Oyster aquaculture shapes intertidal communities: from foundation species to higher trophic levels

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Abstract

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The work presented in this dissertation sits at the intersection of two major global issues, rapidly increasing stressors on marine systems, and simultaneously, a need for sustainable food solutions. Thus, a wicked problem presents itself: how can we support ecosystem function in marine systems, while also leveraging these systems for food production? The first step to addressing this question is to better understand how aquaculture, in this case oyster aquaculture, shapes intertidal communities. Our work was conducted in Willapa Bay and Grays Harbor, Washington, a globally important region for production of the introduced Pacific oyster (*Crassostrea gigas*, now *Magallana gigas*), where we investigated the effects of oyster aquaculture across multiple trophic levels, considering different methods of culture as well as the impacts of associated disturbances. We evaluated the use of oyster culture as habitat to a wide variety of intertidal taxa, focusing first on waterbirds (Chapter 1) and then on nekton (Chapter 2), investigating how organisms respond to culture habitats versus seagrass and mudflat habitats (Chapters 1 & 2). Taxa responses to habitat type were species-specific for birds and nekton

generally, with some taxa utilizing oyster culture while others preferred seagrass or mudflat habitats. Some nekton taxa were found to be structure-generalists, meaning they used seagrass and culture habitats similarly. There were very few instances of negative effects of oyster culture on birds or nekton habitat-use, and we concluded that maintaining a mosaic of habitat types best supports the diverse organisms in the estuary. While structures associated with oyster culture provide habitat to a variety of taxa, disturbance events from mechanical shellfish harvest can cause substantial damage to seagrass (*Zostera marina,* i.e. eelgrass) beds, another habitat-forming foundation species. We investigated how timing of large-scale dredge disturbance interacts with eelgrass phenology and determined that disturbance during the slow-growing overwintering phase or early spring provides the best outcome for eelgrass recovery, with a major contribution from seedlings if the seedbank is given the opportunity to regenerate. Estuaries serve as a resource oasis that link the land to the sea with many organisms spending part of, or the entirety of, their lives as inhabitants. While many estuaries worldwide have been heavily modified anthropogenically and restoration to a "pristine" pre-industrial condition may not be possible, research-based management decisions can conserve or restore function of the ecosystem to better serve native wildlife, commercial fisheries, and aquaculture. Overall, findings from this work can be applied to co-management of estuaries and shellfish aquaculture, with the goal of maintaining ecosystem functioning.

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Acknowledgments

I wouldn’t be where I am today without the opportunities I’ve had to observe the natural world, and the space I’ve been allowed to be curious. From making “pill bug habitats” in the backyard and bringing western tent caterpillars home in my mom’s coffee mug, to helping me keep fish, frogs, birds and rodents, I want to thank my parents, Anna and Ethan, for fostering this curiosity from a young age (even when it was kind of gross; the caterpillars got way out of hand, and my mom definitely consumed a decent amount of fish tank water doing water changes). I’d also like to thank them for introducing me to the ocean at a young age, showing me intertidal critters at low tide and pointing out life beneath the surface from our sailboat. Reflecting on these experiences it is obvious that the seeds of my PhD started sprouting long before five years ago.

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Chapter 1: Taxon-specific habitat and tidal use by birds in an oyster culture estuary

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Abstract

Shorebirds use a variety of intertidal estuarine habitats to rest and refuel during their seasonal migrations. Birds can be found foraging on mud or sandflats, aquatic vegetation, as well as intertidal areas developed for shellfish aquaculture. In Washington State, which contributes substantially to commercial U.S. production of the Pacific oyster (*Crassostrea gigas*), little research has been published about how aquaculture habitats are used by shorebirds relative to surrounding seagrass and mudflat. Using photographic sampling, shore- and waterbird use of mudflat, seagrass and longline oyster culture habitats was studied on an oyster farm in Grays Harbor, WA. Effect of tidal stage (ebb, dry or flood periods) was also evaluated. Thirteen bird taxa were identified and analyzed for effects of habitat on community composition and total bird abundance, while the six most common taxa were used in an analysis of habitat type and tidal stage effects on taxon abundance. Of the six focal taxa, Black bellied plover, American crow and dunlin (*Pluvialis squatarola, Corvus brachyrhynchos, Calidris alpina*, respectively) responded significantly to habitat type—having positive associations with eelgrass and/or longlines—while dunlin, dowitcher and gulls (*C. alpina, Limnodromus* spp., *Larus* spp., respectively) responded significantly to tidal stage—having positive associations with the ebb or flood periods. Total bird observations varied by habitat and through the tidal cycle, where more birds were observed in eelgrass and during ebb and flood periods. There was no strong effect of habitat type on community composition when sampling across

several months. Overall, all three habitat types were used by a variety of shore- and waterbird taxa, with no evidence of a negative effect of longline oyster culture on bird abundance.

Introduction

Estuaries serve as critical stopover and breeding sites for many species of waterbirds. Grays Harbor Estuary, in Washington State, USA, has been designated as a reserve of international significance by the Western Hemispheric Shorebird Reserve Network since 1996 (US Fish & Wildlife Service), due to being an important stopover habitat for migratory birds. Tidal flats of Grays Harbor are also used commercially to farm oysters (mostly non-native *Crassostrea gigas*). As a result, the intertidal zone is a mosaic of three main habitat types: unstructured mudflat, seagrass (native eelgrass, *Zostera marina*), and oyster aquaculture. Despite the recognized importance of the region for shorebirds, how birds interact with the habitat mosaic has not been explored in Grays Harbor, where bird activity could also vary seasonally and through the tidal cycle.

Structured habitats found nearshore, such as seagrasses, reef-forming bivalves, mangroves, and coral reefs, are well known for their ecosystem services, such as providing trophic resources and refuge, as well as their ability to host higher diversity communities than in unstructured habitats (Beck et al. 2003, Kovalenko et al. 2012, Whitfield 2017). Anthropogenic structure, in this case shellfish culture suspended above the sediment, has been documented to have a mix of positive and negative effects on intertidal communities including sea- and shorebirds, with a consensus that the structures can provide resources and refuge (Dumbauld et al. 2009, Callier et al. 2018). In past studies of waterbird use of structures placed for oyster culture, different bird species show distinct habitat associations (Caldow et al. 2003, Kelly et al. 1996, Burger & Niles 2017), and the overall pattern may be attraction (Connolly and Colwell 2005) or avoidance (Hilgerloh et al. 2001). An advance in the current study was to include both unstructured mudflat and eelgrass as comparative habitats to longline oyster culture. With the potential for increased refuge and resource availability, increased use of structured habitats (seagrass and/or suspended oyster culture) by some shorebird taxa was predicted. Shorebird species likely vary in how they use the habitat mosaic, as seen in past studies, based on factors such as foraging strategy (Jing et al. 2007), diet, and predation risk.

Waterbird habitat use could differ over time at multiple temporal scales. Seasonally, migratory birds pass through the region twice a year as they move to and from northern breeding grounds. The physiological needs of the birds are different based on whether they are heading to breed, or returning from breeding, which can be reflected in behavioral changes like flock size, and decisions surrounding caloric intake and predator avoidance (O'reilly & Wingfield 1995. Ydenberg et al. 2002). Other birds, such as the American Crow and certain Gull species use the Grays Harbor habitat continuously throughout the year, but would still be likely to have seasonal changes that reflect pre- and post-breeding, even if they do not migrate. On a much smaller temporal scale, the timing of tides influences access to intertidal flats. In the intertidal habitat mosaic studied here, habitats are exposed 0-2 times a day, depending on neap vs. spring tides. Many shorebirds forage at the waterline (tide-following), where resources are emerging during an ebb tide and before infauna have burrowed down as water drains from sediment (Jiménez et al. 2015), however, tide-following may be abandoned in particularly resource-rich environments (Drouet et al. 2015). Given this, it is hypothesized that taxa who act as habitat-generalists will show strong tide-following tendencies.

Existing work has largely occurred in California and New Jersey (Caldow et al. 2003, Kelly et al. 1996, Burger & Niles 2017, Burger 2018, Connolly and Colwell 2005, Hilgerloh et al. 2001); this study is the first to examine the use of oyster aquaculture habitat by waterbirds in Washington State, a dominant region of commercial oyster production in the United States. This study is also unique in that it compares both bare and eelgrass habitats outside of culture, whereas past studies in other regions have primarily compared inside vs. outside of shellfish aquaculture (a single control habitat), or shellfish reefs versus aquaculture (Burger & Niles 2017, Burger 2018) . The oyster culture featured in the current study is a type of intertidal off-bottom culture known as “longlines”, where clusters of oysters are strung on lines (Fig 1). Including both eelgrass and oyster longlines as two structured habitats in the study allows for differentiation between simply the presence of structure vs. no structure and possible differences based on *type* of structure; i.e. does type of structure matter, or will waterbirds respond to eelgrass and longline habitats similarly? The aims of this study were to determine:

1) Temporal differences in waterbird abundance on an intertidal flat in Grays Harbor through the spring and fall migration periods,
2) How bird taxa respond to habitat type and tidal stage, and
3) How bird community composition responds to habitat type.

Methods

Field Methods

Surveys of waterbirds were performed at an intertidal site with a mosaic of habitat types in Grays Harbor, Washington, USA (46.8667N, 124.0697W). Three focal habitat types were oysters on longlines, eelgrass and adjacent bare mudflat habitat, in patches of ~100 m at equivalent tidal elevation (mean lower low water). There was no eelgrass within the longline habitats (Fig. 1). The design included two true replicates, each of which was a block consisting of three patches (one of each habitat type). Blocks were separated by 1 km. Bird presence was surveyed in the field of view (~7 m diameter) of cameras that captured one image per minute when habitat patches were exposed during a daytime low tide, on average 4.4 hours. Surveys were carried out every two weeks in spring (mid-March to late-May 2020) and in fall (late August to mid-October 2020). Each spring survey consisted of two sampling days, but fall surveys were done on a single day. These two-week intervals coincided with spring tides of relatively high tidal amplitude. In most cases, each patch was surveyed with two cameras. Cameras occasionally failed, resulting in only one camera per patch for that day. Also during mid- and late-March surveys, only longline and eelgrass habitat were surveyed (3 cameras per patch per day; Table 1). Fall surveys were limited at the end of the migration period by low tides no longer occurring during daylight hours, and birds appearing around the farm later than anticipated from their migratory season.

Cameras (GoPro Hero3+ Silver Edition) were placed roughly 60 m apart in each patch and 1m above the substrate, with a bamboo stake placed 7m from the camera to determine the frame of view for data collection. CamDo intervalometers were used to take photos at a 1-minute interval. Cameras were deployed during daylight ebb tides and retrieved during the following flood tide. Sites were accessed by kayak, and camera deployment and retrieval occurred in shallow water, except on some occasions when timing was misjudged, or the authors were constrained by daylight and had to do the final approach to camera position on foot. Photographs were displayed on a computer screen, and birds were identified to species and counted in all images between five minutes before the water ebbed past each camera and then five minutes after the water passed again during the flood. The total interval was divided into equal thirds to create tidal stages: ebb, dry, flood. Some species were combined into taxa groups due to the inability to distinguish species in the photos; for example, all gull species were grouped into "Gull spp.", and long- and short-billed dowitchers were combined into "dowitchers".

Species-specific Abundance and Habitat-use

Samples consisted of all images from each camera per habitat patch and tidal stage on one day. The sum of birds per species for each camera was divided by the number of images examined, providing an index of relative abundance (birds per minute). Any bird that appeared in a frame of view multiple times was counted each time; individuals were unable to be distinguished to overcome this potentially repeated counting. Two analyses were carried out with respect to the six most common bird taxa, addressing Aims 1 and 2:

(1) Seasonality: relative abundance (birds/min on each day) was determined in each block and visualized over time

(2) Habitat type (eelgrass, longlines, bare) and tidal stage (ebb, dry, flood) relative abundance was determined for each habitat patch and tidal stage within a block on days when the species was present (at least one individual sighted at either block).

Generalized linear mixed effects modeling was performed for each taxon (glmmTMB package, R version 4.1.3, R Core Team 2022) to test whether relative abundance (birds/minute on each day) differed by habitat type (eelgrass, longlines, and bare) or tidal stage (ebb, dry, flood). The data were not well described by typical distributions due to the preponderance of zero values and large variability in numbers when birds were present. A tweedie distribution (Smyth 1996, Shono 2008) applied to these data resulted in suitable residual distribution (DHARMa package, R) and was used in linear mixed models. Date and block were included as random effects, with camera position (two or three cameras per habitat per block) as a nested random effect. For dowitchers, dunlin and black bellied plovers, only spring dates were used due to their limited presence during fall sampling, and 5/31/20 was excluded from all models, due to sampling a single bird. Confidence intervals were extracted and visualized with the effect estimates. An additional generalized linear mixed effects model was fit to test the effect of habitat and tidal stage on total bird abundance with date and block as random effects (with camera position nested as before). This model also utilized tweedie distribution and was tested for fit using residual analysis (glmmTMB, DHARMa packages). All observed taxa were combined (total birds/min, no taxa removed), to have a value for each habitat per day and block.

Bird community composition

Abundance standardized by time (birds/min) was calculated for each habitat per block per day, removing taxa that were present in fewer than 5% of the samples, resulting in 11 remaining taxa (Table 1b), and one taxon removed (willet, *Tringa semipalmata*). Rows (birds per day, taxa, tidal stage, habitat and block, averaged across 2-3 cameras [see Table 1a]) that did not have bird sightings were excluded. Including all sampling dates, a distance matrix was calculated (labdsv package, R) using Bray-Curtis distance measures. PERMANOVA was performed (vegan package, R) to test the effect of habitat type (eelgrass, longlines, bare) on shorebird community composition, and visualized using non-metric multi-dimensional scaling (NMDS). For the PERMANOVA, “date” was used as strata to account for the 17 sampling days across several months. Similarity Percentages analysis (SIMPER; vegan package, R) was used to further analyze differences in community composition.

Results

Taxa Presence Over Time

Abundance (birds/minute) over time revealed temporal differences in bird presence (Fig. 2). Dunlin (*Calidris alpina)* and dowitchers (*Limnodromus* spp.) were most frequently observed among the six focal taxa. Dunlin and black bellied plovers (*Pluvialis squatarola*) were most abundant in surveys 2 and 3 (March and April), while dowitchers and whimbrels (*Numenius phaeopus*) were most abundant in survey 4 (end of April and beginning of May). Gulls (*Larus* spp.) were present throughout all surveys, but were most prevalent in the fall, when other taxa were primarily absent. American crows (*Corvus brachyrhynchos*) were present throughout the surveys, at relatively constant abundance.

Taxa Response to Habitat and Tidal Stage

Taxa varied in their response to habitat type. Black bellied plovers, dunlin and American crows had significant responses to habitat type, where Black bellied plovers were positively associated with eelgrass and longlines, while American crow were positively associated with longlines and dunlin with eelgrass (Fig. 3, Table 2). Dunlin, dowitcher and gulls were all significantly affected by tidal stage, where dowitchers were positively associated with the ebb period, while dunlin and gulls were associated with both the ebb and flood periods (Fig. 3, Table 2). When all taxa were combined, there was a significant positive effect of eelgrass relative to bare habitat as well as ebb and flood relative to dry periods on total bird abundance (Table 3, Fig. 4). There was no significant effect of longlines on total bird abundance relative to bare.

Community Response to Habitat

Community composition was not significantly affected by habitat type as a main effect, although a relatively low p-value (0.069) indicates this may be a result of not having enough data and large variability across dates (as assemblages change). Pairwise post-hoc comparisons across habitats indicate that the greatest community differences may be between bare and eelgrass habitats, while bare and longline habitats were the most similar. The similarity of composition across habitat types is corroborated by the NMDS visualization (Fig. 5), showing large overlap of all habitat types. Lastly, SIMPER revealed that gulls, dowitchers and dunlin were primary drivers of the minimal community differences in all pairwise comparisons (Table 4), however none of these were statistically significant.

Discussion

Waterbirds respond to both tidal stage and habitat type. The responses are taxon-dependent, where some taxa respond to habitat type, while others respond more to tidal stage. In the individual analysis of six focal taxa, black bellied plovers, dunlin and American crows responded to habitat type. Black bellied plovers were positively associated with eelgrass and longlines, while dunlin and American crow were positively associated with longlines (Fig. 3, Table 2). Gulls, dunlin and dowitcher all responded significantly to tidal stage, and were positively associated with either ebb or flood periods.

Species-dependent responses to habitat are a common outcome of studies on waterbird habitat use in other regions (Connolly & Colwell 2005, Kelly et al. 1996, Burger & Niles 2017, Hilgerloh et al. 2001, Caldow et al. 2003). In Humboldt Bay, California, in comparisons of eelgrass habitats with and without longlines, whimbrels and dowitchers were more abundant in longline plots than adjacent control plots, while black bellied plovers were more abundant on eelgrass-containing control plots; three additional species, including dunlin, used the habitats differently depending on the site (Connolly & Colwell 2005). This is only mildly consistent with the current study, where black bellied plovers were positively associated with both eelgrass and longlines, dunlin were associated with longlines, and dowitchers were found to be primarily tide followers. Whimbrels did not respond significantly to habitat or tidal factors in this study, but were only present for a brief period of observation.

The current study is unique in that eelgrass is a separate habitat type, and not a continuous variable in control and aquaculture plots, allowing us to differentiate the effects of two types of structured habitats. In another California-based study, Western Sandpipers (*Calidris mauri*) and Dunlin in Tomales Bay avoided suspended bags of oysters, while Willets (*Tringa semipalmata*) were attracted to them (Kelly et al. 1996). Here, dunlin and black bellied plovers were found to be attracted to oyster longline habitats relative to bare, and none of the focal taxa had negative associations with longlines. There was a significant positive effect of eelgrass on total bird observations, but no effect of longline habitat relative to bare was found. Other studies (Kelly et al. 1996, Burger & Niles 2017, Burger 2018) have found positive or negative effects of shellfish aquaculture on bird observations. Differences among studies could come down to factors such as resource distribution, aquaculture type and disturbance amount, surrounding habitats at a landscape scale (e.g. habitat connectivity) (Farmer & Parent 1997), seasonal (and thus behavioral) variation among studies, and even the significance of neighboring stopover sites (Warnock et al. 2004).

Lack of significant response to habitat type is not to say that those taxa *do not* respond to habitat type, as responses may vary at the individual level and therefore would not be captured in this study. Shorebird responses to habitat type are likely to vary with seasonal behavioral changes (related to life history), body condition and age. Migratory shorebirds are often faced with a trade-off between food availability and predation risk. Birds migrating north during the spring (in preparation for breeding) have been found to travel in larger flocks, seeking refuge from predators at the cost of increased competition for food. Conversely, shorebirds traveling south in autumn travel in smaller groups, prioritizing food over predation avoidance (O'Reilly & Wingfield 1995). Similarly, south migrating Western sandpipers responded to the feeding-predation risk tradeoff based on body condition, where individuals in more desperate need of food forage in riskier, high-fattening sites (Ydenberg et al. 2002). With the seasonal variability of bird behavior and physiology, as well as the foraging-predation risk trade-off, habitat use by migrating shorebirds is dynamic and variable, making it difficult to study. While including both spring and fall was attempted in this study, late arrival of birds and the shift of low tides out of daylight hours prevented the study from fully capturing seasonal dynamics at these tidal elevations.

Three of the six taxa—dunlin, gulls and dowitcher—responded to tidal stages, with higher abundances during the ebb or flood periods relative to dry (low water) conditions. Glaucous-winged gulls (*Larus glaucescens*) are known to forage in the lowest intertidal zone available, suggesting they act as tide followers (Irons et al. 1986). This is consistent with the current findings, where gulls were mostly captured as the tide crossed the cameras during ebb and flood, and would have been foraging at a lower tidal elevation during the lowest part of the tide. Similarly, dunlin were most abundant during flood periods. In the Fraser River Estuary, British Columbia, dunlin were found to follow the tide, while Western Sandpipers foraged in the upper intertidal where the microphytobenthos (MPB) was greatest (Jiménez et al. 2015). In the presence of high MPB in Borgneuf Bay, France, Dunlin were found to switch from tide-following behavior to foraging in high MPB areas (Drouet et al. 2015). This suggests that the tide following behavior is plastic, and responds to resource availability for maximum foraging efficiency. This strategy may also interact with the predator risk aversion behavior, in which case an individual may opt for a foraging spot with lower-density prey in exchange for increased refuge.

For community composition, there is overall a very weak, if any, effect of habitat type. Despite being one of two taxa that responded significantly to habitat type, Black bellied plovers did not play a major role in shaping community composition. While the species-specific analyses included only dates when that species was present, the community-level analyses included all dates. This resulted in taxa that were around for a longer duration to have a greater effect on community composition, such as the American crow, dowitchers and gulls (Table 5). Several of the taxa who were present in greatest numbers or longest durations did not have a strong response to habitat type, or used *both* longline and eelgrass more than bare habitats, resulting in an overall weak effect of habitat on community (Table 4,5, Fig. 5). As mentioned previously, response to habitat on an individual basis is important to consider, as well as possible habitat effects on taxa that were present for only short times (whimbrels, red knots, greater and lesser yellow legs). This result is likely dependent on the specific bird assemblage present and could have a different outcome if measured elsewhere. The authors also acknowledge that combining gull and dowitcher species into single taxa may affect community composition findings.

The data indicate that there are species-dependent responses to habitat type and tidal stage, and that habitat type has a weak effect, if any, on community composition of waterbirds. Three of the focal taxa were associated with structurally complex eelgrass or longline habitats relative to bare, while three focal taxa were more affected by tidal stage, utilizing the ebb or flood periods; only dunlin were affected by both habitat type and tidal stage. How birds select habitat likely interacts with factors such as season, life-history behavior/physiology, predation risk, body condition, and resource availability, making trends in small-scale habitat use difficult to detect. The current findings also show that all three habitat types are utilized by the focal taxa, suggesting that all of the habitat types provide functional value. In fact, having a mosaic of intertidal habitat types in close proximity is likely beneficial by providing robust habitat options for foraging.

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Tables and Figures

Table 1a. Total observations of birds on each sampling date

Dates	Habitat Types Surveyed	Cameras per habitat in each of two blocks	Total bird observations per day (including potential repeats)
3/16/20, 3/18/20	Eelgrass, longlines	3	114, 89
3/31/20, 4/2/20	Eelgrass, longlines	3	206, 183
4/13/20, 4/14/20	Eelgrass, longlines, bare	2	103, 246
4/30/20, 5/1/20	Eelgrass, longlines, bare	2	205, 390
5/15/20, 5/17/20	Eelgrass, longlines, bare	2	29, 46
5/30/20, 5/31/20	Eelgrass, longlines, bare	2	20, 1
8/22/20	Eelgrass, longlines, bare	2	47
9/5/20	Eelgrass, longlines, bare	2	26
9/20/20	Eelgrass, longlines, bare	2	40
10/3/20	Eelgrass, longlines, bare	2	18
10/18/20	Eelgrass, longlines, bare	2	81

Table 1b. Total observations of each taxon included in community analysis

Species	Common Name	Total Observations
Corvus brachyrhynchos	American crow	104
Pluvialis squatarola	Black bellied plover	152
Limnodromus scolopaceus & griseus	Dowitcher spp. (long-billed and short-billed)	457
Anas platyrhynchos	Mallard duck	31
Calidris alpina	Dunlin	459
Ardea herodias	Great Blue Heron	6
Larus spp.	Gull spp.	207
Calidris canutus	Red knot	12
Arenaria interpres	Ruddy turnstone	6
Numenius phaeopus	Whimbrel	71
Tringa melanoleuca & T. flavipes	Yellowlegs spp. (greater and lesser)	8

Table 2. Results from six generalized linear mixed effects models of birds/minute of each focal taxon as a function of habitat type and tidal stage. “Bare” habitat and “dry” tidal stage are used as reference variables.

Taxon	Fixed Effect	Estimate	Std. Error	z-value	Pr(>|z|)
Dowitcher (DO)	(intercept)	-6.6389	1.1554	-5.746	9.14e-09
	Eelgrass	0.1211	0.5251	0.231	0.81760
	Longlines	-0.0747	0.5438	-0.137	0.89073
	Ebb	**1.7605**	**0.5372**	**3.277**	**0.00105***
	Flood	1.0670	0.5783	1.845	0.06502
Black Bellied Plover (BBPL)	(intercept)	-8.3949	1.1858	-7.080	1.45e-12
	Eelgrass	**3.3371**	**1.1494**	**2.903**	**0.00369 ***
	Longlines	**2.5530**	**1.1605**	**2.200**	**0.02781 ***
	Ebb	-0.5172	0.4393	-1.177	0.23900
	Flood	0.4099	0.3697	1.108	0.26765
Dunlin (DUNL)	(intercept)	-8.6407	0.9350	-9.242	< 2e-16
	Eelgrass	1.4563	0.7638	1.907	0.0566
	Longlines	**1.5813**	**0.7554**	**2.093**	**0.0363**
	Ebb	**2.5749**	**0.6446**	**3.995**	**6.48e-05**
	Flood	**3.4391**	**0.6209**	**5.539**	**3.04e-08**
Gull spp.	(intercept)	-7.1462	0.6515	-10.968	< 2e-16
	Eelgrass	0.9311	0.5538	1.681	0.09272 .
	Longlines	-0.5055	0.6131	-0.824	0.40966
	Ebb	**1.1746**	**0.4118**	**2.853**	**0.00434 ***
	Flood	**1.2772**	**0.4039**	**3.162**	**0.00157 ***
American Crow (AMCR)	(intercept)	-10.2156	1.3862	-7.370	1.71e-13
	Eelgrass	2.0364	1.3502	1.508	0.13151
	Longlines	**3.3241**	**1.2654**	**2.627**	**0.00862 ***
	Ebb	0.7078	0.4676	1.514	0.13008
	Flood	0.5289	0.4795	1.103	0.26997
Whimbrel (WHIM)	(intercept)	-7.1068	1.0858	-6.545	5.94e-11
	Eelgrass	-0.8110	1.1989	-0.676	0.4987
	Longlines	1.6701	0.9463	1.765	0.0776
	Ebb	0.8443	1.0105	0.836	0.4034
	Flood	1.5447	0.9666	1.598	0.1100

Table 3. Results from generalized linear mixed effects model of total bird abundance as a function of habitat type and tidal stage

Fixed Effect	Estimate	Std. Error	z-value	Pr(>|z|)
(intercept)	-5.0582	0.4033	-12.541	< 2e-16 *
Eelgrass	**0.8165**	**0.2531**	**3.227**	**0.00125 ***
Longlines	0.3405	0.2652	1.284	0.19913
Ebb	**0.7474**	**0.2392**	**3.125**	**0.00178 ***
Flood	**0.9621**	**0.2300**	**4.183**	**0.0000287 ***

Table 4. Permutational analysis of variance (PERMANOVA) results of community composition. Pairwise comparisons include Bonferroni correction.

	DF	SS	R^2	F	P
Habitat	2	1.274	0.04902	1.6751	0.069
Bare vs. eelgrass					0.174
Eelgrass vs. longlines					0.288
Bare vs. longlines					0.537
Residual	65	24.717	0.95098		
Total	67	25.991	1.00000		

Table 5. Similarity Percentages analysis (SIMPER) results showing the percent contribution ("Average") of each taxon to dissimilarity between habitat types, the ordered cumulative sum of contributions, and whether each contribution is statistically significant from permutation.

	Average	Std. Dev.	ratio	ava	avb	Cumulative sum	P
Eelgrass vs. longlines							
DUNL	0.193970	0.248610	0.780500	0.007622	0.014767	0.227	0.114
Gull	0.179340	0.215900	0.830600	0.008379	0.002793	0.438	0.672
DO	0.176790	0.300070	0.589200	0.030274	0.009735	0.645	0.945
AMCR	0.094960	0.159910	0.593800	0.002288	0.003121	0.756	0.075
Eelgrass vs. bare							
DO	0.281910	0.356600	0.970600	0.020698	0.020274	0.329	0.073
Gull	0.232050	0.259600	0.893900	0.007915	0.008379	0.599	0.063
DUNL	0.126760	0.194500	0.651700	0.001783	0.007622	0.747	0.934
BBPL	0.084380	0.150500	0.560600	0.001008	0.004156	0.845	0.549
Longlines vs. bare							
DO	0.267850	0.350500	0.764500	0.020698	0.009735	0.304	0.165
Gull	0.181470	0.264700	0.685500	0.007915	0.002793	0.510	0.601
DUNL	0.168340	0.255100	0.659800	0.001783	0.014767	0.701	0.534
AMCR	0.082260	0.174200	0.472300	0.0000	0.003121	0.794	0.431

Figure 1. Example of photo survey in oyster longline habitat

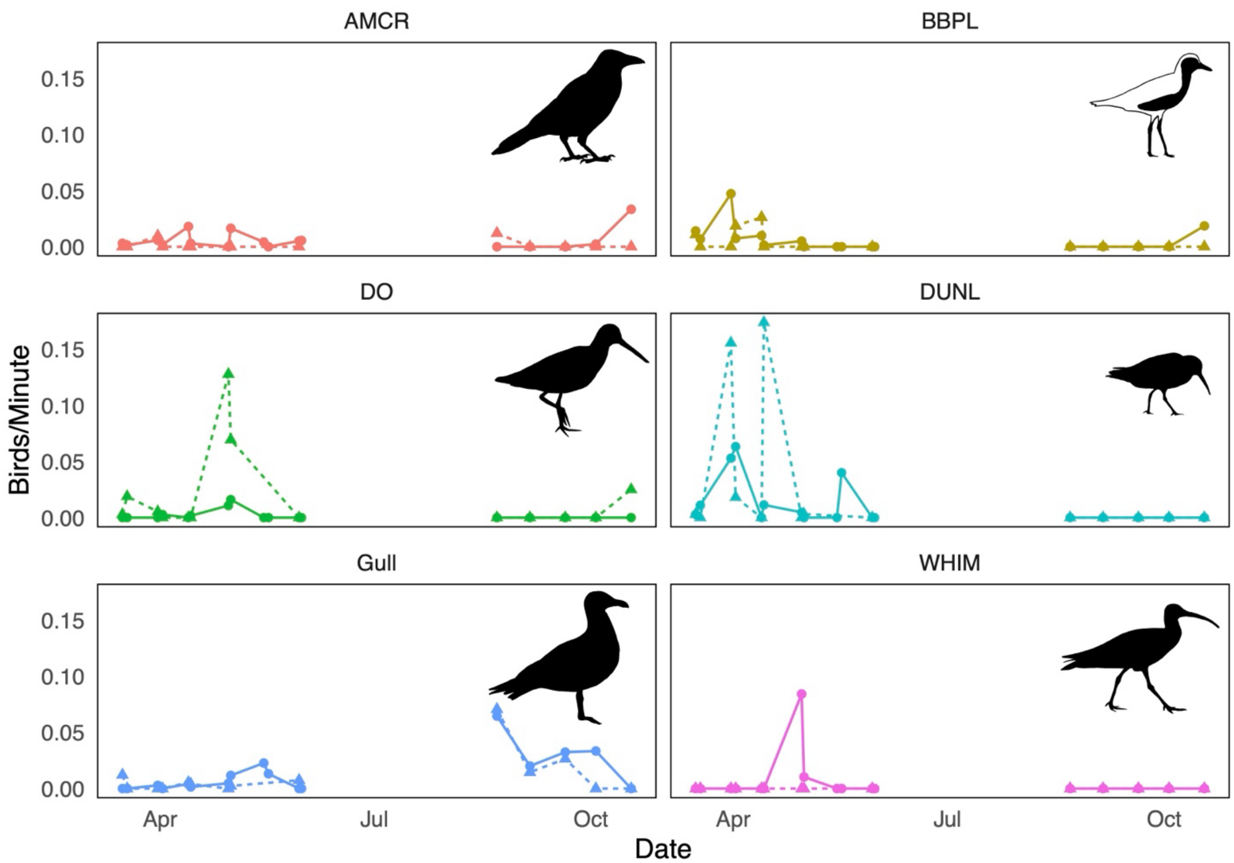

Figure 2. Standardized abundance (birds/min) of the six most prevalent waterbird taxa using intertidal habitat in Grays Harbor, Washington, in 2020. Dotted lines represent the western site, and filled lines represent the eastern site. No data was collected during June or July.

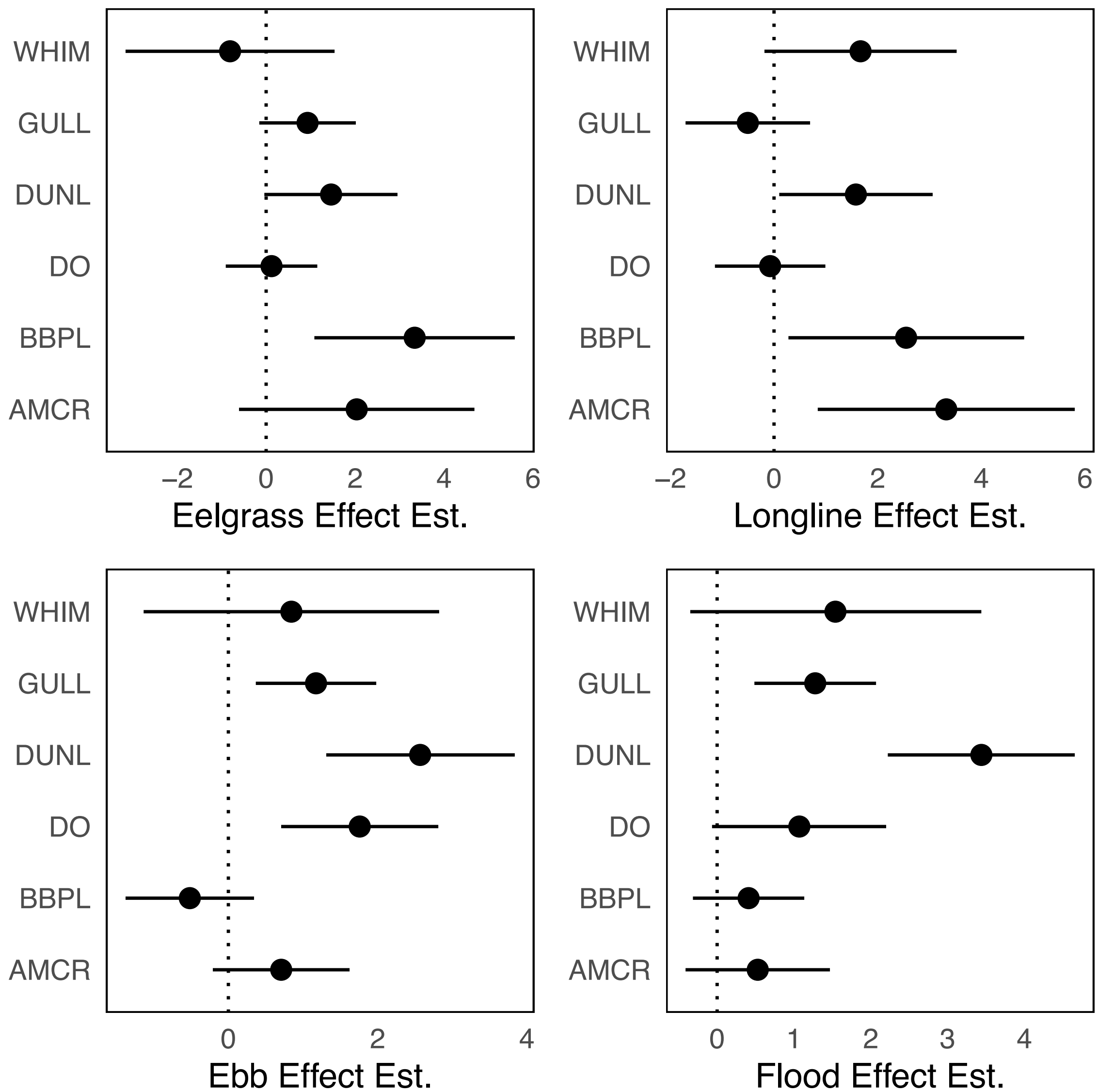

Figure 3. Coefficients and 95% confidence intervals of fixed effects on bird taxa

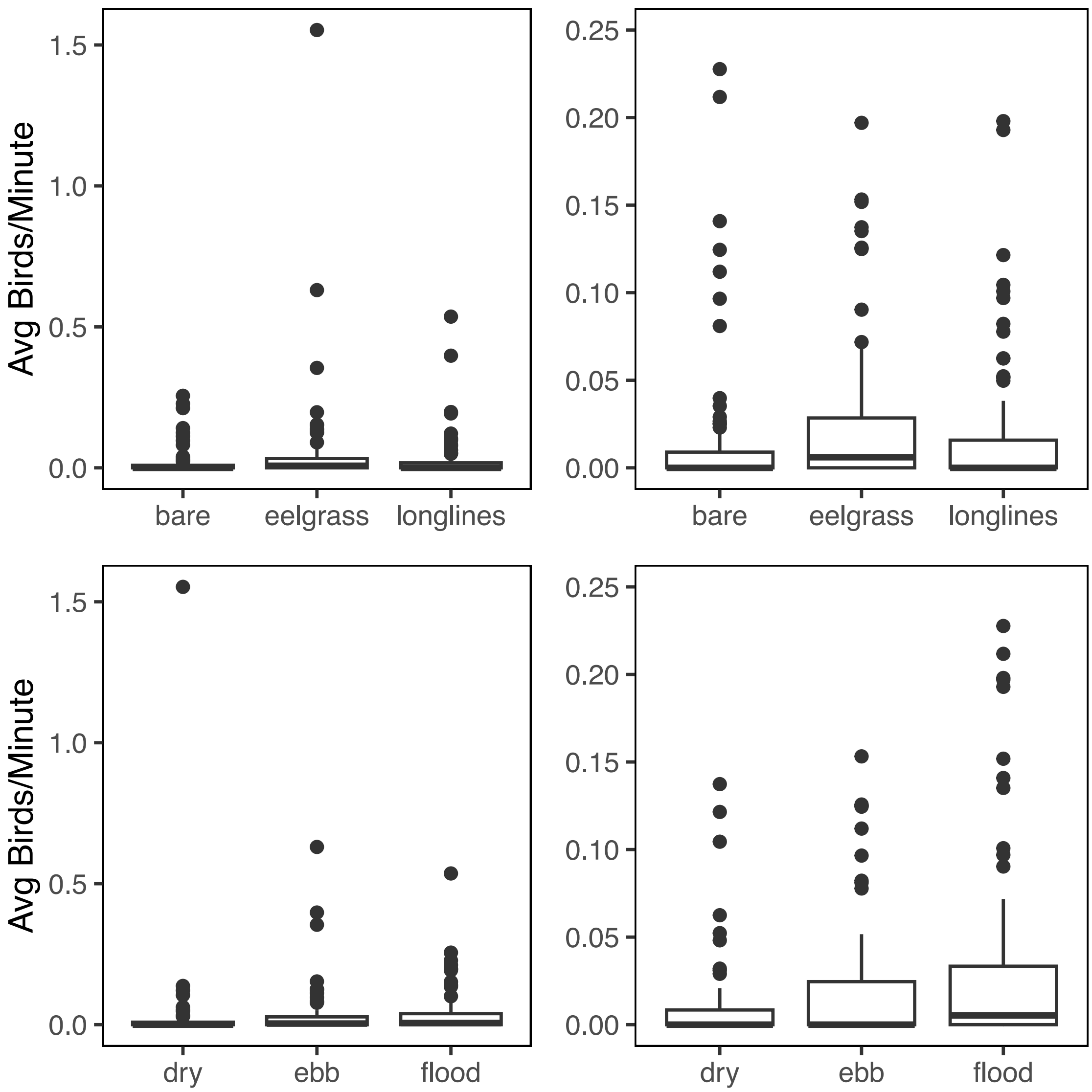

Figure 4. Box plots showing average birds/minute (averaged across cameras within habitat patch/block) in different habitat types and tidal stages. Panels on the left show the entire distribution of data, panels on the right range to y=0.25 to better view trends.

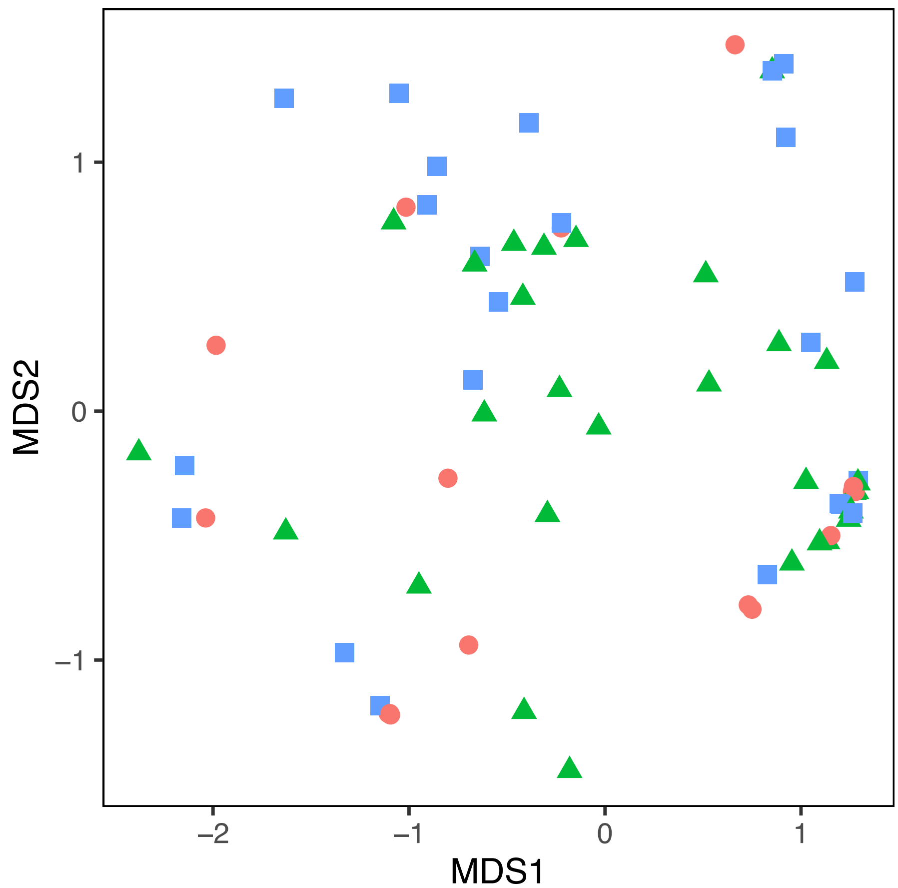

Fig 5. Non-metric multidimensional scaling (NMDS) plot showing community composition grouped by different habitat types. Each point represents one block per day and habitat type. Bare = red circles, eelgrass = green triangles, longlines = blue squares.

Chapter 2: Nekton use of co-occurring aquaculture and seagrass structure on tidal flats

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Abstract

On the extensive tidal flats of Willapa Bay (Pacific coast, USA), oyster culture, seagrass and mudflat create a mosaic of intertidal habitats. Structured habitats are generally considered to increase abundance and diversity of associated species, but less attention has been paid to roles of different kinds of structure (seagrass meadows, reefs, farm infrastructure) or co-occurring structure in shaping nekton assemblage structure. Here, we investigate the effects of different oyster culture methods (suspended culture and bottom culture) on nekton communities and abundance across a gradient of seagrass habitats, during multiple seasons, and using both seine and video sampling methods. Of 23 major estuarine taxa, two generally associated with vertical structure (eelgrass or suspended culture), three were seagrass specialists, and three primarily used habitats lacking vertical structure (mudflat and bottom culture). Where oyster culture was present, five taxa associated with on-bottom and two taxa associated with suspended culture. Assemblage structure responded to co-occurring structure as expected from responses to each structure type independently (i.e. additive effects of seagrass and oyster culture). In contrast to

much empirical evidence in structured habitats, seagrass density was a poor predictor of overall fish abundance. These findings together suggest that maintaining a mosaic of available habitats is favorable for promoting diversity in Willapa Bay.

Introduction

Structured habitats profoundly change the ecological characteristics of nearshore and estuarine systems. Foundation species provide biogenic structure, increase biodiversity and productivity relative to unstructured habitats, as well as modify abiotic conditions such as water chemistry, flow, and sediment properties (Dayton 1972, Ellison 2019). Independently, mangrove forests, coral reefs, shellfish reefs, and seagrass beds generate ecological conditions and support ecological communities that depart from what would be present without them (Beck 2003, Kovalenko 2012, Whitfield 2017). However, few studies have evaluated the ecological effects of co-occurring foundation species (Angelini et al. 2011), and the extent to which these structured habitats may be functionally redundant or act non-additively on assemblage structure. Such studies require a study design in which different foundation species co-occur across a range of relative densities.

Historically, seagrass (*Zostera marina,* eelgrass) and the Olympia oyster (*Ostrea lurida*) were the primary foundation species in estuaries along the northeastern Pacific Ocean, providing structurally complex habitats to estuarine mudflats. Today, southwest Washington state is a globally important producer of the introduced Pacific oyster*, Crassostrea gigas*, and commercial oyster aquaculture makes up about 17% of the total intertidal area (3,876 of 22,699 ha) in Willapa Bay (Feldman et al. 2000). Introduced oysters provide biotic structure when planted directly on-bottom, while other methods involve stakes, lines, and bags extending up to a meter above the sediment surface. Oyster aquaculture is able to provide many of the same ecosystem services as natural oyster ecosystems (Alleway et al. 2019), and in some cases enhanced services (i.e. additional dimension of habitat provisioning in suspended culture methods) (Dealteris et al. 2004), such that oysters in culture have the potential to act as a foundation species. While bottom culture is the traditional method of oyster culture in Washington state, there has been a gradual shift towards suspended culture methods, such as longlines and flipbags, to meet market demands and aquaculture regulations. The resulting seascape is a mosaic of (1) oyster culture (bottom culture or suspended culture), (2) eelgrass, (3) mixed eelgrass and oyster culture, and (4) bare mudflat. These habitats vary by type and complexity of structure, including multiple overlapping structure types, and could host distinct communities.

Globally, shellfish aquaculture (particularly suspended mussel and oyster culture) is recognized to increase macrofaunal abundance and species richness by providing habitat and/or food resources (Dealteris et al. 2004, Pinnex (Usfws), et al. 2005, Dumbauld et al. 2009, Gentry et al. 2020, Theuerkauf et al. 2020, Shinn et al. 2021). This has also been seen in fin-fish aquaculture, where a greater abundance of fish were found in the sandy habitats below sea-cages than nearby submerged aquatic vegetation and sand, with a similar species richness to that found in the vegetated habitats (higher than in bare sand) (Tuya et al. 2005). However, questions remain about how habitat use in shellfish aquaculture compares to seagrass and mixed seagrass-culture habitats, as well as how different methods of oyster aquaculture compare as habitat. In Humboldt Bay, CA, and Rhode Island, shellfish aquaculture gear hosts an even greater overall abundance of nekton than eelgrass (Dealteris et al. 2004, Pinnex (Usfws) et al. 2005). In Willapa Bay, a consistent set of seagrass specialists (Bay pipefish, *Syngnathus leptorhynchus;* Three-spine stickleback, *Gasterosteus aculeatus;* Shiner perch, *Cymatogaster aggregate;* Hippolytid aka “grass” shrimp, *Hippolytidae* spp.) emerges when comparing seagrass and surrounding unstructured habitats (Hosack et al. 2006, Gross et al. 2018, Ruesink et al. 2019), but there is little evidence of whether these taxa would use other vertical structure or mixed seagrass-culture habitats similarly to seagrass. For taxa primarily using structure for refuge, different foundation species may provide functionally redundant habitat. For example, in the Northeast Pacific, suspended aquaculture had similar use to eelgrass habitats by perch species (Muething et al. 2020, Ferriss et al. 2021), a group often associated with seagrass. Alternatively, if taxa are foraging in structure and seeking particular prey, then the resources available could differ between oysters and seagrass as foundation species. While habitat generalists could use any vertically structured habitat, the existence of seagrass (or other aquatic vegetation) and shellfish culture specialists would result in differences of assemblage structure among habitat types, as found in other past studies in the Northwest Atlantic and the Northeast Pacific (Ferriss et al. 2021, Shinn et al. 2021). There is a notable collection of studies comparing habitat use in seagrass to surrounding areas, some including aquaculture (listed above), however there have been no past studies that examine the effects of shellfish aquaculture co-occurring in seagrass habitats, which we accomplish here.

Management decision-making around aquaculture is currently limited because past comparisons of habitat value have addressed eelgrass and oyster culture habitats separately (e.g. Hosack et al. 2006) or have focused on eelgrass as habitat without addressing whether aquaculture within eelgrass changes the associated communities. Many regions have regulations in place that prevent shellfish culture from occurring in seagrass beds. However, in Willapa Bay, oyster culture has overlapped with eelgrass for over a century (Dumbauld & McCoy 2015). While eelgrass densities are typically reduced on oyster beds, certain management strategies of oyster culture can result in co-occurrence of these two foundation species (Tallis et al. 2009, Dumbauld & McCoy 2015). The unusual and historical practice of culturing oysters with co-occurring seagrass in Willapa Bay provides an opportunity to study the additivity, or lack-thereof, of oyster culture and seagrass habitats with regards to macrofaunal assemblage structure.

In this study, we carefully designed a field sampling method to further understanding of the effect of oyster culture within and out of seagrass habitats on nekton communities. First, we employed a crossed design that allows for examination of nekton use in mixed seagrass-aquaculture habitats, which is of significant management interest. Second, we sampled in spring and summer to account for seasonal variation in both seagrass and the structure that it provides, and nekton taxa and life history stages present, which may use habitats differently. Third, we employed two methods of sampling, seining and video, in order to account for potential biases that come with different sampling methods (i.e. performance in different structure types, visibility, disturbance etc.) (Bosch et al. 2017). We asked the following questions:

1) Do eelgrass and oyster culture, either on-bottom or suspended, support distinct suites of associated taxa during spring and summer, and do these associations differ when the habitat types co-occur (i.e. are mixed seagrass and culture habitats non-additive)?
2) How do two commonly used sampling methods, seining and videos, compare in their ability to capture nekton habitat use?

3) Does overall fish abundance increase as a function of eelgrass density, in and out of oyster culture?

Methods

2.1 Study Sites

Willapa Bay, Washington, USA, is one of the larger estuaries along the northeast Pacific coast (240 km^2 at mean sea level, Hickey & Banas 2003) and consists of shallow and extensive mudflats with a mosaic of eelgrass, oyster culture, and mixed eelgrass and culture habitats. In order to determine how oyster aquaculture method and amount of eelgrass affect nekton assemblage structure, we sampled using a crossed design of the three culture methods (no culture, bottom culture, suspended culture) and three eelgrass levels (no eelgrass, sparse eelgrass, dense eelgrass), resulting in nine distinct habitat types that included the full range of eelgrass densities available (Fig. 1). We sampled six regions (Fig. 3) and used a block design in which all habitat types were represented in each of the regions. Three regions had longlines, and three regions had flipbags. The two suspended culture methods were grouped together for the purpose of this analysis, although it is important to note that longlines consist of clusters of oysters strung on lines, while flipbags are mesh bags suspended from one end with a buoy on the other, designed to "flip" with the tide. We included one additional site of 'no eelgrass, no culture' and 'dense eelgrass, no culture' in each region, resulting in 11 sites per region, and a total of 66 sites. In 2020, we sampled three southern Willapa Bay regions in May and again in August. In 2021, we sampled three regions in the northern part of the Bay, during April and July (Fig. 3). Winter sampling was not feasible due to necessary tides occurring overnight as well as poor field and video conditions. Sites within a region were no more than 1km apart and were often neighboring habitat patches (sampling at least 50m apart). Average eelgrass density (shoots/0.25m^2) for each site was determined in each season by running a 100m transect (or two parallel 50m transects) where we counted shoots in twenty 0.25m^2 quadrats, spaced 5m apart (Fig. 1).

2.2 Seining Methods

We used circular beach seines that were customized with a rubber 10" lining along the bottom to operate over sharp oyster shells (6m radius) (Research Nets, Inc), and fished in 0.3-0.7 m deep water, as close to slack tide as possible. Nets were set to cover an area of $11m^2$ and then collapsed to collect the organisms present in that area. In each spring and summer, we set three consecutive seines at each of the 66 sites. In suspended culture, the seine was set from the end of a line by extending the wings into the surrounding rows and carefully collapsing around the central line. All fish, crabs and shrimp were identified to species (or, in some cases, to family), counted and released in the field. The first ten of each taxon caught per seine were measured in centimeters for total length (fish, shrimp) or carapace width (crabs only). Counts and lengths from the three seines were pooled within each site and treated as a single sample.

2.3 Video Methods

At 54 sites (covering the nine distinct habitat-types at each region), we deployed GoPro Cameras (Hero 3+) to capture two-minute videos every ten minutes. Cameras were installed 0.75 m above the sediment at a downward angle, with a marker placed 1 m away to mark the field of view (Fig 2). Camera video settings were set to be consistent across cameras (1080p) to give approximately equal field of view areas. Cameras were deployed during the low tide and collected images during flood tide. Cameras were collected on the next day's low tide. Many sites had two camera deployments within each season, while others only had one due to technical difficulties (i.e. camera failures) or unfavorable weather. Videos were then downloaded and systematically reviewed for mobile organisms in the field of view by two individuals (reproducibility was confirmed during training and spot checks performed). Nekton identification and visitations were extracted from the videos using the MaxCount method (Ferriss et al. 2021), with the modification that individuals that exited the frame and returned within 5 seconds were only counted once to avoid excessive repeated counts. We selected the first 22 two-minute videos after submersion from each camera, excluding videos with complete camera obstruction (i.e. from algae) or extremely poor visibility due to turbidity (could not ID taxa within 1m). Sites with fewer than 22 two-minute videos were excluded for that season, which led to removal of five samples during spring, and three samples during summer. Nekton counts were summed across videos from each camera, and sites with duplicate camera deployments had counts averaged and rounded to the next integer. Rare taxa (occurred in only a single sample) were removed, which resulted in removal of 15 species in the spring, and 9 in the summer.

2.4 Analytical Methods

2.4.1 Taxa habitat associations

First, an initial analysis was performed using the mvabund package (Wang et al. 2012) in R (v4.1.3; R Core Team 2022) to determine overall community response to amount of eelgrass (none, sparse, dense) and type of oyster culture (none, bottom culture, suspended culture), as well as testing for an interaction effect of eelgrass x oyster culture to address a potential non-additive effect of the two habitats (Refer to text S1.1 and Table S1 in Supplement). Upon finding no evidence of an eelgrass x oyster interaction effect on nekton community composition, we proceeded with a Bayesian analysis of taxa habitat associations. A Bayesian approach was suitable due to the hierarchical nature of our data, unaccounted variability (typical for this type of ecological field study), and the ability to provide taxon-specific responses while accounting for the entire community.

Based on datasets with columns for each nekton taxon, the BORAL (Bayesian Ordination and Regression AnaLysis) package (Hui 2016) in R was used to fit correlated column generalized linear mixed models (GLMMs) with latent variables (introduces unmeasured predictors to each sample and accounts for correlations among taxa columns in the response matrix) via Markov Chain Monte Carlo (MCMC) estimation (Hui 2016, Warton et al. 2015 – see Box 1 & Figure 1 for more on latent variable model (LVM) structure). The response matrix included nekton taxon counts for each site (54 sites for videos, 66 for seines), with a separate matrix for each season. Separate analyses were carried out on spring and summer data, due to expected seasonal differences in assemblage, and on seines and videos. For each dataset, we fit two models addressing distinct questions. In the first model, oyster culture (presence/absence) and eelgrass (presence/absence) were included as environmental variables, with region included as a random effect. The second model only included summer data from sites where oyster culture was present, with oyster culture method (bottom culture/suspended culture) and eelgrass (presence/absence) included as environmental variables, and region as a random effect. Eelgrass presence/absence was chosen (instead of "no", "sparse", "dense" categories) because categories were relative to each site and did not represent consistent densities, but did ensure a range of densities were sampled (Fig. 1). All models included two latent variables to account for potential correlation among taxa, and default priors (prior.control) were used. The models were fit using the negative binomial distribution (with log link) and checked with residual analysis. Coefficients were obtained as well as Highest Posterior Density (HPD) intervals and used to create caterpillar plots of taxon-specific responses. Our results were also compared to those in existing literature by performing a literature search of publications that included taxa identified here and at least two of our studied habitat types (*Zostera marina*, mudflat, oyster shell/culture).

2.4.2 Fish abundance across eelgrass densities and culture types

A generalized linear mixed model with negative binomial errors (link = "log") was fit using Stan and the rstanarm package for Bayesian estimation (Goodrich et al. 2020) in R. The response variable was total fish counts (invertebrates removed) from the seining data. Predictor variables included average eelgrass shoot density (shoots/0.25m^2), season (spring or summer), and oyster culture method (none, bottom culture or suspended culture) as fixed effects and region as a random effect. Quantitative shoot density was used instead of density categories as categories were relative to each site, as described above. The model fit and MCMC convergence were checked using posterior predictive check and $\hat{r}$ values, respectively (Table 1, Fig. S1), which were acquired using ShinyStan. The posterior predictive check (ppcheck in ShinyStan) compares the model prediction (y^{rep}) to the observed data (y), and indicates model adequacy (Fig S1.). $\hat{r}$ values < 1.05 indicated no issues of MCMC convergence. Data were also visualized with scatter plots to illustrate distribution.

Results

3.1 Total catch and method comparison

Overall, seining captured a much greater abundance of nekton, as well as greater species richness, relative to videos (see summary of total nekton sampled in spring and summer in Table 2). 30 taxa were sampled in the seines, whereas 17 taxa were identified via video. Furthermore, small and/or cryptic taxa that were abundant in the seines (i.e. gobies, juvenile sole, and small (<3 cm) shrimp species) were not captured in videos. The taxa most sampled by videos were Shiner perch and Dungeness crab (*Metacarcinus magister*), while the taxa most sampled by seine were English sole (*Parophrys vetulus*), Shiner perch, and shrimp of the families *Hippolytidae* (Hippolytid shrimp) and *Crangonidae* (Crangonid shrimp) (Table 2). Seasonal variation in abundances and lengths indicate patterns of nekton migration and reproduction. In particular, English sole and Pacific staghorn sculpin (*Leptocottus armatus*) showed seasonal growth, and Shiner perch migrated into intertidal habitats in summer.

3.2 Taxon habitat associations

3.2.1 Eelgrass and oyster culture

Oyster culture (no, bottom, suspended) and eelgrass density (no, sparse, dense) were statistically significant predictors of nekton community composition (Refer to text S1.1 and Table S1 in Supplement). There was no evidence of an oyster culture x eelgrass interaction effect on nekton community composition, so we proceeded with a more in-depth analysis of taxon-specific habitat responses.

Habitat associations are shown for each taxon as estimated coefficients and posterior densities (Fig. 4 & 5, Table 3). In seines in spring, positive associations with eelgrass occurred for Crangonid shrimp, Hippolytid shrimp, Bay pipefish, Saddleback gunnel (*Pholis ornata*), and Three-spine stickleback. Four of these associations persisted in summer (Hippolytid shrimp, Bay pipefish, Saddleback gunnel, Three-spine stickleback), and additionally Shiner perch were positively associated with eelgrass. Also in seines in spring, positive associations with oyster culture occurred for Arrow goby, *Hemigrapsus* spp., and Hippolytid shrimp. For Hippolytid shrimp and *Hemigrapsus* spp., this positive association persisted into summer. Seining data also indicated negative associations of Arrow gobies with eelgrass and negative associations of Three-spine stickleback and English sole with oyster culture in the spring. In the summer, there were negative associations of English sole with eelgrass, and shiner perch and Three-spine stickleback with oyster culture.

In contrast to seines in which several taxa showed positive associations with eelgrass in both seasons, video data resulted in no positive associations with eelgrass. Video results indicated a negative association of *Hemigrapsus* spp. with eelgrass during the spring, and a negative association of Dungeness crab with eelgrass during the summer, whereas seine results indicated no strong association (negative or positive) with eelgrass for both of these taxa. The video results also found Shiner perch and Staghorn sculpin to be positively associated with oyster culture during the summer, while seine data showed Staghorn sculpin to be neutral and Shiner perch to be negatively associated with oyster culture during summer (see Discussion 4.1: “Method Comparison”). Taxa that were not listed as being associated with habitat had high posterior density (HPD) intervals that included 0. Some of these HPD intervals were quite narrow indicating no habitat association with eelgrass or oyster culture, while wider HPD intervals indicate greater uncertainty of the habitat association estimation.

3.2.2 Bottom vs. suspended culture associations

The second model fit for video and seining data examined summer sites where culture was present, in order to compare associations with bottom culture versus suspended culture. Considering only where culture was present, seining results indicate associations of Arrow gobies with suspended culture, and associations of Chinook salmon (hatchery released, indicated by clipped adipose fin), Dungeness crab, English sole, Saddleback gunnel, Shiner perch and Staghorn sculpin with bottom culture (Fig. 5, Table 3). Videos indicate a similar result for Dungeness crab (associated with bottom culture), but an opposite result for Shiner perch (associated with suspended culture).

3.3 Fish abundance across eelgrass densities and culture types

Total fish in seines ranged from 0 to 598 across samples but was poorly predicted by habitat or season as shown by mean estimates and confidence intervals close to and overlapping zero (Table 1), and an undistinguishable data pattern (both with exception to suspended culture) (Fig.

6). Fewer fish were caught in seines in suspended culture relative to other habitats (Fig. 6, Table 1), however, that is likely an inaccurate reflection of fish density due to challenges of seining around rigid structures (see “Method Comparison”, below).

Discussion

Seagrass and oyster aquaculture both provide habitat to a variety of fish and invertebrate nekton. While certain taxa associate with eelgrass, others associate with oyster culture (one or both methods). Some taxa generally associate with increased vertical habitat structure, in which case the structured habitats (seagrass and suspended culture) may act as functionally redundant. Additionally, some taxa associate with habitats lacking vertical structure and were found primarily in unstructured mudflat (negatively associated with eelgrass/culture) or bottom culture habitats. Nekton respond to co-occurring seagrass and oyster culture habitats as they would when separately occurring (Table S1.1). These different habitat types support distinct nekton assemblage structures and suggest that a mosaic of habitat types can increase overall diversity and support multi-trophic connectivity. Using multiple sampling methods, in this case seining and video footage, is helpful to understand sampling biases, and can reveal strengths and weaknesses of methods in different sampling contexts. Finally, eelgrass density is not a reliable indicator of total fish abundance in Willapa Bay, due to the use of unstructured habitats by highly abundant taxa.

4.1 Method Comparison

Comparison of our results sampling the same sites with both seines and videos reveals important sampling biases, as well as strengths and weaknesses of each method. Seining caught more species of nekton and is not affected by visibility. However, in order to fish multiple sites a day, both ebb and flood were used, which could introduce unaccounted variability. Additionally, while our modified seines operated well in eelgrass and over oyster bottom culture, maneuvering the seine through and around suspended culture provided a challenge that likely reduced our catch (Fig. 6), thus resulting in a negative sampling bias that must be strongly considered when interpreting the results concerning suspended culture. Escapement of small taxa into rigid bottom culture habitats may also be a source of sampling bias with seines. On the other hand, videos provided a more controlled sampling in terms of using footage from one period of the tide, although they were severely affected by visibility in the form of 1) turbidity and 2) view interference via macrophytes. This resulted in likely under-sampling of nekton in highly macrophytic (including eelgrass) or turbid videos, as well as under-sampling of more cryptic taxa, such as juvenile English sole. We found that sampling on clear days with little wind provided the best video quality. Overall, the video method sampled fewer taxa and numbers of nekton than seining (although this is also dependent on sampling effort), but provided a useful tool for capturing habitat use, particularly of pelagic fish, in suspended culture. While seining required a much greater field effort (10-15 minutes per seine pull and three pulls per site), the processing effort required of video analysis is much greater than seining (approximately 45-60 minutes per site), making the two methods of similar magnitude in terms of overall effort. However, many more cameras would have to be deployed to reach a similar number of "captures" as the seining. When selecting methods, we'd suggest considering factors including habitat structure, turbidity and crypticness of taxa. Careful consideration of potential method biases, project goals, and the strengths and weaknesses of different sampling methods is critical when designing a study, which is clearly demonstrated here.

4.2 Taxon habitat associations

Our results illustrate that many nekton species utilize multiple estuarine habitat types, and that both amount of eelgrass and presence/type of oyster culture drive nekton assemblage structure. Where preferences are exhibited, some taxa associate with eelgrass or one method of oyster culture, whereas others respond to degree of structure (i.e. structured vs. unstructured) rather than structure type. Additionally, nekton communities within habitats vary based on season, as organisms migrate and reproduce, thus altering the role of these habitats seasonally. We found no significant statistical interaction of nekton communities in response to eelgrass and oyster culture (Table S1), suggesting that co-occurrence of the two habitats does not affect how taxa respond to the habitats relative to when they are independent; in other words, there is no evidence of a non-additive effect of co-occurring seagrass and oyster culture. Eelgrass associated species (associated spring and/or summer) include Bay pipefish, Three-spine stickleback, Saddleback gunnel and Crangonid shrimp (Fig. 5, Table 3). Three-spine stickleback were the only taxa in the study that were consistently (both seasons, both methods) negatively associated with oyster culture. Positive oyster culture-associated taxa include shore crabs (*Hemigrapsus* spp.), Staghorn sculpin, Arrow gobies and Shiner perch (videos only) (Fig. 5, Table 3); Arrow gobies and Shiner perch were found to be associated with suspended culture, while the other taxa were associated with bottom culture (Fig. 5, Table 3). However, observation suggests gobies reside in holes that occur in the soft sediment under suspended culture, rather than use the culture directly. Another set of taxa showed no overall association with oyster culture presence or absence (i.e. did not prefer or avoid), but were associated with one type of culture when captured within oyster culture habitats. Members of this group are juvenile Chinook salmon (hatchery), Dungeness crab and English sole, which were all found more in bottom culture than suspended culture habitats.

Possibly, opposing associations of taxa with different culture types (i.e. positively associated with bottom culture, negatively associated with suspended culture) could mask an overall association with oyster culture. For example, Chinook salmon were associated with bottom culture relative to suspended culture, and while there was an overall positive association with oyster culture, a large HPD and low number of individuals results in uncertainty around this conclusion. Hippolytid shrimp and Shiner perch showed positive associations with both eelgrass and oyster culture presence (Shiner perch in videos only), suggesting that these taxa may respond generally to increased vertical structure for foraging and refuge (Fig. 4, Table 3). Lastly, English sole, Dungeness crab and *Hemigrapsus* spp. showed tendencies towards habitats without vertical structure (bare or bottom culture), and showed negative correlations with eelgrass in at least one season (Fig. 4, Table 3). English sole also demonstrated seasonal variation in use of oyster culture (negative association in spring), reflecting how different life stages may interact with the habitat mosaic. For example, ~ 2cm, translucent sole may primarily use bare habitats for camouflage in the spring, while slightly older and larger summertime sole may utilize bottom culture habitats for foraging.

When comparing our findings to past studies and evaluating habitat function, it is important to consider sampling methods used. Several recent studies corroborate that videos are an effective method of sampling pelagic nekton such as perch species (Gross et al. 2018), including in suspended culture (Muething et al. 2020, Ferriss et al. 2021). However, these studies using video also sampled relatively low numbers of more cryptic taxa, particularly flatfish such as English sole. We see this discrepancy when comparing our own sampling methods, where seines revealed much higher numbers of cryptic taxa than would have been concluded from using exclusively video methods.

Our results also demonstrate that sampling across multiple seasons is a critical component when evaluating habitat value. Eelgrass and structure-associated organisms tended to be in greater abundance during the summer, when eelgrass itself reaches high biomass (see also Ruesink et al. 2019). In the spring, however, a greater proportion of the community is composed of taxa that prefer unstructured habitats or are habitat generalists, therefore increasing the value of less structured habitats. Seasonal studies of nekton indicate that spring vs. summer encompasses the greatest shift in community composition (Gross et al. 2019b), but many past studies have been restricted to summer sampling. While our findings certainly corroborate that eelgrass and structured habitats (e.g. suspended culture) are of high value to many organisms, using multiple methods of sampling and sampling across seasons allowed us to identify the notable use of unstructured mudflat and bottom culture habitats.

Overall, our results confirm findings from previous work, as well as provide new evidence of habitat associations that expand on past studies. As consistent with past estuarine work (Gross et al. 2018, Ruesink et al. 2019), there are a suite of eelgrass specialist taxa—Bay pipefish, Three-spine stickleback, Saddleback gunnels and Crangonid shrimp—which continuously use eelgrass habitats. Bay pipefish and Crangonid shrimp appeared to use eelgrass regardless of the presence of oyster culture, while Three-spine sticklebacks avoided culture. Our findings confirm that Shiner perch utilize the vertical structure provided by both eelgrass and suspended aquaculture habitats, as previously recorded (Dumbauld et al. 2009, Muething et al. 2020, Ferriss et al. 2021). For taxa found in both eelgrass and suspended culture, such as Shiner perch, the vertical structures could provide redundancy in habitat. It is apparent from videos that Shiner perch may forage among macroalgae attached to longlines or flipbags. Epifaunal abundance is often increased in oyster aquaculture relative to bare areas (Hosack et al. 2006). In keeping with this trophic explanation, grass shrimp (Hippolytidae) were generally structure-associated in our study and could also serve as a food resource for the larger-bodied nekton we captured.

Two species of management importance cannot be considered to use eelgrass as estuarine nursery habitat, because of lack of positive association or statistical negative association. Our findings from both fishing and videos confirm that juvenile Dungeness crabs prefer habitat lacking vertical structure, but utilize bottom culture habitats, which is consistent with past work (Eggleston & Armstrong 1994, Dumbauld et al. 2000, Dumbauld et al. 2009—section 5.3), and suggests that bottom culture is valuable nursery habitat for Dungeness crab. English sole were also found to avoid vertical structure and to use bottom culture habitats during the summer, consistent with past work (Laffargue et al. 2006), Ferriss et al. 2021), The importance of habitats lacking vertical structure, or which have low vertical relief (such as bottom culture) was conspicuous in our study due to the sheer number of juvenile English sole in seines. English sole were our most sampled taxon (followed by Shiner perch), with 2,715 individuals captured via seines during the spring. The lengths of English sole increased between spring and summer (Table 2), suggesting that there is a cohort of newborn English sole in the spring, a fraction of which survive to be larger juveniles by summer. Like Dungeness crab, English sole are commercially important species that use the mudflat and bottom culture habitats as juveniles. Additionally, abundant young flatfish likely provide trophic resources for resident and migratory mesopredators, many of which would be preparing to reproduce, themselves. We highlight the importance of unstructured habitat, not to undermine the value of structured habitats, but to bring attention to an often overlooked feature of estuaries, and to suggest that a mosaic of habitat types supports overall productivity in Willapa Bay.

4.3 Fish abundance across eelgrass densities and culture types

Past studies suggest that overall nekton abundance is higher in structured habitats such as seagrass, oyster culture or reef, and salt marsh habitats (Dumbauld et al. 2009, França et al. 2009, Gross et al. 2019a, Muething et al. 2020, Grabowski et al. 2022). Between structured habitats, nekton abundance has also been documented to be higher in oyster culture habitats than seagrass (Dealteris et al. 2004, Pinnex (Uwfws) et al. 2005), or similar between those habitat types (Hosack et al. 2006—slightly more in seagrass than oyster on average, Muething et al. 2020—more in suspended culture and eelgrass habitats than bottom culture). However, use of structured habitats is also reliant on the surrounding landscape (Grabowski et al. 2022). For example, elevated nekton abundance in seagrass may be more pronounced when fringing along channels than on wide tidal flats where most oyster culture occurs (Gross et al. 2019a), or some demersal fish may be more likely to use structured habitat with adjacent mudflat (Grabowski et al. 2022). Here, we found that eelgrass and oyster culture presence or absence affects nekton assemblage structure, but that eelgrass shoot density (i.e. degree of habitat structure) does not have a strong effect on overall fish abundance. Culture type (no culture, bottom culture, suspended culture) was shown to have a weak effect on fish abundance (Table 1), but was mostly influenced by low fish counts in suspended culture, which were likely affected by negative sampling bias (see above discussion and Fig. 6). These results continue to highlight the importance of less structured habitats for taxa that are often under-represented in studies due to their cryptic nature and common sampling methods. The presence of habitat generalists and those associated with unstructured habitats are likely driving this lack of association between eelgrass density and overall nekton abundance, and it is expected that a similar analysis just including eelgrass-associated species would show a correlation between eelgrass density and abundance, as seen in Belgrad et al. (2021).

4.4 Concluding remarks about habitat values and management applications.

Our findings suggest that the presence of oyster culture in eelgrass habitats is not generally detrimental to the habitat value of eelgrass for nekton. Eelgrass specialists continued to use eelgrass habitat when oyster culture was present (with the exception of Three-spine stickleback), structure-associated taxa used oyster culture with or without the presence of eelgrass, and some taxa that were negatively associated with vertical structure (suspended culture and eelgrass), were found to use oyster bottom culture habitats in addition to bare mudflat. However, in order for eelgrass to co-occur with oyster culture, oyster culture must be managed along two axes: density of oysters (for instance by low stocking densities or gaps in longlines) (Dumbauld et al. 2009, Tallis et al. 2009, Wagner et al. 2012), and intensity and frequency of disturbance. Processes such as dredging and trampling can occur in ways that limit negative impacts on eelgrass, for instance by small reductions in density or capacity for resilience (branching, seedling success) (Cabaço & Santos 2012, Dumbauld and McCoy 2015, Ferriss et al. 2019, Fales et al. 2020, F. Boardman unpubl. data). Existing management of shellfish aquaculture in Willapa Bay has generated a habitat mosaic, on which we capitalized for the treatments in this study. Because of the variety of habitat associations among estuarine nekton, overall composition depends on including patches of bare unstructured mudflat, patches of eelgrass, patches of mixed eelgrass/oyster, and patches of oyster culture of different methods. This farmed seascape contains elements of the original habitat mosaic in this region and supports a diverse group of nekton across multiple life history stages.

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Tables & Figures

Table 1. Summary parameter statistics for model of fish count. (n_eff = effective sample size, $\hat{R}$ = convergence diagnostic, MCSE = Monte Carlo Standard Error,)

	n_eff	$\hat{R}$	mean	mcse	sd	2.50%	25%	50%	75%	97.50%
(Intercept)	1,733	1	4.4	0	0.2	3.9	4.2	4.4	4.5	4.8
Shoot Density	4,089	1	0	0	0	0	0	0	0	0
Season Summer	4,741	1	-0.2	0	0.2	-0.5	-0.3	-0.2	-0.1	0.2
Oysters Bottom	3,816	1	-0.2	0	0.2	-0.5	-0.3	-0.2	0	0.2
Oysters Suspended	3,900	1	-1.1	0	0.2	-1.4	-1.2	-1.1	-1	-0.7
b[(Intercept) Region:Bay_Center]	1,599	1	0.5	0	0.3	0.1	0.3	0.5	0.7	1.1
b[(Intercept) Region:Cutoff]	1,956	1	-0.2	0	0.2	-0.7	-0.4	-0.2	-0.1	0.3
b[(Intercept) Region:Long_Island]	1,690	1	0	0	0.2	-0.4	-0.1	0	0.1	0.5
b[(Intercept) Region:Middle_Sands]	1,896	1	0.1	0	0.2	-0.4	-0.1	0	0.2	0.5
b[(Intercept) Region:Port]	1,790	1	-0.2	0	0.2	-0.7	-0.4	-0.2	-0.1	0.2
b[(Intercept) Region:West_Channel]	2,214	1	0	0	0.2	-0.5	-0.2	0	0.1	0.4
reciprocal_dispersion	4,443	1	1.5	0	0.2	1.2	1.4	1.5	1.6	1.9
Sigma[Region:(Intercept),(Intercept)]	1,646	1	0.2	0	0.2	0	0.1	0.1	0.2	0.8
mean_PPD	4,043	1	68.7	0.1	8.1	54.2	63.1	68.2	73.6	86.2

Table 2. Nekton counts used in analysis. Seine counts are summed catches from all 66 sites. Video counts are summed from the first 22 two-minute videos at each site. Sites with multiple cameras had counts averaged resulting in a single set of counts for each site per season

Taxon	Common name	Spring			Summer		
		Videos	Seine	Size (cm) + SE	Videos	Seine	Size (cm) + SE
Clevelandia ios	Arrow Goby	0	154	4.58 ± 0.29	3	57	3.66 ± 0.13
Lepidogobius lepidus	Bay Goby	0	7	5.00 ± 0.72	1	2	8.00 ±1.00
Syngnathus leptorhynchus	Bay Pipefish	1	66	16.50 ± 0.44	13	141	13.51 ± 0.52
Scorpaenichthys marmoratus	Cabezon	0	4	3.13 ± 0.59	0	0	
Oncorhynchus keta	Chum salmon	0	95	5.70 ± 0.18	0	0	
Oncorhynchus tshawytscha	Chinook salmon	0	0		30	16	8.07 ± 0.30
Crangonidae spp.	Crangon shrimp	0	770	3.56 ± 0.07	0	610	2.40 ± 0.05
Metacarcinus magister	Dungeness crab	57	56	7.75 ± 0.55	106	253	6.92 ± 0.24
Parophrys vetulus	English sole	5	2715	3.55 ± 0.06	25	383	7.54 ±0.13
Carcinus maenas	European green crab	3	1	5.50	0	10	4.94 ± 0.66
Hemigrapsus spp.	Shore crab	5	42	1.01 ± 0.09	2	21	1.27 ± 0.16
Hippolytidae spp.	Grass shrimp	0	1321	1.92 ± 0.04	0	68	1.83 ± 0.11
Pugettia producta	Kelp crab	0	0			2	4.00 ± 0.0
Hexagrammos decagrammus	Kelp greenling	0	6	5.25 ± 0.21	0	27	7.98 ± 0.21
Cottidae sp.	unID juv. sculpin	0	31	2.13	0	0	
Ophiodon elongatus	Lingcod	0	3	10.17 ± 0.17	0	0	
Pandalidae sp.	Pandalus shrimp	0	1	5.00	0	0	
Porichthys notatus	Plainfin midshipman	0	0		0	1	12.00
Cancer productus	Red Rock Crab	0	5	10.50 ± 0.32	1	3	8.33 ±1.20
Pholis ornata	Saddleback gunnel	4	180	6.47 ± 0.18	6	809	7.19 ± 0.13
Cymatogaster aggregata	Shiner Perch	76	75	7.29 ± 0.97	468	2317	5.89 ± 0.10
Hyperprosopon ellipticum	Silver Surfperch	0	0		0	2	7.50 ± 0.50
Hypomesus pretiosus	Surf smelt	1	306	5.79 ± 0.20	14	0	
Citharichthys stigmaeus	Speckled sanddab	0	1	3.00	0	12	6.25 ± 0.29
Leptocottus armatus	Pacific Staghorn sculpin	7	361	5.55 ± 0.15	57	352	10.80 ± 0.16
Platichthys stellatus	Starry flounder	0	11	13.73 ± 0.89	0	32	13.12 ± 0.65
Gasterosteus aculeatus	Three-spine stickleback	1	282	4.72 ± 0.14	26	214	3.18 ± 0.16
Aulorhynchus flavidus	Tubesnout	0	2	13.25 ± 0.25	0	0	
Hyperprosopon argenteum	Walleye surfperch	0	6	7.60 ± 0.19	10	0	
Phanerodon furcatus	White surfperch	0	2	8.50 ± 0.5	7	0	

Taxon	Boardman et al. 2023 (seining / videos)					Reviewed Literature		
	Spring		Summer					
	Eelgrass	Oyster	Eelgrass	Oyster	Culture Pref	Eelgrass	Oyster	Culture Pref
Arrow Goby	- /	+ /			Suspended /			
Chinook Salmon (hatchery)					Bottom /	+ Semmens 2008		
Crangonid shrimp	+ /	- /						
Dungeness Crab			/ -		Bottom / Bottom		+ Dumbauld et al. 2009 + Fernandez et al. 1993	Bottom (Dumbauld et al. 2009)
English Sole		- /	- /		Bottom /	- Ruesink et al. 2019		
Hemigrapsus spp.	/ -	+ /		+ /				
Hippolytid shrimp	+ /	+ /	+ /	+ /		+ Ruesink et al. 2019		
Bay Pipefish	+ /	- /	+ /			+ Ruesink et al. 2019		
Saddleback Gunnel	+ /		+ /		Bottom /	+ Ruesink et al. 2019		
Shiner Perch			+ /	- / +	Bottom / Suspended	+ Dumbauld et al. 2015 + Hosack et al. 2006 + Ruesink et al 2019 + Ferriss et al. 2021 + Gross et al. 2018 + Gross et al. 2017	+ Ferriss et al. 2021 + Muething et al. 2020 F	Suspended (Muething et al. 2020, Ferriss et al. 2021)
Pacific Staghorn Sculpin				/ +	Bottom /		+ Muething et al. 2020 + Ferriss et al. 2021 (regional variation)	Suspended (Muething et al. 2020)

Three-spined stickleback	+ /	- /	+ /	- /		+ Ruesink et al. 2019 + Gross et al. 2018 + Gross et al. 2017		

Table 3. Summary table of taxa habitat associations compared with findings from existing literature

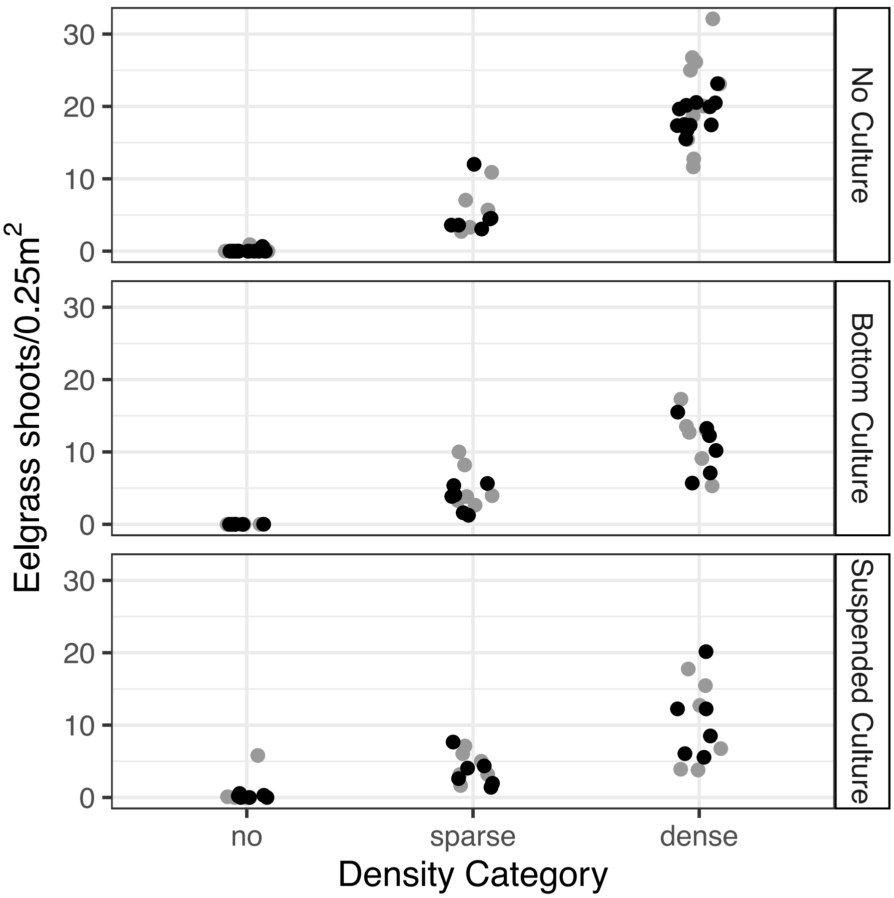

Figure 1. Eelgrass density of each density category at all sampling sites, divided by culture type and season (grey = spring, black = summer). Densities varied across regions and oyster culture methods, so categorizations are relative to within region and culture method (e.g. "Site1- no culture - sparse eelgrass" might be similar eelgrass density to "Site1 - bottom culture - dense eelgrass") resulting in some overlap of categories when viewed across all regions and culture types. Eelgrass density was used as a continuous or binary predictor in all analyses (besides S1.1), but the sampling method ensured we sampled the full spectrum of available densities.

Figure 2. Three-spine stickleback in flip-bags (suspended culture) and sparse eelgrass, starry flounder in bottom culture with sparse eelgrass, and staghorn sculpin in dense eelgrass with no culture (top to bottom).

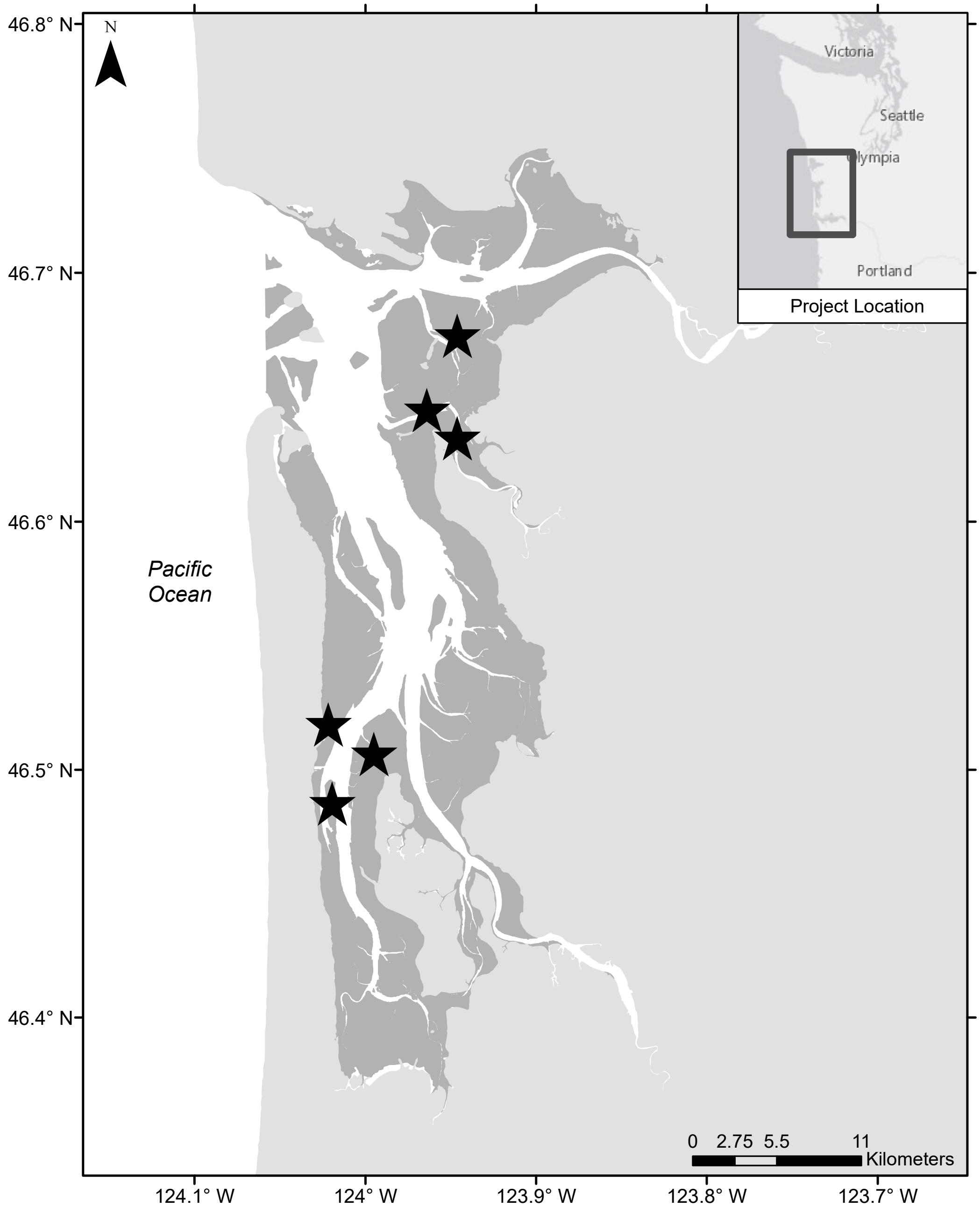

Figure 3. Map of Willapa Bay indicating the six sampling regions with star symbols. The darker grey indicates the intertidal zone. Map modified from Muething et al. (2020) with permission.

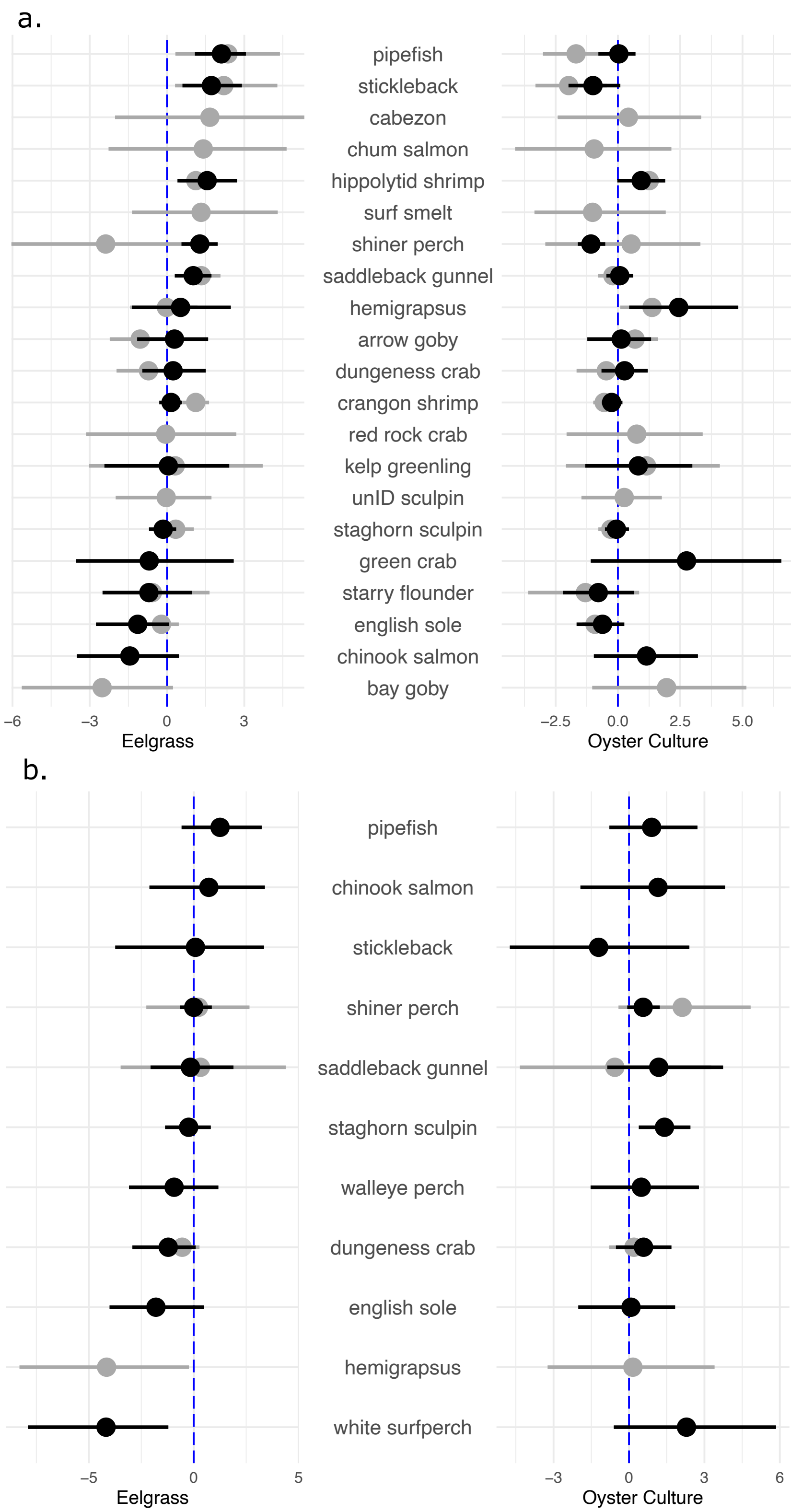

Figure 4. Caterpillar plot showing coefficients with highest posterior density (HPD) interval from seine (a) and video (b) analysis including all sites. Spring in grey, summer in black. Values above zero indicate a positive association with eelgrass (left panels) or oyster culture (right panels). Values below zero indicate negative association with respective habitat structure.

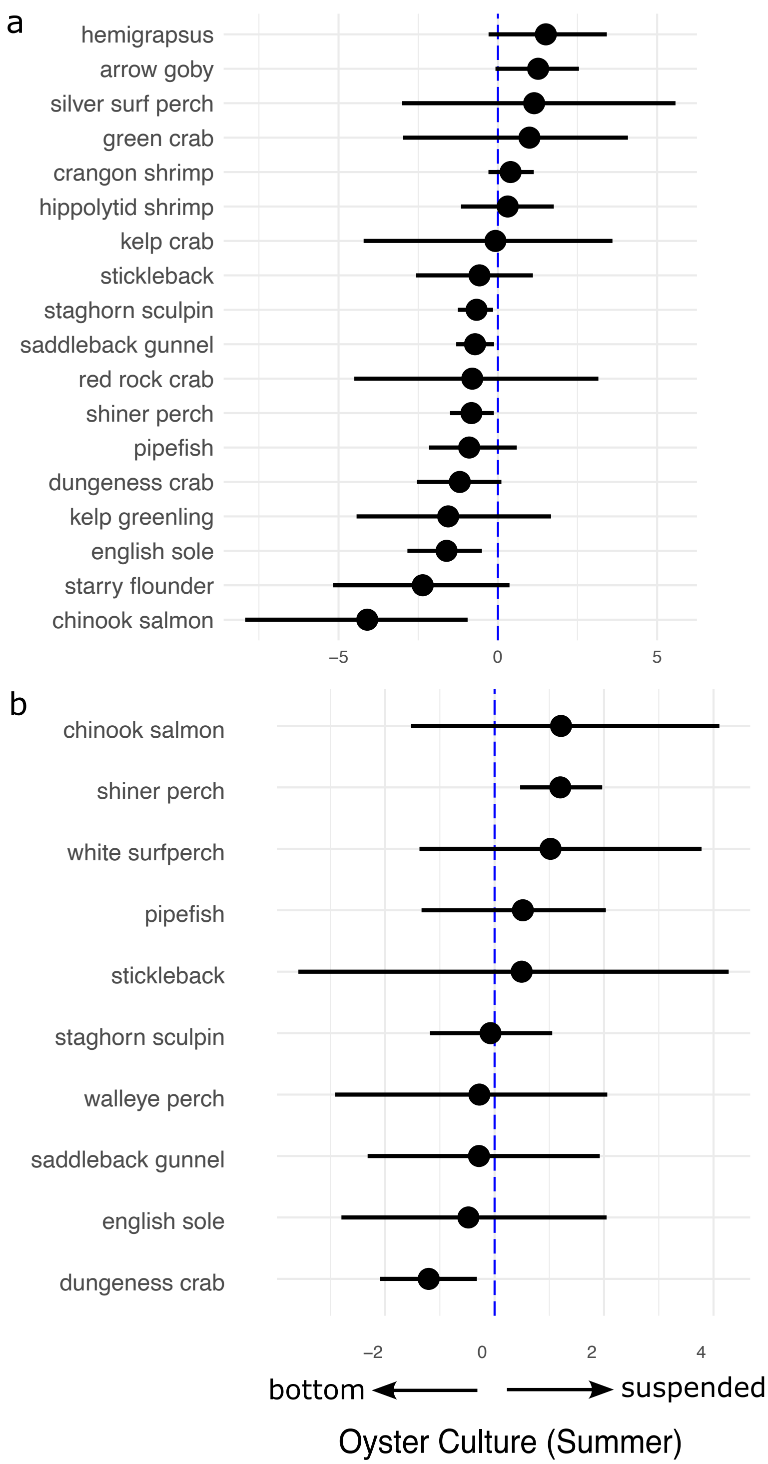

Figure 5. Caterpillar plots showing coefficients and highest posterior density (HPD) intervals from seine (a) and video (b) analysis, including only sites that have oyster culture. Summer data only. Values below zero (left) indicate association with bottom culture, while values above zero (right) indicate association with suspended culture.

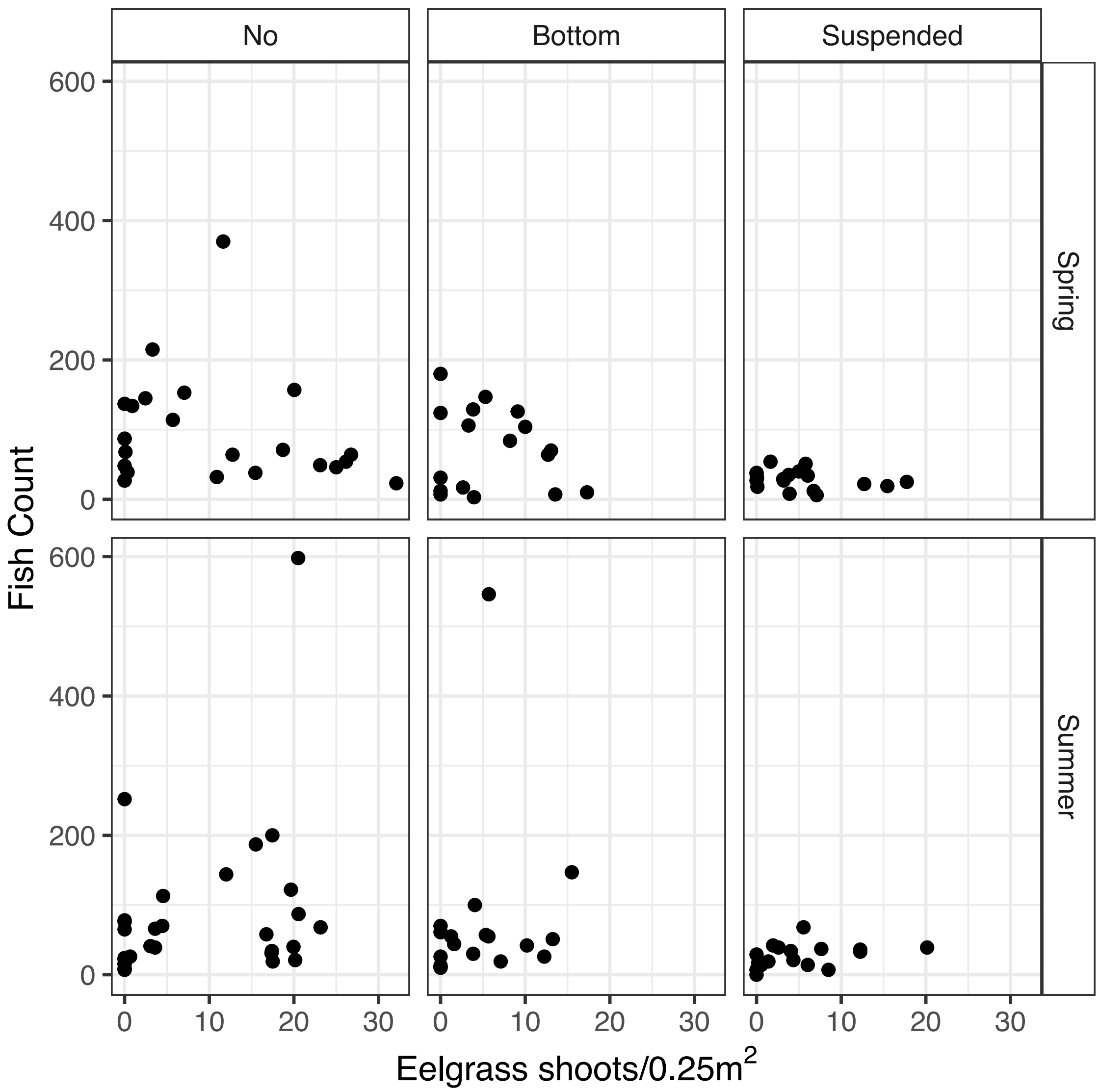

Figure 6. Fish count from seines as a function of eelgrass shoot density in different culture habitats (no culture, bottom culture, suspended culture), and during Spring and Summer. Note that fish count in suspended culture was likely affected by negative sampling bias (see section 4.3)

Supplementary Material

S1.1 Effects of eelgrass and oyster culture on community composition

Methods

To test for the effect of region, eelgrass (no, sparse, dense) and oyster (no, bottom culture, suspended culture) on community composition, we used the mvabund package in R. Rare species (occurring <5% were removed, as in our other analyses. Using the manyglm function, we created GLM models for each season and method. Region, eelgrass & oysters were treated as fixed effects, and the eelgrass x oyster interaction was tested. Models were fit using negative binomial distribution (using a log-link and unknown overdispersion parameter, and with default cor.type = "I", where independence is assumed) and checked with residual analysis using Dunn-Smyth residuals. Analysis of deviance (ANODEV) was run to determine significant effects on community composition, assuming uncorrelated responses and with PIT-trap resampling. See *Methods* section for more details on data processing. A post-hoc test was performed on spring video data to further investigate the effect of eelgrass x culture interaction on community composition. For this univariate test, P-values were adjusted for multiple testing, using a stepdown resampling procedure.

Results

There is a significant effect of eelgrass and oyster culture on nekton community composition, demonstrated by both spring and summer seine data, as well as summer video data. Spring video data was limited due to visibility issues (only four taxa and few observations), resulting in insufficient data when examined alone. There was no strong evidence of an eelgrass x oyster interaction effect on nekton community composition. Interestingly, spring video data indicated a significant effect of this interaction, but for reasons stated above concerning sampling, we do not find this to be strong evidence of an eelgrass x oyster interaction. Furthermore, post hoc tests for spring video data revealed no significant drivers of the possible eelgrass x oyster interaction.

Table S1. Results from ANODEV showing significant effects of oyster culture and eelgrass levels on nekton community composition. The eelgrass x oyster interaction was only significant in spring video data.

Seine Data

SPRING	Res. DF	DF Diff	Dev	Pr(>Dev)
(Intercept)	65			
Region	60	5	277.6	**0.001**
Eelgrass	58	2	144.0	**0.001**
Oysters	56	2	104.9	**0.001**
Eelgrass : Oyster	52	4	106.6	0.104

SUMMER	Res. DF	DF Diff	Dev	Pr(>Dev)
(Intercept)	65			
Region	60	5	278.6	**0.001**
Eelgrass	58	2	103.6	**0.001**
Oysters	56	2	115.6	**0.001**
Eelgrass : Oyster	52	4	81.24	0.281

Video Data

SPRING*	Res. DF	DF Diff	Dev	Pr(>Dev)
(Intercept)	48			
Region	43	5	86.40	**0.001**
Eelgrass	41	2	14.61	0.148
Oysters	39	2	16.96	0.068
Eelgrass : Oyster	35	4	29.05	**0.027**

SUMMER	Res. DF	DF Diff	Dev	Pr(>Dev)
(Intercept)	50			
Region	45	5	103.87	**0.004**
Eelgrass	43	2	55.83	**0.001**
Oysters	41	2	55.61	**0.006**
Eelgrass : Oyster	37	4	34.03	0.781

*Univariate Post-Hoc test for spring video data

SPRING	Dungeness crab		*Hemigrapsus*		Saddleback gunnel		Shiner perch	
	Dev	Pr(>Dev)	Dev	Pr(>Dev)	Dev	Pr(>Dev)	Dev	Pr(>Dev)
Region	47.37	0.001	6.336	0.331	9.294	0.127	23.397	0.001
Eelgrass	3.449	0.559	9.636	0.043	0.61	0.929	0.917	0.929
Oysters	7.486	0.126	5.982	0.204	0.386	0.867	3.105	0.523
Eelgrass : Oyster	11.648	0.195	0	0.820	5.255	0.195	12.148	0.195

S1.2 Posterior predictive check from section 2.4.2

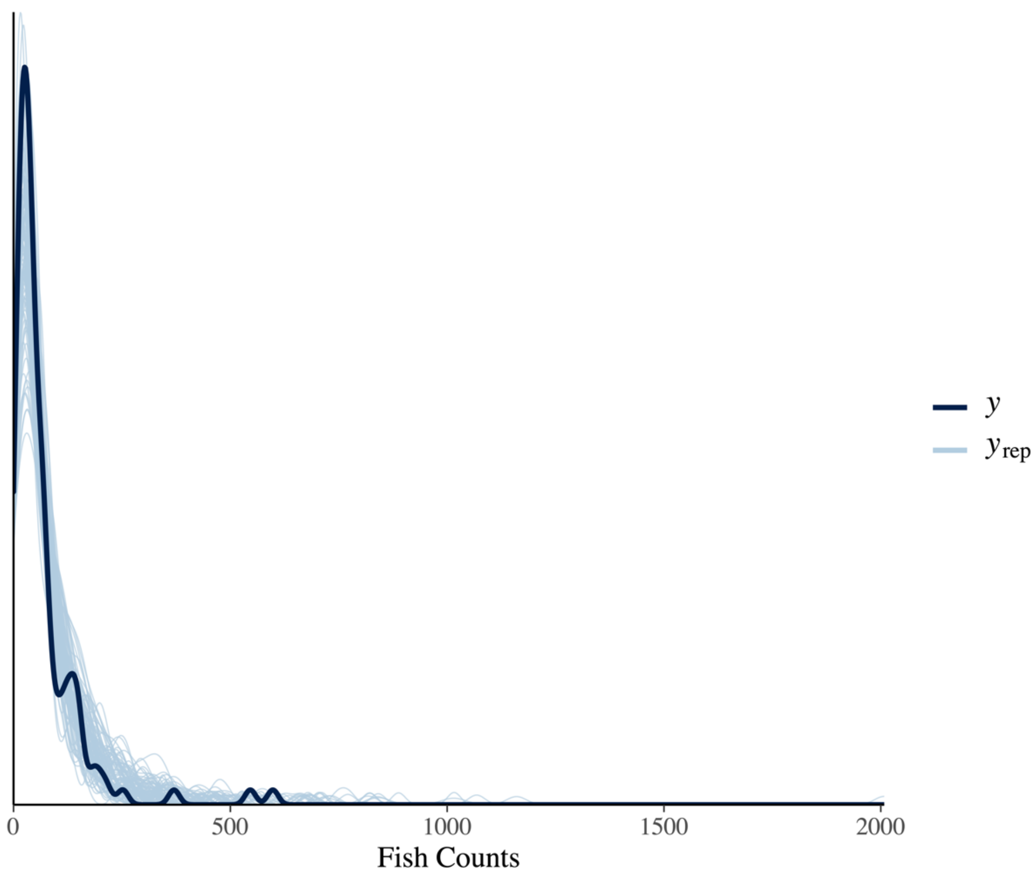

Figure S1. Posterior predictive check for model of fish abundance as a function of eelgrass density, culture type, and season

Eelgrass (*Zostera marina*) recovery affected by disturbance timing on mechanically harvested oyster culture beds

Boardman F.C., Ruesink J.L.

Abstract

Amid global seagrass declines and increasing human demands of coastal habitat, it is critical to mitigate loss of seagrass habitat through understanding seagrass resilience following large scale disturbance. Although seagrasses often respond to disturbance through increased sexual or asexual reproduction, past research on the cosmopolitan *Zostera marina* (eelgrass) is highly variable in terms of whether recovery occurs, and whether seeds or clonal growth is the primary contributor. In Willapa Bay, Washington State, we studied eelgrass recovery following large-scale disturbance on six adjacent oyster culture beds (~6000 m^2 in area) that were harvested using mechanical methods (i.e. dredging). We found that recovery potential and mode is heavily affected by timing of disturbance. Beds disturbed during the non-growing season (October – December) or the early growing season (January – April) showed faster rates of recovery, and higher contribution of new shoots from seedlings relative to beds disturbed during the late growing season (May – September). Consistent with eelgrass life-history, spring seedling densities were positively affected by flowering shoot densities the previous summer. Clonal reproduction (i.e. branching) was negatively affected by disturbance within the past four months, likely from physical damage, but also showed negative density dependence, meaning that higher branching rates were observed at lower shoot densities. Overall, this work provides straightforward guidelines for management of anthropogenic disturbance on eelgrass beds to reduce permanent habitat loss and can also guide restoration efforts of eelgrass beds.

Introduction

Seagrasses are marine angiosperms that provide essential nearshore habitat for fish and invertebrates, as well as offer shoreline protection by sediment stabilization and dissipation of wave energy. Seagrasses are in global decline due to a multitude of stressors including coastline development, nutrient runoff, sedimentation, extreme weather events exacerbated by climate change, fishing and aquaculture practices, disease, as well as physical disturbances (Orth et al. 2006, Short et al. 2014). Between 1879 and 2009, a loss of 29% of known seagrass extent occurred, with rates of decline accelerating since 1990 (Waycott et al. 2009, Dunic et al. 2021). Furthermore, in a world with a growing population, many are looking towards the sea for more sustainable food solutions (Gentry et al. 2017, Costello et al. 2020). Amidst global stressors and expanding mariculture practices, there is a need to enhance the resilience of seagrass ecosystems—the ability to withstand repeated disturbances without switching to an alternative stable state—through research and management (Holling 1973, Horan et al. 2011, Unsworth et al. 2015).

In Washington state, including Willapa Bay, oyster farming and eelgrass (*Zostera marina*) have overlapped for over a century (Dumbauld et al. 2009). The state is unusual in that intertidal lands can be privately owned, contributing to the strong socioeconomic and cultural importance of the shellfish industry that makes Washington a leader in shellfish production in the United States (van Senten et al. 2020). There are multiple methods of growing oysters, including "ground" or "on-bottom" culture, where oysters are grown directly on the sediment, often intermixed with eelgrass. Dredge implements used during harvest or bed maintenance result in disturbance events characterized by removal of eelgrass vegetative tissue as well as damage or removal of below-ground rhizomes (Tallis et al. 2009). Soft, organic-rich surface sediment is also removed in the process to create optimum conditions for growing oysters on the sediment surface. Past research shows that there are many reciprocal effects between eelgrass and oysters (and other bivalves) (Ferriss et al. 2019, Fales et al. 2020) and that space competition between oysters and large morphotypes of eelgrass is minimal below a density of ~20% shell cover (Wagner et al. 2012). While it is known that dredging activity is a substantial disturbance to co-occurring seagrass (Erftemeijer and Lewis 2006), previous work reveals that seagrass can return to full density; however, recovery periods are variable and generally longer than recovery following manual harvest methods (Tallis et al. 2009, Ferriss et al. 2019). Some oyster farmers employ dredging techniques that they observe to be less harmful to eelgrass ("Local" or "Farmers' Ecological Knowledge"), which supports the continued co-occurrence of eelgrass and oysters.

Zostera marina (i.e. eelgrass) is widely distributed across the northern hemisphere*,* with recovery mechanisms that consist of 1) asexual branching from existing shoots, and 2) the production of seeds and seedlings via flowering. Previous work documents variability in recovery potential of *Zostera* species, with seedling contribution ranging from being the dominant recovery mechanism (Olesen & Sand-Jensen 1994, Jarvis & Moore 2010, Johnson et al. 2021), to playing little or no role (Boese et al. 2009, Macreadie et al. 2014). Seagrasses often enhance reproductive investment in response to disturbance (Cabaco and Santos 2012). Similarly, research in Willapa Bay has revealed elevated seedling densities in dredged seagrass beds compared to control and longline aquaculture beds, suggesting that seedlings contribute to bed recovery following dredging disturbance (Wisehart et al. 2007). Because seeds are negatively buoyant and rhizomes extend at <1 m per year (Duarte 1991), these beds of several hectares cannot be rapidly recovered from eelgrass around the edges. Environmental factors certainly affect the success of recovery (Yang et al. 2016), but some baseline biological criteria, such as the existence of a seed bank or remaining adult shoots, must be met for recovery to be likely (Orth and McGlathery 2012). Understanding how timing, frequency and severity of disturbance—the defining features of disturbance regimes (Lytle 2001)—interact with *Z. marina* phenology to determine if clonal shoots and/or seedlings contribute to recovery allows us to hypothesize a two-year framework to predict recovery potential (Fig 1). Eelgrass does not typically flower in the same year as germination, and the seed bank is short lived (<1 year), so a bed needs to be undisturbed for more than a year for the next seed bank to form. Given that a seedbank is present, we predict that seedlings will contribute to recovery following a winter or early spring disturbance, with greatest overall recovery when surviving adult shoots contribute to clonal reproduction (in cases of low or moderate severity disturbance). Following severe disturbance (i.e. no adults shoots remaining), recovery will theoretically be dependent on a seed bank, which may be absent in a high frequency disturbance regime (Fig. 1).

Due to the phenology of *Z. marina* life history, factors such as timing, frequency and severity of a disturbance (i.e. shoot damage versus removal) can all affect whether a seagrass bed will demonstrate resilience or be subjected to a critical ecosystem tipping point (Unsworth et al. 2015, Horan et al. 2011, Holling 1973). Past experimental designs examine recovery of *Z. marina* from shoot removal or leaf damage at the small scale of meters (Fonseca et al. 1984, Boese et al. 2009, Ruesink et al. 2012, Soissons et al. 2016), while other studies track recovery following a single disturbance or die-off event (Plus et al. 2003, Jarvis & Moore 2010, Johnson et al. 2021). Here, we seek to understand recovery of eelgrass at an ecologically relevant scale (thousands of square meters) across multiple areas where disturbance events vary in frequency and seasonal timing. By considering the interaction of phenology of *Z. marina* and disturbance timing, we test the underlying processes that determine if recovery is possible, or whether a seagrass bed has been pushed past an ecosystem tipping point.

We utilized scheduled large-scale disturbances (~10,000 m^2 beds) accompanying mechanical harvest of shellfish to study the recovery mechanisms of eelgrass and determine how season and frequency of disturbance impact resilience. The goal of this study is to quantify and identify how disturbance timing, frequency and eelgrass life-history affect resilience, and to provide key takeaways that inform the management of planned disturbances to mitigate long-term damages to eelgrass populations.

1. How does the timing of disturbance affect recovery of eelgrass density?
2. How does the timing of disturbance affect the contribution of seedlings towards recovery?
3. How do flowering shoot density and oyster shell cover influence seedling recruitment (spring seeding density)?
4. How do the occurrence of recent disturbance, shoot density and season affect branching activity?

While this study has clear implications for the management of shellfish aquaculture practices on seagrass beds, it also contributes to the broader understanding of seagrass resilience by "experimentally" testing the effects of timing and frequency in a semi-controlled setting, at a much larger scale than past experimental work. In a world where seagrasses are threatened globally by anthropogenic disturbances, this work helps to fill an important knowledge gap in understanding seagrass resilience at a scale that is applicable to real-world disturbances.

Methods

Sampling Methods

Our study site, located in the northeastern portion of Willapa Bay, a coastal estuary of Washington state (USA), consists of a series of six contiguous intertidal oyster culture beds, ranging 150-200 meters in length (Fig S1), that are maintained and harvested using mechanical techniques (i.e. dredging by boat at high tide). The beds contain on-bottom cultured oysters that are harvested on a rotating schedule, meaning beds are at varying stages of the crop cycle at one given time. Disturbance severity was relatively consistent, resulting in sparse clusters of remaining adult shoots and rhizomes, which would be the sole source for clonal expansion. From spring 2021 to spring 2024, we measured vegetative shoot density, flowering shoot density, seedling density and oyster shell percent cover (visually estimated, including live oysters and remnant shell) in spring (late April/early May), summer (mid-June) and fall (late August/early September). All samples were taken using two parallel transects, for a total of twenty 0.25 m^2 quadrats per bed, with quadrats 15-20 m apart, depending on the bed width. Transects were set at approximately the same location for each sampling, about 20 m and 40 m from the lower edge of the bed (Fig. S1). Seedlings >15 cm long were considered to be vegetative during summer sampling in terms of density counts. During each sampling, we also collected shoots by excavating half of the quadrats to measure two variables: (1) branching rates (proportion branched of last four rhizome nodes), and (2) in the fall, the proportion of shoots that originated from seed that spring (including branches from seedling-source genets), versus clonal growth from an older plant. In 2021, we only excavated shoots in the fall, and no shoots were excavated in spring 2024. On beds with particularly sparse eelgrass (i.e. lot of empty quadrats), we collected the nearest shoots outside of the quadrats to have additional samples. Genets originated from seed that year are typically still attached to their small coiled rhizome, showing where the seed germinated, whereas plants that are excavated with a thick broken rhizome, were considered to be clones from plants ≥1 year old.

Periods of disturbance lasted from 1 to 5 months, encompassing a handful of acute disturbance events. In addition to harvesting and planting oysters, activities included “cleaning” or maintaining the bed by removing soft sediment from the surface, also done mechanically. We categorized the disturbance events as occurring during different approximate stages of growth for perennial *Z. marina* (Ruesink et al. 2022), where “Early Growing Season” (EGS) is January - April, and “Late Growing Season” (LGS) is May – September (Fig. 2). Seed germination and branching disproportionately occur during EGS, whereas LGS is a period of rapid leaf turnover and increasing shoot size. Seeds typically mature and dehisce from shoots by late July (Ruesink et al. 2022). Thus seed germination occurred during a portion of EGS, and seed production during a portion of LGS (Fig. 2). Beds that had not been disturbed for over a year (from September), were considered controls. We included a “Non-Growing Season” (NGS, biologically, slow-growing), during October - December, but only had one bed during one year that was disturbed during this time (excluding “controls”). The end of each disturbance period was used to determine the category of occurrence.

Data Analysis

Four analyses aligned with the four focal questions of the study and were performed in R (version 4.3.0; R core team 2023) using the glmmTMB package (Brooks et al. 2017) for linear mixed effects modeling and DHARMa package (Hartig 2016) for simulated residual analyses to test model fit. Relevant interactions were tested but not included in the final models; ultimately models were selected based on residual analyses and AIC values.

The first analysis examined the effect of disturbance timing on eelgrass density. We examined vegetative shoot count as a function of ‘days since end of disturbance’, season disturbed, and year, including bed as a random effect to account for repeated sampling; for this analysis, days since end of disturbance was a continuous variable that accommodated beds disturbed more than 12 months before, and there was no “control” category. Here, LGS and NGS disturbance were compared to EGS disturbance (since there was no “control” group), and a gaussian distribution was used. Sample size was 54, as three seasonal samples in each of three years for all six beds were included in this analysis.

Next, to test if timing of disturbance affects the contribution of seedlings towards recovery, we examined the proportion of shoots from seedling origin (excavated in the fall) as a function of season of disturbance and year, with bed as a random effect. This model used a tweedie (link = log) distribution, which accommodates data with zero values and wide variation in the proportion from seedlings when present. Sample size was 18 (six beds sampled in fall over three years).

For the third analysis, to determine which factors influence the density of seedlings in the spring, we modeled spring seedling density as a function of the previous summer’s flowering shoot density, as well as current oyster coverage per bed, and year, with bed as a random effect. Due to the study period of 2021-2024, this analysis only examines seedling densities from 2022, 2023 and 2024 (using flowering shoot densities from 2021, 2022 and 2023), resulting in a sample size of 18. We were unable to reliably test the effect of disturbance during important seed stages (i.e. post-seed settlement, germination), although a higher summer flowering shoot density results from beds being undisturbed in the months leading up to summer sampling (Fig 1). This model uses a tweedie (link = log) distribution family.

Lastly, we tested how branching was related to recent disturbance, shoot density, and season. We determined the average branching activity for each excavated quadrat containing vegetative shoots, and up to 10 quadrats per bed were averaged to calculate proportion of latest four nodes branched. We only examined plants that had gone through at least one winter season (i.e. seedlings were excluded), due to potential differences in phenology. Proportion of nodes branched was analyzed as a function of season, occurrence of disturbance within last four months, vegetative shoot density and year, with bed as a random effect, and used a tweedie distribution (link = log). Sample size was 54 (six beds with three seasonal samples for three years).

Results

Eelgrass on six large areas with different disturbance histories showed a general pattern of lowest vegetative shoot densities at the conclusion of a period of disturbance (Fig. 3). Phenological changes were indicated by the relatively high densities of seedlings in spring, which transitioned to vegetative shoots by the summer sampling time. Some beds returned to about 40 shoots/m^2 in between disturbance events, which is within the range of observed eelgrass bed densities for the large morphotype present in Willapa Bay (Thom et al. 2003), while others failed to recover within the study timeframe (Fig. 3).

Our first analysis determined that days since disturbance had a strong positive effect on vegetative shoot density, while LGS disturbance had a significant negative effect on shoot density relative to EGS disturbance, with EGS disturbance estimated to have over double the shoot density 400 days post-disturbance relative to LGS (Fig. 4). We saw a positive year effect of 2021 (Fig. 4, Table 1), which (including all sampling seasons) had an average overall shoot density of 6.47 per 0.25 m^2, versus 4.05 and 3.67 in 2022 and 2023, respectively.

In the second analysis, relative to controls without disturbance for at least a year, seedling-origin shoots contributed significantly more to beds that were disturbed during EGS and NGS, whereas LGS disturbance (i.e. within the past four months before September sampling) had no effect. Up to 71% of the shoots present on beds at the end of the growing season were only present due to successful seedling establishment earlier in the year (a mean of 20% from seedling contribution). Year also had significant effects, with 2022 having the 0.37 contribution from seedling-origin shoots, with 2021 and 2023 having 0.14 and 0.11 proportion seedling contribution, respectively (Table 2).

The third analysis, testing factors that influence density of seedlings in the spring, revealed that flowering shoot density from the previous summer is a strong predictor of spring seedling density. Percent oyster shell, ranging from 0 to 30% on average, showed a trend of a positive effect on spring seedling density, but this was not statistically significant (Fig. 5). Year was again a significant predictor, with 2023 having a greater spring seedling density, averaging 2.98 per 0.25 m^2, while 2022 and 2024 averaged 1.06 and 1.14 seedlings per 0.25 m^2, respectively (Fig. 5, Table 3).

Lastly, analyzing the effects of recent disturbance (within four months), season, vegetative shoot density and year on branching (proportion of last four nodes branched), we found that branching changed seasonally, with highest rates in spring (significantly greater than in fall) that declined in summer and fall. Additionally, year was a significant predictor, with 2022 having lower branching rates overall. The lowest average branching rate 0.10 during fall 2022, and the highest was 0.40 during spring 2023. There was a negative effect of vegetative shoot density as well as a negative effect of disturbance within the past four months on proportion branched (Fig. 6, Table 4).

Discussion

Our results indicate that timing is a key factor when determining the extent to which an eelgrass bed will recover and by which mechanism. If beds are disturbed at high intensity that removes most shoots and rhizomes, they will be dependent on seedlings to recover, which requires that the bed was allowed to form a seedbank (i.e. flower undisturbed) the previous year. Furthermore, only beds disturbed during the non/slow-growing season or early-growing season will be the appropriate timing for seedlings to emerge at a higher rate, and be able to grow to adult shoots. Beds disturbed during the late growing season risk removal of seedlings that germinated that spring, as well damage to adult shoots before they have flowered and produced seeds. Our findings support and help to explain the inconsistency of dominant recovery mechanisms and recovery success that are reported in the literature from small-scale experimental work or opportunistic studies of natural disturbances. When comparing recovery of *Z. marina* beds following climatic events in Chesapeake Bay during 2005 and 2018, Johnson et al. (2020) report different recovery dynamics, with one case dominated by seedlings while the other was largely recovered via clonal expansion. A collection of other work also documents variability in recovery potential of *Zostera* species, with some reporting seedling contribution as the dominant recovery mechanism (Olesen & Sand-Jensen 1994, Jarvis & Moore 2010, Johnson et al. 2021), while other cases are reliant on clonal reproduction (Boese et al. 2009, Macreadie et al. 2014).

As disturbance intensity increases, two constraints for resilience are 1) fewer shoots for clonal reproduction and 2) reduced flowering shoot density. At the extreme severity of disturbance, where no adult shoots remain, all recovery must occur from the seed bank. If disturbance frequency has a <2 year return time, then any seedlings in the population will not have sufficient time to produce their own seeds, and this timing particularly erodes population resilience if disturbance interferes with seed production, or removes young seedlings post-germination. That is, the period after seed production and before the end of the seedling germination window (i.e. NGS and EGS) is when eelgrass beds show the highest resilience to disturbance. Furthermore, lower intensity disturbance, where some adult shoots remain, offers multiple methods by which eelgrass can recover.

Having two methods of recovery, clonal and via seeds, is a feature that makes *Z. marina*, among other grasses, naturally quite resilient (Unsworth et al. 2015). In the case that adult shoots are removed in a large disturbance event, the seed bank exists as a back-up that can still allow recovery. And in fact, intermediate level of disturbance can cause increased genetic diversity, further contributing to the resilience of that eelgrass population (Hughes and Stachowicz 2011, Foster et al. 2021). It is well established that prescribed disturbance can be used in terrestrial grassland management to promote biodiversity (Edwards et al. 2007, Valko et al. 2014). In these cases, timing and method of disturbance—and how they promote clonal growth versus seed bank contribution—must be considered to achieve desired species composition and not facilitate undesired or invasive taxa that could grow to dominate the landscape (Lewis et al. 2009, Larios et al. 2013, Merou et al. 2013). Unlike most terrestrial grasslands, *Z. marina* regularly exists in monoculture, so cases of high-severity disturbances repeated at high frequency that push eelgrass past the "tipping point" may cause a permanent shift to an unvegetated state, or only gradually be repopulated by nearby *Z. marina* propagules. However, in cases where *Z. marina* is coexisting with or occurring near other seagrass species, ruderal, and in some cases non-native, species may establish after removal of *Z. marina* (Moore et al. 2014, Boardman and Ruesink 2022).

Just as different aspects of disturbance may have positive or negative relationships to recovery via seeds, so too does disturbance affect the ability of adult shoots to produce clonal branches. The pattern of branching due to disturbance was overlain on a typical seasonal pattern, in which densities are lowest in winter, and clonal branching rapidly increases shoot densities as daylength increases (Olesen & Sand-Jensen 1994, Ruesink et al. 2022). Branching rates can increase as a phenotypically-plastic response to shoot thinning or transplant at low density (Ruesink et al. 2012, Ruesink 2018), and the current results involving large-scale disturbances also demonstrated negative density dependence in which branching declined at higher adult shoot density. However, if disturbance had occurred within the last four months, branching activity declined (Fig. 6), likely because physical damage to leaves and stress can inhibit branching (Ruesink et al. 2012). While the survival of adult shoots following disturbance still certainly boosts resilience potential in a bed, it is important to consider that their ability to contribute to clonal repopulation may be hindered following physical damage.

From a management perspective, for aquaculture or otherwise, these findings have straightforward applications for supporting resilience in *Z. marina* beds. If beds are to be disturbed at levels that leave few remnant shoots, ideally it would (1) be during a slow or early growing stage for the local *Z. marina* population (this varies regionally), (2) leave adults shoots with rhizome in place and only disturb a subset of the meadow extent to contribute to clonal expansion, and (3) not be repeated annually in the same location, with breaks of >1 year for beds to recover and for shoots to mature to resupply seed banks. Handpicking of shellfish is also a lower-disturbance (albeit less-efficient) harvest method that can be used to limit disturbance while allowing for harvest during critical time periods. Furthermore, although planting seagrass is not a typical step in shellfish aquaculture, beds with low shoot and seed densities could be restored by spreading *Z. marina* seeds from nearby beds at the end of the summer, supplementing a seedbank if flowering shoots are rare (Marion & Orth 2010). Although not statistically significant, we also observed a positive trend of oyster shells on seedling density, suggesting that low densities of shell may protect delicate seedlings from wave action or drying out. Alternatively, higher cover of shell may have been an index of a late stage in the crop cycle, so that a separate factor was positively related to both shell cover and seedling densities. Similarly, there is the potential for low densities (<30% cover was observed in this study) of live oysters kept on-bottom to support the growth of seedlings by providing both refuge as described above, but also biodeposits that improve conditions for germination or growth (Unsworth et al. 2022).

In a world witnessing global seagrass declines, while simultaneously leveraging the ocean for sustainable food production (Costello et al. 2020, Gentry et al. 2017), it is critical to understand how long-term damage to seagrass beds can be mitigated. While the disturbance documented here on eelgrass is a product of shellfish aquaculture, disturbances generally are becoming increasingly common and widespread due to continued shoreline development and extreme weather events (Orth et al. 2006, Short et al. 2014). Where there is direct anthropogenic cause, management decisions around timing and intensity of disturbance can support long-term resilience of eelgrass beds. In conclusion, the findings from this work contribute to a path forward to support eelgrass resilience during an era of global seagrass declines with increasing nearshore development and climatic extremes. *Z. marina* and other seagrass species have evolved to use resilience mechanisms, which can be leveraged by considering disturbance timing, intensity and seagrass life histories.

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Tables and Figures

Table 1. Results of linear mixed effects model testing shoot density as a function of days since disturbance, season of disturbance and year, with bed as a random effect.

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	2.680	1.024	2.617	0.009*
Days since disturbance	0.016	0.003	6.656	2.82e-11*
LGS Disturbance	-2.152	1.059	-2.032	0.042*
NGS Disturbance	-0.651	1.340	-0.486	0.627
Year 2022	-2.344	1.094	-2.143	0.032*
Year 2023	-1.127	1.145	-0.984	0.325

Table 2. Results of linear mixed effects model testing factors that influence the contribution of seedling-origin shoots towards eelgrass (*Zostera marina*) recovery, as the proportion of seedling-origin shoots at the end of the growing season (September) relative to undisturbed control beds.

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	-3.174	0.377	-8.411	< 2e-16*
Disturbed EGS	1.865	0.371	5.032	4.86e-07*
Disturbed LGS	0.667	0.472	1.415	0.157
Disturbed NGS	1.776	0.574	3.092	0.002*
Year 2022	1.222	0.330	3.706	2.11e-4
Year 2023	-0.649	0.368	-1.765	0.078

Table 3. Results from linear mixed effects model testing spring seedling density as a function of flowering shoot count from the previous summer, percent shell cover and year, with bed as a random effect.

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	-1.320	0.591	-2.233	0.026 *
Flowering Shoot Count (prev. summer)	0.827	0.213	3.891	9.97e-05 *
Percent shell cover	0.026	0.021	1.215	0.224
Year 2023	1.149	0.410	2.802	0.005 *
Year 2024	0.300	0.489	0.613	0.540

Table 4. Results from linear mixed effects model testing proportion of last four shoots branching as a function of season, vegetative shoot density, year and whether or not there was disturbance within the last four months, with bed as a random effect.

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	-1.427	0.152	-9.359	<2e-16*
Spring	0.290	0.100	2.898	0.004*
Summer	-0.028	0.125	-0.223	0.823
Vegetative Shoot Density	-0.049	0.011	-4.641	3.46e-06*
Year 2022	-0.385	0.150	-2.576	0.010*
Year 2023	0.371	0.141	2.638	8.34e-03
Disturbed within past four months	-0.358	0.121	-2.945	3.23e-03*

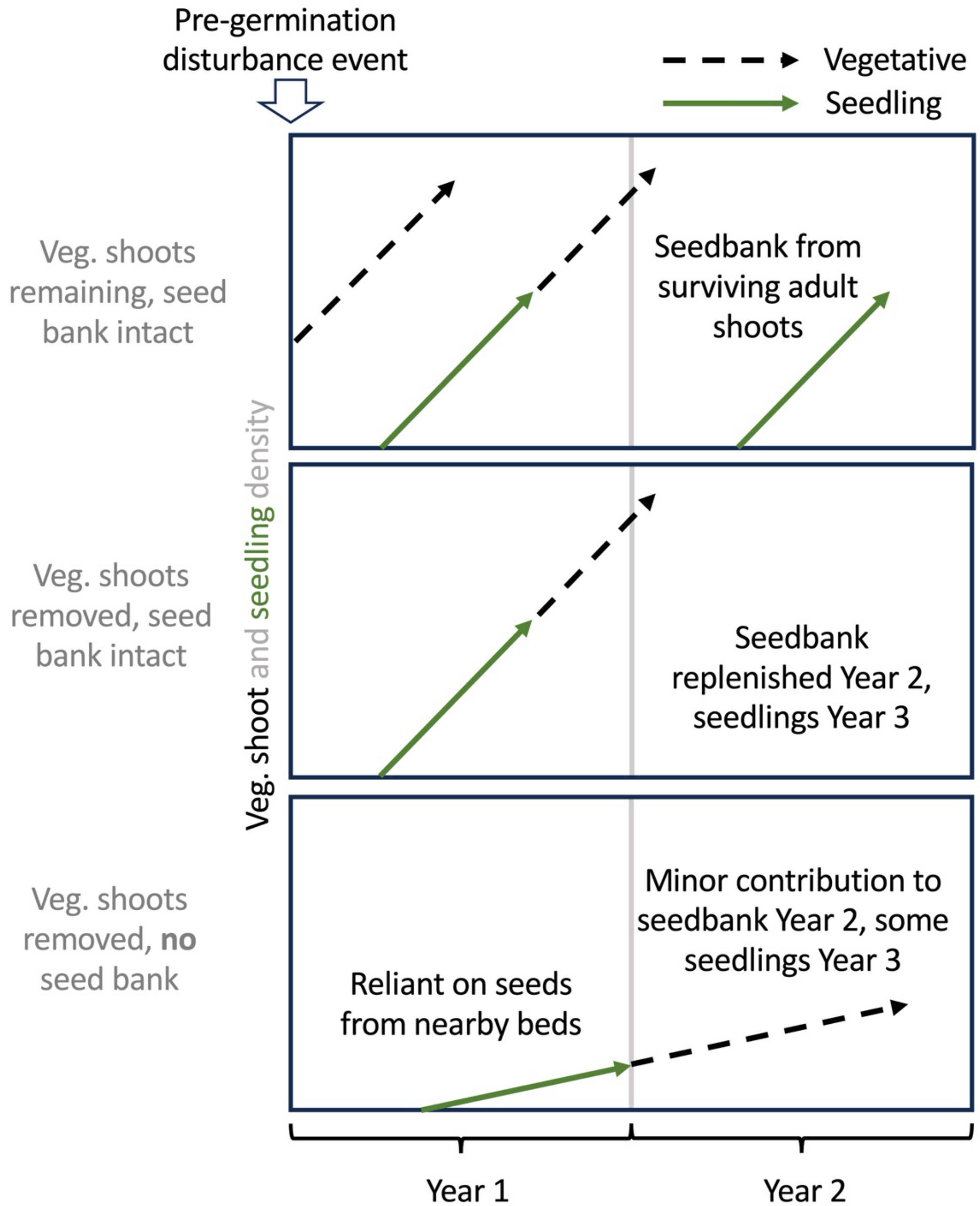

Figure 1. Simplified schematic illustrating two-year framework for eelgrass recovery given different conditions (leftmost) that are determined by timing, severity and frequency of disturbance.

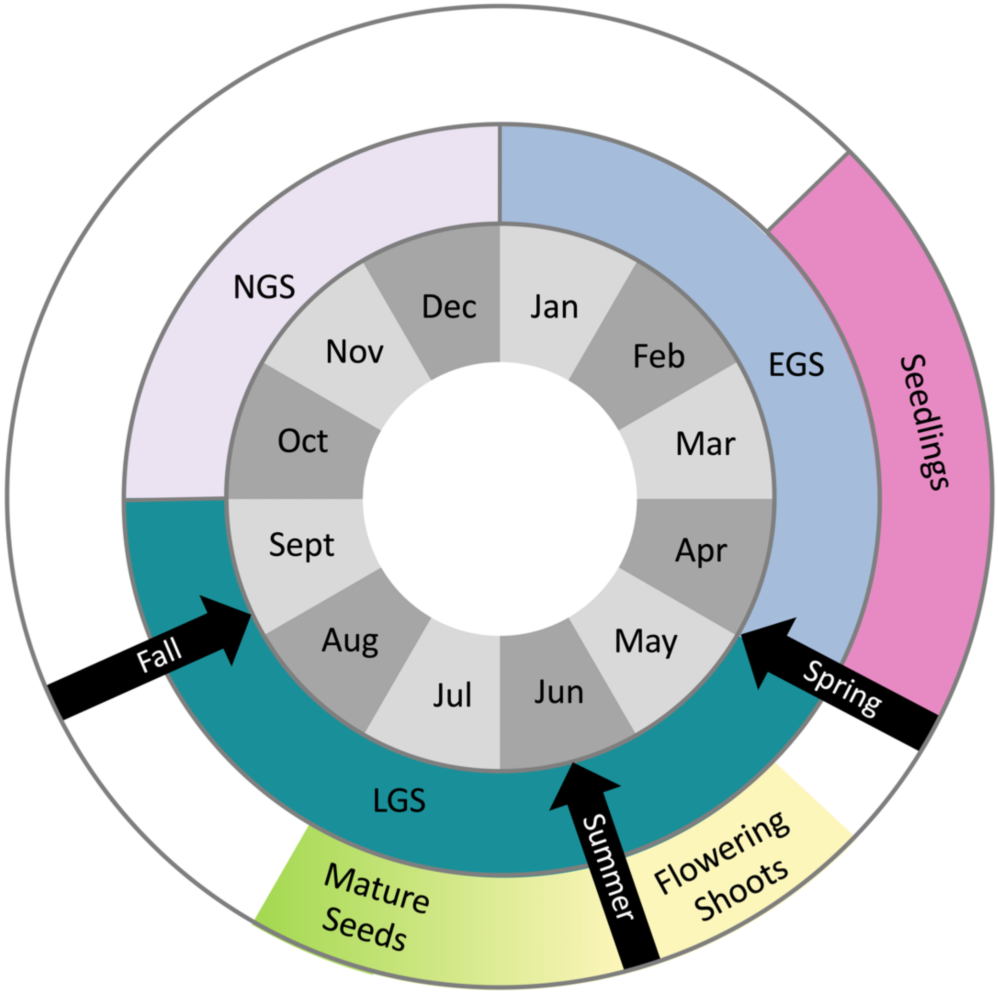

Figure 2. Schematic showing timing of sampling (arrows), as well as disturbance periods (EGS, LGS, NGS) and phenological stages of seedling development.

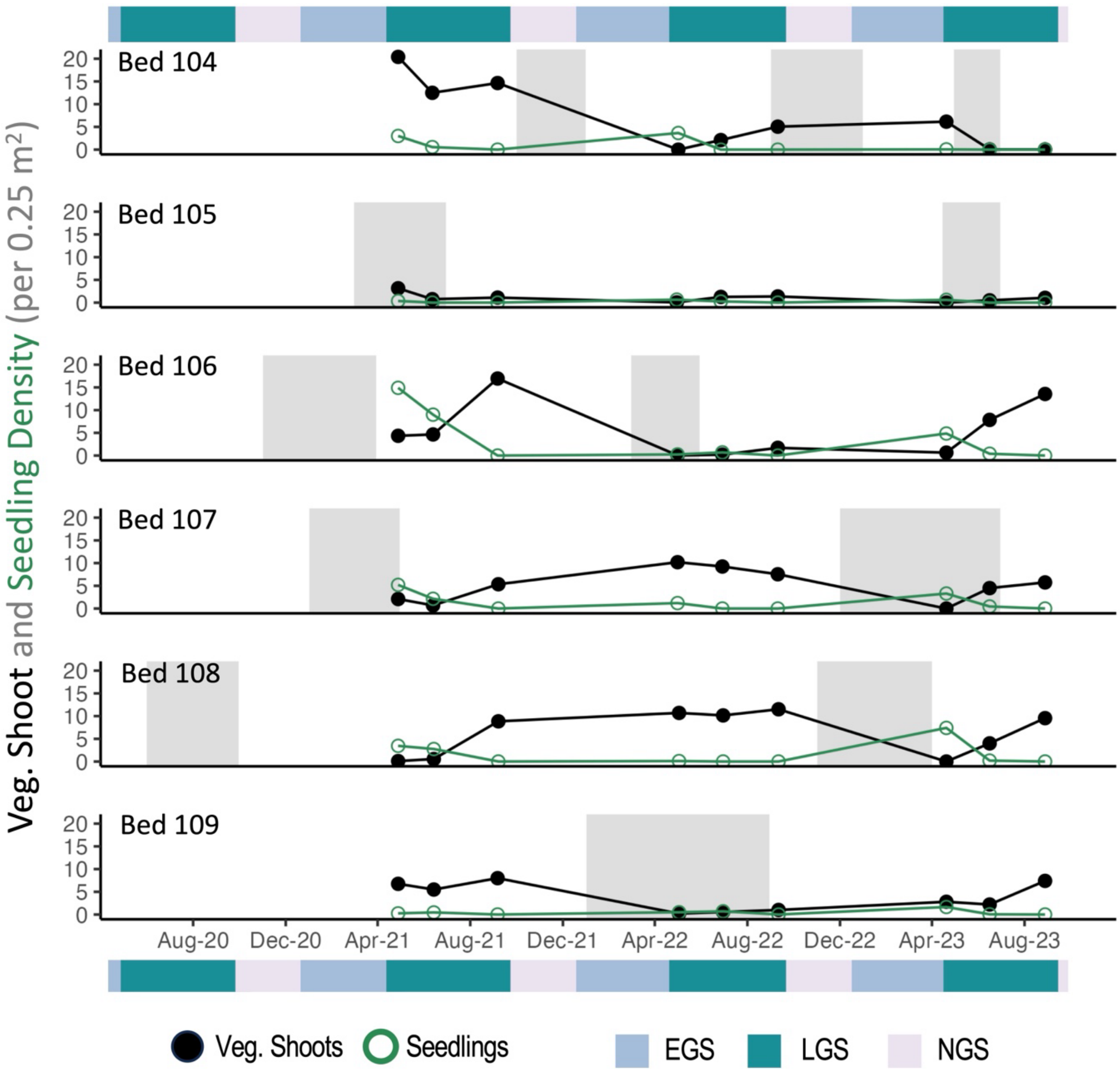

Figure 3. Vegetative shoot and seedling density shown over the three-year sampling period (excluding spring 2024). Gray blocks indicate periods of disturbance. Color strips along top and bottom edges indicate disturbance time categories (EGS, LGS, NGS). For density counts, shoots were considered vegetative once they were >15 cm long, even if they originated as seedlings that spring.

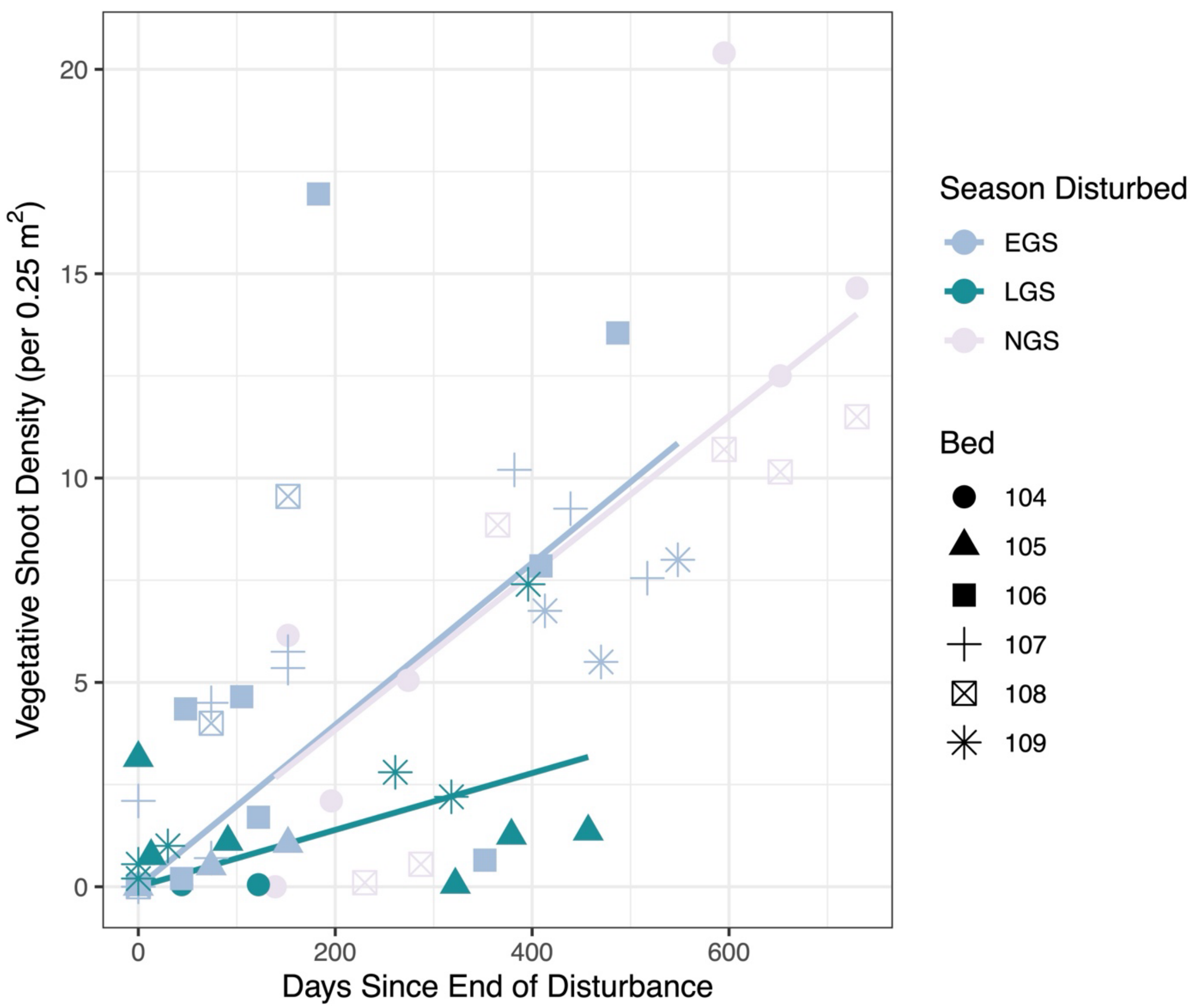

Figure 4. Eelgrass (*Zostera marina*) shoot density as a function of days since disturbance, with color indicating disturbance time category. Best fit lines with a y-intercept at 0 included for visual purposes, see Table 1 for results from generalized mixed model.

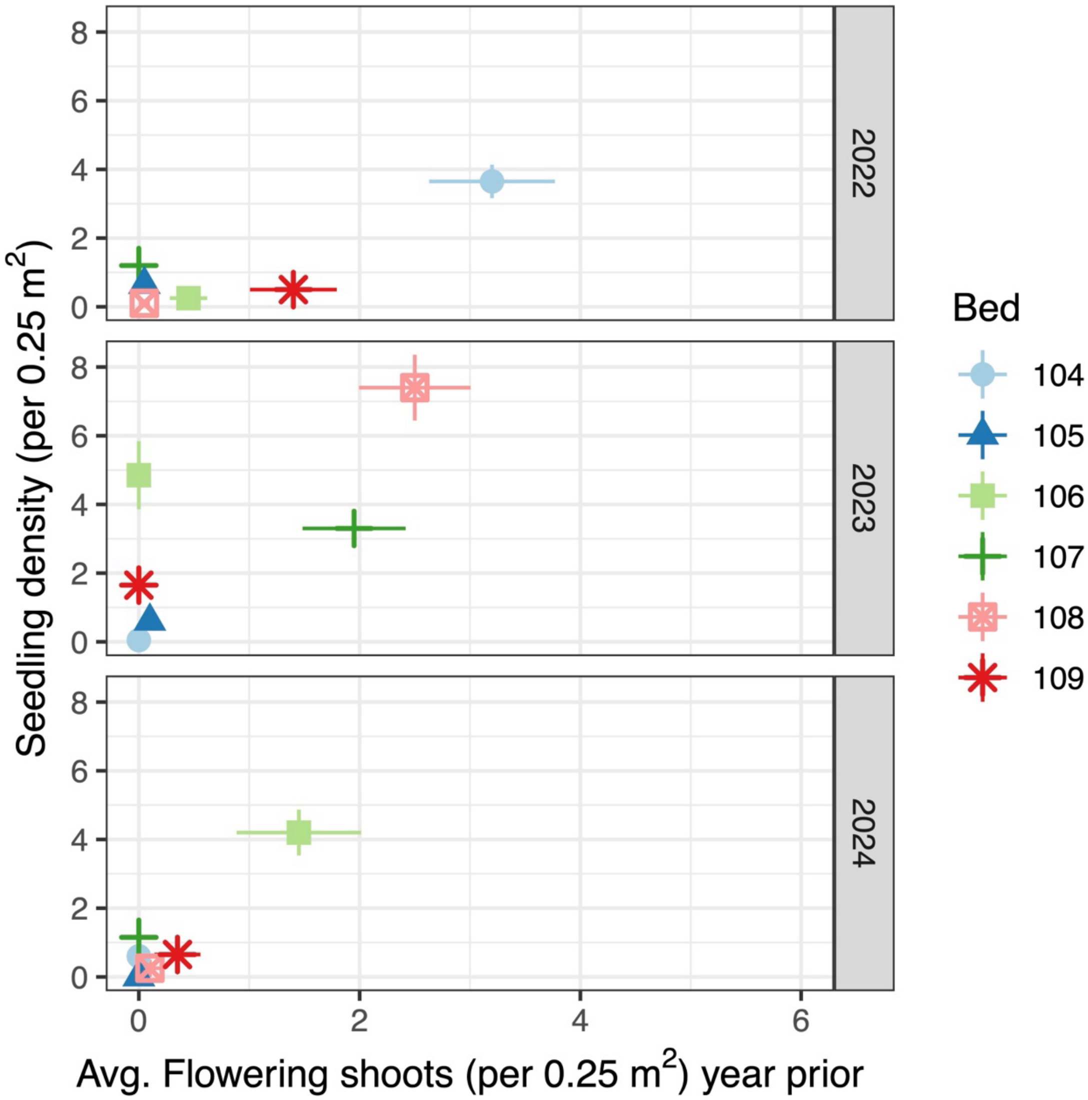

Figure 5. Average seedling density per bed and year as a function of average flowering shoot density from the prior summer. Graph shows the mean values used in the analysis with standard error. See Table 3 for results from generalized mixed model.

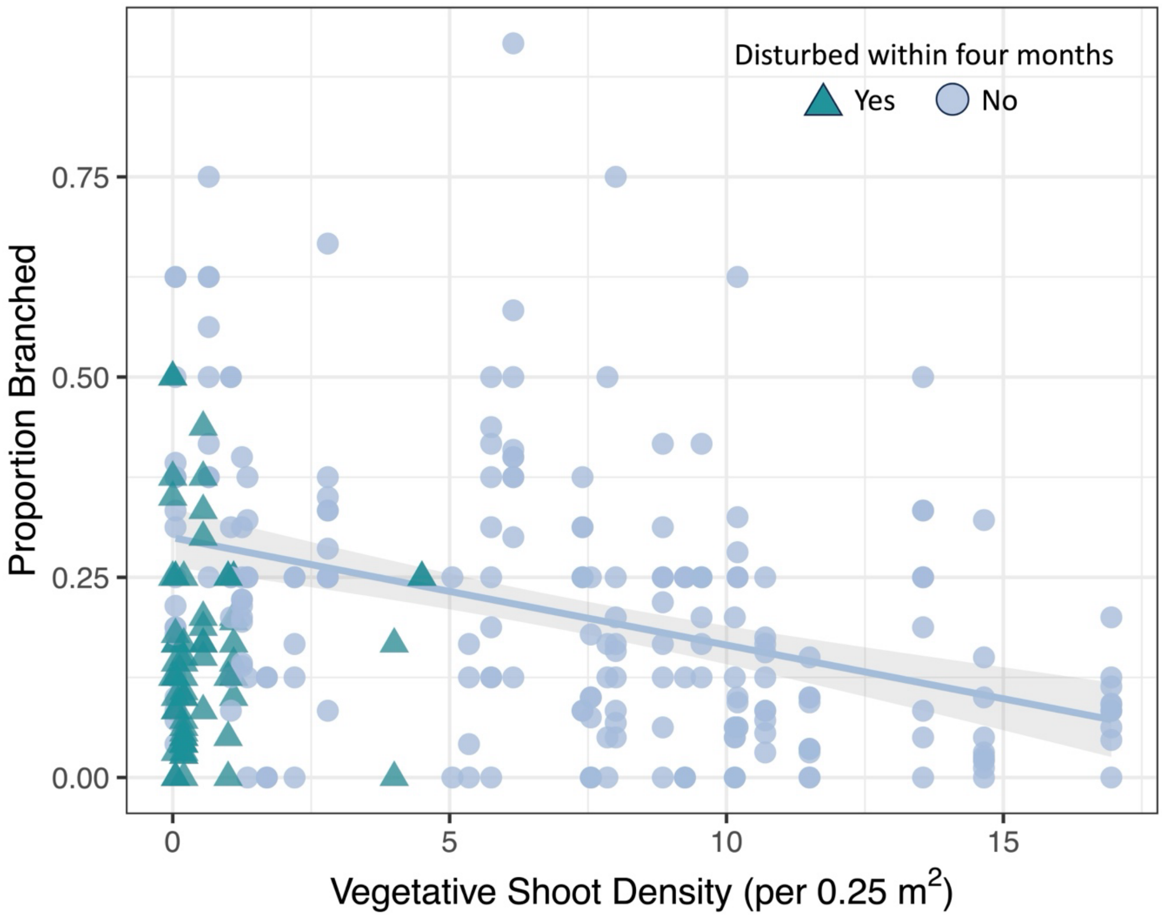

Figure 6. Proportion of the last four nodes branched as a function of vegetative shoot density (per 0.25m^2), with disturbance in the last four months indicated by color and shape. Quadrat values averaged across beds in analysis are shown as individual points; linear best fit line of samples *not* recently disturbed included for visualizing relationship of branching and vegetative shoot density.

Supplementary Material

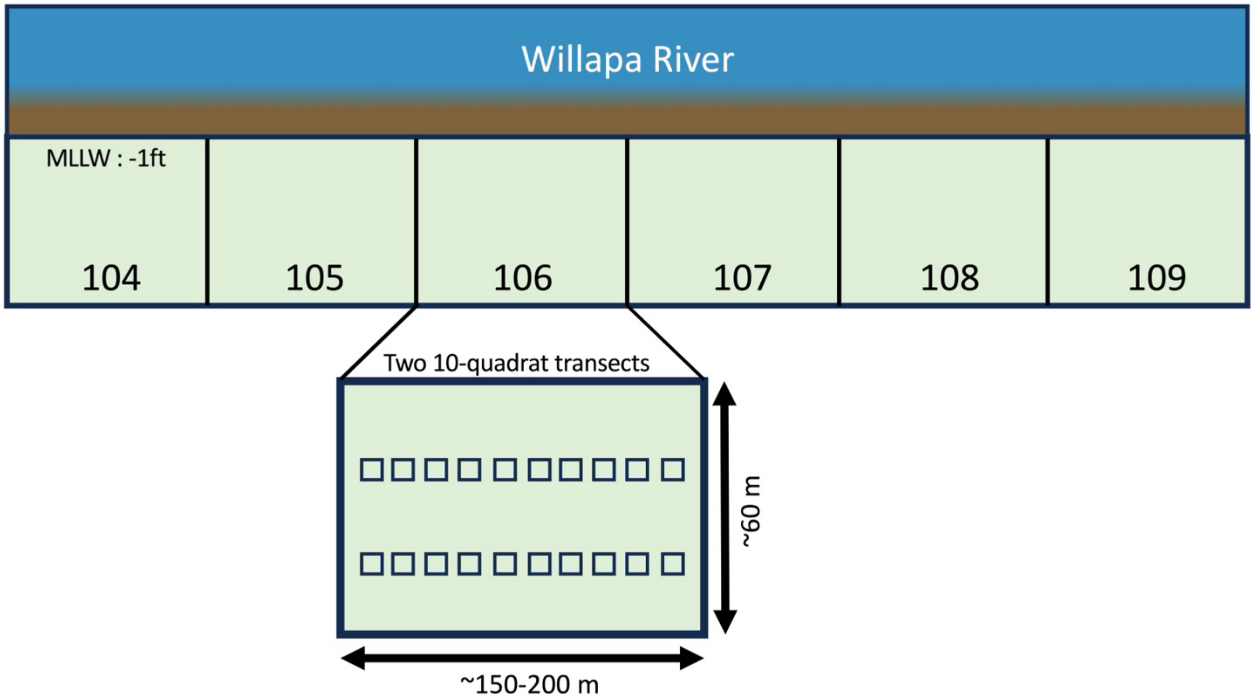

Figure S1. Schematic showing contiguous oyster culture beds that were sampled on as well as the sampling approach.