Quality Assurance Project Plan

Monitoring Fecal Coliform Bacteria in Western Washington Water Bodies

DEPARTMENT OF ECOLOGY
State of Washington

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Publication Information

Studies conducted by the Washington State Department of Ecology (Ecology) that result in new environmental data must have an approved Quality Assurance (QA) Project Plan (QAPP). The purpose of this QAPP is to describe survey work conducted by Water Quality Program (WQP) staff that is designed to measure fecal coliform bacteria (FC) concentrations in surface waters of western Washington. During 2013-2014, some surveys will be funded by the United States Environmental Protection Agency (EPA) through its National Estuary Program (NEP) via an interagency agreement with Ecology as the lead organization for *Toxics and Nutrients Prevention, Reduction and Control* grants. However, contents of the QAPP do not necessarily reflect the views and policies of the EPA, nor does mention of trade names or commercial products constitute EPA endorsement or recommendation for use.

This QAPP is available on Ecology's internet website at https://fortress.wa.gov/ecy/publications/SummaryPages/1410004.html, as well as annual addenda and reports. Data from the surveys described herein and from similar future surveys will be available on Ecology's Environmental Information Management (EIM) website: www.ecy.wa.gov/eim/index.htm.

Waterbody Numbers: Vary by Year
Federal Clean Water Act 303(d) Listings addressed by annual surveys: See Appendix C.

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Cover photo: Michael Martin sampling Swamp Creek at station 0470 in Kenmore above SR 522.

Quality Assurance Project Plan: Monitoring Fecal Coliform Bacteria in Western Washington Water Bodies

September 2013

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Abbreviations: WQP – Water Quality Program; BFO – Bellingham Field Office; NWRO – Northwest Regional Office; SWRO – Southwest Regional Office; HQ – Headquarters; EAP – Environmental Assessment Program

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Abstract

The Washington State Department of Ecology (Ecology) is required by Section 303(d) of the federal Clean Water Act and U.S. Environmental Protection Agency (EPA) regulations to develop and implement Total Maximum Daily Loads (TMDLs) for impaired waters. Ecology's Water Quality Program also surveys water quality in non-TMDL waters. These surveys include ad hoc investigations, complaint-related studies recorded in the agency's Environmental Report Tracking System (ERTS), and surveys related to pollution identification and correction (PIC) programs. The latter usually focus on measuring concentrations of fecal coliform bacteria (FC) in surface waters that indicate the presence of waste from humans or other warm-blooded animals.

The purpose of this Quality Assurance Project Plan (QAPP) is to ensure that non-TMDL surveys conducted in western Washington by staff from Ecology's Northwest and Southwest Regional Offices, as well as the Bellingham Field Office, result in credible FC data. The QAPP does this by describing a programmatic strategy and consistent methods for collecting water samples. It then details procedures for handling and analyzing those water samples.

An addendum to this QAPP will be prepared annually as surveys from prior years are completed and plans are made to survey new watersheds and water bodies. An addendum will also be prepared if new field measurements are made or if water samples are analyzed for new parameters.

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Background

Ecology Water Quality Program (WQP) staff routinely conduct water quality investigations, complaint-related studies (which are recorded in the agency's Environmental Report Tracking System (ERTS)) and pollution identification and correction (PIC) surveys. These surveys often characterize fecal coliform bacteria (FC) concentrations in multiple western Washington watersheds each year. This plan describes sampling methods and the analysis of the water samples that are collected. Three levels of sampling with different purposes are described. Not all projects will require all three levels but all three levels are described so they are consistently applied when needed.

Excess bacteria is the most common pollution problem in regional streams and affects beneficial uses such as swimming, boating, fishing, wading, and other water-related activities. Bacteria water quality standards are set to protect people who work and play in and on the water from waterborne illnesses. In Washington State water quality standards, fecal coliform bacteria are used as "indicator bacteria" for the state's freshwaters (e.g., lakes and streams). FC in water "indicates" the presence of waste from humans and other warm-blooded animals. Warm-blooded animal waste is more likely to contain pathogens that will cause illness in humans than waste from cold-blooded animals. The FC criteria are set at levels that are shown to maintain low rates of serious intestinal illness (gastroenteritis) in people.

The state's Water Quality Standard for bacteria has two criteria: a geometric mean and an upper limit value for no more than 10% of the samples. Randomly collected fecal coliform samples typically follow a lognormal statistical distribution. In Washington State FC TMDL studies, the upper limit statistic (i.e., not more than 10% of samples shall exceed) has been interpreted as a 90^{th} percentile value of the log-normalized values (Cusimano, 1997). If less than ten samples are being evaluated, no samples may exceed the upper limit.

Extraordinary Primary Contact is a use classification for waters capable of "providing extraordinary protection against waterborne disease or that serve as tributaries to extraordinary quality shellfish harvesting areas." To protect these uses: "Fecal coliform organism levels must not exceed a geometric mean value of 50 colonies/100 mL, with not more than 10% of all samples (or any single sample when less than ten samples exist) obtained for calculating the geometric mean value exceeding 100/colonies 100mL". To protect *Primary Contact Recreation* (swimming or water play), FC levels must not exceed a geometric mean of 100 colonies/100 mL, with not more than 10% of all samples (or any single sample when less than ten sample points exist) obtained for calculating the geometric mean exceeding 200 colonies/100 mL [WAC 173-201A-210(3)(b), 2010 edition].

Project Description

Water quality sampling studies conducted by Ecology must have an approved QAPP. This section of the QAPP describes the overall goal and objectives of the study. Subsequent sections provide detailed procedures that will be followed to achieve those objectives. Some sections of the QAPP, especially those related to laboratory analyses, contain technical terms, acronyms, and abbreviations that are defined in Appendix A.

Goal and objectives

The goals of these sampling surveys are to improve stream water quality and to document that improvement.

Specific objectives of the sampling surveys are to:

- Collect credible data of fecal coliform bacteria concentrations in major tributaries, point sources, and drainages throughout selected watersheds under various seasonal or hydrological conditions, including stormwater contributions.
- Narrow the geographic range of primary significant contributors of fecal coliform bacteria.
- Identify the pollution sources within the surveyed watersheds.
- Document stream water quality improvement and determine whether water bodies meet state water quality standards.

The results of the sampling surveys will help Ecology and stakeholders focus efforts on priority pollution sources within each watershed. The project's desired outcomes are:

- Collection of high quality FC data that is reliable for pollution source investigations and useful for measuring general stream quality.
- Public and stakeholder awareness on the level of fecal coliform bacteria in local waters and where corrective actions are needed.
- Management of resources to control point and nonpoint pollution.
- Attainment of Washington State water quality standards for fecal coliform bacteria.

Survey watersheds

Appendix B describes the watersheds and some of the sampling locations in western Washington where Ecology staff plan to collect FC samples in 2013-2014. The appendix also lists Section 303(d) listings that may be addressed by these sampling efforts. An addendum to this QAPP, in the form of a new Appendix B, will be prepared and approved when new watersheds and sampling locations are chosen. This will usually be done on an annual basis and be based on mapping and review of existing FC data in Ecology's Environmental Information Management (EIM) system. An addendum will also be prepared when proposing substantive changes to the QAPP, e.g., new field or laboratory methods.

Organization and Schedule

Project organization

Table 1. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Bellingham Field Office (BFO)		
Steve Hood 360-715-5211	Water Quality Engineer	Recommends watersheds and marine receiving waters in San Juan, Whatcom, and Skagit Counties, for sampling. Helps design, schedule, and conduct sampling. Approves QAPP.
Mak Kaufman 360-715-5221	Senior Water Quality Inspector	Oversees BFO Puget Sound Pollution Source Identification (PIC) program. Performs point and nonpoint source inspections.
Chris Luerkens 360-715-5220	Water Quality Inspector	Performs nonpoint source inspections.
Jessica. Kirkpatrick 360-715-5217	Water Quality Inspector	
Doug Allen 360-715-5200	Manager, BFO	Approves the QAPP and provides regional management direction.
Northwest Regional Office – Water Quality Program		
Dave Garland 425-649-7031	Water Quality Unit Supervisor	Participates in developing sampling design and occasional sample collection. Approves QAPP.
Ralph Svrjcek 425-649-7165	Water Cleanup Area Project Lead	Recommends watersheds for sampling surveys and helps design, schedule, and conduct sampling in Stillaguamish, Island, Snohomish county watersheds, and Cedar River watersheds.
VACANT 425-649-7036	Water Cleanup Area Project Leads	Recommends watersheds for sampling surveys and helps design, schedule, and conduct sampling in the Skagit, Samish, and Kitsap watersheds.
Joan Nolan 425-649-7110		Recommends watersheds for sampling surveys and helps design, schedule, and conduct sampling plans in Cedar-Sammamish and Green-Duwamish watersheds.
Tricia Shoblom 425-649-7288	Lakes Cleanup Project Lead	Recommends lake watersheds in the Northwest Region for sampling surveys. Helps design, schedule, & conduct sampling.
Kevin Fitzpatrick 425-649-7033	Water Quality Section Manager	Approves QAPP and provides management direction.
Southwest Regional Office and Ecology Headquarters – Water Quality Program		
Betsy Dickes 360-407-6296	Stream Sampling Project Leads	Recommends watersheds for regional sampling surveys. Helps design, schedule, and conduct sampling events.
Derek Rockett 360-407-6697		
Andrew Kolosseus 360-407-6368	Water Quality Unit Supervisor	Approves QAPP and provides regional management direction.
Deborah Cornett 360-507-7269	Water Quality Unit Supervisor	
Greg Zentner 360-407-6368	Water Quality Section Manager	
Southwest Regional Office and Ecology Headquarters – Water Quality Program (continued)		
Mike Herold 360-407-6596	Quality Assurance Coordinator	Approves QAPP and annual QAPP addenda, ensuring consistency with WQP QA standards and credible data.

Environmental Assessment Program		
William R. Kammin 360-407-6964	Quality Assurance Officer	Reviews and approves final QAPP.
Tom Gries 360-407-6327	NEP Quality Coordinator	Reviews draft and recommends approval of final 2013-2014 QAPP. Comments on reports describing results of 2013-2014 surveys.
Joel Bird Manchester Laboratory 360-871-8801	Director	Approves QAPP. Provides laboratory staff and resources, sample processing, analytical results, laboratory contract services, and quality assurance/quality control (QA/QC) data.

Project schedule

The schedule for sampling surveys is adaptively managed depending on the watersheds being monitored and status of PIC program efforts. Selected watersheds are typically monitored monthly for one year, but are sometimes monitored for a longer period of time. Table 2 shows the proposed schedule for 2013-2014 and future years.

Table 2. Schedule for completing field and laboratory work, data entry, and reporting.

Field and laboratory work	
Field work	Variable, e.g., collect 24 FC samples in 'selected watersheds' /month.
Field work schedule	Variable, e.g., one regularly-scheduled day / month for approximately 1 year. Typically no more than 3 'selected watersheds' /region.
Laboratory analyses completed and reported to regional office	Preliminary FC values > 200 cfu/100 mL are reported within 48 hrs. Final lab results for all samples reported within 30 days.
Environmental Information System (EIM) system	
EIM data engineer	Variable
EIM user study ID	Variable
EIM study name	Variable
Data due in EIM	Approximately 30 days after laboratory analysis is completed.
Data verification and documentation	
Lead author(s)	Steve Hood (BFO) Ralph Svrjcek (NWRO), and Derek Rockett (SWRO)
Schedule	Approximately 1 month after each sample collection.
Final reports*	
Author(s)	Appropriate Water Quality Program Cleanup Lead or Water Quality Inspector.
Schedule	
Draft due to supervisor	Determined by manager(s).
Draft due to external reviewer	Determined by manager(s), generally 3-4 weeks later.
Final report due on web*	Determined by manager(s), generally 3-4 weeks later.

* - Reports generated from regional sampling surveys may include technical reports, water quality success stories, or other special reports on pollution source correction efforts.

Data Quality Objectives

The overall objectives for data quality are to collect representative water samples of stream and/or lake water in target watersheds, and obtain valid and credible FC concentration data for those samples. Measurement quality objectives (MQOs) describe acceptable levels of error and variability in measurement processes and measured results. Indicators of data quality include precision, sensitivity, bias, representativeness, comparability, and completeness. For example, precision is a measure of random error, usually determined through the use of replicate measurements (Lombard and Kirchmer, 2004). This random error includes errors inherently associated with field sampling and laboratory analysis. Field and laboratory errors are minimized by adhering to strict protocols for sampling and analysis. Precision for replicates will be expressed either as relative percent difference (% RPD) between duplicates or relative percent standard deviation (% RSD) among more than two replicates.

Inspectors will measure latitude and longitude of each site sampled during FC surveys. Occasionally, other incidental field measurements *may* be made (e.g., flow, water temperature, conductivity, turbidity, and dissolved oxygen) but these are usually qualitative in nature, informing the inspector of general conditions in the water body. Inspectors needing these field measurements to be quantitative and recorded in Ecology's EIM database will prepare a separate QAPP, citing quality objectives/controls and protocols described in Ecology standard operating procedures (SOPs) (www.ecy.wa.gov/programs/eap/quality.html).

Precision and sensitivity

Microbiological and analytical methods, precision targets, and method reporting limits or resolution are listed in Table 3. The reporting limits of the methods listed in the table meet the expected range of results and the required level of sensitivity to meet project objectives. The laboratory's MQOs are documented in the Manchester Environmental Laboratory (MEL) Lab Users Manual (MEL, 2008). If using an accredited laboratory other than MEL collect samples for Lab Duplicates in bottles containing at least 250 mL.

Table 3. MQOs for precision and sensitivity of FC measurement systems.

Analysis	Method	Field Replicates	Lab Duplicates	Reporting Limits or Resolution
Fecal Coliform – MF (membrane filtered)	SM 9222D	50% of replicate pairs < 20% RPD 90% of replicate pairs <50% RPD [1]	40% RPD	1 cfu/100 mL

[1] replicate results with a mean of less than or equal to 20 cfu/100 mL will be evaluated separately.
SM = Standard Methods for the Examination of Water and Wastewater, 20th Edition (APHA et al., 1998).

Bias

Bias is defined as the difference between the sample population mean and the true value of the parameter being measured (Lombard and Kirchmer, 2004). Bias is also a component of data accuracy. However, bias from the true value is very difficult to determine for fecal coliform

bacteria. Calibration standards for microbiological analyses are not available. Bias in field measurements will be minimized by strictly following sampling and handling protocols.

Representative sampling

Sampling surveys are designed to have enough sampling sites and sufficient sampling frequency to meet study objectives. FC 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 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.

To reduce the risk of contamination with re-suspended sediment from upstream sampling activities, downstream samples will typically be collected first. Alternatively, sampling upstream locations using pole to hold the sample bottle can reduce this risk. In areas where the transport of New Zealand mud snails between sampling sites is possible, upstream samples may need to be taken first.

The expected range of sample concentrations in most survey watersheds is approximately 10 to over 3,500 colony forming units per 100 milliliters of sample (cfu/100 mL) as shown in Table 4.

Table 4. Expected ranges of sample results and target range of interest.

Parameter	Expected Concentration Range (cfu/100 mL) [1]	Target Concentration range (cfu/100 mL)	Lowest Concentration of Interest (cfu/100 mL)
Fecal Coliform – MF (membrane filtered)	10 – >3,500	1 – 10,000	1

[1] cfu/100 mL – fecal colony forming units per 100 milliliters of sample.

The target range of sample concentrations in the sampling surveys ranges up to 10,000 cfu/100 mL since one of the primary objectives is to identify and locate sources of bacterial pollution. Stream bacteria samples over 1,000 cfu/100 mL are typically anomalous to background levels and can be used to help track high bacteria sources.

Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared to another. This goal is achieved through use of standard techniques to collect and analyze representative samples, along with standardized data verification and reporting procedures. Ecology Northwest and Southwest Regional Office staff will sample some of the same sites currently sampled by local municipalities as well as additional sites. Data from both agencies do not need to be combined for decision-making purposes of this project, but will be compared to ensure similar FC concentrations and trends exist in both datasets for the same sampling station. If FC datasets are not similar, Ecology will further investigate for possible reasons for the discrepancy.

Freshwater FC samples taken by Ecology are usually analyzed using the membrane filtration (MF) method. Ecology typically uses the MF method for stream samples because of its practicality and precision. Some local government monitoring programs use the most probable number (MPN) method of FC analysis. Variation in results derived from MF versus MPN methods will be considered in data comparability analyses where the two different methods are used.

Completeness

EPA defines completeness as a measure of the amount of valid data needed to be obtained from a measurement system (Lombard and Kirchmer, 2004). The completeness goal for Northwest and Southwest Regional Office sampling surveys is to correctly collect and analyze 100% of the FC samples for each of the sites. However, problems occasionally arise during sample collection that cannot be controlled, which can interfere with this goal. Example problems are flooding, inadequate rain for storm sampling, site access problems, or sample container shortages. A lower limit of five samples per season per site will be required for comparison to Washington State criteria, which may be met with the current sampling design depending on sampling frequency at that site. WAC 173-201A states:

> When averaging bacteria sample data for comparison to the geometric mean criteria, it is preferable to average by season and include five or more data collection events within each period….and [the period of averaging] should have sample collection dates well distributed throughout the reporting period.

Sampling Design

Three level sample design

Long-term ambient sampling stations are used to monitor the overall health of large watersheds (e.g., on the scale of 12- digit Hydrologic Unit Codes) over multiple years. If long-term monitoring results indicate poor water quality, one or more focus watersheds may be selected for further investigation. This QAPP describes monitoring and survey work related to the focus watersheds. Long-term ambient monitoring is described in a separate QAPP (Ecology, 2003).

This QAPP defines three additional levels of sampling that may be employed. (1) Short-term ambient stations have a similar purpose as the long-term stations. They are used to characterize smaller water bodies to help identify sources. (2) If short-term stations do not provide sufficient resolution to identify sources, additional source identification samples may be taken. (3) Compliance samples may be taken to verify functionality of best management practices (BMPs) or as part of site inspections.

Short-term ambient stations

These station locations will be chosen to identify highest FC concentrations under different flow regimes. This information is then used to prioritize smaller areas for further sampling work if necessary and to inform cleanup activities.

Short-term ambient stations will be sampled and characterized frequently during both wet and dry seasons, before identifying priority areas for pollution correction actions. Sampling frequency will be influenced by budgets but should result in at least 1-2 monthly samples for at least a 1-year period. Within each month, the sampling interval should be random, but may end up being based on a fixed periodicity.

FC concentrations from these short-term sites will be used to calculate statistics for determining compliance with water quality criteria (e.g., geometric mean, 90^{th} percentile). However, if more than 10% of the short-term ambient site results represent focused sampling events, such as storms and stormwater runoff, then these results must first be excluded from calculations. This ensures targeted sampling results from extreme events are not over-represented. If the sampling plan is random and stormwater events are included, then these events must not be removed. This allows for a low bias.

Short-term (and long-term) ambient sampling stations may also be sampled by other entities. For example, the Washington State Department of Health may contract with a county to collect FC data at a short-term ambient station as part of a PIC grant. If so, the data should be collected under an approved QAPP, consistent with Ecology guidance, and result in the following:

- An estimate of the annual and seasonal geometric mean and 90^{th} percentile statistics for FC concentrations at key stations. At least 12 samples per site are needed to develop these annual statistics, including 5 samples per site during the dry season (generally June – September) and 8 samples per site during the wet season (generally October – May). It

should be noted that 4 samples per a single dry season is not sufficient to list a water body as Category 5 based on the geometric mean criterion unless two or more samples exceed the 90th percentile criterion. A single exceedance of the 90th percentile criterion can lead to a water body being placed in Category 2.

- Results that allow comparison between 1-year and 3-year geometric mean and 90th percentile FC concentrations (if samples collected from short-term ambient stations have also been used in long-term ambient monitoring).
- FC concentrations at short-term sampling sites that represent different reaches and so can be compared to identify areas of increased FC loading. Pollutant loading from sources or tributaries can be estimated if accurate stream flow data are available or can be collected.

Short-term sampling station locations for the current sampling season are listed in Appendix B. These sampling stations are selected based on historical site locations, past FC results, accessibility, safety, ease of access, and to ensure adequate areal coverage of the watershed.

Supplemental source identification stations

Short-term ambient stations will identify areas of interest. However, if short-term station results show elevated FC concentrations, it may be necessary to take supplemental samples to help identify the likely sources (e.g., malfunctioning on-site systems, livestock, wildlife, or manure spreading). If necessary, inspectors will choose supplemental source ID stations to sample after considering relevant information *such as* nearby land use; parcel ownership; other local government records; streamside structures; observed overland flows and seeps; and shoreline vegetation. Stations used for short-term stations may be sampled concurrent with the supplemental source identification samples.

To provide a more quantitative comparison between samples the Poisson ratio test will be used. This test is described in Appendix C. It provides a probability that both samples come from a population with the same average density of bacteria.

Sampling during inspections

For the purposes of this QAPP, a specific location or station where a sample is collected during an inspection, or merely where potential significant contributors of bacteria are suspected, will be termed a confirmation station. These will be identified in field notes as being representative of receiving water, representative of a discharge to receiving waters, or representative of water with a potential to discharge to receiving waters. Samples strongly suspected of having high concentrations of FC should be labeled or otherwise noted as such, and the laboratory should be notified to ensure appropriate dilutions are analyzed.

Sampling, Measurement, and Follow-up Procedures

Upon arrival at a sampling site, latitude and longitude coordinates will be obtained using a hand-held GPS unit following an Ecology SOP (Janisch, 2006) and recorded in a field log. Field sampling protocols will follow SOPs developed by Ecology's Environmental Assessment Program (EAP). Grab samples will be collected following the EAP SOPs for bacteria (Mathieu, 2006) and grab sampling (Joy, 2006). The normal container for bacteria sampling is a 250 mL pre-autoclaved glass bottle (with cork stopper), or polypropylene bottle and cap as shown in Figure 1. The sample bottle normally comes from the lab with aluminum foil wrapped over the cap or stopper to preserve sterility. If working with an accredited laboratory other than MEL they may provide other sterile sealed bottles.

Figure 1. Bacteria water sampling equipment and sample bottles. *Left: Specialized bridge sampler with bottle. Center: 250 mL polypropylene and glass sample bottles. Right: Sampling extension pole.*

Water samples will be collected directly into pre-cleaned containers supplied by the Manchester Environmental Laboratory (MEL), or other laboratory with current accreditation for SM 9222 D. Sample parameters, containers, volumes, preservation requirements, and holding times are listed in Table 6. Bacteria samples will be tagged, stored on ice, delivered to MEL via Ecology courier or regional staff, and analyzed by MEL within 24 hours of collection. Or samples will be labeled, stored on ice and delivered to an accredited laboratory for analysis by the sampler.

Table 5. Containers and holding times for fecal coliform bacteria (FC) samples.

Parameter	Sample Matrix	Container	Preservative	Holding Time
Fecal Coliform	Surface water, stormwater runoff, or other drainage affecting stream quality	120 or 250 or 500 mL glass/poly autoclaved	Cool to 4ºC	24 hours

From MEL (2008)

The following procedures are adapted from the EAP Water Quality Studies Unit SOP for Collection of Fecal Coliform Bacteria Samples in Surface water (Ward and Mathieu, 2011). The departures from the standard SOP relate to the need to use facilities other than Manchester

Environmental Laboratory. These procedures will be followed in sampling surveys for bacteria in wadable streams, ditches, culverts, and other accessible sampling locations. Ten percent of FC samples will be replicated in the field in a side-by-side manner to assess field variability.

When using a laboratory other than MEL it may be necessary to make special provisions to provide samples for laboratory duplicates. Five percent of samples should be collected in containers large enough to provide for lab duplicates.

Supplies

Typical supplies needed for bacteria water sampling include but are not limited to:

- 250 mL or 500 mL pre-autoclaved glass or polypropylene bottles, or other bottles provided by an accredited lab and sealed to ensure no contamination.
- 50 mL sterile syringe for very shallow water bodies or discharges.
- Latex or nitrile gloves (for sites where bacteria level is known or suspected to be high).
- Anti-bacterial hand sanitizer or soap.
- Cooler/s.
- Ice (Regular, or blue ice blocks).
- Tap water.
- Sample tags with work order numbers assigned by MEL, or labels suitable for other accredited laboratories.
- Lab Analysis Request (LAR) forms, or other chain of custody forms for use with accredited laboratory.
- Hip boots or waders (if applicable).
- Sampling extension pole.
- Specialized bridge sampler and line (if applicable).
- Field book.
- Camera.

Scheduling sampling runs

If using MEL

WQP staff will notify laboratory staff (e.g., sample receiving and chain-of-custody, lab analysts) two weeks ahead of sampling dates so they can coordinate schedules and prepare incubation medium. Water samples will be collected on Mondays or Tuesdays to allow time for sample processing and analysis during the work week. Sampling on Thursday through Sunday must be pre-approved by the lab. Prior to sampling, sample tags will be prepared and affixed to sample bottles. Each tag will contain the project name, sample number, samplers, date, and space for site and time. A field book or page will be used for recording similar information and additional notes on sampling conditions in the field.

If using another accredited laboratory

Ecology staff will confirm with lab staff the delivery dates and times needed in order for samples to be processed and analyzed within the allowable holding times. For example, a commercial lab may need to have samples delivered between 1 pm and 4 pm so they can be filtered and

incubations can begin within eight hours of collection. If samples must be delivered after 4 pm, staff will call the laboratory to confirm whether it will be able to filter the sample and start the incubation that work day.

If an expected range of FC concentrations can be provided for new samples before they are collected (e.g., based on past results) the range will be noted in advance on sample tags, on the LAR form, or to the lab microbiologist so dilutions that bracket the range can be prepared. Membrane Filter (MF) method of FC analysis will be used for samples and will be indicated in the proper fields on the Pre-Sampling Notification (PSN) and LAR forms. Both forms can be located in the MEL Lab User's Manual (MEL, 2008).

The lab will be notified as soon as possible if any samples have high enough turbidity (e.g., can filter <25 mL) that they should use the Most Probable Number (MPN) method to measure FC concentrations.

Grab sampling

Samples to characterize loading will be collected only from flowing water and not from pools or ditches that are stagnant. Samples to characterize potential discharges or conditions in a lake may be sampled from stagnant water. Care will be taken not to disturb bottom sediment or let the bottle touch the stream bed, particularly in slow moving or stagnant water. For slow moving streams with easily disturbed sediment, samples will be collected from the stream bank using a sampling extension pole (Figure 1). Sample containers will be filled only once and not pre-rinsed with sample water. The bottle will not be rinsed or filled from another non-sterilized container.

Remove stopper/lid from bottle just before sampling, leaving the aluminum foil over stopper/lid. Be careful not to contaminate the cork (glass bottle), cap (plastic bottle) or the inside of the bottle with fingers, coughing, dirt particles, dripping water from bridges, or other sources of contamination. The sample is collected from the stream thalweg or predominant flow avoiding back eddies and side channels. While facing upstream, hold the bottle near its base and plunge it (mouth down) below the surface, avoiding oversampling the top micro-layer where bacteria tend to concentrate. Collect sample at approximately 40 to 60 percent of the water's depth in wadable water. In lakes, collect the sample from approximately 25 cm depth. While under water, turn the bottle into the current and away from you, the shore, and the side of the sampling platform or boat. If sampling in a lake, move bottle away from you, mouth first to create a small artificial current from mouth to hand. In shallow depths, collect sample from surface if unavoidable and record in field notes.

Fill the sample bottle to the appropriate level, being careful to pull the bottle out of the water as it reaches the point where it is filled to or near the shoulder of the bottle. If the bottle is filled above this level, immediately pour out (downstream of sampler) enough of sample so that the water level is at or near the shoulder of the bottle. This will allow enough air space above the sample for proper mixing and processing for analysis at the lab. After filling the bottle to the appropriate level, securely replace the aluminum covered stopper/lid on sample bottle. Rinse any large amount of dirt or debris from the outside of the container.

Specialized sampling devices

A sampling extension pole such as the one shown in Figure 1 may be used to collect stream samples where feasible. Use of the sampling pole can reduce overall disturbance of the stream and riparian zone, help prevent the spread of New Zealand mud snails, and help ensure a representative sample is collected where wading would be dangerous. The use of a sampling pole can also speed up sample collection times and increase overall staff safety. When using a sampling pole, caution should be taken to prevent the pole from collecting water internally and spilling into the sample bottle. Similarly, if the previous sampling site is suspected to have very high bacteria levels, the end of the pole should be rinsed prior to taking a sample at the next location to avoid contamination.

If sample collection using the sampling pole is not feasible, samples may be collected using a Specialized Bridge Sampler such as shown in Figure 1. In sampling with the Specialized Bridge Sampler, the stopper/lid is removed just before lowering the sampler-with-bottle down on the rope. Hold the stopper/lid via the aluminum foil, or set it somewhere free of dirt or other sources of contamination and out of the wind so it is not disturbed. Lower the sampler so as not to contaminate the open bottle with dirt or dripping water. Lower the base on the sampler to the water surface and raise it up to clean the bottom of the sampler. Lower the sampler about 15 cm and allow sampler to orient into the current. After the sampler is oriented with the bottle upstream of the fin, continue lowering. When approaching the water surface, drop the sampler quickly through the surface to a depth of 25 cm to 50 cm to avoid oversampling the micro-layer. Keep the bottle submerged just long enough for the bottle to fill (or 1-2 inches below the top).

Pull up the sampler and bottle, careful not to contaminate the sample with dirt or water from either the rope or bridge, or other sources of contamination. Pour out sample to allow for the air space needed for proper mixing at the lab. Securely replace the aluminum covered stopper/lid. Rinse any large amount of dirt or debris from the outside of the container.

Where water bodies or discharges to surface water are very shallow, a 50 mL sterile syringe can be used to prevent the introduction of sediments into the sample. The syringe should be filled and emptied into the sample bottle four times to ensure an adequate volume of water/wastewater is sampled. It is preferable to use a new syringe at each location. If an adequate number of syringes is not available then the reused syringe should be flushed at least 3 times at each site and annotations on the use of a reused syringe should be logged in the field notes.

Aquatic invasive species protocols

Special care must be taken to prevent the spread of aquatic invasive species (AIS). Two problem species have been tentatively or definitively identified in western Washington watersheds. These include *Didymopsphenia geminate* (Didymo) and New Zealand Mud Snail (*Potamopyrgus sp.*).

Ecology currently defines problem invasive species areas into two categories: Areas of Extreme Concern and Areas of Moderate Concern. Watersheds with NZ Mud Snails are Extreme Concern Areas while those with Didymo (see brochure in Appendix D) are Moderate Concern Areas. Staff must follow Ecology's standard operating procedures (Parsons et al., 2012, excerpted in Appendix D).

New Zealand Mud Snails

New Zealand Mud Snails have been found in numerous areas of Washington State, where they can potentially cause tremendous environmental and economic impacts. These areas are now considered to be of Extreme Concern. In western Washington they include Marathon Park, Capital Lake (Olympia), and Kelsey and Thornton Creeks in the Seattle area (Figure 2).

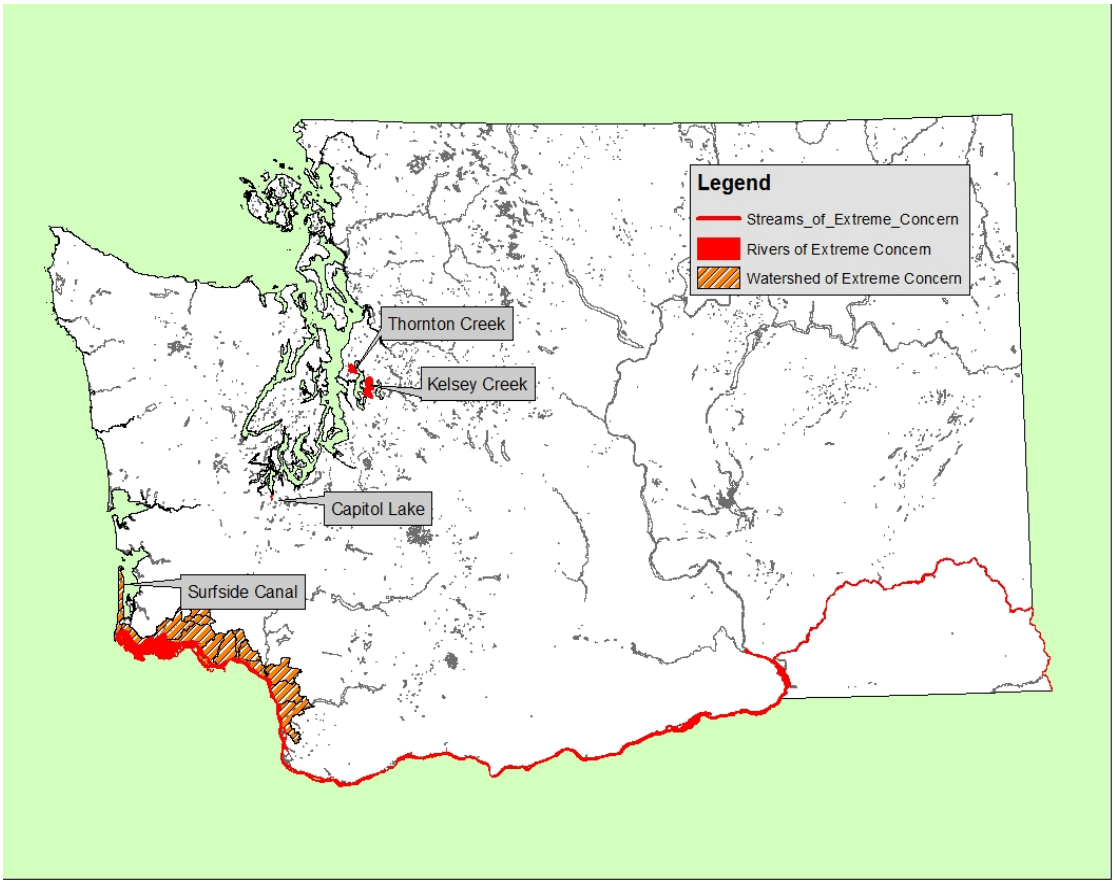

Figure 2. Aquatic Invasive Species Distribution in Washington State.

Consult Ecology's Invasive Species webpage when designing sampling studies in the Puget Sound area.

Staff designing studies in the greater Puget Sound watershed will evaluate two potential sampling sites for the likely presence of mud snails (see Ecology's Invasive Species webpage at www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html and the USGS Nonindigenous Aquatic Species webpage at http://nas2.er.usgs.gov/viewer/omap.aspx?SpeciesID=1008) and contact Jesse Shultz (Washington Department of Fish and Wildlife Invasive Aquatic Species Unit) or Jenifer Parsons (EAP Central Regional Office) with questions that arise.

Any sampling done in a watershed contributing to Capitol Lake or within the drainages to Lake Washington should be followed by decontamination procedures for Areas of Extreme Concern (Parsons et al., 2012, Appendix D).

- Sampling will be done in these watersheds using a pole, if feasible, and avoiding contact with wet streamside soils.
- Sampling will proceed from upstream to downstream.
- Between sampling sites, boots that have contacted stream water or wet streamside soils during sample collection will undergo decontamination procedures using chemicals or heat, especially when cold treatment (4hrs at -4^{0}C) or drying (48 hrs to fully dry) cannot be completed in time.
- Wearing short rubber boots will simplify decontamination, while wearing felt-soled boots will make decontamination more difficult.

Didymo

The Didymo diatom is a single-celled alga that can thrive in cold water and grow to cover stream beds in thick gelatinous mats. These mats can smother various stream organisms and reduce the availability of food to juvenile salmonids.

Ecology staff sampling in areas of the Stillaguamish River Watershed where Didymo may be present will use sample poles wherever feasible and follow the decontamination procedures for Areas of Moderate Concern (Parsons et al., 2012, procedures 6.1 through 6.1.4.5) if not wearing felt-soled boots. Staff wearing felt-soled boots will use an upstream-to-downstream sampling sequence and follow decontamination procedures for Areas of Extreme Concern. Staff will decontaminate all sampling gear using chemicals or heat prior to same-day sampling in uncontaminated watersheds, especially when cold treatment (4hrs at -4^{0}C) or drying (48 hrs to fully dry) cannot be completed in time.

Storage and Transport

If MEL will analyze samples

After collecting the sample, the string or elastic band attached to the sample tag will be looped over stopper/lid until secure. Make sure to attach sample tag beneath, not on top of, the aluminum foil cover, as the covers can be easily separated from the sample bottle during transport and handling. The date and time each station was sampled will be recorded on the sample tag and in the field notes. The filled and labeled sample bottle will be immediately placed in an iced cooler. It is important to cool to 4°C immediately and store in a dark cooler, as bacteria samples are sensitive to light.

Samples will be packed in regular cubed or crushed ice. Lab Analysis Requested (LAR) forms will be left on the ice chest for pick-up and transport to lab. LAR forms at minimum will contain the project name, station names, sample numbers, date, times, and parameters requested. Sample pick-up should be arranged with the lab in advance of the field sampling or samples may be transported to the lab by regional staff early the next day. Samples of ambient contaminated water should be analyzed within 6 to 8 hours (APHA, 1998); however, due to the logistics of sampling over the course of a day, MEL allows a holding time of 24 hours (MEL, 2008). Note: NWRO staff working out of the Bellevue location will place coolers in the Ecology locker at Tukwila Self Storage at 5050 Southcenter Boulevard, Tukwila, WA (206-246-2931).

If another accredited laboratory will analyze samples

After collecting the sample, staff will confirm the sample label contains the correct station, date time and analysis required. The date and time each station was sampled will be recorded in the field notes. The filled and labeled sample bottle will be immediately placed in an iced cooler. It is important to cool to 4°C immediately and store in dark cooler, as bacteria samples are sensitive to light.

Samples will be packed in coolers as soon as possible after collection. Temperature will be maintained below 4 degrees Celsius by ice or blue ice. Use blue ice only after samples have been cooled to storage temperature with regular ice. Chain of custody forms will be completed. Sample labels will verified with log books and chain of custody/lab analysis request forms at the end of the sampling period. If the samples will be dropped off for analysis more than six hours after collection, staff will contact the laboratory to confirm the samples must be filtered and incubation begun in less than two hours.

Records management

Field notes (and occasional photographs) will be taken concurrent with sample collection and labeling, and will be compared to lab analysis reports for accuracy. If samples are analyzed by MEL, sample results will be entered into the Laboratory Information Management System (LIMS), and following data verification, into the EIM database system. If samples are analyzed at another accredited laboratory, the laboratory must report the volume filtered and actual plate counts. Record the information with the laboratory report. After data verification has been completed, the project manager and EIM data engineer will work together to ensure all appropriate project results data, including all FC concentration data, are entered into EIM.

Safety

Gloves should be worn to avoid exposure to water contaminants. If gloves are not worn, hands and anything they touch will be assumed to be contaminated after sampling. In such cases, hands will be cleaned using anti-bacterial soap or hand sanitizer after completing work at each sampling station or, at a minimum, after completing work at sampling stations with known high bacteria counts and before ingesting food or drink. Further field health and safety measures are available in the *Environmental Assessment Program (EAP) Safety Manual* (Ecology, 2009).

Quality Control

Quality control by field staff will first confirm the correct order of sampling stations in each watershed. Where the spread of AIS is not a concern, downstream stations will generally be sampled first to avoid disturbing sediments thereby potentially impacting other sampling efforts. Staff will follow a pre-planned station order unless a sampling pole is used at upstream stations or AIS contamination is a concern. The project manager will review field logs and determine if sampling order is correct. If the sampling order indicated is not as planned, then results will be evaluated for usability and may be rejected.

Quality control for obtaining GPS coordinates for sampling locations will consist of real-time review of instrument calibration records and field logs.

Total variation for field sampling and laboratory analysis will be assessed by collecting replicate water samples. Bacteria samples tend to have a high relative standard deviation (RSD) between replicates compared to other water quality parameters. Bacteria sample precision will be assessed by collecting replicates for approximately 10% of samples in each field survey as shown in Table 7. MEL routinely duplicates sample analyses in the laboratory to determine laboratory precision. If using another accredited laboratory duplicate samples may need to be collected. The difference between field and laboratory variability is an estimate of the sample field variability.

Table 6. Quality Control for FC sampling surveys conducted in western Washington.

Parameter	Field		Laboratory	
	Blanks	Replicates	Method Blanks	Analytical Duplicates
Fecal Coliform bacteria (FC)	N/A	1/10 samples	2/batch	N/A

All water samples will be analyzed at MEL or at another accredited laboratory. MQOs and quality control procedures are documented in the quality assurance manuals or the laboratory will provide a statement that the data met laboratory MQOs or were appropriately qualified. If any of these quality control procedures are not met, the associated results may be qualified by MEL or the project manager and used with caution, or not used at all.

Standard Methods (APHA et al., 1998) recommends a maximum holding time of eight hours for microbiological samples (six hours transit and two hours laboratory processing) for non-potable water tested for compliance purposes. For environmental samples, Standard Methods recommends a holding time of no more than 24 hours. MEL has a maximum holding time for environmental microbiological samples of 24 hours (MEL, 2008). Microbiological samples analyzed beyond the 24-hour holding time are qualified with a “J” qualifier code, indicating an estimated sample result.

Data Management and Interpretation

Sampling station coordinates will be entered into Ecology's EIM database. The data engineer will map station locations to check for anomalies and the project manager will confirm the entry of correct station coordinates.

Results for all types of FC samples, whether collected from a distributor pipe, outfall or receiving water stream will be entered into Ecology's EIM. FC results will be rejected or qualified (as "J") when:

- Samples were collected where water was not flowing, or the SOP of moving the bottle to create a current could not be followed.
- Samples collected from shallow water where the surface film may be over-represented.
- Samples were improperly stored or analyzed outside of acceptable holding time.

Single samples of discharges should be compared to the 90^{th} percentile criterion to determine if a sample exceeds the water quality standards. However, to have 95% confidence that a single sample exceeds the criterion requires knowledge of the quantity of water that was filtered and the actual count. The lower limit of the one tailed 95% confidence range for the count (the value for which there is 95% confidence the value does not exceed the population value) is calculated as the inverse of the gamma cumulative distribution function with probability = 0.05, Alpha = the count and Beta = 1. This can be done in Microsoft Excel using "=GAMMAINV(probability, Alpha, Beta)". The count is then multiplied by 100 and divided by the volume filtered. Values for selected counts and volumes filtered are provided in Table 5. The results where the lower limit of the confidence range exceeds 100 cfu/100 mL are highlighted in yellow. Where the lower limit exceeds 200 cfu/100 mL the cell is highlighted in orange.

Field notebooks will be checked for missing or improbable measurements before leaving each site. Field-generated data will be entered into EXCEL® spreadsheets (Microsoft, 2007) as soon as practical after returning from the field. The EXCEL® Workbook file will be labeled "DRAFT" until data verification are completed. Data entry will be checked by the field assistant against the field notebook data for errors and omissions. Missing or unusual data will be brought to the attention of the project manager for consultation. Verified data will be moved to a separate file labeled "FINAL."

Table 7. Reported FC value with 95% confidence water quality criterion is exceeded. *Yellow and orange show FC greater than 100 cfu/100 mL and 200 cfu/100 mL, respectively.*

Plate	Reported Value Based on Volume Filtered					
	0.1 mL	1 mL	5 mL	10 mL	50 mL	100 mL
1	1000	100	20	10	2	1
2	2000	200	40	20	4	2
3	3000	300	60	30	6	3
4	4000	400	80	40	8	4
5	5000	500	100	50	10	5
6	6000	600	120	60	12	6
7	7000	700	140	70	14	7
8	8000	800	160	80	16	8
9	9000	900	180	90	18	9
10	10000	1000	200	100	20	10
11	11000	1100	220	110	22	11
12	12000	1200	240	120	24	12
13	13000	1300	260	130	26	13
14	14000	1400	280	140	28	14
15	15000	1500	300	150	30	15
16	16000	1600	320	160	32	16
17	17000	1700	340	170	34	17
18	18000	1800	360	180	36	18
19	19000	1900	380	190	38	19
20	20000	2000	400	200	40	20
21	21000	2100	420	210	42	21
22	22000	2200	440	220	44	22
23	23000	2300	460	230	46	23
24	24000	2400	480	240	48	24
25	25000	2500	500	250	50	25
26	26000	2600	520	260	52	26
27	27000	2700	540	270	54	27
28	28000	2800	560	280	56	28
29	29000	2900	580	290	58	29
30	30000	3000	600	300	60	30
31	31000	3100	620	310	62	31
32	32000	3200	640	320	64	32
33	33000	3300	660	330	66	33
34	34000	3400	680	340	68	34
35	35000	3500	700	350	70	35
40	40000	4000	800	400	80	40
50	50000	5000	1000	500	100	50
60	60000	6000	1200	600	120	60

Plate	Reported Value Based on Volume Filtered					
	0.1 mL	1 mL	5 mL	10 mL	50 mL	100 mL
70	70000	7000	1400	700	140	70
80	80000	8000	1600	800	160	80
90	90000	9000	1800	900	180	90
100	100000	10000	2000	1000	200	100
110	110000	11000	2200	1100	220	110
120	120000	12000	2400	1200	240	120
130	130000	13000	2600	1300	260	130
140	140000	14000	2800	1400	280	140
150	150000	15000	3000	1500	300	150
160	160000	16000	3200	1600	320	160
170	170000	17000	3400	1700	340	170
180	180000	18000	3600	1800	360	180
190	190000	19000	3800	1900	380	190
200	200000	20000	4000	2000	400	200
210	210000	21000	4200	2100	420	210
220	220000	22000	4400	2200	440	220
230	230000	23000	4600	2300	460	230

If using MEL

Laboratory-generated data reduction, review, and reporting will follow the procedures outlined in the MEL Users Manual (MEL, 2008). Lab results will be checked for missing and/or improbable data. Variability in lab duplicates will be quantified using procedures outlined in the manual. Any estimated results will be qualified and their use restricted as appropriate. A standard case narrative of laboratory QA/QC results will be sent to the project manager for each set of samples.

As soon as FC data are verified by MEL, the laboratory microbiologist will notify the project manager of FC results greater than 200 cfu/100 mL. The project manager will notify Ecology's NWRO and SWRO Client Staff Contacts and Water Quality Section Manager by e-mail of these elevated counts in accordance with EAP Policy 1-03. The Client Staff Contacts will notify local authorities or permit managers as appropriate.

Data received from MEL by Ecology's Laboratory Information Management System (LIMS) will be checked for omissions against the LAR forms by the field lead. Data can be in EXCEL® spreadsheets (Microsoft, 2007) or downloaded tables from Ecology's Environmental Information Management (EIM) database system. These tables and spreadsheets will be located in a file labeled "DRAFT" until data verification is completed. Field replicate sample results will be compared to quality objectives in Table 3. Data requiring additional qualifiers will be reviewed by the project manager. After data verification and data entry tasks are completed, all field and laboratory data will be copied into a file labeled "FINAL," and then into the EIM system.

If using another accredited laboratory

Confirm that the laboratory report includes QA/QC verification. Enter data from laboratory report into Excel spreadsheet or other file system in use. Label data as DRAFT. For each sampler keep a record of field duplicate results.

Project data in EIM will be independently reviewed by another Water Quality employee for errors at an initial 10% frequency. If any entry errors are discovered, a more intensive review will be undertaken. At the end of the field collection phase of the sampling surveys, the data will be compiled in a data summary or organized on a website. Quarterly progress reports for each of the project areas will be available every 3-4 months throughout the 12-month data collection period.

EIM user study identification numbers will be created for the current sampling survey areas, and all monitoring data will be available via the internet once the project data have been verified. The URL address for this geospatial database is: www.ecy.wa.gov/eim/index.htm. All data will be uploaded to EIM by the EIM data engineer after the data have been reviewed for quality assurance and finalized.

Data Verification

Data verification requires adequate documentation of the data creation and recording process. Data verification involves examining the data for errors, omissions, and compliance with quality control (QC) acceptance criteria. Field log records will be verified by staff before leaving the sampling site. MEL staff or other accredited laboratory staff are responsible for performing laboratory data verification. The project manager will conduct a detailed examination of the data package using professional judgment to determine whether the MQOs for precision, bias, and sensitivity have been met. Field duplicates will be pooled for analysis of precision. The project manager will examine the complete data package following data verification to determine compliance with procedures outlined in this QAPP. Project data will not be reviewed or validated by independent parties.

Data Quality (Usability) Assessment

The field lead or project manager will verify that all measurement and data quality objectives have been met for each monitoring station. The field lead or project manager will make this determination by examining the data and all of the associated QC information. If the MQOs for the data have been met, the quality of the data should be useful for meeting project objectives. If the objectives have not been met (e.g., the percent RPD for sample replicates exceeds the MQO), the project manager will decide how to qualify the data and whether or not it can be used in the technical analysis. For the purposes of the sampling surveys, data that are qualified may still be useable for project objectives.

The project manager will determine if the quality of the data is sufficient to meet project objectives. Although not the focus of this QAPP, the data from the sampling surveys characterizes changes in bacteria water quality over time with enough sensitivity so that sources

of bacteria pollution can be implicated or conclusively identified. The field investigator or project manager will produce a station quality assurance report that will include site descriptions and data quality assurance notes to document this assessment.

Data Analysis

Data can be evaluated using one or more methods. Synoptic sample data for core mainstem stations may be plotted longitudinally by river mile (or smaller scale) to identify problem reaches and tributaries. This method allows problem areas to be considered for additional sampling focus during the next sampling trip to locate pollution sources or source areas. As sufficient sample numbers are achieved at each site, geometric means and 90^{th} percentile values will be calculated for FC data for comparison with water quality standards and with other sampling stations in each watershed. Trend analyses and graphical presentations of the data (box plots, time series, and regressions) may be made using appropriate software as needed.

Ideally, at least 20 FC concentrations that represent a broad range of hydrologic conditions are needed to characterize annual conditions, but fewer data may be adequate for establishing a relative sense of problem stream reaches. While fewer data provide less confidence in FC statistics, individual sample results and small amounts of data may still be useful for the source tracking and identification purposes of this project.

In the event that a value of zero is reported by MEL, staff will use a value of 1 cfu/100 mL in order to facilitate the calculation of a geometric mean and approximate the true lab value. Values of zero cannot be used to calculate a geometric mean.

To determine if two samples are significantly different, they will be compared using the conditional test suggested by Przyborowski and Wilenski (1940) and implemented in R (R Development Core Team, 2012) as the function “poisson.test” or by an EXCEL spreadsheet that performs the same calculations. The analysis method is described in Appendix C with an example calculation.

Laboratory Budget

Annual laboratory budgets for the Bellingham Field Office, Northwest Regional Office, and Southwest Regional Office are provided in Appendices B1, B2 and B3.

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Appendices

Appendix A. Glossary, Acronyms and Abbreviations, Units of Measure

Glossary

90th percentile: A statistical number obtained from a distribution of a data set, above which 10% of the data exists and below which 90% of the data exists.

90th percentile (estimated): A statistical number estimated from the data assuming the data follows a lognormal distribution. It is estimated by log transforming (taking the logarithms) the data. The mean and 1.96 times the standard deviation of the transformed data is added. The antilog of the sum is the estimated 90th percentile.

303(d) list: Section 303(d) of the federal Clean Water Act requires Washington State to periodically prepare a list of all surface waters in the state for which beneficial uses of the water – such as for drinking, recreation, aquatic habitat, and industrial use – are impaired by pollutants.

Ambient: Background or away from point sources of contamination. Characteristic of general conditions.

Aquatic invasive species: Any freshwater or marine species that is not native to an ecosystem and whose introduction does or is likely to cause economic, human health, or environmental harm.

Area of Extreme Concern: Areas of the state documented as having established Aquatic Invasive Species (AIS) that are considered to be a particular environmental or economic threat and hard to remove from sampling equipment, such as areas with New Zealand mudsnail (NZMS) populations. Most equipment and sampling gear used in these areas must undergo rigorous inspection and decontamination procedures to prevent accidental introductions to other waters. (Maps of these areas are available at www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html).

Areas of Moderate Concern: Areas of the state not documented as having established NZMS or other species of extreme concern. These areas may have other invasive species, including plants, animals, fish, invertebrates, and fish pathogens that should not be spread.

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

Confirmation stations: Stations where samples are taken during an initial inspection or a follow-up inspection.

Extraordinary primary contact: Waters providing extraordinary protection against waterborne disease or that serve as tributaries to extraordinary quality shellfish harvesting areas.

Fecal coliform (FC): 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 bacteria 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).

Geometric mean: A mathematical expression of the central tendency (an average) of multiple sample values. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) were calculated. This is helpful when analyzing bacteria concentrations, because levels may vary anywhere from 10 to 10,000 fold over a given period. The calculation is performed by either: (1) taking the nth root of a product of n factors, or (2) taking the antilogarithm of the arithmetic mean of the logarithms of the individual values.

Load allocation: The portion of a receiving waters' loading capacity attributed to one or more of its existing or future sources of nonpoint pollution or to natural background sources.

Loading capacity: The greatest amount of a substance that a waterbody can receive and still meet water quality standards.

National Pollutant Discharge Elimination System (NPDES): National program for issuing, modifying, revoking and reissuing, terminating, monitoring, and enforcing permits, and imposing and enforcing pretreatment requirements under the Clean Water Act. The NPDES program regulates discharges from wastewater treatment plants, large factories, and other facilities that use, process, and discharge water back into lakes, streams, rivers, bays, and oceans.

Nonpoint source: Pollution that enters waters of the state from any dispersed land-based or water-based activities, including but not limited to, atmospheric deposition; runoff from agricultural lands, urban areas, or forest lands; subsurface or underground sources; or discharges from boats or marine vessels not otherwise regulated under the NPDES program. Generally, any unconfined and diffuse source of contamination.

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

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

Point source: Sources of pollution that discharge at a specific location from pipes, outfalls, and conveyance channels to a surface water. Examples of point source discharges include municipal wastewater treatment plants, municipal stormwater systems, industrial waste treatment facilities, and construction sites that clear more than 5 acres of land.

Pollution: Such contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes changes in temperature, taste, color, turbidity, or odor of the waters, and discharge of any liquid, gaseous, solid, radioactive, or other substance into waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare; (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses; or (3) livestock, wild animals, birds, fish, or other aquatic life.

Primary contact recreation: Activities where persons have contact with water to the point of complete submergence including, but not limited to, skin diving, swimming, and water skiing.

Riparian: Relating to the banks along a natural course of water.

Short-term ambient stations: These stations are selected to characterize different reaches within a watershed. They are sampled monthly or twice monthly for the duration of the study.

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Supplemental Source Identification Samples: Samples taken to help narrow location of potential sources.

Surface waters of the state: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and watercourses within the jurisdiction of Washington State.

Synoptic survey: Data collected simultaneously or over a short period of time.

Thalweg: Deepest flowing longitudinal section of a stream.

Total Maximum Daily Load (TMDL): The allocation of a substance in a water body designed to protect it from exceeding water quality standards. A TMDL is equal to the sum of all of the following: (1) individual waste load 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 waste load determination.

Waste load allocation: The portion of a receiving water's loading capacity allocated to existing or future point sources of pollution. Waste load allocations constitute one type of water quality-based effluent limitation.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

Acronyms and abbreviations

BFO	Bellingham Field Office
BMP	Best management practices
DOH	Washington State Department of Health
EAP	Environmental Assessment Program (Ecology)
Ecology	Washington State Department of Ecology
e.g.	For example
EIM	Environmental Information Management database (Ecology)
EPA	U.S. Environmental Protection Agency
et al.	And others
FC	Fecal coliform bacteria
GPS	Geographic Positioning System
i.e.	In other words
MEL	Manchester Environmental Laboratory
MF	Membrane filtration bacteria analysis
MPN	Most probable number bacteria analysis
MQO	Measurement quality objective
NPDES	National Pollutant Discharge Elimination System (see Glossary above)
NWRO	Northwest Regional Office (Ecology)
QA	Quality assurance
QAPP	Quality assurance project plan
QC	Quality control
RM	River mile
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedure
SWRO	Southwest Regional Office
TMDL	Total Maximum Daily Load (see Glossary above)
USGS	U.S. Geological Survey
WAC	Washington Administrative Code
WRIA	Water Resource Inventory Area
WWTP	Wastewater Treatment Plant

Units of Measurement

cfs	cubic feet per second
cfu	colony forming units of bacteria
mg	milligram
mL	milliliters
mg/L	milligrams per liter (parts per million)

Appendix B. Sampling locations by office

The watersheds and some of the target locations in western Washington where Ecology staff will collect FC samples in 2013-2014 are described in Appendix B. Staff will prepare a new Appendix B each year that presents new sampling locations. The current program anticipates samples from:

- Drayton Harbor watershed; Portage Bay and Nooksack River watershed, including Bertrand Creek (Whatcom County).
- Samish River watershed (Skagit County).
- Swamp, Juanita, and Newaukum Creek watersheds (King and Snohomish Counties).
- Various water bodies located in counties of the southwest region.

During the 2013-2014 field season, Ecology's Bellingham Field Office (BFO) staff will survey the Bertrand Creek watershed, which discharges to the Nooksack River, collecting water quality samples from approximately 10 short-term ambient stations and additional samples from stations. BFO staff will also collect FC samples from the Drayton Harbor (Whatcom County) and Samish River watersheds (Skagit County), either in the upcoming year or near future. Northwest Regional Office (NWRO) staff will collect water samples from sites in Swamp, Juanita, and Newaukum Creeks (King and Snohomish Counties). Southwest Regional Office (SWRO) staff will collect FC samples from various water bodies each year. Water bodies, site locations, and number of samples are usually chosen in response to inquiries and complaints.

Appendix B1. Bellingham Field Office Watersheds and sites chosen for FC sampling in 2013-2014

303(d) listings addressed

Table B1-1 shows some sites within the geographic purview of Bellingham Field Office water quality inspectors that have been listed as Category 4A and Category 5 in Washington State's 2008 Water Quality Assessment, at least in part because of FC concentrations in water. These will be addressed in 2013-2014 by the surveys described in this QAPP.

Table B1-1. Some 303(d) listed water bodies in Whatcom County.

Category 5 Bertrand Creek 303(d) Listings – WRIA 1

Site Basis	Township	Range	Section	Parameter	Listing ID#
NWIC-BJB	40N	02E	12	Dissolved Oxygen	7060
B8E	40N	03E	07	Ammonia-N	8629
NWIC-B1	40N	02E	27	Dissolved Oxygen	15428
NWIC-BJ	41N	02E	36	Dissolved Oxygen	47672
NWIC-BH	41N	02E	35	Dissolved Oxygen	47682
NWIC-B2	40N	02E	27	Dissolved Oxygen	47684

Category 4A Bertrand Creek 303(d) Listings – WRIA 1

Site Basis	Township	Range	Section	Parameter	Listing ID#
B1, B3	40N	02E	27	Fecal Coliform	9720
B1	40N	02E	26	Fecal Coliform	39039
NWIC-BH	41N	02E	35	Fecal Coliform	42447
BJB	40N	02E	12	Fecal Coliform	42448
NWIC-B2	40N	02E	27	Fecal Coliform	42497
NWIC-BJ	41N	02E	36	Fecal Coliform	42498
BERTHUSENST1	40N	02E	22	Fecal Coliform	45774
NWIC-BJB	40N	02E	12	Fecal Coliform	46001

Category 5 Duffner Ditch 303(d) Listings – WRIA 1

Site Basis	Township	Range	Section	Parameter	Listing ID#
NWIC-DF3	40N	02E	24	Dissolved Oxygen	47670
NWIC-DF1	40N	02E	26	Dissolved Oxygen	47715
NWIC-DF2	40N	02E	25	Dissolved Oxygen	47716

Category 4A Duffner Ditch Creek 303(d) Listings – WRIA 1

Site Basis	Township	Range	Section	Parameter	Listing ID#
NWIC- DF3	40N	02E	24	Fecal Coliform	6635
B8E	40N	02E	13	Fecal Coliform	6636
NWIC-DF2	40N	02E	25	Fecal Coliform	39086
NWIC-DF1	40N	02E	26	Fecal Coliform	39088

Proposed Short-term Ambient Stations

Table B1-2 and Figure B1-1show target coordinates for short-term ambient station samplings proposed for the 2013-2014 field season.

Table B1-2. Proposed BFO survey sampling sites in Bertrand Creek watershed. (Datum: HARN 1983)

	Site ID	Description	Latitude	Longitude	RM
1	BEJK2.0	Jackman Ditch at border	49.002	-122.501	**2.0**
2	BEDF6.4	Duffner Ditch at Prairie RD	48.98625	-122.485	**6.4**
3	BEDF3.7	Duffner Ditch at Guide Meridian and Main	48.94678	-122.485	**3.7**
4	BEJK0.2	Lower Jackman Ditch at Jackman RD	48.97503	-122.502	**0.2**
5	BE4.3	Bertrand Mainstem at Loomis Trail	48.95021	-122.52	**4.3**
6	BENF2.0	Bertrand North Fork at Loomis Trail	48.95026	-122.542	**2.0**
7	BEMC1.8	McClellan Creek at mailbox of 8895 Wiedkamp RD	48.96434	-122.53	**1.8**
8	BEDF2.2	Duffner Lynden Dentention Pond on Flynn	48.92793	-122.497	**2.2**
9	BE9.1	Bertrand Mainstem at the border (Zero Ave.)	49.00231	-122.523	**9.1**
10	BECC0.2	Cave Creek at the border (Zero Ave.)	49.00222	-122.527	**0.2**

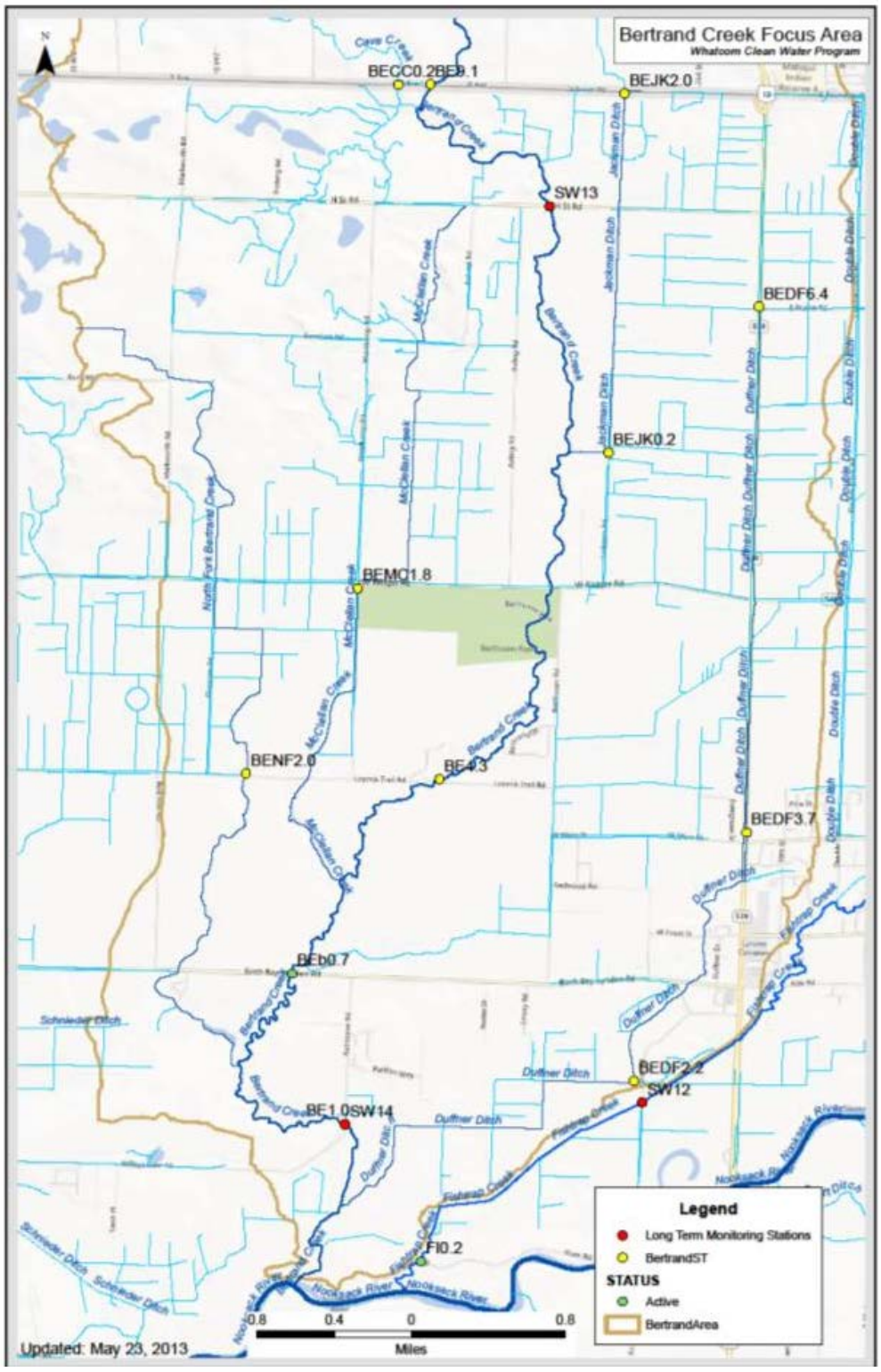

Figure B1-1. FC sampling stations in the Bertrand Creek watershed, 2013-2014.
Short-term stations are shown in yellow.

Budget

Table B1-3 shows the estimated total number of FC samples that will be collected at short-term ambient stations and the approximate laboratory budget associated with their analysis.

Table B1-3. Proposed number of short-term ambient monitoring samples submitted for FC analysis and monthly analytical costs.

Month	FC (MF) Samples	Field Duplicates	Lab Replicates	Cost[1] ($)
January	10	1	1	300
February	10	1	0	300
March	10	1	1	300
April	10	1	0	300
May	10	1	1	300
June	10	1	0	300
July	10	1	1	300
August	10	1	0	300
September	10	1	1	300
October	10	1	0	300
November	10	1	1	300
December	10	1	0	300
Totals	120	12	6	3600

FC = fecal coliform bacteria

MF = membrane filtered
Field Replicates = 10% of the preceding column
Lab Replicates = 5% of field sample number
Cost[1] = estimated cost assumes $25 / sample covered by NEP funds

The budget shown in Table B1-4 is for analysis of FC in samples, plus replicates. It is shown evenly spread out over the year but is likely to be more intense during the wet season. The budget is based on a total of 10 samples per inspector per month, with one field duplicate and one lab replicate per ten samples. Approximately two-thirds of the samples will be collected in the Bertrand watershed, with most of the remaining samples collected from in the Samish Watershed. Some samples may be collected in response to complaints outside of the Samish and Bertrand Watersheds.

Costs associated with collection and analysis of all 2013-2014 FC samples will be covered by NEP funds.

Table B1-4. Estimated number of monthly samples and lab replicates collected by BFO staff for analysis of FC by MF and approximate analytical costs in 2013-14.

Month	FC by MF Samples	Field Duplicates	Lab Replicates	Cost [1] ($)
January	30	3	2	875
February	30	3	1	850
March	30	3	2	875
April	30	3	1	850
May	30	3	2	875
June	30	3	1	850
July	30	3	2	875
August	30	3	1	850
September	30	3	2	875
October	30	3	1	850
November	30	3	2	875
December	30	3	1	850
Totals	360	36	18	10350

FC = fecal coliform bacteria
MF = membrane filtered
Field Reps. = replicates for 10% of the preceding column
Lab Replicates = 5% of field sample number
Cost[1] = estimated cost assumes $25 / sample covered by NEP funds

Appendix B2. Northwest Region Watersheds and Sites Chosen for FC Sampling in 2013-2014

303(d) listings addressed

Table B2-1 (and Figure B1-1) shows some sites within the geographic purview of Northwest Regional Office water quality inspectors that have been listed as Category 5 in Washington State's 2008 Water Quality Assessment, at least in part because of FC concentrations in water. These will be addressed in 2013-2014 by the surveys described in this QAPP.

Table B2-1. Some 303(d) listed water bodies in the Northwest Region.

Swamp Creek 303(d) Listings – WRIA 8

Site	Township	Range	Section	Old ID #	New Listing ID#
SCLU	28N	04E	35	GJ57UL	7464
SCMD	28N	04E	26	--	45282
SCLD	27N	04E	35	GJ57UL	21989
0470	26N	04E	12	GJ57UL	13130

Juanita Creek 303(d) Listings – WRIA 8

Site	Township	Range	Section	Old ID #	New Listing ID#
C446	26N	05E	20	WA69TP	13143
	26N	05E	29	--	46934
0446	26N	05E	30	WA69TP	13127

Newaukum Creek 303(d) Listings – WRIA 9

Site	Township	Range	Section	Old ID #	New Listing ID#
C322	20N	06E	10	JX80LS	13166
H322	20N	06E	12	JX80LS	13971
J322	20N	07E	7	JX80LS	13972
L322	20N	07E	7	LT44JU	13981
322	21N	06E	28	KE55XH	13157
D322	21N	06E	33	JX80LS	13165

Figure B1-1. Ditch tributary to Newaukum Creek northeast of Enumclaw.

Proposed Short-term ambient stations

Table B2-2 shows approximate river mile and/or target coordinates for short-term ambient station samplings proposed for the 2013-2014 field season.

Table B2-2. Northwest Region sampling survey sites in Swamp Creek watershed.

	Site ID	Description	latitude	longitude	RM
1	SCLU	Swamp Creek @ 145th Street SW	47° 30' 10.969"	-122° 20' 43.406"	9.6
2	SWASH	Swamp Creek @ Ash Way (bridge const on 7-14-09)			8.1
3	SWAM	Swamp Creek @ Alder & Maple nr Alderwood Mall			7.3
4	SW195	Swamp Creek @ 195th Place SW			6.0
5	SWLAR	Swamp Creek @ Larch Way bridge #459			5.0
6	SWSCR	Swamp Creek just abv Scriber Cr mouth			4.4
7	SCRLD	Scriber Creek @ mouth, near Bridge #366			
8	LWCK1	Little Swamp Creek @ 192nd St			
9	SCLW	Swamp Creek @ Locust Way bridge # 502 (nr 219 Place SW)			4.3
10	SCLO	Swamp Creek @ Locust Way bridge # 504			3.3
11	SCLD	Swamp Creek @ Lockwood Road, Snohomish-King Co line - bridge # 505			2.3
12	SMKN1	Swamp Creek @ Lockwood Road			1.5
13	470	(or station SWLDK) Swamp Cr @ SR 522			0.4

Budget

The estimated total number of samples that will be analyzed for FC concentrations is shown in Table B2-3. The number is based on monthly sampling at 13 short-term ambient stations (Table B2-2) plus other samples. Table B2-3 also shows the estimated costs. Since all months have more than one survey that occur on different weeks, the monthly and weekly sample loads should not overload staff at MEL. The total sample number submitted for each sampling day will be kept at or below 24.

The greatest uncertainty in the laboratory work load and cost estimates is the number of 'project areas' where regional sampling is being conducted each month. The number of project areas and sampling surveys is expected to vary according to regional and MEL resources available to support the surveys.

Table B2-3. Estimated number of monthly sample submittals and field duplicates for FC by MF analysis and monthly analytical costs.

Month	FC (MF)	Field Duplicates	Cost [1] ($)
January	72	14	2,150
February	72	14	2,150
March	72	14	2,150
April	72	14	2,150
May	72	14	2,150
June	72	14	2,150
July	72	14	2,150
August	72	14	2,150
September	72	14	2,150
October	72	14	2,150
November	72	14	2,150
December	72	14	2,150
Totals	864	168	25,800

FC = fecal coliform bacteria
MF = membrane filtered
Field duplicates = 20% of the preceding column
Cost[1] = estimated cost assumes $25 / sample covered by NEP funds

Appendix B3. Southwest Region Watersheds and Sites Chosen for FC Sampling in 2013-2014

Ecology's Southwest Regional Office usually does not identify or prioritize specific watersheds, water bodies, or sampling sites for FC surveys. Except perhaps for targeted TMDL studies, most FC sampling occurs as a result of complaints. For this reason, it is not possible to provide Section 303(d) listings and proposed sampling sites intended to address them.

303(d) listings addressed

NA

Proposed Short-term Ambient Stations

NA

Budget

Cost for analysis of fecal coliform bacteria in water is $25 / sample.
The total budget is unknown because total number of field and QC samples is unknown.

Appendix C. The Poisson ratio test

There are large variations in fecal samples drawn from a single population. When conducting surveys we have typically had to look at two samples and use professional judgment to determine if we are confident that two samples with different results are likely from the same distribution of bacteria or really represent different conditions.

Standard Methods provides a table with 95% confidence intervals for the counts based on the assumption that the bacteria are from a population with a Poisson distribution. A Poisson distribution has a single parameter λ which is equal to the mean and the variance; it is often expressed as events per unit time. With fecal coliform λ would be counts per 100 mL. The probability of two samples having the same value for λ can be calculated by conditioning the first count on the total count (Przyborowski and Wilenski, 1940). We do this by testing the assumption that, the ratio between the value of λ of the population of the first sample, divided by the value of λ of the population of the second sample equals 1.

To conduct the Poisson ratio test, obtain the actual counts and the volume filtered from MEL or the contract laboratory. For instance, if 50 mL was filtered and 20 colonies were counted, the reported result would be 40 cfu/100 mL. If two plates were evaluated, one with 50 mL filtered and one with 5 mL filtered, and the respective counts were 19 and 1, the results would be reported as 36 cfu/100 mL (19+1/(50+5))*100.

In the free statistical software program R, there is a function in the stats library called "poisson.test" that can be used to test the probability that the ratio of the two samples come from populations with a ratio of λ. The counts of the two samples being considered are entered as a vector of x. The volumes which were filtered are entered as a vector T with a default value of 1. There is a default estimate of 1 for the ratio of λ. The test has alternatives "two.sided" (null hypothesis $\lambda_1 / \lambda_2 = 1$), "less" (null hypothesis $\lambda_1 / \lambda_2 \leq 1$) or "more" (null hypothesis $\lambda_1 / \lambda_2 \geq 1$). The default confidence level for estimated confidence intervals of the ratio is 95%. The usage of the function is defined in the program as:

```
poisson.test(x, T = 1, r = 1,alternative = c("two.sided",
"less", "greater"),
conf.level = 0.95)
```

To compare two samples, one that had 100 mL filtered resulting in a count of 11, and one that had two plates with 5 mL and 50 mL filtered with a count of 0 on the 5 mL plate and a count of 11 on the 50 mL plate, we would enter:

```
poisson.test(x = c(11,11), T = c(1,0.55))
```

And receive the following output:

```
        Comparison of Poisson rates

data:  c(11, 11) time base: c(1, 0.55)
count1 = 11, expected count1 = 14.194, p-value = 0.1818
alternative hypothesis: true rate ratio is not equal to 1
95 percent confidence interval:
 0.2162414 1.3988997
sample estimates:
rate ratio
      0.55
```

This tells us that we cannot reject the null hypothesis that the samples have the same λ at any alpha below 18%. It provides some additional information. The value "expected count1 = 14.194" tells us that if we estimate the λ from all of the samples we should have had a count of 14.194, which is the estimate of λ multiplied by the samples size. We also have an estimate of the 95% confidence interval of the ratio of λ_1 / λ_2. The confidence interval is calculated to be a minimum that is certain to contain 95% of the values, that is, it is slightly oversized.

The calculation of the p-value is done by another function" binom.test". This test performs an exact test of a simple null hypothesis about the probability of success in a Bernoulli experiment. That is, it tests the true probability of success is equal to the proposed probability of success given the number of successes in a number of trials. The number of successes is the first sample count, the number of trials is the total count, and the probability is the proportion of the total filtered water associated with the first sample. So essentially we ask, what is the chance we would get the number of counts we have in the first sample if the probability of a count in the smallest aliquot possible is the same throughout. The Poisson test then converts the confidence interval of the binomial test into a confidence interval on λ_1 / λ_2, the ratio of the two Poisson parameters.

A spreadsheet prepared by Ecology staff, Poisson Comparisons.xls, calculates the p-value. It requires as inputs the counts and volumes filtered for samples 1 and 2. The user then has to check at the bottom of the sheet that the correct binomial counts from the required tail of the binomial distribution are entered. It is currently set up to handle 119 values from the expected count to the total count, or from 0 to the expected count. It will require some modification to the formulae in row 23 to increase the range.

Appendix D. New Zealand Mud Snail Sampling and Decontamination Procedures

The following is an excerpt from Ecology Approved Standard Operating Procedure 070 that addresses decontamination procedures in Areas of Moderate Concern and Areas of Extreme Concern.

6.0 Procedures

6.1 Planning - Prior to Conducting Field Work and During Field Work

6.11 Determine if the field activity is located within an Area of Extreme Concern by checking the current maps at this link: www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html If so, the extra decontamination step (section 6.2.1.2) will need to be followed for all equipment that contacted aquatic sediment, aquatic vegetation or fish. (Note: felt sole wading boots must be decontaminated no matter where they are used).

6.1.2 Use equipment which can be easily inspected and cleaned to both avoid spreading invasive species and reduce impacts to planned field schedules. If possible, bring extra sets of "back up" field equipment in case cleaning and decontamination (if required) can't be done in the field prior to arrival at a new sampling site. Where feasible, especially when working in areas of extreme concern, dedicate gear to be used only in that water body.

6.1.3 Note: wading gear has been implicated in the spread of New Zealand mudsnails as well as other AIS such as didymo (the diatom Didymosphenia geminata) and fish and amphibian diseases. Felt soles can be particularly problematic because of their tendency to stay moist for long periods. The laces and eyelets of lace-up wading boots can also be problem spots because they are difficult to clean. To the extent possible, consider using non-felt soles and boot-foot waders. Information about new boots is available at http://aww.ecology.ecy.wa.gov/programs/eap/InvasiveSpecies/AlternativesToFeltBoots.html Because of these risks from felt sole waders, they must go through the decontamination step (section 6.2.1.2) in all parts of the state.

6.1.4 Conduct field activities to minimize contact between equipment and potential sources of invasive species, particularly aquatic plants, sediment and fish. This can include the following:

6.1.4.1 Sample from least to most contaminated areas, for example, sample upstream to downstream or from areas of less weed growth to dense weed growth.

6.1.4.2 Minimize wading and avoid running boats onto sediment.

6.1.4.3 Avoid getting plants, sediment and fish inside boats or other sampling gear.

6.1.4.4 Use a catch pan underneath dredges, etc., to keep potential AIS off boat decks and out of bilges.

6.1.4.5 Avoid driving or walking through areas of mud and high weed growth

6.2 After Field Work

6.2.1 Inspect, clean and if working in an area of extreme concern, decontaminate equipment – this step is divided into two parts:

6.2.1.1 First – inspect, clean and drain all equipment

6.2.1.1.1 Inspect and clean all equipment that contacted (terrestrial or aquatic) soil, vegetation, or water. Remove any visible vertebrates, invertebrates, plants, algae or sediment. If necessary, use a scrub brush and rinse with clean water either from the site or brought for that purpose. Continue this process until the equipment is clean. Drain all water in bilges, samplers or other equipment that could hold water from the site. Flush areas that can't be seen with clean water until the rinse water is clean. Information on cleaning boats and motors is in Attachment B.

6.2.1.1.2 Do the initial treatment (scrubbing and rinsing) before leaving the sampling site (if possible). If cleaning after leaving the field site, ensure that no debris will leave the equipment and potentially spread invasive species during transit or cleaning. Acceptable interim sites for cleaning include: Ecology OC or Regional Offices, commercial car wash businesses, or other facilities (e.g. WADOT shops), provided drains do not lead to surface waters. A table with commercial car wash locations is available to Ecology employees http://aww.ecology.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-EAPPage.html

6.2.1.2 Second – decontaminate felt sole waders and, in areas of extreme concern, equipment that contacted aquatic sediment, aquatic vegetation, or fish.

6.2.1.2.1 Wipe smooth surfaced sampling equipment that can be easily and fully wiped down until dry. The equipment must be smooth enough so there are no cracks or crevices that could harbor a sand-grain-sized juvenile New Zealand mudsnail while being wiped dry.

6.2.1.2.2 Use one of the decontamination treatments from Attachment A for all other equipment. For additional information on cleaning boats and motors, see Attachment B.

6.2.1.2.3 Decontamination treatments should take place where the procedure can be carried out effectively and safely. Keep in mind that wash and rinse water must not drain to surface water, and all chemicals must be disposed of to a sanitary sewer.

6.3 Relaxing Requirements

6.3.1 Equipment should be cleaned whenever leaving a field site, however, decontamination procedures as described in this SOP need not be followed under the following circumstances.

6.3.2 Documented exceptions:

6.3.2.1 If procedures in this SOP are not workable for a particular project, exceptions may be documented and approved following QAPP guidance.

6.3.3 Moving short distances:

6.3.3.1 If moving by foot within the same watershed, equipment may be used without following procedures in this SOP. Keep in mind to work from upstream to down whenever possible. Procedures laid out in this SOP must be followed when leaving the area.

6.3.4 Sampling by boat:

6.3.4.1 When transiting by boat to different sites within a water body, procedures detailed in this SOP may not be necessary. However, when boating from site to site, don't move water, sediment, organisms or vegetation on sampling gear, boat props, etc. Leaving the water body requires implementing this SOP.

Summary of Field Gear Cleaning and Decontamination Procedure

Prior to field work:

- Check if the sampling will take place in an area of extreme concern – maps at this link: www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html
- Plan field activities to minimize contact between equipment and potential sources of invasive species, particularly aquatic plants and sediment.

After conducting field work:

- **Inspect and clean** all equipment. Remove any visible soil, vegetation, vertebrates, invertebrates, aquatic plants, algae or sediment. If necessary, use a scrub brush and rinse with clean water either from the site or brought for that purpose. Continue this process until the equipment is clean. **Drain** all water in bilges, samplers or other equipment that could harbor water from the site. This step should take place before leaving the sampling site or at an interim site. If cleaning after leaving the sampling site, ensure that no debris will leave the equipment and potentially spread invasive species during transit or cleaning.
- **Additional Requirements for felt sole waders used anywhere in the state and equipment that contacted sediment, aquatic vegetation or fish in areas of extreme concern**:
    - **Smooth surfaced sampling equipment that can be easily and fully wiped down – wipe until dry**. The equipment must be smooth enough so there are no cracks or crevices that could harbor a sand-grain-sized juvenile New Zealand mud snail while being wiped dry.
    - **For all other equipment, use one of the decontamination treatments found in the table below**. Conduct decontamination where the procedure can be carried out effectively and safely. Wash and rinse water must not drain to surface water, and all chemicals must be disposed of to a sanitary sewer.

Equipment Storage:

- **Dry** – Between field sites and upon returning from the field, when cleaning and decontamination requirements are complete store gear to facilitate drying.

Table D1. Decontamination Options

Treatment	Concentration or temperature	Exposure Time	Comments
hot water wash or soak	60° C (140° F)	5 min for felt-soled boots and nets; 10 sec for all other equipment	Ensure all parts of the equipment reach temperature for the full exposure time
	49° C (120° F)	10 min for felt-sole boots and nets; 5 min for other equipment	Ensure all parts of the equipment reach temperature for the full exposure time
cold	-4° C	4 hours minimum	Time starts after the equipment reaches -4 °C
drying	low humidity, in sunlight is best	48 hours	Time starts after the equipment is thoroughly dry
Formula 409 All-Purpose Cleaner[1]	100% (full strength)	10 min	Follow proper procedures for storage and handling.
sparquat 256[2]	3.1% or higher	10 min	Follow proper procedures for storage and handling.
Quat 128	4.60%	10 min	Follow proper procedures for storage and handling.
Hydrogen peroxide[3]	30,000 ppm (3%)	15 min	Spray on until soaked, then keep damp for contact time (cover or place gear in a dry bag)
Virkon Aquatic®	2%	20 min	Must soak (not spray on) Follow proper procedures for storage and handling[4]

[1] Must be antibacterial (make sure it has quaternary ammonia, otherwise it is ineffective)
[2] Sparquat is corrosive; read the MSDS and use with caution.
[3] May be corrosive; read the MSDS and follow safety precautions
[4] Rinse gear after soak to prolong life. Solution degrades, lasts up to 7 days, best if mixed fresh

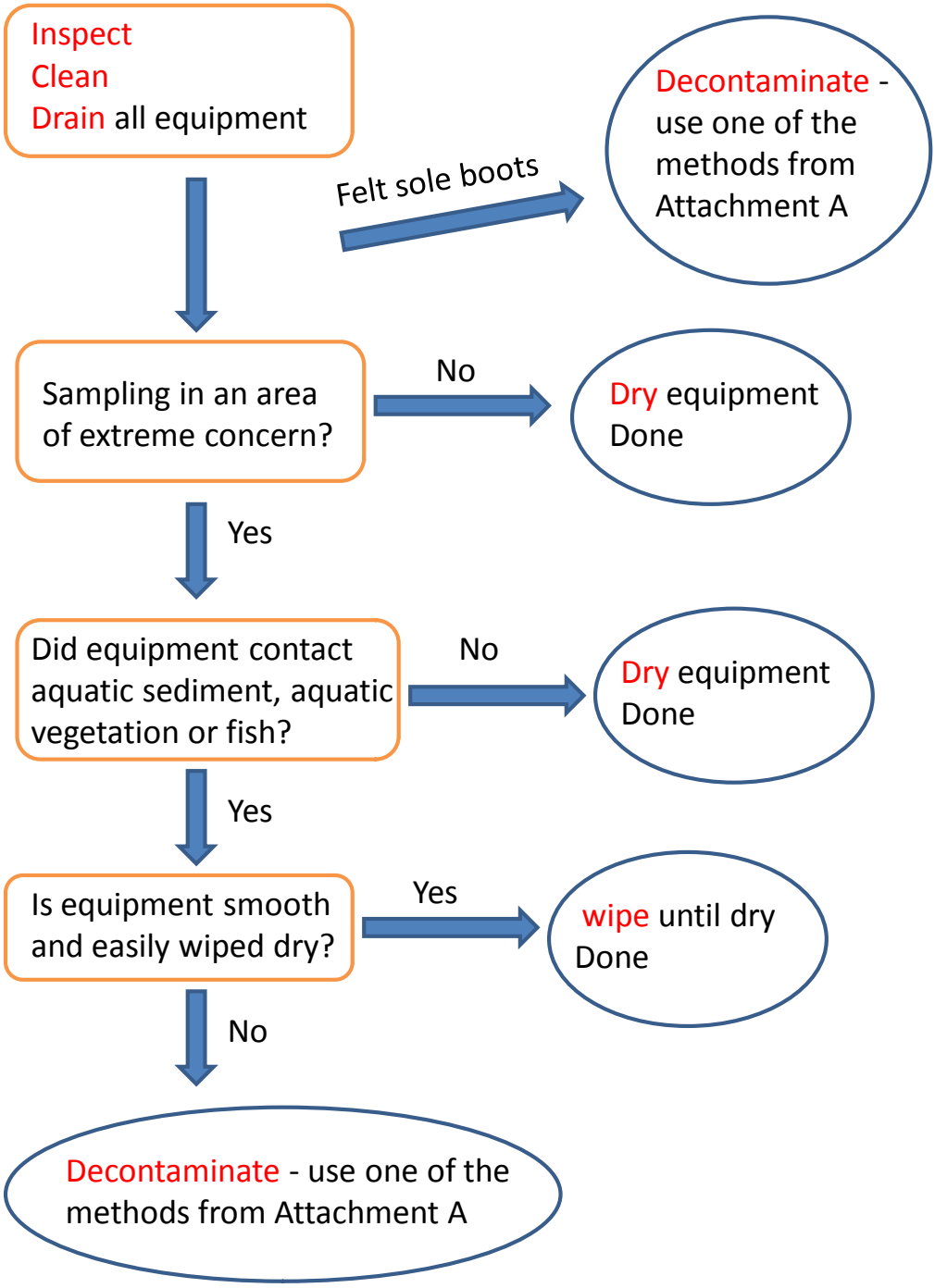

Figure D1. Summary Flow Chart

Figure D2. Fact Sheet on the Invasive Species Didymo