

# **Quality Assurance Project Plan**

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For:

Outfall Assessment and the Effects on Critical  
Nearshore Habitats

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Prepared by:

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Prepared for:

Puget Sound Marine and Nearshore Protection and  
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1.0 Title Page/TOC/Abstract

**Quality Assurance Project Plan**

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**Outfall Assessment and the Effects on Critical Nearshore Habitats**

December 2012

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# Table of Contents

	<u>Page</u>
1.0 Title Page/TOC/Abstract .....	1
2.0 Abstract.....	5
3.0 Background.....	6
4.0 Project Description.....	8
5.0 Organization and Schedule .....	11
6.0 Quality Objectives .....	14
7.0 Sampling Process Design (Experimental Design) .....	20
8.0 Sampling Procedures .....	25
9.0 Measurement Methods.....	30
10.0 Quality Control (QC) Procedures .....	40
11.0 Data Management Procedures .....	41
12.0 Audits and Reports.....	42
13.0 Data Verification.....	42
14.0 Data Quality (Usability) Assessment.....	43
15.0 References.....	45
Appendix A. Project SOW and budget.....	51
Appendix B. Standard Operating Procedures for analysis of nutrients by the UC Davis Stable Isotope Laboratory .....	52
Appendix C. Standard Operating Procedures for analysis of nutrients by the King County Environmental Laboratory .....	53
Appendix E. Glossary, Acronyms, and Abbreviations.....	55
Appendix F. Data Sheets for eelgrass processing.....	63

## List of Figures

Figure 5.1. Project schedule.....	13
Figure 7.1. Eelgrass collection field data sheet. ....	21
Figure 7.2. The proposed sample sites in Puget Sound where eelgrass ( <i>Z. marina</i> ) will be collected. The eelgrass will be analyzed for nutrients, metals, and organic contaminants in above- and belowground plant compartments.....	23
Figure 8.1. Chain of Custody form to track transfer of samples from eelgrass collection to Aquatic Botany Laboratory.....	28
Figure 8.2. Chain of Custody forms to track transfer of samples from Aquatic Botany Laboratory to analytical laboratory. ....	29

## List of Tables

Table 6.1. Eelgrass components collected for chemical analyses. ....	14
Table 6.2.*. Minimum analytical quality assurance criteria for polycyclic aromatic compounds (PACs) and persistent organic pollutants (POPs) by gas chromatography/mass spectrometry (GC-MS). ....	15
Table 6.3*. Required batch quality control measures and quality assurance criteria for mercury via Cold Vapor Atomic Absorption (CVAA). ....	16
Table 6.4. Required batch quality control measures and quality assurance criteria for the ICP-MS metals As, Cd, Cr, Cu, Fe, Pb, Ni, Zn, and V. ....	17
Table 7.1. Ten (10) sample sites throughout greater Puget Sound, their location and latitude and longitude. ....	22
Table 7.2. Five additional sample sites, their location, latitude and longitude and outside funding source. ....	24
Table 9.1. An example of the sample identification labels for the above- and belowground nutrient, metal and organic samples at one site (Thompson Spit – TS). ....	33
Table 9.2. Elements (C:N) to be measured in above- and belowground eelgrass. ....	37
Table 9.3. Metals to be measured in above- and belowground eelgrass. ....	37
Table 9.4. Organics to be measured in above- and belowground eelgrass. ....	38
Table 9.5. Conventional to be measured in this study. ....	38
Table 9.6. Additional samples in Task 2 for Quality Control for the nutrient, metal and organic analyses. ....	39
Table 9.7. Additional samples in Task 3 for Quality Control for the nutrient, metal and organic analyses. ....	39

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## 2.0 Abstract

The goal of the eelgrass – outfall project is to improve our understanding of the concentrations of nutrient, metal and organic contaminants in the dominant seagrass, eelgrass (*Zostera marina* L.), in Puget Sound. A literature review characterized known impacts of outfalls and other sources of loading on eelgrass and how the physical alteration of habitat and effluent affect eelgrass and the greater marine community through trophic transfer (Gaeckle 2012). The literature review also aided in the development of site selection and field and laboratory methods. To determine the concentration of nutrients, metals, and organic contaminants in eelgrass in Puget Sound, eelgrass samples will be collected at sites over a spatial gradient. Eelgrass samples will be analyzed to assess nutrient, metal and organic contaminant concentrations in above- and belowground plant compartments. These data will provide an understanding of nutrient, metal, and organic contaminant concentration in eelgrass and establish a baseline for further research. Field data will also be collected at one outfall scheduled for mitigation actions to describe baseline eelgrass bed characteristics and the extent of nutrient, metal, and organic contaminant concentrations in eelgrass at this site (to be determined). These data will be used as a basis for effectiveness monitoring following changes to the outfall and future projects.

The funds for this project were awarded through the Puget Sound Marine and Nearshore Protection and Restoration Grant Program. The project will be completed by staff in the Nearshore Habitat Program.

## 3.0 Background

Puget Sound has an estimated 23,000 hectares (57,000 acres) of eelgrass (*Zostera marina* L.) (Gaeckle et al. 2011). Eelgrass and other seagrasses are considered indicators of estuarine health (Dennison et al. 1993, Krause-Jensen et al. 2005, Orth et al. 2006) and provide extensive ecosystem services worldwide (Constanza et al. 1997, Green and Short 2003, Larkum et al. 2006). In Puget Sound, eelgrass provides spawning grounds for Pacific herring (*Clupea harengus pallasii*), out-migrating corridors for juvenile salmon (*Oncorhynchus* spp.) (Phillips 1984, Simenstad 1994), and important feeding and foraging habitats for water birds such as the black brant (*Branta bernicla*) (Wilson & Atkinson 1995) and great blue heron (*Ardea herodias*) (Butler 1995). Due to its ecological importance and its rapid response to environmental degradation, eelgrass has been identified as a Vital Sign of ecosystem health and a 2020 eelgrass recovery target was adopted by the Puget Sound Partnership.

Seagrass decline has been observed globally and is primarily attributed to anthropogenic activities such as nutrient loading and shoreline development (Duarte 2002, Orth et al. 2006, Short and Burdick 1996, Waycott et al. 2009). In Puget Sound, there is widespread concern that eelgrass is significantly less abundant than it was historically (Dowty et al. 2010). Human-induced disturbances, assumed to have caused most of the loss and threats to critical nearshore habitats, are expected to increase with population growth and coastal development. However, there are critical uncertainties about the intensity, extent, and reversibility of stressors affecting eelgrass in Puget Sound (Thom et al. 2011).

An improved understanding of key eelgrass stressors is needed to drive management actions in Puget Sound. The 2011 Draft Action Agenda (Proposed Priority Science Questions for 2011-2013 and section B.6.) recommends implementation of coordinated efforts to identify key stressors that affect eelgrass in Puget Sound. The proposed project will further improve the understanding of key eelgrass stressors in Puget Sound.

One area with a significant data gap is the management of loading sources, in particular outfalls, and the impacts outfall infrastructure and discharge have on critical nearshore habitats (e.g., eelgrass and macroalgae). Outfalls discharge municipal or industrial wastewater, stormwater, combined sewer overflows or other effluents. Individual outfalls are potentially associated with multiple known seagrass stressors:

- Excess nutrients promote phytoplankton and algae blooms, and epiphytic growth on eelgrass (Short and Burdick 1996). Eutrophication eventually leads to a reduction in the available light necessary for seagrass to photosynthesize (Ralph et al. 2006, Short et al. 1995) and conditions of low dissolved oxygen (DO) (Simonds et al. 2008) that can affect seagrass (Bricker et al. 2008).
- Contaminants such as heavy metals (Brackup and Capone 1985, Hamoutene et al. 1996, Lewis and Devereux 2009, Pergent-Martini and Pergent 2000), pesticides and herbicides (Bester 2000, Lewis et al. 2002), and trace amounts of other nonessential elements (Prange and Dennison 2000) have been shown to have detrimental effects on seagrass physiology.

- Outfall pipes and their effluent alter physical processes such as hydrology (Neverauskas 1985), salinity (Short 2008), and temperature (Phillips 1982, Thorhaug 1978).
- Additional effects of outfalls include the extent and magnitude of contaminant transfer from seagrass to sediment organic matter and higher trophic organisms (Lewis and Devereux 2009).

Nutrient-related impacts from outfalls have been associated with major losses of seagrass worldwide, yet there are surprisingly few data documenting the extent of nutrient-related impacts on eelgrass in Puget Sound. A basic indicator of water quality degradation - dissolved inorganic nitrogen (DIN; sum of ammonium, and nitrate plus nitrite) loading from anthropogenic sources - is known to have substantial effects on seagrass (Moore and Wetzel 2000). In Puget Sound proper, it is estimated that 59% of the DIN load is from Wastewater Treatment Plants and 14% is from anthropogenic sources carried by rivers from the surrounding watersheds (Mohamedali et al. 2011). Efforts to monitor and manage DIN loads in Puget Sound do not consider thresholds for effects on eelgrass. Research has observed effects of metals and organic contaminants on seagrass physiology (see review by Lewis and Devereux 2009), but there is limited data specific to eelgrass and Puget Sound. In addition, there is limited information on the physical impacts to eelgrass habitat and the trophic transfer of nutrients, metals and organic contaminants in eelgrass in Puget Sound.

While outfalls are hypothesized to have major impacts, there are substantial scientific challenges related to describing and monitoring them. First, it is difficult to adequately capture the broad nature of the direct and indirect physical and chemical effects. Additionally, it is difficult to tease apart the effects of individual outfalls from the cumulative impacts of approximately 100 Wastewater Treatment Plants and thousands of other discharge pipes in Puget Sound (Mohamedali et al. 2011). These challenges notwithstanding, our understanding of the effects of outfalls on eelgrass in Puget Sound could be substantially improved with basic information on the concentration of nutrient, metal and organic contaminants in eelgrass, including:

- A summary of available scientific research on major categories of impacts from loading sources and their effects on eelgrass;
- Information on the extent and magnitude of anthropogenic nutrients, metals, and organic contaminants found in eelgrass tissue in Puget Sound;
- Site-based studies that assess the impacts of individual loading sources (e.g., outfalls) through before-after and/or control-impact research.

Multiple efforts to understand and minimize the impacts of nutrient, metal, and organic contaminant pollution on nearshore habitats are underway in Puget Sound, including:

- DNR seeks sound science to guide management decisions to minimize impacts to critical nearshore habitats from outfalls.
- The Puget Sound Partnership adopted a target to increase eelgrass area by 20% by 2020. Prioritized strategies and collaborations need to be identified in order to reach this goal.
- The 2012/2013 Action Agenda proposed a need for priority information on key eelgrass stressors ([http://www.psp.wa.gov/action\\_agenda\\_2011\\_update\\_home.php](http://www.psp.wa.gov/action_agenda_2011_update_home.php)).
- The Stormwater Work Group is developing an effectiveness monitoring program to evaluate and minimize stormwater issues in Puget Sound. The program has identified

eelgrass and other physical nearshore habitat characteristics as part of its future monitoring parameters. However, work has not yet been initiated.

([http://www.ecy.wa.gov/programs/wq/psmonitoring/ps\\_monitoring\\_docs/SWworkgroupDOCS/3StatusAndTrends051910JFrodge.pdf](http://www.ecy.wa.gov/programs/wq/psmonitoring/ps_monitoring_docs/SWworkgroupDOCS/3StatusAndTrends051910JFrodge.pdf))

- The Washington State Department of Health and Department of Ecology are initiating multiple projects to decrease pathogen and nutrient pollution, using an \$8.5 million grant from the EPA (<http://www.doh.wa.gov/ehp/sf/EPA-grant.htm>). The focus of this work is generally on health-related risks within Puget Sound. However, this work could also benefit other components of nearshore habitat.
- In The Washington Shellfish Initiative, the National Oceanic and Atmospheric Administration (NOAA) and Washington State are creating a private/public partnership to promote the economic and environmental benefits of shellfish, including restoration and protection of water quality ([www.governor.wa.gov/news/shellfish\\_white\\_paper\\_20111209.pdf](http://www.governor.wa.gov/news/shellfish_white_paper_20111209.pdf)).

These efforts could be improved by increased scientific understanding of the concentration of nutrient, metal and organic contaminants in eelgrass and by the identification of specific assessment approaches.

## 4.0 Project Description

The Nearshore Habitat Program (NHP) will complete a series of linked information syntheses and field data collection actions to increase our understanding of the concentration of nutrients, metals, and organic contaminants in eelgrass in Puget Sound. These include:

- A literature review (Gaeckle 2012) summarized available scientific research on four major categories of the effects of loading on eelgrass, including: nutrients, metals and organic contaminants, physical alteration of habitat, and trophic transfer.
- A synthesis of local spatial information and analyses that will evaluate available data on the current status of loading sources and eelgrass in Puget Sound, including the identification of areas with the greatest potential impacts to eelgrass.
- Field data collection at 10 long-term monitoring sites to broadly characterize nutrient, metal and organic contamination concentrations in eelgrass and to establish a baseline at a range of locations over a spatial gradient in Puget Sound. Nutrient, metal and organic contaminant concentrations in Puget Sound eelgrass will be compared to concentrations in other ecosystem components (e.g., mussels, sediment)
- Field data collection at one individual loading source (e.g., outfall) scheduled for mitigation actions in order to describe baseline eelgrass bed characteristics and the extent of nutrient, metal and organic contaminant concentrations in eelgrass. This will serve as the basis for effectiveness monitoring.

Individual technical reports will be distributed describing the methods and findings of each task. Additionally, data and results will be synthesized in a brief white paper that summarizes the concentrations of nutrients, metals and organic contaminants in eelgrass and the potential effects of sources of loading on eelgrass.

#### 4.1 Project goals

The Nearshore Habitat Program (NHP) will complete a series of linked information syntheses, field data collection, and contractual laboratory analyses to increase our understanding of the concentration of nutrients, metals and organic contaminants in eelgrass at a range of sites throughout Puget Sound. The goal of this project is to provide scientifically sound information that can be used by a wide range of government and non-governmental organizations to: 1) understand the impacts of loading on eelgrass; 2) improve shoreline decision making; 3) improve resource protection and restoration efforts; 4) inform assessment approaches considered at individual sites; and 5) compare findings at individual sites to other areas in Puget Sound.

#### 4.2 Project objective

The specific objectives of the project are to assess nutrients, metals and organic contaminants in eelgrass to understand the concentrations of these substances in above- and belowground eelgrass compartments at a range of sites throughout Puget Sound. A similar assessment will occur in the vicinity of a loading source (e.g., outfall) to determine baseline nutrient, metal and organic contaminant concentrations prior to anticipated modifications that will change the effluent at this site (site to be determined). The objectives of this project will provide both scientific and management-oriented results.

The specific objectives of the project include:

- Establish a baseline of nutrient, metal and organic contaminant concentrations in the above- and belowground compartments of eelgrass at a range of sites throughout PS;
- Establish a baseline of nutrient, metal and organic contaminant concentrations in the above- and belowground compartments of eelgrass at a site within the vicinity of an outfall that is scheduled to be modified;
- Provide scientific information and field sampling procedures to the Stormwater Workgroup and the Toxics Workgroup;
- Provide information on the concentrations of nutrient, metal, and organic contaminants in eelgrass for educational, outreach, and management applications;
- Provide recommendations based on sound science to guide DNR conservation measures related to nutrient, metal, and organic contaminants concentrations and the protection of eelgrass in Puget Sound;
- Provide input on restoration and protection priorities for agencies, NGOs, and community groups;
- Identify strategies related to loading sources to support the Puget Sound Partnership's goal to increase eelgrass area 20% by 2020;
- Provide information to the Puget Sound Partnership that summarizes known information on key stressors on eelgrass as described in their Proposed Priority Science Questions for 2011-2013 and section B.6 of the Draft 2011 Action Agenda ('Identify the key stressors on

eelgrass’). The information gathered will also guide future work by DNR’s Eelgrass Stressor-Response Program (ES-RP). And;

- Identify data gaps and recommend next steps for scientific inquiry related to outfalls that will guide resource management strategies.

#### 4.3 Information needed and sources

There are two tasks that will involve the synthesis of existing data. Task 1.2 – Literature Review (Gaeckle 2012) involved searching databases for peer-reviewed manuscripts on the effects nutrients, metals and organic contaminants have on seagrass and specifically eelgrass. A large portion of the literature search was performed by technicians with supervision by the project manager. The literature review was written by the project manager and reviewed by colleagues. Task 1.3 – Spatial Evaluation of Loading Source (e.g., outfall) proximity to Eelgrass Beds will evaluate existing outfall and other loading source data and eelgrass data in ArcGIS to identify areas with the greatest potential impact to the resource. The People for Puget Sound have synthesized up-to-date and thorough outfall location data for Puget Sound, while the DNR has the most complete eelgrass dataset for the area. Task 1.3 will be performed by a Natural Resource Scientist 2 (NRS2) and provided guidance from the project manager.

#### 4.4 Target population

The target population is eelgrass, *Zostera marina* L., which grows throughout the nearshore environment in greater Puget Sound.

#### 4.5 Study boundaries

The study area is the nearshore waters of greater Puget Sound. Greater Puget Sound includes the waters east of Cape Flattery (Neah Bay), and south of Pt. Roberts towards Olympia. However, the extreme reaches of southern Puget Sound (south and west of Pickering and Dana Passage) are excluded from the study because of the sparse distribution of eelgrass in this area. The specific sites to be sampled will coincide with a linked project “Mussel Watch Pilot Expansion Project” (Mussel Watch; Lanksbury et al. 2012) and located in areas that represent a range of shoreline types and development.

For Task 2, the nutrient, metal and organic contaminant concentrations in eelgrass will be assessed at ten (10) sites within the study area. Additional sites may be added through outside funding sources.

#### 4.6 Tasks required

Task 1: Scientific Literature Review (Gaeckle 2012), QAPP preparation and approval, and spatial evaluation of loading source (e.g., outfall) proximity to eelgrass beds in Puget Sound

Task 2: Evaluation of the extent and magnitude of nutrient, metal and contaminant concentrations in eelgrass in Greater Puget Sound

- Collection of eelgrass at sites
- Sample preparation for chemical analyses
- Chemical analyses of eelgrass samples
- Data organization, storage, and analyses

Task 3: Evaluation of eelgrass condition and environmental parameters around an individual outfall

- Assessment of eelgrass habitat at outfall site
- Collection of eelgrass at site
- Sample preparation for chemical analyses
- Chemical analyses of eelgrass samples
- Data organization, storage, and analyses

Task 4: Production of a white paper that synthesizes project results and communication of the results

#### 4.7 Practical constraints

Practical constraints include laboratory analyses and coordination of personnel for sample collection. The predicted laboratory analyses will take considerable time (3 months – 6 months) depending on the back log of the lab and the priority of the project. Finally, samples will be collected by Nearshore Habitat Program colleagues and WDFW colleagues and volunteers. The study sites are co-located with WDFW's Mussel Watch project and eelgrass sample collection will coincide with the collection of the mussel cages in January 2013. A detailed protocol will be provided to insure proper eelgrass sample collection and chain of custody documentation.

#### 4.8 Systematic planning process used

This Quality Assurance Project Plan is a reflection of a systematic planning process that has involved internal and external reviews, comments, and feedback to improve the overall outcome of the project.

## 5.0 Organization and Schedule

### 5.1 Key individuals and their responsibilities (project team, decision-makers, stakeholders, labs, etc.)

Tasks 1-4: Jeff Gaeckle, Lisa Ferrier, Jessica Demetro-Stowe, Kiri Kreamer

#### DNR

Jeff Gaeckle – Project manager, literature review, field work, data management, QA/QC, budget management, QAPP, report and document authorship

Lisa Ferrier – ArcGIS analysis, database management, field work

Jessica Demetro-Stowe and Kiri Kreamer – literature search, field work, sample preparation, data management and QA/QC, and document review and editing

WDFW Cross-cutting Partners

Jim West – WDFW Project manager and principal investigator for “Mussel Watch Pilot Expansion Project” (Mussel Watch; Lanksbury et al. 2012), site selection, sample plan, field work, interpretation of results

Jennifer Lanksbury – site selection, sample plan, field work,

Laurie Niewolny – laboratory facilitator and sample processing

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## 5.2 Project schedule

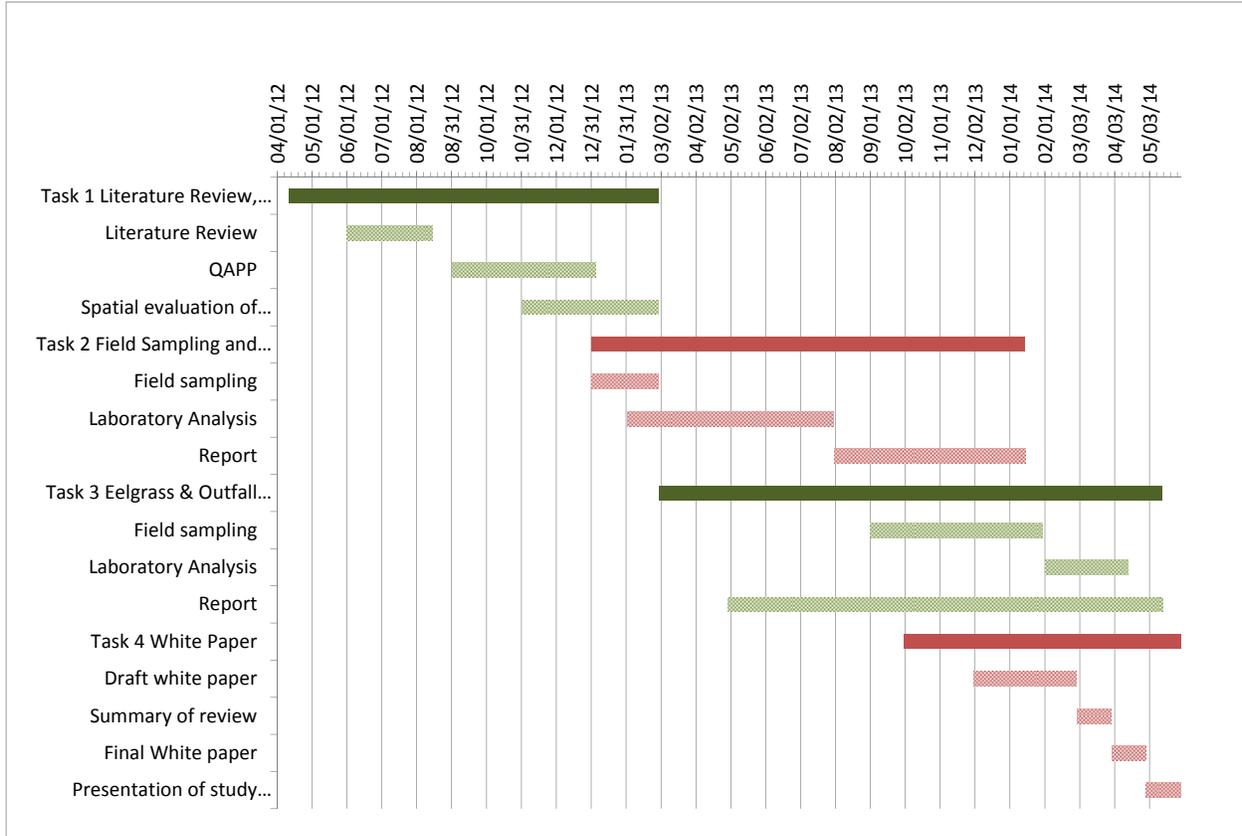


Figure 5.1. Project schedule.

## 5.3 Limitations on schedule

The practical constraints (efficiency of lab analyses) mentioned in Section 4.7 will likely be the primary limitations for the schedule. The Nearshore Habitat Program resources are available to work on the project from March 2012 to March 2014. The proposed schedule may require modification due to a delay by the lab to complete the eelgrass plant tissue chemical analyses.

## 5.4 Budget and funding

The project is funded by the Puget Sound Marine and Nearshore Protection and Restoration Program. The project SOW and detailed budget appear in Appendix A and a summary of the tasks and budget are listed below.

Task 1: Literature review, QAPP, spatial analysis

1.1 Literature Review (Gaeckle 2012, completed) – \$9,410.52

1.2 QAPP –

1.3 Spatial Analysis –

## Task 2: Evaluation of the extent and magnitude of nutrients, metals and contaminants

2.1 Field work – \$23,040.78

2.2 Laboratory analysis – \$83,300.00

2.3 Results report

## Task 3: Eelgrass and Outfall

3.1 Field work – \$24,171.28

3.2 Laboratory analysis – \$23,800.00

3.3 Results report –

## Task 4: White paper

4.1 Draft white paper and data management – \$4,705.26

4.2 Summary of review –

4.3 Final white paper –

4.4 – 4.6 Presentations of study results – \$3,332.00

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## 6.0 Quality Objectives

### 6.1 Measurement Quality Objectives

The quality objective of this project is to provide an understanding of nitrogen, metal, and organic contaminant concentrations in eelgrass at a limited number of sites throughout Puget Sound. The objective for laboratory analyses is to evaluate target analytes in replicate eelgrass compartments (leaves and rhizomes/roots) at sites throughout Puget Sound within detection limits observed in the literature and obtainable by the contracted labs (Table 6.1).

Table 6.1. Eelgrass components collected for chemical analyses.

COLLECTION DATE	EELGRASS COMPARTMENT	SITES	REPLICATES (#/site)	TOTAL	DESCRIPTION
Jan 2013	Leaves	10	3	30	C:N, metal and organic analyses
Jan 2013	Rhizomes / Roots	10	3	30	C:N, metal and organic analyses
Jan 2013	Various			13-19 <sup>a</sup>	QC for C:N, metal and organic analyses
Jan 2014	Leaves	1	10	10	C:N, metal and organic analyses
Jan 2014	Rhizomes / Roots	1	10	10	C:N, metal and organic analyses
Jan 2014	Various			3-7 <sup>a</sup>	QC for C:N, metal and organic analyses
				96 - 106	TOTAL SAMPLES ANALYZED

<sup>a</sup> QC samples described in Tables 9.6 and 9.7.

Sites will be co-located with WDFW's Mussel Watch Pilot Expansion Project (Lanksbury et al. 2012). These sites are identified with specific sites names (Site ID) and GPS coordinates using the North American Datum (NAD83) and recorded in decimal degree format. The accuracy of hand held GPS units is typically within 3-15 meters 95% of the time (<http://www.gps-basics.com>).

The QA/QC procedure for the isotopic analysis of eelgrass samples for stable isotopes of C and N by continuous flow Combustion- Isotope Ratio Mass Spectrometry (C-IRMS) includes physical and analytical checks. The physical check ensures full sample details are received in electronic and hard copies and that all samples arrive intact. Analytical QA/QC requires samples to be analyzed with respect to laboratory standards that have been calibrated against international standards USGS-40 and USGS-41. Every run includes the following lab standards – nylon, glutamic acid or peach leaves (NIST 1547), and USGS-41. In addition bovine liver (NIST 1577) is included as a quality check. Standards are included every twelve samples and at least 6 glutamic acid or peach leaves standards are included per run.

The following tables list the minimum QA/QC criteria for metals and organic compounds analyzed in eelgrass.

Table 6.2.\*. Minimum analytical quality assurance criteria for polycyclic aromatic compounds (PACs) and persistent organic pollutants (POPs) by gas chromatography/mass spectrometry (GC-MS).

QUALITY ASSURANCE ELEMENT	MINIMUM FREQUENCY	ACCEPTANCE CRITERIA
Instrument calibration	Once every batch of samples or once every two batches in one continuous analytical sequence	Analyte concentrations are to be calculated using point-to-point calibration with at least four concentrations levels of calibration standards.
Continuing calibration	At start and end of every analytical sequence and every 10 or fewer field samples	The RSD of the analyte responses to the internal standard is to be $\leq 15\%$ for the repetitions.
Reference materials: Sediment: NIST SRM 1944, NIST SRM 1941b	One with every batch of 20 or fewer field samples.	Concentrations of $\geq 70\%$ of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values. These criteria do not apply to analytes with concentrations below their lower LOQ when the lower LOQ is within or greater than the 95% confidence interval, nor to those analytes known to have coeluting compounds.
Method blank	One with every batch of 20 or fewer field samples	No more than 5 analytes in a method blank are to exceed 2 x lower LOQ. Samples are not corrected for analytes found in the blank.
Sample replicates (i.e., duplicates or triplicates)	One with every 20 or fewer field samples.	RSDs are to be $\leq 15\%$ (equivalent to relative percent differences $\leq 30\%$ for duplicates) for $\geq 90\%$ of the analytes that have concentrations $\geq$ ng/g.
Internal standards/surrogates	At least one internal standard/surrogate is added to every sample	The recoveries are to be 60-130%.
Inter-laboratory comparisons	At least one per year	In conjunction with the NIST or the IAEA.

\* table reproduced from Sloan et al. (2006).

Table 6.3\*. Required batch quality control measures and quality assurance criteria for mercury via Cold Vapor Atomic Absorption (CVAA).

QUALITY CONTROL ELEMENT	DESCRIPTION OF ELEMENT	FREQUENCY OF IMPLEMENTATION	CONTROL LIMIT		
			LIQUID	SOLID	TISSUE
Method Blank (MB)	Interference-free matrix to assess overall method contamination	1 per sample batch	± MDL	± MDL	± MDL
Spike Blank (SB)	Interference-free matrix containing all target analytes	1 per sample batch	85 - 115%	85 - 115%	85 - 115%
Standard Reference Material (SRM)	Certified reference material from NIST or NRCC, which is digested with samples.	1 per solid or tissue sample batch, if applicable	NA	80-120% <sup>c</sup>	80-120% <sup>c</sup>
Laboratory Control Sample (LCS)	Certified reference material from a source other than NIST or NRCC	1 per solid or tissue sample batch, if applicable	NA	80-120% <sup>c</sup>	80-120% <sup>c</sup>
Matrix Spike (MS)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per sample batch	70-130%	75 - 125%	75-125%
Matrix Spike Duplicate (MSD) <sup>a</sup>	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per sample batch	70 - 130% RPD ≤ 20%	75 - 125% RPD ≤ 20%	75 - 125% RPD ≤ 20%
Lab Duplicate (LD) <sup>a, b</sup>	Self-explanatory	1 per sample batch	RPD ≤ 20%	RPD ≤ 20%	RPD ≤ 20%
Filtration Blanks <sup>d</sup>	Method blank for the filtration process, when samples filtered in the lab	2 per sample batch	± MDL		

<sup>a</sup> No calculation performed when both sample and duplicate values < RDL

<sup>b</sup> LD are only analyzed with QA1 sediments and when required by specific projects

<sup>c</sup> Or varies due to control charting

<sup>d</sup> Entered to LIMS as an MB

\* Table reproduced from KCEL SOP 604v6 (2009).

Table 6.4. Required batch quality control measures and quality assurance criteria for the ICP-MS metals As, Cd, Cr, Cu, Fe, Pb, Ni, Zn, and V.

QUALITY CONTROL ELEMENT	DESCRIPTION OF ELEMENT	FREQUENCY OF IMPLEMENTATION	CONTROL LIMIT
			LIQUID
Method Blank (MB)	Interference-free matrix to assess overall method contamination	1 per QC batch	< MDL & > -MDL
Spike Blank (SB)	Interference-free matrix containing all target analytes	1 per QC batch	85% - 115%
Matrix Spike (MS)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch	75% -125%
Matrix Spike Duplicate (MSD)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch or (LD) – Ultra Low level analysis only.	75% -125% %Recovery 20% RPD
Lab Duplicate (LD) <sup>a</sup>	Self-explanatory	1 per QC batch or MSD – Routine level analysis only.	≤ 20% RPD, when at least one value is > RDL
Filtration Blanks (Routine)	Method blank for the filtration process if samples filtered in the lab	2 per QC batch	< MDL & > -MDL
Filtration Blank (Ultra-low)	Method blank for the filtration process	1 per QC batch	< MDL & > -MDL

<sup>a</sup> No calculation performed when both sample and duplicate values < RDL

\* Table reproduced from KCEL SOP 624v2 (2009).

## 6.2.1 Table of targets for:

### 6.2.1.1 Precision

The project is using three labs (UC Davis Stable Isotope Facility, King County Environmental Laboratory, NMFS/Northwest Fisheries Science Center Lab) to perform nutrient, metal and contaminant analyses, respectively. These labs have performed analyses on vegetation, fish and shellfish collected throughout Puget Sound. Precision is monitored and controlled within batches using laboratory replicates of field samples and across batches by analyzing Standard Reference Materials (SRM) of applicable matrix (i.e., eelgrass tissue or a specified NIST matrix).

*Nutrients* – the data are checked for precision of all standards within the accepted sample weight range. The accuracy of the bovine liver (NIST 1577) check is confirmed.

*Metals* – The precision of the metal analyses will be performed using duplicate analyses and a control limit for the relative standard deviation (RSD) of 25%. Mercury analyses use a Relative Percent Difference (RPD), which is the absolute difference of two measurements, divided by the sum of the measurements, multiplied by 100. The RPD is used to evaluate the precision of the replicate measurements.

*Organics* - For this study [NIST SRM 1974b](#) will be used for all organics<sup>1</sup>. Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be  $\leq 15\%$  for the repetitions.

#### 6.2.1.2 Bias

Bias or accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. In addition for POPs, concentrations of  $\geq 70\%$  of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values.

#### 6.2.1.3 Sensitivity

The Lower Limit of Quantitation (LOQ) for all organics in this study is “the concentration that would be calculated if that analyte had a GC-MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ.” (Sloan et al. 2006). Typically LOQ values for POPs that have been reported to Puget Sound Ecosystem Monitoring Program (PSEMP) by this method are in the range of 0.2 to 0.8 ng/g wet weight. In this study, the POPs’ LOQs are given as a range because tissue sample LOQs are affected by the field sample mass used. The LOQ is the lowest concentration at which a POPs sample result will be reported.

EPA defines Method Detection Limit (MDL) in Appendix A to 40 CFR Part 136 as the “minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element”. In this study, the metal’s MDLs are concentrations that cannot be detected or detected at a concentration less than the associated method detection limit considering tissue sample detection limits are affected by the sample mass used, matrix and polyatomic/isobaric interferences. The MDL is the lowest concentration at which a sample result will be reported. Tables 6.3, 6.4 and 9.3 list the respective method detection limits for the metals of concern (As, Cd, Cr, Cu, Fe, Hg, Pb, Ni, Zn, and V).

#### 6.2.2.1 Comparability

The SOPs described in this document (Sloan et al. 2004; Sloan et al. 2006) are consistent with other concurrent and future sampling analytical methods. Although analytical procedures have changed, results of this study will be comparable to results observed in the chemical analyses of eelgrass elsewhere in its range (see literature review, Gaeckle 2012).

#### 6.2.2.2 Representativeness

The sampling design in this study is aimed at assessing contaminants in eelgrass in Puget Sound. To that end the design optimizes spatial coverage that is representative of a range of contamination. However, the sample design is not spatially or temporally comprehensive. Instead, the design is to provide a broad assessment of nutrient, metal and organic contaminant

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<sup>1</sup> SRM 1974b is no longer available from NIST. The NOAA lab has enough matrix on hand for this study, however, a suitable alternative may be substituted, at the chemist’s discretion.

conditions in eelgrass at 10 sites throughout Puget Sound. Additional sites may be added from other funding sources.

The project has considered representing environmental conditions through a number of strategies. These include:

- 1) Samples will be collected during the rainy season (Sep – Feb) to account for the time of year when runoff is greatest and the resource will be impacted the most due to elevated levels of nutrients, metals and organic contaminants.
- 2) Samples will be collected at a number of sites over a large geographical extent to capture a wide range of contamination levels.
- 3) Samples will be collected at a similar tidal elevation (0 m MLLW) at all sample sites.

#### 6.2.2.3 Completeness

The goal of Task 2 is to collect and analyze three (3) replicates of eelgrass (above- and belowground biomass) at 10 sites throughout Puget Sound for nutrients, metals and organic contaminants. The sampling effort will result in 30 replicate samples of above- and belowground biomass for a total of 60 samples. The amount of plant material necessary for analyses varies for each lab:

- a) Nutrients: a minimum of 0.00002 g N and 0.000500 g C dry weight for each plant compartment (leaves and rhizomes / roots)
- b) Metals: 9.0 g wet weight for each plant compartment (leaves and rhizomes / roots)
- c) Organics: 2.5 g (wet weight) for each plant compartment (leaves and rhizomes / roots)

Task 3 will focus on an area of eelgrass in Puget Sound where an outfall is scheduled to be modified (e.g., upgraded treatment, relocated outfall diffuser, decommissioned). The goal of Task 3 is to characterize the nutrients, metals, and contaminants in eelgrass prior to the scheduled outfall modification. Ten samples of above- and belowground biomass will be collected at the selected site (site to be determined). The amount of plant material necessary for nutrient, metal and organic analyses remains the same as in Task 2.

Chemical analysis data will be reviewed to ensure data quality objectives were met. Data will not be released from the labs unless all data quality objectives are met. The residue concentrations will be inspected for lab contamination and expected trends (i.e., nutrient, metal, and organic contaminant levels in urban areas are greater than measured in rural areas).

Task 3 will also survey the existing eelgrass at the selected outfall sites (to be determined) using previously established methods by the Nearshore Habitat Program (Berry et al. 2003, Gaeckle 2009, Gaeckle et al. 2011).

## 7.0 Sampling Process Design (Experimental Design)

### 7.1 Study Design

#### 7.1.1 Sampling location and frequency

Ten (10) sites, co-located with the Mussel Watch Pilot Expansion Project, will be sampled once in January 2013 for above- and belowground eelgrass material. Sites will be located in Greater Puget Sound and sampling will occur at 0 m MLLW (Figure 7.2). Sites were arbitrarily selected, but can be characterized by exposure to loading based on the abundance of discharge sources (Carmichael et al. 2009) and general loading (Mohamedali et al. 2011) throughout Puget Sound (product of spatial analysis of eelgrass and outfall in Task 1). Although these comparisons will not allow one to draw a direct conclusion between loading to Puget Sound and concentrations of nutrients, metals, and organic contaminants in eelgrass, it will provide more information potentially useful for that type of research in the future.

#### 7.1.2 Parameters to be determined

Different parameters will be collected during different components of the project. Project component and related parameters to be collected include:

- a) Field sampling: date, time, location (latitude and longitude) for each sample, depth, sediment type, brief description of the site and weather conditions, and whether other seagrass species are present.
- b) Sample preparation: above- and belowground eelgrass
- c) Analytical laboratory analysis: nutrients, metals, and organic contaminants concentrations in above- and belowground eelgrass material

#### 7.1.3 Field measurements

The variables recorded on waterproof paper and collected in the field include the date (DDMMMYYYY), time (HHMM AM/PM), location (latitude and longitude, NAD83, hddd.ddddd°) for each sample, depth, tidal stage relative to MLLW, sediment type, brief description of the site and weather conditions, and whether other seagrass species are present (Figure 7.1).

**EELGRASS COLLECTION DATA SHEET**

**CONTAMINANTS IN EELGRASS**

SCIENTISTS: \_\_\_\_\_

SITE NAME: \_\_\_\_\_

DATE: \_\_\_\_\_ (DDMMYYYY) TIME: \_\_\_\_\_ (HH:MM) (AM or PM, circle one)

**Sample 1: GPS Coordinates**

Latitude: \_\_\_\_\_ (hddd.dddd°) Longitude: \_\_\_\_\_ (hddd.dddd°) Accuracy: \_\_\_\_\_ (± ft)

**Sample 2: GPS Coordinates**

Latitude: \_\_\_\_\_ (hddd.dddd°) Longitude: \_\_\_\_\_ (hddd.dddd°) Accuracy: \_\_\_\_\_ (± ft)

**Sample 3: GPS Coordinates**

Latitude: \_\_\_\_\_ (hddd.dddd°) Longitude: \_\_\_\_\_ (hddd.dddd°) Accuracy: \_\_\_\_\_ (± ft)

SITE DESCRIPTION: \_\_\_\_\_

Water depth: \_\_\_\_\_ Sediment type: \_\_\_\_\_

Other seagrass present: yes no (circle one) If so, what species: \_\_\_\_\_

Outfalls present: yes no (circle one) If so, how many and approximate distance: \_\_\_\_\_

Distance to Mussel Watch cage (approximate): \_\_\_\_\_

Weather conditions: \_\_\_\_\_

Other observations: \_\_\_\_\_

Figure 7.1. Eelgrass collection field data sheet.

All other variables will be collected in the Aquatic Botany Laboratory, the WDFW Marine Sciences Laboratory, or the analytical laboratories.

## 7.2 Maps or diagram

Ten (10) sites will be sampled throughout greater Puget Sound (Table 7.1, Figure 7.2).

Table 7.1. Ten (10) sample sites throughout greater Puget Sound, their location and latitude and longitude.

SITE NAME	LOCATION	LATITUDE	LONGITUDE
Post Point	Fairhaven-Bellingham	48.7194	-122.5167
March Point	Anacortes	48.4996	-122.5675
Padilla Bay	Mt. Vernon	48.4924	-122.4866
Penn Cove	Coupeville, Whidbey Island	48.2219	-122.6863
Thompson Spit	Miller Peninsula, Gardiner	48.0967	-122.9394
Big Gulch	Mukilteo	47.9107	-122.3222
Duwamish Head	Alki, West Seattle	47.5893	-122.3953
Ruston Way	Puget Creek, Tacoma	47.2811	-122.4771
Sandy Bay	Anderson Island	47.1494	-122.6764
Holly	Hood Canal	47.5706	-122.9715

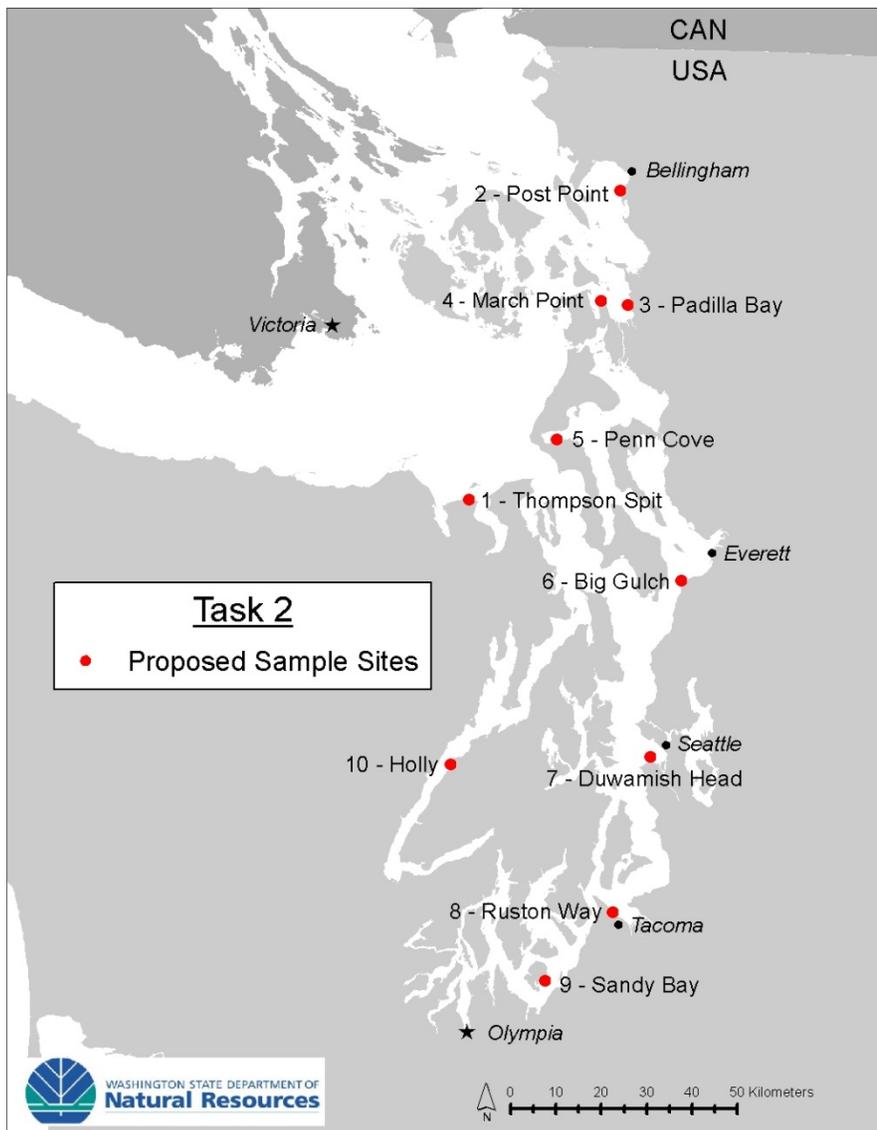


Figure 7.2. The proposed sample sites in Puget Sound where eelgrass (*Z. marina*) will be collected. The eelgrass will be analyzed for nutrients, metals, and organic contaminants in above- and belowground plant compartments.

Five sites may be added through sponsorship by outside sources (Table 7.2).

Table 7.2. Five additional sample sites, their location, latitude and longitude and outside funding source.

SITE NAME	LOCATION	LATITUDE	LONGITUDE	FUNDING SOURCE
Birch Bay	Ferndale	48.8962	-122.7854	DNR Aquatic Reserves
Cypress Island	Strawberry Bay	48.5637	-122.7222	DNR Aquatic Reserves
4-Mile Rock	Magnolia, Seattle	47.6385	-122.4122	ES-RP
Dumas Bay	Federal Way	47.3290	-122.3905	ES-RP
Burley Spit	Purdy	47.6390	-122.6405	ES-RP

ES-RP – Eelgrass Stressor-Response Program

### 7.3 Assumptions underlying design

The main assumption of this study is that nutrient, metal, and organic contaminant concentrations in eelgrass will be measureable as observed in other systems based on the literature review (Gaeckle 2012). The level of contamination will vary depending on numerous factors (e.g., proximity to source, type of contamination at source, hydrodynamics and local sources of contaminants in sediments) throughout greater Puget Sound. However, eelgrass collection will occur shortly after the rainy season begins in the region which will allow substantial exposure to contaminants from stormwater.

### 7.4 Relation to objectives and site characteristics

The objective of the study is to establish a general characterization and baseline of nutrient, metal and organic contaminant concentrations in eelgrass above- and belowground biomass at 10 sites throughout greater Puget Sound. Although additional sites would provide a more in depth understanding of nutrient, metal and organic contaminant concentrations in eelgrass, an increase in field effort and laboratory analyses is beyond the available project resources.

### 7.5 Characteristics of existing data

There is limited existing data on nutrients, metals and organic contaminant concentrations in eelgrass from greater Puget Sound. The only research that assessed metals and organic contaminants in the study area was completed in 1988 at a number of sites in Fidalgo and Padilla Bays (USFWS 1994). The proposed study will fill a data gap and provide baseline information on nutrients, metals and organic contaminants concentrations over a range of sites throughout greater Puget Sound. Other relevant data on nutrients, metals and organic contaminant concentrations in eelgrass from locations throughout the world were presented in the literature review (Gaeckle 2012). A comparison of the data collected in the earlier project (USFWS 1994) conducted in Puget Sound, research conducted elsewhere (e.g., literature review – Gaeckle 2012), and this project will be provided in the final report. There is additional opportunity to

compare nutrient, metal and organic contaminant concentrations measured in eelgrass during this study to concentrations measured in mussels collected as part of the Mussel Watch Pilot Expansion Project (Lanksbury et al. 2012) collected during the same time. No other sampling will co-occur with the eelgrass and mussel cage collection. However, other studies have assessed toxics in biota (West et al. 2001), water (Newton et al. 2002), and sediment (Partridge et al. 2005) throughout Puget Sound and relative comparisons can be made to the results from the eelgrass analyses.

## 8.0 Sampling Procedures

### 8.1 Field measurement and field sampling SOPs

The following sections describe the procedures for collecting eelgrass from field sites, transporting it back to the Aquatic Botany Laboratory (Natural Resources Building, Olympia, WA) for storage prior to sample processing.

### 8.2 Measurement and sample collection and processing

#### *Field Collection*

The collection of eelgrass at the sites will be conducted similar to methods described in UNH-JEL SOP 1.01 (Mueller et al. 1992). Collection of eelgrass may coincide with the retrieval of the mussel cage (Mussel Watch Pilot Expansion Project). A team of eelgrass collectors (minimum of 2 people) will be provided with an insulated box (i.e., cooler) that includes the following equipment:

- 1) 3, 2 gallon labeled Ziploc sample bags or the equivalent. Sample bags will be pre-labeled with the Site ID, Replicate number (1-3). Field collectors will add the date (DDMMYYYY), time (HH:MM AM/PM) and names of collectors).
- 2) Plastic trowels
- 3) 0.25 m<sup>2</sup> (50 cm x 50 cm) stainless steel quadrat
- 4) 0.0625 m<sup>2</sup> (25 cm x 25 cm) quadrat – may be attached to one inside corner of the larger quadrat
- 5) Nitrile gloves – powder free
- 6) Cotton mesh bag for sieving and rinsing eelgrass
- 7) GPS device
- 8) 5 gallon bucket
- 9) Insulated box (i.e., cooler)
- 10) Eelgrass collection data sheet and pencil
- 11) Chain of custody form

Collectors will provide their own field clothing to access 0 m MLLW at each study site. This equipment will vary depending on tidal stage and weather but should not exceed the need for waders or knee boots in addition to warm clothes and rain gear.

### Eelgrass Collection Procedure

- 1) After locating the Mussel Cage, collectors, wearing nitrile gloves, should locate native eelgrass in the vicinity of the Mussel Cage.
- 2) Using their hands or the provided trowel, collectors will need to dig down 10-15 cm into the sediment around a small patch of eelgrass (~ 0.0625 m<sup>2</sup> area) beneath both the above- and belowground compartments of the plants.
- 3) A slight prying, cutting or rocking motion with one's hands or the trowel may be necessary to release the cohesiveness of the sediment and successfully remove the plants, both the above- and belowground eelgrass material.
- 4) As the eelgrass and attached sediment is removed from the substrate, place it in the mesh bag and rinse the above- and belowground material free of sediment and other non-eelgrass material in a pool of water or deeper water at the water's edge.
- 5) Place rinsed plants in the 1.5 gallon Ziploc bag labeled with replicate 1 (or replicates 2 and 3).
- 6) For each sample (replicate) at each site, continue steps 2-5 within a 3 m diameter area until the labeled Ziploc sample bag is 50-75% full of eelgrass, both the above- and belowground material. The number of times steps 2-4 are repeated will depend on the size and density of the eelgrass shoots but should not exceed an area that exceeds 0.25 m<sup>2</sup> of substrate for each replicate.
- 7) Samples (replicates) two (2) and three (3) will be repeated roughly 25-30 m to the left and right of sample (replicate) one (1) which should be in the general vicinity of the Mussel Cage.
- 8) Full Ziploc bags for replicates 1-3 should be sealed and placed in the insulated box after sampling.
- 9) Samples will be transferred from field staff to the project manager (Jeff Gaeckle) or NHP staff with proper signatures for Chain of Custody documentation (Figure 8.1).
- 10) Samples will be stored in WDFW's walk-in refrigerator upon returning to the Aquatic Botany Laboratory (Natural Resources Building, Olympia, WA).

### 8.3 Containers, preservation methods, holding times

#### Eelgrass Collection

Samples will be collected in the field, rinsed free of sediments using seawater at the collection site in a mesh bag. After rinsing, eelgrass from each replicate will be transferred from the mesh rinsing bag to a separate pre-labeled, 2 gallon Ziploc bag. The Ziploc bag will include the following information: site ID, replicate number (1, 2, 3), date (DDMMYY), time (HH:MM AM/PM) and names of collectors. Samples will be stored in an insulated box (e.g., cooler) to minimize the risk of physical damage or thermal stress.

#### Eelgrass samples for nutrient analysis

Milled above- and belowground eelgrass samples will be stored in pre-labeled, 20 mL HDPE scintillation vials and caps. Milled eelgrass will be transferred to tin capsules and 96 well plates for shipment to the nutrient analysis lab. Milled above- and belowground eelgrass samples in 20 mL HDPE scintillation vials will be stored in the Aquatic Botany Laboratory.

#### Eelgrass samples for metal analysis

The above- and belowground eelgrass material will be stored in pre-labeled, 135 mL polystyrene grinding vials with screw-on caps – 135. Vials are 5.4 cm diameter x 6.7 cm long and contain 2-

3 methacrylate, 9.5 mm diameter, grinding balls. Samples will be stored in the WDFW refrigerator until all samples are processed then the samples will be shipped to the KCEL.

#### Eelgrass samples for organic analysis

The above- and belowground eelgrass material that is prepared for organic analysis will be stored in pre-labeled I-Chem certified 200-0250 series, Type III glass with Teflon-line polypropylene lid. Samples will be stored in the WDFW refrigerator until all samples are processed then the samples will be shipped to the KCEL.

#### 8.4 Invasive species evaluation

Eelgrass samples will be collected by hand or in some cases with the assistance of a trowel in the nearshore environment, within  $\pm 0.5$  m of 0 m MLLW, and stored in Ziploc sample bags and insulated boxes. Field equipment will be rinsed thoroughly with seawater at the site and with fresh water upon returning to the lab. Careful rinsing of field equipment, waders, and knee boots will eliminate the possibility of transporting invasive species.

#### 8.5 Equipment decontamination

The excessive rinsing practices performed in 8.4 will eliminate the risk of sample equipment contamination and risk of transporting invasive species.

#### 8.6 Sample ID

Samples will be stored in an airtight container with the following sample identification label:

Survey ID (e.g., Nutrient-Zm, Metal-Zm, Organics-Zm)

Site ID – Replicate – Plant Compartment (e.g., TS – 1 – AG for Thompson Spit, Replicate 1, Aboveground material). Replicates will be collected at the left, center, and right of a deployed Mussel Cage.

GPS coordinates, latitude and longitude (hddd.ddddd°) will be collected for each sample (replicates 1-3).

#### 8.7 Chain-of-custody, if required

Two chain of custody (COC) forms will be used for the project. The field COC (Figure 8.1) will be used to transfer samples from field staff to Aquatic Botany Laboratory staff. The second COC form will be used to track samples from the Aquatic Botany Laboratory to each of the analytical laboratories (e.g., UC Davis Stable Isotope Facility, King County Environmental Laboratory, and the NMFS/Northwest Fisheries Science Center Environmental Conservation Division Laboratory) (Figure 8.2).





## 8.8 Field log requirements

Field personnel will maintain a field log on the Eelgrass Collection Datasheet (Figure 7.1) for each site. Each datasheet will be scanned and saved digitally in a project specific folder on the DNR server and the originals will be stored in a project specific 3-ring binder. A summary of the individual field datasheets will be transcribed into a waterproof Elan Field Book (E 64-8x4 W) maintained by the lead scientist for the field and laboratory components of the project. The field log will include the following information:

1. Project name
2. Site ID
3. Replicate (1-3)
4. Plant compartment (above- and belowground material)
5. Date (DDMMMYYYY)
6. Time (HH:MM AM/PM)
7. GPS coordinates for each sample
8. Field personnel
9. Environmental conditions (e.g., salinity, temperature, tidal stage) at sites
10. Sequence of events
11. Any changes to plan
12. Samples collected or processed
13. Any additional field measurement
14. Unusual circumstances that may affect interpretation of results

A paper and digital copy (pdf format) of the summary field log maintained by the lead scientist will be stored digitally in a project specific folder on the DNR server and in a project specific 3-ring binder. The DNR server is backed-up weekly.

## 8.9 Other sampling-related activities

Task 3 will also survey the existing eelgrass at the selected outfall sites (to be determined) using previously established methods by the Nearshore Habitat Program (Berry et al. 2003, Gaeckle 2009, Gaeckle et al. 2011).

# 9.0 Measurement Methods

## 9.1 Sample preparation methods

Field collected eelgrass samples need to be processed by lab personnel in the Aquatic Botany Laboratory (Room 655, 6<sup>th</sup> floor, Natural Resources Building, Olympia, WA) for each of the three analytical laboratories (nutrients, metals, and organics). The following section describes the necessary equipment and standard operating procedures for eelgrass sample preparation for the three analytical laboratories.

*Eelgrass Sample Preparation in the Aquatic Botany Laboratory*

Equipment and supplies

Paper towels  
Nitrile gloves – powder free  
Eye protection  
Deionized (DI) water  
Tap water  
Teflon squeeze bottles  
6”-8” Ceramic knives  
8”-10” Stainless knives  
Glass microscope slides  
Razor blades  
HDPE processing sheets  
HDPE containers for rinsing eelgrass  
Metric rulers (0.001 m)  
Stainless steel calipers with digital readout (0.01 mm)  
Data sheets  
Pencils  
Fisher Scientific IsoTemp convection drying oven  
Aluminum drying tray  
Desiccator  
Stainless steel forceps  
Refrigerator – walk-in refrigerator (WDFW)  
Freezer – 12 ft<sup>2</sup> chest freezer (DNR)  
Toledo balance (0.0000 g)  
Tin weighing dishes  
50 mL desiccant vials and screw top caps  
Desiccant  
Avery 8167 labels for the 50 mL desiccant vials and caps (pre-printed with office laser printer)  
10 cm x 10 cm paper bags (nutrient samples)  
20 mL HDPE scintillation vials (nutrient samples)  
Avery 8167 labels for the 20 mL scintillation vials (pre-printed with office laser printer)  
Wiley Mill or the equivalent plant grinder (nutrient samples)  
Synthetic paint brush  
Small vacuum  
Stainless spatula or sample spoon  
Tin capsules for solid samples (3.5 x 5 mm) (nutrient samples)  
Well plate, round bottom with lid (nutrient samples)  
Tape – masking tape and packaging tape  
Sample jars – I-Chem certified 200-0250 series, Type III glass with Teflon-line polypropylene lid (organic samples) with provided labels  
Polystyrene grinding vial with screw-on cap – 135 mL polystyrene grinding vial with plastic screw-on cap. Vial is 5.4 cm diameter x 6.7 cm long (metal samples)  
Avery 5960 labels for the polystyrene grinding vials (pre-printed with office laser printer)  
Methacrylate grinding balls – 9.5 mm diameter (metal samples)

Laboratory forms and labels

The following labels will be printed and applied to the sample containers prior to laboratory processing.

*Nutrients* - labels for the 10 cm x 10 cm paper sample bags, 50 mL desiccant vials and caps, and for the HDPE scintillation vials and caps

*Metals* – labels for the polystyrene grinding vials and caps

*Organics* – labels for the Chem certified jars and lids

Sample identification (Table 9.1) will include the following information:

*Survey ID*

Nutrients - Zm

Metals - Zm

Organics - Zm

*Site ID*

Post Point – PP

March Point – MP

Padilla Bay – PB

Penn Cove – PC

Thompson Spit – TS

Big Gulch – BG

Duwamish Head – DH

Ruston Way – RW

Sandy Bay – SB

Holly – HY

Birch Bay – BB

Cypress Island – CI

Four-mile Rock – FR

Dumas Bay – DB

Burley Spit - BS

*Replicates*

1

2

3

*Plant compartment*

Aboveground – AG

Belowground – BG

*Date*

DDMMYYYY

Table 9.1. An example of the sample identification labels for the above- and belowground nutrient, metal and organic samples at one site (Thompson Spit – TS).

NUTRIENTS	METALS	ORGANICS
Nutrients - Zm TS – 1 – AG 07JAN2013	Metals - Zm TS – 1 – AG 07JAN2013	Organics - Zm TS – 1 – AG 07JAN2013
Nutrients - Zm TS – 2 – AG 07JAN2013	Metals - Zm TS – 2 – AG 07JAN2013	Organics - Zm TS – 2 – AG 07JAN2013
Nutrients - Zm TS – 3 – AG 07JAN2013	Metals - Zm TS – 3 – AG 07JAN2013	Organics - Zm TS – 3 – AG 07JAN2013
Nutrients - Zm TS – 1 – BG 07JAN2013	Metals - Zm TS – 1 – BG 07JAN2013	Organics - Zm TS – 1 – BG 07JAN2013
Nutrients - Zm TS – 2 – BG 07JAN2013	Metals - Zm TS – 2 – BG 07JAN2013	Organics - Zm TS – 2 – BG 07JAN2013
Nutrients - Zm TS – 3 – BG 07JAN2013	Metals - Zm TS – 3 – BG 07JAN2013	Organics - Zm TS – 3 – BG 07JAN2013

Sample preparation will occur in the Aquatic Botany Laboratory or in the WDFW Marine Resources Laboratory. Laboratory personnel will create a clean work environment (e.g., work surface, HDPE processing sheets, and equipment) using warm soapy water (Terg-A-Zyme<sup>®</sup>), rinsing with tap water and a final rinse with deionized (DI) water dispensed from a Teflon squeeze bottle.

Between sample replicates, the processing equipment (e.g., HDPE sheets, knives, and nitrile gloves) can be cleaned in soapy water, rinsed in tap water with a final rinse in DI water. Lab personnel should replace nitrile gloves whenever glove integrity is compromised or considered contaminated.

#### Nutrients – sample preparation for analysis at the UC Davis Stable Isotope Facility

##### Pre-drying processing

- 1) Remove an eelgrass sample bag from the refrigerator.
- 2) Record the site ID and date collected on the Nutrient-Zm datasheet.
- 3) Wearing nitrile gloves, remove three (3) eelgrass shoots from the eelgrass sample collection bag and place shoots in HDPE containers containing DI water.
- 4) Carefully rinse eelgrass clean of sediment and other non-eelgrass material.
- 5) Lay rinsed eelgrass on HDPE processing sheets.
- 6) Using a ceramic knife, separate the aboveground eelgrass material from the belowground eelgrass material at the meristem.
- 7) Focusing on the aboveground eelgrass material, carefully separate the leaves and isolate the youngest leaf.
- 8) Remove epiphytes on leaves by pulling each leaf through the thumb and pointer finger or carefully scrape epiphytes using the ceramic knife.

- 9) Measure and cut 10 cm sections of leaf material.
- 10) Repeat steps 6-10 until 5, 10 cm sections (or the equivalent) are collected.
- 11) Measure the width of each section collected and record these values on the Nutrient-Zm datasheet.
- 12) Place measured sections in labeled paper sample bag or 50 mL desiccant vial for drying.
- 13) Place remaining aboveground material back in eelgrass sample bag.
- 14) Focusing on the belowground eelgrass material, remove the third rhizome node using a ceramic knife.
- 15) Collect the equivalent of 20 cm of rhizome length and measure the width of 5 rhizome nodes using calipers and record these values on the Nutrient-Zm datasheet.
- 16) Place measured rhizome sections in the labeled paper sample bag or 50 mL desiccant vial for drying.
- 17) Place remaining belowground material back in the eelgrass sample bag.
- 18) Place above- and belowground eelgrass samples in an aluminum drying tray.
- 19) Repeat steps 1-18 until all eelgrass sample bags are processed for Nutrients-Zm.
- 20) Place samples aluminum drying tray in drying oven set at 60°C for 48 hours or until samples achieve constant weight.

#### Post-drying processing

- 1) Wearing nitrile gloves, transfer approximately 10 sample bags from the drying oven to the desiccator for storage while weighing.
- 2) Tare a tin weighing boat and carefully transfer samples (above- or belowground) onto the weighing boat.
- 3) Record weight (0.0000 g) on Nutrient-Zm datasheet.
- 4) Repeat steps 1-3 until all the above- and belowground samples have been weighed.

#### Grinding processing

- 1) Wearing nitrile gloves, transfer a dried sample (e.g., above- or belowground eelgrass sample) into the Wiley Mill (or equivalent plant processor) hopper and grind to a fine powder. Store ground samples in the labeled 20 mL HDPE scintillation vials.
- 2) Place a check mark in the appropriate cell on the Nutrient-Zm datasheet upon completion of grinding an above- or belowground sample.
- 3) Using a brush and vacuum, clean Wiley Mill between samples.
- 4) Repeat steps 1-3 until all above- or belowground samples are ground.

#### Transfer above- and belowground eelgrass samples into tin capsules

- 1) Wearing nitrile gloves, weigh a tin capsule and record the value on the Nutrient-Zm datasheet. Place the tin capsule in the well plate and record the column – row value on the Nutrient-Zm datasheet.
- 2) Using a spatula, transfer enough ground above- or belowground eelgrass material to fill the tin capsule on the well plate.
- 3) Store the extra vial of the above- or belowground eelgrass sample in the Aquatic Botany Laboratory.
- 4) Repeat steps 1-3 until all samples are transferred into a weighed tin capsule on the well plate, then cover well plate and secure with tape.

### Shipping samples to UC Davis Stable Isotope Facility

- 1) Secured well plates, analysis order form, a copy of the datasheets, and a COC form (Figure 8.2) will be mailed to:

UC Davis Stable Isotope Facility  
Department of Plant Sciences  
Mail Stop #1  
One Shields Ave.  
Davis, CA 95616

### Metals – sample preparation for analysis at the King County Environmental Laboratory

- 1) Remove an eelgrass sample bag from the refrigerator.
- 2) Record the site ID and date collected on the Metal-Zn datasheet.
- 3) Wearing nitrile gloves, remove all the eelgrass plants from the collection bag and place shoots in HDPE containers containing DI water.
- 4) Carefully rinse eelgrass clean of sediment and other non-eelgrass material.
- 5) Lay rinsed eelgrass on HDPE processing sheets.
- 6) Using a ceramic knife, separate the aboveground eelgrass material from the belowground eelgrass material at the meristem. Maintain separate piles of aboveground and belowground eelgrass material.
- 7) Focusing on the aboveground eelgrass material, carefully remove epiphytes on each leaf by pulling leaves through the thumb and pointer finger or carefully scraping epiphytes using a ceramic knife.
- 8) Measure and cut 10-20 cm sections of leaf material until 9 g of eelgrass material or more is collected and place eelgrass in pre-weighed and pre-labeled polystyrene grinding vial with screw-on cap and methacrylate grinding balls.
- 9) Record weight of vial, cap, grinding balls and aboveground eelgrass material on Metals-Zn datasheet.
- 10) Isolate 9 g of belowground eelgrass material and place in a pre-weighed and pre-labeled polystyrene grinding vial with screw-on cap and methacrylate grinding balls.
- 11) Record weight of vial, cap, grinding balls and belowground eelgrass material on Metals-Zn datasheet.
- 12) Store vials in refrigerator in the WDFW Marine Sciences Laboratory.
- 13) Place the remaining above- and belowground eelgrass material in the sample collection bag and return to the refrigerator.

### Shipping samples to King County Environmental Laboratory

- 1) Secured vials, analysis order form, a copy of the datasheets, and a COC form (Figure 8.2) will be mailed to:

Laboratory Project Manager  
King County Environmental Laboratory  
322 W. Ewing Street  
Seattle, WA 98119-1507

Organic analysis – sample preparation for analysis at the NMFS/Northwest Fisheries Science Center Environmental Conservation Division

- 1) Remove an eelgrass sample bag from the refrigerator.
- 2) Record the site ID and date collected on the Organics-Zm datasheet.
- 3) Wearing nitrile gloves, remove the eelgrass material from the collection bag and place it in HDPE containers containing DI water.
- 4) Carefully rinse eelgrass clean of sediment and other non-eelgrass material.
- 5) Lay rinsed eelgrass on HDPE processing sheets.
- 6) Using a ceramic knife, separate the aboveground eelgrass material from the belowground eelgrass material at the meristem. Maintain separate piles of aboveground and belowground eelgrass material.
- 7) Focusing on the aboveground eelgrass material, carefully remove epiphytes on each leaf by pulling leaves through the thumb and pointer finger or carefully scraping epiphytes using a ceramic knife.
- 8) Using a stainless knife mince the aboveground eelgrass into a puree containing 2.5 g of material. Transfer material into pre-weighed and pre-labeled I-Chem certified 200-0250 series, Type III glass with Teflon-line polypropylene lid.
- 9) Record weight of the jar, cap and aboveground eelgrass material on Organics-Zm datasheet.
- 10) Mince the remaining belowground eelgrass into a puree containing 2.5 g of material. Transfer material into pre-weighed and pre-labeled I-Chem certified 200-0250 series, Type III glass with Teflon-line polypropylene lid.
- 11) Record the weight of the jar, cap and belowground eelgrass material on the Organics-Zm datasheet.
- 12) Store jars in refrigerator.

Shipping samples to NMFS/Northwest Fisheries Science Center Environmental Conservation Division

- 1) Secured jars, analysis order form, a copy of the datasheets, and a COC form (Figure 8.2) will be mailed to:

Gina Ylitalo  
Research Chemist  
NMFS/Northwest Fisheries Science Center  
Environmental Conservation Division  
2725 Montlake Boulevard East  
Seattle, WA 98112

Lab Measurement Methods Table. This includes:

## 9.2 Analytes

The following analytes will be measured in the above- and belowground eelgrass samples for nutrients (Table 9.2), metals (Table 9.3), and organics (Table 9.4).

Table 9.2. Elements (C:N) to be measured in above- and belowground eelgrass.

ELEMENTS	# ANALYTES	METHODS	METHOD DETECTION LIMIT	EXPECTED RANGE
		(based on Sharp 2005 <sup>a</sup> )	(µg/g)	(µg/g)
Nitrogen (N)	1	Shick et al. 2012 <sup>b</sup>	20	100
Carbon (C)	1	Shick et al. 2012 <sup>b</sup>	100	1500
δ15N	1	Shick et al. 2012 <sup>b</sup>	20	100
δ13C	1	Shick et al. 2012 <sup>b</sup>	100	1500

<sup>a</sup>Sharp, Z. 2005. *Principles of Stable Isotope Geochemistry*. Prentice Hall.

<sup>b</sup>Shick, E., E. Delgado, J. Matthews. 2012. Isotopic analysis of solid samples for stable isotopes of C and N by continuous flow combustion – isotope ratio mass spectrometry (C-IRMS). UCD-SIF-EACN01.2.

The SOPs for metal analyses incorporates elements of EPA 245.1 revision 3, SW-846 7470, 7471B and PSEP 1997. Arsenic, cadmium, and lead will be analyzed via Thermo Elemental X Series II CCT (Collision Cell Technology) Inductively Coupled Plasma Mass Spectrometer (ICP-MS) following KCEL SOP 624v2. KCEL SOP 624v2 incorporates elements of EPA 200.8 revision 5.4, SW-846 6020A February 2007, ILM05.3 Exhibit D part B, and PSEP 1997. Total solids will be analyzed via KCEL SOP 307v3 to facilitate reporting metals data in both dry and wet weight concentrations.

Table 9.3. Metals to be measured in above- and belowground eelgrass.

METALS	# ANALYTES	METHODS	METHOD DETECTION LIMIT	EXPECTED RANGE
			(wet weight)	(wet weight)
			(µg/g)	(µg/g)
Total mercury (Hg)	1	KCEL SOP 604v6 <sup>a</sup> KCEL SOP 06-01-103-000 <sup>b</sup>	0.00038	MDL to 5
Lead (Pb)	1	KCEL SOP 624v2 <sup>b</sup>	0.004	MDL to 5
Arsenic (As)	1	KCEL SOP 624v2 <sup>b</sup>	0.004	MDL to 5
Cadmium (Cd)	1	KCEL SOP 624v2 <sup>b</sup>	0.002	MDL to 5
Chromium (Cr)	1	KCEL SOP 624v2 <sup>b</sup>	0.008	MDL to 5
Copper (Cu)	1	KCEL SOP 624v2 <sup>b</sup>	0.016	MDL to 5
Iron (Fe)	1	KCEL SOP 624v2 <sup>b</sup>	0.4	MDL to 5
Nickel (Ni)	1	KCEL SOP 624v2 <sup>b</sup>	0.004	MDL to 5
Zinc (Zn)	1	KCEL SOP 624v2 <sup>b</sup>	0.02	MDL to 5
Vanadium (V)	1	KCEL SOP 624v2 <sup>b</sup>	0.003	MDL to 5

<sup>a</sup>KCEL SOP 604v6: King County Environmental Laboratory Standard Operating Procedure 604v6 – Instrumental Analysis for Mercury in Environmental Samples by Cold Vapor Atomic Absorption Spectrometry.

<sup>b</sup> KCEL SOP 06-01-103-000. King County Environmental Laboratory Standard Operating Procedure for Trace Metals Section. Preparation of tissue samples for low tissue concentration range analysis of total mercury by cold vapor atomic absorption spectrometry. Seattle, WA. Pp. 21.

<sup>c</sup> KCEL SOP 604v2: King County Environmental Laboratory Standard Operating Procedure 624v2 – ICPMS Analysis of Water, Wastes, Sediments, and Tissues by the Thermo X Series II CCT.

The SOPs for organic contaminant analyses are based on methods described in Sloan et al. (1993) and Krahn et al. (1988). Both of these documents updated the methods of MacLeod et al. (1985).

Table 9.4. Organics to be measured in above- and belowground eelgrass.

PERSISTENT ORGANIC POLLUTANTS	# ANALYTES	METHODS	LIMIT OF QUANTITATION (LOQ)	EXPECTED RANGE
			(wet weight)	(wet weight)
			(ng/g)	(ng/g)
Polychlorinated biphenyl (PCB) congeners	40	Sloan et al. 2004 <sup>a</sup>	0.2 – 0.8	LOQ to 20
Polybrominated diphenylethers (PBDEs) congeners	11	Sloan et al. 2004 <sup>a</sup>	0.2 – 0.8	LOQ to 20
Organochlorine pesticides (OCPs)	25	Sloan et al. 2004 <sup>a</sup>	0.2 – 0.8	LOQ to 20
Polycyclic Aromatic Hydrocarbons (PAHs)	45	Sloan et al. 2004 <sup>a</sup>	0.2 – 0.8	LOQ to 20

<sup>a</sup> Sloan, C.A., D.W. Brown, R.W. Pearce, R.H. Boyer, J.L. Bolton, D.G. Burrows, P. Herman, M.M. Krahn. 2004. Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants. NOAA Technical Memorandum NMFS-NWFSC-59. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Seattle, WA. Pp. 63.

Table 9.5. Conventionals to be measured in this study.

CONVENTIONAL	# ANALYTES	METHOD	METHOD DETECTION LIMIT	EXPECTED RANGE
			(%)	(%)
Dry Weight	1	Gravimetric	0.1	2-15

### 9.2.1 Matrix

Above- and belowground native eelgrass (*Zostera marina* L.) tissue is the only matrix analyzed for nutrients, metals, and organics.

### 9.1.3 Number of samples

For Task 2, the project plans to sample 3 replicates of eelgrass at 10 sites. Each sample will be separated into above- and belowground eelgrass material for a total of 60 samples. Additional samples will be added to each analysis for Quality Control purposes (Table 9.6). Similarly, in Task 3, additional samples will be added for Quality Control purposes (Table 9.7).

Table 9.6. Additional samples in Task 2 for Quality Control for the nutrient, metal and organic analyses.

ANALYSIS	SAMPLES	QUALITY CONTROL SAMPLES:FIELD SAMPLES	QUALITY CONTROL SAMPLES
	Total (aboveground:belowground)		
Nutrients	60 (30:30)	2:12	5
Metals	60 (30:30)	3:20	3-9 <sup>a</sup>
Organics	60 (30:30)	2:12	5
TOTAL			13-19

<sup>a</sup>KCEL requires 3 QC samples per ICPMS, Hg, and Total Solids analysis consisting of 19, 15 and 11 g for each QC sample respectively. However, 3 samples of 27 g (wet weight) will cover all QC requirements.

Table 9.7. Additional samples in Task 3 for Quality Control for the nutrient, metal and organic analyses.

ANALYSIS	SAMPLES	QUALITY CONTROL SAMPLES:FIELD SAMPLES	QUALITY CONTROL SAMPLES
	Total (aboveground:belowground)		
Nutrients	20 (10:10)	2:12	1-2
Metals	20 (10:10)	3:20	1-3
Organics	20 (10:10)	2:12	1-2
TOTAL			3-7

#### 9.1.4 Expected range of results

The expected range of results for some of the analyses related to eelgrass is outlined in Appendices A and B of the literature review (Gaeckle 2012). Certain analytes have not been assessed in eelgrass but have been assessed in other seagrass species (Gaeckle 2012).

#### 9.1.5 Analytical method

Laboratory SOPs attached.

#### 9.1.6 Sensitivity/Method Detection Limit (MDL)

The Lower Limit of Quantitation (LOQ) for all organic analyses in this study is “the concentration that would be calculated if that analyte had a gas chromatography – mass spectrometry (GC-MS) response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ.” (Sloan et al. 2006).

EPA defines Method Detection Limit (MDL) in Appendix A to 40 CFR Part 136 as the “minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element”. In this study, the metal’s MDLs are concentrations that cannot be detected or detected at a concentration less than the associated method detection limit considering tissue sample detection limits are affected by the sample mass used, matrix and polyatomic/isobaric interferences. The MDL is the lowest concentration at which a sample result will be reported. Table 9.3 lists the respective method detection limits for the four metals of concern.

## 9.2 Sample preparation method(s)

Section 9.1 describes in detail the procedure required to prepare eelgrass samples necessary for the nutrient, metal and organic contaminant analyses.

## 9.3 Field procedures table field analysis table

Section 8.0 describes in detail the eelgrass sample collection procedures.

## 9.5 Lab(s) accredited for method(s)

Laboratory SOPs attached.

# 10.0 Quality Control (QC) Procedures

## 10.1 Table of lab and field QC required

Quality control procedures, quality assurance criteria and corrective actions for organic contaminant data are detailed in the SOPs provided by the NMFS/Northwest Fisheries Science Center Laboratory (Sloan et al. 2006). Precision is monitored and controlled within batches using laboratory replicates of field samples (2 replicates run for every batch of 12 samples) and across batches by analyzing Standard Reference Materials (SRMs – one per batch). Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be  $\leq 15\%$  for the repetitions. For organic contaminant analysis, accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. A SRM of applicable matrix will be selected to be analyzed i.e., tissue. Concentrations of  $\geq 70\%$  of individual analytes are to be within 30 % of either end of the 95% confidence interval of the reference values. One method blank is run for every 20 or fewer field samples. No more than 5 analytes in a method blank are to exceed 2x the lower LOQ before corrective action is taken. The corrective action will be to re-extract and re-analyze the affected samples and if necessary, qualify the sample data. At least one internal standard (surrogate) is added to each sample, with acceptable recoveries ranging from 60 to 130%.

Quality control measure and quality assurance criteria for metals data are detailed in Table 6.3 and Table 6.4. Precision is monitored and controlled within batches using laboratory replicates of

field samples and matrix spike duplicates (one per batch). Accuracy of analysis is evaluated by comparing measured standard reference material (SRM) values and a laboratory control sample (LCS) with the respective certified values. A SRM of applicable matrix will be selected to be analyzed. Method blanks and spikes are evaluated for overall run and process contamination. These are run every batch as is applicable.

Since there is currently no standard laboratory accreditation for stable isotope analysis, the UC Davis Stable Isotope Facility performs regular calibration checks against international standards USGS-40 and USGS-41. Every run includes the following lab standards – nylon, glutamic acid or peach leaves (NIST 1547), and USGS-41. In addition bovine liver (NIST 1577) is included as a quality check. Standards are included every twelve samples and at least 6 glutamic acid or peach leaves standards are included per run.

#### 10.2 Corrective action processes

The contracted laboratory is responsible for demonstrating that analytical results fall within the acceptable QC criteria.

## **11.0 Data Management Procedures**

#### 11.1 Data recording/reporting requirements

Data are received from analytical laboratories in Excel spreadsheets. Data will be examined by Nearshore Habitat Program staff to identify gross formatting or transcription errors. Raw analyte concentrations are compared with expected ranges (Gaeckle 2012) to identify potential outliers. In addition preliminary tables of summary statistics, scatter plots, and time trend plots are created to examine the new data.

The Nearshore Habitat Program staff will format these data into a structure compatible with an ArcGIS geospatial database. The database will be stored on a DNR server, which is backed up nightly as part of an automated network backup service provided by DNR Information Technology (IT) Services.

#### 11.2 EIM data upload procedures

All data generated by this project will be submitted to Ecology's EIM database.

## 12.0 Audits and Reports

### 12.1 Number, frequency, type, and schedule of audits

The NWFSC analytical lab participates in annual NIST or IAEA inter-lab comparison studies. The King County Environment Lab is accredited with Washington Department of Ecology (ECY) and is audited based on the ECY schedule. There is currently no standard laboratory accreditation standard for stable isotope analysis, therefore the UC Davis Stable Isotope Facility performs regular calibration checks against international standards USGS-40 and USGS-41. Every run includes the following lab standards – nylon, glutamic acid or peach leaves (NIST 1547), and USGS-41. In addition bovine liver (NIST 1577) is included as a quality check. Standards are included every twelve samples and at least 6 glutamic acid or peach leaves standards are included per run.

### 12.2 Responsible personnel

Nearshore Habitat Program staff will submit final reports and data packages to Ecology's EIM database as detailed in the Scope of Work. The project data will be automatically uploaded to EPA's STORET database from Ecology's EIM database. Jeff Gaeckle is responsible for these products.

### 12.3 Frequency and distribution of project reports

Each task has detailed reports and documents listed as deliverables. The frequency and distribution of these products is outlined in the project SOW.

### 12.4 Responsibility for reports

Nearshore Habitat Program staff will submit final reports and data packages to Ecology's EIM database as detailed in the Scope of Work. The project data will be automatically uploaded to EPA's STORET database from Ecology's EIM database. Jeff Gaeckle is responsible for these products.

## 13.0 Data Verification

### 13.1 Field data verification, requirements, and responsibilities

All sample location data for this study will be verified with GIS-plotted latitude and longitude data and corresponding field notes provided by field collectors. Field verification can also be validated with photographic evidence from the Mussel Watch Pilot Expansion Project. Field verification of sample collection will be confirmed based on completed COC forms (Figure 8.1).

### 13.2 Lab data verification

Data generated by the analytical lab are reviewed for outlier values, transcription errors and other problems by at least two chemists. Final review is conducted by a lab manager who approves data before they are released to the client and documented with the COC form (Figure 8.2). Prior to database entry the client reviews data by comparing results with similar matrices the literature (Gaeckle 2012). Data, means, and standard deviations are plotted and putative outliers evaluated for validity. Evaluation of the validity of putative outliers includes reviewing all collection, biological, and analytical data for potential transcription errors, communication with analytical labs to verify reported values are correct, and evaluation of biological covariates that might explain otherwise unanticipated values.

### 13.3 Validation requirements, if necessary

There is no anticipated plan to conduct data validation due to limited resources.

## 14.0 Data Quality (Usability) Assessment

### 14.1 Process for determining whether project objectives have been met

The success of meeting data quality objectives is evaluated based on the outcome of quality control procedures during analytical procedures. Typically if QC criteria are not met the problem is identified, corrected, and sample (or extract) re-run. In cases where QC criteria have not been met and there is not enough tissue to be reanalyzed, the data are to be censored with appropriate qualifiers to allow an objective evaluation of the usability of the final record. Rejected data are censored with an “R” or equivalent qualifier. Based on the range of data values expected in this study and the appropriate limits of quantitation rejected data should be rare with the exception of some organic contaminants. Adequacy of sample number will be evaluated during the statistical analysis of analytes for subsequent studies.

### 14.2 Data analysis and presentation methods

Data collected for the Outfall Assessment and the Effects on Critical Nearshore Habitats study is designed to provide a preliminary understanding of the amount of nutrients, metals, and organic contaminants in eelgrass throughout Puget Sound. Analysis and presentation of data will be conducted using programs commonly employed by the Nearshore Habitat Program and PSEMP to compare spatial distribution of these analytes in Puget Sound.

### 14.3 Treatment of non-detects

Non-detected analytes are censored with a “<LOQ” or “U” qualifier. The value reported for non-detected analytes will be the LOQ or Method Detection Limit, depending on analytical procedure. It is the responsibility of data users to decide how to use data censored as non-detected. The current study will primarily report *analyte averages* ( $\pm$  error) for plant compartments at each site and compared across a range of conditions from highly contaminated

to relatively pristine. We anticipate substituting zero for “U” qualified data in contaminant-class summations. Data from literature suggests the target analytes in eelgrass could be dominated by substantial concentrations of a number of individual analytes (Gaeckle 2012). Substituting zero, or any trivial or nominal concentration, is not anticipated to change comparison results for summed analytes.

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## **16.0 Appendices**

### **Appendix A. Project SOW and budget**

## Statement of Work

### Outfall Assessment and the effects on Critical Nearshore Habitats WA Dept of Natural Resources

## Statement of Work

### I. Scope

The Nearshore Habitat Program (NHP), part of the Washington State Department of Natural Resources, will complete a series of linked information syntheses and field data collection actions to increase our understanding of the effects of outfalls on eelgrass in Puget Sound. These include:

- A literature review that will summarize available scientific research on four major categories of the effects of outfalls on eelgrass, including: nutrients, metals and other contaminants, physical alteration of habitat, and trophic transfer.
- A synthesis of local spatial information and analyses that will evaluate available data on the current status of outfalls and eelgrass in Puget Sound, including the areas of greatest potential impacts.
- Field data collection at 10 long-term monitoring sites to broadly characterize nutrient and contamination concentrations in eelgrass and to establish a baseline at a range of locations over a spatial gradient in Puget Sound. Nutrient and contaminant concentrations in eelgrass will be compared to concentrations in other ecosystem components.
- Field data collection at one individual outfall scheduled for mitigation actions in order to describe baseline eelgrass bed characteristics and the extent of nutrient and contaminant concentrations in eelgrass. This will serve as the basis for effectiveness monitoring.

Individual technical reports will be distributed describing the methods and findings of each task. Additionally, data and results from each task will be synthesized in a brief white paper that summarizes the potential effects of outfalls on eelgrass.

### II. Objectives

The goal of this project is to provide scientifically sound information that can be used by a wide range of government and non-governmental organizations to: 1) understand the impacts of outfalls on eelgrass; 2) improve shoreline decision making; 3) improve resource protection and restoration efforts; 4) inform assessment approaches considered at individual sites; and 5) compare findings at individual sites to other areas in Puget Sound. Specifically, the information produced will be used in the following activities:

- Provide a foundation for local and regional analyses of nutrient, metal and contaminant impacts on eelgrass, including both scientific and management-oriented studies;

- Provide scientific information and field sampling approaches to the Stormwater Work Group related to their planned eelgrass marine parameter;
- Establish a baseline for assessing change in outfall impacts at one individual site;
- Create more complete information on the impacts of outfalls on eelgrass for educational, outreach, and management applications;
- Provide recommendations based on sound science to guide DNR conservation measures related to outfalls and the protection of eelgrass in Puget Sound;
- Provide input on restoration and protection priorities for agencies, NGOs, and community groups;
- Identify strategies related to outfalls to support the Puget Sound Partnership's goal to increase eelgrass area 20% by 2020;
- Identify data gaps and recommend next steps for scientific inquiry related to outfalls that will guide resource management strategies;
- Provide information to the Puget Sound Partnership that summarizes known information on key stressors on eelgrass as described in their Proposed Priority Science Questions for 2011-2013 and section B.6 of the Draft 2011 Action Agenda; and
- Identify data gaps in knowledge about the relative importance of outfalls as stressors on eelgrass. This will inform the Partnership's Proposed Priority Science Questions for 2011-2013, 'Identify the key stressors on eelgrass' (2011 Draft Action Agenda). It will also guide future work by DNR's Eelgrass Stressor-Response Project.

### III. Tasks and Deliverables

The project will be completed in three phases. The first phase, to be completed as Task 1 in fall 2012, will summarize information on the effects of nutrient, metal, and contaminants in eelgrass through a literature review, perform a spatial analysis of known eelgrass and outfall data in Puget Sound, and develop the Quality Assurance Project Plan (QAPP) required for GIS analysis, field work, and eelgrass plant tissue analysis. The first deliverable of Task 1, to be completed in spring 2012, will synthesize existing information on the impacts of outfalls, nutrients, metals and contaminants on eelgrass through a literature review. The information obtained in the literature review will guide the development of the (QAPP) required for GIS analysis, field work, and eelgrass plant tissue analysis. A synthesis of the literature review and the existing spatial data and analysis to summarize the current scientific knowledge related to impacts of outfalls on eelgrass beds in Puget Sound will complete phase 1.

The second phase, to be completed by late 2013, will characterize nutrient, metal and contaminant concentrations in eelgrass at selected sites in Puget Sound (Task 2). In addition, the second phase will characterize environmental parameters and establish the extent and magnitude of nutrient, metal and contaminant concentrations in eelgrass tissue at a select priority outfall that is scheduled to have an upgrade or modification that results in a change in effluent discharge (Task 3). The work will provide an important baseline from which to evaluate the effectiveness of future outfall management actions.

The third phase (Task 4), to be completed in winter of 2014, will be to synthesize data and results into a white paper that provides DNR, managers, and decision makers with a science assessment of potential

outfall impacts on eelgrass in Puget Sound as well as management considerations that may minimize these impacts.

Management of the project, experimental design, field work, and completion of deliverables and guidance documents will be carried out by the NHP's Eelgrass Stressor-Response Project lead, Jeff Gaeckle. Nearshore Habitat Program scientist, Lisa Ferrier, will be responsible for GIS support, database management and field work. A NHP technician will assist with literature review, field data collection, and data management and QA/QC. Plant tissue chemical analysis will be contracted out to an approved lab.

**Task 1. Scientific Literature review, QAPP Preparation, and Spatial Evaluation of Outfall Proximity to Eelgrass Beds in Puget Sound.**

DNR will characterize the known impacts of outfalls on eelgrass beds through a scientific literature review. The literature review will summarize the effects of outfalls on eelgrass in the context of changes in nutrients, metals and contaminants, physical alteration, and trophic transfer. The results of the literature review will be used to prepare and complete the Quality Assurance Project Plan (QAPP) for the GIS, field, and laboratory components of this project. A synthesis of local spatial information and analyses of outfalls and eelgrass distribution in Puget Sound will be performed to characterize the extent of overlap between existing outfalls and eelgrass data in the study area. These findings will be used to identify areas of greatest concern for outfall impacts to eelgrass in Puget Sound.

**Deliverables 1.1.** A review of literature that summarizes the effects of outfalls on eelgrass in the context of changes in nutrients, metals and contaminants, physical alterations, and trophic transfer.

**Estimated Cost: \$9,410.52** + (DNR Match: \$10,906.50 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$20,317.02 (total cost)

Target Completion Date: June 30, 2012

**Deliverables 1.2.** A Quality Assurance Project Plan (QAPP) for the spatial analysis (GIS), field, and laboratory components of the project.

**Estimated Cost: \$0.00** + (DNR Match: \$3,635.50– this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$3,635.50 (total cost)

Target Completion Date: August 31, 2012

**Deliverables 1.3.** A report on the spatial evaluation of outfall proximity to eelgrass beds that characterizes the extent of overlap between existing outfalls and eelgrass in Puget Sound. The report will include the spatial analysis methods, data sources and metadata, and an analysis of map products.

**Estimated Cost: \$0.00 + (DNR Match: \$9,481.25— this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$9,481.25 (total cost)**

**Target Completion Date: November 30, 2012**

## **Task 2. Evaluation Of The Extent and Magnitude of Nutrient and Contaminant Concentrations in Eelgrass in Greater Puget Sound.**

Task 2 will assess the magnitude of anthropogenically derived nutrients, metals, and organic contaminants in eelgrass over a broad geographical range. The 10 proposed sample sites for assessment of nutrients, metals and contaminants in eelgrass plant tissue are located in areas that have been monitored since 1989 as part of an effort to assess toxic contaminants in Puget Sound fish (West et al. 2001), water quality (Newton et al. 2002), and sediments (Partridge et al. 2005). The 10 sites will be co-located with WDFW's Mussel Watch sites and will represent a wide range of shoreline types and contaminant levels over a large geographical extent. Additional site selection criteria, described in the "Blue Mussels as Indicators of Stormwater Pollution in Nearshore Marine Habitats in Puget Sound" report will rely on an assessment of upland land-use (a proxy for impervious surfaces) and potential contaminant sources (e.g., stormwater outfalls, marinas, or combined sewer overflows (CSO)). Sites may also be selected based on other known funded projects (e.g., FY 2012 NEP Toxics and Nutrients Preventing Polycyclic Aromatic Hydrocarbon (PAH) Pollution), where there are point-sources of contamination and planned cleanup. DNR and WDFW will coordinate field sample efforts during the fall months to correspond with seasonal increases in contamination loads that are associated with winter stormwater runoff. Sample collection will occur during the fall of 2013 and consist of the following:

- 10 sites (existing long-term contaminant monitoring sites) with a broad geographical range and contaminant levels throughout Greater Puget Sound,
- 3 replicates per site,
- 2 different samples per replicate to assess above ground biomass (leaves) and below ground biomass (root plus rhizomes).

DNR and WDFW will also coordinate the supervision of a technician to process and prepare samples for shipping to the contracted lab for analysis. Laboratory samples will be prepared based on specifications required by the contracted lab. Laboratory analysis costs approximately \$1,000 per sample to measure nutrients (total nitrogen, carbon, C:N ratio, and stable isotopes  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ), metals (e.g., As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn), and contaminants (e.g., PAHs, PBDEs and PCBs) of concern in each eelgrass sample (leaves and roots plus rhizomes). A total of 70 samples would be analyzed for this task; 60 site samples and 10 QA/QC and calibration samples.

Results will be analyzed and presented in a report that describes observed spatial patterns in extent and magnitude of the listed nutrients, metals, and contaminants in eelgrass at each sample site. This effort will constitute the first assessment of contaminants in eelgrass at a broad scale in Puget Sound. Additionally, the report will compare observed levels in Puget Sound with known thresholds for effects in eelgrass from published literature and explore food web impacts through estimating total amount of

contaminants in eelgrass and the potential trophic transfer. The report will provide a broad assessment of how pervasive the effects of nutrient, metal, and contaminant pollution are on Puget Sound eelgrass as well the linkages between contaminants in different components of the system.

**Deliverables 2.1.** A field sampling summary that describes the field effort to collect eelgrass samples at ten study sites. The field sampling report will include the methods, dates, sites, and data collected at each study site. In addition, a copy of the field notes will be included in the appendix of the report.

**Estimated Cost: \$23,040.78 + (DNR Match: \$29,929.00 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$52,969.78 (total cost)**

**Target Completion Date: February 28, 2013**

**Deliverables 2.2.** Laboratory analysis results of eelgrass tissue for nutrients, metals, and contaminants that were collected from each study site (sampled in Task 2.1). The laboratory analysis results will include the data produced by the contracted lab.

**Estimated Cost: \$83,300.00 + (DNR Match: \$3,635.50 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$86,935.50 (total cost)**

**Target Completion Date: July 31, 2013**

**Deliverables 2.3.** A report documenting nutrient and contaminant concentrations in eelgrass at Puget Sound study sites sampled in Task 2.1.

**Estimated Cost: \$0.00 + (DNR Match: \$10,906.50 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$10,906.50 (total cost)**

**Target Completion Date: January 15, 2014**

### **Task 3. Evaluation of Eelgrass Condition and Environmental Parameters around an Individual Outfall.**

Task 3 will conduct field evaluations of eelgrass and environmental parameters adjacent to an outfall where modifications are expected in the near future (e.g., Port Townsend outfall or another outfall identified by the Pollution Identification and Correction program, PIC). High resolution assessment of eelgrass distribution and abundance, plant tissue nutrient, metal and contaminant concentration, and environmental parameters will provide an understanding of the extent and magnitude of impacts across a spatial gradient from the selected outfall of interest. This work will also provide important baseline information that can be used to evaluate project effectiveness of the outfall modification. The site-level assessments will include:

- Eelgrass bed distribution (extent, minimum and maximum depths) and characteristics.
- Plant tissue nutrient, metal and contaminant concentrations in leaves, and roots plus rhizomes.

- Environmental parameters such as light (photosynthetically available radiation, PAR), temperature, and salinity.

**Deliverable 3.1.** A field sampling summary that describes the field effort to collect eelgrass samples and environmental data at one study site in Puget Sound. The field sampling report will include the methods, dates, and data collected at the study site. In addition, a copy of the field notes will be included in the appendix of the report.

**Estimated Cost: \$24,171.28 + (DNR Match: \$19,197.75 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$43,369.03 (total cost)**

**Target Completion Date: September 30, 2013**

**Deliverables 3.2.** Laboratory analysis results of eelgrass tissue from the study site (sampled in Task 3.1) for nutrients, metals, and contaminants. The laboratory analysis results will include the data produced by the contracted lab.

**Estimated Cost: \$23,800.00 + (DNR Match: \$1,817.75 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$25,617.75 (total cost)**

**Target Completion Date: October 31, 2013**

**Deliverables 3.3.** A report documenting the environmental parameters and eelgrass nutrient, metal, and contaminant concentrations at one study site (sampled in Task 3.1) in Puget Sound.

**Estimated Cost: \$0.00 + (DNR Match: \$10,906.50 – this will be counted as programmatic match to WDFW’s Cooperative Agreement with EPA, not separately) = \$10,906.50 (total cost)**

**Target Completion Date: January 15, 2014**

#### **Task 4. Production of a White Paper That Synthesizes Project Results and Communication of the Results.**

Task 4 will produce a short white paper targeted toward decision makers that summarizes the findings of Tasks 1, 2 and 3. The report will summarize the literature review performed in Task 1, present the results of the broad scale and site level research performed in Tasks 2 and 3, and provide management recommendations to DNR and decision makers on data gaps and steps for future scientific inquiry that will develop and improve outfall management on state-owned aquatic lands (SOAL) in Puget Sound. In addition to a white paper, this task will also present the results of this project at local, regional, national and international conferences and meetings.

**Deliverable 4.1.** A draft white paper summarizing the effects of outfalls on eelgrass, spatial patterns in eelgrass nutrient, metal, and contaminant concentrations in Puget Sound and management recommendations to minimize impacts to eelgrass from

outfalls. Early stages of this deliverable will require thorough data quality assurance and quality control and data entry into STORET.

**Estimated Cost: \$4,705.26** + (DNR Match: \$12,380.00 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$17,085.26 (total cost)

Target Completion Date: January 31, 2014

**Deliverable 4.2.** A summary of the internal and external review of the draft white paper that includes reviewer information, reviewer comments, and the author's responses to comments.

**Estimated Cost: \$0.00** + (DNR Match: \$3,635.50 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$3,635.50 (total cost)

Target Completion Date: February 28, 2014

**Deliverable 4.3.** A final white paper and presentation prepared for internal and external distribution.

**Estimated Cost: \$0.00** + (DNR Match: \$7,271.00 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$7,271.00 (total cost)

Target Completion Date: March 31, 2014

**Deliverable 4.4.** Study results will be presented at local, regional, national, and international conferences. Specific conferences will be determined based on project status, abstract acceptance, and the time within the project period that the conference(s) will convene. The final list of conferences will be based upon mutual acceptance by the project sponsor and the grant program.

**Estimated Cost: \$3,332.00** + (DNR Match: \$3,635.50 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = 6,967.50 (total cost)

Target Completion Date: March 31, 2014

**Deliverable 4.5.** In collaboration with the WDFW Mussel Watch project, a briefing of project findings will be presented to the Stormwater Work Group, the Toxic Work Group, and the Puget Sound Partnership's Science Panel.

**Estimated Cost: \$0.00** + (DNR Match: \$908.88 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$908.88 (total cost)

Target Completion Date: March 31, 2014

**Deliverable 4.6.** In collaboration with the WDFW Mussel Watch project, a briefing of project findings will be presented to the Washington State Departments of Fish and Wildlife (WDFW), Natural Resources (DNR), and Ecology.

**Estimated Cost: \$0.00 + (DNR Match: \$908.88 – this will be counted as programmatic match to WDFW's Cooperative Agreement with EPA, not separately) = \$908.88 (total cost)**

**Target Completion Date: March 31, 2014**

#### **IV. Reporting Requirements:**

##### **Biannual Progress Reports:**

The Project Sponsor will provide progress reports describing the work completed for the Puget Sound Marine and Nearshore Protection and Restoration Grant Program in the reporting period. The reporting period is synced to inform the Grant Program's EPA reporting schedule; therefore it is critical that the Project Sponsor submit these reports to the Grant Program according to the following schedule:

First Reporting Period: Project Start - September 30, 2012 **Report due by October 15, 2012**

Second Reporting Period: October 01, 2012 - March 31, 2013 **Report due by April 15, 2013**

Third Reporting Period: April 0, 2013 - September 30, 2013 **Report due by October 15, 2013**

Fourth Reporting Period: October 01, 2013 - March 31, 2014 **Report due by April 15, 2014**

Progress Reports shall include, at a minimum:

- A description of the work completed in the reporting period.
- The status and completion date for the project activities.
- Description of any problem or circumstances affecting the completion date, scope of work, or costs.

##### **Final Performance Report:**

The Project Sponsor will submit a final performance report to the Grant Program, due April 15, 2014, or if the project ends before December 31, 2013, then within 90 calendar days of completion or termination of the project. The report will summarize the basic project accomplishments, and identify key lessons related to planning, design, execution and evaluation.

#### **V. Performance Period**

Funding will be provided through April 30, 2014.

Project Sponsor: Washington State Department of Natural Resources (DNR)														
Project Title: Outfall Assessment and the Effects on Critical Nearshore Habitats														
	Deliverable 1.1	Deliverable 1.2	Deliverable 1.3	Deliverable 2.1	Deliverable 2.2	Deliverable 2.3	Deliverable 3.1	Deliverable 3.2	Deliverable 3.3	Deliverable 4.1	Deliverable 4.2	Deliverable 4.3	Deliverable 4.4	
Describe deliverable	Scientific Literature Review	QAPP (GIS, field & laboratory)	Summary report of spatial evaluation of outfall proximity to eelgrass beds in Puget Sound	Field sampling summary (10 sites)	Laboratory analysis results	Report documenting nutrient, metal, and contaminant concentrations in eelgrass	Field sampling summary (1 site)	Laboratory analysis results (1 site)	Report evaluating impacts of an individual outfall on eelgrass and environmental parameters that support eelgrass	Draft white paper	Summary of the white paper review (internal and external comments, responses to comments, and information on reviewers)	Final white paper	Present Results	
Due date	30-Jun-12	31-Aug-12	30-Nov-12	28-Feb-13	31-Jul-13	15-Jan-14	30-Sep-13	31-Oct-13	15-Jan-14	31-Jan-14	28-Feb-14	31-Mar-14	31-Dec-15	Total
Personnel	9410.52			11862			15721.28			4705.26				41699.06
Technician, Vacant	5166			7749			7749			2583				23247.00
Fringe Benefits	2742			4113			4113			1371				12339.00
Indirect Costs	1502.52			3678.78	13300		3859.28	3800		751.26			532	27423.84
Travel				7500									2800	10300.00
Equipment														0.00
Supplies														0.00
Contracts							8450							8450.00
Other <sup>1</sup>					70000			20000						90000.00
<b>Total</b>	<b>9410.52</b>	<b>0</b>	<b>0</b>	<b>23040.78</b>	<b>83300</b>	<b>0</b>	<b>24171.28</b>	<b>23800</b>	<b>0</b>	<b>4705.26</b>	<b>0</b>	<b>0</b>	<b>3332</b>	<b>171759.84</b>

Add deliverable columns as needed

<sup>1</sup> Describe any "other" costs per deliverable as a narrative below

## **Appendix B. Standard Operating Procedures for analysis of nutrients by the UC Davis Stable Isotope Laboratory**

Shick, E., E. Delgado, J. Matthews. 2012. Isotopic analysis of solid samples for stable isotopes of C and N by continuous flow combustion – isotope ratio mass spectrometry (C-IRMS). Stable Isotope Facility, Department of Plant Sciences, University of California, Davis. Pp. 14.

## **Appendix C. Standard Operating Procedures for analysis of nutrients by the King County Environmental Laboratory**

KCEL SOP 604v6. 2009. King County Environmental Laboratory Standard Operating Procedure for Trace Metals Section. Instrumental analysis for mercury in environmental samples by cold vapor atomic absorption spectrometry. Seattle, WA. Pp. 37.

KCEL SOP 624v2. 2009. King County Environmental Laboratory Standard Operating Procedure for Trace Metals Section. ICPMS analysis of water, wastes, sediments and tissues by the thermo X series II CCT. Seattle, WA. Pp. 39.

KCEL SOP 06-01-103-000. 2001. King County Environmental Laboratory Standard Operating Procedure for Trace Metals Section. Preparation of tissue samples for low tissue concentration range analysis of total mercury by cold vapor atomic absorption spectrometry. Seattle, WA. Pp. 21.

KCEL SOP 307v3. 2008. King County Environmental Laboratory Standard Operating Procedure for Total Solids and Total Volatile Solids. Seattle, WA. Pp. 15.

## **Appendix D. Standard Operating Procedures and Quality Assurance Plan for analysis of nutrients by the NMFS/Northwest Fisheries Science Center Laboratory**

- Sloan, C.A., D.W. Brown, G.M. Ylitalo, J. Buzitis, D.P. Herman, D.G. Burrows, G. Yanagida, R.W. Pearce, J.L. Bolton, R.H. Boyer and M.M. Krahn. 2006. Quality assurance plan for analyses of environmental samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and metabolites of polycyclic aromatic compounds. NOAA Technical Memorandum NMFS-NWFSC-77. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Seattle, WA. Pp. 43.
- Sloan, C.A., D.W. Brown, R.W. Pearce, R.H. Boyer, J.L. Bolton, D.G. Burrows, P. Herman, M.M. Krahn. 2004. Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants. NOAA Technical Memorandum NMFS-NWFSC-59. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Seattle, WA. Pp. 63.

## Appendix E. Glossary, Acronyms, and Abbreviations

### Quality Assurance Glossary

**Accreditation** - A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data. For Ecology, it is "Formal recognition by (Ecology)...that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin, 2010)

**Accuracy** - the degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms precision and bias be used to convey the information associated with the term accuracy. (USGS, 1998)

**Analyte** - An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e. g. fecal coliform, Klebsiella, etc. (Kammin, 2010)

**Bias** - The difference between the population mean and the true value. Bias usually describes a systematic difference reproducible over time, and is characteristic of both the measurement system, and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI). (Kammin, 2010; Ecology, 2004)

**Blank** - A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process. (USGS, 1998)

**Calibration** - The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured. (Ecology, 2004)

**Check standard** - A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards, but should be referred to by their actual designator. (i. e. CRM, LCS, etc.) (Kammin, 2010; Ecology, 2004))

**Comparability** - The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator. (USEPA, 1997)

**Completeness** - The amount of valid data obtained from a data collection project compared to the planned amount. Completeness is usually expressed as a percentage. A data quality indicator. (USEPA, 1997)

**Continuing Calibration Verification Standard (CCV)** - A QC sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run. (Kammin, 2010)

**Control chart** - A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system. (Kammin, 2010; Ecology 2004)

**Control limits** - Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean. (Kammin, 2010)

**Data Integrity**- A qualitative DQI that evaluates the extent to which a dataset contains data that is misrepresented, falsified, or deliberately misleading. (Kammin, 2010)

**Data Quality Indicators (DQI)** - Data Quality Indicators (DQIs) are commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity. (USEPA, 2006)

**Data Quality Objectives (DQO)** - Data Quality Objectives are qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions. (USEPA, 2006)

**Dataset** - A grouping of samples, usually organized by date, time and/or analyte. (Kammin, 2010)

**Data validation** - An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment, and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability and integrity, as these criteria relate to the usability of the dataset. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation
- Use of third-party assessors
- Dataset is complex
- Use of EPA Functional Guidelines or equivalent for review

Examples of data types commonly validated would be:

- Gas Chromatography (GC)
- Gas Chromatography-Mass Spectrometry (GC-MS)
- Inductively Coupled Plasma (ICP)

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier, data is usable for intended purposes
- J (or a J variant), data is estimated, may be usable, may be biased high or low
- REJ, data is rejected, cannot be used for intended purposes (Kammin, 2010; Ecology, 2004)

**Data verification** - Examination of a dataset for errors or omissions, and assessment of the Data Quality Indicators related to that dataset for compliance with acceptance criteria (MQO's). Verification is a detailed quality review of a dataset. (Ecology, 2004)

**Detection limit** (limit of detection) - The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero. (Ecology, 2004)

**Duplicate samples** - two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis. (USEPA, 1997)

**Field blank** - A blank used to obtain information on contamination introduced during sample collection, storage, and transport. (Ecology, 2004)

**Initial Calibration Verification Standard (ICV)** - A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples. (Kammin, 2010)

**Laboratory Control Sample (LCS)** - A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples. (USEPA, 1997)

**Limit of Quantitation (LOQ)** – In organic analyses, the LOQ is the concentration that would be calculated if that analyte had a GC-MS response area equal to the area of the lowest level calibration standard used in that calibration. Similar to a Detection Limit (DL) in metals analyses.

**Matrix spike** - A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects. (Ecology, 2004)

**Measurement Quality Objectives (MQOs)** - Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness. (USEPA, 2006)

**Measurement result** - A value obtained by performing the procedure described in a method. (Ecology, 2004)

**Method** - A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed. (EPA, 1997)

**Method blank** - A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples. (Ecology, 2004; Kammin, 2010)

**Method Detection Limit (MDL)** - This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero. (Federal Register, October 26, 1984)

**Nutrient:** Substance such as carbon, nitrogen, and phosphorus used by organisms to live and grow. Too many nutrients in the water can promote algal blooms and rob the water of oxygen vital to aquatic organisms.

**Organic** - Material derived from the remains or products of living entities.

**Parameter:** A physical chemical or biological property whose values determine environmental characteristics or behavior.

**Percent Relative Standard Deviation (%RSD)** - A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

Percent relative standard deviation,  $\%RSD = (100 * s)/x$  where  $s$  = sample standard deviation, and  $x$  = sample mean (Kammin, 2010)

**Parameter** - A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene, nitrate+nitrite, and anions are all “parameters”. (Kammin, 2010; Ecology, 2004)

**Population** - The hypothetical set of all possible observations of the type being investigated. (Ecology, 2004)

**Precision** - The extent of random variability among replicate measurements of the same property; a data quality indicator. (USGS, 1998)

**Quality Assurance (QA)** - A set of activities designed to establish and document the reliability and usability of measurement data. (Kammin, 2010)

**Quality Assurance Project Plan (QAPP)** - A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives. (Kammin, 2010; Ecology, 2004)

**Quality Control (QC)** - The routine application of measurement and statistical procedures to assess the accuracy of measurement data. (Ecology, 2004)

**Relative Percent Difference (RPD)** - RPD is commonly used to evaluate precision. The following formula is used:

$$\text{Abs}(a-b)/((a+b)/2) * 100$$

Where a and b are 2 sample results, and abs() indicates absolute value

RPD can be used only with 2 values. More values, use %RSD.

(Ecology, 2004)

**Replicate samples** - two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled. (USGS, 1998)

**Representativeness** - The degree to which a sample reflects the population from which it is taken; a data quality indicator. (USGS, 1998)

**Sample (field)** – A portion of a population (environmental entity) that is measured and assumed to represent the entire population. (USGS, 1998)

**Sample (statistical)** – A finite part or subset of a statistical population. (USEPA, 1997)

**Sensitivity** - In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit. (Ecology, 2004)

**Spiked blank** - A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method. (USEPA, 1997)

**Spiked sample** - A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency. (USEPA, 1997)

**Split Sample** – The term split sample denotes when a discrete sample is further subdivided into portions, usually duplicates. (Kammin, 2010)

**Standard Operating Procedure (SOP)** – A document which describes in detail a reproducible and repeatable organized activity. (Kammin, 2010)

**Surrogate** – For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis. (Kammin, 2010)

**Systematic planning** - A step-wise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning. (USEPA, 2006)

### **Glossary References**

Ecology. 2004. Guidance for the Preparation of Quality Assurance Project Plans for Environmental Studies. <http://www.ecy.wa.gov/biblio/0403030.html>

USEPA. 1997. Glossary of Quality Assurance Terms and Related Acronyms. <http://www.ecy.wa.gov/programs/eap/qa.html>

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Kammin, W.R. 2010. Definition developed or extensively edited by William Kammin, 2010.

USGS. 1998. Principles and Practices for Quality Assurance and Quality Control. Open-File Report 98-636. <http://ma.water.usgs.gov/fhwa/products/ofr98-636.pdf>

## Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

AHs	Aromatic Hydrocarbons
ASE	Accelerated solvent extraction
AU	Assessment Unit
CHs	Chlorinated Hydrocarbons
COC	Chain of Custody
DNR	Washington Department of Natural Resources
DOH	Washington State Department of Health
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
GC-MS	Gas Chromatography - Mass Spectrometry
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words or that is
LOQ	Limit of Quantitation
MQO	Measurement quality objective
NIST	National Institute of Standards and Technology
OCPs	Organochlorine pesticides
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
POPs	Persistent organic pollutants
PSEMP	Puget Sound Ecosystem Monitoring Program
PSP	Puget Sound Partnership
PSWQA	Puget Sound Water Quality Authority
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SEC	HPLC Size-exclusion high-performance liquid chromatography
SOP	Standard operating procedure
SRM	Standard reference material
STORET	STOrage and RETrieval data warehouse - EPA's repository and framework for sharing ecological monitoring data
WDFW	Washington Department of Fish and Wildlife

### *Units of Measurement*

°C	degrees Centigrade
dw	dry weight
ft	feet
g gram	a unit of mass
kg kilograms	a unit of mass equal to 1,000 grams.
km kilometer	a unit of length equal to 1,000 meters.
m	meter
mg	milligram
mg/Kg	milligrams per kilogram (parts per million)
mm	millimeter
ng/g	nanograms per gram (parts per billion)
ng/Kg	nanograms per kilogram (parts per trillion)
pg/g	picograms per gram (parts per trillion)
ug/g	micrograms per gram (parts per million)
ug/Kg	micrograms per kilogram (parts per billion)
ww	wet weight

## **Appendix F. Data Sheets for eelgrass processing**

### **F.1. Nutrient-Zm**

### **F.2. Metal-Zm**

### **F.3. Organics-Zm**

SURVEY ID: NUTRIENTS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	# 10 cm LEAF SECTIONS	WIDTH OF 10 cm SECTIONS					Dry WEIGHT of AG MATERIAL	GROUND?	CAPSULE WEIGHT	CAPSULE + SAMPLE WEIGHT	CAPSULE WELL PLATE LOCATION
					(mm)	(mm)	(mm)	(mm)	(mm)					

BB	1													
BB	2													
BB	3													

PP	1													
PP	2													
PP	3													

CI	1													
CI	2													
CI	3													

MP	1													
MP	2													
MP	3													

PB	1													
PB	2													
PB	3													

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: NUTRIENTS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	# 10 cm LEAF SECTIONS	WIDTH OF 10 cm SECTIONS					Dry WEIGHT of AG MATERIAL	GROUND?	CAPSULE WEIGHT	CAPSULE + SAMPLE WEIGHT	CAPSULE WELL PLATE LOCATION
					(mm)	(mm)	(mm)	(mm)	(mm)					

PC	1													
PC	2													
PC	3													

TS	1													
TS	2													
TS	3													

BG	1													
BG	2													
BG	3													

FR	1													
FR	2													
FR	3													

DH	1													
DH	2													
DH	3													

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: NUTRIENTS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	# 10 cm LEAF SECTIONS	WIDTH OF 10 cm SECTIONS					Dry WEIGHT of AG MATERIAL	GROUND?	CAPSULE WEIGHT	CAPSULE + SAMPLE WEIGHT	CAPSULE WELL PLATE LOCATION
					(mm)	(mm)	(mm)	(mm)	(mm)					

HY	1													
HY	2													
HY	3													

BS	1													
BS	2													
BS	3													

DB	1													
DB	2													
DB	3													

RW	1													
RW	2													
RW	3													

SB	1													
SB	2													
SB	3													

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: NUTRIENTS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	TOTAL RHIZOME LENGTH <small>(min 20 cm total length)</small>	WIDTH OF RHIZOME SEGMENTS					DRY WEIGHT of BG MATERIAL <small>(g)</small>	GROUND? <small>(✓)</small>	CAPSULE WEIGHT <small>(g)</small>	CAPSULE + SAMPLE WEIGHT <small>(g)</small>	CAPSULE WELL PLATE LOCATION <small>(Column - Row)</small>
					(mm)	(mm)	(mm)	(mm)	(mm)					

BB	1													
BB	2													
BB	3													

PP	1													
PP	2													
PP	3													

CI	1													
CI	2													
CI	3													

MP	1													
MP	2													
MP	3													

PB	1													
PB	2													
PB	3													

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: NUTRIENTS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	TOTAL RHIZOME LENGTH <small>(min 20 cm total length)</small>	WIDTH OF RHIZOME SEGMENTS					DRY WEIGHT of BG MATERIAL <small>(g)</small>	GROUND? <small>(✓)</small>	CAPSULE WEIGHT <small>(g)</small>	CAPSULE + SAMPLE WEIGHT <small>(g)</small>	CAPSULE WELL PLATE LOCATION <small>(Column - Row)</small>
					(mm)	(mm)	(mm)	(mm)	(mm)					

PC	1													
PC	2													
PC	3													

TS	1													
TS	2													
TS	3													

BG	1													
BG	2													
BG	3													

FR	1													
FR	2													
FR	3													

DH	1													
DH	2													
DH	3													

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: NUTRIENTS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	TOTAL RHIZOME LENGTH <small>(min 20 cm total length)</small>	WIDTH OF RHIZOME SEGMENTS					DRY WEIGHT of BG MATERIAL <small>(g)</small>	GROUND? <small>(✓)</small>	CAPSULE WEIGHT <small>(g)</small>	CAPSULE + SAMPLE WEIGHT <small>(g)</small>	CAPSULE WELL PLATE LOCATION <small>(Column - Row)</small>
					(mm)	(mm)	(mm)	(mm)	(mm)					

HY	1												
HY	2												
HY	3												

BS	1												
BS	2												
BS	3												

DB	1												
DB	2												
DB	3												

RW	1												
RW	2												
RW	3												

SB	1												
SB	2												
SB	3												

Processed by date:

Entered by date:

Checked by date:

SURVEY ID: METALS - Zm

ABOVEGROUND - LEAF						
SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)
BB	1					
BB	2					
BB	3					
PP	1					
PP	2					
PP	3					
CI	1					
CI	2					
CI	3					
MP	1					
MP	2					
MP	3					
PB	1					
PB	2					
PB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: METALS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

PC	1					
PC	2					
PC	3					

TS	1					
TS	2					
TS	3					

BG	1					
BG	2					
BG	3					

FR	1					
FR	2					
FR	3					

DH	1					
DH	2					
DH	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: METALS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

HY	1					
HY	2					
HY	3					

BS	1					
BS	2					
BS	3					

DB	1					
DB	2					
DB	3					

RW	1					
RW	2					
RW	3					

SB	1					
SB	2					
SB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: METALS - Zm

BELOWGROUND - RHIZOMES + ROOTS						
SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)
BB	1					
BB	2					
BB	3					
PP	1					
PP	2					
PP	3					
CI	1					
CI	2					
CI	3					
MP	1					
MP	2					
MP	3					
PB	1					
PB	2					
PB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: METALS - Zm

BELOWGROUND - RHIZOMES + ROOTS						
SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)
PC	1					
PC	2					
PC	3					
TS	1					
TS	2					
TS	3					
BG	1					
BG	2					
BG	3					
FR	1					
FR	2					
FR	3					
DH	1					
DH	2					
DH	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: METALS - Zm

BELOWGROUND - RHIZOMES + ROOTS						
SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	VIAL + CAP + GRINDING BALL WEIGHT	VIAL + CAP + GRINDING BALLS + SAMPLE WEIGHT	WET WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)
HY	1					
HY	2					
HY	3					
BS	1					
BS	2					
BS	3					
DB	1					
DB	2					
DB	3					
RW	1					
RW	2					
RW	3					
SB	1					
SB	2					
SB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

BB	1					
BB	2					
BB	3					

PP	1					
PP	2					
PP	3					

CI	1					
CI	2					
CI	3					

MP	1					
MP	2					
MP	3					

PB	1					
PB	2					
PB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

PC	1					
PC	2					
PC	3					

TS	1					
TS	2					
TS	3					

BG	1					
BG	2					
BG	3					

FR	1					
FR	2					
FR	3					

DH	1					
DH	2					
DH	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**ABOVEGROUND - LEAF**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF AG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

HY	1					
HY	2					
HY	3					

BS	1					
BS	2					
BS	3					

DB	1					
DB	2					
DB	3					

RW	1					
RW	2					
RW	3					

SB	1					
SB	2					
SB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

BB	1					
BB	2					
BB	3					

PP	1					
PP	2					
PP	3					

CI	1					
CI	2					
CI	3					

MP	1					
MP	2					
MP	3					

PB	1					
PB	2					
PB	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

PC	1					
PC	2					
PC	3					

TS	1					
TS	2					
TS	3					

BG	1					
BG	2					
BG	3					

FR	1					
FR	2					
FR	3					

DH	1					
DH	2					
DH	3					

Processed by:

Entered by:

Checked by:

SURVEY ID: ORGANICS - Zm

**BELOWGROUND - RHIZOMES + ROOTS**

SITE ID	REPLICATE	DATE COLLECTED	DATE PROCESSED	JAR + CAP WEIGHT	JAR + CAP + SAMPLE WEIGHT	WEIGHT OF BG MATERIAL
	(1-3)	(DDMMYYYY)	(DDMMYYYY)	(g)	(g)	(g)

HY	1					
HY	2					
HY	3					

BS	1					
BS	2					
BS	3					

DB	1					
DB	2					
DB	3					

RW	1					
RW	2					
RW	3					

SB	1					
SB	2					
SB	3					

Processed by:

Entered by:

Checked by: