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Quality Assurance Project Plan

Fecal Coliform Method Comparison Study in Estuarine Waters of Washington State

October 2012

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Publication Information

Each study conducted by the Washington State Department of Ecology (Ecology) must have an approved Quality Assurance Project Plan. The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completing the study, Ecology will post the final report of the study to the Internet.

The plan for this study is available on Ecology's website at https://fortress.wa.gov/ecy/publications/SummaryPages/1203124.html.

Data for this project will be available on Ecology's Environmental Information Management (EIM) website at www.ecy.wa.gov/eim/index.htm. Search User Study ID, NMat0005.

Ecology's Activity Tracker Code for this study is 12-023.

Waterbody Numbers:

WA-14-0110	Oakland Bay
WA-01-0020	Drayton Harbor
WA-22-0020	Grays Harbor (outer)
WA-22-0030	Grays Harbor (inner)
WA-18-0010	Strait Of Juan De Fuca (east) (Dungeness Bay)

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Signatures are not available on the Internet version.
EAP: Environmental Assessment Program
EIM: Environmental Information Management database

Table of Contents

List of Figures and Tables

Page

Figures

Tables

Abstract

The Washington State Department of Ecology (Ecology) is performing a study to compare two methods used to determine the concentration of fecal coliform bacteria in a waterbody. The goal of this study is to compare the Colilert®-18 method (Idexx Laboratories, 2012) to the primary multiple tube-fermentation method (MTF) used to quantify FC levels in marine and brackish waters of Washington State.

Ecology and Washington State Department of Health (DOH) will collect approximately 120 samples from 4 shellfish growing areas throughout Washington State's coastal waters. Samples will be split, with one portion of the split analyzed by the DOH laboratory using an MTF method and the other portion analyzed by Ecology's Manchester Environmental Laboratory using the Colilert®-18 CS method.

Ecology will then conduct a statistical comparison between the two methods to determine if they are relatively comparable for quantifying fecal coliform concentrations in marine waters of Washington State.

This project plans describes the study goal, objectives, methods, and quality control procedures for the study.

Background

Overview

High concentrations of fecal coliforms (FC) and other fecal indicator bacteria (FIB) in fresh and marine waters indicate the potential presence of harmful pathogens that pose a public health risk to the people that recreate in these waterbodies. In addition, elevated pathogen levels in marine or estuarine waters can accumulate in shellfish tissue, making them unsafe to eat. Consequently, it is important to accurately and consistently monitor FIB in public waters.

A relatively new approach to identifying FIB in water samples relies on the color or fluorescence produced by the reaction between different strains of bacteria and specific enzymes; the methods that utilize this approach are often referred to as enzyme-based or chromogenic substrate (CS) methods. Several studies have demonstrated that CS methods can be comparable to traditional methods, cost-effective, and reproducible (Yakub et al., 2002; Palmer et al., 1996; Redman, 2003). The goal of this study is to compare the Colilert®-18 CS method (Idexx Laboratories, 2012) to the primary multiple tube-fermentation (MTF) method used to quantify FC levels in marine and brackish waters of Washington State.

Both Washington State's water quality standards and the National Shellfish Sanitation Program (NSSP) (FDA, 2009) set limits for bacteria in surface waters based on FC concentrations. The Washington State Department of Health (DOH) implements the NSSP standards and is responsible for evaluating all commercially harvested shellfish areas to determine their suitability for harvest. The Washington State Department of Ecology (Ecology) develops and implements the state's water quality standards.

Washington State Fecal Coliform Methods

DOH analyzes FC samples using a "direct" MTF method that utilizes an A-1 medium for culture and a most-probable-number (MPN) method of enumeration (APHA, 2012; SM9221E2). This test is referred to as "direct" because it allows the analyst to read results after only 24 hours of incubation, with no confirmation step necessary.

Ecology currently analyzes FC samples using two methods:

1. For freshwater samples, a membrane filtration (MF) method with a plate count of colony forming units (CFU) method of enumeration (APHA, 2012; SM9222D);
2. For estuarine or marine samples, an MTF-MPN test with a confirmation step (EC medium) (APHA, 2012; SM9221E1).

Ecology uses an MF-CFU method in freshwater because the method is less expensive and more precise than an MTF-MPN method (APHA, 2012). DOH uses the A-1 medium MTF-MPN method in marine water, because it is one of the few methods approved by the U.S. Food and Drug Administration for growing area classification sampling (NSSP approved lab methods;

FDA, 2009). Ecology uses an MTF-MPN method in marine and estuarine waters to provide data comparable to the DOH shellfish growing area monitoring data.

Ecology does not use the A-1 test for MTF-MPN, because the media has a short shelf life and the laboratory may need to analyze samples on short notice. For example, with triggered storm sampling the lab is able to prepare the EC medium at the beginning of a month (in which sampling is anticipated) and is then prepared to analyze storm samples on short notice. The A-1 medium has only a 7 day shelf life, requiring preparation of media on a weekly basis. Given that Ecology utilizes an MTF-MPN method infrequently, this results in a more wasteful, costly approach. DOH analyzes MTF-MPN samples routinely in large numbers, making the A-1 medium method a more efficient, cost-effective approach.

Chromogenic Substrate Methods

The Colilert®-18 method is a CS-MPN method that is approved by the U.S. Environmental Protection Agency (EPA), as well as numerous other countries and organizations, for analysis of Total Coliforms and E. coli (EPA, 2003).

With an adaptation to the incubation temperature, the method can also be used to estimate FC concentrations. The FC method has been recommended by EPA for approval for analysis of wastewater samples (Oshiro, 2010), but has not yet been formally approved as part of 40 CFR Part 136 under the Clean Water Act. Some regional EPA offices have approved the use of Colilert-18 for detection of FC in wastewater as an Alternate Test Procedure (ATP) in the interim (EPA, 2010). In addition, the Oregon Department of Ecology has switched to the Colilert-18 CS method as the primary method used to analyze ambient water samples for E. coli in state laboratories (Redman, 2003).

Project Description

The purpose of this study is to test whether the Colilert®-18 CS method can provide comparable results to the current MTF method used to quantify fecal coliform (FC) levels in marine and brackish waters of Washington State.

Ecology and DOH will collect approximately 120 samples from 4 shellfish growing areas throughout Washington State's coastal waters. Samples will be split, with one portion of the split analyzed by the DOH laboratory using an MTF method and the other portion analyzed by Ecology's Manchester Environmental Laboratory (MEL) using the Colilert®-18 CS method.

Ecology will then conduct a statistical comparison between the two methods to determine if they are comparable. If the methods are determined to be comparable, Ecology may submit an ATP request to EPA, for approval of the method in ambient marine waters. Or Ecology may use the methodology to perform non-compliance based investigations of fecal contamination in brackish and marine waters.

Organization and Schedule

Table 1 lists the people involved in this project. All are employees of the Washington State Department of Ecology. Table 2 presents the proposed schedule for this project.

Table 1. Organization of project staff and responsibilities.

Staff (all are EAP except client)	Title	Responsibilities
Nuri Mathieu Directed Studies Unit Western Operations Section Phone: 360-407-7359	Project Manager; Principal Investigator	Co-authors the QAPP. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Writes the draft and final reports.
Nancy Jensen Manchester Environmental Laboratory Phone: 360-871-8810	Lead Microbiologist	Co-authors QAPP. Performs/supervises CS analysis, including processing, calculations, and quality control procedures.
George Onwumere Directed Studies Unit Western Operations Section Phone: 360-407-6730	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Robert F. Cusimano Western Operations Section Phone: 360-407-6596	Section Manager for the Project Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Joel Bird Manchester Environmental Laboratory Phone: 360-871-8801	Director	Approves the final QAPP.
William R. Kammin Phone: 360-407-6964	Ecology Quality Assurance Officer	Reviews and approves the draft QAPP and final QAPP.

EAP: Environmental Assessment Program
EIM: Environmental Information Management database
QAPP: Quality Assurance Project Plan

Table 2. Proposed schedule for completing field and laboratory work, data entry into EIM, and reports.

Field and laboratory work	Due date	Lead staff
Field work completed	May 2013	Nuri Mathieu
Laboratory analyses completed	May 2013	
Environmental Information System (EIM) database		
EIM user study ID	NMat0005	
Product	Due date	Lead staff
EIM data loaded	July 2013	Nuri Mathieu
EIM quality assurance	August 2013	George Onwumere
EIM complete	September 2013	Nuri Mathieu
Final project memo		
Author lead / Support staff	Nuri Mathieu	
Schedule		
Draft due to supervisor	January 2014	
Draft due to client/peer reviewer	February 2014	
Draft due to external reviewer(s)	February 2014	
Final (all reviews done) due to publications coordinator	March 2014	
Final report due on web	May 2014	

Study Design

Ecology will accompany DOH on their routine shellfish area classification sampling runs to collect split samples between July 2012 and April 2013. Sample collection will be spread out over the dry season (June to October) and the wet season (November to April), with additional comparison samples collected during targeted storm events.

Ecology will collect samples from 4 different shellfish growing areas along Washington State's coastal shoreline (Table 3 and Figure 1).

Each area will be sampled once during the dry season and once during the wet season. Field staff will collect approximately 10 samples per area per sampling event for a total of 80 samples.

Approximately 20 samples will be reserved for two storm runoff sampling events. Storm sampling events will occur opportunistically, based on a minimum of 0.5" of rainfall in a 24-hour period with negligible (<0.1") rainfall in the preceding 24 hours. Storm sampling may occur at any of the over 100 Washington shellfish growing areas, based on staff availability and storm criteria being met. The goal will be to target storm events at growing areas with known contamination problems during runoff events.

Table 3. Sampling locations for the 2012-13 study.

Region Name	Growing Area Name	County	Latitude[1]	Longitude[1]
North Puget Sound	Drayton Harbor	Whatcom	48.97833	-122.76335
South Puget Sound and Hood Canal	Oakland Bay	Mason	47.22359	-123.06183
Strait of Juan de Fuca	Dungeness Bay	Clallam	48.16053	-123.15686
Pacific Coast, Grays Harbor, and Willapa Bay	Grays Harbor	Grays Harbor	47.11605	-124.20998

[1] Coordinates for approximate centroid of sampling area.

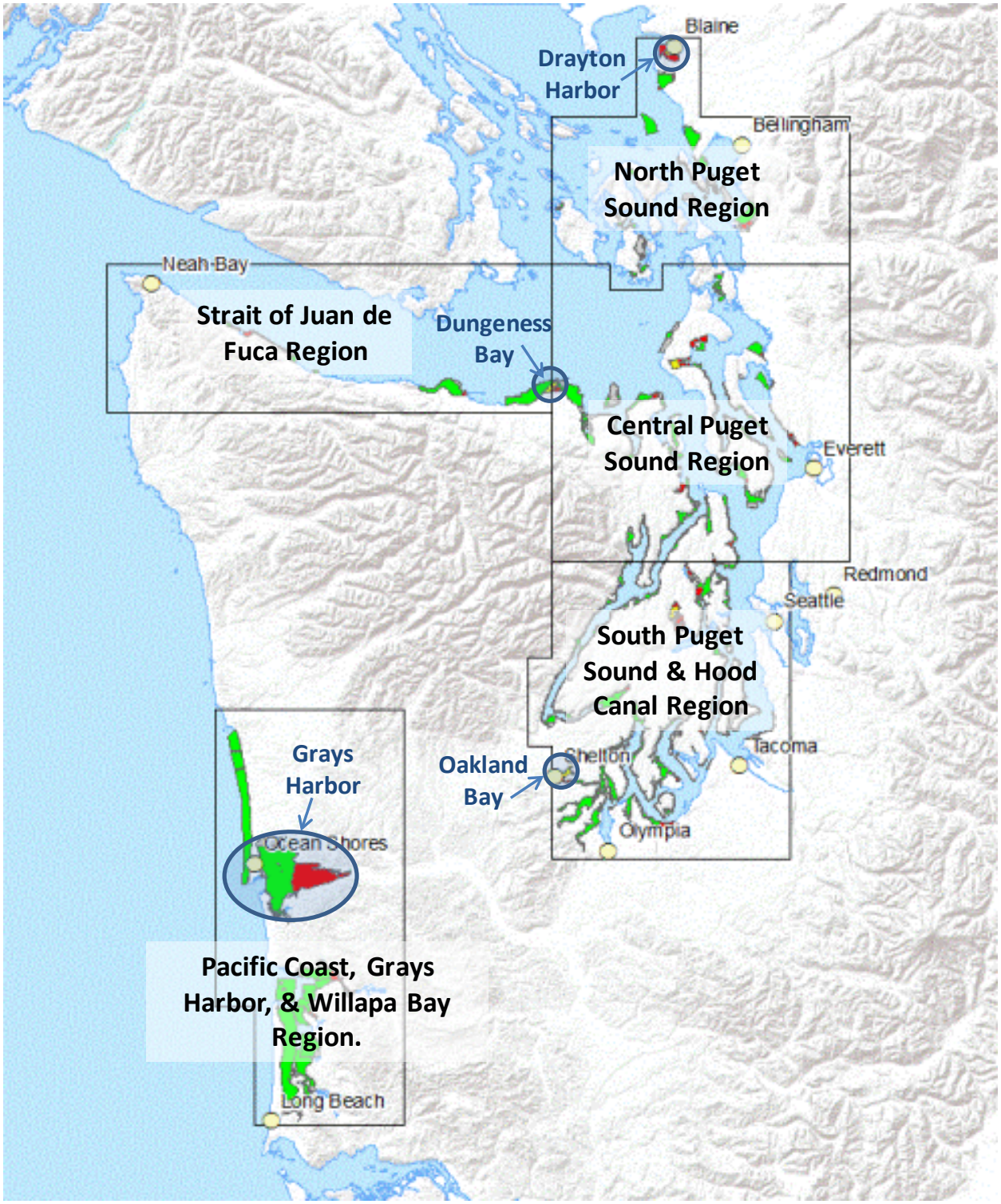

Figure 1. Shellfish growing area sampling location map for the 2012-13 study.

Sampling Procedures

Field staff will collect water samples following standard protocols for DOH, NSSP, and Ecology. Water samples will be collected with a sampling wand from the boat deck following the Environmental Assessment Program's Directed Studies Unit Standard Operating Procedures (SOPs) for bacteria (Ward and Mathieu, 2011) and grab sampling (Joy, 2006), as well as DOH Procedure #003 (DOH, 1996).

Ecology or DOH field staff will collect samples into sterile 500 mL containers provided by Ecology's Manchester Environmental Laboratory (MEL). Samples will be *immediately* split three ways as follows:

- Approximately 100 mL sample into a 125 mL bottle provided by DOH for MTF-MPN sample.
- Approximately 200 mL sample into a 250 mL bottle provided by MEL for CS-MPN.
- Approximately 200 mL sample will be retained in the original 500 mL bottle, in case additional sample is needed for quality control procedures, bacterial identification, or alternate analysis methods.

Split samples will be labeled and then immediately placed on ice, delivered to the laboratory by the end of the day, and incubated within 24 hours of collection.

Analytical Procedures

MEL will analyze CS samples for FC following the enzyme substrate test (multiple-well procedure) described by Standard Methods (SM9223B), the manufacturer's test kit (Idexx, 2012), and the manufacturer's Colilert-18 FC protocol addendum to test for FC in wastewater. The FC protocol is identical to the total coliform protocol, with the exception that the sealed trays are incubated at a temperature of 44.5°C (±0.2°C), in place of the 35°C incubation temperature.

The general procedure involves:

1. Add contents of one Colilert-18 snap pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 44.5˚C ±0.2˚C incubator for 18 hours (prewarming to 35˚C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.

5. Read results by comparing the color of each well to a comparator provided by the manufacturer. Count the number of positive wells and refer to the MPN table provided with the Quanti-Tray to obtain a Most Probable Number.

DOH will analyze samples following standard methods for MTF using an A-1 medium (SM9221E2). The general procedure involves preparing the A-1 medium, inoculating 15 tubes with 3 different sample dilutions (5 tubes for each dilution), incubating tubes for 3 hours at 35˚C ±0.5˚C, and then incubating tubes for an additional 21 hours at 44.5˚C ±0.2˚C. Any gas production within a tube of the A-1 medium is considered a positive result. The MPN value is obtained from the MPN index tables provided in Standard Methods, using the combination of positive tubes from each dilution.

Quality Control Procedures

Field

Field sampling and measurements will follow quality control protocols described in Ecology's field sampling protocols. If any of these quality control procedures are not met, the associated results will be qualified and used with caution or not used at all. Total variation from field sampling and analytical processes will be assessed by collecting and analyzing replicate samples. Sample precision will be assessed by collecting replicates for approximately 20% of samples in each survey. The difference between field variability and laboratory variability is an estimate of the sample field variability.

Laboratory

MEL routinely duplicates sample analyses in the laboratory to determine the presence of bias in analytical methods. In addition to MEL's normal QC procedures for microbiological samples, for each sampling event the analyst will:

- Analyze laboratory blanks using sterile de-ionized water.
  - If the sterile water exhibits a faint yellow color (positive result) the substrate batch will be discarded and a new batch will be used for subsequent analyses.
  - Previously analyzed samples within the same batch will be qualified as estimates.
- Perform false positive tests by analyzing a non-coliform strain of *Pseudomonas aeruginosa*.
- Perform false negative tests by analyzing an *E. coli* FC strain and a non *E.coli* FC strain of *Klebsiella pneumonia*.
- Perform coliform bacterial identification following Standard Methods (APHA 2012; SM9225) on isolates extracted from ~5-10% of collected samples. Isolates will be extracted by wiping the back of the quanti-tray with isopropyl alcohol, puncturing the well with sterile scalpel, and removing sample for isolation.

Table 4 contains the laboratory budget for the sampling and QC procedure for the study.

Table 4. Laboratory budget.

Event/Sample type	# of Samples per Area	# of Areas	Field Replicates	Field Blanks	# of samples	$/sample	Subtotal
Field Samples							
Wet Season	10	4	8	1	49	$ 30.00	$1,470.00
Dry Season	10	4	8	1	49	$ 30.00	$1,470.00
Storm Events	10	2	4	1	25	$ 30.00	$ 750.00
Lab QC							
Lab Blanks					10	$ 30.00	$ 300.00
False Positive - *Pseudomonas aeruginosa*					10	$ 30.00	$ 300.00
False negative - *E. coli*					10	$ 30.00	$ 300.00
False negative - *Klebsiella pneumonia*					10	$ 30.00	$ 300.00
Bacterial Identification					25	$ 75.00	$1,875.00
						Total =	$6,765.00
						Total Budgeted =	$7,000.00

Costs include 50% discount for Manchester Laboratory.

Quality Objectives

Field sampling procedures and laboratory analysis inherently have associated error. Measurement quality objectives (MQOs) state the allowable error for a project. Precision and bias provide measures of data quality and are used to assess agreement with MQOs.

Table 5 outlines analytical methods, expected precision of sample replicates, and method reporting limits and/or resolution. The targets for analytical precision of laboratory analyses are based on historical performance by MEL for environmental samples taken around the state by EAP (Mathieu, 2006). The reporting limits of the methods listed in the table are appropriate for the expected range of results, and the required level of sensitivity to meet project objectives. The laboratory's quality control procedures are documented in the MEL *Lab Users Manual* (MEL, 2008) and *Quality Assurance Manual* (MEL, 2010).

Table 5. Measurement quality objectives for field measurements and laboratory analyses.

Analysis	Method/ equipment	Field replicate MQO (median)	Lab duplicate MQO	Reporting limits and resolution
Field Measurements				
Water temperature	YSI-30®	+/- 0.2° C	n/a	0.01° C
Specific conductivity/ Salinity	YSI-30®	5% RSD	n/a	0.1 umhos/cm
Laboratory Analyses				
FC – MPN Enzyme-based Substrate Test	SM 9223B	50% of replicate pairs < 50% RSD 90% of replicate pairs <100% RSD[1]	40% RPD	1.8 MPN/100 mL

[1] This is the MQO based on the historically poor precision results obtained from MTF-MPN replicate samples. Precision is expected to improve significantly with the CS-MPN method based on the results of previous studies.

Precision

Precision is defined as the measure of variability in the results of replicate measurements due to random error. Random error is imparted by the variation in concentrations of samples from the environment as well as other introduced sources of variation (e.g., field and laboratory procedures). Precision for replicates will be expressed as percent relative standard deviation (%RSD) and assessed following the MQOs outlined in Table 5.

Bias

Bias is defined as the difference between the population mean and true value of the parameter being measured. Field and laboratory quality blanks provide a measure of ~~any~~ bias affecting sampling and analytical procedures. Field staff will minimize bias in field measurements and samples by strictly following measurement, sampling, and handling protocols

EAP staff will assess bias in field samples by submitting field blanks. Field staff will prepare blanks in the field by filling the bottles directly with sterile deionized water. Field staff will then handle and transport the samples to MEL in the same manner that the rest of the samples are processed.

Comparability

Comparability to the DOH shellfish growing area data will be established by strictly following routine DOH procedures for sample collection. All Ecology field staff will review the appropriate SOPs and receive project specific training for sample collection, splitting, and processing from the project manager, prior to sample collection. SOP review, project specific training, and strict adherence to Ecology and DOH protocols will ensure comparability between samples collected at different sites, during different seasons, or by different staff.

Representativeness

FC bacteria values are known to be highly variable over time and space. Sampling variability can be somewhat controlled by strictly following standard procedures and collecting quality control samples, but natural spatial and temporal variability can contribute greatly to the overall variability in the parameter value. Resources limit the number of samples that can be taken at one site spatially or over various intervals of time. Laboratory and field errors are further expanded by estimate errors in seasonal loading calculations and modeling estimates.

Ecology designed the sampling regime to provide a wide range of sampling conditions for comparison of the two methods including: (1) spatially throughout each growing area and throughout each of five shellfish growing regions in Washington State; (2) temporally during the regions dry season (June to October), wet season (November to April), and during storm runoff events.

Completeness

EPA has defined completeness as a measure of the amount of valid data needed to be obtained from a measurement system (Lombard and Kirchmer, 2004). The goal for this study is to correctly collect and analyze a minimum of 95% of the samples for all sites. Problems occasionally arise during sample collection that cannot be controlled, including inclement weather delaying sampling collection or analysis.

Data Management Procedures

Field measurement data will be entered into a field book with waterproof paper in the field and then entered into Excel® spreadsheets (Microsoft, 2007) as soon as practical after returning from the field. This database will be used for preliminary analysis and to create a table to upload data into Ecology's Environmental Information Management (EIM) System.

Sample result data received from MEL by Ecology's Laboratory Information Management System (LIMS) will be exported prior to entry into EIM and added to a cumulative spreadsheet for laboratory results. This spreadsheet will be used to informally review and analyze data during the course of the project.

An EIM user study (NMat0005) has been created for this study and all monitoring data will be available via the internet once the project data has been validated. The URL address for this geospatial database is: www.ecy.wa.gov/eim/. All data will be uploaded to EIM by the EIM data engineer once it has been reviewed for quality assurance and finalized.

All spreadsheet files, paper field notes, and Geographic Information System software products created as part of the data analysis will be kept with the project data files.

Audits and Reports

Audits on field work and data analysis may be conducted by the project manager's unit supervisor at any time during the course of the project. The project manager will be responsible for submitting a short technical report according to the project schedule. The report will describe the results of the statistical comparison and include recommendations for future application of the method in marine waters of Washington State.

Data Verification and Validation

MEL will provide verification for laboratory-generated data. Data reduction, review, and reporting will follow the procedures outlined in the MEL *QA Manual* (MEL, 2012). Lab results will be checked for missing or improbable data. Variability in lab duplicates will be quantified using the procedures outlined in the MEL *QA Manual* (MEL, 2006). Any estimated results will be qualified and their use restricted as appropriate. A standard case narrative of laboratory Quality Assurance/ Quality Control (QA/QC) results will be sent to the project manager for each set of samples.

The Excel® Workbook file containing field data will be labeled "DRAFT" until data verification and validation are completed. Data entry will be checked against the field notebook data for errors and omissions.

Field replicate sample results will be compared to quality objectives in Table X. Data requiring additional qualifiers will be reviewed and verified by the project manager.

The project manager will validate data received from LIMS by:

- Checking for omissions against the "Request for Analysis" forms.
- Checking result values against expected range of results and data from previous surveys.

After data verification is complete, all field, laboratory, and flow data will be entered into Ecology's EIM system. An independent data reviewer will validate the EIM data by checking for errors following standard EAP protocols.

Once the EIM data has been validated, the project manager will compile all project data in a data summary report. Internal (within Ecology) and external (project stakeholders) reviewers will provide validation of the report.

Data Quality (Usability) Assessment

The project manager will verify that all data quality objectives have been met for all samples collected. If the objectives have not been met (such as percent RSD for sample replicates exceeds the MQO), then the project manager will decide how to qualify the data and how it should be used in the analysis or whether it should be rejected. Documentation of the data quality assessment will be summarized in the final report and all assessment files will be archived with the project data.

The project manager will summarize data in the results section of final report and present the data analysis in the discussion section of the report. Results will be compared and summarized using standard statistical measures and presented using tables and charts. During data analysis, the project manager will evaluate the adequacy of the study design, based on the results, to draw conclusions and make recommendations.

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Appendix. Glossary, Acronyms, and Abbreviations

Glossary

Ambient: Background or away from point sources of contamination.

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Fecal coliform: That portion of the coliform group of bacteria which is present in intestinal tracts and feces of warm-blooded animals as detected by the product of acid or gas from lactose in a suitable culture medium within 24 hours at 44.5 plus or minus 0.2 degrees Celsius. Fecal coliform are "indicator" organisms that suggest the possible presence of disease-causing organisms. Concentrations are measured in colony forming units per 100 milliliters of water (cfu/100 mL).

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

Pathogen: Disease-causing microorganisms such as bacteria, protozoa, viruses.

Total Maximum Daily Load (TMDL): A distribution of a substance in a waterbody designed to protect it from not meeting (exceeding) water quality standards. A TMDL is equal to the sum of all of the following: (1) individual wasteload allocations for point sources, (2) the load allocations for nonpoint sources, (3) the contribution of natural sources, and (4) a margin of safety to allow for uncertainty in the wasteload determination. A reserve for future growth is also generally provided.

Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

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
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
NPDES	(See Glossary above)
QA	Quality assurance
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials
TOC	Total organic carbon
WAC	Washington Administrative Code
WRIA	Water Resource Inventory Area

Units of Measurement

°C	degrees centigrade
mL	milliliters