NATURAL RESOURCES

Effects of Outfalls and Effluent on Eelgrass (*Zostera marina* L.): A Literature Review

August 17, 2012

WASHINGTON STATE DEPARTMENT OF
Natural Resources
Peter Goldmark - Commissioner of Public Lands

Effects of Outfalls and Effluent on Eelgrass (*Zostera marina* L.): A Literature Review

August 17, 2012

Jeffrey Gaeckle

Nearshore Habitat Program
Aquatic Resources Division

WASHINGTON STATE DEPARTMENT OF
Natural Resources
Peter Goldmark - Commissioner of Public Lands

Acknowledgements

The Nearshore Habitat Program is part of the Washington State Department of Natural Resources' (DNR) Aquatic Resources Division, the steward for state-owned aquatic lands. A special thanks to colleagues Helen Berry, Pete Dowty, Fred Short, Lisa Ferrier, Jessica Demetro Stowe, Andrew Ryan and Heather Gibbs for their careful review and thoughtful comments that greatly improved earlier versions of this document.

The study was made possible through a grant with the Puget Sound Marine and Nearshore Protection and Restoration Program under Interagency Agreements 12-1177 (WDFW) and 12-292 (DNR).

This project has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement PC 00J29801 to Washington Department of Fish and Wildlife. The contents of this document do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Washington State Department of Natural Resources
Aquatic Resources Division
1111 Washington St. SE
P.O. Box 47027
Olympia, WA 98504-7027

www.dnr.wa.gov

Contents

EXECUTIVE SUMMARY

The Washington State Department of Natural Resources (DNR) is steward of 2.6 million acres of state-owned aquatic land. DNR manages these aquatic lands for the benefit of current and future citizens of Washington State. DNR’s stewardship responsibilities include protection of eelgrass (*Zostera marina* L.), an ecologically important nearshore habitat in greater Puget Sound. In 2010, DNR became the monitoring lead for the Puget Sound eelgrass ecosystem indicator (PSP Indicator Action Team 2010), and contributes to efforts that aim to achieve the goals set by the Puget Sound Partnership and supports the conservation and restoration of eelgrass.

There is little knowledge available in regard to the effects of certain stressors on eelgrass in Puget Sound (Thom et al. 2011), and the effects of nutrients (nitrogen and phosphorus), metals (e.g., Cd, Cu, Cr, Hg, Ni, Pb, and Zn), and contaminants (e.g., PAHs, PCBs, PBDEs) from outfalls is of particular interest. In an effort to meet its land stewardship responsibilities and to support the Partnership’s goal to increase eelgrass area by 20% by 2020, DNR has identified a need for more information on the effects nutrients, metals, and contaminants have on eelgrass in greater Puget Sound. The purpose of this document is to provide an overview of available scientific literature on the changes outfalls and effluent have on the physical environment and the observed concentrations and effects of nutrients, metals and contaminants on eelgrass. It is also intended to provide guidance to the design of field surveys and biogeochemical analysis of eelgrass to advance the understanding of outfalls and effluent in greater Puget Sound.

Outfalls that discharge residential, commercial, and industrial wastewater as well as upland stormwater are abundant throughout Puget Sound. The construction of the outfall, the chemical composition and volume of the discharge waters, and the properties of the receiving waters all determine what effect the effluent will have on the marine environment. For seagrasses, the uptake rates and concentrations of nutrients, metals, and contaminants are subject to a high degree of variability due to a number of factors. Research has demonstrated the uptake of these substances and the physiological effects on seagrass, but these data tend to be species and location specific. Little is known about the concentrations of nutrients, metals, and contaminants in eelgrass in the Pacific Northwest (Kaldy 2006) and more specifically, in greater Puget Sound. Basic nutrients, nitrogen and phosphorus, are likely abundant in Puget Sound, but whether these substances are at levels that cause toxicity in eelgrass is unknown. The nearshore environment where eelgrass grows will experience the greatest affects from metals and contaminants loading because of the proximity to input sources (Ecology and King County 2011, Mohamedali et al. 2011). Yet no research has been done to understand the concentration of nutrients, metals, and contaminants in eelgrass and the potential effects they have on the health of this dominant flora.

Although tissue nutrient content values for eelgrass are considerably more variable than other seagrasses, it is recommended that an initial baseline assessment of nutrients and $\delta^{15}N$ in eelgrass be performed to determine whether plants are exposed to nutrient enrichment (Duarte 1990, Udy and Dennison 1997). In addition, the assessment of metals (e.g., Cd, Cu, Cr, Hg, Ni, Pb, and V) in eelgrass compartments (leaves, rhizomes/roots, epibiota) would characterize metal uptake by eelgrass in Puget Sound and identify whether metal concentrations are at levels to cause physiological impacts to seagrass (Appendices A and B; Lewis and Devereux 2009). Finally, an assessment of PAHs, PCBs, and other organic contaminants (e.g., herbicides, antifouling paints), substances that affect seagrass metabolic processes individually and when combined (Appendix C; Lewis and Devereux 2009), would provide more information on factors that impact eelgrass in Puget Sound. The recommended assessments of nutrient, metal and contaminant concentrations in eelgrass will provide valuable baseline information for decision makers to effectively manage and protect this ecologically significant resource in Puget Sound.

1 Introduction

The Washington State Department of Natural Resources (DNR) stewards 2.6 million acres of state-owned aquatic land for the benefit of current and future citizens of Washington State. Eelgrass (*Zostera marina* L.), the dominant seagrass in the Pacific Northwest region, is an important component of both public and private nearshore aquatic lands in greater Puget Sound. Eelgrass and other seagrasses are known to provide extensive ecosystem services worldwide (Costanza et al. 1997, Green and Short 2003, Larkum et al. 2006). It has been well documented that eelgrass stabilizes sediments and filters marine waters (Short and Short 1984). In Puget Sound specifically, eelgrass provides spawning grounds for Pacific herring (*Clupea harengus* pallasi), shelter for egg-bearing Dungeness Crab (*Cancer magister*), out-migrating corridors for juvenile salmon (*Oncorhynchus* spp.), and important feeding and foraging habitats for waterbirds such as the Black Brant (*Branta bernicla*) and Great Blue Heron (*Ardea herodias*) (Armstrong et al. 1988, MacKay 1942, Phillips 1984, Simenstad 1994, Wilson and Atkinson 1995, Butler 1995).

As steward of state-owned aquatic lands, DNR is committed to monitor eelgrass in Puget Sound through the Puget Sound Ecosystem Monitoring Program (PSEMP) and supports efforts to achieve the Puget Sound Partnership goals. The Partnership has recognized the global ecological significance of eelgrass (Dennison et al. 1993, Krause-Jensen et al. 2005, Orth et al. 2006) and identified it as one of 21 Dashboard of Ecosystem Indicators. Through scientific support by DNR, the Partnership established a target for 20% more eelgrass by 2020.

Limited knowledge is available in regard to the effects of certain stressors on eelgrass in Puget Sound (Thom et al. 2011), but evidence has linked losses and impacts in eelgrass and other seagrass populations to pollution (Grady 1980, Marshall et al. 1993). To date, there has only been one study in Puget Sound that assessed the concentration of metals and organic compounds in eelgrass (USFWS 1994). One area with a significant data gap is the management of outfalls, in particular, the impacts outfall infrastructure and effluent have on eelgrass. Outfalls are considered structures that convey wastewater and surface and stormwater to Puget Sound (Carmichael et al. 2009). Some examples of outfalls include: municipal or industrial wastewater outfalls, surface and stormwater outfalls, and combined sewer overflows outfalls. The effluent discharged from outfalls can be treated from wastewater treatment facilities or untreated in the case of stormwater outfalls or overburdened combined sewer overflow outfalls. To date there has been some assessment of outfalls (Carmichael et al. 2009) and contaminant loading (Hart Crowser et al. 2007, EnvironVision et al. 2008) and nutrient loading (Mohamedali et al. 2011) from outfalls into Puget Sound, but there is limited information how eelgrass is affected by outfall construction and outfall effluent.

Therefore, DNR has identified a need for more information on the effects of the construction and physical presence of outfalls and the impacts their effluent (e.g., nutrients, metals, and organic contaminants) have on eelgrass throughout the Sound. Baseline information, such as the concentration of nutrients, metals and organic compounds in eelgrass, will provide value in understanding the presence of these substances in Puget Sound and their potential to reach levels that will affect eelgrass. The additional eelgrass stressor data will improve management of this resource on state-owned aquatic lands and provide support in the Partnership's goal to increase eelgrass area by 20% by 2020.

The initial effort of the project is to conduct a literature review to understand how outfall construction and effluent (e.g., nutrients, metals, and contaminants) affects eelgrass. The literature review summarized in this report will be used to prepare and complete the Quality Assurance Project Plan (QAPP) for the GIS, field, and laboratory components of the project (Deliverable 1.2 of the Interagency Agreement between DNR and WDFW).

2 Review

The literature review focuses on the effects of outfalls and effluent on seagrass in general and eelgrass specifically. The broad categories in the review will include: 1) changes outfalls have on physical habitat, 2) the effects nutrients, metals and contaminants have on eelgrass, and 3) the effects the trophic transfer of metals and contaminants have on seagrass ecosystems.

2.1 Physical Alteration

The installation of an outfall into the marine environment will cause physical alteration to that habitat during the construction and operation phase of the outfall. However, the impacts to the marine environment related to each phase will be quite different. Construction of an outfall into the marine environment entails installation of a discharge pipe on or beneath the seafloor with a diffuser head at the terminal end where effluent mixes with the receiving waters.

The areas where outfalls have the greatest impact during construction and installation phase include: 1) the transition from land to water, 2) the distance along or beneath the seafloor, and 3) at the location where effluent is discharged (e.g., end of outfall pipe or diffuser head). During operation, the greatest impact will occur in the vicinity of the diffuser head where the effluent mixes with the receiving waters. The area of impact in the receiving waters is dependent upon the volume, flow rate, concentration and type of discharge from the outfall.

2.1.1 Outfall Construction and Installation

The construction of an outfall through a seagrass meadow will have direct impacts because of physical damage to the plants, removal of the suitable sediments, and a reduction of available light from turbidity and construction platforms necessary for pipe installation. Seagrass meadows located within the vicinity of an outfall construction zone risk impacts related to light reduction from turbidity and work platforms. Below are a few examples from Puget Sound of completed or proposed outfall installation or upgrade projects that highlight some of the effects to seagrass beds.

Brightwater

In 2008, the installation of the Brightwater Wastewater Treatment Plant, Point Wells, Snohomish – King County, outfall required the discharge pipe to be trenched and buried across a narrow nearshore area vegetated with patchy eelgrass beds that ranged in depth from -0.5 to -3.5 m (relative to Mean Lower Low Water, MLLW) (King County 2003). An Eelgrass Study Area of 10,300 m^2 was designated to include the Marine Outfall Corridor, a 7 m wide swath for the outfall trench, plus an additional 64 m on either side to account for damage from trenching and construction activities (e.g., excess sediment resuspension and turbidity from dredging and prop wash, light inhibition from turbidity and work construction platforms) (King County 2008). Prior to trenching, all the eelgrass in the Marine Outfall Corridor was removed and stockpiled in outdoor mesocosms to propagate 8,000 shoots for the post-construction transplant. The 2009 transplant followed the preliminary eelgrass survey pattern of two distinct bands of eelgrass, and surveys performed after transplanting found similar distribution of eelgrass to pre-construction surveys but higher shoot densities due to intentional over-planting.

Eastsound Sewer Outfall Pipe Replacement Project

The Eastsound Sewer and Water District, Orcas Island, plans to replace its current outfall pipe (20 cm diameter, 520 m offshore, -12 m MLLW deep) with a new one (30 cm diameter, 160 m offshore, -3 m MLLW deep) placed 30 cm below grade (Jen Jay, Inc. 2012a, b). The proposed location of the upgraded outfall diffuser is on a band of bare sediment 60 m wide between two eelgrass beds. The hydrodynamics of the receiving waters places the eelgrass in the chronic mixing zone which extends to a distance of 64 m from the diffuser head. Although the proposal indicates the new outfall will be installed via directional drilling, thereby avoiding direct removal or destruction of plants, there remains the potential of nutrient, metal, and contaminant loading to the existing eelgrass from the effluent. Furthermore, turbidity and construction platforms at the outfall could cause a reduction in available light to the eelgrass and create a potential long-term impact.

Port Townsend outfall replacement Project

The city of Port Townsend needs to replace an existing outfall pipe (45 cm diameter, 245 m offshore, -7 m MLLW deep) with an upgraded pipe (60 cm diameter, 225 m offshore, -4.5 m MLLW) (CH2MHill 2009). The proposed outfall will be trenched 225 m offshore with direct impacts to roughly 1,376 m^2 of seagrass habitat (CH2MHill 2009). The result will be short-term eelgrass and *Phyllospadix* spp. destruction which will require mitigation and possible long-term enrichment and contamination of the seagrass beds.

Post Point Outfall

In 2008, the City of Bellingham replaced its deteriorating secondary outfall (76 cm diameter) with a new outfall (140 cm diameter) that caused direct physical damage to approximately 110 m^2 of eelgrass habitat (Hart Crowser 2006, Fleming and Stutes 2011). Eelgrass transplants in the impact area had poor success due to liquefaction of backfill sediments (Fleming and Stutes 2011). Although a greater area of eelgrass was planted as part of the outfall project mitigation, there was a net loss of area 1 to 3 years after transplanting (Fleming and Stutes 2011).

2.1.2 Outfall Operation

In addition to the direct impact to seagrass from the installation and construction of the outfall pipe (Section 2.1.1) the physical impacts caused by outfall effluent include changes to the marine habitat due to scour and changes to the properties of the receiving waters (e.g., clarity and chemistry - nutrients, metals, contaminants, temperature, and salinity). The impacts to the marine environment during the operation of an intact, non-failing outfall are focused in the location of terminal end of the outfall pipe (e.g., diffuser head) and dependent upon the characteristic of the effluent being discharged. The location of the diffuser head will affect the amount of dilution based on the hydrodynamic properties of the receiving waters at the point of discharge. The source, whether residential, commercial, or industrial, and the discharge rate and concentration will determine the characteristics of the effluent and affect its impact on the environment (e.g., receiving waters). The criteria for discharge water from wastewater treatment facilities in WA State are regulated by Washington Administrative Code (WAC) Chapter 173-201A and the U.S. Environmental Protection Agency (EPA 1992).

Erosion
The most direct physical impact from outfall operation is likely a result of scour from the effluent and a direct loss of nearshore habitats (e.g., substrate, seagrass, and macroalgae). The excessive water movement may cause mechanical damage and mortality to seagrass, enhance the potential for erosion, and alter the sediment regime in the immediate vicinity of an outfall diffuser. The intricate matte produced by seagrass rhizome stabilizes sediments (Phillips 1978, McRoy and Hellferich 1980) and loss of seagrass and its belowground biomass makes these sediments susceptible to erosion (EPA-SA 1998). Although the type of seagrass and site specific conditions will determine the rate of rhizome matte degradation, there are additional concerns that once seagrass loss is initiated, subsequent erosion will lead to further seagrass loss.

Water Quality
It has been well documented that an increase in nutrients to marine systems promotes the growth of macroalgae, epiphytes, and phytoplankton causing a reduction of available light reaching seagrass and in turn affecting seagrass maintenance and growth (Bulthuis and Woelkerling 1983, van Montfrans et al. 1984, Borum 1985, Twilley et al. 1985, van Vierssen and Prins 1985, Cambridge et al. 1986, Tomasko and Lapointe 1991, Tomasko et al. 1996). The prolific growth of macroalgae, epiphytes, and phytoplankton in response to nutrient loading and the detrimental effects on eelgrass have been demonstrated in numerous field and mesocosm studies (Harlin and Thorne-Miller 1981, Short et al. 1991, 1995, Short and Burdick 1996, Silberstein et al.

1986, Neckles et al. 1993, Williams and Ruckelshaus 1993, Hauxwell et al. 2001, 2003) and in the assessments of outfalls (Vaudrey et al. 2010).

Changes to water quality due to an increase in nutrients will affect seagrass over wide spatial and long temporal scales. In Port Adelaide, South Australia, an outfall was commissioned in 1978 in an area with 85% seagrass cover consisting of four species (Neverauskas 1987a, Bryars and Neverauskas 2004). One year after the outfall pipe was commissioned roughly 0.25 ha of area around the terminal end of the outfall was denuded of the dominant seagrasses, *Posidonia* spp. and *Amphibolis* spp., and cover dropped to 50% within a 5 ha area (Neverauskas 1987a). Subsequent monitoring four years after the outfall began operation detected 365 ha of seagrass was lost and impacts were observed over an area of 1,900 ha.

The ability for seagrass ecosystems to recover from impacts caused by outfalls depends on a number of factors including the severity of the impact and the recolonization efficiency of the seagrass ecosystem. Nine years after the Port Adelaide sewage outfall was decommissioned, there were approximately 2 ha of seagrass, at 28% cover, growing around the old diffuser (Bryars and Neverauskas 2004). The recovery of habitat near the diffuser suggests seagrass loss was due to poor water quality rather than a loss of suitable sediments from scour or erosion (Bryars and Neverauskas 2004). Although there are limited accounts of seagrass recolonization and recovery from improved water quality due to decommissioned or upgraded outfalls in the Pacific Northwest, there have been documented cases from the Mediterranean Sea (Pergent-Martini and Pergent 1996), South Australia (Bryars and Neverauskas 2004, EPA-SA 1998), Long Island Sound, CT (Vaudrey et al. 2010), and Tampa Bay, FL (Greening 2000, Tampa Bay NEP 2009).

Turbidity

Turbidity, caused by suspended sediments in the water column, attenuates the photosynthetically available radiation necessary for seagrass to survive. Research has demonstrated the detrimental effects of increased turbidity on eelgrass (Moore et al. 1997) and other seagrass systems (Erftemeijer et al. 2006). Excessive turbidity can also lead to particulate fall out and seagrass burial causing total loss (Erftemeijer et al. 2006). An increase in turbidity from outfall operations can reduce the available light necessary for seagrass maintenance and growth (Neverauskas 1987b). In another case, monitoring found a 10-80% reduction in available light due to the effluent from a chemical plant that discharged 200 tons year^{-1} of silt and clay (Balestri et al. 2004).

Temperature

The optimal water temperature range for eelgrass productivity in Puget Sound is between 7ºC and 13ºC (Thom et al. 2003). However, Biebl and McRoy (1971) found that tide pool and subtidal forms of eelgrass in Izembek Lagoon, AK, can tolerate exposure to temperatures as low as -6ºC and as high as 30ºC for up to 12 h before plant mortality sets in. However, it is not uncommon for eelgrass to experience and survive short exposures (≤ 30 min) to temperatures outside this range (Biebl and McRoy 1971). In the Gulf of California, Mexico, eelgrass grows as an annual because of the unfavorably high temperatures (~28ºC) during the summer months (Santamaría-Gallegos et al. 2000).

The temperature of effluent, which varies depending upon its source and the controls dictated by permit requirements, has the potential to change the temperature of the receiving waters and ultimately impact seagrass. In Cockburn Sound, Western Australia, a refinery outfall elevated receiving water temperatures by 0.6ºC within a 100 m area and likely did not cause temperature stress to the seagrass (Cambridge et al. 1986). However, an energy generation plant outfall has a greater potential to raise receiving water temperatures due to the constant demands for electricity and the need to cool operations. A power generation facility on the Ohio River routinely observed discharge water temperatures above 40ºC with a peak at 57ºC with effects of the elevated temperatures observed 8 km from the outfall (Dayton Power & Light J.M. Stuart Station 2008). On two dates, August 9 and September 24, 2007, discharge temperatures from the power generation facility were 49ºC and 37ºC, respectively. Upstream reference sites on the respective dates recorded water temperatures of 29ºC and 25ºC, while readings as high as 35ºC and 30ºC were recorded a mile down river (Dayton Power & Light J.M. Stuart Station 2008). At Turkey Point, Biscayne Bay, FL, an electric generation plant caused seawater temperatures to increase 4˚C above the optimal temperature of 26-28 ˚C for species diversity and denuded approximately 30 ha of *Thalassia* seagrass habitat (Roessler 1974). Many variables affect the dynamics of temperature plumes in receiving waters, but it is evident that changes in the temperature of the receiving waters have physiological effects on seagrass (Marsh et al. 1986) and could cause plants to be more susceptible to disease (Rasmussen 1977).

Salinity

Although seagrass can tolerate varying environmental conditions, there is a range of salinity for optimal growth. The general range of salinity for eelgrass is 5 – 35 psu (Moore and Short 2006) and in Puget Sound the salinity range for optimal growth is 10 - 30 psu (Thom et al. 2003). Research has demonstrated prolonged salinities greater than 32 psu (Kamermans et al. 1999) or less than 5 psu (Moore et al. in review, Sand-Jensen and Borum 1983) will affect eelgrass growth and survival. One indirect effect of an increase in salinity is the enhanced prevalence of *Labyrinthula zosteracea*, a marine slime mold, which causes wasting disease and varying degrees of eelgrass die off (Burdick et al. 1993, Burdick and Short 1999, Short et al. 1988).

The change in salinity related to outfall discharge has been linked to declines in seagrass. In Alicante, Spain, the dense, high salinity plume from a desalination plant stratifies near the sea floor and has been shown to negatively affect *Posidonia oceanica* growth and productivity (Fernández-Torquemada and Sanchez-Lizaso 2005a, b). The effects of salinity on eelgrass may be a concern in the San Juan Archipelago where potable water is scarce and requests for permits to install private or public desalination plants could increase from the 12 active plants that produce an average 23,528 gallons per day of treated water (San Juan County Resources Advisory Committee 2009).

pH

There is evidence of an inverse relationship between pH and seagrass photosynthesis. Subtle increases in pH will reduce photosynthesis due to a limitation in HCO_3^- concentrations whereas a decrease in pH increases photosynthesis (Invers et al. 2001, Invers et al. 1997). The observed increase in photosynthesis due to a decrease in pH is greater in the temperate species *Z. marina* and *Phyllospadix torreyi* compared to subtropical species *Posidonia oceanica* and *Cymodocea nodosa* (Invers et al. 2001).

Wastewater from treatment facilities in WA State are allowed to discharge wastewater with a pH range from 6.5 to 8.5. Seawater pH naturally ranges from 7.5 to 8.4 and the combined effects of ocean acidification and the potential for lower pH from outfalls could have long term, positive benefits for eelgrass in Puget Sound. Although lower pH could make conditions more favorable for seagrass in Puget Sound, there may be detrimental consequences to other marine organisms (e.g., reduction in calcification for shellfish).

2.2 Carbon and Nutrients

Seagrasses require carbon, nitrogen, and phosphorus to support plant maintenance, photosynthesis and growth (Ralph et al. 2006). Eelgrass typically has a C:N:P ratio of 255:15:1 (Duarte 1990). However, excess nitrogen and other nutrients from upland sources create eutrophic conditions (N:P>16; Kaldy 2006), reduce available light, increase interspecific competition with algae, and subsequently cause seagrass decline. Finfish and shellfish aquaculture facilities can also cause excess nitrogen and ammonia buildup that lead to detrimental effects on seagrass (Cancemi et al. 2003, Deslous-Paoli et al. 1998, Plus et al. 2003). Light attenuation caused by excess growth of epiphytes, macroalgae, and phytoplankton from nitrogen loading has significantly affected temperate (Borum 1985, Burkholder et al. 1992, Short and Burdick 1996, Twilley et al. 1985) and tropical seagrass (Bulthuis and Woelkerling 1983, McGlathery 1995).

In addition to light attenuation from excess nutrients, there is evidence that excess nitrogen can reach toxic levels in seagrass and cause other essential nutrients to become limited (Burkholder et al. 1992, van Katwijk et al. 1997).

The level of nutrients that can be discharged into marine waters is regulated, subject to U.S. EPA guidelines, by the Washington State Department of Ecology. State and federal permits are required to discharge wastewater into Puget Sound and specific concentrations are dependent upon the type of discharge source (residential, industrial, stormwater), technology based categorical limits, and characteristics of the receiving waters.

2.2.1 Carbon, nitrogen and phosphorus

In a nitrate enrichment experiment, eelgrass shoots experienced a loss of structural integrity and showed a decrease in eelgrass shoot growth and density (Burkholder et al. 1992). It was hypothesized that the mechanism that affected eelgrass was the imbalance in carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) ratios. Plants that readily take up nitrate and convert it to ammonia risk depleting carbon resources for amino acid production and plant maintenance even in carbon rich environments (Salisbury and Ross 1978, as cited in Burkholder et al. 1992). The ammonia produced reaches toxic levels and the plant is unable to fix enough carbon to maintain metabolic processes and provide structural integrity to the seagrass leaves (Burkholder et al. 1992). Carbon limitation is more of an issue in sandy substrates compared to muddy or terrigenous environments as the latter tend to have greater abundance of available carbon (van Katwijk et al. 1997). Further implications can arise in muddy environments where the addition of nutrients can lead to sulfide toxicity and seagrass decline (Holmer et al. 2005, Frederiksen et al. 2006, 2008).

The abundance of nitrate could also lead to a N:P imbalance as there may not be adequate P to maintain metabolic processes (Tilman et al. 1982 as cited in Burkholder et al. 1992). However, in areas with terrigenous sediments such as the PNW, phosphorus is found in organic matter or concentrated mineral pools (Hemminga and Duarte 2000, Marbà et al. 2006). Seagrass beds tend to be sinks for phosphorus where it accumulates in organic material or where becomes bound to other minerals (Pedersen et al. 1997). Eelgrass can become stressed even further when temperatures increase in nitrate enriched environments and plants utilize more carbon and phosphorus due to higher metabolic output at these temperatures (Zimmerman et al. 1989).

The nitrogen, carbon, and phosphorus content (% dry weight) of eelgrass have been measured in numerous studies throughout its range and the values are considerably more variable than observed in other seagrass (Duarte 1990). Based on previous research, the range of nitrogen content in eelgrass is 1.2 – 5.6 % DW, phosphorus is 0.15-0.78 % DW and carbon is 28 – 43 % DW (Duarte 1990). Multiple site specific factors affect nutrient availability and potential toxicity in seagrass. Although there is no specific concentration of nitrogen, carbon, or phosphorus in eelgrass that will indicate plant stress, there are tools designed to suggest the presence of nutrient enrichment. The assessment of the seagrass physiological characteristics (amino acid composition, tissue nutrient content, and $\delta^{15}N$) is one method to determine if plants are exposed to nutrient enrichment (Udy and Dennison 1997). Hemminga and Duarte (2000) suggest that C:N ratios less than 20 and C:P ratios less than 1000 indicate nutrient enriched systems. Another method used to indicate nutrient over enrichment is the nutrient pollution indicator (NPI) which is used to compare plant condition to nutrient loading (Lee et al. 2004).

Puget Sound receives natural and anthropogenic sources of carbon and nutrients through many pathways including atmospheric deposition, oceanic upwelling, upland

and stormwater runoff, and various types of outfalls. Due to the natural sources of carbon and nutrients available in Puget Sound, the addition of certain elements, such as nitrogen, could be detrimental to eelgrass in certain areas. In Puget Sound, it is estimated the average annual dissolved inorganic nitrogen (DIN) loading from anthropogenic sources is 2.7 times the natural loading conditions (Mohamedali et al. 2011). Annual DIN loads were greatest in the main basin of Puget Sound and almost entirely a result of discharge from residential wastewater treatment facilities (Mohamedali et al. 2011). The DIN loads between Edmonds and the Tacoma Narrows bridge, an area with the greatest concentration of outfalls (Carmichael et al. 2009), were 3.6 times the average for greater Puget Sound, an area not including the Straits (Mohamedali et al. 2011). The continued addition of DIN in excess of natural conditions will likely shift the carbon and nutrient balance in Puget Sound and develop conditions (e.g., eutrophication) less suitable for eelgrass.

2.3 Metals and Contaminants

Metals and contaminants can be incorporated into seagrass leaves and vascular tissue through assimilation from the water column and the sediment (Bester 2000, Brinkhuis et al. 1980, Schwartzschild et al. 1994). Studies have demonstrated that metal and contaminant concentrations in seagrass tissue often reflect the availability of these substances in the environment based on the similarities between concentrations in plant compartments (e.g., leaves, rhizomes, and roots) and the environment (sediment and water) (Bester 2000, Pergent-Martini and Pergent 2000, Marín-Guirao et al. 2005, Lyngby and Brix 1982, Sanchiz et al. 2001). The uptake of excess metals can cause toxicity and metabolic or morphological effects to seagrass.

2.3.1 Metals

Metal uptake in seagrass

There are numerous studies that demonstrate the uptake, accumulation and translocation of metals in and between seagrass compartments (e.g., leaves, rhizomes, roots and epibiota) (Appendix A summarizes results from selected studies that measured metal concentrations in seagrass tissue). The rhizome and roots of a plant are often a sink for metals and other contaminants, while the seagrass leaves are a source, since they senesce and decompose elsewhere, eventually transferring metals and contaminants back into the environment (Drifmeyer et al. 1980). The metal content of epibiota have also been evaluated in seagrass systems (Schlacher-Hoenlinger and Schlacher 1998, Sanz-Lázaro et al. 2012), and have been shown to have high concentrations of metals indicating efficient absorption rates and transfer rate between leaves and epibiota (McRoy and Goering 1974, Sanz-Lázaro et al. 2012).

The rate of metal uptake and translocation between compartments and the potential for metal toxicity depend on a number of factors including the specific metal and its form, the morphology and growth rate of seagrass (rapid growth causes a dilution of metals in plant tissue) (Lyngby and Brix 1982), translocation dynamics and available binding sites (Das et al. 1997), and environmental conditions (e.g., climatic, sediment, and water properties) (Rozan and Hunter 2001, Prange and Dennison 2000). Often metal concentrations in seagrass correlate well with metal availability in the environment (water and sediments) (Besar et al. 2008, Pergent-Martini and Pergent 2000, Sanchiz et al. 2001) and proximity to a source (Brix et al. 1983, Hoven et al. 1999, Pergent et al. 2011, Stenner and Nickless 1975), but these data provide no information on the physiological effects on seagrass (Ralph et al. 2006).

Although it does not hold true for every case, studies have identified cadmium (Cd), copper (Cu), and zinc (Zn) as metals that tend to accumulate in higher concentrations in seagrass leaves (Sanchiz et al. 2000, Campanella et al. 2001), while higher concentrations of chromium (Cr), iron (Fe), and lead (Pb) are found in the roots and rhizomes of seagrass (Lewis and Devereux 2009, Prange and Dennison 2000).

In the Mediterranean Sea, the dominant seagrass, *Posidonia oceanica*, can live up to 30 years, has a slow leaf turnover rate and creates massive belowground root and rhizome mattes (Gobert et al. 2006) making it a valuable species to track for metal contamination (Costantini *et al.* 1991, Catiski and Panayotidis 1993, Pergent-Martini and Pergent 2000). Sanz-Lázaro et al. (2012) found *P. oceanica* meadows were sinks for Fe, Ni, Cr, As, Ag, and Cs. Pergent and others (2011) also observed higher concentrations of Ag, Hg, and Pb in *P. oceanica* at an industrial, city site along the coast of France in the northwestern Mediterranean Sea compared to a more pristine sample station. At the industrial, city site Ag was 1.4 times higher, Hg was 3.8 times higher and Pb was 2 times higher in concentration compared to the reference site.

In Venezuela, scientists found Cd and Cu in higher concentrations in the roots and rhizomes of *Thalassia testudinum* compared to the leaves (Alfonso et al. 2008). The metal allocation within the seagrass was consistent between multiple study sites with Zn and vanadium (V) concentrations upwards of 2 to 7 times higher in the roots and rhizomes compared to the leaves (Alfonso et al. 2008). The high levels of V indicate possible petroleum contamination as V is found in fossil fuels and petroleum refinery processes.

Another study along the west coast of Florida investigated the concentrations of Arsenic (As) in *Thalassia testudinum* and its relationship to phosphorus (P) availability (Fourqurean and Cai 2001). Arsenic and P concentrations ranged between 0.9 to 3.4 and 544 to 6294 $\mu g\ g^{-1}$ respectively in six estuaries along the western Florida coastline. In an attempt to understand the seagrass tissue content of metals related to the sediments, *T. testudinum* and *Halodule wrightii* leaves, roots and rhizomes were analyzed along the coastal lagoons of Texas and results found metals in seagrass tissue correlated closely to sediment metal levels and highest concentrations of Cd and Zn were associated with contaminated sites (Pulich 1980, Whelan *et al.* 2005).

Aluminum (Al) concentrations were not elevated in seagrass growing near a source of contamination and results suggested seagrass does not accumulate Al in its tissues

(Prange and Dennison 2000, Malea 1993, Malea and Haritonidis 1995). Accumulations of chromium (Cr) and zinc (Zn) have also been quite low in some studies (Prange and Dennison 2000) further emphasizing that site specific factors and seagrass species play important roles in metal uptake and accumulation.

Metal uptake in eelgrass

The metallic content of eelgrass has been assessed in a number of locations throughout its range to understand the uptake of these substances in eelgrass compartments, the concentrations that cause toxic effects, and the utility of eelgrass as a bio-indicator of metals in the environment (Appendix A, Lewis and Devereux 2009). The metallic composition of eelgrass analyzed in these studies included: antimony (Sb), arsenic (As), cadmium (Cd), calcium (Ca), cesium (Ce), cobalt (Co), copper (Cu), chromium (Cr), gold (Au), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), nickel (Ni), potassium (K), samarium (Sm), scandium (Sc), selenium (Se), silver (Ag), sodium (Na), strontium (Sr), vanadium (V), and zinc (Zn). It is evident when comparing results of these studies that metal uptake by eelgrass depends on a number of physical and chemical factors (Appendix A; Brix and Lyngby 1984, Lewis and Devereux 2009). A few factors that affect the uptake rate of metals in eelgrass include the proximity of eelgrass to a source of metal contamination (e.g., outfall), the contaminant concentration, and climatic conditions which affect outfall discharge rates and eelgrass growth rates.

In the Thau Lagoon, France (Mediterranean Sea), researchers found the order of metal concentrations in eelgrass leaves to be Fe > Cu > Pb > Ni > Cr and in the rhizomes and roots it was Fe > Cu > Pb > Cr > Ni (DeCasabianca et al. 2004). In Limfjord, Denmark, a suite of similar metals were analyzed and the order of descending metal concentration in the leaves was Na ≥ K > Ca > Mg > Mn > Fe > Zn > Cu > Pb > Cd and the concentrations were similar in the rhizome-roots except Fe > Mn (Brix and Lyngby 1984). The strong correlation between metal concentrations in leaves compared to the rhizome-root compartment observed by Brix and Lyngby (1984) demonstrated the ability of eelgrass to absorb metals from its environment (water and sediments) and to translocate these substances between different compartments in the plant. Two other studies showed a similar order of metal concentrations in eelgrass leaves and rhizome-roots. In Yaquina Bay, OR, the order of concentration of metals in leaves was Fe > Zn > Ni > Cu > As > Cr > Cd and similar in rhizome-roots except Cr > As (Kaldy 2006). In Alaska, McRoy (1970) assessed fewer metals but found a nearly similar order of concentration in eelgrass leaves Fe > Zn > Cu > Ni > Cd.

As with other seagrasses, the concentration of metals in eelgrass compartments can vary depending on plant growth and the proximity to and type of contamination source. Brix and Lyngby (1982) showed that Cd, Pb, and Zn appeared to bio-accumulate in eelgrass while Cu concentrations declined with age due to translocation or leaf senescence. In another study in Limfjord, Denmark, the concentrations of four metals were similar between the leaf and rhizome-root compartments measured across a gradient of pollution (Zn > Cu > Pb > Cd), while the levels of three metals (Zn, Cu, and Pb) were significantly elevated near an industrial city center and a wastewater discharge (Brix et al. 1983). Elevated levels of Cd, Cu, Pb, and Zn were also observed in eelgrass at a site within close proximity of mining activities in southern Spain compared to sites in more pristine areas (Stenner and Nickless 1975).

In the Piscataqua River and Great Bay Estuary, a research and monitoring program was designed to assess the waste released from the Portsmouth Naval Shipyard in Kittery, ME (Johnston et al. 1994a, b, Short 1994). An assessment of eelgrass leaf and rhizome-root tissue identified Cr, Hg, Ni, Pb, and Zn as contaminants of concern in the estuary (Appendices A and B).

Hoven and others (1999) measured Pb uptake in leaves of hydroponically deployed eelgrass to identify the source of pollution. Using Pb isotope ratios ($^{206}Pb/^{207}Pb$) the researchers were able to link Pb in eelgrass to a nearby pollution source confirming the utility of eelgrass as an indicator species for Pb pollution. The Pb concentrations measured in leaves within the vicinity of the pollution were 2 to 5 times greater than concentrations measured at the reference site (Hoven et al. 1999), but considerably lower than levels observed in eelgrass at other sites contaminated with Pb (Brix et al. 1983, Stenner and Nickless 1975). Hoven and others (1999) may have observed lower Pb concentrations because the eelgrass in their study was not grown in contact with sediments laden with high Pb concentrations which could increase Pb translocation to leaves. Research has showed a positive correlation of metals between the above- and belowground parts of eelgrass and between the availability of metals in the water and sediment (Brix and Lyngby 1982, Brix et al. 1983). Brix and Lyngby (1982) demonstrated that the content of trace metals in eelgrass reflected levels in sediments, however, a majority of the metals in the leaves were absorbed from the water. A comparison of metal concentration results in eelgrass from Limfjord, Denmark (Brix et al. 1983), southern Spain (Stenner and Nickless 1975), and Yaquina Bay, OR (Kaldy 2006) found a close overlap in values except at sites that were elevated due to contamination (Table 1). The one study in Puget Sound, WA, assessed metal concentrations in eelgrass near an oil refinery (USFWS 1994). The values observed in this study were within the ranges observed elsewhere throughout the world (Table 1).

Table 1. A comparison of metal concentrations (ppm dry weight) measured in eelgrass from sites in Limfjord (Denmark), Oregon and Washington (USA), and southern Spain.

LOCATION	Cd	Cu	Pb	Zn	REFERENCE
	ppm	ppm	ppm	ppm	
Limfjord, Denmark*	2.9	16.7	37.5	175	Brix et al. 1983
Limfjord, Denmark (means)	0.6	4.8	1.1	78	Brix et al. 1983
Yaquina Bay, OR	1.7	10	-	29	Kaldy 2006
Ayamonte, Spain	2.0	36	16	215	Stenner & Nickless 1975
La Rábida, Spain*	5.3	1350	1800	1480	Stenner & Nickless 1975
Cadiz Bay, Spain	2.0	9	6	100	Stenner & Nickless 1975
Padilla Bay, WA	1.1-3.6	5.1-11.8	0.9-2.7	21.5-34.8	USFWS 1994

* (asterisk) indicates maximum values due to close proximity to contamination.
Brix et al. 1983 – close proximity to city center and wastewater treatment discharge
Stenner and Nickless 1975 – close proximity to mining activities
USFWS 1994 – close proximity to an oil refinery

Metal toxicity in seagrass

There is substantial evidence in the literature that shows metal toxicity in seagrass from field and laboratory experiments. While *in situ* experiments will capture environmental conditions and the physiological effects of metal toxicity in seagrass, field experiments are often confounded by varying characteristics of the water, sediment, plants and metal (Ward 1989, Macinnis-Ng and Ralph 2002).

In two separate studies, roughly 1300 km apart on the east coast of Australia, scientists found Cu concentrations of 0.5 mg L^{-1} and 1.0 mg L^{-1} had negative effects on *Halophila ovalis* morphology, growth and photosynthesis (Ambo-Rappe et al. 2011, Prange and Dennison 2000). A third study performed in a mesocosm observed a reduction of photosynthetic activity in *H. ovalis* exposed to 1 mg L^{-1} of Cu and plant mortality at Cu concentrations above 5 mg L^{-1} (Ralph and Burchett 1998). In addition to toxic effects of Cu observed in *H. ovalis*, high concentrations of Cu affected photosynthetic activity in *Halophila spinulosa* (Prange and Dennison 2000) while another species, *Halodule uninervis*, showed no signs of toxicity due to the high Cu concentrations. Excess Cu in *Cymodocea serrulata* was stored in the roots and rhizomes and showed no observed physiological complications (Prange and Dennison 2000). Toxic concentrations of Cu in excess of 100 ppm (mg L^{-1}) have typically caused changes in photosynthetic activity of *Halophila*, *Halodule*, *Zostera* and *Posidonia* (Macinnis-Ng and Ralph 2002, Prange and Dennison 2000), and seagrass growth rates (Hamoutene et al. 1996, Malea et al. 1995, Ward 1989).

Even though Pb is considered a non-essential element, stored in structural components of the leaf tissue making it unavailable to the plant, there is evidence of it causing toxic effects to some seagrasses (Ralph and Burchett 1998a, Ambo-Rappe et al. 2011). Although low concentrations of Pb showed minor effects on plant physiological and metabolic processes (Ambo-Rappe et al. 2011), concentrations in excess of 100 ppm (mg L^{-1}) impacted photosynthetic activity (Macinnis-Ng and Ralph 2002, Prange and Dennison 2000), and growth rates (Hamoutene et al. 1996, Malea et al. 1995, Ward 1989) in *Halophila*, *Halodule*, *Zostera* and *Posidonia*.

Iron, Fe, is utilized for primary production whereas other elements can be considered pollutants in low concentrations. Even so, high doses of Fe can induce stress in seagrass. Working in Queensland, Australia, Prange and Dennison (2000) noticed a reduction in amino acids and photosynthetic capacity in *Zostera muelleri* (formerly *Z. capricorni*) as a response to increased Fe.

Certain seagrasses have responded differently to increased concentrations of Al. In some cases, research found limited Al accumulation in seagrass (Prange and Dennison 2000, Malea 1993, Malea and Haritonidis 1995), while Bucalossi and others (2006) found that Al affected *Posidonia* reductase activity.

Metal toxicity in eelgrass

While it has been demonstrated that eelgrass can be used as an indicator of metals in the marine environment (e.g., Brix et al. 1983, Brix and Lyngby 1984, Hoven et al. 1999, Lewis and Devereux 2009), a concern is the level of bio-accumulation that can cause toxic effects on the plant. And, yet, there are a limited number of studies that have assessed the toxic thresholds and effects of metal contamination on eelgrass (Table 2; Lewis and Devereux 2009).

Table 2. List of metals toxicity thresholds and the effects on eelgrass. Table has been modified slightly from Table 6 in Lewis and Devereux (2009).

METAL	RESPONSE PARAMETER	TEST DURATION	EFFECT CONCENTRATION	REFERENCE
		days (d), hours (h)		
Cu, Cd, Hg	growth rate	19 d	5 µM	Lyngby and Brix 1984
Cr, Pb, Zn	growth rate	19 d	50 µM	Lyngby and Brix 1984
Hg, Ni	nitrogen fixation	24 h	10 mg L^{-1}	Brackup and Capone 1985
Pb	nitrogen fixation	24 h	100 mg L^{-1}	Brackup and Capone 1985
Cu, Cd, Zn, Pb	growth rate	10 d	10 mg L^{-1}	Conroy et al. 1991 as cited in Lewis and Devereux 2009

2.3.2 PAHs, PCBs, and other organic contaminants

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other organic contaminants (e.g., herbicides and pesticides) enter the nearshore system through various types of outfalls and accumulate in sediments and biota or transfer throughout the food web. The presence of most contaminants depends on land use patterns and point source pollution resulting in an abundance of contaminants that originate from petroleum based activities, industrial sources, and agriculture. A recent assessment of toxic loading to Puget Sound found the highest loading rates of PAHs from residential sources such as surface and stormwater runoff (EnvironVision et al. 2008, Hart Crowser et al. 2007). Although seagrass is shielded from certain contaminants because of the structural integrity of the plant and antibacterial biofilm on the leaves (Gunnarsson et al. 1999), impacts from contaminants can range from smothering (oils spills) to various physiological implications (herbicides). Although some of these contaminants (e.g., oil) typically enter the marine environment through sources other than outfalls, it is important to document baseline levels and consider their effects on eelgrass.

Oil

Seagrasses are susceptible to damage from petroleum by means of a direct spill, continuous, low concentration input from recreational and commercial vessel activities or stormwater runoff. The greatest impact from oil is when it comes in direct contact with seagrass causing a reduction in growth, senescence of leaves, and mortality (Jacobs 1988, Runcie et al. 2006). Other effects of direct contact between oil and seagrass include a significant reduction in photosynthesis due to partial or total light inhibition (Howard et al. 1989), inhibition of thylakoid membrane oxidation which affects photosynthesis (Marwood et al. 2001), and impacts to gas exchange and rhizome expansion rates (Fonseca et al. 2009). However, certain effects were generally not persistent over the long term (Burns et al. 1993, Dean et al. 1998, Jacobs 1980), due to the limited duration of contact between oil and seagrass as the oil is transported away when the plants become submerged during flood tide.

Beyond the impacts produced by the direct contact of oil, there is evidence of other effects to seagrass systems caused by oil. Monitoring after an oil spill in Panama found total loss of *Thalassia testudinum* beds and associated invertebrate communities (Jackson et al. 1989). The *Exxon Valdez* oil spill caused a range of short term impacts from a reduction in eelgrass biomass and density of reproductive shoots (Dean et al. 1998) to a total loss of eelgrass in some areas (Juday and Foster 1990). Whereas the assessment of oil spills on seagrass in the Persian Gulf show no effect on plant photosynthesis (Durako et al. 1993) or species composition, abundance and distribution (Kenworthy et al. 1993).

There are a few studies on the physiological effects of oil and oil dispersants to seagrass and the results suggest that dispersed oil has a greater impact on *Thalassia testudinum*, *Syringodium filiforme*, and *Halodule wrightii* (Thorhaug et al. 1986, Thorhaug and Marcus 1987), *Posidonia australis* (Hatcher and Larkum 1982), and *Halophila ovalis* (Ralph and Burchett 1998b) than oil or dispersants alone. A mesocosm study in Western Australia found *P. australis* had significantly reduced leaf growth rates when exposed to oil residue from refinery effluent, equivalent to a maximum of 1 ppm ($\mu g\ g^{-1}$) of hydrocarbons, over a 32 day period (Cambridge et al. 1986).

In Florida, levels of PAHs, byproduct of fuel combustion, were below the detectable limit in most seagrass samples (Lewis et al. 2007), a common occurrence in some waters (Haynes et al. 2000). Pergent and others (2011) found higher values of PAHs in *Posidonia oceanica* closer to anthropogenic sources (e.g., harbor, city). There were also greater concentrations in 3 of the 14 detected PAHs in the *Posidonia* leaves observed at the industrial, city site compared to a pristine, control site. A mesocosm study found the uptake of PAHs by seagrass to a point where above- and belowground biomass concentrations matched sediment levels was determined to occur within a 60 day period (Huesemann et al. 2009). In Puget Sound, highest PAH concentrations were measured in eelgrass at sites within close proximity to oil transfer structures and outfalls adjacent to an oil refinery (USFWS 1994). However, the assessment of PAH concentration in eelgrass did not evaluate whether the observed concentrations were toxic to eelgrass and affected plant viability (USFWS 1994).

PCBs

Polychlorinated biphenyls (PCBs) were assessed along the Queensland coast of Australia, to determine levels in *Cymodocea serrulata*, *Halodule uninervis*, and *Zostera muelleri*, however, the concentrations were below detectable levels (Haynes et al. 2000). Similarly, undetectable results for PCBs were found in a study that assessed

PCBs in *Thalassia testudinum* and *Halodule wrightii* at 13 sites along the Florida pan handle (Lewis et al. 2007) and PCBs in eelgrass at the mouth of the Piscataqua River, Maine (Johnston et al. 1994).

Herbicides, pesticides and other organic compounds

Seagrass beds located near agricultural regions likely come in contact with effluent from outfalls that contains herbicides and pesticides. Chemicals such as atrazine and pentachlorophenol (at concentrations of 1 ppm) have been shown to affect photosynthesis and respiration in *Thalassia testudinum* (Walsh et al. 1982), while higher concentrations of atrazine (30 ppm) significantly reduced *Halodule wrightii* productivity and survival (Mitchell 1987). In some cases, *H. wrightii* appeared to be more tolerant to pollution than other species (Grady 1981, Mitchell 1987).

Bester (2000) sampled seagrass along the Wadden Sea and found a correlation between high levels of pesticides and damaged and declining seagrass beds. One of the pesticides, triazine, is known to inhibit photosystem II and thus have an effect on all marine plants (e.g., seagrass, phytoplankton, macroalgae). Another study in Roskilde fjord, Denmark, showed that eelgrass photosynthetic processes were affected by glyphosate, bentazone and MCPA (2-methyl-4-chlorophenoxyacetic acid) (Nielsen et al. 2007). The herbicides alone were found to affect chlorophyll *a* and *b* and RNA:DNA ratios in eelgrass but had greater effects as mixtures (Nielsen et al. 2007). Bester (2000) acknowledged that more research was necessary to tease out the direct effects of these compounds and the effects of multiple compounds impacting seagrass. A similar concern was expressed by Correll and Wu (1982) on the synergistic effects of multiple herbicides. Correll and Wu (1982) found very low concentrations of atrazine (12 $\mu g\ L^{-1}$) inhibited growth and productivity of *Vallisneria americana* (freshwater eelgrass) in the Chesapeake Bay and were concerned of the combined effect from the addition of the herbicide, alachlor. A summary of other compounds presented in the study by Correll and Wu (1982) and how these substances affect seagrass are presented in Appendix B.

Correll and Wu (1982) also exposed eelgrass and other common Chesapeake Bay macrophytes to atrazine concentrations of 75 $\mu g\ L^{-1}$ and 650 $\mu g\ L^{-1}$; maximum levels plants were exposed to in the Bay. At 75 $\mu g\ L^{-1}$ of atrazine, oxygen production increased slightly in a controlled environment. However, a significant decrease in oxygen production was observed when plants were exposed to atrazine concentrations of 650 $\mu g\ L^{-1}$. The effects of different doses of atrazine were tested on eelgrass in three other studies (Table 3; Lewis and Devereux 2009).

Table 3. The effects of different concentrations and exposures of atrazine, an agricultural herbicide, on eelgrass. Table has been modified slightly from Table 6 in Lewis and Devereux (2009).

RESPONSE PARAMETERS	TEST DURATION	EFFECT CONCENTRATION	REFERENCE
	days (d), hours (h)	µg L^{-1}	
oxygen production	24 h	100 (reduced) 1000 (total inhibition)	Kemp et al. 1982
adenine nucleotides growth mortality	6 h 21 d	10 (reduced ATP and total adenylates) 100 (reduced ATP and total adenylates, growth inhibition and total mortality)	Delistraty & Hershner 1984
growth mortality chlorophyll	10-40 d	1,900 (1st effect, whole plant)	Schwartzschild et al. 1994
oxygen production	21-42 d	75 (stimulation) 650 (inhibition)	Correll & Wu 1982

Brackup and Capone (1985) found that N_2 fixation (C_2H_2 reduction) activity of the bacteria associated with the roots and rhizomes of eelgrass was negatively affected by naphthalene, pentachlorophenol, Temik and Kepone at 100 ppm. Their study was lab based with high contaminant loading, not uncommon in the environment, but the researchers stressed concern that the bacteria and behavior of the contaminants may respond differently in a natural setting (Brackup and Capone 1985). These results imply that not only was seagrass being affected by contaminant loading but the associated organisms that improve conditions for the seagrass were also affected.

The contaminants in *Cymodocea serrulata*, *Halodule uninervis*, and *Zostera muelleri* along the Queensland, Australia, coast between Cape York and Moreton Bay were assessed to determine levels of organochlorines, chlorpyrifos, and atrazine (Haynes et al. 2000). Only the herbicide diuron was detected in the seagrass samples at a level that ranged between 0.0006 to 0.0017 µg g^{-1}. With the exception of a few spiked diuron readings from the sediment analysis, the diuron concentration observed in the seagrass was typically higher than the level observed in the sediments (Haynes et al. 2000). The concentrations of other organochlorines, chlorpyrifos, and atrazine in the seagrass samples were below detectable levels.

Additional research is needed to identify the concentration and toxicity thresholds of organic compounds, particularly herbicides, that affect seagrass productivity and to understand the synergistic effects of multiple organic contaminants in the system. A similar assessment is also necessary to better understand the ability of seagrass to uptake PCBs and to determine the toxic thresholds of these contaminants in seagrass.

2.4 Trophic Transfer

It is uncertain how the trophic connectivity of a marine system will respond to loading of excess nutrients, metals, and contaminants from outfalls. Certain species may be better suited to the new environment, colonize the outfall area, and create a shift in trophic components and associations. In the case of an outfall discharging into an area dominated by seagrass, the excess nutrients will likely cause a shift to an algae dominated system with additional changes in other flora and faunal communities.

Community Shifts

A change in the composition of marine species within the vicinity of outfalls has been well documented. The most obvious change can be observed in systems dominated by seagrass and replaced by algae, an organism better adapted for low light, high nutrient conditions (Johansson and Lewis 1992).

In Hillsborough Bay, FL, a sub-basin of Tampa Bay, seagrass was replaced by blue-green algae from the 1950s to the 1980s due to nutrient loading from point source pollution. Similarly, in Tampa Bay, a 1982 survey estimated 80% of the original 31,000 ha of seagrass was replaced by blue-green algae (Lewis et al. 1985), again an impact of eutrophication from excessive loading from waste water treatment facilities. Significant efforts in the 1970s to reduce loading by modifying discharge standards improved water quality in the Tampa Bay system and the algal dominated system has since recovered with seagrass to near historical levels (Greening 2000, Tampa Bay NEP 2009). Changes in macrophyte communities due to outfalls can be quite obvious as in the case of a seagrass to algal dominated transition or less so with changes in algal species composition alone (Golubic 1970, Littler and Murray 1975, Munda 1980, Murray and Littler 1984, Brown et al. 1990, Fairweather 1990). Although recovery from an algal dominated community to a seagrass dominated community is possible (e.g., Tampa Bay, Tampa Bay NEP 2009; Long Island Sound, CT, Vaudrey et al. 2010; Port Adelaide, Neverauskas 1987a), little is known about the fate of metals and contaminants as the dominant macrophyte changes in a system.

Other research compared the macroinvertebrate communities between sediments contaminated from mining operations and non-contaminated sites and found differences in community composition with no observable difference in seagrass parameters (Marín-Guirao et al. 2005). In a separate mesocosm experiment, *Thalassia testudinum* was grown in sediments from a drilling operation to assess the response of the invertebrate community to ferrochrome lignosulfonate and barium (Morton et al. 1986, Kelly et al. 1987). Results found the contaminated sediment affected invertebrate abundance and species richness (Morton et al. 1986, Kelly et al. 1987). In addition, the *T. testudinum* experienced a reduction of chlorophyll *a* which subsequently caused a reduction in shoot productivity and decomposable biomass – two critical components that support the detrital food web (Morton et al. 1986, Price et al. 1986, Kelly et al. 1987).

There is also evidence of differences in epiphyte assemblages between sites with seagrass impacted by an outfall and control sites (Brahim et al. 2010). Outfall discharge has also caused changes in species abundance and composition in other marine communities, including finfish (Azzurro et al. 2010), corals (Reopanichkul et al. 2010), and nematode and polychaete assemblages (Fraschetti et al. 2006).

Fate of metals and contaminants

Metals and contaminants in seagrass can be transferred across trophic levels through direct grazing and the decomposition of the plant material and associated epibiota (McComb et al. 1981, Ward 1987). In eelgrass, metals are bound in leaves for 55-83 days, the estimated life of the aboveground biomass, and 193 days in the belowground biomass (Jacobs 1979, Sand-Jensen 1975). The concentration of contaminants in seagrass tissue can be several times higher than levels found in the sediment and water (Lewis and Devereux 2009). In Florida, the metal concentrations in above- and belowground *Halodule wrightii* and *Thalassia testudinum* biomass were 6 and 11 times the concentration in the sediment, respectively (Lewis et al. 2007). The range of metal concentrations in seagrass tissue can be tens to thousands of times background water concentrations (Lewis and Devereux 2009).

Plants also have the ability to phytoremediate hydrocarbons, such as PAHs and PCBs, as demonstrated in the wetland genus *Juncus* (Lin & Mendelssohn 2009) and in eelgrass (Huesemann et al. 2009). A concern is the residual concentration of PAHs and PCBs in plants and the potential for these substances to enter the food web. The detrital community consists of a suite of organisms (e.g., bacteria, benthic algae, phytoplankton, polychaetes, and amphipods) that are efficient at breaking down organic material and acquiring the nutrients, metals, and contaminants (Price et al. 2012) and there is a significant amount of seagrass biomass produced yearly. Kaldy (2006) estimated a carbon export rate from eelgrass in Yaquina Bay, OR, at 1.8×10^5 kg y^{-1}, an amount equivalent to 180 g C m^{-2} y^{-1}. Therefore, in some areas the potential for trophic transfer of metals and contaminants is a concern, particularly in systems where seagrass is the dominant food source for certain species (See reviews by Valentine and Duffy 2006 and Valentine and Heck 1999). For example, species whose diet consists of primarily seagrass (e.g., manatee and dugongs, Haynes et al. 2005, Jackson et al. 2001, Lanyon et al. 1989, Lefebvre et al. 2000, Valentine and Heck 1999; sea turtles, Jackson et al. 2001, Lanyon et al. 1989; and waterfowl, Baldwin and Lovvorn 1994), could risk health related issues due to metal and contaminant bio-accumulation.

The transfer of metals and contaminants found in eelgrass to organisms managed for consumption is also important to consider. The accumulation of metals and contaminants in finfish and shellfish has been monitored for a number of years throughout Puget Sound (Kimbrough et al 2008, Lanksbury and West 2011, O'Neill and West 2009, West et al. 2008, 2011). However, it is not well understood to what extent metals and contaminants transfer from eelgrass to primary consumers. A potential path in which metals and contaminants could be transferred to finfish is through the consumption of invertebrates that forage on eelgrass detritus laden with metals and contaminants, but no research has been completed to understand this trophic connection and path for metal and contaminant transfer.

There are some preliminary results that suggest a portion of the organic matter consumed by shellfish is from eelgrass (Howe and Simenstad 2011). Therefore shellfish will accumulate metals and contaminants found in the eelgrass, substances shellfish are not effective at depurating, and increase the potential for bio-accumulation and human related health risks (Lee et al. 2008). Further research is recommended to understand and track the fate of metals and contaminants in eelgrass and to what extent these substances transfer to and bio-accumulate in finfish and shellfish.

Although not necessarily found in residential outfalls, tributyltin, a component of anti-fouling paint, could be a major component in the effluent of stormwater drains or industrial outfalls near marine facilities. In an attempt to understand the fate of tributyltin, it was added to microcosms to demonstrate the potential ecosystem-wide effects in tropical seagrass beds and the associated organisms (Kelly et al. 1987, 1990a, and 1990b). The tributyltin accumulated in sediments and *Thalassia testudinum* and had significant, negative effects on associated fauna (molluscs, annelids, and arthropods). There is also evidence that tributyltin accumulates in eelgrass (Francois et al. 1989) and *T. testudinum* (Levine et al. 1990) posing a threat to bio-accumulate further up the food chain.

It is evident that metals and contaminants from outfalls could potentially transfer between trophic levels and trigger changes in community diversity, create sinks with high concentrations of contaminants, and cause health risks. There is currently inadequate research that quantifies metal and contaminant transfer routes, rates and the eventual fate of these substances in eelgrass ecosystems (Lewis and Devereux 2009).

3 Conclusions

Eelgrass is an important habitat in Puget Sound and supports numerous ecosystem functions. It is considered a significant indicator of ecosystem health (Dennison et al. 1993, Krause-Jensen et al. 2005, Orth et al. 2006), and has been identified as an indicator to track the recovery of Puget Sound. To further assess stressors that cause eelgrass decline in the Sound (Thom et al. 2011), it is critical to understand the effects of outfall construction and effluent on eelgrass. Outfalls that discharge residential, commercial, and industrial wastewater along with upland stormwater are abundant throughout developed coastal areas, particularly in Puget Sound. It has been shown that the construction of outfalls and the discharged effluent affect marine organisms and processes, and specifically eelgrass. The impacts to eelgrass range from physical effects on the environment where it grows to physiological effects on the plants.

The assessment of nutrients, metals, and organic contaminants in seagrass has been performed in many coastal areas throughout the world (Lewis and Devereux 2009, Ralph et al. 2006). The focus of many of these investigations has been to determine the concentration of nutrients, metals and contaminants in seagrass and to understand their effect on plant physiology and survival. More recently there have been efforts to understand the fate of nutrients, metals, and contaminants incorporated into seagrass tissue (Kaldy 2006) throughout the broader environment, how much of a role seagrass plays in phytoremediation (Huesemann et al. 2009), and if bio-accumulation of metals or contaminants is a concern in trophic processes (Scarlett et al. 1999).

Research has demonstrated the physiological effects, particularly on photosynthetic processes, that nutrients, metals, and contaminants cause to eelgrass. However, many factors affect the availability, uptake, and toxicity of nutrients, metals, and contaminants and little is known about the concentrations of these substances in eelgrass in the Pacific Northwest (Kaldy 2006) and more specifically greater Puget Sound (USFWS 1994). It is assumed that basic nutrients, nitrogen and phosphorus, are abundant in Puget Sound, but whether these substances are at levels that cause toxicity in eelgrass is unknown. Similarly, it is likely that metals and contaminants abound in Puget Sound, particularly in the nearshore environment. Eelgrass grows within close proximity to a wide range of contaminant sources (e.g., outfalls, rivers, upland runoff from residential, commercial and industrial area), yet only one, geographically limited, study has been done to capture baseline concentrations of these substances in eelgrass (USFWS 1994). Furthermore, no research has been conducted in Puget Sound to evaluate the potential effect nutrients, metals, and organic contaminants have on the health of this dominant flora.

An initial baseline assessment of nutrients, metals, and contaminants in eelgrass is necessary to understand baseline concentrations of these substances in eelgrass, to determine whether these concentrations are approaching toxic levels relative to similar research in the literature (Appendices A and B), and to understand their fate to effectively manage nearshore systems (Peters et al. 1997). Of particular interest are the concentrations of metals, organic contaminants and herbicides because of their potential as phytotoxins, their multi-chemical interactions on seagrass (Lewis and Devereux 2009), and their likely abundance in Puget Sound (Ecology and King County 2011, Mohamedali et al. 2011). Baseline data on nutrient, metal and contaminant concentrations in eelgrass will provide valuable information for decision makers to effectively protect this ecologically significant resource in Puget Sound.

4 References

Alfonso, J.A., J. Azocar, J.J. LaBrecque, B. Garcia, D. Palacios and Z. Benzo. 2008. Trace metals in bivalves and seagrass collected from Venezuelan coastal sites. International Journal of Tropical Biology 56:215-222.

Ambo-Rappe, R., D.L. Lajus and M.J. Schreider. 2011. Heavy metal impact on growth and leaf asymmetry of seagrass, *Halophila ovalis*. Journal of Environmental Chemistry and Ecotoxicology 3:149-159.

Armstrong, D.A., J.L. Armstrong and P.A. Dinnel. 1988. Distribution, abundance and habitat associations of Dungeness Crab, *Cancer magister*, in Guemes Channel, San Juan Islands, Washington. Journal of Shellfish Research 7:147-148.

Azzurro, E., M. Matiddi, E. Fanelli, P. Guidetti, G. LaMesa, A. Scarpato and V. Axiak. 2010. Sewage pollution impact on Mediterranean rocky-reef fish assemblages. Marine Environmental Research 69:390-397.

Augier, H., G. Giles and G. Ramonda. 1978. Recherce sur la pollution mercurielle dans le golfe de Foss (Méditerranée, France): Degre de contamination par le mercure des phanorogames marines *Posidonia oceanica* Delile *Zostera noltii* Horneman et *Zostera marina* (L.) Revue Internationale d'Océanographie Médicale. Tomes LI-LII:55-69.

Baldwin, J.R. and J.R. Lovvorn. 1994. Expansion of seagrass habitat by the exotic *Zostera japonica* and it use by dabbling ducks and brant in Boundary Bay, British Columbia. Marine Ecology Progress Series 103:119-127.

Balestri, E., L. Benedetti-Cecchi and C. Lardicci. 2004. Variability in patterns of growth and morphology of *Posidonia oceanica* exposed to urban and industrial wastes: contrasts with two reference locations. Journal of Experimental Marine Biology and Ecology 308:1-21.

Besar, S.N.T., N.A.M. Shazili, S.A. Sbdulah, and A.S. Mamat. 2008. Experimental and field study on accumulation of heavy metals in seagrass (*Halodule pinifolia and Halophila minor*) in Setiu Wetland, Terengganu. Journal of Sustainability Science and Management 3:41-73.

Bester, K. 2000. Effects of pesticides on seagrass beds. Helgoland Marine Research 54:955-98.

Biebl, R. and C.P. McRoy. 1971. Plastmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. Marine Biology 8:48-56.

Bojanowski, R. 1973. The occurrence of major and minor chemical elements in the more common Baltic seaweed. Oceanologia 2:81-152.

Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. Marine Biology 87:211-218.

Brackup, I. and D.C. Capone. 1985. The effect of several metal and organic pollutants on nitrogen fixation (acetylene reduction) by the roots and rhizomes of *Z. marina* L. Environmental and Experimental Botany 25:145-151.

Brahim, M.B., A. Hamza, I. Hannachi, A. Rebai, O. Jarboui, A. Bouain and L. Aleya. 2010. Variability in the structure of epiphytic assemblages of *Posidonia oceanica* in relation to human interferences in the Gulf of Gabes, Tunisia. Marine Environmental Research 70:411-421.

Brinkhuis, B.H., W.F. Penello and A.C. Churchill. 1980. Cadmium and magnesium flux in eelgrass *Zostera marina.* II. Metal uptake by leaf and root-rhizome tissues. Marine Biology 58:187–196.

Brix, H. and J.E. Lyngby. 1982. The distribution of cadmium, copper, lead and zinc in eelgrass (*Zostera marina* L.). Science of the Total Environment 24:51-63.

Brix, H., J.E. Lyngby and H-H. Schierup. 1983. Eelgrass (*Zostera marina* L.) as an indicator organism of trace metals in the Limfjord, Denmark. Marine Environmental Research 8:165-181.

Brix, H., J.E. Lyngby. 1984. A survey of the metallic composition of *Zostera marina* (L.) in the Limfjord, Denmark. Archives of Hydrobiology 99:347-359.

Brown, V.B., S.A. Davies and R.N. Synnot. 1990. Long-term monitoring of effects of treated sewage effluent on the intertidal macroalgal community near Cape Schanck, Victoria. Australia. Botanica Marina 33:85-98.

Bryars, S. and V. Neverauskas. 2004. Natural recolonization of seagrasses at a disused sewage sludge outfall. Aquatic Botany 80:283-289.

Bucalossi, D., C. Leonzio, S. Casini, M.C. Fossi, L. Marsili, S. Ancora, W. Wang, M. Scali. 2006. Application of a suite of biomarkers in *Posidonia oceanica* (L.) delile to assess the ecotoxicological impact on the coastal environment. Marine Environmental Research 62:S327-S331.

Buchsbaum, R.N., F.T. Short, D.P. Cheney. 1990. Phenolic-nitrogen interactions in eelgrass (*Zostera marina* L.): possible implications for disease resistance. Aquatic Botany 37:291-297.

Bulthuis, D.A. and T. Shaw. 1993. Effects of application of glyphosate on the eelgrasses *Zostera marina* and *Zostera japonica* in Padilla Bay, Washington. Padilla Bay National Estuarine Research Reserve Technical Report No. 8, Washington State Department of Ecology, Mount Vernon, WA.

Bulthuis, D.A, and W.J. Woelkerling. 1983. Biomass accumulation and shading effects of epiphytes on leaves of the seagrass *Heterozostera tasrnanica* in Victoria, Australia. Aquatic Botany 16:137-148

Burdick, D.M., F.T. Short and J. Wolf. 1993. An index to assess and monitor the progression of wasting disease in eelgrass *Zostera marina*. Marine Ecology Progress Series 94:83-90.

Burdick, D.M. and F.T. Short. 1999. The effects of boat docks on eelgrass beds in coastal waters of Massachusetts. Environmental Management 23:231-240.

Burkholder, J.M, K.M. Mason and H.B. Glasgow Jr. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. Marine Ecology Progress Series 81:163-178.

Burns, K.A, M.G. Ehrhardt, B.L. Howes and C.D. Taylor. 1993. Subtidal benthic community respiration and production near the heavily oiled Gulf Coast of Saudi Arabia. Marine Pollution Bulletin 27:199-205.

Burrell, D.C. and J.R. Schubel. 1977. Seagrass ecosystem oceanography. *In:* McRoy, C.P. and Helfferich, C. (eds). Seagrass Ecosystems: A scientific perspective. Dekker, New York, pp. 195-232.

Butler, R.W. 1995. The patient predator: Foraging and population ecology of the great blue heron, *Ardea herodias*, in British Columbia. *Occasional Papers for Canadian Wildlife Service* No. 86.

Cambridge, M.L. and A.J. McComb. 1984. The loss of seagrass from Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. Aquatic Botany 20:229-243.

Cambridge, M.L. A.W. Chiffings, C. Brittan, L. Moore and A.J. McComb. 1986. The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. Aquatic Botany 24:269-285.

Campanella, L., M.E. Conti, F. Cubadda and C. Sucapane. 2001. Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. Environmental Pollution 111:117-126.

Cancemi, G., G. De Falco and G. Pergent. 2003. Effects of organic matter input from a fish farming facility on a *Posidonia oceanica* meadow. Estuarine, Coastal and Shelf Science 56:961-968.

Carmichael, R., L. Green, C. O'Meara and M. Yarrow. 2009. Project Documentation Report: Public Stormwater Outfalls to Puget Sound. UW Extension, University of Washington, Seattle, WA, for the People for Puget Sound. Pp. 21. (http://pugetsound.org/policy/stormwater/outfalls)

Catiski, V.A. and P. Panayotidis. 1993. Copper, chromium and nickel in tissues of the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* (Potamoetonaceae) from Greek coastal areas. Chemosphere 26:963- 978.

CH2MHill. 2009. Port Townsend Outfall Replacement Project Seagrass, and Geoduck Clam Monitoring and Mitigation Plan. Prepared for the City of Port Townsend. 16 pp.

Chesworth, J.C., M.E. Donkin and M.T. Brown. 2004. The interactive effects of the antifouling herbicide Irgarol 1051 and Diuron on the seagrass *Zostera marina* (L.) Aquatic Toxicology 66:293-305.

Correll, D.L. and T.L. Wu. 1982. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Botany 14:151–158.

Costantini, S., R. Giordano, L. Ciaralli and E. Becaloni. 1991. Mercury, cadmium and lead evaluation in *Posidonia oceanica* and *Codium tomentosum*. Marine Pollution Bulletin 22:362-363.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Sutton and M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387:253-260.

Damyanova, A., M. Lazarova and G. Dechev. 1981. Application of destructive neutron-activation analysis for determination of microelements in *Zostera marina* and *Ulva lactuca*. Isotopenpraxis 17:256-257.

Das, P., S. Samantaray and G.R. Rout. 1997. Studies of cadmium in plants: a review. Environmental Pollution 98:29-36.

Dean, T.A., M.S. Stekoll, S.C. Jewett, R.O. Smith and J.E. Hose. 1998. Eelgrass (*Zostera marina* L.) in Prince William Sound, Alaska: effects of the Exxon Valdez oil spill. Marine Pollution Bulletin 36:201-210.

DeCasabianca, M.L., P.S. Tari, R. Gauchet, C. Raynaud and V. Rigollet. 2004. Relationships between heavy metal concentrations in sedimetns and eelgrass and environmental variables (*Zostera marina*, Thau Lagoon, France). Vie et Milieu 54:231-238.

Delistraty, D.A. and C. Hershner. 1983. Effects of the herbicide atrazine on adenine nucleotide levels in *Zostera marina* L. (eelgrass). Aquatic Botany 18:353–369.

Dennison, W.C., R.J. Orth, K.A. Moore, J.C. Stevenson, V. Carter, S. Kollar, P.W. Bergstrom and R.A. Batiuk. 1993. Assessing water quality with submerged aquatic vegetation: habitat requirements as barometers of Chesapeake Bay health. BioScience 43:86-94.

Deslous-Paoli, J-M., P. Souchu, N. Mazouni, C. Juge, and F. Dagault. 1998. Relations milieu-ressources: impact de la conchyliculture sur un environnement lagunaire méditerranéen (Thau). Oceanologica Acta 21:831-843.

Dieckmann, G.S. 1982. Jahresgang, aufnahme und verbleib von cadmium im seegrss *Zostera marina* L. aus der Kieler Förde (Westliche Ostsee). Report Sonderforschungsbereich 95 Wechselwirkung Meer. Meeresboden, Nr. 62, Nov. 1982, 94 pp.

Drifmeyer, J.E., G.W. Thayer, F.A. Cross and J.C. Zieman. 1980. Cycling of Mn, Fe, Cu, and Zn by eelgrass *Zostera marina* L. American Journal of Botany 67:1089–1096.

Duarte, C.M. 1990. Seagrass nutrient content. Marine Ecology Progress Series 67:201-207.

Durako, M.J., W.J. Kenworthy, S.M.R. Fatemy, H. Valavi and G.W. Thayer. 1993. Assessment of the toxicity of Kuwait crude oil in the photosynthesis and respiration of seagrasses of the northern Gulf. Marine Pollution Bulletin 27:223–227.

Ecology and King County. 2011. Control of Toxic Chemicals in Puget Sound: Assessment of Selected Toxic Chemicals in the Puget Sound Basin, 2007-2011. Washington State Department of Ecology, Olympia, WA and King County Department of Natural Resources, Seattle, WA. Ecology Publication No. 11-03-055. www.ecy.wa.gov/biblio/1103055.html

EnviroVision Corporation, Herrera Environmental Consultants, Inc. and Washington Department of Ecology. 2008. Phase 2: Improved Estimates of Toxic Chemical

Loadings to Puget Sound from Surface Runoff and Roadways. Ecology Publication Number 08-10-084. Olympia, Washington. Pp. 183.

EPA-SA (Environmental Protection Agency – South Australia). 1998. Changes in seagrass cover and links to water quality off the Adelaide metropolitan coastline. Department for Environment, Heritage and Aboriginal Affairs GPO Box 2607 ADELAIDE SA 5001. Pp 33.

EPA. 1992. United States Environmental Protection Agency (U.S. EPA) - National Toxics Rule. Federal Register 57(246):60848.

Erftemeijer, P.L.A. and R.R. Lewis III. 2006. Environmental impacts of dredging on seagrasses: A review. Marine Pollution Bulletin 52:1553-1572.

Fairweather, P.G. 1990. Sewage and the biota on seashores: assessment of impact in relation to natural variability. Environmental Monitoring and Assessment 14:197-210.

Fernández-Torquemada, Y. and J.L. Sánchez-Lizaso. 2005a. Effects of salinity on leaf growth and survival of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. Journal of Experimental Marine Biology and Ecology 320:57-63.

Fernández-Torquemada, Y., J.L. Sánchez-Lizaso and J.M. González-Correa. 2005b. Preliminary results of the monitoring of the brine discharge produced by the SWRO desalination plant of Alicante (SE Spain). Desalination 182:395-402.

Fleming, A. and J. Stutes. 2011. Planning and building a marine outfall in a sensitive ecosystem: lessons learned. Proceedings of Pipelines 2011: A sound conduit for sharing solutions. American Society of Civil Envirnners. Pp.903-918.

Fonseca, M.S., C.M. Addison, G.A. Piniak and N. Cosentino-Manning. 2009. Draft – Demographic and photosynthetic characteristics of eelgrass after an oil spill in San Francisco Bay, USA. Pp. 32.

Fourqurean, J.W. and Y. Cai. 2001. Arsenic and phosphorus in seagrass leaves from the Gulf of Mexico. Aquatic Botany 71:247-258.

Fraschetti, S., C. Gambi, A. Giangrande, L. Musco, A. Terlizzi and R. Danovaro. 2006. Structural and functional response of meiofauna rocky assemblages to sewage pollution. Marine Pollution Bulletin 52:540-548.

Francois, R., F.T. Short and J.H. Weber. 1989. Accumulation and persistence of tributyltin in eelgrass (*Zostera marina* L.) tissue. Environmental Science and Technology 23:191-196.

Frederiksen, M.S., M. Holmer, E. Díaz-Almela, N. Marbà and C.M. Duarte. 2007. Sulfide invasion in the seagrass *Posidonia oceanica* at Mediterranean fish farms: assessment using stable sulfur isotopes. Marine Ecology Progress Series 345:93-104.

Frederiksen, M.S., M. Holmer, M. Perez, O. Invers, J.M. Ruiz and B.B. Knudson 2008. Effect of increased sediment sulfide concentrations on the composition of stable sulfur isotopes (δ^{34}S) and sulfur accumulation in the seagrasses *Zostera marina* and *Posidonia oceanica*. Journal of Experimental Marine Biology and Ecology 358:98-109.

Gobert, S., M.L. Cambridge, B. Velimirov, G. Pergent, G. Lepoint, J-M. Bouquegneau, P. Dauby, C. Pergent-Martini and D.I. Walker. 2006. Biology of *Posidonia*. *In:* Larkum, A.W.D., Orth, R.J., Duarte, C.M. (eds). Seagrasses: Biology, Ecology and Conservation. Pp. 387-408. Springer, Netherlands.

Golubic, S. 1970. Effect of organic pollution on benthic communities. Marine Pollution Bulletin 1:56-57.

Gorham, J., Hughes, L.L. and Jones, R.G.W. 1980. Chemical composition of salt-marsh plants from Ynys Môn (Anglesey): the concept of physiotypes. Plant, Cell Environment 3:309-318.

Grady, J.R. 1981. Properties of seagrass and sand flat sediments from the intertidal zone of St. Andrews Bay, Florida. Estuaries 4:335–344.

Green, E.P. and F.T. Short. 2003. *World atlas of seagrasses*. Prepared by the UNEP World Conservation Monitoring Centre. University of California Press, Berkeley, USA. 298 pp.

Greening, H.S. 2000. Seagrass management: it’s not just nutrients! Proceedings of a Symposium. St. Petersburg, FL. Pp. 257.

Gunnarsson, J.S., M.E. Granberg, H.C. Nilsson, R. Rosenberg and B. Hellman. 1999. Influence of sediment-organic matter quality on growth and polychlorobiphenyl bioavailability in echinodermatat (*Amphiura filiformis*). Environmental Toxicology and Chemistry 18:1534-1543.

Hamoutene, D., M. Romeo, M. Gnassia and M. Lafaurie. 1996. Cadmium effects of oxidative metabolism in a marine seagrass: *Posidonia oceanica*. Bulletin of Environmental Contamination and Toxicology 56:327-334.

Harlin, M.M. and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. Marine Biology 65:221-229.

Hart Crowser. 2006. Final: Post-point outfall repair/replacement conservation and habitat monitoring plan, Bellingham, Washington. Prepared for the City of Bellingham, Department of Public Works. Report #: 12627-01-03.

Hart Crowser, Inc., Washington Department of Ecology, U.S. Environmental Protection Agency and Puget Sound Partnership. 2007. Phase 1: Initial Estimate of Toxic Chemical Loadings to Puget Sound. Ecology Publication Number 07-10-079. Olympia, Washington. Pp. 188.

Hatcher, A.I. and A.W.D. Larkum. 1982. The effects of short term exposure to Bass Strait crude oil and Corexit 8667 on benthic community metabolism in *Posidonia australis* Hook f. dominated microcosms. Aquatic Botany 12:219-227.

Hauxwell, J., J. Cebrián, C. Furlong and I. Valiela. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82:1007–1022.

Hauxwell, J., J. Cebrián and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Marine Ecology Progress Series 247:59–73.

Haynes, D., J. Muller and S. Carter. 2000. Pesticide and herbicide residues in sediments and seagrasses from the Great Barrier Reef World Heritage area and Queensland coast. Marine Pollution Bulletin 41:279-287.

Haynes, D., S. Carter, C. Gaus, J. Müller and W. Dennison. 2005. Organochlorine and heavy metal concentrations in blubber and liver tissue collected from Queensland (Australia) dugong (*Dugong dugon*). Marine Pollution Bulletin 51:361-369.

Hemminga, M.A. and C.M. Duarte. 2000. Seagrass Ecology. Cambridge University Press, Cambridge. Pp. 298.

Holmer, M., M.S. Frederiksen and H. Møllegaard. 2005. Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. Aquatic Botany 81:367-379.

Hoven, H.M., H.E. Gaudette and F.T. Short. 1999. Isotope ratios of $^{206}Pb/^{207}Pb$ in eelgrass, *Zostera marina*, indicate sources of Pb in an estuary. Marine Environmental Research 48:377-387.

Howard, S., J.M. Baker and K. Hiscock. 1989. The effects of oil and dispersants on seagrasses in Milford Haven. *In:* Dicks, B. (ed). Ecological Impacts of the Oil Industry, pp 61-98. John Wiley and Sons, Ltd., Chichester.

Howe, E.R. and C.A. Simenstad. 2011. Differences in food web connectivity across intertidal gradients in embayment and fluvial dominated estuaries. 21st Biennial Conference of the Coastal and Estuarine Research Federation, Daytona Beach, FL.

Huesemann, M.H., Hausmann, T.S., Fortman, T.J., Thom, R.M. and Cullinan, V. 2009. *In situ* phytoremediation of PAH- and PCB-contaminated marine sediments with eelgrass (*Zostera marina*). Ecological Engineering 35:1395-1404.

Invers, O., Pérez, M., and Romero, J. 1997. Effects of pH on seagrass photosynthesis: a laboratory and field assessment. Aquatic Botany 59:185–194.

Invers, O., R.C. Zimmerman, R.S. Alberte, M. Pérez and J. Romero. 2001. Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. Journal of Experimental Marine Biology and Ecology 265:203-217.

Jackson, J.B.C., J.D. Cubit, B.D. Keller, V. Batista, K. Burns, H.M. Caffey, R.L. Caldwell, S.D. Garrity, C.D. Getter, C. Gonzalez, H.M. Guzman, K.W. Kaufmann, A.H. Knap, S.C. Levings, M.J. Marshall, R. Steger, R.C. Thompson and E. Weil. 1989. Ecological effects of a major oil spill on Panamanian coastal marine communities. Science 243:37-44.

Jackson, J.B.C., M.X. Kirby, W.H. Berger, K.A. Bjorndal, L.W. Botsford, B.J. Bourque, R.H. Branbury, R. Cooke, J. Erlandson, J.A. Estes, T.P. Hughes, S. Kidwell, C.B. Lange, H.S. Lenihan, J.M. Pandolfi, C.H. Peterson, R.S. Steneck, M.J. Tegner and R.R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 297:629-637.

Jacobs, R.P.W.M. 1979. Distribution and aspects of the production and biomass of eelgrass, *Zostera marina* L., at Roscoff, France. Aquatic Botany 7:151-172.

Jacobs, R.P.W.M. 1980. Effects of the 'Amoco Cadiz' oil spill on the seagrass community at Roscoff with special reference to the benthic infauna. Marine Ecology Progress Series 2:207-212.

Jacobs, R.P.W.M. 1988. Oil and the seagrass ecosystem of the Red Sea. Oil Chemical Pollution 5:21–45.

Jen Jay, Inc. 2012a. Eastsound Sewer Outfall Pipe Replacement Project NWS# 2011-879: Eelgrass Macro Algae Monitoring Plan.

Jen Jay, Inc. 2012b. Eastsound Sewer Outfall Pipe Replacement Project NWS# 2011-879: Addendum to: eelgrass Macro Algae Monitoring and Mitigation Plan

Johansson, J.O.R. and R.R. Lewis III. 1992. Recent improvements of water quality and biological indicators in Hillsborough Bay, a highly impacted subdivision of Tampa Bay, Florida, USA. Science of the Total Environment 1992:1199-1215.

Johnston, R.K., W.R. Munns Jr., L. Mills, F.T. Short and H.A. Walker. 1994a. Estuarine ecological risk assessment for Portsmouth Naval Shipyard, Kittery, Maine (ERLN Contribution No. 1471). Naval Command, Control and Ocean Surveillance Center. San Diego, CA.

Johnston, R.K., M. Muzzio, D. Cullen and D. Anne. 1994b. Chemical contamination in marine sediments, tissues, and water samples from the Piscataqua River and Great Bay estuary. *In:* Johnston, R.K., W.R. Munns Jr., F.T. Short and H.A. Walker (eds). 1994. Estuarine ecological risk assessment for Portsmouth Naval Shipyard, Kittery, Maine Phase I: problem formulation. Technical Report 1627, Naval Command, Control and Ocean Surveillance Center, San Diego, CA.

Juday, G.P. and N.R. Foster. 1990. A preliminary look at effects of the Exxon Valdez oil spill on Green Island Research natural area. Agroborealis 22:10-17.

Kaldy, J.E. 2006. Carbon, nitrogen, phosphorus and heavy metal budgets: how large is the eelgrass (*Zostera marina* L.) sink in a temperate estuary? Marine Pollution Bulletin 52:332-356.

Kamermans, P., M.A. Hemminga and D.J. de Jong. 1999. Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). Marine Biology 133:527-539.

Kelly, J.R., D.T. Rudnick, R.D. Morton, L.A. Buttel, S.N. Levine and K.A. Carr. 1990a. Tributyltin and invertebrates of a seagrass ecosystem: Exposure and response of different species. Marine Environmental Research 29:245–276.

Kelly, J.R., S.N. Levine, L.A. Buttel, K.A. Carr, D.T. Rudnick and R.D. Morton. 1990b. Tributyltin effects within a *Thalassia* seagrass ecosystem. Estuaries 13:301–310.

Kelly, J.R., T.W. Duke, M.A. Harwell and C.C. Harwell. 1987. An ecosystem perspective on potential impacts of drilling fluid discharges on seagrasses. Environmental Management 11:537–562.

Kemp, W.M., J.C. Means, T.W. Jones and J.C. Stevenson. 1982. Herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation. *In:* Macalaster, E.G., D.A. Baker and M. Kasper (eds). Chesapeake Bay Program Technical Studies: A Synthesis. pp 502–567. U.S. Environmental Protection Agency, Washington, DC.

Kenworthy, W.J., M.J. Durako, S.M.R. Fatemy, H. Valavi and G.W. Thayer. 1993. Ecology of seagrasses in northeastern Saudi Arabia one year after the Gulf War oil spill. Marine Pollution Bulletin 27:213–222.

Kimbrough, K.L., W.E. Johnson, G.G. Lauenstein, J.D. Christensen and D. A. Apeti. 2008. An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 74. 105 pp.

King County. 2003. Appendix 7-F Eelgrass Survey Results for the Brightwater Marine Outfall Alternatives. Final. Prepared for the Brightwater Project, Seattle, WA. September 2003.

King County. 2008. Eelgrass restoration and biological resources implementation workplan. Revised. Prepared for the Brightwater Project, Seattle, WA by Grette Associates, Battelle Marine Sciences Laboratory and King County Department of Natural Resources and Parks. April 2008.

Krause-Jensen D., T.M. Greve and K. Nielsen. 2005. Eelgrass as a bioindicator under the European Water Framework Directive. *Water Resources Management* 19(1):63-75.

Lanksbury, J. and J.E. West. 2011. 2011/2012 mussel watch phase 1 sampling summary and progress report. Puget Sound ecosystem monitoring program toxics in biota. Washington Department of Fish and Wildlife, Olympia, WA. Pp. 75.

Lanyon, J., C.J. Limpus and H. Marsh. 1989. Dugong and Turtles: Grazers in the Seagrass System. *In*: Larkum, A.W.D., A.J. McComb and S.A. Shepherd (eds). Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region. pp. 610-627. Elsevier, Amsterdam.

Larkum, A.W.D., R.J. Orth and C.M. Duarte (eds). 2006. *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, Netherlands. 691 pp.

Lee, K-S., F.T. Short and D.M. Burdick. 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. Aquatic Botany 78:197-216.

Lee, R., A. Lovatelli and L. Ababouch. 2008. Bivalve depuration: fundamental and practical aspects. *FAO Fisheries Technical Paper*. No. 511. Food and Agriculture Organization of the United Nations (FAO), Rome. 139p.

Lefebvre, L.W., J.P. Reid, W.J. Kenworthy and J.A. Powell. 2000. Characterizing manatee habitat use and seagrass in Florida and Puerto Rico: implications for conservation and management. Pacific Conservation Biology 5:289-298.

Levine, S.N., D.T. Rudnick, J.R. Kelly, R.D. Morton, L.A. Buttel and K.A. Carr. 1990. Pollutant dynamics as influenced by seagrass beds: Experiments with tributyltin in *Thalassia* microcosms. Marine Environmental Research 30:297–332.

Lewis, M.A. and R. Devereux. 2009. Nonnutrient anthropogenic chemicals in seagrass ecosystems: fate and effects. Environmental Toxicology and Chemistry 28:644-661.

Lewis, M.A., D.D. Dantin, C.A. Chancy, K.C. Abel and C.G. Lewis. 2007. Florida seagrass habitat evaluation: a comparative survey for chemical quality. Environmental Pollution 146:206-218.

Lewis, R.R., M.J. Durako, M.D. Moffler and R.C. Phillips. 1985. Seagrass meadows of Tampa Bay – a review. *In:* Treat, S.F., J.L. Simon, R.R. Lewis and R.L. Whitman (eds.). Proceedings Tampa Bay Area Scientific Information Symposium, pp. 210-246. Bellweather Press.

Littler, M.M. and S.N. Murray. 1975. Impact of sewage on the distribution, abundance and community structure of rocky inter-tidal macro-organisms. Marine Biology 30:277-291.

Lin, Q.X. and I.A. Mendelssohn. 2009. Potential of restoration and phytoremediation with *Juncus roemerianus* for diesel-contaminated coastal wetlands. Ecological Engineering 35:85–91.

Lyngby, J.E. and H. Brix. 1982. Seasonal and environmental variation in cadmium, copper, lead and zinc concentratinos in eelgrass (*Zostera marina* L.) in the Limfjord, Denmark. Aquatic Botany 14:59-74.

Macinnis-Ng, C.M.O and P.J. Ralph. 2002. Towards a more ecologically relevant assessment of the impact of heavy metals on the photosynthesis of the seagrass *Zostera capriconi*. Marine Pollution Bulletin 45:100-106.

MacKay, D.C.G. 1942. The Pacific edible crab, *Cancer magister*. Bulletin of the Fisheries Research Board of Canada 62:1-32.

Malea, P. and S. Haritonidis. 1995. Local distribution and seasonal variation of Fe, Pb, Zn, Cu, Cd, Na, K, Ca, and Mg, concentrations in the seagrass *Cymodocea nodosa* (Ucria) Aschers in the Antikyra Gulf, Greece. Marine Ecology 16:41-56.

Malea, P. 1993. Bioaccumulation of aluminium in the seagrass *Cymodocea nodosa* (Ucria) Aschers. and *Posidonia oceanica* (L.) Delile and in macroalgae of the Gulf of Antikarya (Greece). Botanica Marina 36:423-431.

Malea, P., T. Kevrekidis and S. Haritonidis. 1995. The short-term uptake of zinc and cell mortality of the seagrass *Halophila stipulacea* (Forsk.) Aschers: Israel. Journal of Plant Sciences 43:21-30.

Marbà, N., M. Holmer, E. Gacia and C. Barrón. 2006. Seagrass beds and coastal biogeochemistry. *In:* Larkum, A.W.D., Orth, R.J., Duarte, C.M. (eds). Seagrasses: Biology, Ecology and Conservation. Pp. 135-157. Springer, Netherlands

Marín-Guirao, L., A.M. Atucha, J.L. Barba, E.M. López and A.J.C. Fernández. 2005. Effects of mining wastes on a seagrass ecosystem: metal accumulation and bioavailability, seagrass dynamics and associated community structure. Marine Environmental Research 60:317-337.

Marsh, J.A., Dennison, W.C. and Alberte, R.S. 1986. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). Journal of Experimental Marine Biology and Ecology 101:257-267.

Marwood, C.A., Solomon, K.R. and Greenberg, B.M. 2001. Chlorophyll fluorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of polycyclic aromatic hydrocarbons. Environmental Toxicology and Chemistry 20:890-898.

Mayer, J.R. and N.R. Elkins. 1990. Potential for agricultural pesticide runoff to a Puget Sound estuary: Padilla Bay, Washington. Bulletin of Environmental Contaminants and Toxicology 45:215-222.

McComb, A.J., M.L. Cambridge, H. Kirkman and J. Kuo. 1981. The biology of Australian seagrass. *In:* Plate, J.S. and A.J. McComb (eds). The Biology of Australian Plants, pp. 258-293. University of Western Australia Press, Perth.

McGlathery, K.J. 1995. Nutrient and grazing influences on a subtropical seagrass community. Marine Ecology Progress Series 122:239-252.

McRoy, C.P. 1970. On the biology of eelgrass in Alaska. Ph.D. thesis. University of Alaska, Fairbanks, AK.

McRoy, C.P. and J.J. Goering. 1974. Nutrient transfer between seagrass *Zostera marina* and its epiphytes. Nature 148:173-174.

McRoy, C.P. and C. Helfferich. 1980. Applied aspects of seagrasses. *In:* Phillips, R.C. and C.P. McRoy (eds). Handbook of Seagrass Biology: An Ecosystem Perspective. pp. 297-343. Garland STPM Press, New York.

Mitchell, C.A. 1987. Growth of *Halodule wrightii* in culture and the effects of cropping, light, salinity and atrazine. Aquatic Botany 28:25–37.

Mohamedali, T., M. Roberts, B. Sackmann and A. Kolosseus. 2011. Puget Sound Dissolved Oxygen Model – Nutrient Load Summary for 1999-2008. Washington State Department of Ecology, Olympia, WA. Publication No. 11-03-057. Pp 172.

Moore, K.A. and F.T. Short. 2006. *Zostera*: Biology, Ecology and Management. pp. 361-386. *In:* Larkum, A.W.D, R.J. Orth and C.M. Duarte (eds.). Seagrasses: Biology, Ecology and Conservation. Springer, Netherlands.

Moore, K.A., R.L. Wetzel and R.J. Orth. 1997. Seasonal pulses of turbidity and their relations to eelgrass (*Zostera marina* L.) survival in an estuary. Journal of Experimental Marine Biology and Ecology 215:115-134.

Moore G.E, F.T. Short and P.D. Sokoloff. In Review. The influence of salinity on growth and biomass in eelgrass, *Zostera marina* L. Marine Ecology Progress Series.

Morton, R.D., T.W. Duke, J.M. Macauley, J.R. Clark, W.A. Price, S.J. Hendricks, S.L. Owsley-Montgomery and G.R. Plaia. 1986. Impact of drilling fluids on seagrasses: An experimental community approach. *In:* Cairns Jr., J. (ed). Community Toxicity Testing. STP 920. American Society for Testing and Materials, Philadelphia, PA, USA, pp. 199–212.

Munda, I.M. 1980. Changes in the benthic algal associations of the vicinity of Rovinj (Istrian coast, north Adriatic) caused by organic wastes. Acta Adriatica 21:299-332.

Neckles, H.A., R.W. Wetzel and R.J. Orth. 1993 Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. Oecologia 93.285-295.

Neverauskas, V.P. 1987a. Monitoring seagrass beds around a sewage sludge outfall in south Australia. Marine Pollution Bulletin 18:158-164.

Neverauskas, V.P. 1987b. Port Adelaide sewage treatment works sludge outfall. Effect of discharge on the adjacent marine environment. Progress report. July 1982-May 1984. EWS Report 85/6.

Nielsen, L.W. and I. Dahllöf. 2007. Direct and indirect effects of the herbicides glyphosate, bentazone and MCPA on eelgrass (*Zostera marina*). Aquatic Toxicology 82:47-54.

Nienhuis, P.H. 1986. Background levels of heavy metals in nine tropical seagrass species in Indonesia. Marine Pollution Bulletin 17:508-511.

O'Neill, S.M. and J.E. West. 2009. Marine distribution, life history traits and the accumulation of polychlorinated biphenyls (PCBs) in Chinook salmon (*Oncorhynchus tshawytscha*) from Puget Sound, Washington. Transactions of the American Fisheries Society 138:616-632.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott and S.L. Williams. 2006. A global crisis for seagrass ecosystems. BioScience 56:987-996.

Pedersen, M.F., C.M. Duarte and J. Cebrián. 1997. Rates of changes in organic matter and nutrient stocks during seagrass *Cymodocea nodosa* colonization and stand development. Marine Ecology Progress Series 159:29-36.

Peirano, A., V. Damasso, M. Montefalcone, C. Morri and C.N. Bianchi. 2005. Effects of climate, invasive species and anthropogenic impacts on the growth of the seagrass *Posidonia oceanica* (L.) Delile in Liguria (NW Mediterranean Sea). Marine Pollution Bulletin 50:817-822.

Pergent-Martini, C. and G. Pergent. 1996. Spatio-temporal dynamics of *Posidonia oceanica* beds near a sewage outfall (Mediterranean – France). *In:* Kuo, J., R.C. Phillips, D.I. Walker and H. Kirkman (eds.), Seagrass Biology: Proceedings of an InternationalWorkshop. Rottnest Island,Western Australia, January 25–29, 1996, Sciences WA, Nedlands, pp. 299–306.

Pergent-Martini, C. and G. Pergent. 2000. Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. International Journal of Environment and Pollution 13:1-6.

Pergent, G., C. Labbe, C. Lafabrie, R. Kantin and C. Pergent-Martini. 2011. Organic and inorganic human-induced contamination of *Posidonia meadows*. Ecological Engineering 37:999-1002.

Peters, E.C., N.J. Gassmann, J.C. Firman, R.H. Richmond and E.A. Power. 1997. Ecotoxicology of tropical marine ecosystems. Environmental Toxicology and Chemistry 16:12-40.

Phillips, R.C. 1978. Seagrasses and the Coastal Marine Environment. Oceanus. Woods Hole Oceanographic Institution. 21**:**30-40.

Phillips, R.C. 1984. *The ecology of eelgrass meadows in the Pacific Northwest: a community profile*. U. S. Fish and Wildlife Service FSW/OBS-84/24. 85pp. Available online: http://www.nwrc.gov/library.html.

Plus, M., J.-M. Deslous-Paoli and F. Dagault. 2003. Seagrass (*Zostera marina* L.) bed recolonisation after anoxia-induced full mortality. Aquatic Botany 77:121-134.

Price, W.A., J.M. Macauley and J.R. Clark. 1986. Effects of drilling fluids on *Thalassia testudinum* and its epiphytic algae. Environmental Experiment Botany 26:321–330.

Price, A., W. Maher, J. Kirby, F. Krikowa, E. Duncan, A. Taylor and J. Potts. 2012. Distribution of arsenic species in an open seagrass ecosystem: Relationship to trophic groups, habitats and feeding zones. Environmental Chemistry 9:77-88.

PSP Indicator Action Team. 2010. Development of the dashboard of ecosystem indicators for Puget Sound. The Puget Sound Partnership's Indicators Action Team. http://www.psp.wa.gov/pm_dashboard.php

Pulich, W.M. 1980. Heavy metal accumulation by selected *Holodule wrightii* Asch. populations in the Corpus Christi bay area. Contributions in Marine Science 23:90-100.

Prange, J.A. and W.C. Dennison. 2000. Physiological responses of five seagrasses species to trace metals. Marine Pollution Bulletin 41:327-336.

Ralph, P.J. and M.D. Burchett. 1998a. Photosynthetic response of *Halophila ovalis* to heavy metal stress. Environmental Pollution 103:91-101.

Ralph, P.J. and M.D. Burchett. 1998b. Impact of petrochemicals on the photosynthesis of Halophila ovalis using chlorophyll fluorescence. Marine Pollution Bulletin 36:429-436.

Ralph, R.J., D. Tomasko, K. Moore, S. Seddon and C.M.O. Macinnis-Ng. 2006. Human impacts on seagrasses. *In:* Larkum, A.W.D., R.J. Orth and C.M. Duarte (eds). Seagrasses: Biology, Ecology and Conservation. Pp. 567-593. Springer, Netherlands.

Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. *In:* McRoy, C.P. and O. Helfferich (eds)., Seagrass Ecosystems: A Scientific Perspective. Marcel Dekker, New York 1-51.

Reopanichkul, P., R.W. Carter, S. Worachananant and C.J. Crossland. 2010. Wastewater discharge degrades coastal waters and reef communities in southern Thailand. Marine Environmental Research 69:287-296.

Roessler, M.A. 1974. Studies of effects of thermal pollution in Biscayne Bay, Florida. Office of Research and Development. U.S. EPA, EPA-660/3-74-014. Pp. 149. Washington, D.C.

Rozan, T. F. and K.S. Hunter. 2001. Effects of discharge on silver loading and transport in the Quinnipiac River, Connecticut. The Science of the Total Environment 279:195-205.

Runcie, J., C. Macinnis-Ng and P. Ralph. 2006. The toxic effects of petrochemicals on seagrasses. Literature review. Institute for Water and Environmental Resource Management and Department of Environmental Sciences. University of Technology, Sidney. Pp. 20.

Sanchiz, C., A.M. Garcia-Carrascosa and A. Pastor. 2000. Heavy metal contents in soft-bottom marine macrophytes and sediments along the Mediterranean coast of Spain. P.S.Z.N. Marine Ecology 21:1–16.

Sanchiz, C., A.M. Garcia-Carrascosa and A. Pastor. 2001. Relationships between sediment physico-chemical characteristics and heavy metal bioaccumulation in Mediterranean soft-bottom macrophytes. Aquatic Botany 69:63-73.

Sand-Jensen, K. 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. Ophelia 14:185-201.

Sand-Jensen, K. and J. Borum. 1983. Regulation of growth of eelgrass (*Zostera marina* L.) in Danish coastal waters. Marine Technology Society Journal 17:15-21.

San Juan County Water Resources Advisory Committee. 2009. The current status of desalination systems in San Juan County, Washington - executive summary and technical supplement. Pp. 88. (http://sanjuanco.com/health/ehswrm.aspx).

Santamaría-Gallegos, N.A., J.L. Sánchez-Lizaso and E.F. Félix-Pico. 2000. Phenology and growth cycle of annual subtidal eelgrass in a subtropical locality. Aquatic Botany 66:329-339.

Sanz-Lázaro, C., P. Malea, E.T. Apostolaki, I. Kalantzi, A. Marín and I. Karakassis. 2012. The role of the seagrass *Posidonia oceanica* in the cycling of trace elements. Biogeosciences Discussions 9:2623-2653.

Scarlett, A., P. Donkin, T.W. Fileman, S.V. Evans and M.E. Donkin. 1999. Risk posed by the antifouling paint Irgarol 1051 to the seagrass, *Zostera marina*. Aquatic Toxicology 45:159-170.

Schlacher-Hoenlinger, M.A. and Schlacher, T.A. 1998. Accumulation, contamination, and seasonal variability of trace metals in the coastal zone - patterns in a seagrass meadow from the Mediterranean. Marine Biology 131:401-410.

Schwartzschild, A.C., W.G. MacIntyre, K.A. Moore and E.L. Libelo. 1994. *Zostera marina* L. growth response to atrazine in root-rhizome and whole plant exposure experiments. Journal of Experimental Marine Biology and Ecology 183:77–89

Short, F.T. 1994. Eelgrass collection and analysis. *In:* Johnston, R.K., Munns Jr., W.R., Short, F.T. and Walker, H.A. (eds). 1994. Estuarine ecological risk assessment for Portsmouth Naval Shipyard, Kittery, Maine Phase I: problem formulation. Technical Report 1627, Naval Command, Control and Ocean Surveillance Center, San Diego.

Short, F.T. 1995. Status of eelgrass in Portsmouth Harbor and its use in assessing heavy metal contaminants in the Great Bay Estuary near the Portsmouth Naval Shipyard. Report submitted to: Johnston, R.K., Portsmouth Naval Shipyard Ecological Rish Assessment. 19 pp.

Short, F.T. and D.M. Burdick. 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading on Waquoit Bay, Massachusetts. Estuaries 19:730-739.

Short, F.T. and C.A. Short. 1984. The seagrass filter: purification of coastal waters. In: V.S. Kennedy (ed). The Estuary as a Filter. Pp. 395-413. Academic Press, Orlando.

Short, F.T., B.W Ibelings and C. den Hartog. 1988. Comparison of a current eelgrass disease to the wasting disease of the 1930's. Aquatic Botany 30:295-304.

Short, F.T., G.E. Jones and D.M. Burdick. 1991. Seagrass decline: problems and solutions. Coastal Wetlands Coastal Zone 1991 Conference-ASCE. Long Beach, CA.

Short, F.T., D.M. Burdick and J.E. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina* L. Limnology and Oceanography 40:740-749.

Silberstein, K., A.W. Chiffings and A.J. McComb. 1986. The loss of seagrass in Cockburn Sound, Western Australia. Aquatic Botany 24:355-371.

Simenstad, C.A. 1994. Faunal associations and ecological interactions in seagrass communities of the Pacific Northwest coast, pp.11-17. *In*: Wyllie-Echeverria, S., A.M. Olson and M.J. Hershman (eds). 1994. *Seagrass Science and Policy in the Pacific Northwest: Proceedings of a Seminar Series*. U.S. Environmental Protection Agency, Seattle, WA. (SMA 94-1). EPA 910/R-94 004. 63 pp.

Stenner, R.D. and G. Nickless. 1975. Heavy metals in organisms of the Atlantic coast of S.W. Spain and Portugal. Marine Pollution Bulletin 6:89-92.

Tampa Bay NEP. 2009. *Tampa Bay Estuary Program Progress Report 2009*. Technical Publication #04-09. 13 pp.

Thom, R.M., G.D. Williams and A.B. Borde. 2003. Conceptual Models as a Tool for Assessing, Restoring, and Managing Puget Sound Habitats and Resources. *In*: 2003 Georgia Basin/Puget Sound Research Conference, 31 March - 3 April 2003, Vancouver, BC. Georgia Basin/Puget Sound Research Conference, Vancouver, BC, Canada.

Thom, R.M., K.E. Buenau, C. Judd and V.I. Cullinan. 2011. Eelgrass (*Zostera marina* L.) stressors in Puget Sound. Prepared for Washington State Department of Natural Resources by Pacific Northwest National Laboratory, Sequim, WA. PNNL-20508.

Thorhaug, A., J. Marcus and F. Booker. 1986. Oil and dispersed oil on subtropical and tropical seagrasses in laboratory studies. Marine Pollution Bulletin 17:357–361.

Thorhaug, A. and J. Marcus. 1987. Oil spill clean-up: The effect of three dispersants on three subtropical/tropical seagrasses. Marine Pollution Bulletin 18:124–126.

Tomasko, D.A. and B.E. Lapointe. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. Marine Ecology Progress Series 75:9-17.

Tomasko, D.A., C.J. Dawes and M.O. Hall. 1996. The effects of anthropogenic nutrient enrichment on turtle grass (*Thalassia testudinum*) in Sarasota Bay, Florida. Estuaries 19:448-456.

Twilley, R.R., W.M. Kemp, K.W. Staver, J.C. Stevenson and W.R. Boynton. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. Marine Ecology Progress Series 23:179-191.

Udy, J.W. and W.C. Dennison. 1997. Growth and physiological response of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. Journal of Experimental Marine Biology and Ecology 217:253-277.

USFWS. 1994. Trace elements and oil-related contaminants in sediment, bivalves, and eelgrass from Padilla and Fidalgo Bays, Skagit County, Washington, 1988. U.S. Fish and Wildlife Service, Ecological Services, Washington State Office, Olympia, WA. Pp. 27.

Valentine, J.F. and J.E. Duffy. 2006. The central role of grazing in seagrass ecology. *In:* Larkum, A.W.D., R.J. Orth and C.M. Duarte. (eds). Seagrasses: Biology, Ecology and Conservation. Pp. 463-501. Springer, Netherlands.

Valentine, J.F. and K.L. Heck Jr. 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. Marine Ecology Progress Series 176:291-302.

van Katwijk, M.M., L.H.T. Vergeer, G.H.W. Schmitz and J.G.M. Roelois. 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157:159-173.

van Montfrans, J.J., R.L. Wetzel and R.J. Orth. 1984. Epiphyte-grazer relationships in seagrass meadows: consequences for seagrass growth and production. Estuaries 7:289-309.

van Vierssen, W. and T.C. Prins. 1985. On the relationship between growth of algae and aquatic macrophytes in brackish water. Aquatic Botany 21:165-179

Vaudrey, J.M.P, J.N. Kremer, B.F. Branco and F.T. Short. 2010. Eelgrass recovery after nutrient enrichment reversal. Aquatic Botany 93:237-243.

Walsh, G.E., D.L. Hansen and D.A. Lawrence. 1982. A flow-through system for exposure of seagrass to pollutants. Marine Environmental Research 7:1–11.

Ward, T.J. 1987. Temporal variation of metals in the seagrass *Posidonia australis* and its potential as a sentinel accumulator near a lead smelter. Marine Biology 95:315–321.

Ward, T.J. 1989. The accumulation and effects of metals in seagrass habitats. *In:* Larkum A.W.D., McComb A.J., Shepherd S.A. (eds.) Biology of seagrasses: A treatise on the biology of seagrasses with special reference to the Australian region. Elsevier, New York, pp. 797-820.

Water Quality Institute. 1978. Akkumulering af tungmetaller i sediment, bundvegetation, bundfauna og fisk. Vandkvalitetsundersøgelse i Køge Bugt 1977/78. Rapport til Hovedstadsrådet, 78 pp.

West, J.E., J. Lanksbury, S. O'Neill and A. Marshall. 2011. Control of toxic chemicals in Puget Sound Phase 3: persistent bioaccumulative and toxic contaminants in pelagic marine fish species from Puget Sound. Washington Department of Ecology, Lacey, WA. Publication No. 11-10-003. Pp. 60.

West, J.E., S.M. O'Neill and G.M. Ylitalo. 2008. Spatial extent, magnitude, and patterns of persistent organochlorine pollutants in Pacific herring (*Clupea pallasi*) populations in the Puget Sound (USA) and the Georgia Basin (Canada). Science of The Total Environment 394:369-378.

Whelan III, T., J. Espinoza, X. Villareal and M. CottaGoma. 2005. Trace metal partitioning in *Thalassia testudinum* and sediments in the Lower Laguna Madre, Texas. Environmental International 31:15-24.

Williams, S.L. and M.H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74:904-918.

Wilson, U.W. and J.B. Atkinson. 1995. Black brant winter and spring-stages use at two Washington coastal areas in relation to eelgrass abundance. *The Condor* 97:91-98.

Wolfe, D.A., G.W. Thayer and S.M. Adams. 1976. Manganese, iron, copper and zinc in an eelgrass (*Zostera marina*) community. Radioecology and energy resources. Proc. Fort. Ecological Society of America. Special Publication 1:256-270.

Zimmerman, R.C., R.D. Smith and R.S. Alberte. 1989. Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). Journal of Experimental Marine Biology and Ecology 130:93-109.

Appendix A Metals in Seagrass

Appendix A includes results from other studies that assessed metal concentrations in seagrass compartments (e.g., leaves, rhizomes, roots, and epibiota). These data will be useful to compare to the results of measured metal concentrations in eelgrass from Puget Sound.

The data are copied directly from the published manuscript, however, when indicated these tables have been modified slightly for clarity or to fit the format of the Appendix.

Table A-1. Mean concentration of metals in *Thalassia testudinum* collected along the northeastern coast of Venezuela. Table modified from Alfonso et al. 2008.

SAMPLE SITE	**SEAGRASS PARAMETER**	**Cd**	**Cr**	**Cu**	**Ni**	**V**	**Zn**
		(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)
Isla Bucha	Leaf	1.79 (0.89)	<dl	3.80 (2.61)	3.88 (0.25)	1.60 (0.61)	16.11 (3.48)
	rhizome/roots	1.52 (0.53)	2.14 (0.30)	5.86 (0.48)	4.62 (2.53)	6.51 (0.25)	25.11 (5.93)
Pertigalete	Leaf	1.6 (0.37)	<dl	6.01 (1.62)	4.83 (2.34)	2.20 (0.81)	14.07 (2.14)
	rhizome/roots	1.49 (0.24)	1.81 (0.48)	4.73 (1.28)	4.27 (0.18)	15.14* (1.65)	32.47 (4.68)
Isla Larga	Leaf	1.62 (0.26)	<dl	5.41 (0.52)	2.80 (1.44)	1.09 (0.92)	12.3 (3.10)
	rhizome/roots	1.70 (0.40)	<dl	6.26 (2.22)	3.14 (1.17)	4.49 (0.15)	32.58 (7.02)

* = mean value of elements with significant concentration differences between sample sites.
< dl = below detection limit.

Table A-2. The metal concentrations (µg/g dry weight) for 10 metals in the leaves and rhizome/roots compartments of *Thalassia testudinum* from 9 sites in Florida. Table modified from Lewis et al. 2007. Dash (-) equals no data.

SITE		PLANT COMPARTMENT	Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Zinc	Selenium	Silver
			As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Se	Ag
Santa Rosa Sound	Canal	roots/rhizomes	<2	0.7	0.5	<5	0.009	0.6	0.3	<50	1.3	0.2
	Canal	leaves	<2	0.8	0.1	7.1	0.018	3.5	0.3	<50	1.6	0.5
	Bayou	roots/rhizomes	<2	0.6	0.5	<5	0.007	0.8	0.3	<50	1.6	0.3
	Bayou	leaves	<2	1.2	<0.1	8.2	0.014	2.8	0.4	<50	1.7	0.6
	Golf Complex	roots/rhizomes	2.6	1.3	0.5	<5	0.009	0.7	0.9	<50	1.6	0.3
	Golf Complex	leaves	8.2	0.6	0.2	5.9	0.014	2.1	3.7	<50	2.2	0.6
	National Seashore	roots/rhizomes	<2	0.7	0.3	<5	-	0.6	0.4	<50	1.0	0.2
	National Seashore	leaves	<2	0.8	<0.1	<5	-	3.1	0.7	<50	<0.1	0.6
	Marina	roots/rhizomes	<2	0.9	0.8	<5	0.010	1.1	1.0	52	1.2	0.7
	Marina	leaves	<2	0.9	0.1	20.5	0.019	3.0	1.7	73	<1.0	0.5
Little Sabine Bay		roots/rhizomes	2.0	0.6	0.2	15.3	0.006	0.6	1.9	<50	1.3	0.2
		leaves	<2	0.9	<0.1	<5	0.014	1.9	0.6	<50	<1.0	0.5
Bonito Bay		roots/rhizomes	<2	0.5	0.4	<5	0.010	0.9	1.3	<50	<1.0	0.2
		leaves	<2	0.7	<0.1	<5	0.020	3.4	1.1	<50	<1.0	0.4

St. Joseph Bay Marina		**roots/rhizomes**	**<2**	**<0.1**	**<5**	**<5**	**-**	**<1**	**<1**	**8.4**	**<1.0**	**<0.1**
		leaves	**<2**	**0.11**	**<5**	**<5**	**-**	**<1**	**<1**	**6.1**	**<1.0**	**<0.1**
Little Duck Key		**roots/rhizomes**	**<2**	**<0.1**	**<5**	**<5**	**-**	**<1**	**<1**	**3.0**	**<1.0**	**<0.1**
		leaves	**<2**	**<0.1**	**<5**	**<5**	**-**	**<1**	**<1**	**4.8**	**<1.0**	**<0.1**

Table A-3. The metal concentrations (µg/g dry weight) for 10 metals in the leaves and rhizome/roots compartments of *Halodule wrightii* from 9 sites in Florida. Table modified from Lewis et al. 2007. Dash (-) equals no data.

SITE		PLANT COMPARTMENT	Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Zinc	Selenium	Silver
			As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Se	Ag
Santa Rosa Sound	Bayou	roots/rhizomes	2.7	0.4	2.9	8.3	0.013	1.3	1.3	<50	1.6	0.18
	Bayou	leaves	3.8	0.4	0.6	7.5	0.012	2.2	1.2	<50	2.5	0.29
	Golf Complex	roots/rhizomes	3.7	0.4	2.4	8.7	0.016	1.9	2.2	<50	1.9	<0.1
	Golf Complex	leaves	6.3	0.5	0.6	6.7	0.013	2.8	2.7	<50	2.3	0.21
	National Seashore	roots/rhizomes	<2	0.4	2.0	<5	0.012	1.3	1.1	<50	1.6	0.19
	National Seashore	leaves	2.6	0.4	0.2	<5	0.020	1.2	1.3	<50	1.7	0.65
	Wastewater Outfall	roots/rhizomes	<2	0.4	0.3	<5	0.019	<1	<1	<50	<1.0	0.13
	Wastewater Outfall	leaves	<2	0.3	3.5	9.1	0.038	3.5	1.5	<50	1.9	0.23
Choctawhatchee Bay		roots/rhizomes	<2	<0.1	<5	<5	-	<1	<1	2.9	<1.0	0.14
		leaves	<2	<0.1	<5	<5	-	<1	<1	3.5	<1.0	<0.1
Bonito Bay		roots/rhizomes	3.1	0.2	0.6	<5	0.009	2.7	4.1	<50	<1.0	<0.1
		leaves	7.4	0.3	0.4	<5	0.017	1.8	4.8	<50	1.4	0.32
St. Joseph Bay	Bayou	roots/rhizomes	<2	<0.1	<5	<5	-	<1	<1	6.8	<1.0	<0.1
		leaves	<2	<0.1	<5	<5	-	<1	<1	6.1	<1.0	<0.1
St. Joseph Bay	Marina	roots/rhizomes	<2	<0.1	<5	<5	-	<1	<1	6.0	<1.0	<0.1

		leaves	**<2**	**<0.1**	**<5**	**<5**	**-**	**<1**	**<1**	**6.2**	**<1.0**	**<0.1**
Ohio Key		**roots/rhizomes**	**<2**	**0.12**	**<5**	**<5**	**-**	**<1**	**<1**	**2.5**	**<1.0**	**<0.1**
		leaves	**<2**	**0.13**	**<5**	**<5**	**-**	**<1**	**<1**	**3.4**	**<1.0**	**<0.1**

Table A-4. Mean concentration (ppm dry weight) and range of four metals measured in the aboveground (leaves) and belowground (rhizome/roots) compartments of eelgrass in Limfjord, Denmark. Table modified from Brix et al. 1983.

METAL	ABOVEGROUND - BELOWGROUND	MEAN	RANGE	RANGE
	Leaves Rhizomes/roots		(low)	(high)
			(ppm)	(ppm)
Pb	Leaves	1.07	0.47	37.46
	Rhizome/roots	1.04	0.35	29.77
Cu	Leaves	4.79	1.86	16.63
	Rhizome/roots	3.33	1.82	19.29
Cd	Leaves	0.62	0.09	2.92
	Rhizome/roots	0.30	0.13	0.92
Zn	Leaves	78	41	175
	Rhizome/roots	55	25	125

Table A-5. Geometric mean concentration (ppm dry weight) of and range of metals measured in the aboveground (leaves) and (rhizomes/roots) compartments of eelgrass in Limfjord, Denmark. Table modified from Brix and Lyngby 1984.

METAL	COMPARTMENT	n	GEOMETRIC MEAN (x)	RANGE	RANGE	C.V.
			(ppm)	(low)	(high)	(%)
Ca	Leaves	40	13,100	4,600	36,300	48
	Rhizome/roots	40	13,500	5,200	51,800	73
Cd	Leaves	40	0.62	0.09	2.92	98
	Rhizome/roots	40	0.30	0.13	0.92	62
Cu	Leaves	38	4.91	2.74	10.71	34
	Rhizome/roots	36	3.33	1.82	5.80	35
Fe	Leaves	40	390	80	2,990	113
	Rhizome/roots	40	835	101	4,080	129
K	Leaves	39	35,000	19,3000	49,800	30
	Rhizome/roots	40	28,000	21,000	41,000	21
Mg	Leaves	39	7,960	6,900	9,000	7
	Rhizome/roots	40	6,,870	4,800	9,000	17
Mn	Leaves	40	1,820	480	5,770	76
	Rhizome/roots	40	260	74	1,200	81
Na	Leaves	40	33,300	24,400	49,500	19
	Rhizome/roots	40	35,100	26,600	39,400	20
Pb	Leaves	36	1.07	0.47	3.09	52
	Rhizome/roots	36	1.04	0.35	2.79	73
Zn	Leaves	40	78	41	175	45
	Rhizome/roots	40	55	25	125	50

Table A-6. Metal concentration (µg/g) in eelgrass leaves, rhizome/roots and sediments in the Thau Lagoon, France. Although some of these values are repeated in the next table (Lewis and Devereux 2009), the data here are valuable because they compare the metal concentrations between above- and below-ground biomass and the sediments. Table modified from DeCasabianca et al. 2004.

		Fe	**Zn**	**Cr**	**Cu**	**Pb**	**Ni**
		(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)
Content	Sediment	6100 (1090)	36 (8)	21 (8)	19 (4)	13 (4)	9 (3)
Content	Leaves	186 (82)	83 (29)	0.3 (0.2)	10 (4)	1 (1)	0.6 (0.4)
Content	Roots	921 (87)	44 (19)	2 (2)	9 (5)	2 (1)	1 (1)
Ratio	Leaves/Sediment	0.03	2.3	0.01	0.5	0.1	0.1
	Root / Sediment	0.15	0.5	0.08	0.5	0.1	0.1
Sig Diff		Root > leaves	Leaves > root	Root > leaves	No	No	No

Table A-7. Metallic composition (ppm dry weight) of eelgrass (*Z. marina*) compartments (leaves, rhizomes, roots) from the literature. Table modified from Lewis and Devereux (2009) and Brix and Lyngby (1984) with the addition of other data as listed.

REFERENCE	LOCATION	COMPARTMENT	VALUE	Al	As	Au	Ca	Cd	Ce	Co	Cr
		(leaves, rhizome/roots)	(single, mean, range)	Aluminum	Arsenic	Gold	Calcium	Cadmium	Cesium	Cobalt	Chromium
Augier et al. (1983)	Mediterranean Sea	leaves									
Bellester et al. (1980), as cited in Lewis and Devereux (2009)	Catalonia Coast (Spain)	whole plant	single					0.26			
Bojanowski (1973)	Baltic (Poland)	leaves	mean				11,800			1.91	
			range				9,300-21,800			0.27-6.80	
Brix et al. (1983)	Bay of Aarhus (Denmark)	leaves	mean					1.03			1.4
		rhizome/roots	range					0.1-0.9			
Brix and Lingby (1984)	Limfjord (Denmark)	leaves	mean				13,000	0.62			2.2
			range				4,600-36,300	0.09-2.92		2.5-15.7	0.07-9.8
Damyanova et al. (1981)	Black Sea (Bulgary)	leaves	mean		0.21	0.0044		7.15	0.72	6.34	1.53
			range		0.07-0.43	0.0024-0.0072		6.00-9.20	0.53-0.90	5.66-7.10	1.25-1.75
DeCasabianca et al. (2004)	Thau Lagoon (France)	leaves	mean								0.3
DeCasabianca et al. (2004)	Thau Lagoon (France)	rhizome/roots	mean								2
Dieckmann (1982	Kiel Fjord (Germany)	leaves	mean					0.49-2.22			
Drifmeyer et al. (1980)	Beaufort (North Carolina)	leaves	mean								
Gorham et al. (1980)	English Channel	leaves	mean				5130				
Güven et al. (1993), as cited in Short	Bosphorus Strait (Turkey)	leaves						2.3			13.6

(1995)											
Güven et al. (1993), as cited in Lewis and Devereux (2009)	Bosphorus Strait (Turkey)	whole plant	mean					1.9-2.3			8.3-13.6
Johnston et al. 1994a, 1994b, and Short 1994	Piscataqua River and Great Bay Estuary (Maine/New Hampshire	leaves	mean	51.3	0.9			0.9			0.6
		leaves	range	9.0-120.0	0.6-1.4			0.3-1.9			0.3-0.9
		rhizome/roots	mean	577.7	4.1			0.5			4.5
		rhizome/roots	range	203.0-938.0	1.5-10.9			0.3-0.8			1.7-9.7
Kaldy (2006)	Yaquina Bay (Oregon)	leaves	range								3-15
McRoy (1966) , as cited in Burrell and Schubel (1977)								0.23		0.03	
US FWS (1994)	Padilla and Fidalgo Bays		Range	1,905-7,320	1.6-7.8			1.1-3.6			5.5-35.2
Water Quality Institute (1978)	Bay of Køge (Denmark)	leaves	mean					0.5			0.8
			range					0.1-1.7			<0.4-1.8
Wolfe et al. (1975), as cited in Lewis and Devereux (2009)	Newport River estuary (Oregon)	whole plant	mean								
Wolfe et al. (1976)	Beaufort (North Carolina)	leaves	mean								

REFERENCE	LOCATION	COMPARTMENT	VALUE	Cu	Fe	Hg	K	Mg	Mn	Na	Ni
		(leaves, rhizome/roots)	(single, mean, range)	Copper	Iron	Mercury	Potassium	magnesium	manganese	sodium	nickel
Augier et al. (1983)	Mediterranean Sea	leaves									
Bellester et al. (1980), as cited in Lewis and Devereux (2009)	Catalonia Coast (Spain)	whole plant	single	5.6							2.1
Bojanowski (1973)	Baltic (Poland)	Leaves	mean	15.2	480		34,700	9,900	990	24,300	4.6
			range	8.0-33.5	120-1,540		12,800-53,500	8,200-11,200	130-2,270	10,000-34,800	1.3-11.8
Brix et al. (1983)	Bay of Aarhus (Denmark)	leaves		5.86	296						
		rhizome/roots	range	1.8-19.3							
Brix and Lingby (1984)	Limfjord (Denmark)	leaves	mean	4.91	390	0.012	35,000	7,960	1,820	33,300	
			range	1.86-16.6	80-2,990	0.005-1.14	13,600-49,800	6,900-11,000	480-5,770	24,400-49,500	
Damyanova et al. 1981	Black Sea (Bulgary)	leaves	mean	9.89	670	0.48			50		
			range	7.76-11.65	559-789	0.38-0.59			41-60		
DeCasabianca et al. (2004)	Thau Lagoon (France)	leaves	mean	10	186						0.6
DeCasabianca et al. (2004)	Thau Lagoon (France)	rhizome/roots	mean	9	921						1
Dieckmann (1982	Kiel Fjord (Germany)	leaves	mean	7.9	1,240				154		
Drifmeyer et al. (1980)	Beaufort (North Carolina)	leaves	mean	6.4	810						
Gorham et al. (1980)	English Channel		mean				29,100	12,000		55,900	

Güven et al. (1993), as cited in Short (1995)	Bosphorus Strait (Turkey)	leaves		39.8							17.5
Güven et al. (1993), as cited in Lewis and Devereux (2009)	Bosphorus Strait (Turkey)	whole plant	mean	23.4-39.8							12.9-17.5
Johnston et al. 1994a, 1994b, and Short 1994	Piscataqua River and Great Bay Estuary (Maine/New Hampshire)	leaves	mean	20.0	294.3	0.01			96.2		1.4
		leaves	range	8.8-62.6	58.0-590.0	0.01-0.02			14.0-265.0		0.4-2.3
		rhizome/roots	mean	17.6	3,624.4	0.03			57.2		2.1
		rhizome/roots	range	8.3-36.7	1,280.0-6,200.0	0.01-0.05			15.0-240.0		1.1-3.0
Kaldy (2006)	Yaquina Bay (Oregon)	leaves	range	10-20							2-120
McRoy (1966), as cited in Burrell and Schubel (1977)				7.50	34-345	1.33			34-1,845		0.4
US FWS (1994)	Padilla and Fidalgo Bays		range	5.05-11.8	2,835-10,300	0.009-0.022			118-324		4.6-21.0
Water Quality Institute (1978)	Bay of Køge (Denmark)	leaves	mean	4.5		0.19					
			range	2.0-9.3		0.07-0.50					
Wolfe et al. (1975), as cited in Lewis and Devereux (2009)	Newport River estuary (Oregon)	whole plant	mean	7.9							
Wolfe et al. (1976)	Beaufort (North Carolina)	leaves	mean								

REFERENCE	LOCATION	COMPARTMENT	VALUE	Pb	Sb	Sc	Se	Sm	Sr	V	Zn
		(leaves, rhizome/roots)	(single, mean, range)	lead	antimony	scandium	selenium	samarium	strontium	vanadium	zinc
Augier et al. (1983)	Mediterranean Sea	leaves									
Bellester et al. (1980), as cited in Lewis and Devereux (2009)	Catalonia Coast (Spain)	whole plant	single	0.79							
Bojanowski (1973)	Baltic (Poland)	Leaves	mean						240		300
			range						155-480		80-820
Brix et al. (1983)	Bay of Aarhus (Denmark)	leaves	mean								
		rhizome/roots	range	0.4-30							25-125
Brix and Lingby (1984)	Limfjord (Denmark)	leaves	mean	1.07							78
			range	0.47-37.5							41-175
Damyanova et al. (1981)	Black Sea (Bulgary)	leaves	mean		0.91	0.018	0.22	0.38			37
			range		0.75-1.01	0.014-0.021	0.10-0.33	0.27-0.53			31-45
DeCasabianca et al. (2004)	Thau Lagoon (France)	leaves	mean	1							83
DeCasabianca et al. (2004)	Thau Lagoon (France)	rhizome/roots	mean	2							44
Dieckmann (1982	Kiel Fjord (Germany)	leaves	mean								70
Drifmeyer et al. (1980)	Beaufort (North Carolina)	leaves	mean								

Gorham et al. (1980)	English Channel		mean								
Güven et al. (1993), as cited in Short (1995)	Bosphorus Strait (Turkey)	leaves		32							91
Guven et al. (1993), as cited in Lewis and Devereux (2009)	Bosphorus Strait (Turkey)	whole plant	mean	26.1-32.1							48.7-91.3
Johnston et al. 1994a, 1994b, and Short 1994	Piscataqua River and Great Bay Estuary (Maine/New Hampshire	leaves	mean	1.3							63.7
		leaves	range	0.8-2.1							51.4-79.2
		rhizome/roots	mean	7.4							48.4
		rhizome/roots	range	1.7-14.0							24.2-75.9
Kaldy (2006)	Yaquina Bay (Oregon)	leaves	range								20-40
McRoy (1966), as cited in Burrell and Schubel (1977)											27-56
US FWS (1994)	Padilla and Fidalgo Bays		range	0.9-2.7			<0.2-0.4				21.5-34.8
Water Quality Institute (1978)	Bay of Køge (Denmark)	leaves	mean	3.4							66
			range	0.4-13.0							33-120
Wolfe et al. (1975), as cited in Lewis and Devereux (2009)	Newport River estuary (Oregon)	whole plant	mean								70
Wolfe et al. (1976)	Beaufort (North Carolina)	leaves	mean								

Table A-8. Chemical characteristic of *Z. marina*. Table modified from Gunnarsson et al. 1999.

SUBSTRATES	TN	TOC	C/N	AMINO ACID	LIPID	POLYPHENOL
	Total nitrogen (% dry wt)	Total organic carbon (% dry wt)	carbon-nitrogen ratio	Total amino acid content (µg/mg dry wt)	nonpolar lipids (% dry wt)	Polyphenolic substances (% dry wt)
Z. marina	1.32 (0.03)	27.2 (0.7)	22	63.4	0.5 (0.1)	0.5

Table A-9. Trace metal concentrations (µg g^{-1} dry wt, mean ± S.E.) measured in *Posidonia oceanica* leaves at an industrialized city site (Toulon) and a pristine site (Calvi). All concentrations of metals between Toulon and Calvi were significantly different, $p<0.05$. Table modified from Pergent et al. 2011.

METAL	TOULON	CALVI
	(industrialize city site)	(pristine site)
	(µg g^{-1} dry wt)	(µg g^{-1} dry wt)
Ag	0.7 (0.0)	0.5 (0.0)
Hg	0.15 (0.03)	0.04 (0.01)
Pb	3.0 (0.1)	1.5 (0.3)

Appendix B PAHs, PCBs, and Organic Contaminants in Seagrass

Appendix B includes results from other studies that assessed contaminant concentrations in seagrass compartments (e.g., leaves, roots, rhizomes, and epibiota). These data will be useful to compare to the measured contaminant concentrations in eelgrass from Puget Sound.

The data are copied directly from the published manuscript, however, when indicated these tables have been modified slightly for clarity or to fit the format of the Appendix.

Table B-1. The following compounds have been found in the North Sea and have been shown to cause impacts to seagrass. Table modified from Bester 2000.

COMPOUND	EFFECT	PRESENCE	REFERENCE
Triazines	e.g. Photosynthesis	North Sea and Wadden Sea	
Phenylureas	e.g. Photosynthesis	Wadden Sea	Meerendonk et al. 1994 as cited in Bester 2000.
Acetanilides	e.g. Photosynthesis	Wadden Sea	Meerendonk et al. 1994 as cited in Bester 2000.
Glyphosate/gluphosinate	e.g. Photosynthesis	Likely most commonly used pesticide in Germany and the Netherlands	
Chlorophenols/chlorophenoxycarbonic acids	e.g. growth stimulant	German Bight and North Sea	Hühnerfuss et al. 1990 as cited in Bester 2000
PAH	e.g. DNA-adducts/cancer	Wadden Sea	de Jong et al. 1993 as cited in Bester 2000
Benzothiazoles	e.g. Toxic to microorganism	German Bight	
Polycyclic musk fragrances	e.g. Bioaccumulation	German Bight	
Chlorinated anilines	e.g. Mutagensis	German Bight	
Nitro-aromatic compounds	e.g. Mutagensis	German Bight	
HCH (lindane)	Unknown/Neuro toxic	German Bight and Wadden Sea	

Table B-2. PAH concentrations (mean ± (S.E.) measured in *Posidonia oceanica* leaves at an industrialized city site (Toulon) and a pristine site (Calvi). Table modified from Pergent et al. 2011.

PAH	PAH	TOULON	CALVI
	(abbr.)	(industrialize city site)	(pristine site)
		(µg g^{-1} dry wt)	(µg g^{-1} dry wt)
Low Molecular Weight	*Low Molecular Weight*		
Naphthalene	Nap	-	-
Acenaphtylene	Acy	-	-
Acenaphthene	Ace	4.4 (0.6)	5.2 (1.1)
Fluorine	Flr	19.3 (1.9)	20.8 (5.7)
Medium Molecular Weight	*Medium Molecular Weight*		
Phenanthrene	Phe	84.6 (13.0)	53.2 (6.6)
Anthracene	Ant	2.3 (0.3)	2.7 (0.5)
Fluoranthene	Flt	18.7 (1.6)	15.8 (2.7)
Pyrene	Pyr	18.6 (1.0)	15.0 (3.0)
Benzo(a)anthracene	BaA	22.8 (1.2)	20.1 (2.0)
Chrysene	Chry	12.0 (1.1)	11.7 (2.5)
High Molecular Weight	*High Molecular Weight*		
Benzo(b)fluoranthene	BbF	23.4 (1.8)	19.3 (4.1)
Benzo(k)fluoranthene	BkF	3.0 (0.6)	1.9 (0.4)
Benzo(a)pyrene	BaP	3.3 (0.1)	2.2 (0.6)
Dibenzo(a,h)anthracene	DahA	1.4 (0.1)	1.1 (0.3)
Benzo(g,h,i)perylene	BghiP	4.9 (0.2)	0.8 (0.3)
Indeno(1,2,3-cd)pyrene	Ind	8.9 (0.4)	0.7 (0.2)

Concentrations of Phe, BghiP, and Ind were significantly different between the two sites.

Table B-3. PAH concentrations (ppb) measured in *Z. marina* leaves and roots in the Piscataqua River and Great Bay Estuary, Maine/New Hampshire. Table has been modified from Johnston et al. 1994a, b, and Short 1994.

LOCATION	DATE	% H20	FLRENE	PHEN	ANTH	C1	C2	C3	C4
DESCRIPTION	YYMMDD	Percent Moisture	Fluorene	Phenanthrene	Anthracene	C1-phenanthrene + anthracene	C2-phenanthrene + anthracene	C3-phenanthrene + anthracene	C4-phenanthrene + anthracene
EELGRASS LEAVES									
3	910916	15.0	10.00 [a]	11.00 [b]	20.00 [a]	20.00 [a]	20.00 [a]	20.00 [a]	20.00 [a]
3	910916	15.0	10.00 [a]	10.00 [a]	10.00 [a]	20.00 [a]	20.00 [a]	20.00 [a]	20.00 [a]
19	910917	10.0	10.00 [a]	12.00 [b]	20.00 [a]	20.00 [a]	29.00 [b]	20.00 [a]	20.00 [a]
12A	910922	10.0	4.10 [c]	21.00 [b]	3.70 [c]	27.00 [b]	52.00 [b]	16.00 [c]	22.00 [a]
12A	910922	10.0	2.60 [c]	27.00 [b]	4.90 [c]	40.00 [b]	50.00 [b]	17.50 [c]	22.00 [a]
EELGRASS RHIZOMES/ROOTS									
3	910916	12.0	10.00 [b]	22.00 [b]	20.00 [a]	55.00 [b]	72.00	30.00 [b]	20.00 [a]
19	910917	10.0	10.00 [a]	20.00 [b]	20.00 [a]	20.00 [a]	45.00 [b]	20.00 [a]	20.00 [a]

LOCATION	DATE	% H20	FLUORAN	PYRENE	BAA
DESCRIPTION	YYMMDD	Percent Moisture	Fluoranthene	Pyrene	Benz(a)anthracene
EELGRASS LEAVES					
3	910916	15.0	25.00 [b]	26.00 [b]	20.00 [a]
3	910916	15.0	18.00 [b]	18.00 [b]	20.00 [a]
19	910917	10.0	34.00 [b]	44.00 [b]	20.00 [a]
12A	910922	10.0	60.00	49.00	12.00 [c]
12A	910922	10.0	57.00	49.00	10.60 [c]
EELGRASS RHIZOMES/ROOTS					
3	910916	12.0	79.00	79.00	40.00 [b]
19	910917	10.0	76.00	90.00	68.00

<u>Data qualifier codes:</u>

a analyte was not detected below the method detection limit (MDL) shown.

b reported value was below the limit of quantification (LOQ)

c reported value was below the MDL

Table B-4. Polycyclic aromatic hydrocarbons (PAH) concentrations (ppb) measured in *Z. marina* at sites in Padilla and Fidalgo Bays, Skagit County, WA. Table has been modified from US FWS (1994).

LOCATION	**DATE**	**% H20**	**NAPTH**	**FLRENE**	**PHEN**	**ANTH**	**FLUORAN**	**PYRENE**	**1,2-BEN**
DESCRIPTION	YYMMDD	Percent Moisture	Napthalene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	1,2-benzanthracene
EELGRASS SAMPLE									
PB1-EG	26 Jul – 4 Aug 2012	84.0	<0.01	<0.01	0.06	<0.01	<0.01	<0.01	<0.01
PB2-EG	26 Jul – 4 Aug 2012	82.0	<0.01	<0.01	0.06	<0.01	<0.01	<0.01	<0.01
SB2-EG	26 Jul – 4 Aug 2012	76.0	<0.01	<0.01	0.11	<0.01	<0.01	<0.01	<0.01
FB2-EG	26 Jul – 4 Aug 2012	84.5	<0.01	0.01	0.13	<0.01	0.02	<0.01	0.01
FB4-EG	26 Jul – 4 Aug 2012	83.0	<0.01	0.01	0.13	<0.01	<0.01	<0.01	<0.01

LOCATION	**DATE**	**CHRY**	**BENZO(b)**	**BENZO(k)**
DESCRIPTION	YYMMDD	Chrysene	Benzo(b)-fluoranthrene	Benzo(k)-fluoranthrene
EELGRASS SAMPLE				
PB1-EG	26 Jul – 4 Aug 2012	<0.01	<0.01	<0.01
PB2-EG	26 Jul – 4 Aug 2012	<0.01	<0.01	<0.01
SB2-EG	26 Jul – 4 Aug 2012	<0.01	<0.01	<0.01
FB2-EG	26 Jul – 4 Aug 2012	<0.01	<0.01	<0.01
FB4-EG	26 Jul – 4 Aug 2012	<0.01	<0.01	<0.01

Table B-5. Aliphatic hydrocarbons (AH) concentrations (ppb) measured in *Z. marina* at sites in Padilla and Fidalgo Bays, Skagit County, WA. Table has been modified from US FWS (1994).

LOCATION	DATE	N-DODE	N-TRI	N-TETRA	OCTYL	N-PENTA	NONYL	N-HEXA	N-HEPTA
DESCRIPTION	YYMMDD	N-dodecane	N-tridecane	N-tetradecane	Octylcyclohexane	N-pentadecane	Nonylcyclohexane	N-hexadecane	N-heptadecane
EELGRASS SAMPLE									
PB1-EG	26 Jul – 4 Aug 2012	0.05	0.12	0.29	0.07	13.5	0.21	3.2	14.0
PB2-EG	26 Jul – 4 Aug 2012	0.08	0.13	0.30	0.09	7.1	0.27	2.4	9.6
SB2-EG	26 Jul – 4 Aug 2012	0.05	0.15	0.39	0.13	6.3	0.39	1.8	6.9
FB2-EG	26 Jul – 4 Aug 2012	0.05	0.20	0.50	0.16	9.0	0.50	2.2	8.0
FB4-EG	26 Jul – 4 Aug 2012	0.11	0.21	0.52	0.17	13.0	0.51	2.3	8.2

LOCATION	DATE	PRIS	N-OCTA	PHYT	N-NONA	N-EICO	TOTAL
DESCRIPTION	YYMMDD	Pristane	N-octadecane	Phytane	N-nonadecane	N-eicosane	Total
EELGRASS SAMPLE							
PB1-EG	26 Jul – 4 Aug 2012	0.97	1.2	0.48	3.3	0.22	37.61
PB2-EG	26 Jul – 4 Aug 2012	1.2	1.3	0.59	3.0	0.24	26.50
SB2-EG	26 Jul – 4 Aug 2012	1.9	1.6	0.83	3.3	0.27	24.01
FB2-EG	26 Jul – 4 Aug 2012	2.1	2.1	1.1	4.3	0.32	30.53
FB4-EG	26 Jul – 4 Aug 2012	2.0	2.1	1.0	4.3	0.55	34.97

Appendix C Field and Analysis Methods

Appendix C summarizes methods from other studies that may be useful to consider when developing the sample plan and procedures for eelgrass collection and processing in Puget Sound for this project.

Alfonso et al. 2008 – Seagrass separated into leaf blades and rhizome/roots, triplicate samples collected at each sampling site. Samples were rinsed, epiphytes removed and tissue dried at 80ºC for 24 h. Dried samples were ground into a fine powder with an agate mortar and pestle. Method to detect metals was Inductively Coupled Plasma Optical Emission Spectroscopy.

Brix et al. 1983 – Plants were fractionated into above-ground (leaves and stems, likely sheaths) and below-ground parts rhizomes and roots). Epiphytes were removed by scraping and washing. Samples dried at 105ºC until constant weight, then ground in a porcelain mortar.

DeCasabianca et al. 2004 – Sampled monthly from February 1994 to March 1995 at a 1.7 m depth. Eelgrass was sampled in triplicate from a 0.25 m^2 quadrat. Samples dried at 70ºC for 48 h. After sample prep, metal analysis was performed with the Flame Atomic Absorption Spectrophotometry method.

Hoven et al. 1999 and Bester 2000 – Both studies mention, for the sake of comparative studies between metal and contaminant concentration in seagrass and the environment, that sampling the sediment is better than sampling the water. Metals and contaminants accumulate in the sediment whereas their concentration in water is highly variable due to tidal exchange.

Johnston et al 1994a, b, and Short 1994 – Authors investigated the concentration of metals and contaminants in the Piscataqua River and Great Bay Estuary. Drying samples on aluminum foil prior to analysis caused elevated Al concentrations in samples. The study also found that sample handling affected inorganic concentrations in the dry and wet samples. The authors concluded that the concentrations of As, Cr, Fe, Mn, Ni, and Pb were all likely elevated due to the contamination of the aluminum foil. It was concluded that Ag, Cd, Cu, and Zn were not contaminated.

Marín-Guirao et al. 2005 – Used a toothbrush to remove all sediments from the root/rhizomes and checked effectiveness of the toothbrush procedure under a scope.