

FISH ASSEMBLAGES IN SOUTH PUGET SOUND *Z. MARINA*

BY

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ABSTRACT

Fish Assemblages in South Puget Sound *Z. marina*

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Seagrass is a marine flowering plant that is an important indicator of ecosystem health by providing vital habitat for fish in marine ecosystems. Unfortunately, seagrass is currently experiencing a worldwide decline due to multiple stressors including shoreline development and pollution. Eelgrass (*Zostera marina*), a seagrass species native to the Salish Sea, provides vital habitat for important commercial fishes including herring (*Clupea pallasii*) and salmon (*Oncorhynchus spp.*). A loss of eelgrass coverage could potentially harm these vital food fish stocks. Washington Department of Natural Resources (DNR) manages approximately 500 m² of transplanted eelgrass at Joemma State Park (JSP) in South Puget Sound as part of their goal to increase total eelgrass coverage in Puget Sound 20% by 2020. To observe fish abundance and diversity at a transplanted eelgrass site, video data was collected to compare fish assemblages at JSP and a natural eelgrass bed at Dupont Warf. Unbaited underwater video cameras were deployed biweekly from June to August 2017. A two-minute video was recorded every ten minutes after initial camera deployment at low tide. Video was recorded for 24 hours, but footage after sunset was discarded due to low light. The data were analyzed to quantify fish abundance and diversity at each site. Of the 12 fish groups identified, 2 were not present at either JSP site (spiny dogfish and striped surfperch). Bay pipefish were the only fish group significantly associated with transplanted eelgrass, while shiner perch, gunnels, tube-snout, striped surfperch, and spiny dogfish were significantly associated with the natural eelgrass. The remaining 5 groups (salmonids, forage fishes, pacific snake prickleback, pacific stag sculpin, and pile perch) had no significant association with a site type. Despite the lack of a significant association between site and 5 fish groups, each of these fishes has been shown to associate with eelgrass in previous studies. This research can be treated as a pilot study for using non-invasive video recording to assess fish assemblages in South Puget Sound eelgrass beds.

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Introduction

Puget Sound is a temperate estuarine system spanning over one thousand square miles along the inland waters of Washington State. The Puget Sound is a diverse ecosystem containing 211 fish species among its marine waters and 19 river drainage basins (Washington State Department of Ecology, 2016). Fishes in Puget Sound range from small gobies and blennies to larger salmonids and cod. Fish communities in Puget Sound can vary based on seasonal patterns, with certain fishes like salmonids transitioning from streams to open ocean at different life stages (Simenstad et al., 1982). Fish habitat in Puget Sound includes oyster mudflats, tidal floodplains, and kelp beds. Puget Sound's development and pollution has caused fish populations to decline due to loss of habitat (Toft et al., 2007). Adequate habitat is essential for fishes to survive predation, as well as find sources of food. One critically important habitat for fishes in Puget Sound is seagrass.

Seagrass is an important marine macrophyte that is considered an indicator of ecosystem health (Macreadie et al., 2017), and provides habitat, nursery and shelter habitat for a variety of fishes. Among the thousands of miles of marine waters in Puget Sound are patches of eelgrass (*Zostera marina* L.), a species of seagrass, which can range from a few square meters of hundreds of meters in both intertidal and subtidal areas of the Sound. *Z. marina* can inhabit depths between +1.4 and -11 meters relative to mean lower low water, and is commonly found in fringe or tideflat habitats (Christiaen et al., 2017). The abundance and diversity of fishes found in *Z. marina* is extensive, supporting fishes like salmonids and herring (Phillips, 1984; Simenstad et al., 1982), making it a critical habitat for preserving biodiversity in Puget Sound. Unfortunately,

seagrasses populations are declining globally due to a suite of anthropogenic factors (Duarte, 2002). Restoration efforts of global seagrass populations have met mixed results, but advances in technology and habitat modeling have proven to be successful in recent restoration efforts (Fonseca, 1998). In Puget Sound, the local species of seagrass, eelgrass, plays important roles in the nearshore environment ranging from binding sediment to carbon sequestration (Bos et al., 2007; Duarte et al., 2005).

Z. marina populations face many of the same anthropogenic stressors that seagrasses face globally, along with increased development along Puget Sound's shoreline (Li et al., 2007). With declining *Z. marina* coverage comes declining ecosystem services that benefit marine fauna and humans alike. Reduced *Z. marina* coverage also means less biodiversity in Puget Sound, as there are fish species that depend on the habitat for specific parts of their lives or the entirety of their lives (Phillips, 1984). Restoration of *Z. marina* throughout the United States is mixed in terms of success in creating large seagrass beds that are resilient over time; issues stem from similar problems that global seagrass restoration efforts have faced, including large die-offs of *Z. marina* populations (Robblee et al., 1991; Short, Muehlstein, & Porter, 1987) and an inability to recruit seagrass at restoration sites (Bell et al., 2008; Fonseca, 1998). However, trends of growth and restoration efforts in Puget Sound have been positive within the past five to ten years based on overall stable populations and successful small-scale restorations (Christiaen et al., 2017). The restoration of *Z. marina* is a critical component of ensuring nearshore ecosystems do not lose important habitat. An important component of seagrass restoration activities is looking at how seagrasses are used by fishes.

Examining fish abundance and diversity, also known as fish assemblages, in relation to seagrasses, helps scientists determine what ecosystem services seagrasses are providing for

marine fauna. Research on fish assemblages in *Z. marina* in the Pacific Northwest found that fish assemblages varied based on time of day and season (Garwood et al., 2013; Obaza et al., 2015; Robinson et al., 2013). In addition, research comparing fish assemblages in transplanted seagrass to natural seagrass has been observed throughout the country with a variety of seagrass types (Brown-Peterson et al., 1993; Sheridan, 2004). However, a combination of these two bodies of work (fish assemblages in the Pacific Northwest and comparing fish assemblages between restored and natural seagrass) is lacking.

The Washington Department of Natural Resources (DNR) manages approximately 500 m² of transplanted *Z. marina* at Joemma State Park (JSP), on Case Inlet in South Puget Sound. The DNR transplanted *Z. marina* to JSP with the hopes of creating new beds to provide ecosystem services and functions for the nearshore environment. Understanding what fish are present in *Z. marina* in South Puget Sound is one important measure of how well transplanted *Z. marina* is providing ecosystem services in relation to natural seagrass, a term known as “functional equivalency” (Fonseca, 1998). Determining if transplanted seagrass provides ecosystem services for the local environment can dictate future restoration efforts within Puget Sound, and provide future restoration efforts with important data on fish assemblages in recently transplanted seagrass. Ensuring that restored *Z. marina* beds provide ecosystem services is critical because of the alarming rate that *Z. marina*, and seagrasses all over the world, are losing coverage (Waycott et al., 2009). Restoring seagrass communities is crucial to prevent further loss of this important marine fauna, their habitat, and the ecosystem services that seagrasses, including *Z. marina*, provides.

To assess the diversity and abundance of fishes present in transplanted seagrasses, studies on fish assemblages were conducted in South Puget Sound (SPS). The use of unbaited

underwater video to study fish assemblages is a common technique in coral reefs and seagrasses (Smith et al., 2011; Watson et al., 2005). Studies using underwater video collect continuous footage ranging from periods of 15 minutes to 90 minutes to examine fish assemblages, although there is no standard methodology for underwater video census. Other conventional methods of sampling fish assemblages (seine nets and baited underwater video cameras) have raised concerns about unbiased results or potential damage to seagrass. Although unbaited underwater video to study fish assemblages has been used for coral reef and seagrass fish assemblages in the past, its use for fish assemblages of restored *Z. marina*, specifically in Puget Sound, is lacking. Therefore, this fieldwork will passively collect data on fish use of transplanted *Z. marina* that will add to the knowledge of fish communities in SPS and the potential uses of transplanted *Z. marina* for said communities.

The results of this study will provide information on fish abundance and diversity at transplanted *Z. marina* sites at JSP, bare sediment sites at JSP, and natural *Z. marina* sites at Dupont. Evidence of fish presence in transplanted *Z. marina* at JSP can elucidate the ecosystem services the restored *Z. marina* is providing at JSP for fishes. Video footage showing fish abundance and diversity at bare sediment and natural *Z. marina* sites will provide data on fish use of both habitats. The results of this thesis will help determine if transplanted *Z. marina* at JSP is providing ecosystem services like natural *Z. marina*, by being used by fishes commonly found in SPS. Data from fieldwork will aggregate hours of collective footage, showing which fish are present in transplanted *Z. marina* in relation to nearby natural *Z. marina*.

Literature Review

Overview of Seagrasses

Seagrasses are aquatic flowering plants consisting of 70 differing species found in marine and estuary environments on every continent except Antarctica (Green, 2003). Found in both intertidal and subtidal environments, seagrass (which are taxonomically distinct from seaweed and alga), has an aboveground portion that consists of leaves connected to a sheath and a belowground portion that the sheath connects to the root/rhizome complex (Figure 1). Seagrasses can form large underwater meadows capable of altering the surrounding water current, nutrient dynamics, and sediment level (Bos et al., 2007; Heiss et al., 2000), and can even filter bacterial pathogens from the water column (Lamb et al., 2017). These meadows are also home to a myriad of aquatic vertebrates and invertebrates that use seagrass as shelter and nursery habitat (McDevitt-Irwin et al., 2016; Ruso & Bayle-Sempere, 2006). Seagrass also an important food source for large vertebrates including black brant geese (Wilson & Atkinson, 1995), dugong (Yamamuro & Chirapart, 2005), and sea turtles (Bjorndal, 1979).

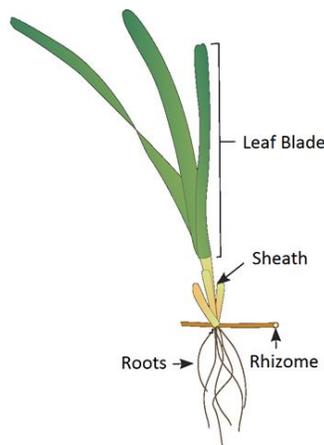


Figure 1. Seagrass anatomy. Modified from (Collier, 2004)

Nursery/Shelter

Seagrasses provide essential nursery habitat for many juvenile and larval stages of important commercial and recreational fishes and shellfishes (Bertelli & Unsworth, 2014; Short et al., 2011). Multiple meta-analyses conclude the aboveground biomass of seagrass meadows provides complex habitat that provides better shelter when compared to bare sediment (Heck Jr. et al., 2003; McDevitt-Irwin et al., 2016; Whitfield, 2017). Marine organisms use seagrass blades as refuge from predators, which have reduced maneuverability and visual acuity for prey hiding in seagrass blades, increasing the likelihood of survival for juvenile or residential organisms (Horinouchi et al., 2009). Seagrass blades also provide surface area for sessile organisms like encrusting algae to attach, which utilize the blade tips as a means of having better access to sunlight (Trautman & Borowitzka, 1999). Alga attached to seagrass also provide a source of food for invertebrate mesograzers that inhabit seagrass beds (Ebrahim et al., 2014), while sessile organisms and invertebrate mesograzers in seagrass beds also provide fishes with a source of food (Horinouchi et al., 2012; Kwak et al., 2015). In addition, epiphytic algae attached to seagrass blades provide further structural complexity in seagrass beds by creating additional housing for microcrustaceans and vertebrates (Adams et al., 2004; Corona et al., 2000). Seagrass also provides an important connecting habitat between other marine habitats like mangroves and coral reefs. The distance between seagrass, mangroves, or coral reefs can dictate fish abundance and diversity based on availability of shelter from predation, nursery habitat, and migration distance (Dorenbosch et al., 2007; Unsworth et al., 2008).

Sedimentation

The seagrass canopy is capable of reducing wave action, in turn filtering particles out of the water current that drop into the seagrass bed, promoting sediment accretion (Bos et al., 2007). Removing particles from the water current can reduce erosion risk (Potouroglou et al., 2017) while also reducing opacity, effectively filtering the water (Fonseca et al., 1998). The canopy can further prevent erosion by reducing the likelihood of sediment resuspension. By attenuating waves, the seagrass canopy prevents upwelling of deposited sediments within its meadow (Ros et al., 2014; Terrados & Duarte, 2000). Seagrass meadows create a positive feedback loop i.e. promoting sediment accumulation that allows more seagrass to grow in the surrounding environment that otherwise would be unable to do so, which in turn promotes more sediment accumulation (Heide et al., 2011). This ability to reduce coastal erosion is not only based on aboveground canopy length, but can be promoted via the root/rhizome system's ability to hold sediment in place (Christianen et al., 2013).

Carbon Sequestration

The filtration of particulate from the water column not only promotes sedimentation, but also the burial of organic carbon within seagrass beds. The carbon stored in seagrass biomass and soil is referred to as "blue carbon" (Howard et al., 2014). Despite only occupying less than 0.2% of the world's oceans, it's estimated that the global seagrass biomass contains 75.5 – 151 TgC, while the top meter of global seagrass soils contains between 4.2 and 8.4 PgC (Fourqurean et al., 2012). Seagrasses are responsible for 20% of global carbon sequestration in marine sediments (Duarte et al., 2013). Reduced flow of water within seagrass canopies causes detritus, seston, and other allochthonous carbon to drop out of the water current and deposit within the meadow

(Greiner et al., 2013; Kennedy et al., 2010). Organic carbon that settles in seagrass meadows is retained for centuries to a millennium due to multiple mechanisms including poor metabolism in sediments due anaerobic conditions and dissipation of wave action due to seagrass canopy reducing resuspension (Duarte et al., 2013). However, seagrasses also play an important role in spreading carbon throughout the ocean. Seagrass exports a large amount of carbon to both nearshore and deep ocean environments (Duarte & Krause-Jensen, 2017), indicating a significant role in carbon burial throughout aquatic environments.

Global Seagrass Decline

Despite the critical ecosystem services that seagrasses provide, seagrasses are currently facing a global decline. A review by Waycott et al. (2009) revealed alarming values in seagrass loss, including a rate of decline at 110 km per year since 1980, with 1.5% of mean seagrass area per year disappearing. This rate is comparable to the loss of mangroves (1.8% mean area per year) and even faster than tropical forest loss per year (0.5% mean area per year). The same study found that the rate of loss increased from 0.9% per year in 1940 to 7% per year in 1990. Overall, 80-100% of seagrass species are in decline (Green, 2003), with at least 10 species at an elevated risk of extinction (Short et al., 2011). Declining seagrass coverage has a slew of detrimental environmental affects, ranging from increased carbon returning to the ocean-atmosphere system (between 11.3 and 22.7 TgC per year) (Fourqurean et al., 2012), increased resuspension of sediment, and reduced biodiversity (Duarte, 2002). Loss of seagrass also affects the organisms that inhabit it, including 115 IUCN Red List species of marine invertebrates, fishes, sea turtles, and mammals that rely on seagrass (Short et al., 2011).

Causes of seagrass decline range from anthropogenic factors including coastal pollution and dredging to environmental issues including sea level rise and wasting disease (Duarte, 2002; Waycott et al., 2009). Seagrass decline has been linked to lower fishery production in multiple cases (Gillanders, 2006; McArthur & Boland, 2006; Tuya et al., 2014), indicating an economic stake in preserving seagrass ecosystems. The decline of seagrass can have immediate effects on smaller communities that rely on artisanal fisheries for sustenance (de la Torre-Castro & Rönnbäck, 2004). In addition, the nursery function that seagrasses provide for commercial fishes means seagrasses are an important source for future fisheries as much as current ones (Tuya et al., 2014). Seagrass ecosystem services for fisheries is valued at \$34,000/ha per year (in 2010 USD) (Short et al., 2011), making their loss not only an environmental issue, but an economic one too. Other estimates of the economic savings seagrass provide humans includes up to 13.7 billion per year collectively in carbon sequestration (Pendleton et al., 2012).

Restoration of Seagrass

Restoration of seagrasses is critical to preserving the myriad of ecosystem services it provides for animals and humans alike. It has been shown that restored seagrass can improve the surrounding environment, including storing more carbon, nitrogen, and sediment compared to unvegetated areas. Restoration not only has benefits for ecosystem services, but tangible monetary benefits for modern efforts to store carbon and nitrogen. A restored *Z. marina* bed in Virginia Coast Reserve Long Term Ecological Research site was estimated to store approximately \$4.10 (in 2011 USD) per hectare per year of carbon (Greiner et al., 2013). However, improvements from restoration are not immediate, as the same study found in their experiment showing restored seagrass beds 4 years old were not as good at storing carbon and sediment as 10-year-old seagrass beds. Their experiment shows that seagrass restoration efforts

are an ongoing process that requires habitual monitoring to determine successful restoration of ecosystem services. The definition of “success” for seagrass restoration commonly focuses on increased coverage of seagrass, but often overlooks the importance of seeing if restored seagrasses are providing the ecosystem services for organisms that natural seagrass does (Lefcheck et al., 2017). Investigating the effectiveness of restored seagrass sites at providing ecosystem services similar to natural seagrass, a term known as “functional equivalency” (Fonseca et al., 1998).

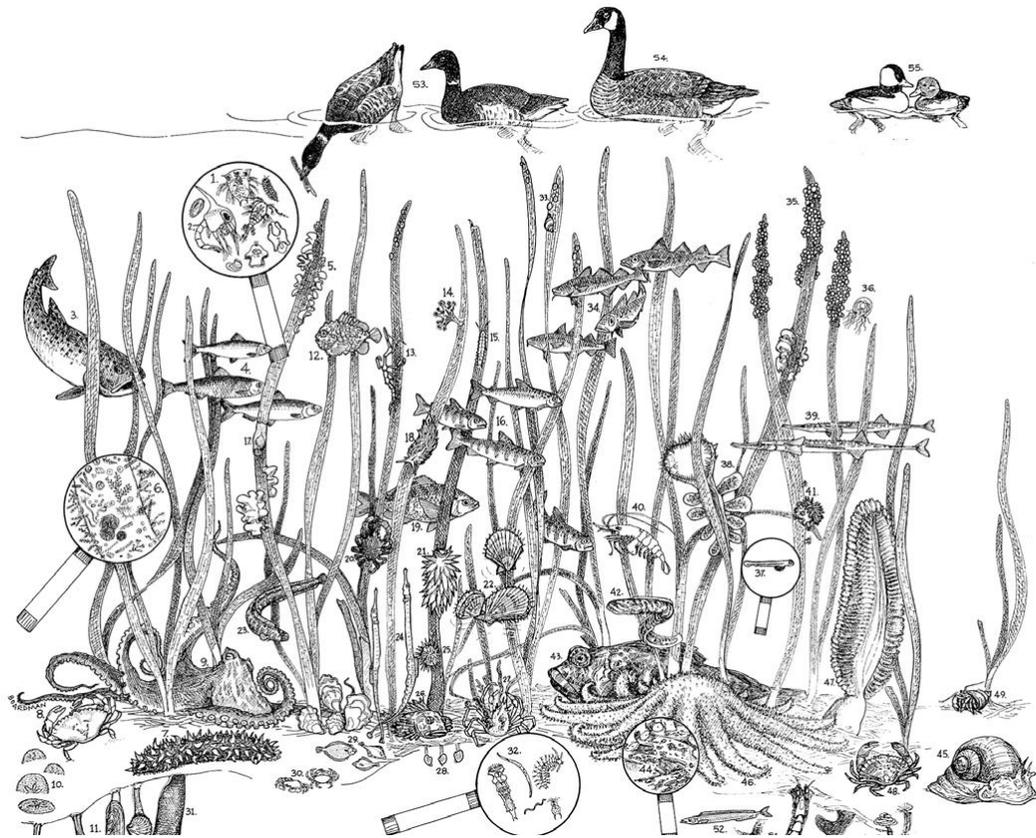
***Z. marina* and Puget Sound**

Z. marina is the dominant biomass found on the Pacific Coast of North America between Baja California and Alaska’s Yukon Delta; in addition, *Z. marina* is found throughout the world including the east coast of the United States, Japan, and the Wadden Sea (Green, 2003). Puget Sound’s seagrass population is predominately *Z. marina*, but has populations of the introduced Japanese eelgrass (*Zostera japonica*) as well (Christiaen et al., 2016). Phillips' (1984) ecological profile on the Pacific Northwest provides a thorough description of *Z. marina*’s features. Found in muddy or mixed sand/mud substrate, *Z. marina*, a perennial seagrass, is characterized by having between two and five round-tipped and strap-like leaves per shoot, depending on the environment. In addition, *Z. marina* has thin mesh of rhizome commonly buried between 3 and 4 cm below the sediment surface. *Z. marina* is capable of both vegetative (rhizomatic expansion) and reproductive (flowering and pollination) growth. *Z. japonica*, although similar in appearance, is discernable from *Z. marina* by its smaller sized leaves and shallow rhizome (no deeper than 3 cm).

The Washington State Department of Fish and Wildlife (WDFW) has classified seagrass beds as habitats of special concern (WAC 220-110-250) when dealing with hydraulic projects (RCW 77.55.021), while the Washington Department of Ecology lists seagrass as a critical habitat in its Shoreline Management Act (RCW 90.58), making seagrass beds federally protected within the state of Washington (Christiaen et al., 2016). Monetizing the importance of *Z. marina* for Puget Sound rests on one study by Batker et al. (2008), which valued *Z. marina*'s nutrient cycling in Puget Sound at \$5 - \$15 million (in 2006 USD) . However, the economic value of *Z. marina* consists of a slew of other ecosystem services that are not quantified in their evaluation. The importance of *Z. marina* has been acknowledged long before colonization of the Pacific Coast by Europeans. *Z. marina* had significant cultural value to coastal native groups including the Haida and Kwakwaka'wakw peoples, which used eelgrass as a food source and a material for basket weaving (Wyllie-Echeverria et al., 1994).

The *Z. marina* ecosystem of Puget Sound is an intricate and complex system connecting zooplankton to fishes to black brant geese (Figure 2). *Z. marina* provides important commercial food fishes like Pacific herring (*Clupea pallasii*) and Chinook salmon (*Oncorhynchus tshawytscha*) with habitat used for nursery and shelter. Pacific herring use *Z. marina* blades as a substrate to attach eggs, with up to 20,000 eggs being deposited on blades by a single female pacific herring. Pacific herring are a valuable commercial food fish as well as an important forage fish within Puget Sound (Phillips, 1984). Salmonids use *Z. marina* as a migration corridor as well as a refuge from predators during juvenile stages (Simenstad et al., 1982). However, the association between juvenile salmon and *Z. marina* is contested by Murphy (2000), who claims that direct evidence is lacking to make such a claim. Instead, he found that juvenile salmon likely have been found in *Z. marina* not because of the *Z. marina* itself, but the reduced exposure to

currents and waves within the beds. However, seagrasses are known to reduce wave action through blade attenuation (Fonseca & Cahalan, 1992), which provides a service to juvenile salmonids. The effectiveness of *Z. marina* as a source of denitrification has been observed as well (Zarnoch et al., 2017). Top down predation by fishes that frequent *Z. marina* is also an important controlling factor in reducing potentially harmful mesograzers from consuming *Z. marina* biomass, reducing coverage and production (Lewis & Anderson, 2012).



1. Zooplankton	14. Stalked jellyfish	29. Juvenile flounder	41. Brooding anemone
2. Larval crab	15. Eelgrass isopod	And sole	42. Prickleback
3. Salmon	16. Juvenile salmon	30. Juvenile crab	43. Sculpin
4. Herring	17. Bubble shell	31. Geoduck	44. Bacteria on detritus
5. Epiphytic macroalgae	18. Opalescent nudibranch	32. Sediment microfauna	45. Moon snail
6. Epiphytic microalgae, Hydzoa, and bryozoa	19. Perch	33. Snail and snail eggs	46. Sunflower seastar
7. Sea cucumber	20. Juvenile kelp crab	34. Juvenile cod, tomcod	47. Sea pen
8. Dungeness crab	21. Alabaster nudibranch	And wall-eyed pollock	48. Red rock crab
9. Octopus	22. Scallop	35. Herring eggs	49. Hermit crab
10. Sand dollars	23. Gunnel	36. Jellyfish	50. Worms
11. Clams and cockles	24. Bay pipefish	37. Larval fish	51. Ghost shrimp
12. Pacific spiny Lumpsucker	25. Sea urchin	38. Melibae-hooded nudibranch	52. Sand lance
13. Caprellid amphipod	26. Juvenile sculpin	39. Tubesnout	53. Black Brant
	27. Decorator crab	40. Shrimp	54. Canada Goose
	28. Juvenile clams		55. Bufflehead

Figure 2. Fauna associated with *Z. marina* (Mumford Jr, 2007)

Z. marina Coverage in Puget Sound

It is difficult to ascertain the exact acreage of *Z. marina* historically present in Puget Sound due a lack of reliable information regarding potential or historic coverage; estimates of historic coverage between 45,000 and 50,000 hectares have issues with methodology and conflicting data from more recent analysis (Dowty et al., 2010). A lack of reliable historic *Z. marina* population information is further exacerbated by the fact that it is unknown if south Puget Sound ever had a historic presence of *Z. marina* (Thom et al., 2011). Defining seagrass as “natural” can become an issue when a historical presence is not known. In 2000, Washington’s Department of Natural Resources (DNR) created the submerged vegetation monitoring program in order to address spatial and temporal trends in *Z. marina* (Berry et al., 2003). As of 2014, the total Puget Sound eelgrass coverage was 24,300 ha (Christiaen et al., 2016). The depth distribution of Puget Sound’s *Z. marina* can range from +1.4 m to -12.4 m in relation to the mean lower low water tide (Thom et al., 2014). The size and density of patches can have differing effects on community assemblages, with larger, contiguous beds capable of housing more permanent, residential species (Hensgen et al., 2014). Despite being smaller in size, fringe *Z. marina* beds play an important role in connecting habitats for migrating fishes like salmon; fringe habitat accounts for approximately 50% of *Z. marina* in Puget Sound while the other half is larger tidal flat sites (Christiaen et al., 2016).

Z. marina Decline in Puget Sound

Although a majority of seagrasses are in decline worldwide, Puget Sound’s seagrass population remains stable at the large scale (100s of kms), despite a shift in coverage at a smaller scale (10s of kms) (Shelton et al., 2016). This stability should not create the assumption that

there is no need for restoration, as small-scale changes in seagrass coverage could create unknown shifts in ecosystem productivity and sediment transport. Furthermore, evidence in contemporary monitoring shows a larger amount of *Z. marina* sites with declining populations than sites with increasing populations (Dowty et al., 2010).

Z. marina populations in Puget Sound are declining from factors common among all seagrass declining populations (Duarte, 2002; Walker et al., 2007); however, local loss of *Z. marina* coverage in Puget Sound is attributed to indirect changes in water quality including increased sediment and nutrient inputs into Puget Sound (Li et al., 2007; Orth et al., 2006) and limited light due to overhanging structures (Rehr et al., 2014). Seagrass are photosynthetic organisms dependent on adequate amounts of light to survive (Thom et al., 2008), making overhanging structures detrimental for seagrasses. Nightingale & Simenstad's (2001b) review found the construction of overhanging structures like docks and ferry terminals can reduce the amount of light in the water column to a point that effectively kills off *Z. marina*. In addition, direct disturbances include dredging (Nightingale & Simenstad, 2001a), and aquaculture reduce *Z. marina* populations in Puget Sound. Seagrass restoration in Puget Sound is in direct contact with the shellfish aquaculture.

The shellfish industry brought in over 50 million dollars in South Puget Sound alone (Washington Sea Grant, 2015); however, the practices of shellfish growing/harvest can have negative effects on seagrass beds. Dumbauld et al. (2009) provides a review of shellfish aquaculture's effects on *Z. marina* including boat anchor scars, dredging and filling. Their review also cites the negative effects of hard clam harvest methods like "clam kicking" and hand digging have on eelgrass; furthermore, his review shows clam harvest with shovels was found to reduce eelgrass coverage and biomass in the short term. However, a recent study of oyster

aquaculture effects on eelgrass in Willapa Bay showed no long-term negative effects on eelgrass coverage, and could potentially enhance their presences on larger scales (Dumbauld & McCoy, 2015).

Population growth and associated development in the Puget Sound region is another factor that puts *Z. marina* at risk of decline. Coastal populations are predicted to increase throughout Puget Sound, with projected increases in the counties surrounding South Puget Sound (Thurston, Pierce, and Mason counties) ranging from 34% to 52% between 2000 and 2025 (Puget Sound Action Team, 2007). Increased populations can have negative effects on *Z. marina*, as the increase in coastal infrastructure due to booming populations have been cited as a critical threat to *Z. marina* (Grech et al., 2012). This increasing coastal population leads to an increase in coastal development, which can take the form of shoreline armoring. Puget Sound has approximately 27% of its coast armored, about 13 million feet (Thom et al., 2011). Shoreline armoring can change the sediment regime, starving beaches of sediments required to promote *Z. marina* colonization/growth (Simenstad et al., 2008). Poor seedling survival due to increased hydrogen sulfide levels (Dooley et al., 2013; Thom et al., 2011) and wasting disease (Groner et al., 2014; Short et al., 1987) have also reduced *Z. marina* populations in Puget Sound.

Climate change is predicted to increase *Z. marina* losses due to higher water levels reducing the amount of light able to penetrate the water column and reach seagrass blades (Stevens & Lacy, 2012). The predicted increase in oceanic temperatures due to climate change will also create additional stressors for seagrass, as *Z. marina* production is optimal in a narrow range of 5-8 degrees Celsius, with die off more apparent at temperatures 15 degrees Celsius or higher (Thom et al., 2001). However, changes in climate can potentially benefit *Z. marina*, as La Niña and El Niño events have already proven to drastically increase production of *Z. marina* in

Washington state (Thom et al., 2003); the same study found that climate-induced changes in sea-level can potentially affect *Z. marina* coverage. Rising sea levels can potentially open new areas to *Z. marina* colonization by inundating upper tidelands, where desiccation was once a limiting of a factor (Duarte, 2002). Links between changes in climate and *Z. marina* abundance have been observed in areas including Sequim bay during El Niño conditions, with *Z. marina* growth lower during colder years and growing faster at the beginning of the 1997 El Niño, one of the strongest in the 20th century (Puget Sound Action Team, 2007).

Z. marina Restoration

Efforts to restore global *Z. marina* populations have met mixed success. Common methods employed to restore *Z. marina* globally include conventional seagrass restoration methods like staples (Davis & Short, 1997; Park & Lee, 2007) and nutrient enrichment (Orth et al., 2006), as well as alternative methods like seed dispersal (Busch et al., 2010; Orth et al., 2006) and “Transplanting Eelgrass Remotely with Frame Systems,” known as TERFS (Short et al., 2002). Issues with success in restoration of *Z. marina* populations predominately focus on flaws in the methodology itself or stochastic environmental factors. Despite roadblocks with previous restoration attempts, there are efforts underway to restore *Z. marina* populations in South Puget Sound. The Washington DNR has a goal to increase eelgrass coverage by 20%, approximately 4,000 hectares, by 2020 (Thom et al., 2014). The 4,000-hectare restoration criteria is based on baseline *Z. marina* coverage documented in 2000 and 2008. A transplant suitability model was created in 2014 to establish specific locations in Puget Sound where large-scale restoration plantings would succeed. This suitability model was based on a combination of biophysical factors, historical presence of seagrass, and results from test plantings (Thom et al., 2014). It was concluded that Joemma Beach State Park (JSP) was a suitable site for large-scale

seagrass restoration. Currently, approximately 500 m² of *Z. marina* is present at JSP (J. Gaeckle, *pers. comm.*).

Fishes in Puget Sound

Puget Sound is home to a wide array of marine life. Phillips (1984) summarized the communities found in the Pacific Northwest's *Z. marina* into four categories: 1) permanent residents (syngnathids, gobies, blennies, etc.) 2) seasonal residents consisting of either juveniles (sea bass, rock fishes, greenlings, etc.) or residents during spawning seasons (squid, portunid crabs, some shrimps) 3) transient species (puffers) and 4) casual species (undefined). Puget Sound is also home to important migratory fishes including salmonid species like Chinook salmon (Rice et al., 2011; Rice et al., 2012) and Pacific herring (Penttila, 2007; Phillips, 1984). Information on fish stocks within Puget Sound are mixed, with forage fish stocks including sand lance and pacific herring documented throughout the Sound (Penttila, 2007) as well as salmon (Rice et al., 2012). Common fishes found in South Puget Sound (SPS) that use eelgrass during the summer include surf smelt (*Hypomesus pretiosus*), sand lance (*Ammodytes hexapterus*), Pacific herring, and juvenile salmonids including Chum (*Oncorhynchus keta*) and Chinook (*Oncorhynchus tshawytscha*) salmon (Rice et al., 2012). Each of these fishes utilizes eelgrass as a means of shelter, migration corridor, or nursery habitat during one or all of their life stages (Phillips, 1984).

Common practices of observing fish assemblages involve active data collection methods including seine netting and baited underwater video cameras. Seine nets are the preferred method of net-based seagrass fish assemblage collection (Guest et al., 2003), especially when waters are too turbid to use visual census or fishes sampled are small enough to pass through the seine net

mesh (Nagelkerken et al., 2001). Studies using underwater video collect continuous footage ranging from periods of 15 minutes to 90 minutes to examine fish assemblages, although there is no standard methodology for underwater video census. Baited cameras, although effective at attracting fish to the field of view of the camera, can potentially bias results by attracting scavenger fish to the experiment site that normally might not be present (Harvey et al., 2007; Murphy & Jenkins, 2010) and can potentially attract fish from greater distances, creating bias in fish assemblage data at the experiment site. Information on fish assemblages in South Puget Sound are sparse, with Rice et al. (2012) being one of the first studies to document pelagic fishes and jellyfish in Puget Sound. Their study found South Puget Sound dominated by jellyfish in the southern sampling sites (Main basin and South Sound) while pelagic fish i.e. salmonids, herring, flatfish, etc. were the dominant sample in the northern sampling sites (Whidbey and Rosario basins). Species richness in South Sound was found to be highest between June and July, although the species richness was much lower than richness during most months of sampling in the Rosario and Whidbey basins (Figure 3). An important note in this study is that although published in 2012, the sampling occurred in 2003, which indicates if it is one of the first studies to assess forage fish and jellyfish in Puget Sound, there is a decade plus gap in sampling. Differences in fish assemblages between night and day has also been observed for fish assemblages in eelgrass (Thedinga et al., 2011) and seagrasses globally (Kopp et al., 2007; Robertson, 1980; Unsworth et al., 2007). Abundance of fish can stay the same during day and night, but diversity of fishes can be found to alter depending on the time of day (Thedinga et al., 2011). Investigating differences in fish assemblages based on time of day can elucidate other important ecosystem services seagrasses provide marine life at different times of day. Fish assemblages in relation to *Z. marina* in parts of the greater pacific northwest such as California

(Garwood et al., 2013; Obaza et al., 2015), Oregon (Ferraro & Cole, 2010), and Alaska (Murphy, 2000) are recorded as well.

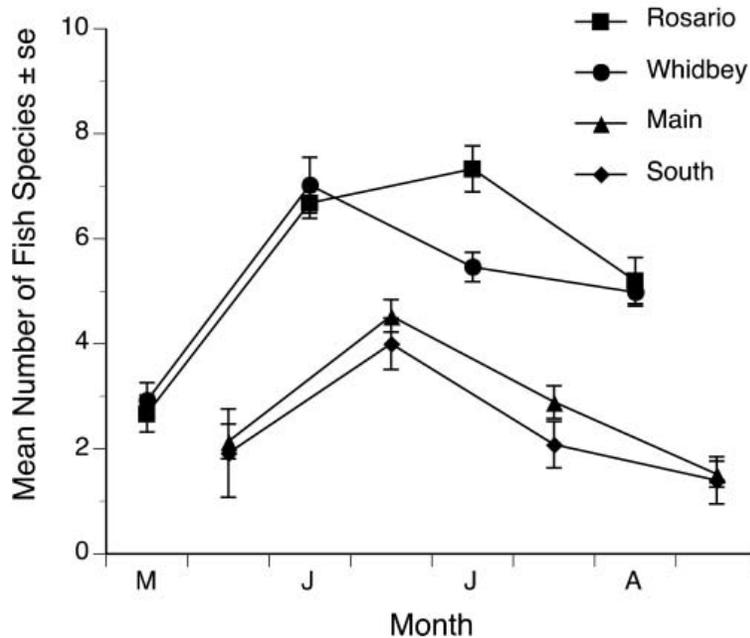


Figure 3. Mean (\pm SE) fish species richness per tow at four Puget Sound locations (Rice et al., 2012)

Assemblage Theory and Seagrass

Understanding why and how areas are colonized by species requires examining the theory behind assemblage formation and understanding the relationship between colonizing organisms and their environment. Two prominent assemblage-based theories are neutral theory, also known as universal neutral theory (UNT), and niche theory.

Neutral theory assumes all ecological communities of similar trophic levels are structured by stochastic events like ecological drift, random migration, and random speciation; organisms are identical in every other way i.e. they all have the same chances of reproduction and death

regardless of their species (Hubbell, 2001). MacArthur and Wilson's island biogeography theory is a type of neutral theory, according to Hubbell. His work was based on their island biogeography and species area relationship work (MacArthur & Wilson, 1963). Neutral theory is not universally accepted, sparking a widespread debate on the logistics of treating all organisms as equal in terms of reproductive success, which some argue is too simplified of a notion that cannot work at every spatial scale (McGill et al., 2006).

Niche theory is a group of theories that assert the importance of competition between species as a defining factor in species distribution (Bruno et al., 2003). Niche theory, according to Chase (2003), can be divided into two main components, the Grinnellian component, consisting of the requirements of a species for survival in an environment (Grinnell, 1917), and the Eltonian component, how a species affects its surrounding environment (Elton, 1927). Local processes like environmental filtering, biotic interactions, and interspecific tradeoffs are considered the determining factors in local assemblages (Chase & Myers, 2011). Niche theory makes multiple assumptions to work, as summarized by Weiher et al. (2011): 1) Functional traits are the currency of assemblages 2) species traits are fixed due to trait evolution being slower than community assembly, and speciation takes much longer than competitive exclusion. Aspects from both theories are important to structuring communities, as stochastic and deterministic forces both play parts in structuring communities at various scales (Chase & Myers, 2011).

Assemblage theory in relation to seagrass and the fauna that inhabit it can be used to examine issues of genetic resilience in relation to connectivity of beds in the wake of disturbances like climate change (Chust et al., 2013). Unlike animals, seagrass, a plant, is not capable of rapid expansion through movement; spreading genetic material involves pollination or clonal reproduction (Hemminga & Duarte, 2000). The lack of physical movement makes aquatic

plants reliant on ocean currents for connections to other macrophyte populations for reproduction or colonization (Filipe et al., 2011; Coleman et al., 2011). Connectivity between habitats, for both macrophytes and the fauna that inhabit them, is critical for promoting genetic diversity and resilience. This can prove problematic when looking at conservation of seagrass, as dispersal limitation makes sudden habitat loss potentially critical for seagrass populations (Chust et al., 2013). Incorporating assemblage theory into seagrass conservation can help shareholders examine how seagrass will respond to disturbances.

Using assemblage theory to investigate fish assemblages within seagrass is based on similar principles to seagrass assemblage theory (reliance on currents for some dispersal, importance of genetic resilience etc.), but fishes that inhabit seagrass are not always permanent residents. Only certain fishes spending the entirety of their lives within beds, while some fishes spend specific life stages in seagrass beds (Phillips, 1984). Stochastic forces like ocean currents are important for larval distribution of fishes (Cowen & Sponaugle, 2009), while patch size, edge effects, and proximity to other environments (seagrass, mangroves, mudflats, etc.) can affect competition and predation of fish assemblages in seagrasses (Figure 4).

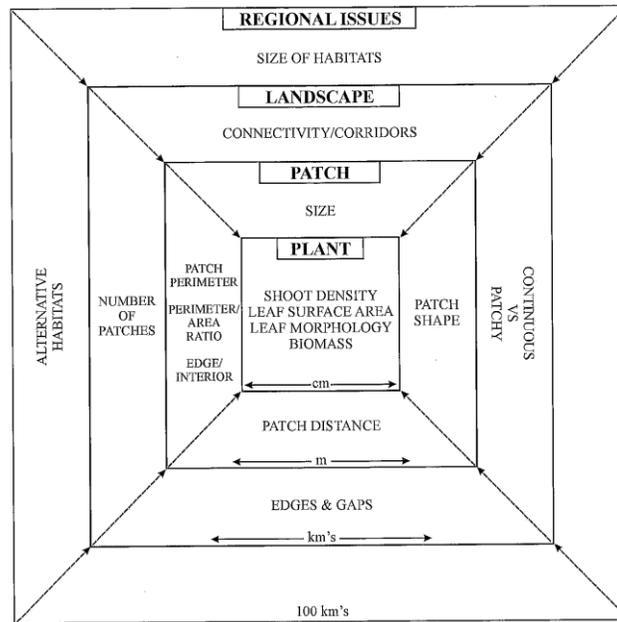


Figure 4. Model representing different predator/prey interactions at various scales in seagrass beds (Heck Jr. & Orth, 2007)

Seagrasses provide nearshore environments with a variety of ecosystem services that are essential to the health of global marine waters. They are capable of absorbing and holding onto large amounts of carbon (Duarte et al., 2013), help support nearshore sedimentation (Fonseca & Cahalan, 1992), and provide fishes with complex habitat that translates to food sources for both higher and lower trophic levels (Mumford Jr, 2007). Puget Sound’s resident species of seagrass, *Z. marina*, is a vital component of the nearshore ecosystem, providing Puget Sound’s important commercial fishes with critical habitat. Seagrasses are facing a global decline, and local *Z. marina* populations are not exempt. Restoration efforts of *Z. marina* are underway in Puget Sound, and one way to examine the effectiveness of restored sites is by examining fishes present. Fish assemblages are an indicator of how restored seagrasses are potentially being used, and the use of underwater video is a non-invasive way of examining what fishes are present in seagrass.

By looking at fish assemblages in seagrasses, stakeholders can assess if restoration efforts are providing nearshore ecosystem services. Restoration efforts of *Z. marina* at Joemma State Park are currently underway to assess if transplanted *Z. marina* is providing habitat functions for fishes in a manner like other parts of Puget Sound.

Methods

Study Area

Joemma State Park

Joemma State Park (JSP), located at southeast Key Peninsula, is a popular destination for camping, boating, and shellfish harvesting. A local shellfish farm uses a section of the tidelands for aquaculture. As of 2017, approximately 500 m² of *Z. marina* has been transplanted at Joemma State Park by the Washington Department of Natural Resources (DNR). The focus of this thesis is on two specific sites at JSP: 1) two rectangular strips of *Z. marina* transplanted in 2015 (Figure 5), referred to as transects three and four (T3 and T4) and 2) two bare sediment transects of similar proportion to T3 and T4. “Bare” is defined as an unvegetated tide flat.



Figure 5. Aerial image of Joemma State Park *Z. marina* restoration site. Green transects lines (not drawn to scale) labeled T3 and T4 represent approximate location of transplanted *Z. marina* transects three and four. Red transects lines (not drawn to scale) represent approximate location of bare sediment transects three and four (labeled BS3 and BS4). Red arrow indicates North (Image courtesy of Department of Ecology, 2016).

Dupont Warf

The city of Dupont is home to a large (300+ m²) naturally occurring *Z. marina* bed at Dupont Warf, located at the end of Sequalitchew creek (Figure 6). The continuous *Z. marina* bed is large enough where it is impossible to measure its full size on foot. Notable environmental features of the area include the freshwater input from the Sequalitchew creek (red circle in Figure 6) and human presence due to being a scenic destination at the end of the Sequalitchew creek hiking trail. The two locations of the fieldwork, JSP and Dupont, are 48 miles apart. The purpose of collecting data at Dupont's natural *Z. marina* bed was to provide summary information on fish diversity and abundance at a different location with a natural *Z. marina* bed. Dupont's information was studied in relation to JSP's data to assess any similarities or differences in fish assemblages between the two sites. Dupont and JSP's fish assemblages were compared per four-day tide series, as comparison of assemblages per day was not possible due to the inability to deploy cameras at the same time at each location (see below).



Figure 6. Aerial shot of Dupont Warf. Red circle indicates freshwater input from Sequelitchew creek. Green line represents approximate area of thesis fieldwork camera deployment. Red arrow indicates North (Image courtesy of Department of Ecology, 2016)

Grid Creation

Using a 100m transect tape, the dimensions of the two rectangular strips of the DNR's transplanted seagrass were measured (T3: 20 m x 1.75 m, T4: 25 m x 2.25 m). To establish the location for each camera along the edge of the transplanted and bare sediment sites at JSP, a grid was created with 9 cells (numbered 1-9), each representing a square 5 meters long (Figure 7). Each cell of the grid represented a potential location for one camera, to be placed at the midpoint of the length of the cell. Camera placement at a grid cell would be along the outer edge of the cell (blue line in Figure 7), facing towards the inner substrate, i.e. seagrass (green color in Figure 7) or bare sediment. Each cell was assigned a number (1-9) and, using www.random.org, the placement of each camera was randomized based on the number selected (the first 5 numbers chosen are the assigned locations for the 5 cameras at the site). An identical grid was used to

randomly assign camera deployment in a measured bare sediment area at JSP, referred to as BS3 and BS4. BS3 and BS4 were parallel strips (to imitate the shape of T3 and T4) and were 13.7 meters apart based on the top left corner of BS3 to the bottom left corner of BS4, which was also based on an approximate distance T3 and T4 were spaced apart. BS3 and BS4 were both west of T3 and T4.

The direction each camera faced (towards the shore or away from the shore) was randomized as well, assigned either a 1 or a 2 on random.org. A '1' represented placing the camera at the edge of the transect so that it faced the shore (West), while '2' represented placing the camera at the edge so it faces out towards the open water (East). Cameras were never deployed in the same cell to ensure duplication of footage did not occur.

At the natural seagrass site at Dupont, creating a transect of the natural *Z. marina* bed was not possible due to the large size of the bed. Therefore, each of the 5 GoPro cameras were placed 5 meters apart along one edge of the *Z. marina* bed. The screw anchors were left in the sediment at Dupont for the duration of the fieldwork to ensure camera deployment was in the same spots.

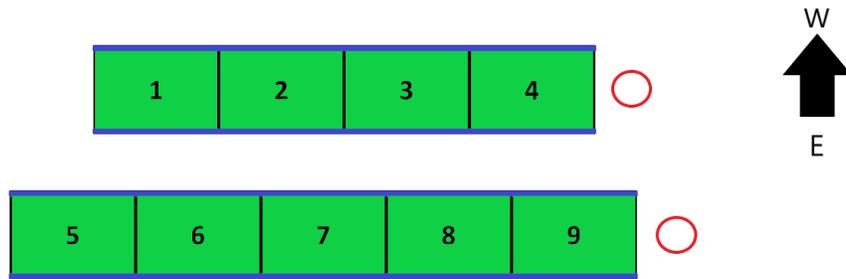


Figure 7. Example of T3 and T4 grid created to randomize camera placement. Red circle indicates screw anchor placement, where transect line was measured from. arrow indicates approximate cardinal direction.

Camera Setup

A GoPro Hero 3+ camera with a programmable time-lapse intervalometers (CamDo Solutions) in waterproof housing was used for each camera/PVC rig. Cameras were secured on top of a 55 cm long $\frac{3}{4}$ " PVC pipe. A 0.4 cm diameter hole was drilled 0.5 cm from the top portion of the PVC to allow the fastening of a black plastic zip tie through the PVC and underwater housing to secure the camera to the PVC (Appendix A.1, A.2). An angle grinder was used to remove a portion of the top of the PVC to allow the GoPro underwater housing and camera lens to be level (Appendix A.3). The bottom of the PVC was cut at an angle to fashion a needle-like point to allow insertion of the PVC into sediment to be easier (Figure 8). Due to large rocks in Dupont's sediment, the simple insertion of the PVC rig used at JSP was not feasible. Instead, each camera was attached to a garden screw anchor (see Figure 9) using three black

plastic zip ties to secure the camera in the sediment. The total length of the PVC was shorter (30 cm) to have the cameras at each location sit at the same height above the sediment (30 cm) without inserting the PVC directly into the rocky sediment. A yellow tag with the DNR's contact information was attached to each camera's PVC tubing by drilling a 0.4 cm diameter hole approximately 5.5 cm from the top. A black plastic zip tie was used to secure the tag to the PVC.

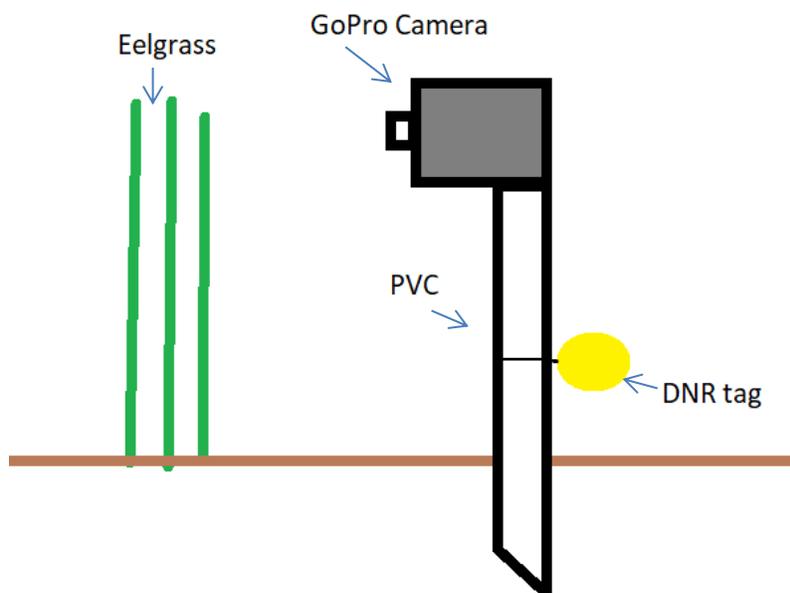


Figure 8. Camera setup for JSP at transplanted *Z. marina* site.

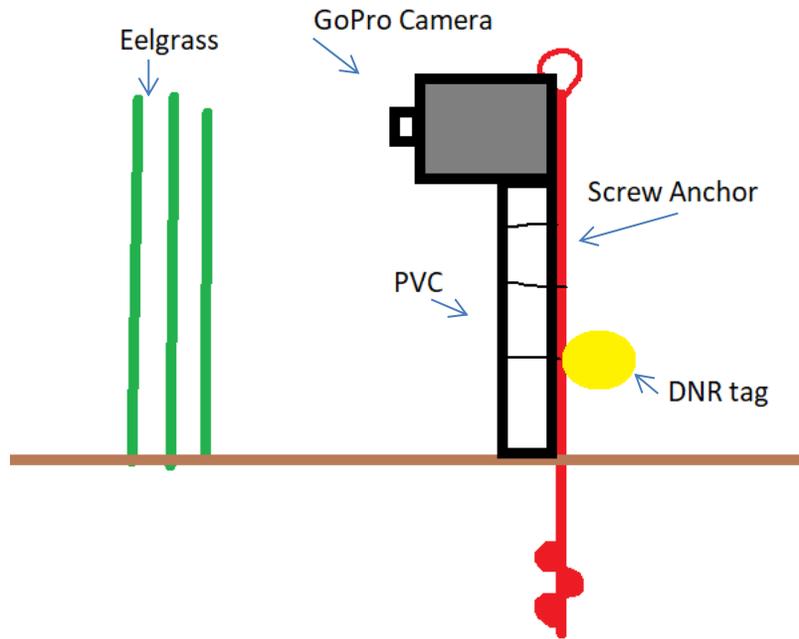


Figure 9. Diagram representing camera setup at Dupont natural *Z. marina* site.

Camera Deployment

A total of 15 GoPro Hero 3+ cameras, each installed with a CamDo™ intervalometer and encased in a waterproof housing were deployed. At JSP, five cameras were placed in the randomized grid at T3 and T4 while 5 cameras were placed in the randomized grid at BS3 and BS4. During each deployment, a 100m transect tape was secured to the screw anchor on the southern end of the *Z. marina* strip, and the tape was stretched across the strip to create the length of the grid on site. Camera placement was determined prior to deployment based on randomization.

The natural seagrass bed at Dupont was too far away from JSP to deploy cameras the same day as the JSP site (Figure 10), so cameras were deployed within two days of camera deployment at JSP to collect data during the same tide series.

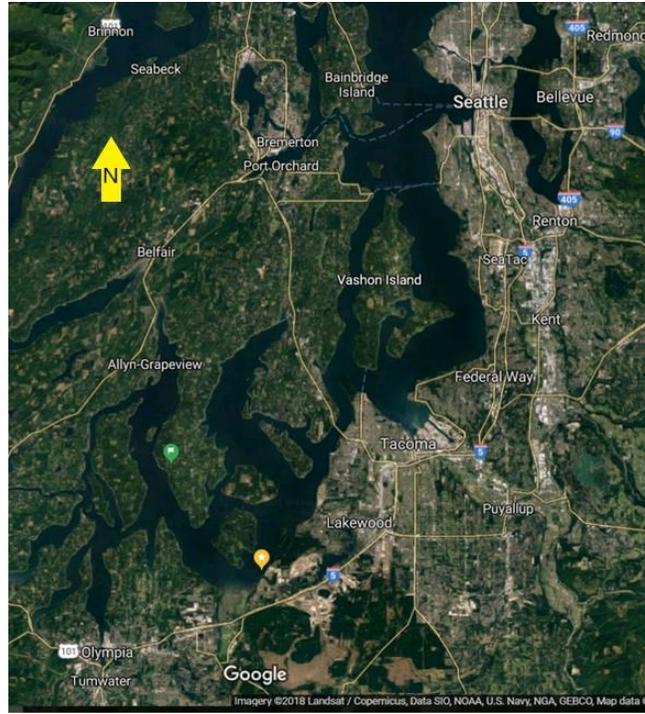


Figure 10. Location of both study sites in Puget Sound Green marker indicates JSP, yellow marker indicates Dupont (Image courtesy of Google Maps, 2018).

The five cameras at Dupont were secured to the screw anchors using three black plastic zip ties securing the PVC around the stem of the screw anchor. Due to the rocky sediment, the camera's PVC was unable to penetrate the rock; instead, the bottom of the PVC was placed on the sediment surface. Camera placement was not randomized like at JSP, instead, the 5 screw anchors remained at the location to ensure repetition with each camera deployment. The lack of randomization was due to a lack of enough edge/shallow enough *Z. marina* edge to warrant randomizing the deployment of the cameras every time.

Each camera was placed 30 cm away from the edge of the bed at all three sites, with the lens of the camera facing towards the bed. 30 cm was chosen as the ideal distance from the *Z. marina* bed to view a majority of the bed (within the cameras viewpoint) and minimize wasted

recording of bare sediment areas outside of transects. At both sites, the PVC was inserted 25 cm deep at JSP, putting the camera approximately 30 cm above the sediment. Camera deployment occurred during low tide. At the time of deployment, the time of each camera's deployment was recorded, as well as weather and temperature.

Measuring visibility

Visibility was measured after camera deployment. A printed/laminated secchi disk (35 cm diameter) was held at camera height (30 cm above sediment) and moved away from a Go-Pro Hero 3+ camera installed with a CamDo™ intervalometer (not one of the cameras used during deployments) at 0.5-meter increments until the 4 or 5-meter mark. The intervalometer was programmed so it would not activate, letting the camera record continuous video footage. Distance away from the camera was determined by holding a measuring tape and walking away from the camera holding the secchi disk at the approximate height of the camera. The location for determining visibility was at a bare sediment location at least 30 meters away from deployed cameras.

Camera Programming

A 2-minute video was recorded every 10 minutes on each camera once cameras were deployed in the field. The Cam-Do™ intervalometer attached to the back of each Go-Pro camera was programmed to turn on the camera every 10 minutes, while a coding script on the SD memory card was programmed to auto-record the first 2 minutes of video once the camera was turned on via the intervalometer. 2-minute clips were recorded for 24 hours after deployment.

Camera Retrieval

Cameras were retrieved approximately 24 hours after deployment during low tide. At the time of retrieval, average canopy height of the square meter approximately one meter away from the camera measured at each retrieval. Average canopy height was measured by averaging the tallest blade (leaf) measurement of three different seagrass shoots and taking 80% of that value per Washington DNR protocol (J. Gaeckle, *pers. comm.*). After average canopy height was measured at each camera's deployment location, the camera was removed. Zip ties fastening the cameras to the PVC were cut to access the camera's memory cards. Cameras (in underwater housing) and PVC were rinsed with fresh water after retrieval to prevent salt water damage.

Data Analysis

Footage after nightfall was discarded due to lack of visibility. In addition, clips became increasingly shorter as time went on due to reduced battery life but clips shorter than 30 seconds were discarded. Clips with 1/4th of the screen obstructed by sea lettuce (*Ulva lactuca*) for 20 or more seconds were discarded. Every fish present within the field of observation was identified to species (if possible) or to one of four groupings (flatfishes, forage fishes, gunnels, and salmonids). The number of fish that enter the field of view was counted, with fish that left the field of view and returned within 10 seconds being considered the same fish, and not recounted.

The dependent variables measured were fish abundance, fish diversity, vertical location of fishes (pelagic, above seagrass, within seagrass, benthic), and foraging behavior based on buccal expansion and/or lunging (yes/no). This thesis presented results based on fish abundance and diversity, but not on vertical location or foraging behavior. Independent variables measured

at each deployment included canopy height, tidal current (rising, high, falling, or low), and weather at time of deployment (cloud coverage, temperature), and major environmental issues (large presence of algae, etc.).

Fish groupings were created based on ability to identify down to species. If species identification was not possible, fish were identified to groups based on body type (flatfishes, salmonids, etc). Fish count data was converted into count/hour data to reduce the number of zeros. Count/hour data was analyzed using a Kruskal-Wallis test with a Dunn's test for pairwise differences to determine abundance associations based on site type. Count data was also analyzed for abundance in relation to time of day. Graphs showing abundance in relation to time of day were selected based on data having a majority of non-zero values (Appendix B). Fish species/groups that were not recorded at a site during majority of the research period (example: spiny dogfish abundance at JSP was zero for entire research period) are not presented. Graphs were evaluated qualitatively for patterns relating abundance to time of day or tidal period. Serial correlation between time and abundance was examined by calculating a correlogram in R statistical software (R Core Team 2018). Shiner perch was the only fish group examined using the serial correlation because the it had the largest amount of count data of all the fish groupings.

Results

A total of 78,963 fish were counted over a total of 2,276 clips. 705 clips were from the natural site, 753 from bare sediment, and 818 from the transplanted site. The majority of the clips (92.3%) lasted two minutes, only 176 (7.7%) of all clips had a recording time of less than two minutes. 12 fish species or groups were recorded that included most of fishes seen between all three sites. Eight species could be reliably identified (shiner perch, pacific snake prickleback, pacific staghorn sculpin, bay pipefish, tube-snout, pile perch, striped surfperch, and spiny dogfish, see Table 1 for scientific names) while an additional four groups were identified by their body shape (flatfishes, gunnels, forage fishes, and salmonids). Shiner perch were the most abundance fish across all three sites, while bay pipefish and tube-snout were the least common species seen between all three sites (Table 1). Of the 12 species or groups, only spiny dogfish and striped surfperch were not viewed in any clips at JSP (transplanted and bare sediment sites); all 12 species or groups were viewed at the natural site.

Table 1. Median Fish Count/Hour for each Fish Species or Group

Fish	Bare Sediment	Transplanted	Natural
<i>Fish Identified to Species</i>			
Shiner Perch (<i>Cymatogaster aggregata</i>)	302.17	278.51	1632.75
Pacific Snake Prickleback (<i>Lumpenus sagitta</i>)	2.83	0.64	0.25
Pacific Staghorn Sculpin (<i>Leptocottus armatus</i>)	0.45	0.73	1.25
Bay Pipefish (<i>Syngnathus leptorhynchus</i>)*	0.00	1.00	0.00
Tube-snout (<i>Aulorhynchus flavidus</i>)*	0.00	0.00	2.25
Pile Perch (<i>Rhacochilus vacca</i>)	0.22	0.22	2.25
Striped Surfperch (<i>Embiotoca lateralis</i>)	0.00	0.00	2.39
Spiny Dogfish (<i>Squalus acanthias</i>)	0.00	0.00	0.32
<i>Fish Identified to Body Type</i>			
Forage Fishes	14.13	6.85	207.27
Flatfishes	18.7	4.16	2.89
Salmonids	1.19	0.43	5.96
Gunnels	0.23	0.14	4.81

* For the bay pipefish and tube-snout, a zero median count/hour does not mean a complete absence of that species in that site. There was one tube-snout observed at the transplanted site and one at the bare sediment site, and one bay pipefish observed at the natural and bare sediment sites.

Abundance Based on Site Type

Natural Z. marina

Five fish species or groups (shiner perch, gunnels, tube-snout, striped surfperch, and spiny dogfish) had varying abundance across the three sites (Figures 11-15). Abundance of shiner perch and gunnels was higher in natural than transplanted *Z. marina* (Shiner perch: Kruskal-Wallis $\chi^2_{(2)} = 6.74$, $P = 0.034$, Dunn test $Z = -2.33$, $P = 0.059$. Gunnels: Kruskal-Wallis $\chi^2_{(2)} = 6.72$, $P = 0.035$, Dunn test $Z = -2.51$, $P = 0.036$). Abundance of tube-snouts, striped surfperch and spiny dogfish was higher in the natural site compared to the bare sediment and transplanted sites (Tube-snout: Kruskal-Wallis $\chi^2_{(2)} = 10.19$, $P = 0.0061$, transplanted and natural site Dunn test $Z = -2.76$, $P = 0.017$, natural and bare sediment site Dunn test $Z = 2.61$, $P = 0.027$).

Striped surfperch: Kruskal-Wallis $\chi^2_{(2)} = 9.88$, $P = 0.0071$, transplanted and natural site Dunn test $Z = -2.63$, $P = 0.026$, natural and bare sediment Dunn test $Z = 2.63$, $P = 0.026$. Spiny dogfish: Kruskal-Wallis $\chi^2_{(2)} = 13.29$, $P = 0.0013$, transplanted and natural site Dunn test $Z = -3.07$, $P = 0.0064$, natural and bare sediment site Dunn test $Z = 3.07$, $P = 0.0064$).

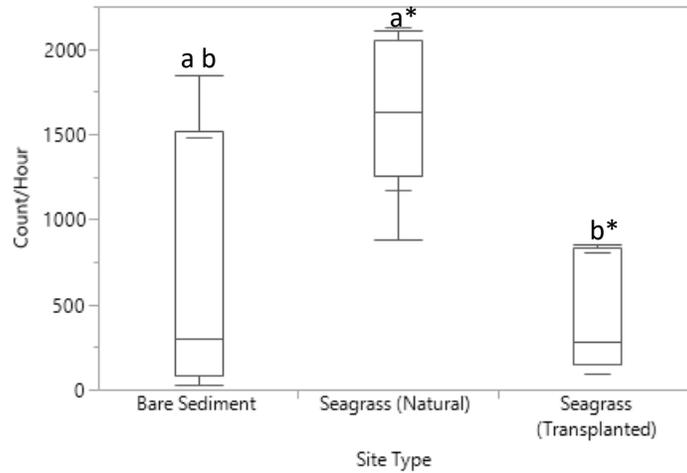


Figure 11. Shiner Perch abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

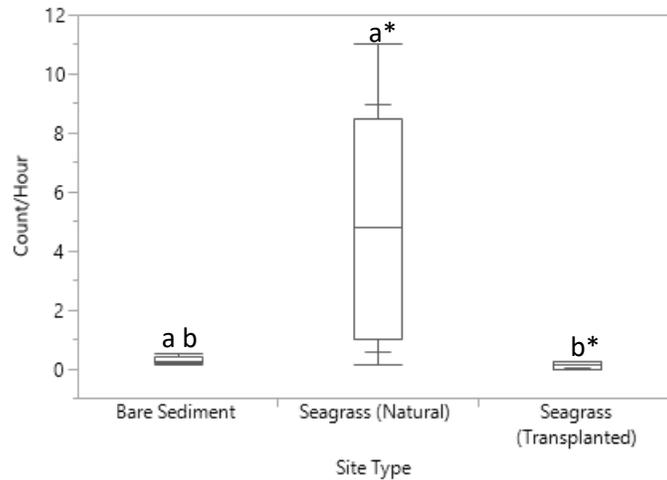


Figure 12. Gunnel abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

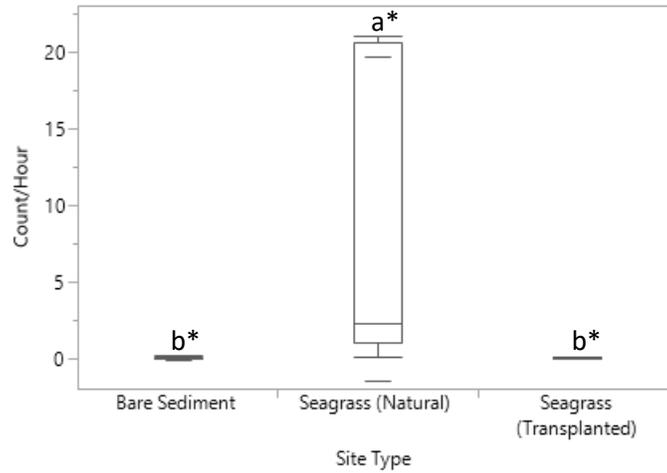


Figure 13. Tube-snout abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

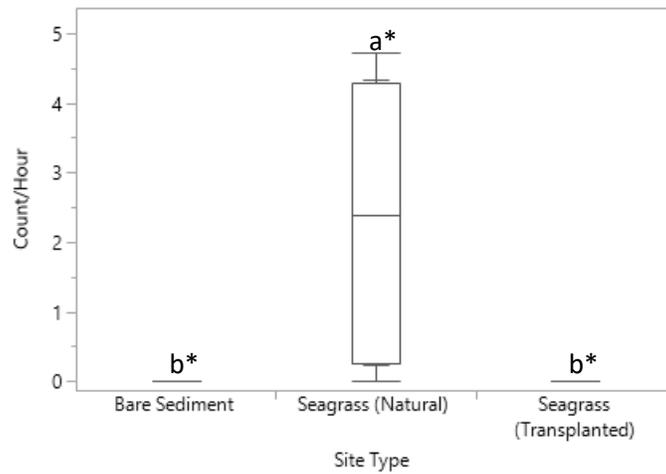


Figure 14. Striped surfperch abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

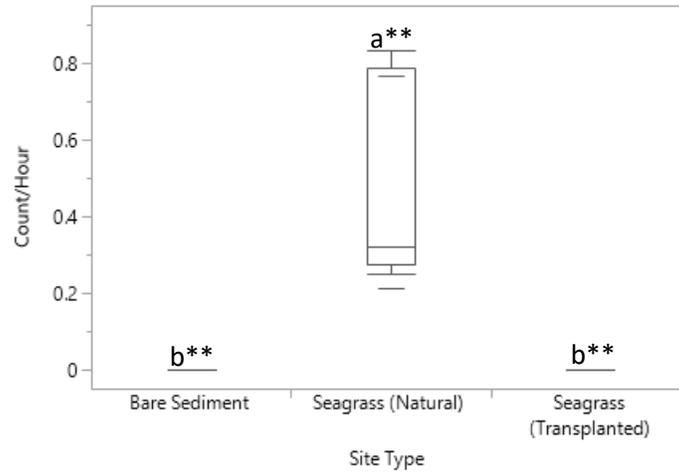


Figure 15. Spiny dogfish abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $**P < 0.05$, or $0.05 < P < 0.10$.

Transplanted Z. marina

The abundance of bay pipefishes varied across the three sites (Figure 16, Kruskal-Wallis $\chi^2_{(2)} = 8.42$, $P = 0.015$). The abundance of bay pipefishes was higher in transplanted seagrass compared to the natural seagrass (Dunn test $Z = 2.68$, $P = 0.022$).

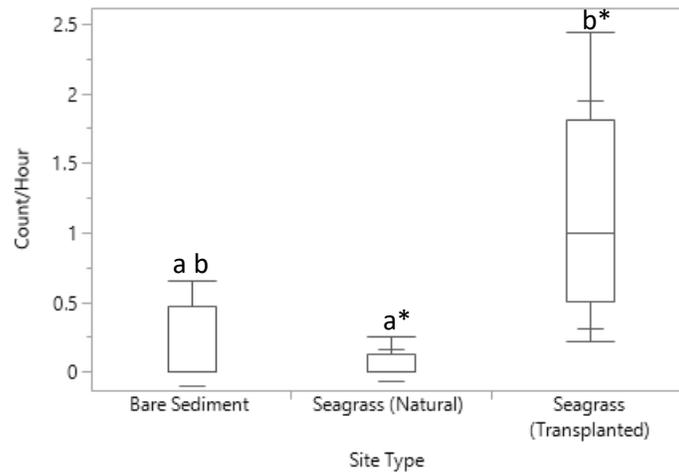


Figure 16. Bay pipefish abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

Bare Sediment

The abundance of flatfishes varied across the three sites (Figure 17, Kruskal-Wallis $\chi^2_{(2)} = 10.22$, $P = 0.0060$). The abundance of flatfishes was lower in natural seagrass compared to the bare sediment (Dunn test $Z = -3.04$, $P = 0.0071$).

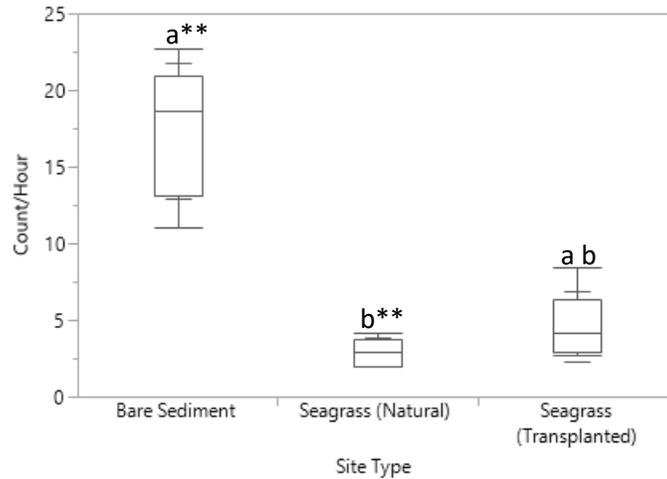


Figure 17. Flatfish abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $**P < 0.05$, or $0.05 < P < 0.10$.

No Observable Relationship

Five fish species or groups (pacific snake prickleback, forage fishes, pacific staghorn sculpin, pile perch, and salmonids) had no variation across the three sites. These five groups (Figures 18-22) also had no pairwise differences in abundances between the three sites (Pacific Snake Prickleback Kruskal-Wallis $\chi^2_{(2)} = 4.94$, $P = 0.085$. Forage fish Kruskal-Wallis $\chi^2_{(2)} = 2.39$, $P = 0.30$. Pacific Staghorn Sculpin Kruskal-Wallis $\chi^2_{(2)} = 2.66$, $P = 0.26$. Pile perch Kruskal-Wallis $\chi^2_{(2)} = 4.50$, $P = 0.11$. Salmonid Kruskal-Wallis $\chi^2_{(2)} = 3.39$, $P = 0.18$).

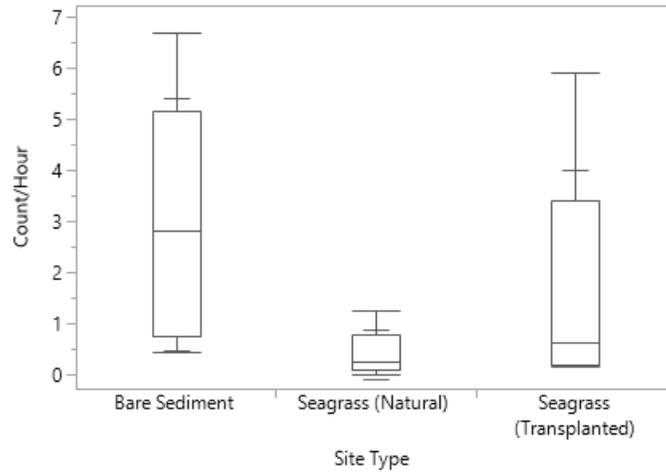


Figure 18. Pacific Snake Prickleback abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

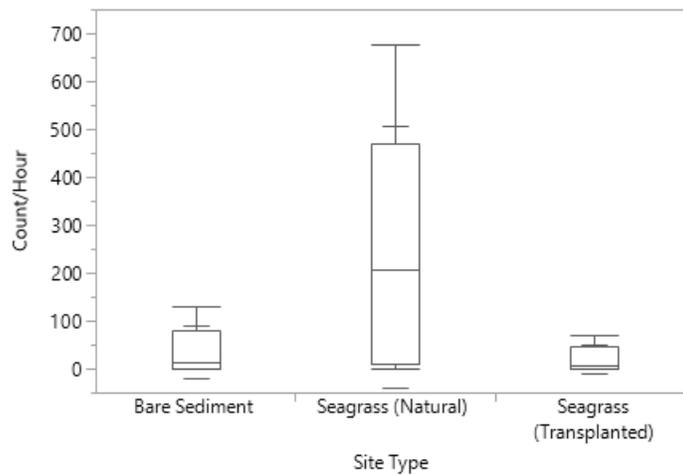


Figure 19. Forage fish abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

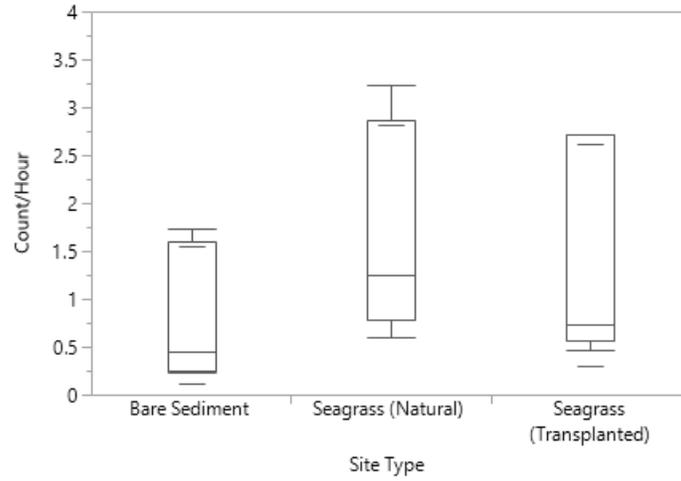


Figure 20. Pacific staghorn sculpin abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

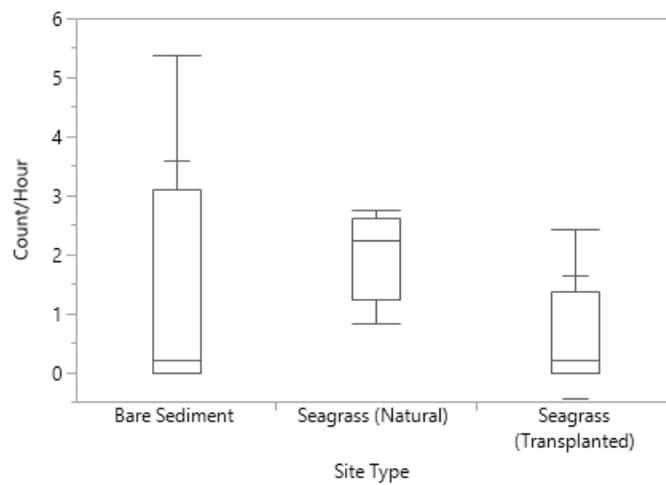


Figure 21. Pile perch abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

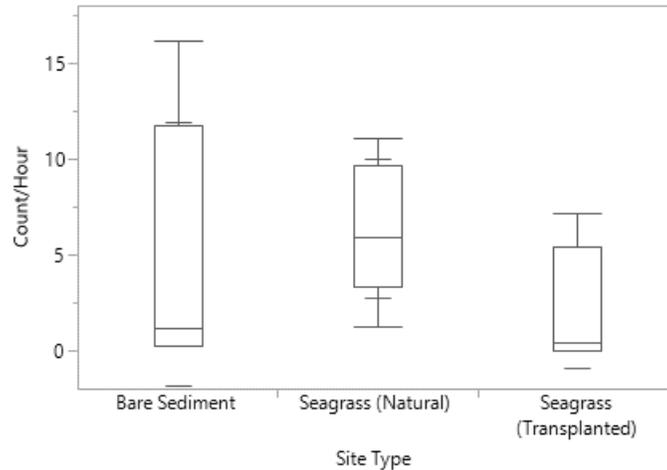


Figure 22. Salmonid abundance based on site type. Different letters indicate a significant difference based on Dunn's test at $*P < 0.05$, or $0.05 < P < 0.10$.

Abundance Based on Time of Day

The abundance of fish over the course of a single day did not have statistically significant autocorrelation for any species x day combination, with one exception (shiner perch on 7/22/2017 at the transplanted eelgrass site, see Appendix B). Therefore, the following summary should be taken as a qualitative description based on the plots alone (Appendix B). Nine fishes (shiner perch, flatfishes, pacific snake prickleback, forage fishes, gunnels, pipefish, pile perch, striped surfperch, spiny dogfish) at the natural site had a majority (3 or more of the 5 research days) of their peak count/efforts (abundance) between low and high tides, with a decline in abundance generally after the peak value; flatfishes at the bare sediment site also had a peak abundance between tides that generally decreased afterwards. Similarities between JSP and Dupont in terms of abundance in relation to time of day were not apparent. Peak abundance was earlier in the day (closer to low tide) for shiner perch (JSP transplanted), flatfish (both sites JSP), and tubenose (natural site) with a decline in abundance throughout the day. Peaks in abundance around specific hours of the day were apparent for a majority of pacific snake prickleback days

(close to 7 PM at natural site) and forage fishes days of observation (close to 6 or 7 PM at natural site). Other noticeable relationships between abundance peaks and time of day were an increasing abundance past the high tide period for flatfishes at the transplanted site; in addition, pacific staghorn sculpin had an early peak in abundance and then a decline throughout the observation period for most of the research days.

Discussion

Out of the 12 fish species or groups observed in this study, two species (striped surfperch and spiny dogfish) were not observed Joemma State Park (JSP) sites (bare sediment and transplanted *Z. marina*). All 12 fish species or groups were observed at the natural eelgrass site at Dupont Warf. Shiner perch was the most abundant fish observed throughout the study. Shiner perch abundance at the natural site was significantly greater than in transplanted seagrass. These results concur with previous studies that have shown a relationship between shiner perch and *Z. marina* when investigating fish assemblages in *Z. marina* (Obaza et al., 2015; Robinson & Yakimishyn, 2013). Gunnels, striped surfperch, and tube-snout had significantly higher abundances with the natural *Z. marina* when compared to transplanted *Z. marina*; bay pipefishes had a significantly higher abundance in transplanted *Z. marina* when compared with natural *Z. marina*. Each of these fishes have been observed to inhabit *Z. marina* and prefer said habitat to unvegetated areas elsewhere on the west coast of the United States (Hosack et al., 2006; Johnson et al., 2003; Johnson et al., 2010). Together these results may indicate a preference for vegetated habitat over unvegetated habitat.

Z. marina provides a source of refuge and nursery habitat for fishes (Bertelli & Unsworth, 2014; Lazzari, 2015), as well as a food source from the macroinvertebrates and other epifauna that inhabit *Z. marina* (Reum & Essington, 2008). The role of a nursery, refuge, and a location that prey inhabit are all potential explanations for the association of these fishes with the *Z. marina* recorded during this study. Preference for natural or transplanted *Z. marina* in this study compared to bare sediment could be dictated by food availability (Macreadie et al., 2010), edge effects, predation, or patch scale variables (size of *Z. marina* patch, closeness to other environments i.e. other *Z. marina* patches, algae, bare sediment (see Gillanders, 2007 for

review), all of which have been shown to influence fish abundance and diversity within seagrass beds. Fish assemblages have also been observed to be influenced by seagrass shoot density and blade length (McCloskey & Unsworth, 2015), as well as predation that can change based on the scale of observation (Heck Jr. & Orth, 2007). Macroinvertebrate abundance in relation to seagrass can also be influenced by shoot density (Attrill et al., 2000) and blade surface area (De Troch et al., 2005), in addition to patch dynamics and edge effects (Dorenbosch et al., 2007; Hensgen et al., 2014). A mix of these factors could be influencing the associations of the 12 fish species/groupings between the three site types, but this study was not designed to assess which factors specifically affected fish assemblages.

Flatfishes were found in greater abundance at the bare sediment site when compared to the natural eelgrass site. Flatfishes are benthic fishes, predominately resting on the sediment surface. Flatfishes are also cryptic, making use of their flat body shape and crypsis to hide on the sediment surface, often burying themselves within the sediment to avoid predation (Ryer et al., 2008; Ryer et al., 2004). Observing fewer flatfish in *Z. marina* compared to bare sediment could be due to the flatfishes' commonly being found in sandy and muddy substrate, which it uses for camouflage and protection (Gibson et al., 2014). However, juvenile flatfishes have been observed to prefer vegetation like *Z. marina* as habitat, likely to avoid predation by hiding in the vegetation (Murphy, 2000). Flatfishes in the Pacific Northwest are known to have preferences for certain sand types and grain size (Moles & Norcross, 1995; Rooper et al., 2003), however, habitat structures like shells and sponges can also affect flatfish habitat preference (Ryer et al., 2004). Sediment preference of certain Pacific Northwest flatfishes, including *Lepidopsetta polyxystra* and *Platichthys stellatus* (the latter of which was identified as one of the flatfishes in the flatfish grouping) can shift during their first year of development (Moles & Norcross, 1995;

Ryer et al., 2004). Open sediment, i.e. bare sediment with no structural complexity, can benefit flatfishes by having higher foraging opportunity, but also increase predation risk (Ryer et al., 2004). The life stage of the flatfishes identified in this study was not determined, so it's difficult to know whether any differences in abundances observed were due to differences in their stage of development.

Salmonids did not have a significant association with any site type; however, salmonids were observed at all three sites. Salmonids were also the third most abundant fish out of the 12 groups and species. Although it has been shown that juvenile salmonids use seagrass for feeding and shelter (Simenstad et al., 1982), and salmonid association with *Z. marina* has been observed in other studies (Harris et al., 2008), there was no association between salmonid abundance and a site type in our study. Salmonids are highly mobile predator has been noted to use *Z. marina* beds. Juvenile chinook salmon travel through estuaries from April through July (Healey, 1982) while coho, sockeye, and some chinook travel through estuaries from April through June (Duffy et al., 2005). These months coincide with the time frame of our study, and evidence of salmonids traveling through *Z. marina* beds during recordings aligns with those months as well. The age of salmonids recorded on video was not possible, as they were moving too fast past cameras to assess potential age based on image alone.

Spiny dogfish were found to have higher abundance in natural *Z. marina* at Dupont Warf, in comparison to both transplanted seagrass and bare sediment at JSP. Spiny dogfish have been observed in Puget Sound during summer months (Reum & Essington, 2008), explaining their presence for the duration of this study. Spiny dogfish are a higher trophic level than the other fish groupings recorded, all of which can be considered lower mesopredators. Spiny dogfish feed on pacific herring (Harvey et al., 2012; Jones & Geen, 1977; Tanasichuk et al.,

1991), a fish that uses *Z. marina* beds for spawning habitat in Puget Sound (Phillips, 1984). In addition, they have been caught in/near *Z. marina* beds in other parts of Puget Sound, and are considered as a part of the *Z. marina* food web (Penaluna & Bodensteiner, 2015). Spiny dogfish's presence at the natural seagrass could have been dictated by a larger presence of its food source at that site. Although pacific herring was not one of the fish identified in the fish groupings, other forage fishes were observed in larger numbers at the natural site, indicating a possibility that pacific herring, a forage fish, would also have been in larger numbers at that site.

There were no significant associations between fish and site type for pacific snake pricklebacks, forage fishes, pacific staghorn sculpin, and pile perch. These fish are all mobile mesopredators, and the use of non-baited video cameras to record fish assemblages means it is inevitable that some fishes were not recorded, or those fish that were recorded were not representative of the entire population. Evidence of these fishes and their association with *Z. marina* for habitat has been observed in other studies (Johnson et al., 2003; Johnson et al., 2010; Murphy, 2000; Robinson & Yakimishyn, 2013), so the lack of evidence from this study should not be taken to mean that these fishes do not in fact have a significant association with *Z. marina*.

Fish assemblages in seagrass have been previously observed to change in abundance in relation to time of day. A change in tides changes the amount of water above seagrass beds: higher tides mean more room for fishes to move around seagrass beds, which can be beneficial for fishes feeding on pelagic invertebrates or predator fishes maneuvering through seagrass beds for prey. Lower tides can often leave seagrass exposed, as was the case for the first two sampling days in this research, preventing fishes from inhabiting seagrass beds. Lower tides also make it harder for larger predator fish to maneuver through seagrass beds, giving smaller prey fish a better chance at survival.

Changes in seagrass fish assemblages based on time of day can also be caused by fish migrations to/from seagrass beds from nearby habitats. Fishes have been observed to migrate from coral reefs and mangroves to/from seagrass beds at night, suggesting diel changes in assemblages based on nearby habitats (Kopp et al., 2007; Unsworth et al., 2007). The change in habitat selection could be due to differences in protection from predators (Thedinga et al., 2011) and/or differing food availability based on time of day (Unsworth et al., 2007). Fishes in tropical regions have been observed to migrate to seagrass beds to feed at specific times of the day based on prey availability (Robblee & Zieman, 1984; Unsworth et al., 2007). Diel changes in assemblages can also be caused by trophic dynamics, with the presence/absence of a few select species of fishes affecting the assemblages in seagrass (Thedinga et al., 2011). Migration can also take place within vertical water column above seagrass beds, traveling vertically based on food availability and protection from predators (Ruso & Bayle-Sempere, 2006).

The presence of a peak in abundance between low and high tide for mesopredator fishes (shiner perch, flatfishes, pacific snake prickleback, forage fishes, gunnels, pipefish, pile perch, and striped surfperch) could be dictated by food availability and/or protection from predators. Incoming and outgoing tides could affect the presence of pelagic zooplankton and small invertebrates that these mesopredators feed on. Incoming tides could bring more nekton and seston from deeper waters, increasing food availability which could cause increased abundance due to feeding. Differences in fish groups/species peaks based on time of day could be due to different peaks in food availability as well, as the diets of these mesopredators are not necessarily identical and could consist of invertebrates that have higher abundances based on different times of day. Another potential factor in mesopredator abundance could be the amount of water above the *Z. marina*. More water above *Z. marina* as the tide heightens means larger predator fishes

(spiny dogfish, salmonids, etc.) can move through the beds to hunt mesopredator fishes. The explanation for the rise to a peak mesopredator abundance then a decline afterwards could be the time of day trade-off mesopredator fishes make between foraging in *Z. marina* and being vulnerable to predation. Spiny dogfish peak abundance between tides could also be related to diel changes in prey abundance at the study sites, but peak abundance was scattered between low and high tide, making it difficult to ascertain if their abundance was in relation to peaks or declines in abundance for other fishes examined in this study. It is not possible to determine if nearby habitat affected fish assemblages at the study sites, as various sized patches of *Z. marina*, bare sediment, and drifting algae were present at each study site but were not examined in the scope of this research. In addition, diel changes in assemblages based on trophic could not be examined due to the inability to record at night.

Limitations of the Study Design

The lack of replication limits the ability to draw conclusions about differences in abundance across sites. Any differences cannot be directly attributed to the presence or absence of *Z. marina*, due to the lack of replicates in the research design. More replicates (sites) would create a more robust picture of fish assemblages in each site. However, due to limited resources and time this study was not able to examine multiple sites.

In addition, the lack of a bare sediment site at the natural eelgrass site (Dupont) to serve as a comparison was another design flaw. With only one bare sediment site at JSP, it is difficult to draw robust conclusions about fish assemblages between both JSP and Dupont's seagrass sites without a baseline bare sediment site to reference for Dupont. The bare sediment site at JSP had statistically significant differences in fish counts/hour for flatfishes, so having a bare sediment

site at the natural seagrass site would have been beneficial to compare the two bare sediment sites for flatfish significance. Adding a bare sediment site at the natural seagrass site would have also provided more information about fish assemblages present near said seagrass at Dupont.

In this study it was not possible to mimic the dimensions of the transplanted bed with the natural bed surveyed, which meant any differences in fish activity/presence could be due to size of the eelgrass bed alone. The large amount of seagrass edge habitat present at the transplanted site was not matched at the natural site section used for the study. It is well documented that fish have different uses of edge vs bed habitat, with edges providing different habitat for predator/prey interactions (Bologna, 2006; Bologna & Heck, 2002; Moore & Hovel, 2010). The position of a fish within a seagrass patch and the location of said seagrass patch in relation to other patches has been observed to affect predation, as predation at seagrass edges is greater than other locations in some seagrass beds (Smith et al., 2011). The larger amount of continuous *Z. marina* at the natural site could have potentially created different predator/prey interactions compared to the transplanted site, which could bias the assemblages.

Fish are influenced by the complexity of their environment, and it became apparent that there was a noticeably larger amount of *Ulva lactuca* and *Laminaria* was present at the natural site. Algae can provide additional habitat and food sources for fish and invertebrates (Adams et al., 2004; Gartner et al., 2013), especially when compared with habitat lacking any type of vegetation (Sogard & Able, 1991), which could potentially influence their presence in the area. Gunnels were often recorded hiding within *Ulva lactuca*, which could explain their higher abundance at the natural site compared to the other two sites. *Ulva lactuca* presence was observed at all three sites, but was seen more at the natural site, while *Laminaria* was only observed at the natural site.

The inability to deploy cameras at both JSP and Dupont on the same day makes it difficult to ascertain any conclusions about time-related fish assemblages. Although cameras often covered similar timeframes on different days, there are a myriad of possibilities that could occur in a 24-hour period that could affect which fish are recorded, including weather events, human presence, and other stochastic factors. Another issue with camera placement lies in the fact that it is possible for double counting to have occurred. It is possible that fish counted during one clip were present during the unrecorded 10-minute segments between recordings and counted again for the following clip at any of the cameras at each site. The lack of synchronization between cameras at all sites also means it is possible for fishes to have swum between cameras during recordings, causing them to be counted multiple times as separate fishes. Taking continuous footage between each camera was one way to solve the issue of double counting, but it would have resulted in less overall footage due to constraints with battery life.

Completing this study in an open environment meant dealing with uncontrollable environmental factors. Drifting algae was present at each site, often obstructing camera lenses. Loss of footage related to *Ulva lactuca* covering camera lenses caused large portions of certain days footage to be thrown out. Another issue with recording came from multiple cameras failing to record entire days' worth of clips in the first few deployments, likely an issue caused by human error in programming the intervalometers. Issues were also apparent with fish identification. Video quality was not fine enough to identify smaller species of fishes, or fishes over 1.5 to 2 meters away from camera. Color patterns of fishes, often used for identification, proved to be difficult to identify due to water coloration and camera grain (Figure 23). Recordings after 7:00 PM often magnified these difficulties, as image quality significantly deteriorated after sunset. Identification (to the species level) of small flatfishes, gunnels, and

salmonids proved to be the most difficult for these reasons. Smaller flatfishes and gunnels were too small for the cameras to identify markings, while the salmonids often moved past cameras too quickly to be able to identify. Cryptic fishes or fishes hiding within seagrass beds were also difficult to identify, as viewing within the *Z. marina* benthos was not possible due from the angle of the cameras. Cryptic species like flatfishes proved to be difficult to identify in the bare sediment site due to their nature to blend into the surrounding sediment. Despite this array of issues with fish identification, the 12 groupings of fish were based on fishes predominately seen at each site, with unidentified species being a minority of the fishes observed.



Figure 23. Example of difficulties when identifying fish species due to time of day and color of water.

Future Improvements

This thesis can be considered a pilot study in the use of non-invasive underwater video to examine fish assemblages. However, there is room to improve the methodology. The addition of a bare sediment site at the natural seagrass site would have made this study's results more robust, as comparisons could have been made between both *Z. marina* sites and both bare sediment sites.

In addition, it would have provided supplementary information on fishes present in Dupont's bare sediment. Fish assemblages could be quantitatively compared based on time of day between each of the sites, as some fish are known to have diurnal patterns of behavior (Michael B. Robblee & Zieman, 1984; Robertson, 1980; Thedinga et al., 2011). This data could help determine if South Puget Sound fish assemblages are using seagrass differently between different times of day. Temporal patterns in fish assemblages could also be investigated, as *Z. marina* fish assemblages undergo changes based on season (Garwood et al., 2013). Investigating each of these influences on fish assemblages would help create a better understanding of fish abundance and diversity in Puget Sound's *Z. marina* communities, which would contribute to the greater understanding of how *Z. marina*, both natural and transplanted, supports fish communities.

Conclusion

Fish diversity was comparable between natural and transplanted *Z. marina* in south Puget Sound, with only 2 of the 12 fish species or groupings not present in all three sites. Abundance of fishes was significantly associated with natural *Z. marina* (Dupont Warf) or transplanted *Z. marina* (JSP) for shiner perch, gunnels, pipefish, Tube-snout, Striped surfperch, and Spiny dogfish. The lack of significant association between the other fish groupings (Pacific snake prickleback, pacific staghorn sculpin, salmonids, forage fishes) could have been due to random chance or lack of replicates, as each of these fishes have been observed to have associations with *Z. marina* habitat as a means of refuge, food, or nursery. The significant association of flatfishes with bare sediment habitat was potentially due to increased foraging potential in bare sediment habitat as well as preference for specific types of sediment. Future efforts to examine fish assemblages using a passive method could consider these results as a pilot study to guide methodology for future research.

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Appendices

Appendix A. Camera/PVC rig setup: 1 & 2) GoPro underwater housing attachment to PVC. 3) Top portion of PVC modified with angle grinder for underwater housing to sit level on PVC



Appendix A.1

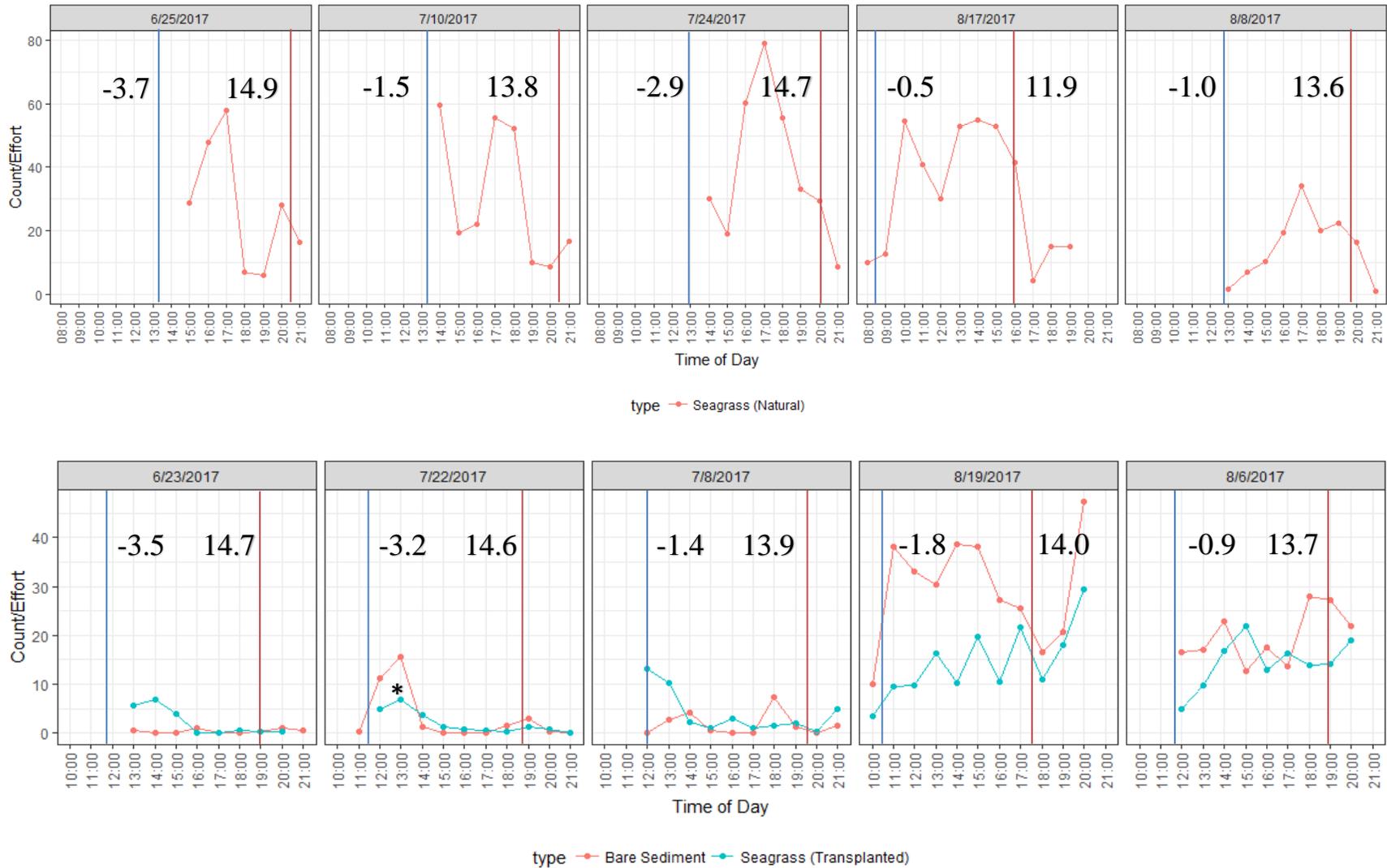


Appendix A.2

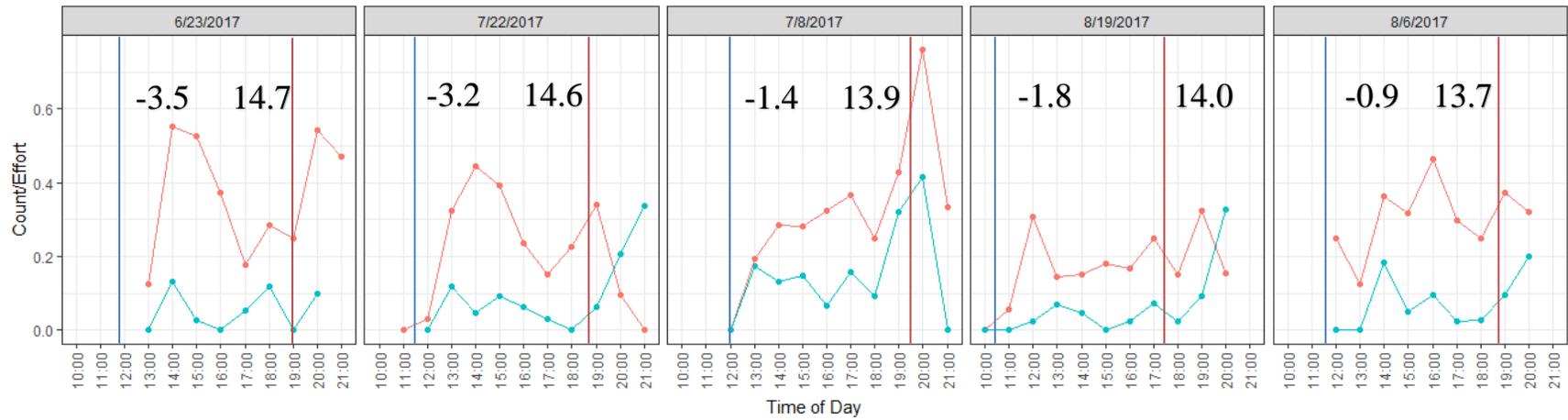
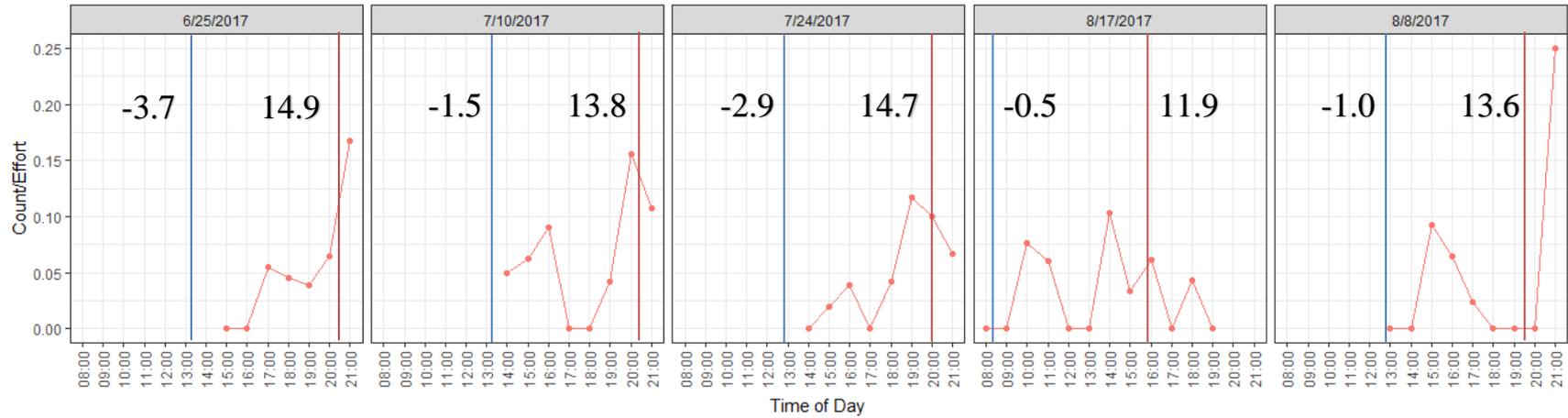


Appendix A.3

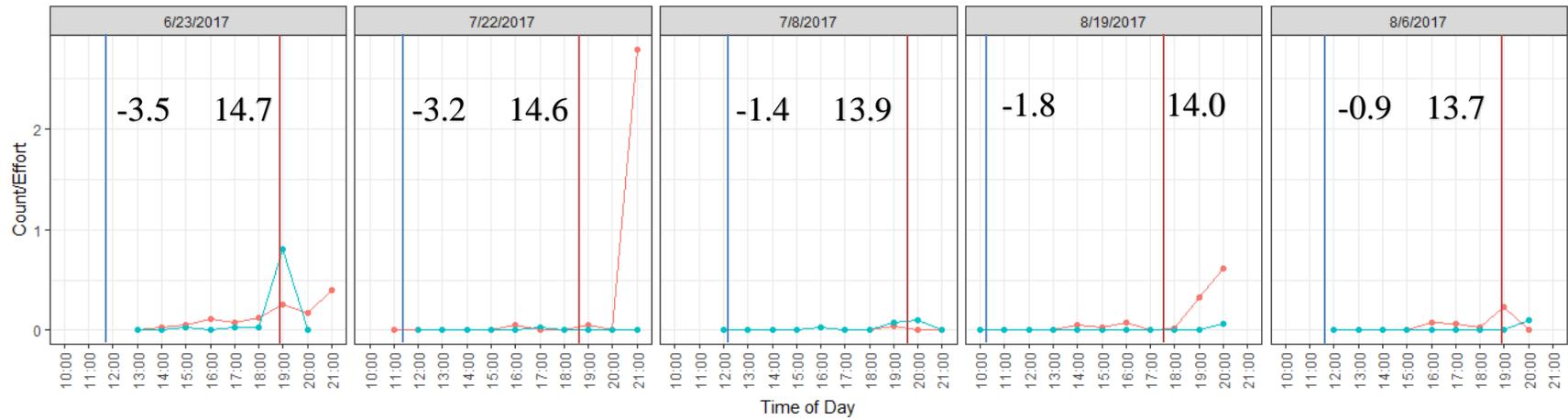
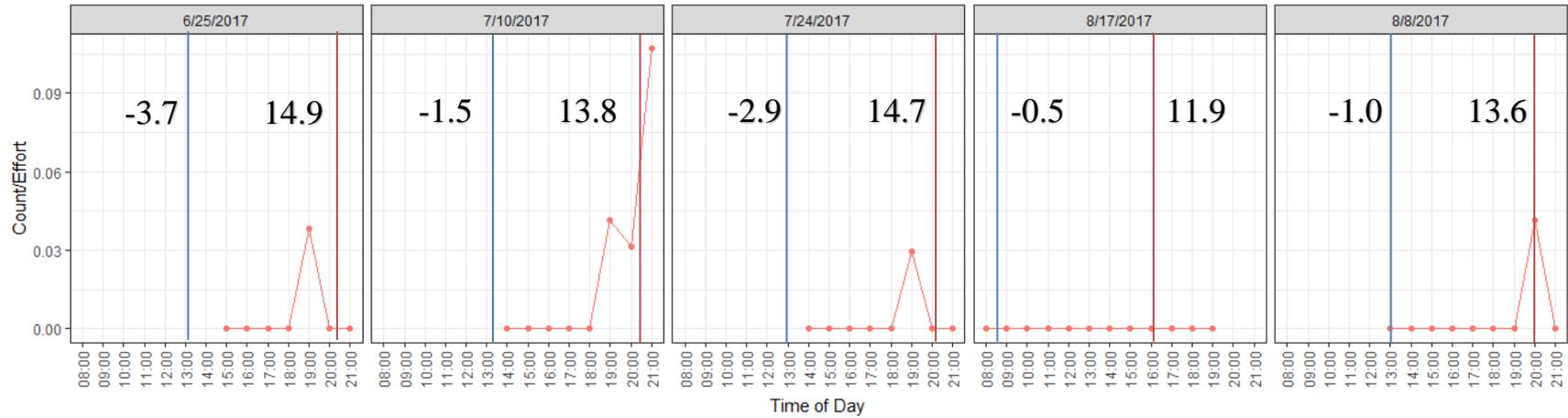
Appendix B. Fish abundance based on time of day. Blue line indicates low tide, red line indicates high tide. Tide values are in Feet. The asterisk on 7/22/2017 for shiner perch indicates significant autocorrelation at lag 1 (0.643), based on the correlogram.



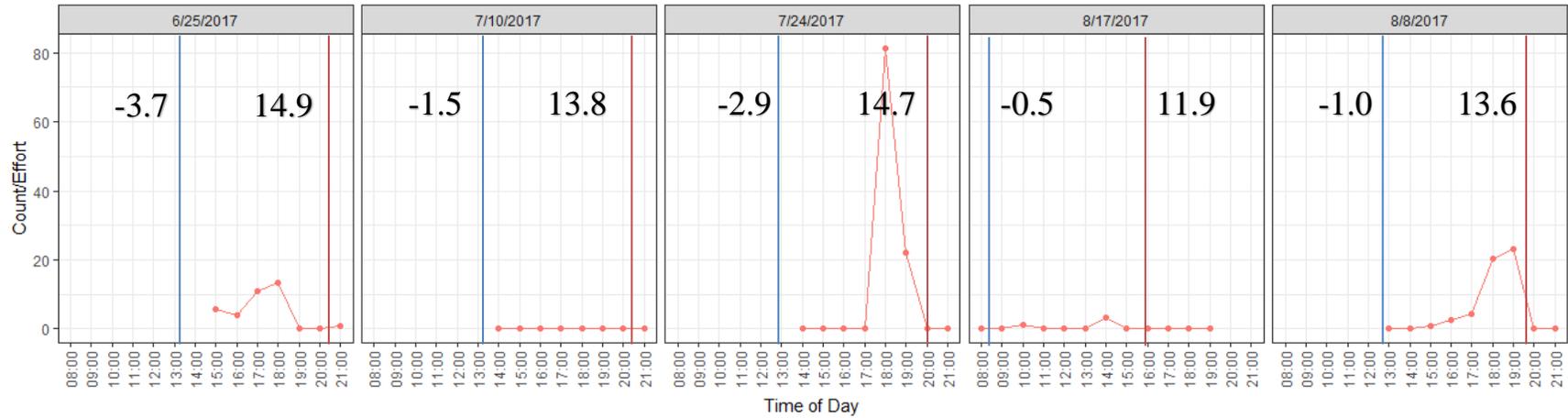
Flatfish



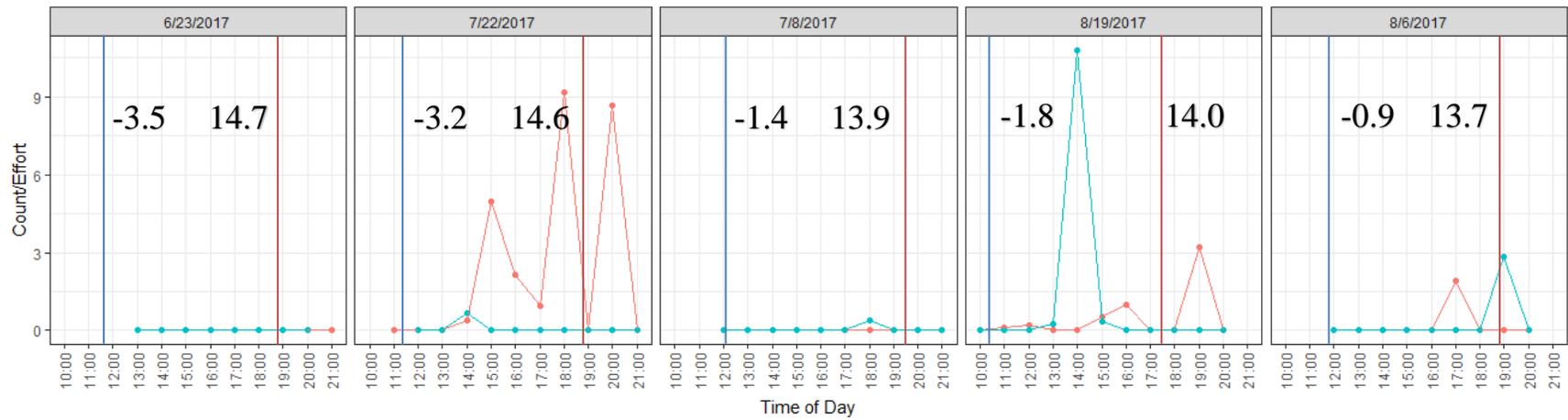
Pacific Snake Prickleback



Forage Fishes

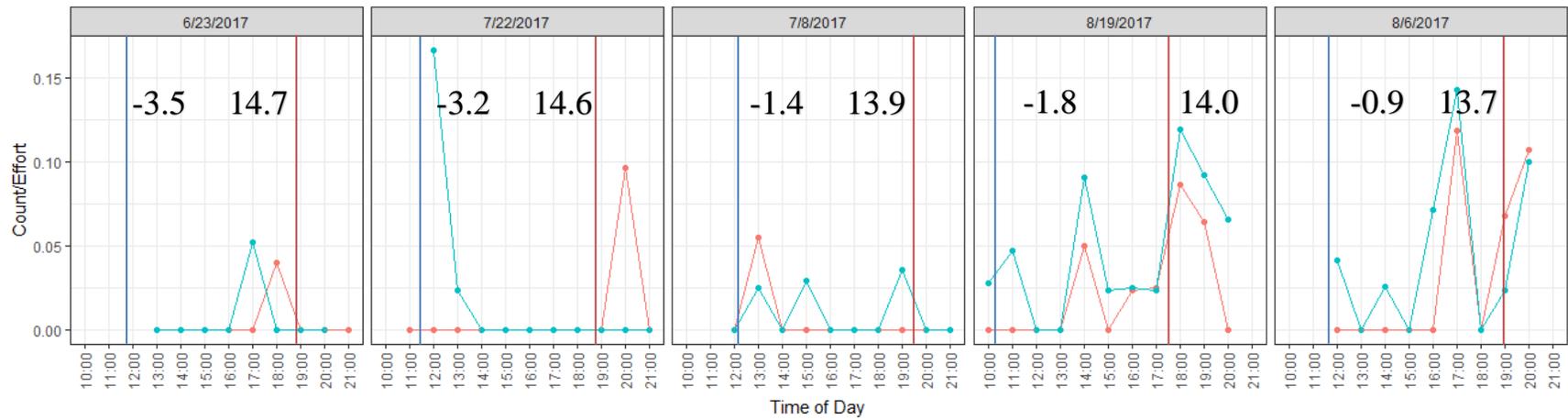
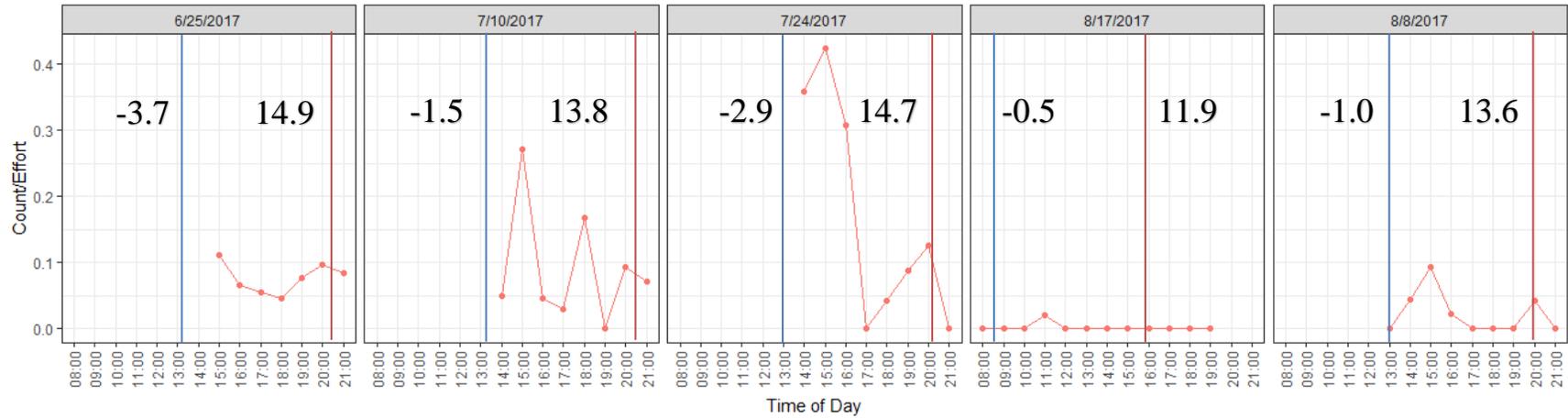


type — Seagrass (Natural)

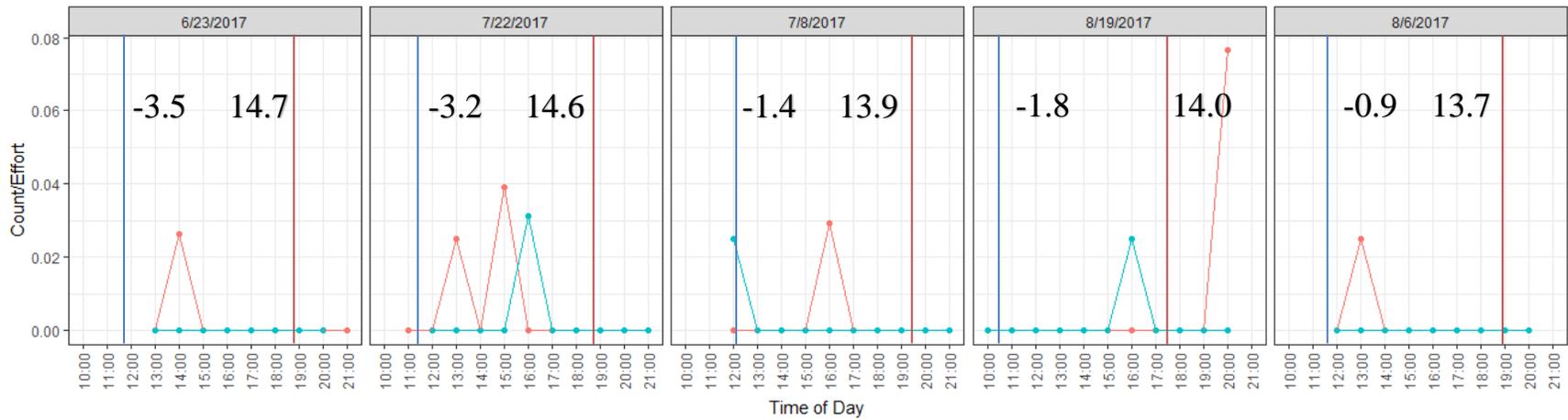
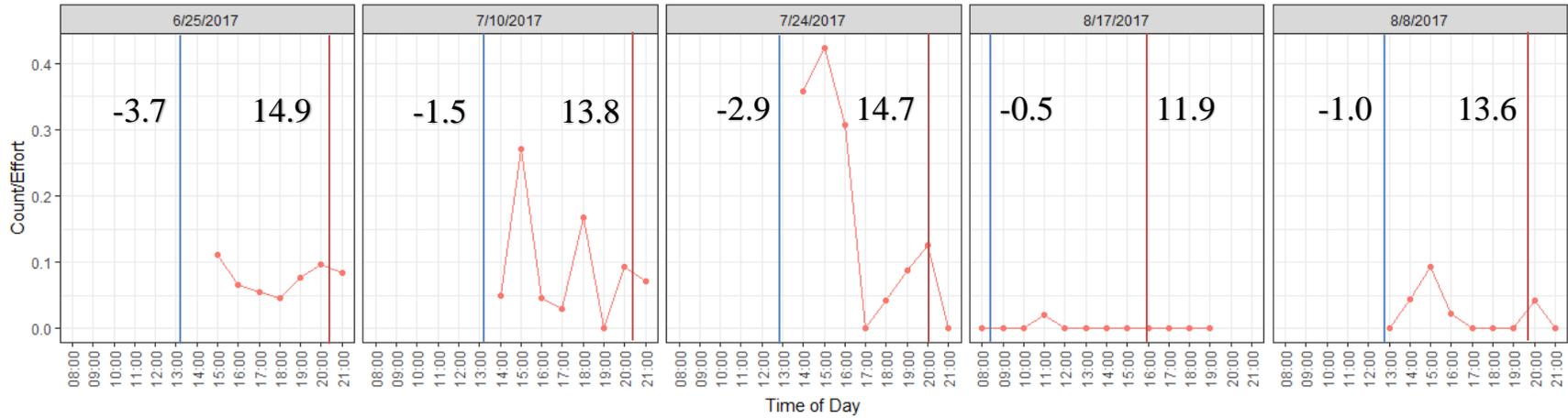


type — Bare Sediment — Seagrass (Transplanted)

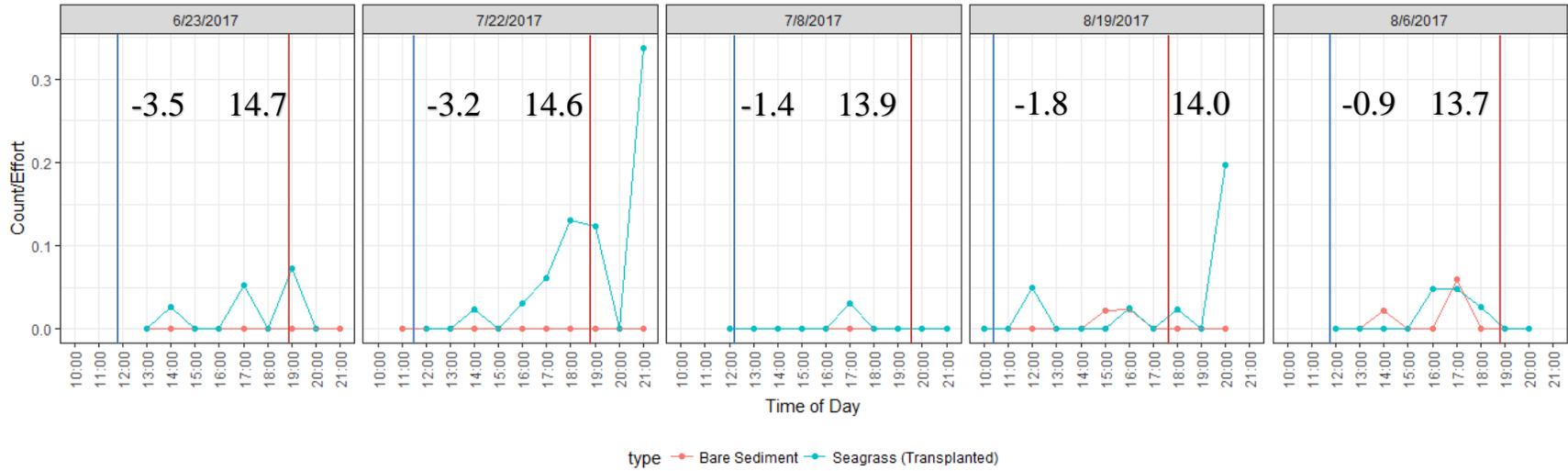
Pacific Staghorn Sculpin



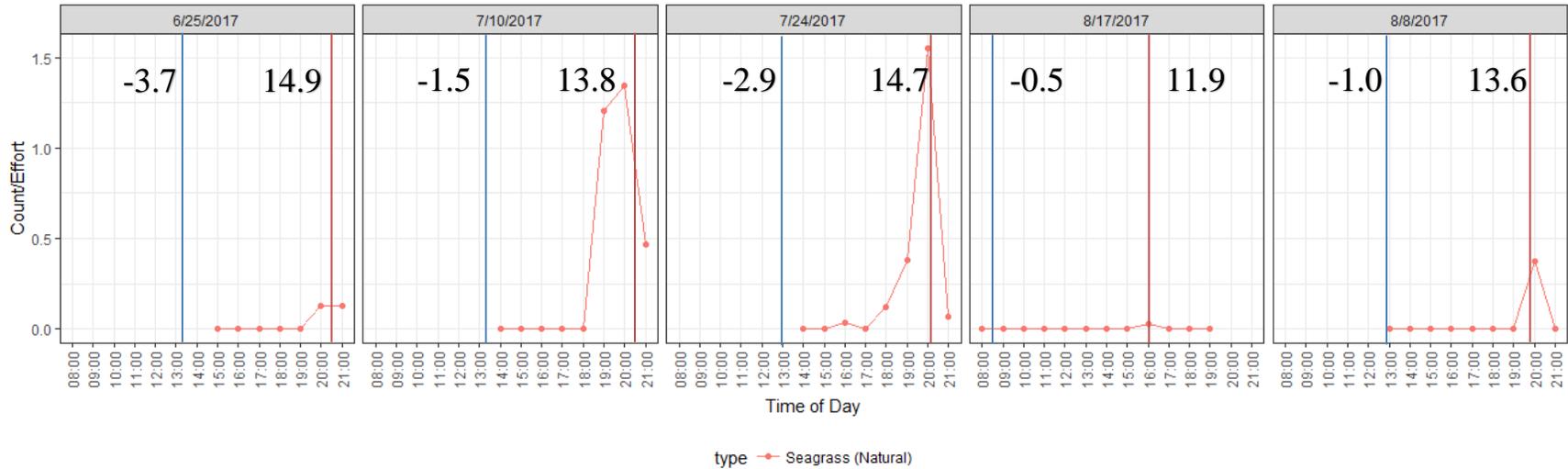
Gunnels



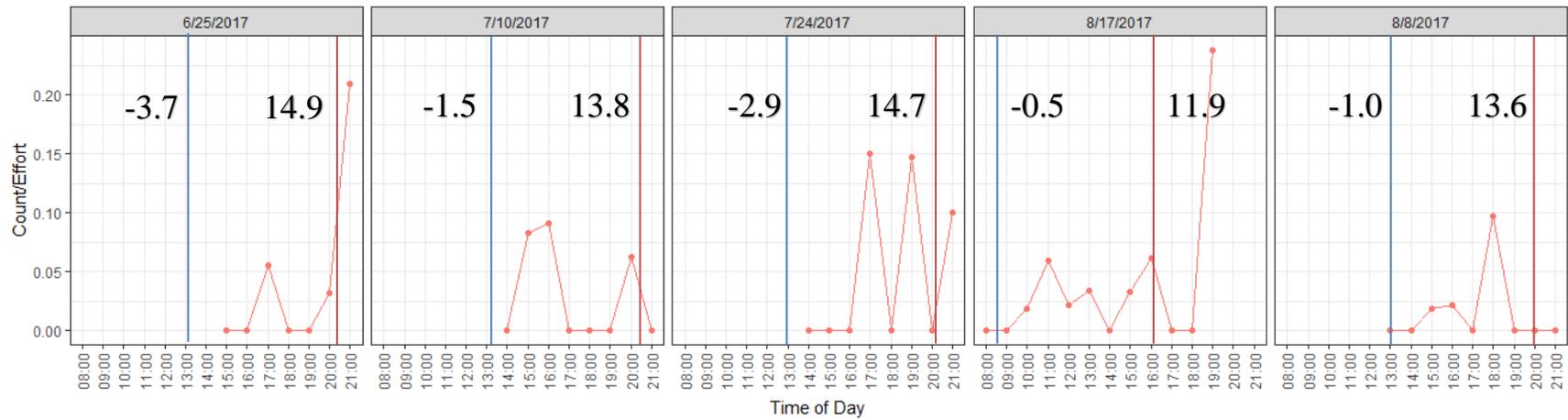
Pipefish



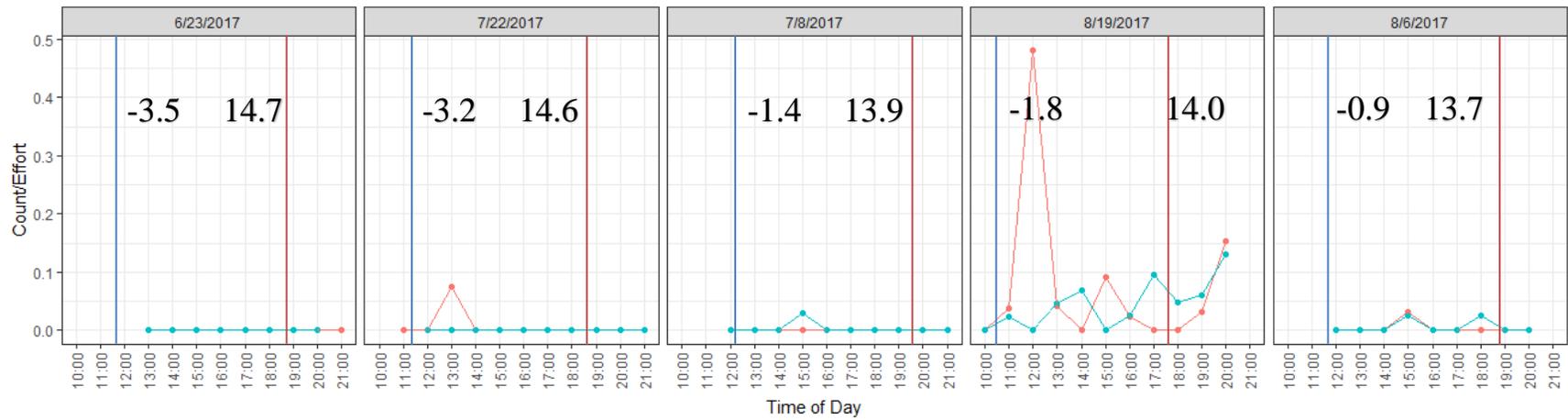
Tube-snout



Pile Perch

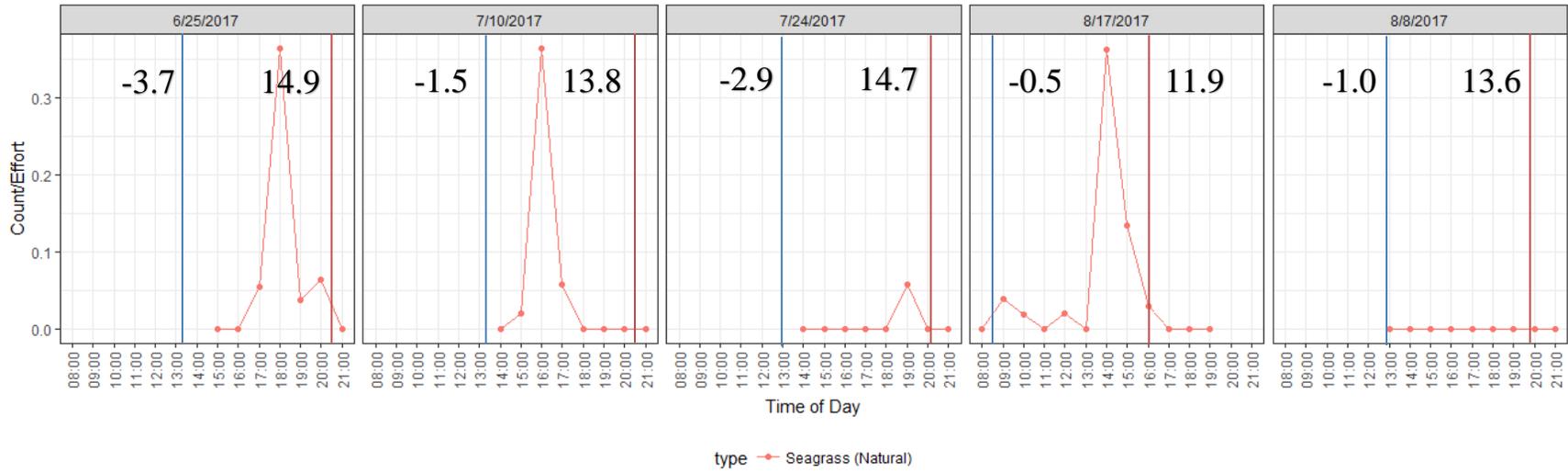


type — Seagrass (Natural)

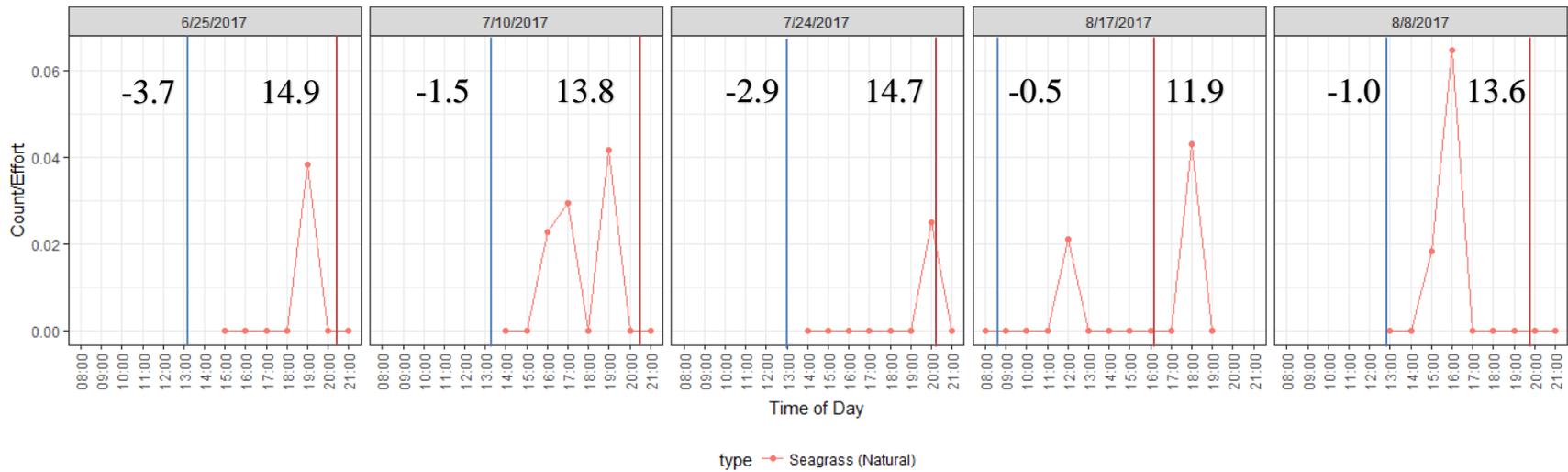


type — Bare Sediment — Seagrass (Transplanted)

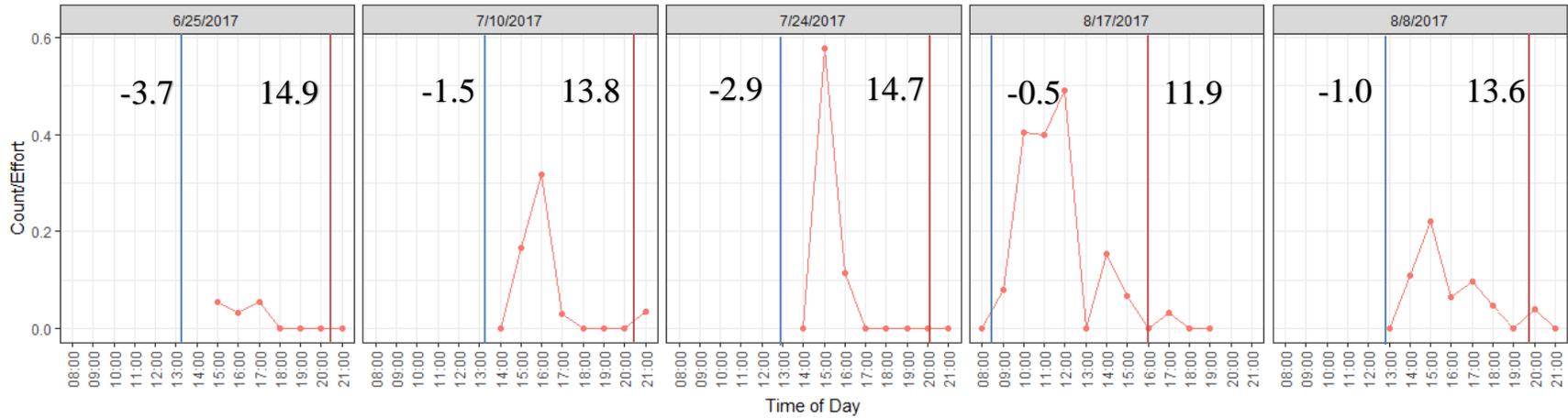
Striped Surferper



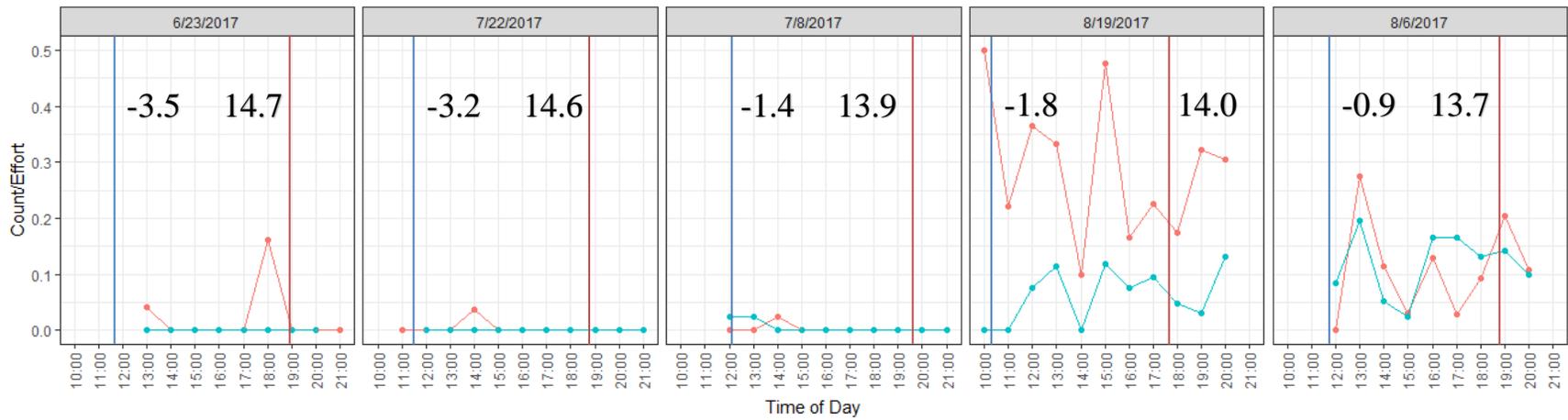
Spiny Dogfish



Salmonids



type — Seagrass (Natural)



type — Bare Sediment — Seagrass (Transplanted)