AN EVALUATION OF EELGRASS *(Zostera marina)* EPIFAUNAL COMMUNITIES

FOLLOWING LARGE-SCALE RESTORATION of the

NISQUALLY RIVER DELTA, WASHINGTON

by

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Abstract

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Zostera marina, a species of eelgrass native to the Puget Sound, serves as an ecosystem engineer in marine environments, supports an abundance of commercially and culturally important species, and is widely considered an indicator of ecosystem health. Eelgrass beds located along estuary boundaries benefit outmigrating juvenile salmon by providing refugia and hosting a diverse community of invertebrate prey, thus facilitating the transition from freshwater to saltwater. In particular, the federally-threatened Nisqually fall Chinook *(Oncorhynchus tshawytscha)* stock stands to benefit from eelgrass habitat. Here I evaluate the epifaunal invertebrate prey composition of four distinct eelgrass beds located along the Nisqually River Delta in Puget Sound, Washington for abundance and diversity during the spring outmigration season (March - September 2014). Epifaunal abundance increased through time at all sites but one, and community structure experienced a shift in dominance from amphipods to increasing proportions of polychaetes and annelids. Invertebrate abundance was most significantly influenced by site ($p<0.0001$) and month ($p<0.001$), and eelgrass shoot complexity was positively correlated with increased abundance for many key species. These data help to validate and quantify the contribution of Nisqually eelgrass beds as a valuable source of prey for juvenile Chinook salmon, and reinforce the ecological value of these habitats for the management of threatened salmon stocks in Puget Sound.

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This study would not have been possible without the help from a great many people:

I. INTRODUCTION

The 2009 restoration of the Nisqually River Delta in South Puget Sound, Washington, USA, completed a multi-stage restoration effort that reconnected over 300 hectares of previously-diked land to tidal inundation, allowing for the reclamation of approximately 75% of historic tidal marsh habitat (Barham, 2010). The removal of the historic Brown Farm Dike at Nisqually National Wildlife Refuge represents the largest estuary restoration in the Pacific Northwest, and has increased salt marsh habitat in Southern Puget Sound by 50% (Woo et al., 2011). Restoration was stimulated by the need to protect habitat for migratory birds and enhance forage opportunity for salmon, including the federally listed Nisqually fall Chinook salmon (U.S. Fish and Wildlife Service, 2005). Four eelgrass beds are located near the mouth of the restored Nisqually River Delta, each experiencing varying, indirect effects of restoration from alterations to sediment transport and water flow rates (Davenport, 2012).

Zostera marina, or common eelgrass provide a wide range of ecological services that benefit both humans and wildlife along coastal boundaries (Plummer et al. 2013). Eelgrass plants produce and sequester high volumes of organic carbon, aid in nutrient cycling and help stabilize fine sediments to reduce erosion (Boström et al. 2006; Duarte & Chiscano 1999; Duarte et al. 2005). Eelgrass beds form a structurally complex habitat that support a higher diversity and abundance of ecologically and economically important species as compared to unvegetated habitats (Bell & Westoby 1986; Orth et al. 1984). Eelgrass blades provide spawning substrate for marine forage fishes (Penttila 2007), create a rich nursery habitat for juvenile organisms, and support outmigrating

anadromous fish during their transition from freshwater to saltwater (Beck et al. 2001; Costa et al. 1994; Carr et al. 2011).

Eelgrass beds are highly sensitive to environmental stressors that alter light attenuation, and are often the first to respond to habitat disturbances that alter light availability in the nearshore environment (Short & Wyllie-Echeverria 1996). Increasing development along coastal areas and increased pollution inputs have contributed to an unprecedented decline of eelgrass species (Waycott et al. 2009). Moreover, this decline is accelerating, and may have negative impacts on organisms that rely on these habitats (Hyndes et al., 2003; Irlandi & Crawford, 1997). Eelgrass beds in Puget Sound have been monitored since 2000 by the Washington Department of Natural Resources (WA DNR). No long-term (multi-decadal) data exists for these stocks, but preliminary population data (2000 – 2013) indicate that eelgrass beds in Puget Sound are following this global negative trend, with some variability between years (Christiaen et al., 2015; Gaeckle, et al., 2011; Orth et al., 2006).

Eelgrass beds that occur along estuary boundaries provide a valuable link between freshwater and marine habitats for outmigrating juvenile Pacific salmon by offering refuge from predators and providing a rich food base before fish enter the open ocean (Takekawa et al., 2013). The largest proportion of prey consumed in eelgrass beds is thought to come from epifaunal species that attach to eelgrass blades, though the full extent of how salmon utilize the invertebrate and habitat resources of eelgrass beds is still poorly understood. This study represents a preliminary effort to evaluate the biological contribution of eelgrass beds to outmigrating salmon in the restored Nisqually River Delta. To quantify this contribution, we sought to measure the availability and

composition of invertebrate communities found in the eelgrass beds on the Nisqually Delta by assessing patterns of biodiversity and abundance of epifaunal invertebrates, conducting key species analysis for several known invertebrate prey species, and examining how site characteristics such as temperature, salinity and plant complexity and density impact these patterns of diversity both spatially and temporally.

Assessing changes in the distribution and community characteristics of associated invertebrate populations will provide a necessary baseline to quantify the forage opportunity of resident eelgrass beds to salmon populations. Eelgrass epifaunal communities have not yet been studied at Nisqually, despite growing evidence that these nearshore habitats provide a vital transitional habitat for salmon growth. Understanding these patterns will allow managers to better identify benchmarks for eelgrass recovery at Nisqually. A comprehensive evaluation of epifaunal invertebrates on the Nisqually river delta will inform adaptive management strategies to protect and enhance eelgrass habitat that supports outmigrating salmon populations. Lastly, this project will contribute to a more comprehensive understanding of food web interactions in delta habitats, allowing for a targeted allocation of resources to species critical for salmon forage.

This project was conducted in collaboration with the U.S. Geological Survey (USGS), U.S. Fish and Wildlife Service (USFWS) and the Nisqually Indian Tribe at the Nisqually National Wildlife Refuge. This research served as a pilot project to evaluate the ability of nearshore eelgrass habitats to provide enhanced opportunity to juvenile salmon populations in Puget Sound. The study falls within the larger research aims of the Nisqually National Wildlife Refuge's plan to document post-restoration recovery rates and habitat characteristics of the Nisqually River Delta.

II. LITERATURE REVIEW

Overview of Seagrasses

Seagrasses are a globally distributed class of marine flowering plants characterized by long, narrow leaf blades that form underwater meadows along shallow, coastal margins and within estuaries (Figure 1). Seagrass species have relatively low species diversity with only 58 extant species worldwide (Larkum, 2007). These plants evolved from a terrestrial plant ancestor 100 million years ago, and represent the only plant that has successfully transitioned from land back into the marine environment (Larkum, et al. 2007). Seagrass plants increase habitat complexity by forming dense, meadow-like mats of submersed vegetation. These meadows support higher density and abundance of invertebrates when compared to adjacent unvegetated habitat, and provide critical habitat for many commercially and recreationally important species (Beck et al., 2001). Seagrasses have some of the most substantial light requirements of any submersed aquatic vegetation, requiring up to 29% of incident radiation to reach blades for growth (Dennison et al., 1993; Short & Wyllie-Echeverria, 1996). As a result, they are acutely sensitive to environmental changes that reduce light availability. Furthermore, seagrasses may act as a 'coastal canary' to highlight preliminary impacts of environmental degradation (Orth et al., 2006).

Seagrass beds are a critical driver of nearshore carbon sequestration. Despite occupying 0.2% of the seafloor, seagrass species cumulatively store 50% of total organic carbon (C_{org}) in ocean sediments and serve as one of the most effective carbon sinks on Earth (Duarte et al., 2005; Waycott et al., 2009). Seagrasses are estimated to bury 27.4

tetragrams of C_{org} per year, or approximately 10% of yearly C_{org} sequestration in the

oceans (Fourqurean et al., 2012). It is estimated that seagrass ecosystems store between

4.2 – 8.4 petagrams of organic carbon overall, and possibly as much as 19.9 petagrams

(Fourqurean et al., 2012). For reference, one petagram represents over two trillion

pounds. In an environment where anthropogenic climate change threatens our ocean's

ability to uptake CO_2 through increased temperatures and ocean acidification, the

contribution of seagrass to the global carbon sequestration budget may become

increasingly important in the years to come (Doney, 2009).

Despite growing recognition of the importance of these habitats, seagrasses are

experiencing an unprecedented decline. Since the late 19th century, seagrass meadows

have declined in all areas of the globe where data exists (Waycott et al. 2009). Aerial

cover of seagrass beds has declined 29% since 1879, at a rate of 1.5% per year through

1980 (Fourqurean et al., 2012). The rate of this decline has accelerated since 1980 to 5%

loss of seagrass extent per year, and the causes of this acceleration are still poorly

understood (Hughes et al., 2008; Short et al., 2011; Short & Wyllie-Echeverria, 1996;

Waycott et al., 2009). Nearly 15% of all seagrass species are considered threatened by the

International Union for Conservation of Nature (IUCN) (Hughes et al., 2008; Short et al.,

2011), including eelgrass.

Life History of Eelgrass

Common eelgrass (*Zostera marina)* is the most prolific of six species of

seagrasses along the Pacific Coast of North America. Eelgrass plants grow in soft

substrates along shallow embayments and coastal margins ranging from the Bearing Sea

in Alaska to the Gulf of California (Larkum et al., 2007; Phillips, 1983). Eelgrass plants propagate through the lateral spread of perennial root-rhizomes and annual seed dispersal. Its leaves are long thin blades ranging from 2 – 20 millimeters wide and up to 53 centimeters long that radiate upward from the root rhizome (Phillips, 1983).

Figure 1. Technical illustration of Zostera marina blades, seeds and root rhizomes. Reprinted from: The Families of Flowering Plants (website) (Watson & Dallwitz, 2015).

Eelgrass is a powerful ecosystem engineer that greatly enhances community diversity and biomass. Much like terrestrial plants, rhizome root structures stabilize soft sediment and reduce rates of erosion (Larkum, 2007). Eelgrass blades form a thick understory of vegetation that provides refugia for clinging epifauna and macroalgae and greatly enhances the diversity and biomass of fish and invertebrate species (Attrill et al., 2000; Blackmon et al., 2006.; Edgar & Robertson, 1992; Orth et al., 1984; Curras et al., 1993). For example, Carr et al. (2011) compared the abundance and diversity of associated mesograzers with eelgrass shoot density over time in San Francisco Bay. They found that eelgrass plants with flowering shoots supported higher densities of epifauna as compared to non-flowering blades or unvegetated sediment.

Eelgrass shoots alter the physical characteristics of water parcels that interact with eelgrass beds, further enhancing nursery habitat. Eelgrass blades reduce water flow rate, wave action, and sediment re-suspension, trapping suspended sediment particles and organic matter. This results in the largest sediment accretion rate of any aquatic vegetation (Duarte et al., 2005; Orth et al., 2006). While some organic matter is transported to adjacent environments, the majority is buried within these soft sediments, and is a significant source of carbon sequestration among types of submerged aquatic vegetation (Larkum, 2007; Duarte et al., 2005).

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

Figure 2. The eelgrass meadow: a world of microhabitats. Reprinted from Kelp and Eelgrass in Puget Sound (Mumford, 2007).

Salmon Utilization of Eelgrass Habitat

Nearshore habitats such as eelgrass beds provide an important link between freshwater and marine habitats for multiple species of juvenile Pacific salmon. These habitats are especially important for ocean-bound species of Chinook salmon, *Oncorhynchus tshawytscha*, which have a longer residency time in nearshore and

estuarine habitats than any other salmon species (Magnusson & Hilborn, 2003). Chinook salmon are one of 27 stocks of salmon in Puget Sound, and are listed as threatened under the Endangered Species Act (National Marine Fisheries Service Northwest Region, 2005). Eelgrass beds that occur along estuary boundaries are used by outmigrating juvenile salmon to help enhance their early growth and development (Figure 3). During salmon life cycles, the largest proportion of natural mortality for salmon occurs in the first few months spent in the marine environment. Since predation dynamics are largely size-selective, juvenile salmon that are able to rapidly increase their size are afforded a competitive advantage during their early life cycle (Brodeur et al., 2007). Juvenile salmon utilize the nearshore environment for most of the year, with eelgrass beds used consistently from May through September (Thom, et al., 1989). Salmon are not bound by their natal stream, and migrate extensively around Puget Sound to access different nearshore areas (Shaffer, 2004).
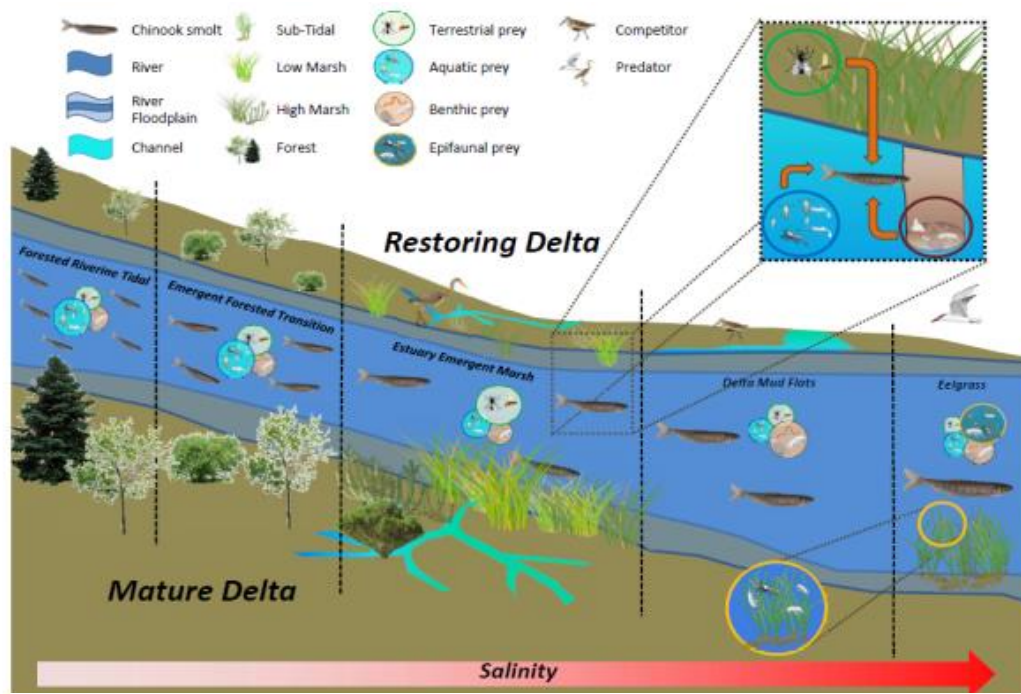
Figure 3. Conceptual model of salmon habitat use in a restored estuarine wetland. The largest proportion of prey consumed in eelgrass beds is thought to come from epifaunal species that attach to eelgrass blades. Model created by USGS Western Ecological Research Center (2013).

Thom et al. (1989) evaluated the abundance of fish and prey species in four habitat types in Puget Sound to evaluate the relative importance of different habitat types to fisheries resources. For eelgrass beds, they found that salmon abundance varied over time and showed a quick response to changes in preferred prey abundance. Chinook salmon preferentially selected copepods, and overall prey biomass was positively correlated with eelgrass biomass such that larger eelgrass plants supported greater abundances of prey species. Chinook prey utilization on the Nisqually Delta nearshore before estuary restoration varied with salmon body size, and was largely composed of amphipods and copepods (Pearce, et al., 1982). Elsewhere in Puget Sound, euphausiid shrimp were also an important prey species (Brodeur, 1990).

Patterns of Eelgrass Decline

In all coastal regions of the United States, eelgrass populations have experienced large fluctuations in range and extent in response to environmental stressors over the last 100 years (Orth et al., 2006). North Atlantic eelgrass populations experienced a debilitating epidemic in the 1930s that killed 90% of all eelgrass within two years (Short & Wyllie-Echeverria, 1987). Many populations were completely extirpated along the east coast of North America, ranging from Maine to Florida (Dexter, 1985). This wasting disease was caused by a pathogenic form of *Labyrinthula zosterae*, a marine slime mold that had significant economic and ecological impacts on dependent eelgrass species (Dexter, 1985; Short et al., 1987).

Labyrinthula zosterae spreads through direct contact between infected blades. It presents as black or brown spots that spread to cover eelgrass blades at a rate of 0.8mm

per hour. The pathogen acts as a secondary decomposer on living and senesced blades, dramatically reducing photosynthetic capacity of the infected blades and decreasing plant fitness (Larkum, 2007; Ralph & Short, 2002). Both pathogenic and non-pathogenic strains of *L. zosterae* have been found on eelgrass beds in the Atlantic and Pacific oceans (Short et al., 1987). Wasting disease continues to be observed in eelgrass meadows throughout North America and Europe including the northeastern Pacific ocean, although no infestation since has led to the precipitous declines observed in the 1930s (Ralph & Short, 2002). Risk of infection is believed to be greater in plants that are already subject to other stressors such as increased temperatures, disturbance or pollution (Larkum, 2007). As coastal areas are increasingly subject to environmental stressors, beds infected with *L. zosterae* are at a heightened risk of widespread decline.

Loss of eelgrass beds from the 1930s collapse catastrophically altered nearshore ecosystem processes and negatively impacted many dependent species (Short et al., 1987). Areas with widespread eelgrass loss observed a decline of migratory waterfowl, including the Brant goose *Branta bernicla,* which rely on eelgrass as a preferred forage material (>80%) in the winter (Addy & Aylward, 1944), as well as the collapse of a commercial scallop fishery in Chesapeake Bay. This decline also resulted in the first recorded extinction of an eelgrass dependent limpet species *(Lottia aleveus)* (Larkum et al., 2007). Eelgrass populations of the Atlantic largely recovered after 30 years, but remained stagnant or nonexistent in areas where erosion was strengthened in absence of eelgrass beds (Larkum et al., 2007). From the 1960s to present day populations have declined from historic extent due to anthropogenic stressors caused by shoreline development and nearshore pollution (Short et al., 2011). This decline is not evenly

distributed and can cause severe negative impacts on urbanized coastal ecosystems (Orth et al., 2006).

Eelgrass Trends in Puget Sound

In Puget Sound, Washington, the greatest threat to eelgrass beds comes from aggressive coastal development due to the rapid and accelerating urbanization of metropolitan areas (Gaeckle, et al., 2011). Development-associated stressors to eelgrass include construction of overwater structures such as docks and marinas that shade benthic habitats, and shoreline armoring. Armored shorelines alter the energy budgets of nearshore systems by disrupting habitat connectivity and flow of water and nutrients from terrestrial to nearshore environments (Rehr et al., 2014). Armored shorelines are frequently at a sediment deficit as compared to natural shorelines (Rehr et al., 2014). Twenty-seven percent of shorelines in in Puget Sound are armored, thereby changing the sedimentation pattern and characteristics of adjacent nearshore habitats (Shipman, et al., 2009).

Eelgrass beds occupy approximately 43% of shorelines and 9% of the sea floor in Puget Sound, ranging from 1 – 3,000 hectares in size (Gaeckle, et al., 2011). The Puget Sound Partnership (PSP) has tracked eelgrass abundance and distribution since 2000 in a comprehensive monitoring effort through the Submerged Vegetation Monitoring Project (SVMP; Figure 4). In 2010, the PSP identified eelgrass as one of 20 dashboard ecosystem indicators of ecosystem health using trend data compiled from the SVMP (Gaeckle et al., 2011). The following year, PSP identified a target goal to increase eelgrass extent by 20% in 2020, using a combination of passive and active restoration

methods (Gaeckle et al., 2011). In addition, the Washington Department of Fish and Wildlife has designated eelgrass beds as a habitat of special concern under their authority over hydraulic projects (WAC 220-110-250), and the Washington Department of Ecology designated eelgrass areas as critical habitat through the Washington Shoreline Management Act (WAC 172-26-221) (Gaeckle et al., 2011).

It is difficult to predict long-term trends without multi-decadal data, but preliminary trends of eelgrass populations have been evaluated throughout Puget Sound. Since 2000, the total area occupied by eelgrass in Puget Sound has remained stable. Site-wide trends are more difficult to interpret. In 2009, overall populations were stable, yet site data showed a greater proportion of sites with declining eelgrass populations, which suggests that a net loss of eelgrass area is being obscured by a few stable populations (Gaeckle et al., 2011). From 2009 – 2013 site populations experienced an overall increase, especially in South Puget Sound and at the site of two large-scale delta restoration projects. Both the Skokomish River Delta and the Nisqually River Delta showed significant increases in eelgrass bed extent, suggesting that changing sedimentation patterns from dike removal are benefitting the nearshore environment (Christiaen et al., 2015).
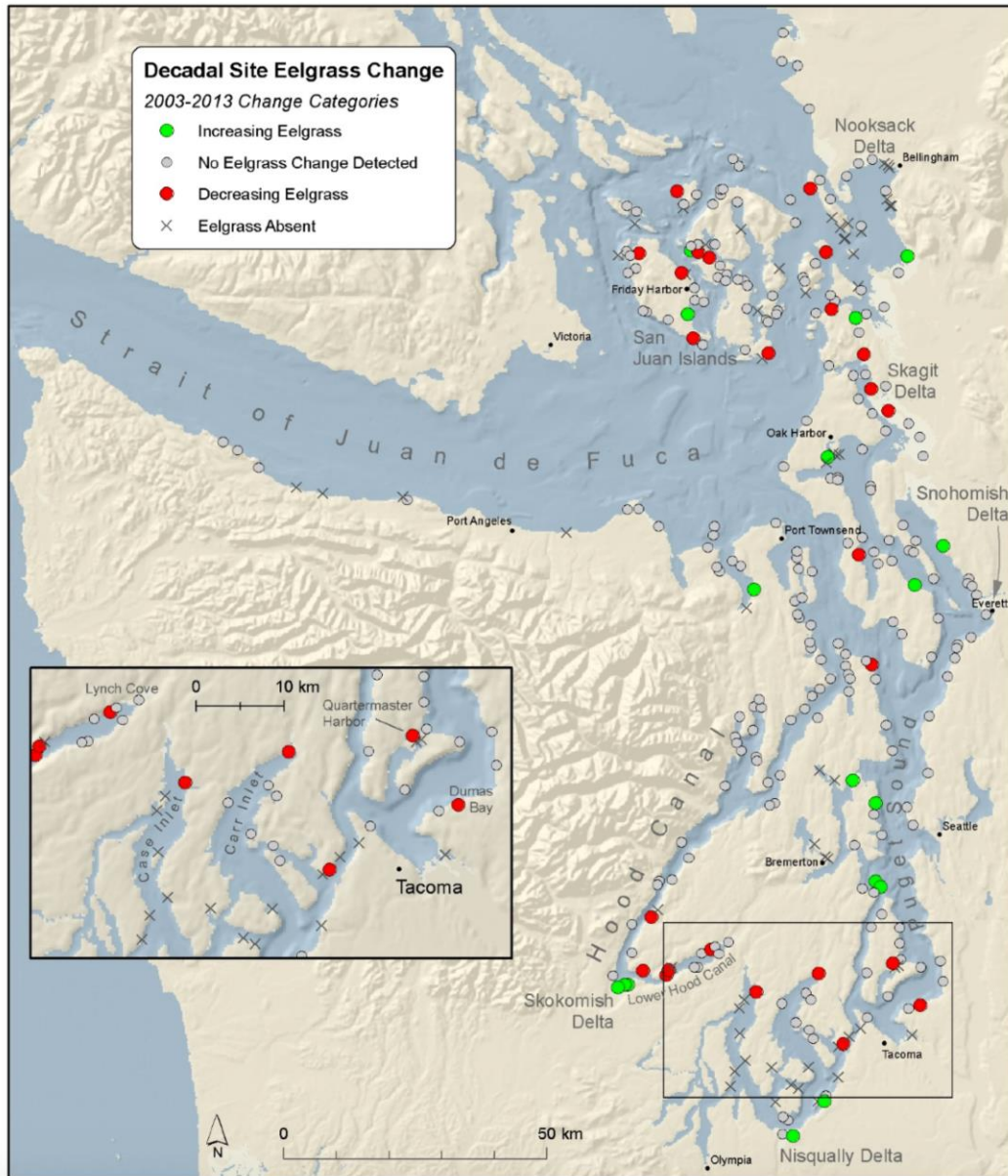
Figure 4. Site-wide decadal trends in eelgrass abundance, 2003 – 2013. Adapted from the Puget Sound Submerged Vegetation Monitoring Project (Christiaen et al., 2015).

Eelgrass Restoration

As eelgrass populations continue to decline, research has increasingly shifted towards methods to mitigate stressors and restore existing eelgrass beds (Orth et al., 2006). The mechanics of eelgrass restoration bear many similarities to terrestrial restoration projects, where seed dispersal and erosion are influenced by water currents

instead of air currents (Seddon, 2004). Seagrass restoration presents a unique challenge in that restoration is often strongly limited by access, since these habitats are submerged most of the time. Restoration projects involve costly and specialized equipment, often requiring certified SCUBA divers to complete basic restoration techniques (Seddon, 2004).

Active restoration methods include physical interventions that are used to boost or enhance eelgrass recruitment in degraded habitats by seeding or replanting diminished eelgrass beds (Seddon, 2004). These methods have mixed success rates, and can produce especially low survival rates when used as the only restoration intervention (Short & Wyllie-Echeverria, 1996). Passive restoration include natural enhancements of eelgrass habitat, through reductions to factors that stress or inhibit eelgrass growth and recruitment by enhancing preferred habitat conditions (Seddon, 2004).

Moore & Short (2006) found that "improvement of water clarity is the single greatest factor that will aid in the restoration of *Zostera* species." Interventions to improve water clarity include steps to reduce nutrient loading, eutrophication, and stormwater runoff, or to limit sediment discharge into coastal waters (Short & Wyllie-Echeverria, 1996). Improving water clarity encourages eelgrass to expand laterally and re-establish historic depth limits (Larkum, 2007). Research into restoration interventions grew in interest during the 1930s in response to decimation of eelgrass populations by wasting disease (Addy, 1947; Addy & Aylward, 1944), however the lack of plant response to reseeding and planting efforts was acknowledged even then and has not improved since (Fonseca, 2011).

Despite this stagnation in restoration success, scientists have broadened their understanding of the key factors that influence success and failure rates in restoration projects. McGlathery et al. (2012) mapped functional and structural recovery trajectories in eelgrass beds by evaluating metrics of primary productivity, sediment deposition, shoot density and plant biomass of large experimental beds in successive years. Unvegetated beds were seeded in 0.4 hectare plots with a total of 4.4 million seeds. Habitat characteristics were compared to adjacent unvegetated sites for 9 years. The authors observed an initial 4-year delay in shoot density across all sites, with a linear increase from 4 – 9 years. Compared to eelgrass beds at 3-4 years after planting, 9-year old beds had 20 times more productivity, 2 times more organic matter, 3 times more carbon and 4 times more nitrogen sequestered in progressively finer sediment. Such results are encouraging for eelgrass restoration efforts.

Impact of Dikes on Coastal Habitats

Removal of shoreline armoring is one method used to enhance preferred eelgrass habitat and restore habitat connectivity between terrestrial and marine environments (Heerhartz & Toft, 2015; Shipman, et al., 2009). Diking along river deltas prevents the formation of distributary channels that help to slow rates of coastal erosion by depositing sediment along the delta mouth. Without these distributary channels, water enters the delta at a faster rate, increasing turbulence and decreasing light penetration and water clarity (Giesen, et al., 1990). Armored deltas limit the distribution of sediment both behind and in front of the dike, and a higher percentage of sediment is lost offshore (Stevens & Lacy, 2012).

Hood (2004) evaluated changes to adjacent diked habitat on the Skagit River, Washington, and found that dikes are directly and indirectly responsible for estuarine and nearshore habitat loss. Dike construction reduced the tidal prism, resulting in an increase of channel filling and deposition along seaward dike boundaries, and a decrease in channel sinuosity with enhanced erosion landward. Dikes are both directly and indirectly responsible for estuarine and nearshore habitat loss. Coastal development has increased over the past 200 years, primarily due human interventions to preserve and extend arable land. These actions reduce tidal flow to a thin ring along manipulated boundaries, and have significant negative impacts on delta extent and function of critical habitats (Boumans, et al., 2002).

Removal of shoreline armoring has been shown to alter sedimentation dynamics, water hydrology and geomorphology for adjacent nearshore environments, while increasing estuary tidal prisms (Hood, 2004). Restoration of armored deltas modifies patterns of sedimentation at river mouths, leading to accretions of soft sediment characteristics in the nearshore environment (Davenport, 2012; McGlathery et al., 2012). Restoration can also have a direct positive benefit to juvenile salmon species, especially Pacific salmon (*Oncorhynchus spp.*) that utilize nearshore seagrass habitats as a staging ground before heading to the open ocean (David et al., 2014; Gray et al., 2002; Lind-Null & Larsen, 2010; Shreffler et al., 1992).

Initial trends from the 2009 Nisqually Delta restoration indicate that the tidal flat geomorphology has changed since restoration. Sediment grain size within Nisqually has decreased with time, likely as a result of increased sediment transport through reconnected tidal channels (Davenport, 2012). The addition of sediment to the nearshore

has the opportunity to support expanded eelgrass habitat. Preliminary trends of eelgrass

abundance indicate that the eelgrass beds at Nisqually are increasing in size following

dike removal, further reinforcing the positive benefit of dike removal in this estuary

environment (Christiaen et al., 2015).

III. METHODS

Study Area

The Puget Sound is a polyhaline fjord encompassing 1.6 million acres in western Washington, USA. Freshwater input from multiple large river systems combines to create a mixed-salinity system. Shorelines are moderate to steeply-sloped, and substrates range from rocky shoreline common in northern Puget Sound to softer sand and clay sediments in southern Puget Sound. Puget Sound coastlines are primarily nourished by inputs from nearby feeder bluffs or deposition of sediment from river deltas, which contribute to support a diverse assemblage of flora and fauna, including five species of commercially and culturally important Pacific salmon (*Oncorhynchus spp.*) and top predator species such as orca whales, California sea lions, and seals.

The Nisqually River Delta is a river valley estuary located at the southern end of Puget Sound. The Nisqually River originates from the Nisqually Glacier on Mount Rainier, and flows northwest for 125 kilometers before entering into Puget Sound (47.08°N, 122.70°W; Figure 5). As the largest river flowing into southern Puget Sound, and the largest restored estuary in this region, the Nisqually River Watershed covers approximately 1,900 square kilometers and includes one national park, a United States military base, two counties, and a rapidly-growing urban population (Karlstrom, 1971). The Nisqually River Delta lies within the boundaries of the Nisqually National Wildlife Refuge, which was designated as a national natural landmark in 1971 due to its ecological significance. It is operated by the U.S. Fish and Wildlife Service (USFWS)

and managed by both the USFWS and the Nisqually Indian Tribe (U.S. Fish and Wildlife Service, 2005).

In 1905, construction of the 5-mile Brown Farm Dike converted emergent marsh and tidal slough habitat into agricultural and pasture land, isolating 600 hectares of wetland habitat from tidal influence. Land behind the dike subsided over time due to a lack of sediment inputs and decomposition of previously submerged organic matter such as peat (Barham, 2010).

Figure 5. The Nisqually River Delta, Washington with study area and refuge boundary identified. Map created by Sierra Blakely, May 2015, ESRI Basemap.

Removal of five-miles of earthen dike in October 2009 restored 308 hectares of previously diked habitat to tidal inundation, allowing reclamation of 75% of historic tidal habitat. Restoration of the Nisqually River Delta reconnected over 35 kilometers of

historic tidal channels, improved upstream riparian habitats and enhanced habitat for

wildlife, migratory birds and federally threatened salmon including the Nisqually fall

Chinook stock. The Nisqually Delta restoration increased salt marsh habitat in Southern

Puget Sound by 50%, and ranks as the largest estuary restoration in the Pacific Northwest

(Davenport, 2012, Figure 6).
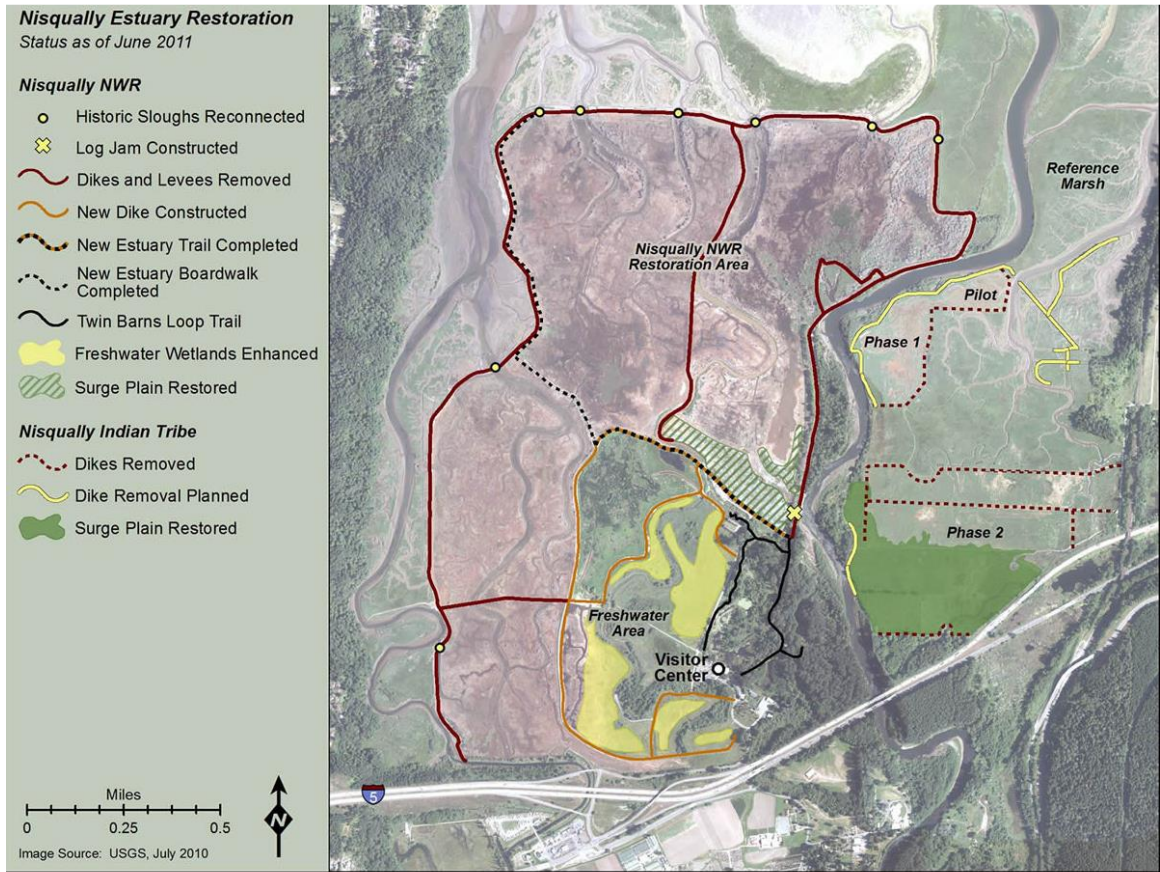
Figure 6. Nisqually National Wildlife Refuge restoration map, created by J. Cutler, Nisqually Indian Tribe.

We conducted eelgrass site surveys at four *Zostera marina* beds located along the

Nisqually River Delta: Hogum Bay (HGB), McAllister Creek (MCA), Red Salmon

Slough (RSS) and Cormorant Passage (CMP) (Figure 7). These eelgrass beds were

identified by the Washington Department of Natural Resources as part of the Submerged

Vegetation Monitoring Project (Gaeckle et al., 2011) and are positioned along the West

and East sides of the Nisqually Delta. McAllister Creek is located on the West side of the Nisqually Delta at the mouth of McAllister Creek, a spring-fed creek (47.06°N, 122.43°W). Red Salmon Slough is located on the East Side of the Nisqually Delta at the mouth of Red Salmon Slough on the Nisqually River (47.06°N, 122.41°W). Hogum Bay is located northwest of the Delta and just north of an aquaculture farm operated by Taylor Shellfish Farm (47.07°N, 122.44°W). Cormorant Passage is the farthest site from the Delta, and occurs along a steeper shoreline closer to the main channel opposite Anderson Island (47.09°N, 122.37°W).

Figure 7. Location and approximate size of Nisqually River Delta eelgrass beds. Blue imagery represents 2014 aerial imagery of delta landforms, ESRI basemap used. Map created by Sierra Blakely, February 2015.

All eelgrass samples were collected by the U.S. Geological Survey Western Ecological Research Center (USGS WERC) as part of the Estuary and Salmon

Restoration Program (ESRP), an ongoing monitoring project that aims to assess the capacity of the Nisqually delta to support invertebrate prey following restoration. We chose sites to align with fish capture sites used by th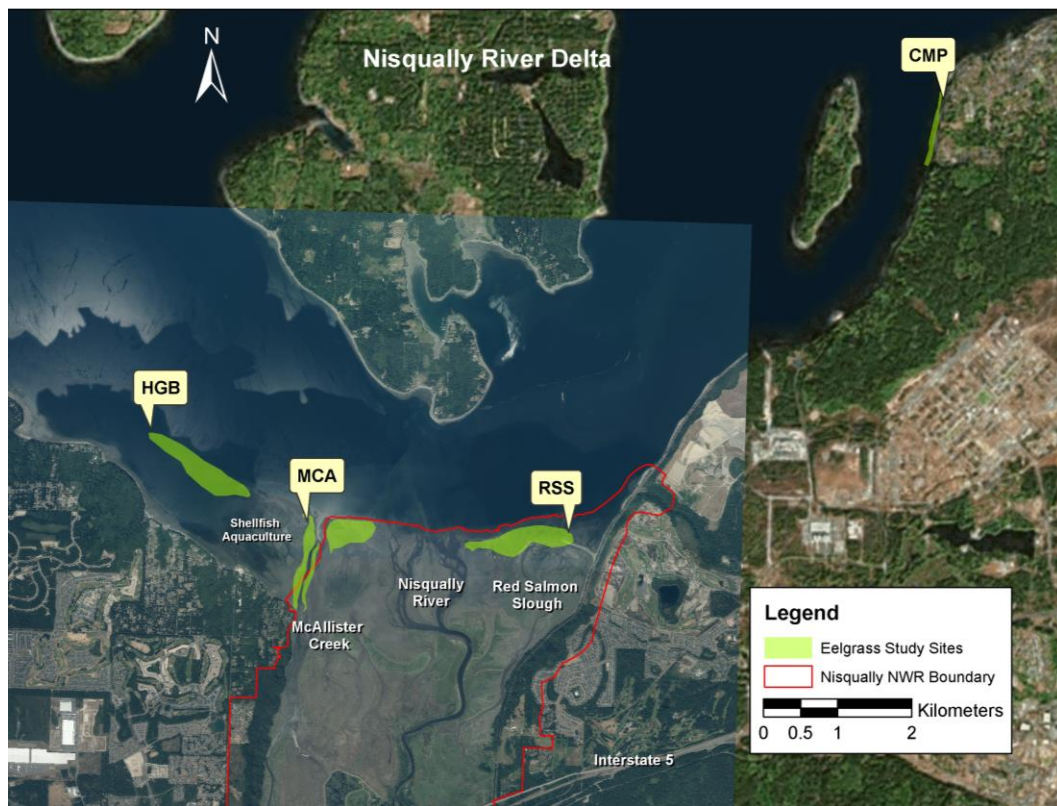e Nisqually Indian Tribe for stock assessment purposes. McAllister Creek was sampled from March through September 2014, and the additional three sites (Hogum Bay, Red Salmon Slough & Cormorant Passage) were sampled from May through July 2014.

Experimental Design and Data Analysis

We measured temperature and salinity six times per site using a YSI Pro 2030 (Yellow Springs, Ohio USA), and averaged these values across each sample. Shoot density was collected once in June 2014 by Steve Rubin (USGS Western Fisheries Research Center), from the center of each eelgrass bed during low tide when the beds were exposed. To determine changes in biophysical variables, we used a general hierarchical cluster analysis to evaluate each site's compositional response to specific predictor variables including temperature, salinity, number of nodes and mean blade length. Month was used as a covariate to account for sampling variation and seasonal differences. We used a multivariate analysis of variance (MANOVA) to identify which predictor variables explained the most variation between sites. Prior to running each analysis, a histogram of the data distribution was evaluated, and a Shapiro-Wilk normality test was run to evaluate normality. Data were log-transformed when necessary. Several candidate models were compared using the Akaike Information Criterion (AIC) to determine the best fit model. Model selection was performed using R version 3.1.3 (R Core Team, 2015) and Excel.

Plant Collection

We collected ten eelgrass plants per site during the lowest tide of the sampling cycle (0.0 to -3.42 feet, Mean lower low water). Eelgrass shoots collected in March and April were gathered opportunistically from plants caught on the boat anchor, as the combined water and air temperature was too cold to safely collect samples by hand. From May and continuing through September, plants were collected by hand by separating the rhizome from the shoot at the sediment surface, with care taken to avoid jostling the plants to reduce loss of epifauna during collection. No distinction was made between vegetative and flowering eelgrass shoots. Collected shoots were transferred to an 8-ounce jar filled with ambient seawater, then placed into a cooler until processing. All samples were processed within 24 hours of collection. Once rinsed for invertebrates, each eelgrass plant was measured for blade length (mm) and number of nodes per plant.

Invertebrate Collection

We processed epifaunal samples following the protocol established by Carr et al. (2011) and Holmlund et al. (1990). Each jar was emptied onto a 500 μm sieve, before the eelgrass shoot was placed in three 1-minute freshwater baths to remove clinging epifaunal. This technique has been shown to remove $92 - 100\%$ of epifauna from algae and 90% from eelgrass plants. We poured each freshwater bath through a 500 μm sieve to capture invertebrates, which were preserved in 95% ethanol and identified to the lowest possible taxa by technicians at the USGS San Francisco Bay Estuary field station in Vallejo, California.. We used a subset (75) of the total samples that had been processed

by February 2015. That subset contained 29,504 invertebrates, identified to various taxonomic levels.

We used a multiple analysis of variance (MANOVA) to identify patterns of invertebrate community characteristics between sites. Amphipods, copepods, nematodes, ostracods, polychaetes, and tanaids were selected as key species groups, due to greater abundances of these groups across all months, as well as their established function as prey for juvenile salmon (Brennan, et al., 2004). To understand which predictor variables (temperature, salinity, mean blade length, number of nodes and mean shoot density) had the strongest effect on community structure, we used generalized linear models to compare both the additive and interactive effects of our predictor variables. Both temperature and salinity showed a unimodal distribution and were therefore compared using a polynomial model. All candidate models were compared using AIC to determine which site variables were the best predictors of species abundance.

Invertebrate community similarity was calculated using multi-response permutation procedures (MRPP) for each site using PC ORD (McCune & Mefford, 2011), and a Sorenson distance measure. The primary matrix of species abundances was relativized by species maximum to control for the effect of overabundant species in our results. Due to the non-normality of our data, we performed a non-metric multidimensional scaling (nMDS) using PC ORD to linearize the relationships of species abundance and our predictor variables of site, temperature, salinity, mean blade length, number of nodes per plant and mean shoot density.

IV. RESULTS

Community Structure

Six unique phyla of epifaunal invertebrates were identified from our samples collected March – July 2014. Twenty five unique taxa were observed at Cormorant Passage, 29 unique taxa observed at McAllister Creek, 28 unique taxa observed at Hogum Bay and 19 unique taxa observed at Red Salmon Slough (Appendix A). We observed a shift in abundance across all sites, from a community dominated by arthropods in March through May, to a community dominated by nematodes, annelids and molluscs in June and July (p<0.001, Figures 9a-b).
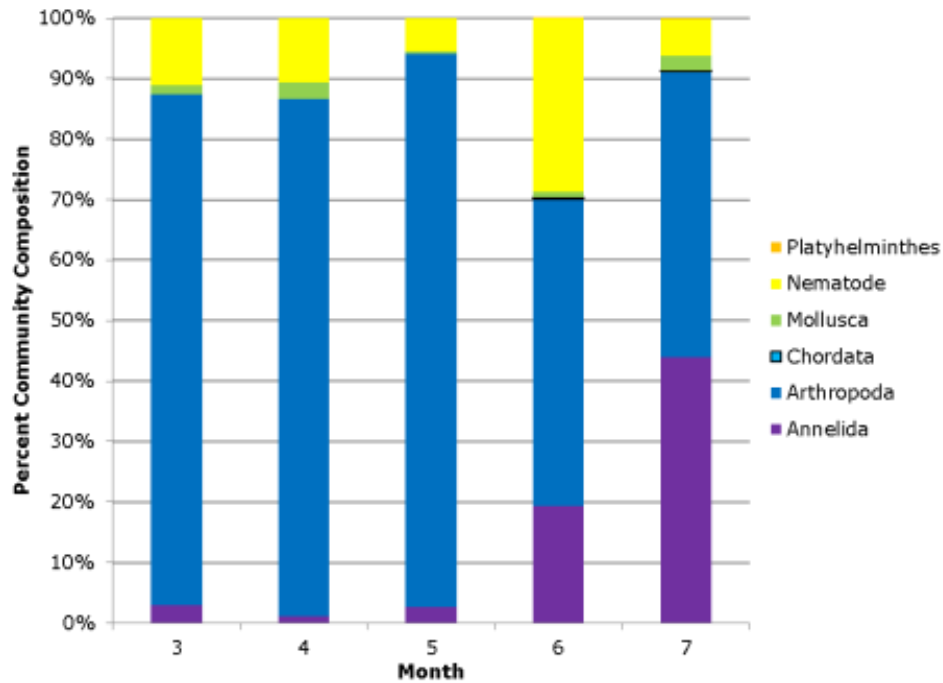
Figure 9a. Percent community composition of total phylum abundance observed.
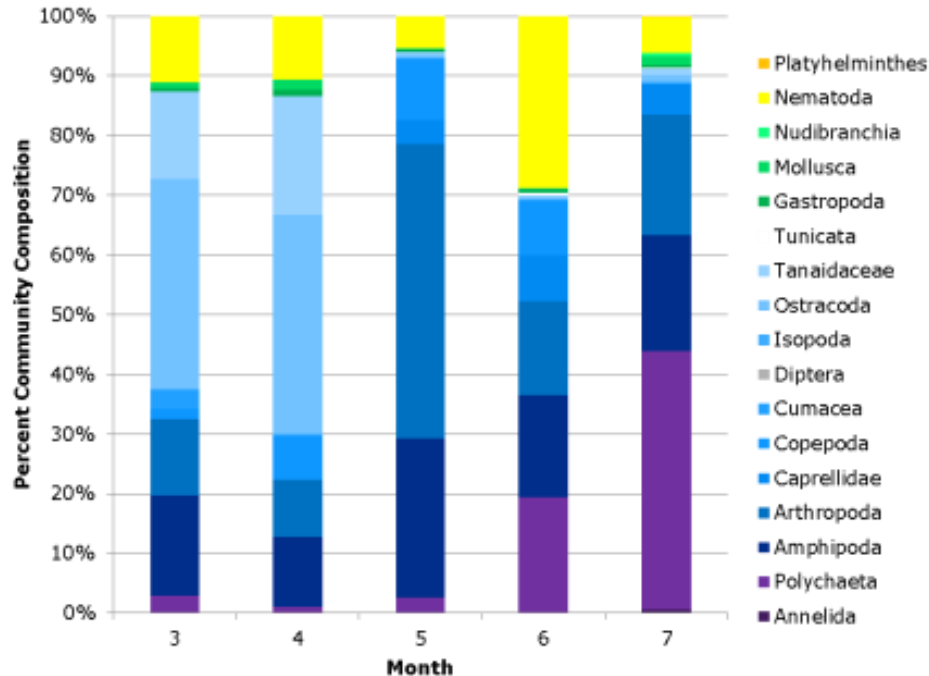
Figure 9b. Percent community composition of total species abundance observed to lowest taxonomic identification level.

Abundance varied significantly by both site and time (Figure 10). Hogum Bay had significantly higher abundances than any other site, and supported abundances at least one magnitude higher than those observed at other sites ($p < 0.05$). This finding was visualized using a hierarchical cluster analysis that aggregated the predictor values of site and month to identify compositional responses. Hogum Bay in June was the most divergent site for community composition, and McAllister Creek was the site most similar across months (Figure 11). Mean epifaunal abundance increased between March and July ($p < 0.05$, Figure 10). Among months, March and April were showed the greatest difference of phylum abundance from June and July. McAllister creek was the only site sampled during March and April, therefore abundance for these months represent a smaller sample size with less representation across the four sampling sites.
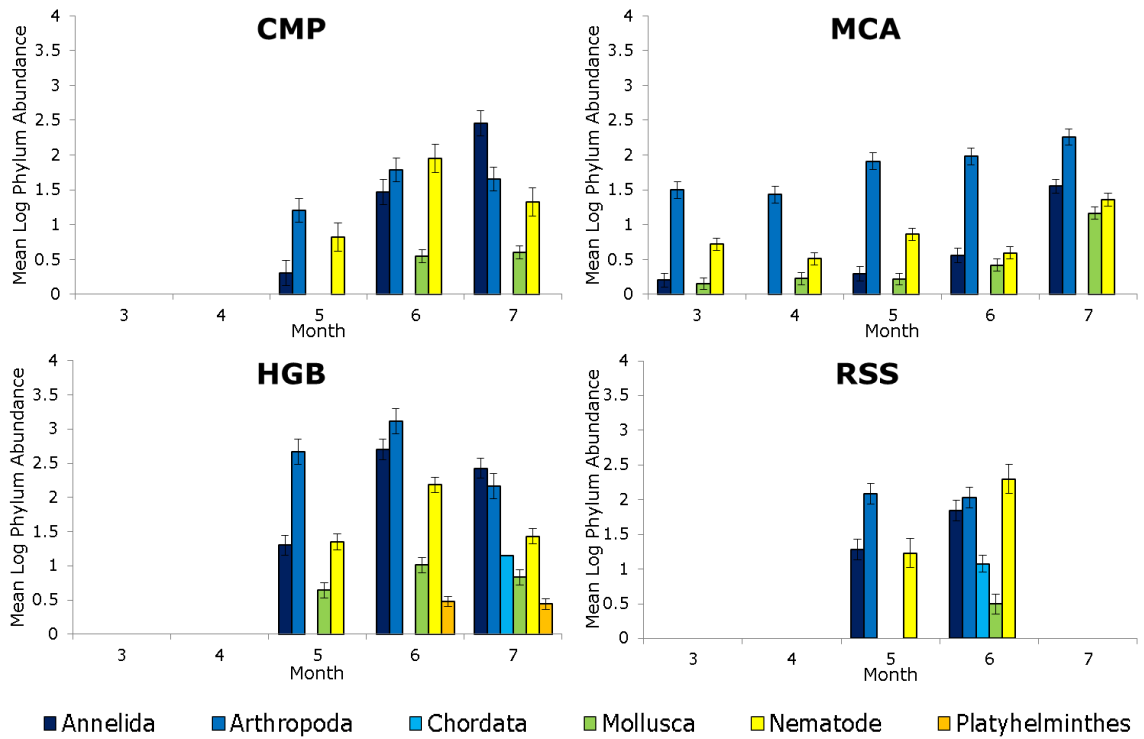
Figure 10. Mean epifaunal abundance by site and month, +/- s.e. Data was log-transformed for ease of visualization. No July samples were counted for RSS.
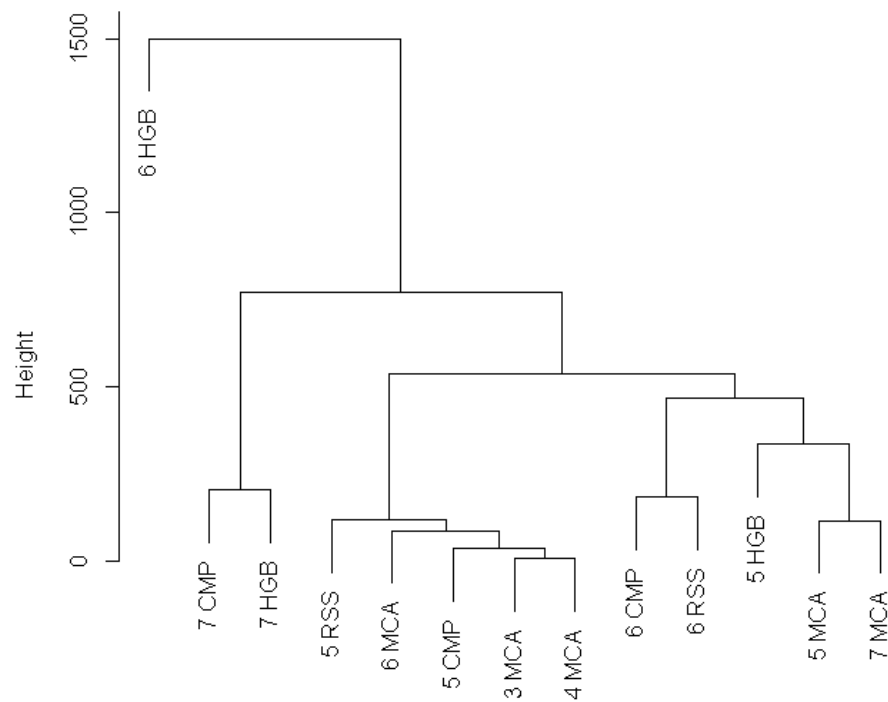
Figure 11. Hierarchical clustering analysis of differences in phylum abundance by month and site. Branches indicate degrees of separation using Euclidian distance measure.

We confirmed this significance using a multiple analysis of variance of site characteristics of site + month + nodes + mean blade length (Table 1). While all site characteristics exhibited statistical significance, site was the strongest predictor of phylum abundance (p<0.0001).

A multi-response permutation procedure with Sorenson's distance measure showed a significant difference between sites (A=10462810, p<0.0001). We visualized this using non-metric multidimensional scaling (nMDS) to linearize the relationship of species abundance and site, temperature, salinity, mean blade length, nodes and mean shoot density. nMDS showed a strong distinction between groups in the species space (Figure 12).
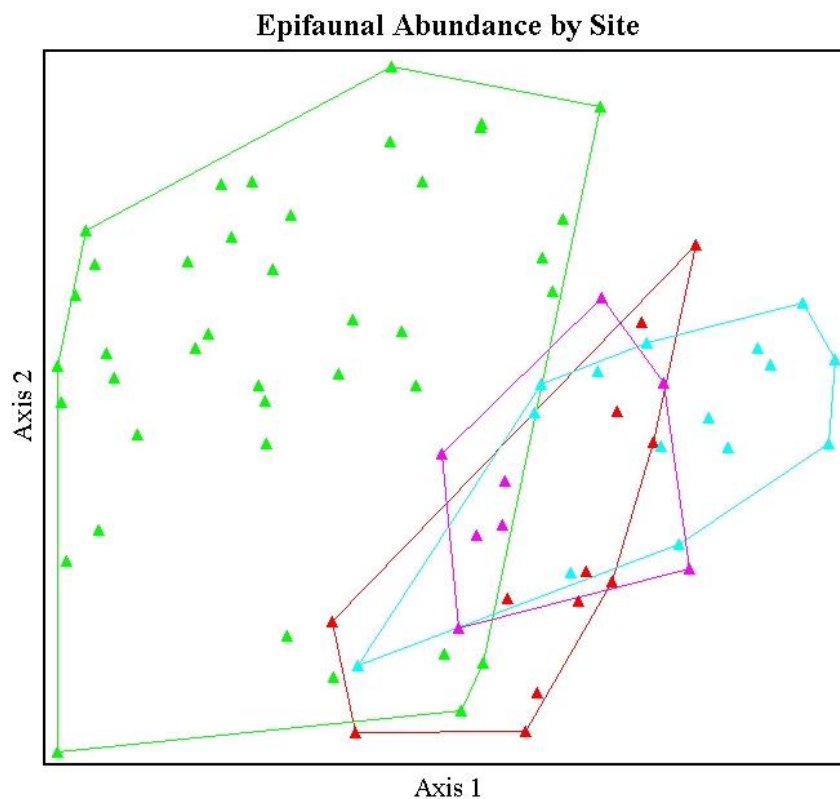
Figure 12. nMDS ordination analysis of taxa abundance by site characteristics using Sorenson's (Bray-Curtis) distance measure. MCA = Green triangles, HGB = Blue triangles, CMP = Red triangles, RSS = Pink triangles.

Biodiversity

Biodiversity varied significantly across site and time (Figure 13). An ANOVA of site, month, number of nodes and mean blade length showed that month was the most significant predictor variable of biodiversity ($p<0.005$, Table 2). Mean blade length and the interactive effect between month x site were also significant ($p<0.01$). Mean biodiversity at McAllister Creek was on average 40% higher than any other site, and this relationship differed depending on month. McAllister Creek experienced the lowest biodiversity during May and June, while Cormorant Passage, Hogum Bay and Red Salmon Slough had the greatest diversity during those months. Analysis using AIC shows that a combination of predictor variables of month, site, nodes and mean blade length explains more of the pattern of biodiversity than month alone (Table 3).
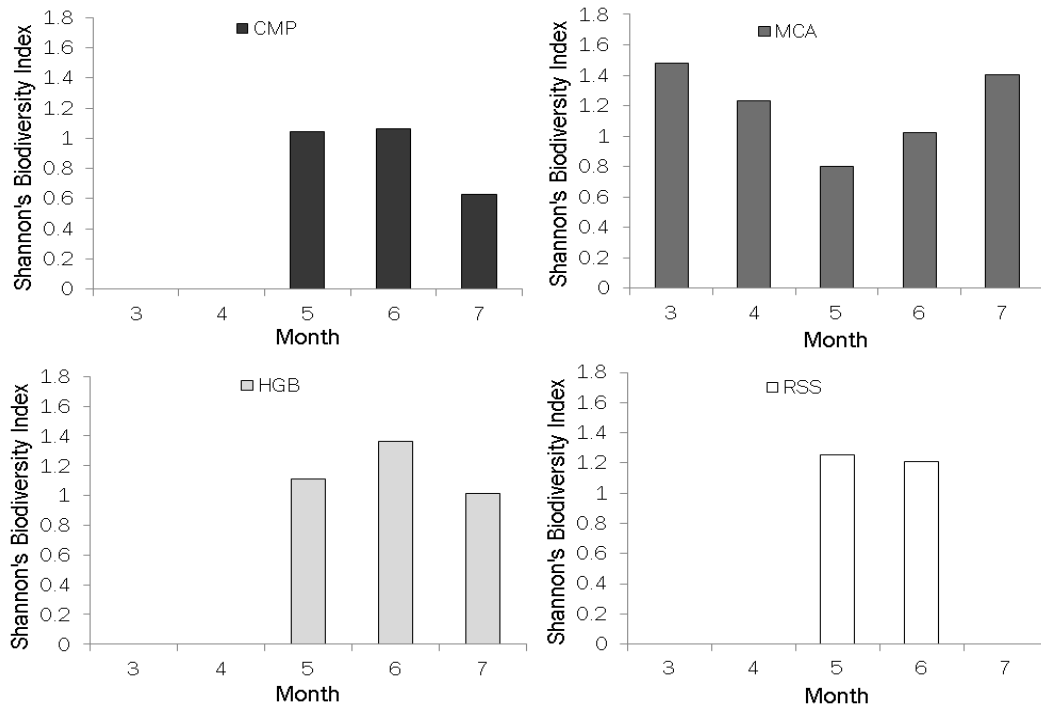
Figure 13. Mean sample diversity (as measured by Shannon's Diversity Index) for epifaunal abundance by site.

Key Species Analysis

Arthropoda

Arthropods exhibited both highest overall abundances and contained the largest number of individuals identified to a lower taxonomic level, allowing for additional analysis of community structure for this phylum. Arthropod abundance increased in May and remained high through July, with the exception of McAllister Creek, where arthropod abundance dipped in June. Hogum Bay populations were two magnitudes higher than any other site (Figure 14). Arthropod abundance varied seasonally by site, and was positively correlated with number of nodes per plant ($p < 0.05$, Table 4). The interactive effect of month, site and nodes was also found to be significant, but was rejected due to the small sample size and high volume of null values in the generalized linear model.
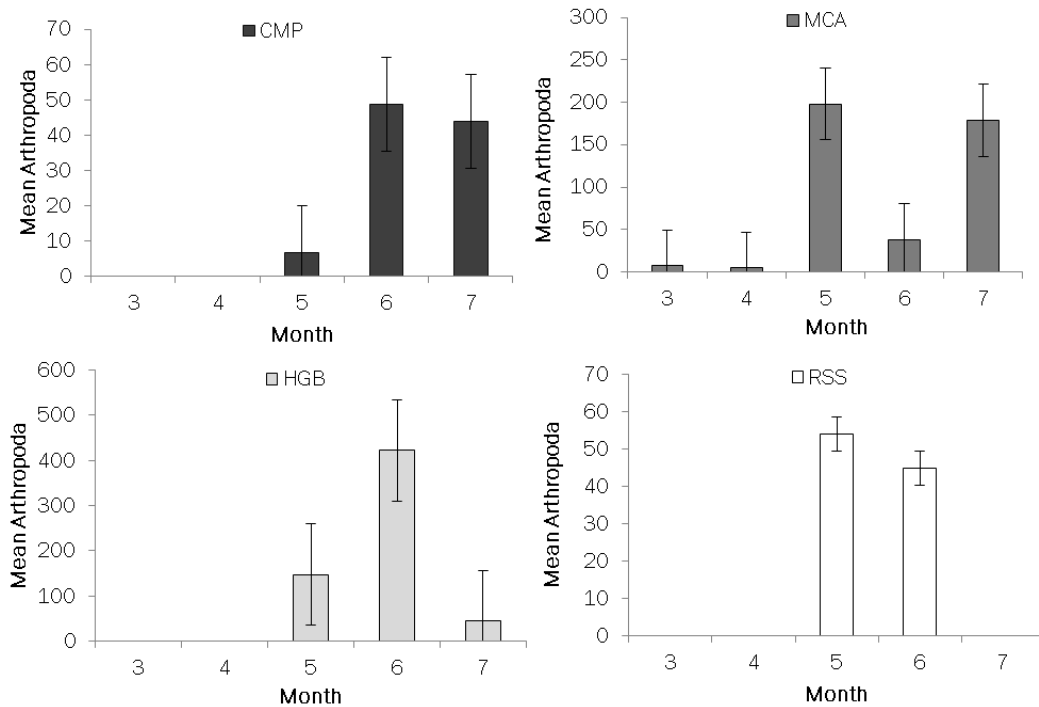
Figure 14. Mean Arthropoda abundance by site & month, +/- s.e.

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Arthropod community structure varied significantly across months (p<0.05). March and April were dominated by high densities of ostracods and tanaids, which shifted shifted to a population dominated by high densities of copepods, caprellids and amphipods from May through July (p<0.05, Figure 15).
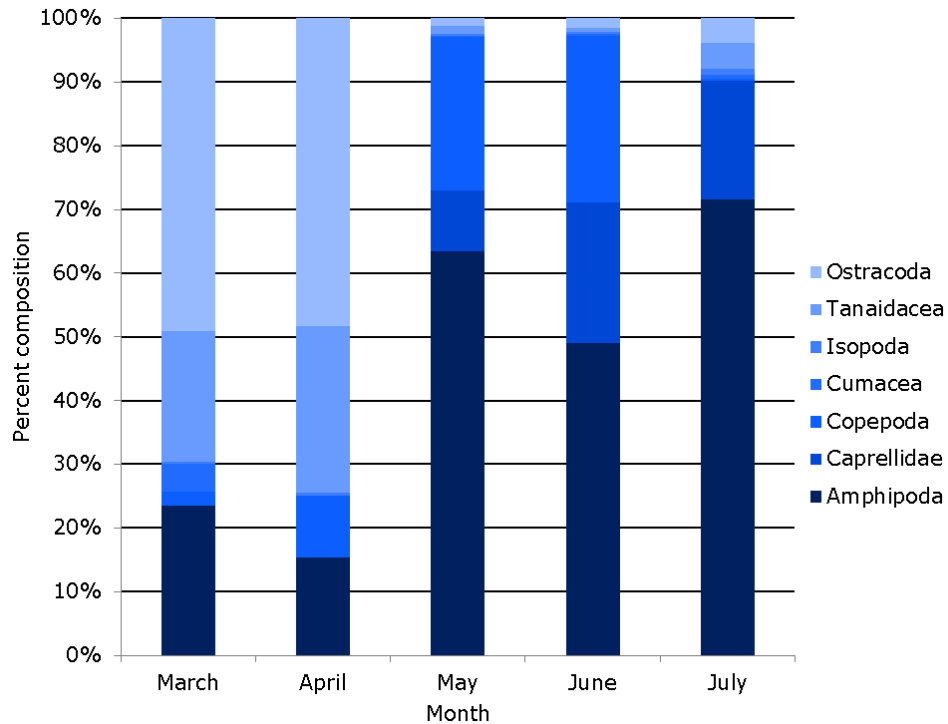
Figure 15. Percent composition of Arthropoda taxa by month across all sites and sampling months.

Amphipod abundance varied seasonally, and the degree of variation was dependent upon site. Populations were low in March and April, and then increased to a maximum mean abundance of 687 individuals in July. Abundance varied dramatically by site, with lowest overall abundance observed at Cormorant Passage. Hogum Bay had the highest overall abundances, and was two orders of magnitude higher (p<0.005, Figure 16). Analysis of candidate generalized linear models showed that site was the most

significant predictor variable in amphipod abundance (p<0.05, Table 5). We observed a

positive relationship between blade length and amphipod abundance. Any difference in

amphipod abundance due to number of nodes was explained by site differences, since

node number varied widely across sites from a minimum mean of 6.5 nodes at Cormorant

Passage to a maximum mean of 7.6 nodes at Red Salmon Slough (p<0.05).
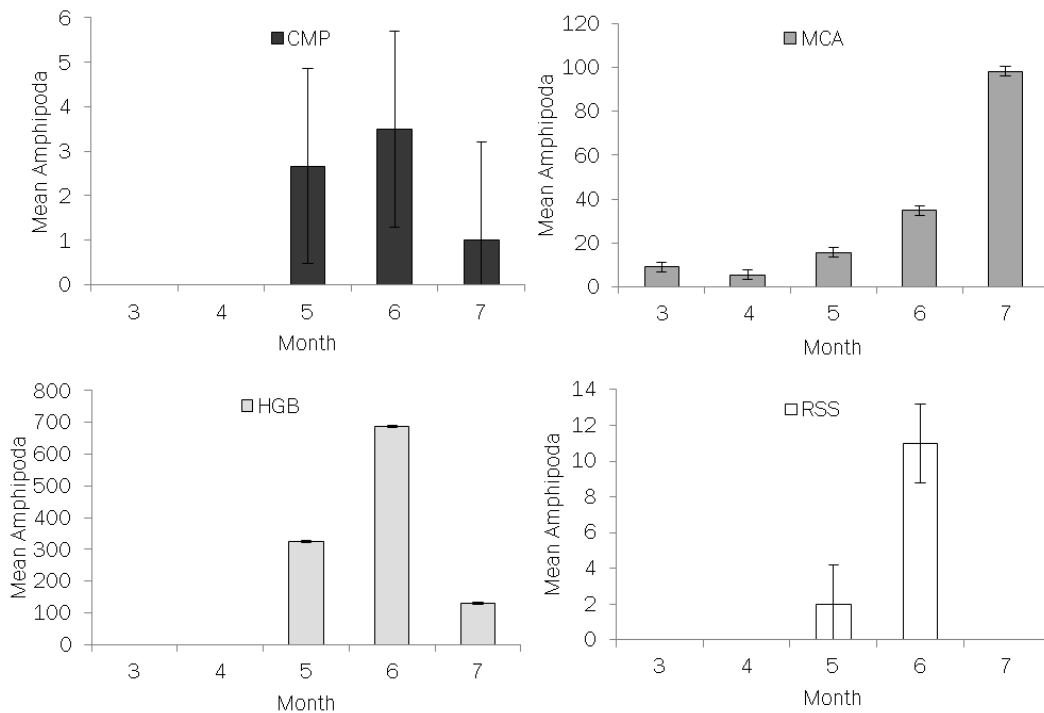
Figure 16. Mean Amphipoda abundance by site and month, +/- s.e.

Copepoda

Copepod abundance varied significantly over time, and the degree of this

variation was dependent on site (p<0.05). Populations at Cormorant Passage and

McAllister Creek steadily increased across the sampling period, while Hogum Bay

populations declined in July. Red Salmon Slough abundance was only recorded for May,

although this is likely due to a lack of samples processed for July at this time (Figure 17).

Copepod abundance was positively correlated with blade length and number of nodes. A

full interactive model of month x site x nodes was indicated as a better fit for abundance

patterns, but was rejected due to a small sample size that limited statistical certainty

(Table 6).
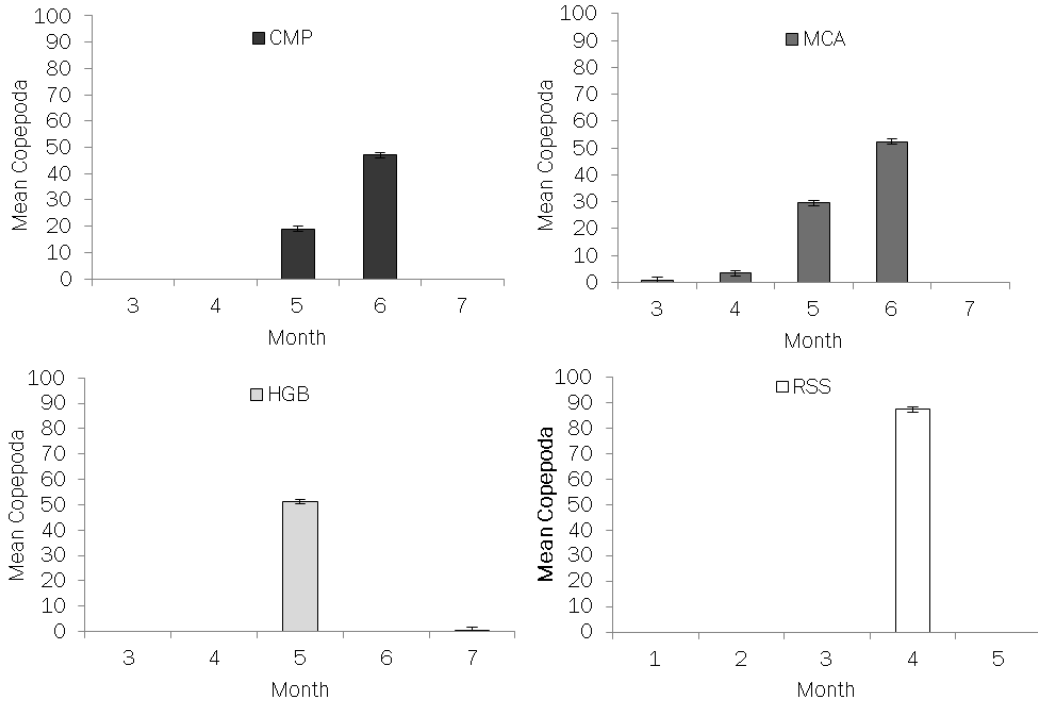
Figure 17. Mean Copepoda abundance by site & month, +/- s.e.

Polychaeta

Polychaete abundance varied significantly over time, and the degree of this

variation was dependent on site (p<0.05). Abundance increased through time for

Cormorant Passage, McAllister Creek and Red Salmon Slough, while abundance peaked

in June at Hogum Bay (Figure 18). The interactive model of month, site and nodes was

the best predictor of polychaete abundance (Table 7).
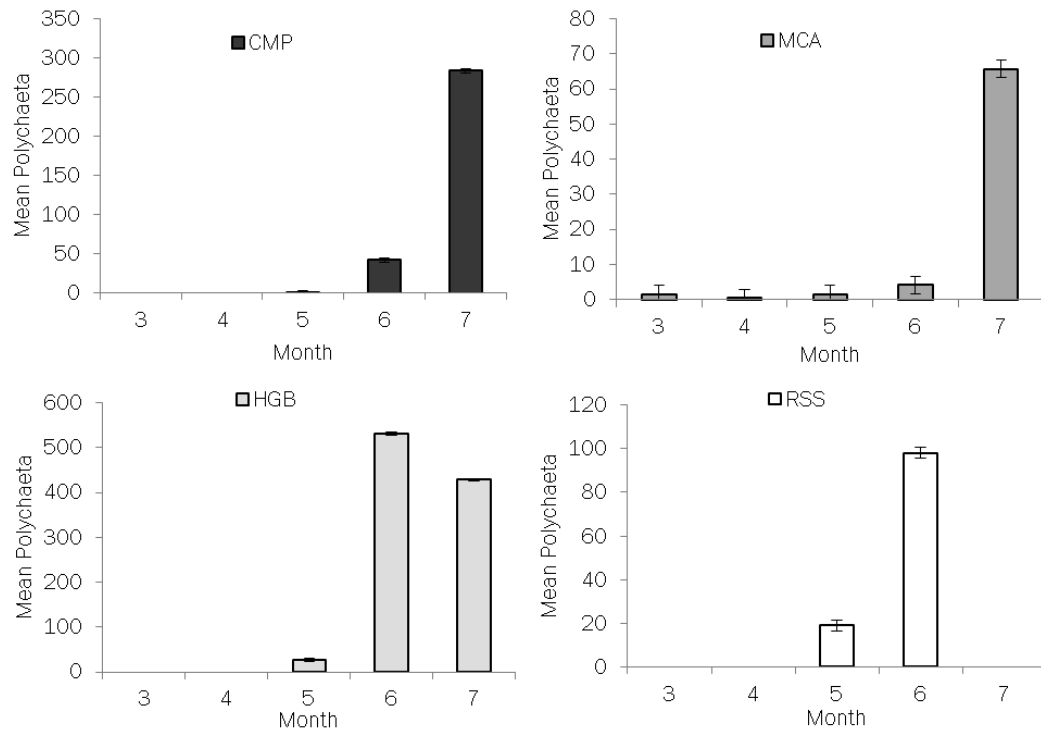
Figure 18. Mean Polychaeta abundance by site & month, +/- s.e.

Ostracoda

McAllister Creek was sampled over a longer period of time, and exhibited two peaks in abundance both early in the season (March/April) and late in the season (July) with low abundances of ostracods between those months. Conversely, Cormorant Passage, Hogum Bay and Red Salmon Slough only had ostracods present in June, when abundance was lowest at McAllister (Figure 19). Ostracod abundance varied over time, and the degree of this variation was again dependent on site differences. A full interactive model incorporating month x site + mean blade length + salinity^2 was a better fit based on the AIC index, but this model was rejected due to a high proportion of blanks in the output model summary, which suggests that the sample size for this analysis was too small.
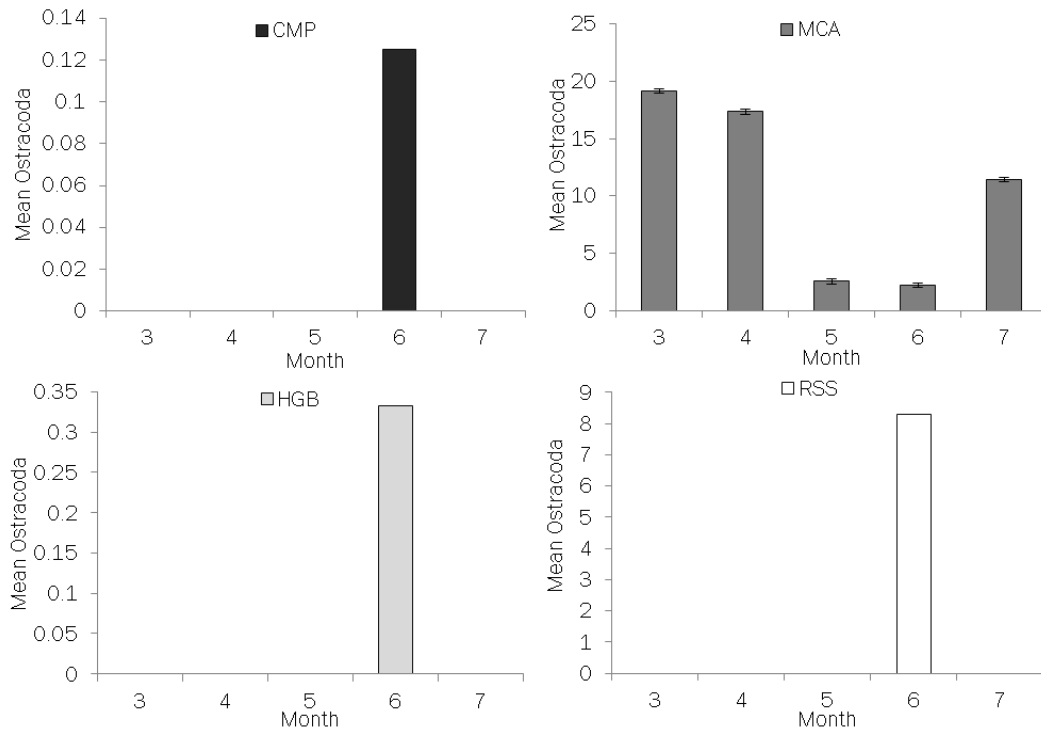
Figure 19. Mean Ostracoda abundance by site & month, +/- s.e.

Tanaidacea

Tanaid abundance followed a similar pattern to that of ostracods. McAllister Creek was the only site to exhibit two peaks of abundance early (March/April) and later in the season (July) with low abundances in the intervening months. Cormorant Passage, Hogum Bay and Red Salmon Slough all peaked in abundance in June or July, while McAllister Creek abundances were 2 magnitudes greater than the other three sites (Figure 20). This variation was dependent on an interaction effect of month and site, and was positively correlated to mean number of nodes ($p < 0.05$).
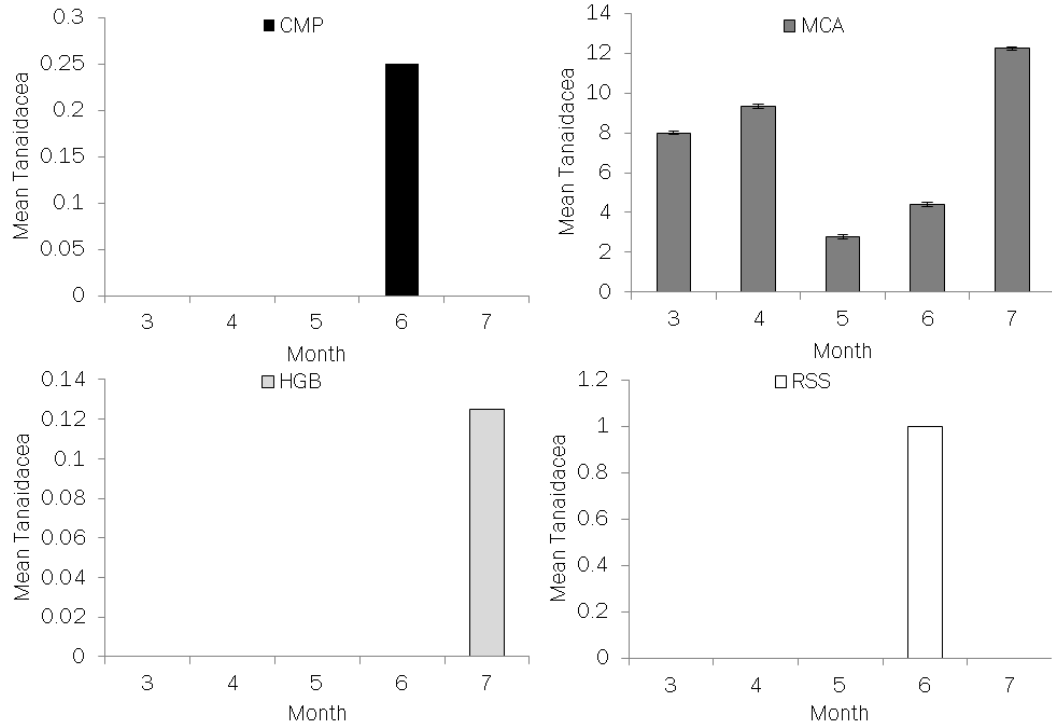
Figure 20. Mean Tanaidacea abundance by site & month, +/- standard error.

Biophysical Variables by Site

Temperature increased steadily from March through July across all sites (Figure 21). We observed a significant change in water temperature through time that was strongly dependent on site ($p<0.01$). A one-way analysis of variance showed that these seasonal differences varied by site (Table 9). McAllister Creek showed the greatest variation over time of any other site ($p<0.05$). Temperature through time was dependent on site, and month was the greatest predictor of temperature.
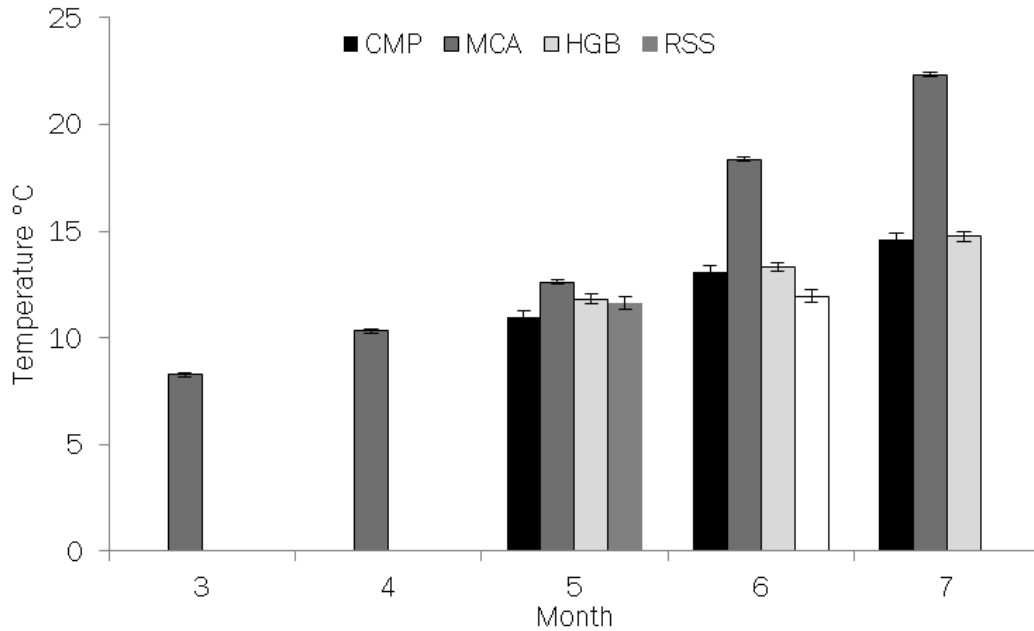
Figure 21. Mean temperature by site and month. Error bars signify +/- s.e.

Salinity varied significantly over time by month and site (p<0.01, Figure 22). An overall trend towards decreasing salinity measurements supports our assumption that sites located closer to the Nisqually Delta (McAllister Creek & Red Salmon Slough) experienced greater freshwater input than sites located farther from the Nisqually River and McAllister Creek (Cormorant Passage & Hogum Bay). A one-way ANOVA found that both month (p<0.05) and site (p<0.01) were significant predictors of salinity (Table 11). Of the candidate linear models used, the full interactive model using month x site was the best predictor of salinity at each site (p<0.05; p<0.01). We conducted the same analysis for node number and mean blade length, and found no statistically significant results, indicating that month and site were not significant predictors of eelgrass node number or mean blade length.
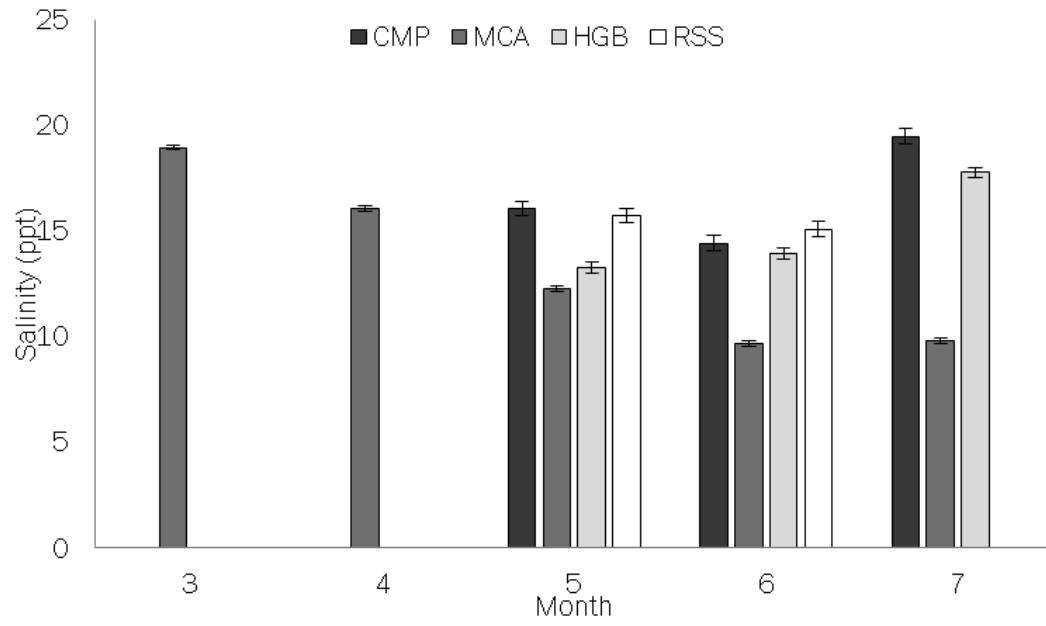
Figure 22. Mean Salinity by site and month. Error bars signify +/- standard error.

V. DISCUSSION

Site Variations

Epifaunal communities play an important role in providing foraging opportunity for outmigrating juvenile salmon in Puget Sound In this study, we observed an abundance of epifaunal invertebrates across all four sampling sites, including known salmon prey species such as amphipods, copepods, ostracods, tanaids and polychaetes. These abundances varied widely for each species through time, and were not evenly distributed across sites. Among the four eelgrass beds sampled, Hogum Bay exhibited the greatest difference in monthly invertebrate community structure with peak densities in June, while the remaining sites of McAllister Creek, Cormorant Passage and Red Salmon Slough saw increases in available prey biomass throughout the sampling period.

Hogum Bay site characteristics of mean shoot density, nodes, mean blade length, temperature and salinity were all similar to the biological ranges observed at the other three eelgrass beds. This suggests that there are other drivers beyond site characteristics that support higher epifaunal abundances. Hogum Bay is adjacent to 300 acres of commercial shellfish aquaculture property operated by National Fish and Oyster that produces oysters, manila clams and geoduck. Studies have shown that filter feeders such as bivalves exert a top-down control on aquatic vegetation by removing phytoplankton and particulate organic matter from the water column, thereby increasing light penetration and resulting in a more amenable environment for aquatic plant growth (Newell, 2004). This enhancement could be responsible for the greater surface area and epifaunal invertebrate abundances at Hogum Bay.

Our site at McAllister Creek is also located adjacent to the aquaculture farm; however, this site did not support epifaunal abundances as great as Hogum Bay. Invertebrate densities at McAllister Creek were at least one magnitude higher than at Cormorant Passage or Red Salmon Slough. This site is also impacted by its proximity to the mouth of the spring-fed McAllister Creek, which runs north into Puget Sound. Unlike rivers, spring-fed creeks supply a seasonally constant source of freshwater, which may limit any impact of the shellfish aquaculture from interacting with this site. This was reinforced by the ordination results of our site epifaunal communities, which confirmed a clear separation of McAllister Creek site from the other delta sites. Anecdotal observations indicated that water clarity was lowest at McAllister Creek, likely due to the constant current from the creek mouth and associated turbidity. Further studies that assess light penetration depth in Nisqually eelgrass beds are recommended for a clearer understanding of these dynamics.

Patterns of Epifaunal Diversity

Eelgrass beds on the Nisqually River Delta varied strongly in epifaunal community composition and total abundance, and this abundance varied widely over time and among all sites. Overall community structure was characterized by a greater abundance of relatively few dominant taxa per site, and followed patterns observed at eelgrass sites in the San Francisco Bay Estuary (Carr & Boyer, 2011). Abundance was significantly impacted by the interactive effect between time of year and site for all invertebrates. Taxa dominance can be attributed to a number of factors of biological factors that can facilitate the availability of select species during different months. Biological communities are largely influenced by the accessibility of prey resources and

nutrient sources. Many ostracod species have greater abundances during March and April with a dip in populations during May and June when species reproduce (Hull, 1997) We observed this pattern with ostracod populations across our four eelgrass sites, although ostracod populations did not regain dominance later in the season. Rates of development for epifaunal species have also been linked to temperature fluctuations, where increased temperatures can negatively affect hatch and development rates for juveniles due to increasing water temperature and salinity fluctuations over the spring to summer period (Hull, 1997).

Suggestions for Further Research

This project serves as a starting point for examining post-restoration epifaunal invertebrate abundance within and among Nisqually eelgrass beds. These data have a variety of applications to help strengthen the link between invertebrate prey communities and patterns of juvenile salmon abundance in nearshore vegetated habitats, but additional studies are needed to identify the direct contribution of eelgrass prey sources to salmon during periods of outmigration. Lampara netting is a technique used to sample fish in nearshore areas inaccessible to beach seining, and was conducted at all eelgrass sites during 2014. It is our hope that catch data can be compared to our findings of epifaunal species composition to evaluate whether juvenile salmon occur in eelgrass beds during periods when preferred prey species are present in high abundances. Fish gut contents were also collected in 2014, and have the potential to be used in gut content analysis to calculate a percent similarity index of observed prey species within eelgrass beds and salmon diets. Since salmon have been shown to move rapidly into habitats with greater preferred prey abundances, we would expect to see an increase in salmonid abundance

during periods dominated by high abundances of amphipods and copepods (Brennan et al., 2004).

Eelgrass bed size was mapped at Nisqually during 2014, and could be used to explore the relationship between eelgrass patch size and epifaunal abundance, which has the potential to inform which eelgrass beds may provide the greatest benefit to resident salmon for prioritizing ongoing management projects. The UGSG is sampling the same parameters of epifaunal invertebrates and eelgrass site characteristics for 2015, which will allow for a multi-year comparison of abundance and diversity that may help predict how Nisqually eelgrass invertebrate communities change over time.

The abundance of amphipods, copepods and polychaetes observed in May through July overlaps with periods of delta utilization during salmon outmigration (May through September), and supports our conclusion that eelgrass beds of the Nisqually delta have the potential to provide a valuable source of prey for juvenile salmon that utilize these habitats as a transitional habitat during outmigration. We can expect this forage opportunity to increase through time, as eelgrass bed extent continues to increase following the 2009 delta restoration (Christiaen et al., 2015). These data reinforce the ecological value of restoration of these habitats for the management and support of threatened salmon stocks in Puget Sound.

Table 1. Multivariate Analysis (MANOVA) of the effects of site, month, nodes and mean blade length on epifaunal invertebrate abundance.

	Df	Pillai	Approx F	Num DF	Den DF	Pr(>F)
Month	4	1.4263	1.9234	68	236	0.0003129*
Site	3	1.4686	3.2717	51	174	3.943e-09 *
MSD	1	0.32094	1.6125	17	58	0.09059
Nodes	1	0.49584	3.3555	17	58	0.0002946 *

Table 2. One-way ANOVA output for Shannon's biodiversity index and site characteristics of month, site, nodes, mean blade length and Month x Site. Asterisk denotes significant values.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Month	4	1.918	0.4794	5.171	0.00118*
Site	3	0.14	0.0468	0.505	0.68055
Nodes	1	0.138	0.1383	1.492	0.22659
Mean Blade Length (MBL)	1	0.937	0.9372	10.108	0.00232*
Month x Site	5	1.546	0.3093	3.335	0.00996*
Month x MBL	4	0.528	0.132	1.281	0.28954
Site x MBL	3	0.505	0.1683	1.634	0.19283
Month x Site x MBL	3	0.17	0.0567	0.551	0.64997

Table 3. AIC model selection of one-way ANOVA fit of Shannon's Biodiversity Index. Asterisk denotes best fit model.

	Df	AIC
Month x Site	14	58.02555
Month x Site + Nodes	15	54.4779
Month x Site x Nodes	25	62.82086
Month x Site + MBL	15	59.51478
Month x Site x MBL	25	64.12012
Month x Site + Nodes + MBL	16	50.22716 *

Table 4. AIC test of linear fit models of influences on Arthropoda abundance. Asterisk denotes best fit model.

	Df	AIC
Site	2	15269.89
Month + Site	8	15133.92
Month x Site	13	12683.14
Nodes + Site	5	14101.55
Nodes x Site	8	12361.37 *
MBL + Site	5	18213.26
MBL x Site	8	17577.63

Table 5. AIC model selection of linear fit models of influences on Amphipoda abundance. Asterisk denotes best fit model.

	Df	AIC
Site	4	5931.25
Month + Site	8	4218.57
Month x Site	13	2565.841*
Nodes + Site	5	5294.481
Nodes x Site	8	5262.67
MBL+ Site	5	5932.53
MBL x Site	8	5906.374

Table 6. AIC model selection of linear fit models of influences on Copepod abundance. Asterisk denotes best fit model.

	Df	AIC
Site	4	6648.059
Month + Site	8	5454.787
Month x Site	2	3837.772 *
Nodes + Site	13	4898.091
Nodes x Site	5	6368.736
MBL + Site	8	8725.354
MBL x Site	5	6633.271

Table 7. AIC test of linear fit models of influences on Polychaeta abundance. Asterisk denotes best fit model.

	Df	AIC
Nodes	2	18231.517
Month x Site	12	3818.25
Month x Site + Nodes	8	8735.551
Month x Site + MBL	14	3737.537
Month x Site x Nodes	24	3054.403 *
Month x Site + MBL	14	3820.183
Month x Site + Temp^2	15	3301.505

Table 8. One-way ANOVA of the effect of month, site and month x site on temperature

	Df	F	P
Month	4	91.248	< 2e-16 *
Site	3	31.914	1.14e-12 *
Month x Site	5	6.646	5.00e-05 *

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Appendices

Appendix A. Epifaunal invertebrate species observed, broad taxonomic group and classification by site, March – July 2015.

Phylum	Species	Taxonomic Group	Classification Level	CMP	MCA	HGB	RSS
Annelida	Eteone	Polychaeta	Genus	X		X	
	Neanthes	Polychaeta	Genus	X	X	X	
	Oligochaeta	Annelida	SubClass	X	X	X	X
	Piscolidae	Annelida	Family		X		
	Polychaeta	Polychaeta	SubClass	X	X	X	X
Arthropoda	Americorophium	Amphipoda	Genus				X
	Ampeliscidae	Amphipoda	Family	X			
	Amphipoda	Amphipoda	Order	X	X	X	X
	Ampithoe lacertosa	Ampithoidae	Species	X	X	X	X
	Ampithoe valida	Amphipoda	Species		X		
	Ampithoidae	Amphipoda	Family		X		
	Aoridae	Amphipoda	Family			X	
	Aoroides columbiae	Amphipoda	Species			X	
	Caprellidae	Caprellidae	Family	X		X	X
	Chironomidae	Diptera	Family	X			
	Cirripedia	Arthropoda	InfraClass		X	X	
	Copepoda	Arthropoda	SubClass	X	X	X	X
	Corophiidae	Amphipoda	Family		X	X	X
	Cumacea	Cumacea	Order		X	X	X
	Eobrolgus	Amphipoda	Genus		X		
	Eogammarus	Amphipoda	Genus	X	X	X	
	Grandidierella japonica	Amphipoda	Species	X	X	X	X
	Harpacticoida	Copepoda	Order	X	X	X	X
	Idotea	Isopoda	Genus	X	X	X	
	Isaeidae	Amphipoda	Family	X		X	
	Isopoda	Isopoda	Order	X		X	
	Leptochelia	Arthropoda	Genus		X		
	Lysianassoidea	Amphipoda	Superfamily			X	
	Monocorophium	Amphipoda	Genus	X	X		X
	Ophelina	Amphipoda	Genus		X		
	Ostracoda	Ostracoda	Order	X	X	X	X
	Pagarus	Arthropoda	Genus		X		X
	Tanaidaceae	Tanaidaceae	Order	X	X	X	X
Chordata	Ascidiacea	Tunicata	Class		X	X	X
Mollusca	Bivalvia	Mollusca	Class	X	X	X	X
	Gastropoda	Gastropoda	Class	X	X	X	X
	Mytilus	Mollusca	Genus		X		
	Nudibranchia	Nudibranchia	Order	X		X	
Nematoda	Nematoda	Nematoda	Phylum	X	X	X	X
Platyhelminthes	Platyhelminthes	Platyhelminthes	Phylum	X	X	X	
	Trematoda	Platyhelminthes	Class	X			

Appendix B. Eelgrass field collection techniques: a-b) Collecting eelgrass in the field. c) Freshwater station & sieve. d). Measuring eelgrass blade length and node number. d) Processed eelgrass prepared for drying.
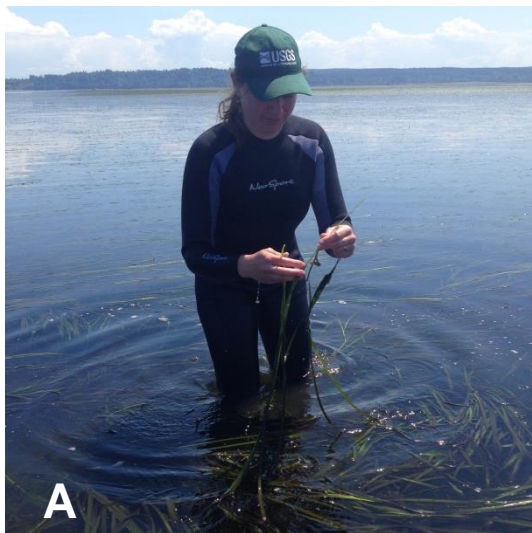
Appendix C. Eelgrass beds at a) Hogum Bay and b) Cormorant Passage.