipated differences in carbon and nitrogen isotopic signatures between farmed and unfarmed tidelands will allow us to model the corresponding diet proportions between these areas for several common species of nearshore crab and fish.

Objectives

I will augment the video analysis by collecting new fish and crab stable isotope data to identify the foraging potential of shellfish aquaculture for nearshore marine species, and to explore the implications of this potential at a seascape scale. I will characterize crab and fish feeding through the collection of stable isotopes to determine if diets and energy flow differ among seascapes with varying proportions of eelgrass, bare sediment, and shellfish aquaculture habitat. I will collect my data in Puget Sound in collaboration with tribes and industry (farms at which the video was collected, including Taylor Shellfish, Drayton Harbor Oyster Co, and Padilla Bay National Estuarine Research Reserve.

My goal is to quantify foraging behavior using underwater video and stable isotope mixing models to generate estimates of percent diet that originate from shellfish farms and percent diet that originate from unfarmed natural areas in the short term (video data) and longer term (stable isotope data). My specific hypotheses are:

1) The contribution of consumed prey originating from aquaculture sites will be highest in seascapes dominated by relatively unproductive (e.g., bare bottoms vs. eelgrass) or developed (e.g., armored shorelines vs. beaches, agricultural vs. vegetated backshore) habitats.
2) The amount of time fish and crabs are observed feeding will be higher in aquaculture and natural areas with relatively more structure (e.g., oyster flipbags, eelgrass) than in areas with less structure (e.g., clam nets, bare sediment).

Methods

Data for chapter 1 was collected in the spring and summer of 2017 and 2018. GoPro cameras were placed at seven different shellfish aquaculture sites across North Puget Sound, South Puget Sound, and Hood Canal. Sites were selected based on the shellfish species being farmed, the grow gear present, and the accessibility of the site. The three gear types studied were oyster flipbags (also known as tumble bags), clam nets, and loose oyster bottom culture with is roughly comparable to a natural oyster reef. These gear types are the predominant grow-out gear type used by most oyster and clam farms in Puget Sound. Reference sites were selected to be of similar tidal elevation to the farmed areas and consisted of unfarmed eelgrass meadows and mudflats that were located 30 to 60 meters away from the edge of the shellfish farm. This distance from the farms was chosen to minimize potential environmental influences from the shellfish farms, while maintaining similarity in environmental conditions between the farmed and unfarmed reference sites.

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

The cameras were mounted on individual pieces of PVC pipe approximately 30cm 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. Each site had two video collections per summer between June and August of 2017 and 2018. 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, morning high tides were used for analysis due to improved visibility in morning versus afternoon tides. During tidal cycles where daylight morning high tides were not available, afternoon high tides were used for analysis instead.

After cameras were retrieved, 10 video segments were selected for analysis from each habitat type. Of these, five videos were from timelapse that were recorded over the course of an hour prior to high tide, and five videos 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. In all, several thousand hours of video were collected, of which approximately 75 hours was analyzed for species and behavioral observations. For more information on how this data was collected, see (Ferriss et al. 2021).

Using the video analysis software BORIS (Friard and Gamba 2016), counts of fish and crabs observed within a one-meter square area were counted from each video. Each organism observation was also assigned a feeding behavior classification. Organisms were grouped as either observed to be actively feeding or observed to not be feeding. Videos were ranked as either low, medium, or high visibility to account for potential observer bias resulting from different water quality conditions across the study sites which could have obscured observations or behaviors in lower visibility videos.

Observations from each 2-minute video were summed across the 10 videos analyzed from each habitat type on a given date, to calculate the sum of each species and behavior combination observed. We obtained a total of 393 crab observations, 244 demersal fish observations, and 2,043 pelagic fish observations across all seven farm sites. Crabs were not separated further into species or other groupings due to visual difficulty with correctly identifying crab species on video.

Video data consists of behavior count data derived from underwater video of species observed in different nearshore environments across several sites in North Puget Sound. Behavior data will be analyzed to examine the extent to which species feed more frequently in farmed versus unfarmed control areas. Generalized linear mixed models will be used for modeling. Predictor variables will include fixed effects of habitat type and visibility, and a random effect of site. Models will be ranked based on AICc values, and model averaging will be employed if there are multiple competing models within delta AICc 2.0 of the top model.

I conducted initial sampling for chapter 2 in Padilla Bay, Samish Bay, and Drayton Harbor during the summer of 2021, with larger scale final sampling completed in the summer of 2022. At each location, I will estimate the percent of an organism's diet that originates from farmed areas versus natural areas for several nearshore species, including Dungeness crabs (*Metacarcinus magister*), graceful crabs (*Metacarcinus gracilis*), shiner perch (*Cymatogaster aggregata*), English sole (*Parophrys vetulus*), staghorn sculpin (*Leptocottus armatus*), and stickleback (*Gasterosteus aculeatus*). Because Padilla Bay does not have any shellfish farms, this site will serve as a reference site. Nearshore species were targeted with beach seines at low tide, along with opportunistic hand collection of crabs when possible. Crab pots were deployed concurrently with beach seining to try to trap additional crabs while we were in the field.

I also collected diet sources for these species concurrently within shellfish farms, as well as in unfarmed reference areas within each bay. Collection at each site included common prey items such as amphipods, along with common invertebrates such as shore crabs and several species of intertidal snail. Eelgrass, algae, epiphytes, and gut balls from filter feeders (Pacific oysters and manila clams) will serve as indicators of primary production at each site. Alternatively, mud and bubble snails, which graze on epiphytic algae, can be used to quantify the primary productivity (Scheuerell, personal communication). These snails are ideal because they are ubiquitous across the sample sites, and provide a consistent proxy of primary productivity across the sites.

All samples will be dissected to obtain tissue samples for stable isotope analysis. Dorsal muscle plugs will be extracted from each fish, and claw muscle taken from each crab. All samples will be freeze dried, pulverized, weighed into tin capsules, and analyzed on a mass spectrometer at UW to measure their carbon and nitrogen isotope ratios. Using the stable isotope mixing model MixSIAR (Stock and Semmens 2018), I will estimate the percent diet that originates from farmed areas and unfarmed areas for each target species using individual mixing models for each species. A fixed effect of site will be added to the mixing model to quantify differences across different habitat types.

Results

<u>Chapter 1 - Frequency of feeding by species functional group at different habitat types</u>

Results from the GLMM will allow me to evaluate and rank which habitats were most frequently used for feeding by the different species functional groups. Preliminary results suggest that pelagic fish such as surf perch fed most frequently in eelgrass and clam net habitat, demersal fish such as flatfish fed most frequently in mudflats and oyster flipbag habitats, and crabs (primarily larger cancer crabs) fed most frequently in mudflat habitat. These results are in line with several previous studies on structure preferences for common species within each of these functional groups.

<u>Chapter 2 – Diet composition as a function of habitat</u>

Samples from Padilla Bay (which has no aquaculture, and where 100% of the diet is assumed to be from unfarmed areas) will be used as a reference to determine if the model over-assigns feeding to farms. Estimates can be corrected if needed based on this data. For example, if consumers from Padilla Bay are estimated to derive 20% of their diets from aquaculture, then we know that the models are over-estimating consumption from aquaculture by about 20%. Estimates from the other sites that do contain aquaculture can be adjusted accordingly.

Results from this chapter will consist of estimates of diet proportions coming from farmed oyster flipbags and unfarmed eelgrass meadows. Whereas results from chapter 1 were derived from behavioral observations and consisted of behavioral ranking by habitats, chapter 2 results will be estimated from the isotopic makeup of diet items (primary productivity) collected from oyster flipbag and nearby eelgrass meadow habitat, and consumers (fish and crabs) collected in the same overall area. A hypothetical example result could be: Staghorn sculpin living in proximity to oyster farms are estimated to derive 60% of their overall diet from farmed areas, and 40% of their diet from unfarmed eelgrass meadows.

Interpretation and Significance

My findings will help determine if aquaculture habitats can enhance nearshore foraging opportunities for fish and invertebrates, especially within seascapes that are less naturally productive or already highly modified. Thus, managers charged with protecting nearshore habitats may prioritize the introduction of aquaculture in unproductive or degraded seascapes, but employ a more precautionary approach in more productive, intact seascapes. Increasing shellfish aquaculture is a policy priority for NOAA, but there are lingering questions about the scale of ecosystem services provided by shellfish farms, and their impacts to the nearshore ecosystem. This translates to management uncertainty about how much shellfish aquaculture to authorize, and where to locate it.

Timeline:

Chapter 1: The behavior data that will be used to write chapter 1 has already been collected and organized. I currently have the analysis completed for this chapter. I anticipate having a complete chapter 1 draft to share with committee by mid-winter quarter 2023.

Chapter 2: Initial sample collection for the stable isotope project began in the summer of 2021. I collected a final batch of samples in summer 2022 to supplement the initial collection and acquire a sufficient sample size to conduct analysis. Stable isotope samples collected in summer of 2021 were run in early summer 2022. Samples collected in the summer of 2022 were processed and analyzed in fall 2022. I am 100% finished with running all the samples as of writing this proposal. I anticipate having a complete chapter 2 draft by the end of spring quarter 2023.

My goal is to have drafts of both chapters by the end of spring quarter. Summer quarter 2023 I want to finalize the drafts for submission to journals and hopefully submit them. Thesis defense will be at the end of July, with a UW submission deadline of August 18th.

Table 1. Anticipated timeline of progress:

Quarter & year	Tasks
Spring 2022	• Formed committee
Summer 2022	• Completed fieldwork • Plan of study submitted • First committee meeting
Fall 2022	• Finished sample processing • Finished isotope sample analysis • Complete initial chapter 1 draft
Winter 2023	• Chapter 1 draft cleaned up and edited with committee • Chapter 2 data analysis
Spring 2023	• Complete chapter 2 draft
Summer 2023	• Finish chapters and defend thesis

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