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State of Washington

Quality Assurance Project Plan

Pilot Study: Monitoring the Effectiveness of Pollution Control Activities on Agricultural Lands

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

This Quality Assurance Project Plan is available on Ecology's website at https://fortress.wa.gov/ecy/publications/SummaryPages/1403124.html

Author and Contact Information

Scott Collyard
P.O. Box 47600
Environmental Assessment Program
Washington State Department of Ecology
Olympia, WA 98504-7710

Communications Consultant: phone 360-407-6834.

Washington State Department of Ecology - www.ecy.wa.gov

o	Headquarters, Olympia	360-407-6000
o	Northwest Regional Office, Bellevue	425-649-7000
o	Southwest Regional Office, Olympia	360-407-6300
o	Central Regional Office, Yakima	509-575-2490
o	Eastern Regional Office, Spokane	509-329-3400

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Quality Assurance Project Plan

Pilot Study: Monitoring the Effectiveness of Pollution Control Activities on Agricultural Lands

December 2014

Approved by:

Signature:	Date:
Ron Cummings, EAP Client, WQP	
Signature:	Date:
Scott Collyard, Author / Project Manager, and Principal Investigator, EAP	
Signature:	Date:
George Onwumere, Author's Unit Supervisor, EAP	
Signature:	Date:
Robert F. Cusimano, Author's Section Manager, EAP	
Signature:	Date:
Doug Allen, Section Manager for Project Study Area, WQP	
Signature:	Date:
Markus Von Prause, Ambient Monitoring Data Lead, EAP	
Signature:	Date:
Joel Bird, Director, Manchester Environmental Laboratory	
Signature:	Date:
Bill Kammin, Ecology Quality Assurance Officer	

Signatures are not available on the Internet version.
EAP: Environmental Assessment Program
WQP: Water Quality Program

1.0   Title Page and Table of Contents

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2.0 Abstract

As part of Washington State's Shellfish Initiative, the state departments of Health and Ecology are funding local and Puget Sound health programs to implement pollution control activities in order to reduce nutrient and pathogens entering surface waters flowing into the sound. As part of this effort, the Washington State Department of Ecology (Ecology) will conduct a pilot study that uses two stations monitored by Ecology's Freshwater Monitoring Unit to assess the cumulative effects of pollution control measures on several water quality parameters. Specifically, Ecology will employ an upstream/downstream monitoring design in the Bertrand Creek watershed, an agriculturally dominated sub-watershed of the Nooksack River in Whatcom County, Washington.   Implementation of pollution control measures and changes in land uses within the treatment area will be tracked over the study period and results will be compared to the upstream monitoring station's results.

The primary goal of this study is to evaluate changes in several common water quality indicators in relation to implementation of pollution control measures and land use changes in the watershed. The resulting monitoring data will also be used to determine compliance with water quality standards and identify sources of nutrient and pathogen sources over the study period. Also, because implementation and effects of pollution control measures will occur beyond the project study period, a long-term watershed-scale effectiveness monitoring project plan will be developed for the study area.

3.0   Background

Located in Whatcom County of northwestern Washington, the Bertrand Creek watershed originates in British Columbia, Canada and is part of the larger Nooksack River watershed, which ultimately drains to Bellingham Bay and Puget Sound. Bertrand Creek and several of its tributaries do not meet the state water quality standards for ammonia, bacteria, dissolved oxygen, and temperature and are on Washington's 303(d) list of impaired waters for not meeting those criteria.

A Total Maximum Daily Load study report for the lower Nooksack River basin, conducted by Ecology in 2000, identified Bertrand and the neighboring Fishtrap Creek as contributing 44% of the annual fecal coliform (FC) bacteria load to the lower Nooksack River Basin (Joy, 2000). Also, a recent assessment (Whatcom County, 2013) of FC data taken from two stations in Bertrand Creek found bacteria levels have been increasing since 2003 (Figure 1).
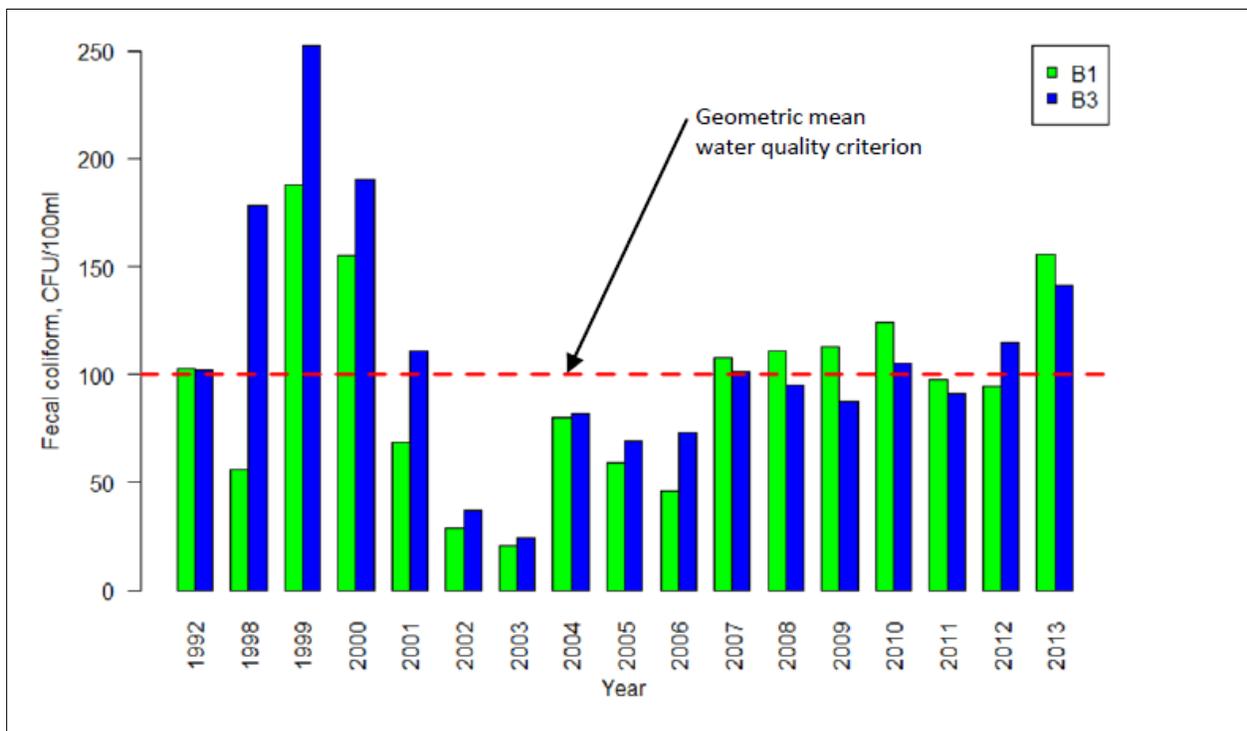
Figure 1. Bertrand Creek fecal coliform geometric mean annual historical comparison of two monitoring stations.

Station B1 is located upstream from the confluence with the Nooksack River, and B3 is located ~1 mile upstream of B1. Data source: Whatcom County.

As part of Washington State's Shellfish Initiative, the state departments of Health and Ecology are funding local and Puget Sound-wide health programs to reduce nutrients and pathogens entering surface waters flowing into the sound. Effectiveness of water cleanup efforts, e.g., implementing best management practices (BMPs), and other pollution control activities, will be measured through a series of monitoring and data analysis actions. These actions are outlined in *Guidance for Effectiveness Monitoring of Total Maximum Daily Loads in Surface* (Collyard and Onwumere, 2013). All monitoring activities will support the broad-based goals for determining the effectiveness of watershed-based pollution control plans. These are to determine if: (1) water quality standards are being met and/or progress is being made towards meeting standards, (2) improvements in water quality are linked to water cleanup activities and (3) the current implementation strategy is sufficient.

3.1 Study area and surroundings

Bertrand Creek is a small sub-watershed of the Nooksack River Water Resource Inventory Area (WRIA 1) and is a part of the Wiser Lake Creek-Nooksack River 12-digit Hydrologic Unit Code (HUC). Bertrand Creek is a perennial stream approximately 18.8 miles long and originating in British Columbia, Canada. Bertrand Creek enters the United States in Whatcom County and flows approximately 9.5 miles before entering the Nooksack River. The drainage area encompasses a total of 42.5 square miles, of which 20.9 square miles is located in Whatcom County. Extensive networks of surface ditches crisscross the watershed and are used primarily for drainage of uplands and irrigation fields for agriculture. In addition to the mainstem of Bertrand Creek, Cave Creek and several surface ditches flow from the Canadian into the United States side of the watershed. Many of these ditches go dry during the summer time.

The Bertrand watershed lies on the flat Lynden Terrace, a glacial outwash plain that slopes gradually southward to the Nooksack River (Figure 2). Repeated glacial advances and retreats have left deposits of gravel and cobble near the Canadian border. These deposits have been redistributed over time, resulting in cobble and gravel deposits in upstream areas of Bertrand Creek and sand and silt downstream (Easterbrook, 1971).

Major land uses on the Canadian and United States side of the watershed include rural residential housing, agricultural production, forest lands and urban areas. Agriculture has historically been dominated by dairy farming in the watershed; however, berry and poultry production have replaced most of the dairy land in British Columbia. In the United States, dairy production has been predominant in the watershed; however, berry production has been more prominent in the recent past (Embertson, 2010).
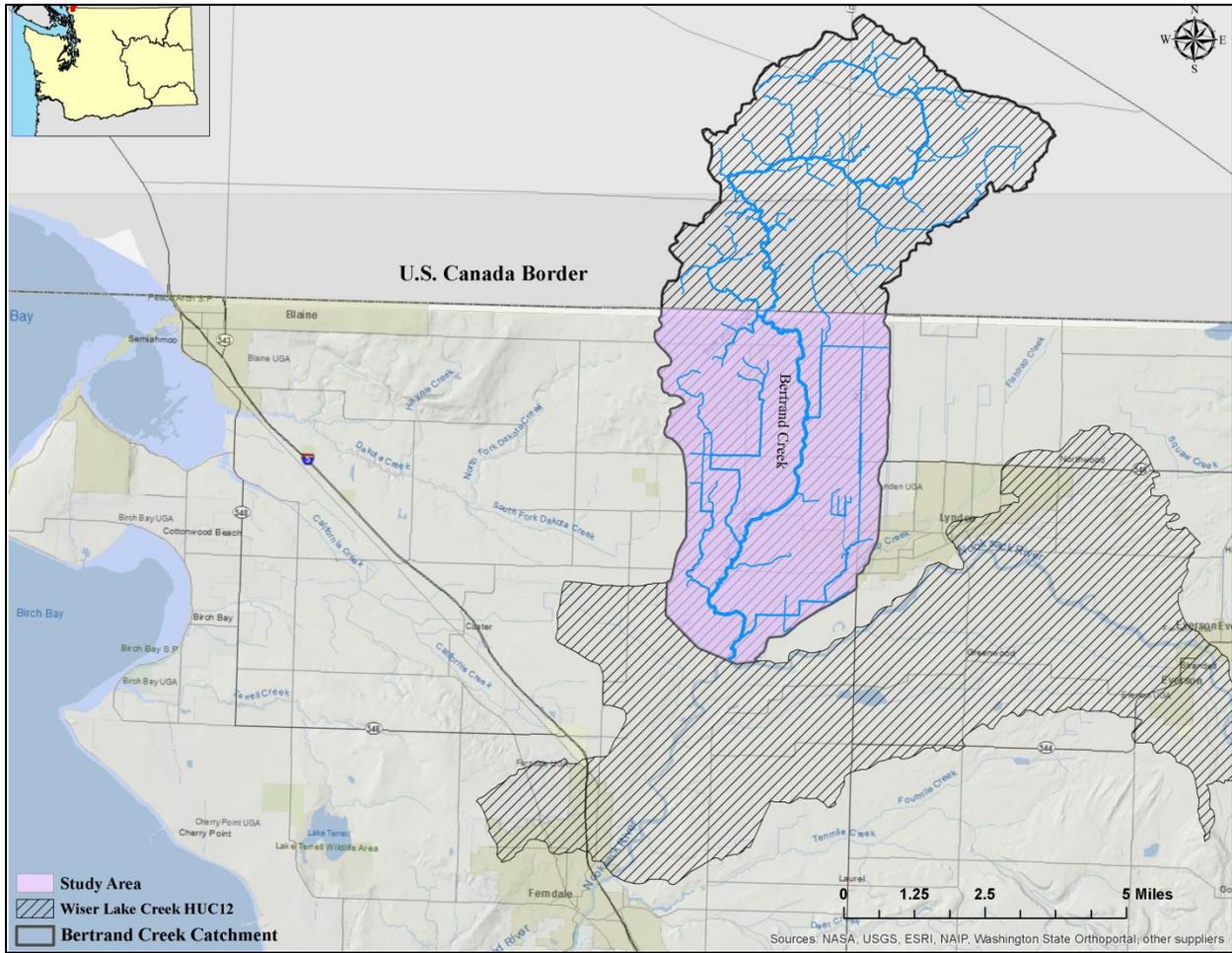
Figure 2. Bertrand Creek study area for the effectiveness monitoring study.

3.1.1 Logistical problems

Although rare, logistical problems such as excessive precipitation during typically dry periods, scheduling conflicts, sample bottle delivery errors, vehicle or equipment problems, site access issues, or the limited availability of personnel or equipment may interfere with sampling. Any circumstance that interferes with data collection and quality will be noted and discussed in the final report.

3.1.2 History of study area

The Bertrand watershed contains a substantial portion of Whatcom County's priority agricultural resource lands. These areas also contribute to important watershed processes. They provide habitat for endangered fish that spawn and rear in tributaries that flow through them to the Nooksack River, and they provide habitat for shellfish in the Puget Sound.

Shellfish harvest closures in Portage Bay by the Lummi Nation and the Washington Department of Health (DOH) have prompted an urgent need to control bacteria and nutrient inputs in the Lower Nooksack River.

3.1.3 Parameters of interest

This study will assess effectiveness of water cleanup activities by monitoring 303(d) listed parameters (Table 1). Each listing documents a specific location in Bertrand Creek where the impairment was measured (Figure 2). Staff will be looking at this watershed more thoroughly and may find other impaired locations. This study will also measure other surrogate water quality parameters that will assist with measuring the effects of land use activities.

Bertrand Creek and its tributaries are considered *Extraordinary* quality water and are protected for the designated uses of salmon and trout spawning, core rearing, and migration. Bertrand Creek and its tributaries are also protected for *Extraordinary* primary contact recreation.

Table 1 and Figure 3 show the Category 5, 4A, and 2 listings on the state Water Quality Assessment in the Bertrand Creek Watershed, approved by EPA in 2012 (Ecology, 2014). A full list of water quality impairments is available in Washington's Water Quality Assessment 303(d)/305(b) Integrated Report Viewer (http://apps.ecy.wa.gov/wats/Default.aspx).

Table 1. Bertrand Creek and tributaries on the 2012 303(d) list of impaired water bodies that do not meet water quality standards.

Water body Name	Category	Parameter	WBID Code	NHD Reach Code	Assessment Listing ID	Township/ Range/Section
Bertrand Creek	2	Bacteria	46498	17110004000399	1225334489122	40N/2E/13
Bertrand Creek	2	Bacteria	46707	17110004000396	1225334489122	40N/2E/13
Bertrand Creek	2	Ammonia-N	8624	17110004001168	1224843489611	40N/2E/27
Bertrand Creek	2	Bioassessment	19878	17110004000399	1225334489122	41N/2E/35
Bertrand Creek	2	Dissolved Oxygen	38951	17110004000400	1225334489122	40N/2E/12
Bertrand Creek	2	Temperature	9094	17110004000396	1225334489122	40N/2E/14
Bertrand Creek	4A	Bacteria	9720	17110004000396	1225334489122	40N/2E/2
Bertrand Creek	4A	Bacteria	39039	17110004000396	1225334489122	40N/2E/27
Bertrand Creek	4A	Bacteria	42447	17110004000400	1225334489122	40N/2E/12
Bertrand Creek	4A	Bacteria	42448	17110004000399	1225334489122	40N/2E/11
Bertrand Creek	4A	Bacteria	42497	17110004001195	1225416489286	40N/2E/1
Bertrand Creek	4A	Bacteria	42498	17110004001297	1225059489745	41N/2E/36
Bertrand Creek	4A	Bacteria	45774	17110004000437	1225335489362	40N/2E/27
Bertrand Creek	4A	Bacteria	46001	17110004001297	1225059489745	40N/2E/24
Bertrand Creek	5	Ammonia-N	8629	17110004004391	1226832488025	40N/2E/27
Bertrand Creek	5	Dissolved Oxygen	7060	17110004001297	1225059489745	40N/3E/7
Bertrand Creek	5	Dissolved Oxygen	15428	17110004000396	1225334489122	40N/2E/26
Bertrand Creek	5	Dissolved Oxygen	47672	17110004001297	1225059489745	40N/2E/26
Bertrand Creek	5	Dissolved Oxygen	47682	17110004000400	1225334489122	40N/2E/24
Bertrand Creek	5	Dissolved Oxygen	47684	17110004001195	1225416489286	40N/2E/24
Duffner Ditch	2	Bacteria	45405	17110004006781	1224878489406	41N/2E/35
Duffner Ditch	2	Dissolved Oxygen	7076	17110004001168	1224843489611	40N/2E/27
Duffner Ditch	4A	Bacteria	6635	17110004004396	1226832488025	40N/2E/12
Duffner Ditch	4A	Bacteria	6636	17110004001168	1224843489611	40N/2E/12
Duffner Ditch	4A	Bacteria	39086	17110004004396	1226832488025	41N/2E/36
Duffner Ditch	4A	Bacteria	39088	17110004004397	1226832488025	40N/2E/22
Duffner Ditch	5	Dissolved Oxygen	47670	17110004006781	1224878489406	40N/2E/25
Duffner Ditch	5	Dissolved Oxygen	47715	17110004004397	1226832488025	40N/2E/26
Duffner Ditch	5	Dissolved Oxygen	47716	17110004004396	1226832488025	40N/2E/25

WBID: Water body Identification
NHD: National Hydrography Dataset
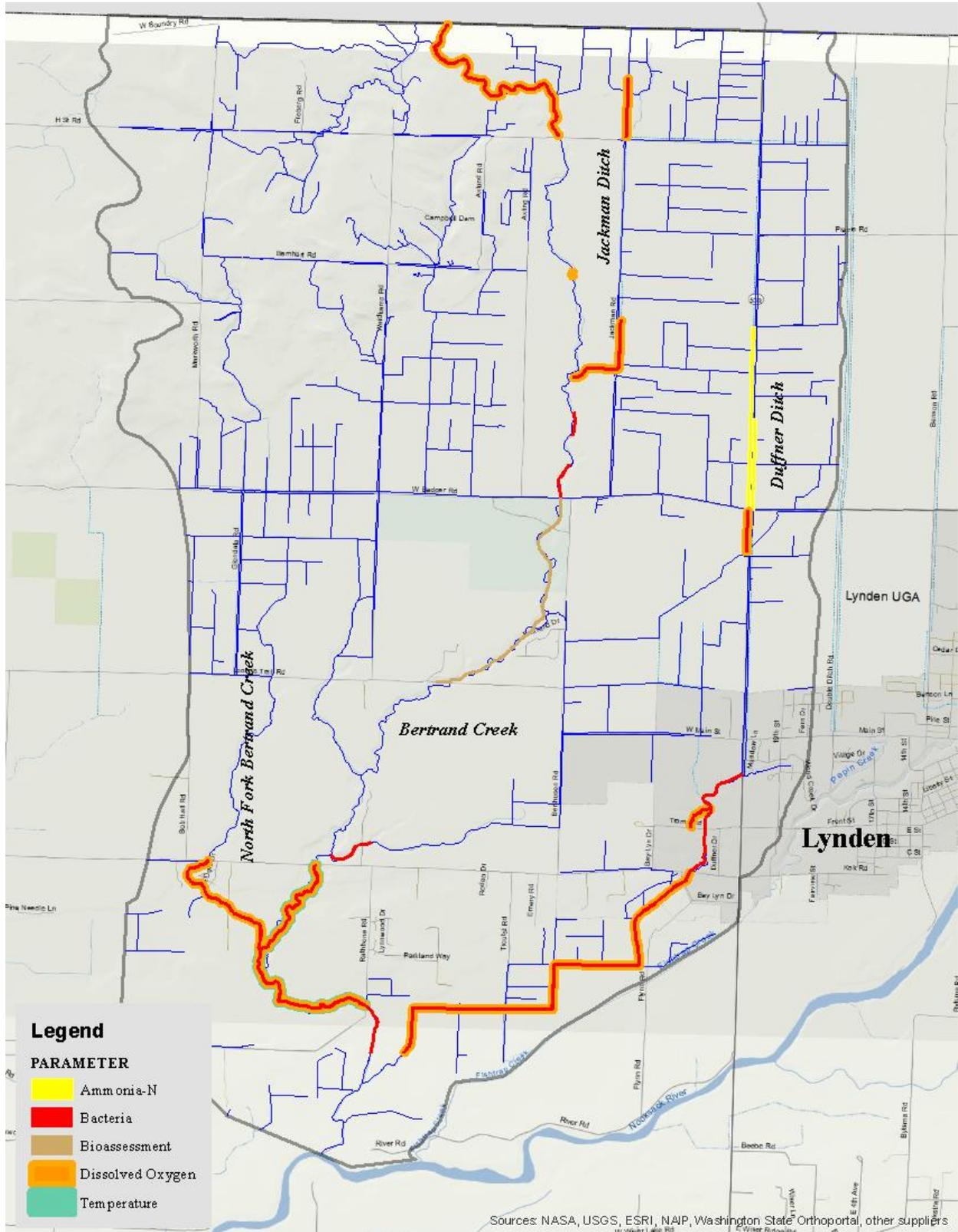
Figure 3. Bertrand Creek watershed 2012 303(d) list of impaired water bodies.

3.1.4 Results of previous studies

Table 2 outlines previous water quality studies conducted within the Bertrand Creek watershed that were in Ecology's Environmental Information Management (EIM) system. A summary of data from these studies is presented in Table 3. These data may be used in the final report to evaluate water quality trends within the study area.

Table 2. Bertrand Creek water quality data in EIM used in data assessment.

Study Name	Years	Study ID
Statewide River and Stream Ambient Monitoring-WY 2000 through WY 2009	1966 - 2009	AMS001E
Water Quality Monitoring Implementation	2004 - 2006	G0300021
Water Quality in Dakota, Bertrand, and Fishtrap Creek Watersheds	1992 - 1992	BEDI0002
Recover Bertrand Creek, WRIA 1	2002 - 2004	G0100188
Lower Nooksack River Bacteria TMDL	1997 - 1998	JJOY0004
TMDL Implementation Monitoring in WRIA 1 Project	2002 - 2004	G0200112
Whatcom County Shellfish Protection Plan	1997 - 2002	G9900096
Continuous Stream Monitoring	2001 - 2010	AMS004
Ecology's Freshwater Ambient Biological Assessment Program	1993 - 1994	fwbenth1
Manure as Fertilizer Groundwater Study	2004 - 2011	bcar0003

Table 3. Summary statistics from historic water quality studies found in EIM.

Parameter	Date Range	Number of samples	Median Value	Minimum value	Maximum value
Ammonia-N (mg/L)	1992-2007	42	0.05	0.01	0.23
Fecal Coliform (FC) Bacteria (colonies/100 mL)	1992-2010	226	95	1	3560
Specific Conductivity (umhos/cm)	1992-2010	39	243	148	370
Dissolved oxygen (mg/L)	1992-2007	83	9.01	1.9	13.1
Nitrate+Nitrite-N (mg/L)	2004-2007	42	3.4	2.2	5.4
Ortho-Phosphate (mg/L)	2004-2007	38	0.02	0.0055	0.11
pH	1992-2007	41	7.05	6.01	7.87
Temperature (°C)	1992-2007	83	10.7	3.9	18.3
Total Kjeldahl Nitrogen (mg/L)	2004-2007	26	0.265	0.05	0.81
Total Persulfate Nitrogen (mg/L)	2006-2007	12	3.75	2.58	4.99
Total Phosphorus (mg/L)	2004	26	0.05	0.05	0.38
Total Suspended Solids (mg/L)	2004-2007	38	4.5	1	35
Turbidity (NTU)	2004-2007	37	5.8	0.95	37

Current Monitoring Efforts

Below is a description of the current monitoring efforts underway in the Bertrand Creek watershed to support the shellfish initiative. Locations are summarized in Table 4. The data resulting from these efforts may be used to supplement data collected under this QAPP to assess effectiveness of pollution control activities on water quality.

Quarterly sampling

Nonpoint source inspectors from Ecology's Water Quality Program are conducting ambient water quality sampling for FC bacteria six times at 10 established short-term locations (Ecology, 2014). Inspectors sample twice monthly, according to a systematic random sampling schedule. See Figures 3 and 4 for a map of 2012 303(d) list of impaired water bodies and current sampling locations. Three of these sampling sites (Cave Creek, Bertrand Creek and Jackman Ditch (BECC0.2, BE9.1 and BEJK2.0)) are located at the Canadian-U.S. border and are used to measure water quality entering the United States.

Ecology inspectors are collecting additional samples six times each quarter, from multiple publicly accessible sites in other drainages to help identify specific pollution sources. Follow-up for identified pollution sources may involve enforcement action by Ecology, referral to an NPDES-permitted municipal jurisdiction, referral to the local Conservation District for farm planning or other technical assistance, or referral to the appropriate local Health District or Department.

The Washington Department of Agriculture (WSDA) is also conducting supplemental bacteria monitoring in response to chronically high FC results in the basin. The supplemental sampling is being used by WSDA to investigate pollution sources related to manure management. Also, WSDA inspectors are using the information to identify nutrient management activities in the basin that present a risk of affecting water quality.

Long-term sampling

Through the end of 2013, Whatcom County Public Works (WCPW) and Northwest Indian College (NWIC) staff collected routine FC samples at two long-term monitoring stations on the mainstem of Bertrand Creek (Douglas, 2013). In 2014, the NWIC ended their sampling program and the monitoring at one of the stations (B3) has ended. The Nooksack Indian Tribe staff also collects FC samples at one long-term sample location on the mainstem of Bertrand Creek (Table 4). In addition to FC, the Tribe also collects E.coli, pH, water temperature, total phosphorus, total kjeldahl nitrogen (TKN), nitrite-N, nitrate-N, and total nitrogen monthly at this location (Nooksack Natural Resources Department, 2013).

Table 4. Current sampling efforts underway in the Bertrand Creek watershed.

Station ID	Water body	Data Originator	Monitoring Type	Sampling frequency	Timeline
BECC-0.2	Cave Creek	Ecology	Systematic random/source ID	2 times/month	2013-2014
BES-0.1	Bertrand Creek	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEMC-1.8	Jackman Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEJK-2.0	Jackman Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEJK-0.2	NF Bertrand Creek	Ecology	Systematic random/source ID	2 times/month	2013-2014
BENF-2.0	Bertrand Creek	Ecology	Systematic random/source ID	2 times/month	2013-2014
BE-4.3	Duffner Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEDF-6.4	Duffner Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEDF-3.7	Duffner Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
BEDF-2.2	Duffner Ditch	Ecology	Systematic random/source ID	2 times/month	2013-2014
SW-13	Bertrand Creek	NWIC	Long-term trend monitoring	2 times/month	1998-present
B-1	Bertrand Creek	Whatcom County	Long-term trend monitoring	2 times/month	1998-present
B-2	Bertrand Creek	Nooksack Indian Tribe	Long-term trend monitoring	1 time/month	2009-present
B-3	Bertrand Creek	NWIC	Long-term trend monitoring	2 times/month	1998-2014

NF: North Fork
NWIC: Northwest Indian College
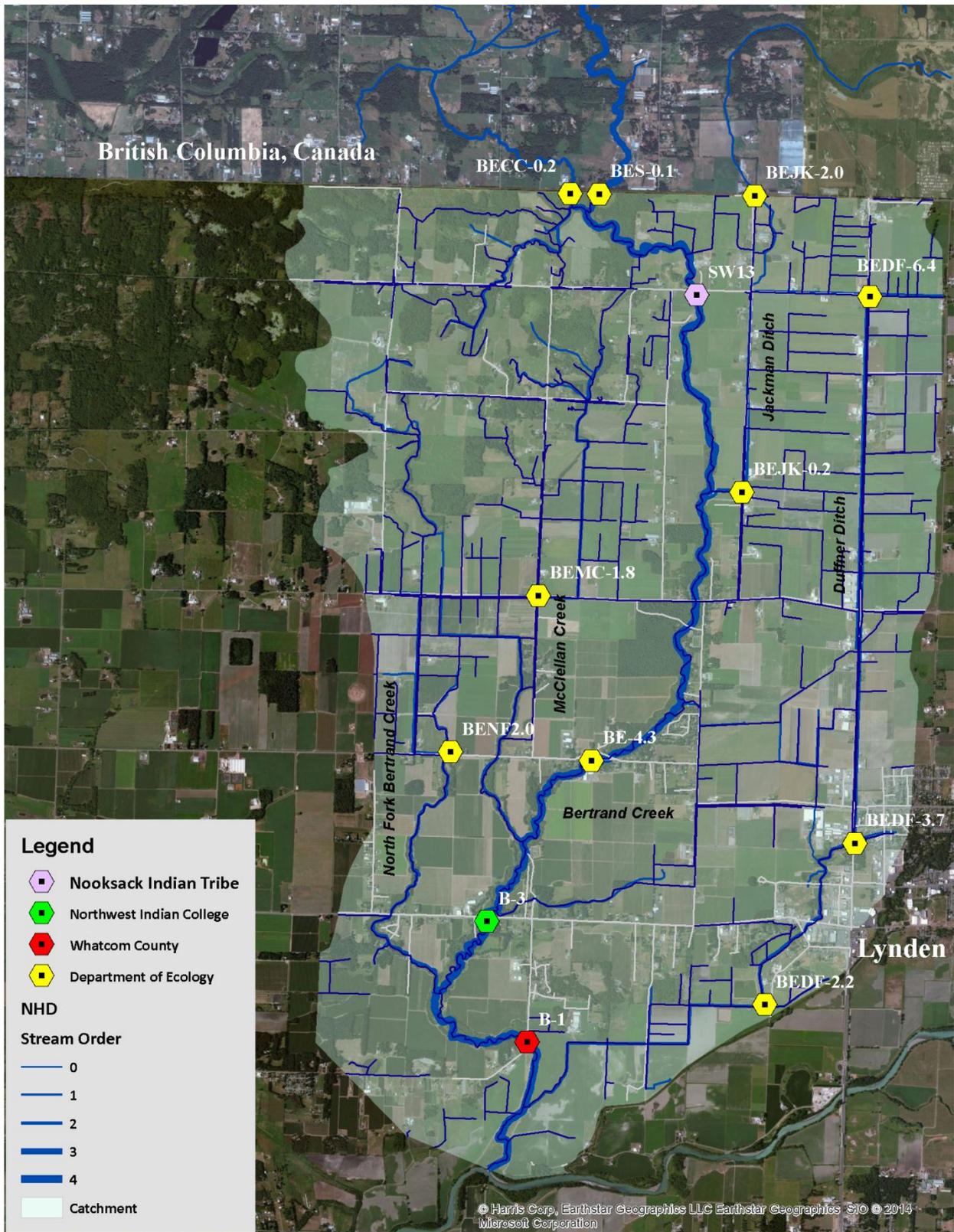
Figure 4. Current water quality monitoring locations in the Bertrand Creek watershed.

3.1.5 Regulatory criteria or standards

Beneficial uses in Bertrand Creek and its tributaries are listed as core summer salmonid habitat and primary contact recreation. Applicable water quality criteria for this study include ammonia, dissolved oxygen, bacteria, and temperature, (Table 5).

Table 5. Applicable water quality criteria for Bertrand Creek.

Ammonia (mg N/L)	Shall not exceed the numerical value in total ammonia nitrogen give by: For salmonids present: $\dfrac{0.275}{1+10^{7.204-pH}} + \dfrac{39.0}{1+10^{pH-7.204}}$
Dissolved oxygen	Lowest 1 day minimum 9.5 mg/L
Fecal coliform bacteria	Fecal coliform organism levels must not exceed a geometric mean value of 100 colonies/100 mL, with not more than 10% of all samples (or any single sample when less than 10 sample points exist) obtained for calculating the geometric mean value > 200 colonies/100 mL.
Temperature	Highest 7-DADMAX (7 day average of the daily maximum temperatures) 16.0º C, supplemental criteria: 13°C from February 15 through June 15 Bertrand Creek above Berthusen Park.

Ammonia

Ammonia is considered a toxic substance. Water quality standards specify that toxic substances shall not be introduced above natural background levels in waters of the state that have the potential, either singularly or cumulatively, to adversely affect characteristic water uses, cause acute or chronic toxicity to the most sensitive biota dependent upon those waters, or adversely affect public health, as determined by Ecology.

Dissolved oxygen

Aquatic organisms are very sensitive to reductions in the level of dissolved oxygen (DO) in the water. While inadequate oxygen can cause direct mortality, the state designed the criteria to maintain conditions that support healthy populations of fish and other aquatic life. The health of fish and other aquatic species depends on maintaining an adequate supply of DO in the water. Oxygen levels affect incubation success, growth rates, swimming ability, susceptibility to disease, and the relative ability to endure other environmental stressors and pollutants.

Low DO can release toxic metals and phosphorus from sediments, and cause increased availability of toxic substances like ammonia and hydrogen sulfide. These problems may contaminate the habitat of aquatic organisms, reducing the overall health of the water body.

Oxygen levels can fluctuate over the day and night (diurnal fluctuation) in response to changes in climatic conditions as well as the respiratory requirements of aquatic plants and algae. Since the health of aquatic species is tied predominantly to the pattern of daily minimum oxygen concentrations, the criteria are the lowest 1-day minimum oxygen concentrations that occur in a water body.

Nutrient enrichment may lead to low DO levels and increase the occurrence of excessive primary productivity leading to harmful algal blooms and macrophyte growth. Large biomass of primary producers may be associated with severe diurnal swings in DO concentrations. Water quality criteria described in Table 5 are used to ensure that where a water body is naturally capable of providing full support for its designated aquatic life uses, that condition will be maintained. The criteria recognize, however, that not all waters are naturally capable of staying above the fully protective DO criteria. When a water body is naturally lower in oxygen than the criteria, the state provides an additional allowance for further depression of oxygen conditions due to human activities. In this case, the combined effects of all human activities must not cause more than a 0.2 mg/L decrease below that naturally lower DO condition.

While the numeric criteria generally apply throughout a water body, they are not intended to apply to discretely anomalous areas such as in shallow stagnant eddy pools where natural features unrelated to human influences are the cause of not meeting the criteria. For this reason, the standards direct that one take measurements from well-mixed portions of rivers and streams. For similar reasons, samples should not be taken from anomalously oxygen-rich areas. For example, in a slow-moving stream, focusing sampling on surface areas within a uniquely turbulent area would provide data that are erroneous for comparing to the criteria.

Fecal coliform (FC) bacteria

Bacteria criteria are set to protect people who work and play in and on the water from waterborne illnesses. In Washington, the Department of Ecology's (Ecology) water quality standards use FC 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. Waste from warm-blooded animals 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.

Other indicators, such as Escherichia (E.) coli and enterococci, have been evaluated as alternative or additional surrogates for pathogens under the triennial review of state water quality standards. However, at the time of this publication, FC bacteria remain the designated indicator.

During sufficient precipitation events, rainwater washes the surface of the landscape and the impervious surfaces, saturates soils, and raises water tables. Runoff from the stormwater can accumulate and transport fecal matter. This stormwater loaded with fecal matter may often drain to receiving water bodies and potentially degrade water quality.

The criteria for FC outlined in Table 5 and described below are based on allowing no more than the pre-determined risk of illness to humans that work or recreate in a water body. Once the concentration of FC in the water reaches the numeric criterion, human activities that would

increase the concentration above the criteria are not allowed. If the criterion is exceeded, the state will require that all known and reasonable technologies and targeted BMPs be implemented to reduce human impacts and bring FC concentrations into compliance with the standard.

- The *Primary Contact* use is intended for waters "where a person would have direct contact with water to the point of complete submergence including, but not limited to, skin diving, swimming, and waterskiing." More to the point, however, the use is designated to any waters where human exposure is likely to include exposure of the eyes, ears, nose, throat, and urogenital system. Since children are also the most sensitive group for many of the waterborne pathogens of concern, even shallow waters may warrant primary contact protection. To protect this use category: "Fecal coliform organism levels must not exceed a geometric mean value of 100 colonies/100 mL, with not more than 10 percent of all samples (or any single sample when less than ten sample points exist) obtained for calculating the geometric mean value exceeding 200/colonies mL" (WAC 173-201A, 2011 edition).

Compliance is based on meeting both the geometric mean criterion and the 10% of samples (or single sample if less than ten total samples) limit. These two measures used in combination ensure that bacterial pollution in a water body will be maintained at levels that will not cause a greater risk to human health than intended. While some discretion exists for selecting sample averaging periods, compliance will be evaluated for both monthly (if five or more samples exist) and seasonal (dry season versus wet season) data sets.

The criteria for FC bacteria are based on allowing no more than the pre-determined risk of illness to humans that work or recreate in a water body. The criteria used in the state standards are designed to allow seven or fewer illnesses out of every 1,000 people engaged in primary contact activities. Once the concentration of FC in the water reaches the numeric criterion, human activities that would increase the concentration above the criteria are not allowed. If the criterion is exceeded, the state will require that human activities be conducted in a manner that will bring FC concentrations back into compliance with the standard.

If natural levels of FC (from wildlife) cause criteria to be exceeded, no allowance exists for human sources to measurably increase bacterial pollution. While the specific level of illness rates caused by animal versus human sources has not been quantitatively determined, warm-blooded animals (particularly those that are managed by humans and thus exposed to human-derived pathogens as well as those of animal origin) are a common source of serious waterborne illness for humans.

Temperature

Many types of fish species rely on the watershed for spawning, rearing, migration, and residence. Anadromous fish of the watershed include chinook, coho, chum, and trout (Herger, 1997). Temperature and supplemental spawning criteria have been established in order to protect aquatic life uses within the watershed.

Temperature affects the physiology and behavior of fish and other aquatic life. Temperature may be the most influential factor limiting the distribution and health of aquatic life and can be greatly influenced by human activities.

Temperature levels fluctuate over the day and night in response to changes in climatic conditions and river flows. Since the health of aquatic species is tied predominantly to the pattern of maximum temperatures, the criteria are expressed as the highest 7-day average of the daily maximum temperatures (7-DADMax) occurring in a water body.

In the water quality standards, aquatic life use categories are described using key species (salmon versus warm water species) and life-stage conditions (spawning versus rearing) (WAC 173-201A-200, 2011 edition).

1. To protect the designated aquatic life uses of "Core Summer Salmonid Habitat" the highest 7-DADMax temperature must not exceed 16°C (60.8°F) more than once every ten years on average.

Washington uses the criteria described above and in Table 5 to ensure that where a water body is naturally capable of providing full support for its designated aquatic life uses, that condition will be maintained. The standards recognize, however, that not all waters are naturally capable of staying below the fully protective temperature criteria. When a water body is naturally warmer than the above-described criteria, the state provides an allowance for additional warming due to human activities. In this case, the combined effects of all human activities must not cause more than a 0.3°C (0.54°F) increase above the naturally higher (inferior) temperature condition.

In addition to the maximum criteria noted above, compliance must also be assessed against criteria that limit the incremental amount of warming of otherwise cool waters due to human activities. When water is cooler than the criteria noted above, the allowable rate of warming up to, but not exceeding, the numeric criteria from human actions is restricted to:

1. Incremental temperature increases resulting from individual point source activities must not, at any time, exceed 28/T+7 as measured at the edge of a mixing zone boundary (where "T" represents the background temperature as measured at a point or points unaffected by the discharge).

2. Incremental temperature increases resulting from the combined effect of all nonpoint source activities in the water body must not at any time exceed 2.8°C (5.04°F).

4.0   Project Description

A *Nested paired* study design will be used to determine the effectiveness of pollution control measures on Bertrand Creek watershed. *Nested paired* study designs are very useful for quickly assessing the effectiveness of BMPs in improving water quality in a water body. A nested paired watershed design is sometimes referred to as an "above and below" design where one monitoring station is located above a treatment area and one station is located below the treatment area (Collyard and Onwumere, 2013).

Water quality monitoring stations will be established in Bertrand Creek at the border with British Columbia, Canada and 0.5 miles above the confluence of the Nooksack River. The area between these monitoring locations will be the treatment area where pollution control measures are expected to occur. Additional synoptic water quality, biological and habitat monitoring will occur within the treatment area to further access water quality conditions.

Although the primary focus in the Bertrand Creek watershed is to implement pollution control measures to reduce FC and nutrient levels, it is expected that other surrogate parameters will respond to pollution control actions. Thus, this study will look at a suite of water quality, biological, and habitat parameters that are likely to respond to pollution control measures aimed at reducing pathogen and nutrient loading. This may increase the likelihood that changes in water quality can be measured and linked to pollution control measures.

During the project, additional sites and/or samples will be added or sampled at the project manager's discretion to provide information that will help meet the goals and objectives of the study. The project manager will immediately review laboratory results to determine the possible need for source identification sampling to verify unexpected laboratory results or to isolate specific sources of pollution.

4.1 Project goals

The primary goal of this study is to measure changes of water quality indicators in relation to implementation of pollution control measures and land use changes in the watershed. The secondary goal of this study is to determine compliance with water quality standards and identify sources of nutrient and pathogen sources over the study period.

4.2 Project objectives

Objectives of the proposed study are as follows:

1. Establish two ambient and continuous water quality monitoring stations above and below where pollution control actions are expected to occur in the Bertrand Creek watershed.
2. Conduct synoptic sampling of biological, habitat, and water quality parameters during low flow conditions.
3. Collect data consistent with sampling requirements of delisting, as specified in Washington's Water Quality Program Policy 1-11 (Ecology, 2012).

4. Document historical and current pollution control measures.
5. Monitor changes in monitoring parameters overtime.
6. Establish link between pollution control efforts and changes in monitoring parameters.

4.3 Information needed and sources

Meeting these goals requires a comprehensive list of pollution control measures implemented to protect or restore water quality. This information will be needed from Whatcom County, Whatcom Conservation District, and non-profit organizations involved in implementing pollution control measures. Also required are historical and current fecal coliform, precipitation, salinity, and other covariate data from regional monitoring programs, to assess trends over time.

4.4 Target population

The target population for this study is surface waters within the Bertrand Creek watershed (Figure 5).

4.5 Study boundaries

Water Resource Inventory Area (WRIA) and 8 and 10-digit Hydrologic Unit Code (HUC) numbers for the study area:

WRIA
- 1-Nooksack River

HUC numbers
- 17110004-Nooksack
- 171100040503-Wiser Lake Creek-Nooksack River
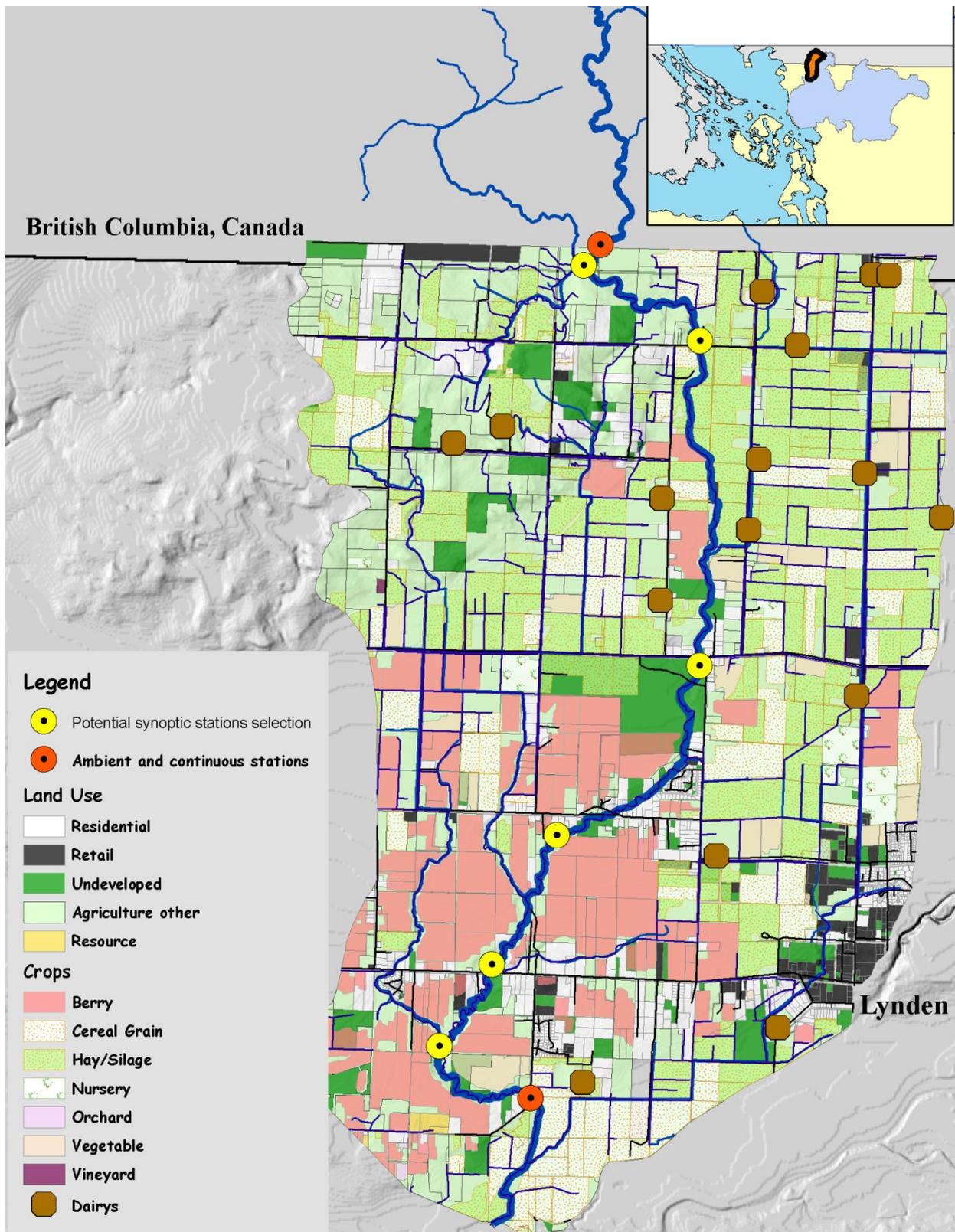
Figure 5. Bertrand Creek watershed resource land use and proposed sampling locations.

4.6 Tasks required

The following types of data will either be obtained from other sources or collected for this project:

- Continuous and ambient hydrology, meteorology, and water quality data collected within the study area to provide continuous and discrete inputs to establish baseline water quality conditions.

- Periodic synoptic surveys to collect higher resolution data to characterize water quality and biological productivity in Bertrand Creek and its tributaries.

- Inventory of current and past land uses, land use practices, and pollution control measures.

4.7 Practical constraints

Not applicable.

4.8 Systematic planning process

Not applicable.

5.0　Organization and Schedule

5.1　Key individuals and their responsibilities

Table 6 lists the key people involved with this project and their responsibilities.

Table 6. Organization of project staff and responsibilities.

Staff (all are EAP except client)	Title	Responsibilities
Ron Cummings WQP headquarters Phone: 360-407-6795	EAP Client	Clarifies scope of the project. Provides internal review of the QAPP and approves the final QAPP.
Scott Collyard Directed Studies Unit, WOS Phone: 360-407-6455	Project Manager / Principal Investigator	Writes the QAPP. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Writes the draft and final report.
Markus Von Prause Freshwater Monitoring Unit WOS Phone: 360-407-6681	Ambient Monitoring Lead	Collects samples and records field information.
Paul Anderson Directed Studies Unit, WOS Phone: 360-407-7548	Field Lead	Collects samples and records field information.
George Onwumere Directed Studies Unit, WOS Phone: 360-407-6730	Unit Supervisor for the Project Manager	Reviews and approves project scope and budget and tracks progress. Provides internal review of the QAPP and approves the final QAPP.
Robert F. Cusimano WOS Phone: 360-407-6596	Section Manager for the Project Manager	Reviews the draft QAPP and approves the final QAPP.
Joel Bird Manchester Environmental Laboratory Phone: 360-871-8801	Director	Approves the final QAPP.
Tom Gries Statewide Coordination Section	NEP QA Coordinator	Reviews and comments on draft QAPP and project report. Recommends approval of QAPP.
William R. Kammin Phone: 360-407-6964	Ecology QA Officer	Reviews the draft QAPP and approves the final QAPP.

EAP: Ecology's Environmental Assessment Program
EIM: Environmental Information Management database
NEP: National Estuary Program
QAPP: Quality Assurance Project Plan
WQP: Water Quality Program
WOS: Western Operations Section

5.2 Special training and certifications

Not applicable.

5.3 Organization chart

See Table 6.

5.4 Project schedule

Table 7 lists the complete project schedule.

Table 7. Proposed schedule for completing field and laboratory work, data entry into EIM, and reports.

Field and laboratory work	Due date	Lead staff
Field work completed	December 2016	Paul Anderson
Laboratory analyses completed	December 2016	
Environmental Information System (EIM) database		
EIM Study ID	scol005	
Product	Due date	Lead staff
EIM data loaded	February 2017	Paul Anderson
EIM quality assurance	March 2017	Scott Collyard
EIM complete	March 2017	Paul Anderson
Final report		
Author lead / Support staff	Scott Collyard /Paul Anderson	
Schedule		
Draft due to supervisor	May 2017	
Draft due to client/peer reviewer	June 2017	
Draft due to external reviewer(s)	July 2017	
Final (all reviews done) due to publications coordinator	August 2017	
Final report due on web	September 2017	

5.5 Limitations on schedule

Not applicable.

5.6 Budget and funding

The estimated laboratory budget and number of lab samples shown in Table 8 is based on the proposed schedule in Table 7. The greatest uncertainties in the cost estimate are with the storm event sampling, sites where streams are ephemeral, and source identification sampling. We plan to keep the submitted number of samples within the estimate; however, because not all storm and investigation sites have been selected yet, we can only estimate.

Table 8. Project budget and funding.

Parameter /Analysis	Sites	Surveys	Field Dupes	Field Blanks	Total Samples	Cost per Sample ($)	Subtotal ($)
Water Quality							
Ammonia-N	4	6	1	1	25	26.02	651
Alkalinity	4	6	1	1	25	18.43	461
Chloride	4	6	1	1	25	14.09	352
Hardness	4	6	1	1	25	23.48	587
Nitrate + nitrite-N	4	6	1	1	25	14.09	352
Persulfate Nitrogen, Total	4	6	1	1	25	14.09	352
Phosphorus, soluble reactive	4	6	1	1	25	14.09	352
Phosphorus, total	4	6	1	1	25	16.26	407
Solids, total suspended	4	6	1	1	25	19.5	488
NO_3^- ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	4	6	1	1	25	n/a	n/a
Biological							
Periphyton Identification	2	12	1	0	25	300	7500
Ash Free Dry Weight	2	12	1	0	25	24.93	623
Chlorophyll <u>a</u>	2	12	1	0	25	46.6	1165
Percent Total Solids	2	12	1	0	25	11.92	298
Total Metals[1]	2	12	1	0	25	206	5150
Percent Total Organic Carbon	2	12	1	0	25	45.52	1138
Total Carbon/Nitrogen	2	12	1	0	25	58.25	1456
Watershed Health Assessment							
Biological and habitat (Merritt, 2010)	4	3	1	n/a	13	800.00	10,400
Subtotal =							31,732

[1]Al, As, Cd, Cu, Fe, Mn, Ni, P, Pb, Zn

6.0   Quality Objectives

6.1   Decision Quality Objectives (DQOs)

Not applicable.

6.2   Measurement Quality Objectives (MQOs)

Field sampling procedures and laboratory analyses inherently have associated uncertainty, which results in data variability. Measurement quality objectives (MQOs) state the acceptable data variability for a project. *Precision* and *bias* are data quality criteria used to indicate conformance with MQOs. The term *accuracy* refers to the combined effects of precision and bias (Lombard and Kirchmer, 2004).

Field sampling precision and bias will be addressed by submitting replicate samples. Ecology's Manchester Environmental Laboratory (MEL) will assess precision and bias in the laboratory through the use of duplicates and blanks.

Table 9 outlines analytical methods, expected precision of sample duplicates, and method reporting limits. The targets for precision of field replicates are based on historical performance by MEL for environmental samples taken around the state by Ecology's Environmental Assessment Program (Mathieu, 2006). The reporting limits of the methods listed in the table are appropriate for the expected range of results and the required level of sensitivity to meet project objectives. The laboratory's MQOs and QC procedures are documented in the MEL *Lab Users Manual* (MEL, 2008).

Table 9. Measurement quality objectives for field and laboratory analyses.

Parameter	Accuracy (deviation or % deviation from true or replicate value)[*]	Precision (% relative standard deviation)	Sensitivity (reporting limit)
Field parameters			
Oxygen	± 0.5 mg/L	10	0 to 50 mg/L
Temperature	± 0.4 ° C	10	0 to 30ºC
pH	± 0.3 standard units	10	6 to 14 s.u.
Conductivity	± 5 uS/cm or 10%, whichever is greater	10	0 to 100,000 uS/cm
Nitrate-nitrite (Satlantic SUNA)	± 0.028 mg/L or ±10% of reading, whichever is greater (under laboratory conditions)	10	0.028 to 56 mg/L
Turbidity	2% (1-499 NTU), ±4% (500-1600 NTU)	15	1 to 1600 NTU
Synoptic parameters			
Ammonia-N	80-120	10 %	0.01 mg/L
Alkalinity	80-120	10%	5 mg/L
Chloride	80-120	10%	0.1 mg/L
Hardness	80-120	10%	0/1 mg/L
Nitrate + nitrite-N	80-120	10 %	0.01 mg/L
Persulfate Nitrogen, Total	80-120	10%	0.01 mg/L
Phosphorus, soluble reactive	80-120	10 %	1 ug/L
Phosphorus, total	80-120	10 %	0.005 mg/L
Solids, total suspended	80-120	15 %	0.5 mg/L
$NO3^-$ (δ^{15}, $\delta^{17}O$, $\delta^{18}O$)	NA	20%	0.1%
Periphyton tissue			
Chlorophyll a	80-120	20%	0.1 ug/L
Percent Total Solids	80-120	20%	0.1%
Percent Total Organic Carbon	80-120	20%	0.1%
Total Metals[1]	80-120	20%	0.1-5 mg/Kg
Ash Free Dry Weight	NA	20%	0.05 ug/L
Total Carbon	85-115	20%	0.01% Total C
Total Nitrogen	85-115	20%	0.01% Total N
Total Phosphorus	85-115	20%	5.0 mg/Kg
$NO3^-$ ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	NA	20%	0.01%
Bioassessment			
Periphyton Taxonomy	Barbour 1999	20% RSD	NA
Macroinvertebrate Taxonomy	Barbour 1999	20% RSD	NA

[1]Al, As, Cd, Cu, Fe, Mn, Ni, P, Pb, Zn

6.2.1 Targets for precision, bias, and sensitivity

6.2.1.1 Precision

Precision is a measure of the variability in the results of replicate measurements due to random error. Random error is imparted by the variation in concentrations of samples from the environment as well as other introduced sources of variation (e.g., field and laboratory procedures). Precision for laboratory duplicate samples will be expressed as relative percent difference (RPD). Precision for field replicate samples will be expressed as the relative standard deviation (RSD) for the group of duplicate pairs (Table 9).

6.2.1.2 Bias

Bias is defined as the difference between the sample value and true value of the parameter being measured. Bias affecting measurement procedures can be inferred from the results of quality control (QC) procedures. Bias in field measurements and samples will be minimized by strictly following Ecology's measurement, sampling, and handling protocols (Table 14).

6.2.1.3 Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance. It is commonly described as detection limit (Table 9). In a regulatory sense, the method detection limit (MDL) is usually used to describe sensitivity. This should be done in terms of the lowest quantity of a physical or chemical parameter detectable (above background noise) by each field instrument or laboratory method.

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

Comparability will be achieved by assuring the same methods and Standard Operating Procedures (SOPs) are used in all synoptic, ambient, and continuous monitoring efforts.

All data used in statistical comparisons and trend analysis from all agencies will be assessed for precision before analysis. If data sets do not meet standards for precision and biases, they will not be used in any analysis.

6.2.2.2 Representativeness

The study is designed to have enough sampling sites at sufficient sampling frequency to meet study objectives. Water quality values are known to be highly variable over time and space. Sampling variability can be somewhat controlled by strictly following standard operating procedures and collecting QC samples, but natural spatial and temporal variability can contribute greatly to the overall variability in the results. Resources limit the number of samples that can be taken at one site spatially or over various intervals of time.

6.2.2.3 Completeness

EPA has defined completeness as a measure of the amount of valid data needed to be obtained from a measurement system (Lombard and Kirchmer, 2004). The goal for the Bertrand Creek watershed study is to correctly collect and analyze 100% of the samples for each of the sites. However, problems occasionally arise during sample collection that cannot be controlled; thus, a completeness of 95% is acceptable. Potential problems are flooding, site access problems, or sample container shortages.

7.0 Sampling Process Design (Experimental Design)

7.1 Study design

Ecology will employ a nested paired "above and below" sampling approach to evaluate the impact of a system of BMPs in the Bertrand Creek watershed. In the nested paired watershed design, one monitoring station is located above the treatment area and one station is located below the treatment area. We will monitor before, during, and after BMP implementation so that differences between nested areas can be evaluated. Also, trends in water quality will be evaluated during the study to assess progress of implementation activities.

Synoptic sampling for water quality, biological, and habitat parameters will also occur "within" the treatment area at or near locations where land uses change. Synoptic sampling will occur during low flow periods.

Water samples to be analyzed for FC and nutrients will be collected monthly, while samples to be analyzed for metals will be collected bi-monthly. Sampling will follow *Standard Operating Procedures for the Collection, Processing, and Analysis of Stream Samples* (Ward, 2007). Biological and habitat assessments will be conducted one time/year and will follow protocols outlined in *Quality Assurance Monitoring Plan, Ambient Biological Monitoring in Rivers and Stream: Benthic Macroinvertebrates and Periphyton* (Merritt, 2009).

Technical specifications for the continuous stations are included in Appendix A. The sensor array includes Satlantic SUNA™ and Hydrolab sensors. Sensors will be installed for continuous monitoring per specifications to the manufacturer's guidelines. Other equipment at the study site will follow Standard Operating Procedures for continuous telemetry site installation established by the Freshwater Monitoring (FMU) and the Stream Hydrology Unit SHU and *the Standard Operating Procedures for Turbidity Threshold Sampling*. Sensor-derived water quality parameters (e.g., nitrate-nitrite, oxygen, temperature, pH, conductivity, and turbidity) will be sampled continuously every 15 minutes.

7.1.1 Field measurements

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Meteorology and Hydrology

Air temperature and relative humidity data will be recorded at two stations near Bertrand Creek. Meteorological data will be obtained from the Ag WeatherNet station at Lynden. Table 10 shows nearby weather stations and available data. Continuous hydrology data for Bertrand Creek will be obtained from two existing flow gages (above 0 Ave, BC Canada, and above Rathbone Road near the confluence with the Nooksack River (Table 11).

Table 10. Summary of weather stations, location, and available data.

Station ID	Location	Network/ Origin	Air Temp	Dew Point	Relative Humidity	Precipitation	Wind Direction
Lynden	Lynden	Washington State University AgWeatherNet	x	x	x	x	x
Bertrand Creek @ 0 Ave	Bertrand Creek at Boarder	DOE	x			x	
Bertrand Creek @ Rathbone Rd	Bertrand Creek at Rathbone Road	DOE	x			x	

Table 11. Current stream gauge location in the Bertrand Creek watershed.

Location	Data Originator	Period of Record
Bertrand Creek at Boarder	USGS (12212390)	2007-present
Bertrand Creek at Rathbone Road	DOE	2002-present

Ecology will estimate continuous hydrology for other sources based on regression with gage stations or interpolation between flows measured during the synoptic surveys. Known water withdrawals will be subtracted from the flow balance. Groundwater gains (or losses) will be estimated as the residual in the flow balance.

Ambient and Continuous Water Quality Monitoring

Beginning October of 2013, Ecology's Freshwater Monitoring Unit added two monitoring sites on its network of statewide monitoring stations. Both continuous and ambient water quality data are being collected on Bertrand Creek at 0 Ave in British Columbia, Canada and at Rathbone Road near the confluence with the Nooksack River. Continuous measurements include temperature, dissolved oxygen, pH, turbidity, and nitrate-nitrite. Monthly grab samples include ammonia-N, FC, nitrate+nitrite-N, soluble reactive phosphorus, soluble reactive, suspended solids, total nitrogen, total phosphorus, turbidity, metals, and hardness.

Biological and Habitat Assessment

Biological communities provide information about environmental conditions based on the individual taxa ranges of sensitivity to environmental conditions. Ecology's Biological Monitoring staff will assess benthic macroinvertebrate and periphyton communities to assess stream conditions.

Where Ecology collects biological samples, we will also take physical habitat measurements and water and sediment samples, to describe the environment at the time of sampling. Biological and habitat assessments will be conducted by Ecology's Biological Monitoring staff following their methodology and protocols (Merritt, 2009).

Nitrogen Isotope Monitoring

In collaboration with the University of Washington (UW), school of Aquatic and Fishery Sciences, Ecology will collect NO_3^- triple isotopes that will identify NO_3^- sources and how NO_3^- loading varies with geophysical, habitat, and land use patterns. Monthly NO_3^- sampling in both surface waters and periphyton samples will occur at both ambient monitoring sites and synoptic survey sites. This sampling is part of a larger Puget Sound-wide study that will identify sources of nitrogen and sinks and could assist in identifying potential measures to mitigate NO_3^- pollution to Puget Sound.

Source identification and Optical Brightener (OB) Surveys

In conjunction with targeted sampling, Ecology staff plans to use fluorometry as an inexpensive and practical bacterial source tracking (BST) method to identify or confirm *human* sources of fecal contamination. Fluorometry is a chemical BST method which identifies human fecal contamination by detecting OBs, also known as fluorescent whitening agents. Optical brighteners are added to most laundry detergents and represent about 0.15% of the total detergent weight (Hartel et al., 2008). Because household plumbing systems mix with effluent from washing machines and toilets together, OBs are associated with human sewage in septic systems and wastewater treatment plants (Hartel et al., 2008).

Synoptic Surveys

Based on results of continuous and ambient water quality monitoring results, Ecology will conduct synoptic surveys during the low flow period within treatment areas to evaluate water quality impacts from different land uses or land use practices. Surveys will be conducted at breaks in land uses, e.g., urban, residential, and agricultural. Data will be used to determine if further pollution control measures are needed.

Synoptic data collection will include:

- Multiple probes deployed to collect continuous diel data (at 15-minute intervals) for temperature, pH, dissolved oxygen, and specific conductance at the mainstem sites and other significant sources.

- Grab samples for ammonia-N, FC, nitrate + nitrite-N, soluble reactive phosphorus, soluble reactive, suspended solids, total nitrogen, nitrogen isotopes, and total phosphorus. Grab samples collected in early morning and later afternoon to assess diurnal swings in continuous data.
    - Streamflow measured manually at sites sampled when and where logistically feasible, depending on safe conditions.
- Biological and habitat assessments possibly conducted at all sites one time each year with the following parameters collected:
    - Macroinvertebrates, periphyton, biomass, periphyton metals, periphyton nutrients, sediment metals, and other instream habitat parameters.

Implementation monitoring

For the purposes of this study, implementation monitoring will document the pollution control activities that were implemented in the watershed over the study period. Additionally, Ecology will attempt to document existing pollution control measures. The most common use of implementation monitoring is to determine what and where BMPs were implemented. Typically this is carried out as a review or site inspection and does not involve any water quality measurements.

Implementation monitoring itself cannot directly link management activities to water quality changes. It must be supported by adequate water quality monitoring design that is capable of providing reasonable assurances of progress being made towards meeting water quality standards. These monitoring activities are a critical part of an evaluation and are necessary to meet many of the objectives outlined in this study.

Implementation of pollution control activities in the Bertrand Creek watershed fall primarily under the jurisdiction of Whatcom County Health Department, Ecology, and Washington Department of Agriculture (WDA). All agencies have been actively involved in water quality protection and cleanup actions through site inspections and enforcement of state water quality law. Additionally, the Whatcom Conservation District (WCD) plays an active role in the watershed by providing assistance to landowners who wish to implement BMPs that protect surface waters.

Ecology will identify present pollution control measures and regulatory actions throughout the study area over time. This information will be used to develop implementation metrics which will be associated with improvements in water quality over time. The Whatcom Clean Water Program (http://www.ecy.wa.gov/water/WhatcomCleanWater.html) and the Whatcom Conservation District will be the primary sources of data. Other potential sources of water quality improvement project data are identified in Collyard and Onwumere (2013).

7.1.2 Sampling location and frequency

Sampling locations are described in Table 12 and Figure 5. The FMU will conduct monthly ambient monitoring at Bertrand Creek at Ave 0 and Rathbone Road. Continuous monitoring of selected water quality parameters will also be collected at these stations throughout the course of this study. Bioassessment and habitat monitoring will be conducted during Ecology's biological assessment index period (between July and October). Synoptic sampling will be conducted when flows are expected to be the lowest (between August and September).

Field staff will perform reconnaissance on these sites before the first synoptic survey. In the course of the synoptic surveys, additional stations may be added to the sampling network. Table 12. Proposed sampling locations for 2013 Bertrand Creek baseline monitoring.

Primary Land use	Station Name	Description	Latitude	Longitude
Ambient and Continuous Monitoring Stations (all season)				
Agriculture	Bertrand Creek @ 0 Ave	Located on the U.S./Canadian Border near USGS gage# 12212390	49.00250	-122.52320
Agriculture	Bertrand Creek @ Rathbone Rd	Located on Bridge at river mile 1	48.92400	-122.52990
Synoptic Monitoring Stations (low flow season)				
Agriculture	Bertrand Creek at H St	Private access upstream of H St bridge crossing	48.9939	-122.509
Agriculture	Bertrand Creek below Jackman Ditch	Private access downstream of Jackman Ditch	48.9739	-122.5078
Mixed residential	Bertrand Creek at Berthusen Park	Public access downstream of Berthusen Memorial Drive	48.9575	-122.5096
Mixed residential	Bertrand Creek at Loomis Trail Road	Private access downstream of Loomis Trail Road	48.9482	-122.5272
Agriculture	Bertrand Creek at Birch Bay Lynden Road	Private access upstream of Birch Bay Lynden Road	48.9362	-122.5358

7.1.3 Parameters to be determined

See Table 13.

7.2   Maps or diagram

See Figure 5.

7.3   Assumptions underlying design

Not applicable.

7.4   Relation to objectives and site characteristics

Not applicable.

7.5   Characteristics of existing data

Not applicable.

8.0   Sampling Procedures

8.1   Field measurement and field sampling SOPs

Field sampling and measurement protocols will follow Ecology's SOPs (Table 13). The sampling procedures will follow those described in *Standard Operating Procedures for Hydrolab® DataSonde® and MiniSonde® Multiprobes* (Swanson, 2007), modified as necessary in accordance with users manuals to account for luminescent-type oxygen probes.

Sampling procedures for lab-analyzed samples will follow procedures in Ward (2007). Biological and habitat samples will be collected at selected, using Ecology protocols (Merritt, 2009). Biological samples will be collected in riffle areas within stream reaches. The stream reach will be defined as 20 times bankfull width. In addition, monthly periphyton samples will be collected using Ecology SOP EAP085 (Mathieu et al., 2013). Greater than ten percent of the biological samples will be replicated in the field in a-side-by side manner, to assess field and laboratory variability.

Table 13. Field sampling and measurement methods and protocols.

Parameter	Measurement/Sample Type	Lab Method	Field Protocol
Ambient Water Quality Samples	Grab samples	See Appendix A	Hallock and Ehinger (2003)
Continuous Water Quality Monitoring	Hydrolab multi-parameter sonde	See Appendix A	Hallock (2009)
Synoptic Water quality samples (see Table for list)	Grab samples	See Table 11	Ward (2007)
Synoptic Continuous DO, pH, Conductivity and Temperature	Hydrolab multi-parameter sonde	n/a	EAP033 (Swanson, 2010)
Flow	Instantaneous	n/a	EAP024 (Kardouni, 2013)
Periphyton	In stream	See Table 11	EAP073 (Mathieu et al., 2013)
Bioassessment and Habitat	In stream	n/a	Merritt (2009)
Optical Brighteners	Cyclops 7 sensor	n/a	EAP091 (Anderson and Swanson, 2014)

8.2 Containers, preservation methods, holding times

Field staff will collect grab samples directly into pre-cleaned/sterilized containers supplied by Manchester Environmental Laboratory (MEL) and described in their *Lab Users Manual* (MEL, 2008). Table 14 lists the sample parameters, containers, volumes, preservation requirements, and holding times. Field staff will store samples for laboratory analysis on ice and deliver to MEL within 24 hours of collection via either the Ecology courier or direct drop-off after sampling. MEL follows standard analytical methods outlined in their *Lab Users Manual* (MEL, 2008). Isotope samples will be delivered to the UW within 48 hours of collection by Ecology staff. UW will follow a standard analytical methods outlined in Appendix B.

Table 14. Container type, required water volume, method of preservation, and maximum permissible holding times for synoptic lab-analyzed samples.

Analyte	Container Type	Sample Volume (mL)	Preservation	Holding Time
Water quality				
Ammonia-N	Poly	125	adjust to pH<2 w/ H2SO4 and cool to 4C	28 days
Alkalinity	Poly	500 mL no headspace	Cool to ≤6°C; Fill bottle completely; DO NOT agitate sample	14 days
Chloride	Poly	100 mL	Cool to 6°C	28 days
Hardness	Poly	125	H2SO4 to pH<2, cool to≤6°C	6 months
Nitrate + nitrite-N	Poly	125	adjust to pH <2 w/ H2SO4 and cool to <4C	28 days
Nitrogen, total	Poly	125	adjust to pH<2 w/ H2SO4 and cool to <4C	28 days
Persulfate Nitrogen, total	Poly	125	adjust to pH<2 w/ H2SO4 and cool to <4C	28 days
Phosphorus, soluble reactive	Brown poly	125	filter in field and cool to <4C	48 hrs
Phosphorus, total	Poly	60	adjust to pH<2 w/ H2SO4 and cool to <4C	28 days
Solids, total suspended	Poly	1000	cool to <4C	7 days
NO3$^-$ (δ^{15}N, δ^{17}O, δ^{18}O)	Poly	125	adjust to pH<2 w/ H2SO4 and cool to <4C	28 days
Periphyton				
Chlorophyll <u>a</u>	Glass test tube w/acetone	1000 mL	Cool to <6C keep in dark	28 days post
Ash Free Dry Weight	Poly	200 mL	Cool to <6C	24 hr pre-filtration: 28 days post
Percent Total Solids	Poly centrifuge tube	1 g ww	Cool to <6C	7 days
Metals[1]	Poly centrifuge tube	1 g ww	Cool to <6C	6 months
Percent Total Organic Carbon	Poly centrifuge tube	1 g ww	Cool to <6C	28 days
Total Carbon & Nitrogen	Poly centrifuge tube	1 g ww	Cool slurry to ≤4°C; keep in dark; dry filter at 103-105°C & store in desiccator	24 hr pre-filtration; 100 days post
Phosphorus	Poly centrifuge tube	1 g ww	Cool to <4C keep in dark	14 days pre-acidification; 6 months post
NO3$^-$ (δ^{15}N, δ^{17}O, δ^{18}O)	Poly centrifuge tube	5 1g ww	Cool to <6C keep in dark	28 days

8.3 Invasive species evaluation

Field staff will follow EAP's SOP070 on minimizing the spread of invasive species (Parsons et al., 2012). The Bertrand Creek study area is not in an area of extreme concern. Areas of extreme concern have or may have invasive species like New Zealand mud snails that are particularly hard to clean off equipment and are especially disruptive to native ecological communities. For more information, please see Ecology's website on minimizing the spread of invasive species at www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html.

8.4 Equipment decontamination

At the end of each field visit, field staff will follow procedures specified in *Standard Operating Procedure for Areas of Moderate Concern* to minimize the risk of spreading aquatic invasive species. If stations are located in Areas of Extreme Concern, staff will follow procedures in *Standard Operating Procedures to Prevent Accidental Introductions of Aquatic Organisms from Areas of Extreme Concern Through Aquatic Plant Monitoring Activities* (Parsons, 2012).

8.5 Sample ID

MEL will provide the field lead with work order numbers for all scheduled sampling dates. The work order number will be combined with a field ID number that is given by the field lead. This combination of work order number and field ID number constitute the sample ID. All sample IDs will be recorded in field logs and in an electronic spreadsheet for tracking purposes.

8.6 Chain-of-custody, if required

Once collected, samples will be stored in ice-containing coolers in the sampling vehicle. When field staff are not in the sampling vehicle, it will be locked to maintain chain-of-custody. Upon return to the Operations Center, staff will complete the chain-of-custody portion of the Laboratory Analysis Required sheet and will place the coolers in the walk-in cooler.

8.7 Field log requirements

A field log will be maintained by the field lead and used during each sampling event. The following information will be recorded during each visit to each site:
- Name of location
- Field staff
- Environmental conditions
- Date, Time, Sample ID, samples collected, identity of QC samples
- Field measurement results
- Pertinent observations
- Any problems with sampling

Data collected using the OB sensor and/or logger will be recorded electronically. However, a separate log sheet will be maintained for each location that the OB sensor is used. If the OB sensor is being used to collect real time data, the following information will be recorded:

- Name of location
- Field staff
- Environmental conditions
- Date, start and stop times
- Location of deployment (logger only)
- Description of area covered
- Pertinent observations
- Any problems with the OB sensor

8.8 Other activities

Any field staff new to the type of sampling being conducted for this study will be trained by senior field staff or the project manager, following relevant Ecology SOPs. Any maintenance needed for the YSI Exo, Turner Designs Cyclops 7 or Hydrolab MiniSonde® will be performed by trained field staff, following Ecology's SOP EAP033 and manufacturer instructions and recommendations. Before sampling begins, staff will send MEL a schedule of sampling events. This will allow the lab to plan for the arrival of samples. The lab will be notified immediately if there will be any deviations from the scheduled date of sampling. To ensure that the appropriate number and type of required sample containers are available, the field lead will work with the laboratory courier to develop a schedule for delivery of sampling containers.

9.0 Measurement Methods

9.1 Field procedures table/field analysis table

Manchester Environmental Laboratory (MEL) conducts laboratory analyses and laboratory procedures following Standard Operating Procedures and other guidance documents. Analytical methods and lower reporting limits are listed in Table 15. University of Washington Seattle will conduct the nitrogen isotope analyses and laboratory procedures following methods provided in Appendix B.

Field sampling and measurement protocols will follow SOPs developed by the Environmental Assessment Program (Table 15).

9.2 Lab procedures table

Table 15. Laboratory analytical methods and reporting limits for lab-analyzed synoptic samples.

Analyte	Sample Matrix	# of Samples	Expected Range of Results	Method[2]	Method Detection Limit
Water Quality					
Ammonia-N	Water	25	0.01-0.25 mg/L	SM4500NH3H	0.01 mg/L
Alkalinity	Water	25	5.0-300 mg/L	EPA Method 310.2	5 mg/L
Chloride	Water	25	0.10-20 mg/L	EPA method 300.0	0.1 mg/L
Hardness	Water	25	10-300 mg/L	SM2340B	0/1 mg/L
Nitrate + nitrite-N	Water	25	0.01-6 mg/L	SM4500NO3I	0.01 mg/L
Persulfate Nitrogen, Total	Water	25	0.005-0.5 mg/L	SM2540D	0.005 mg/L
Phosphorus, soluble reactive	Water	25	0.003-0.15 mg/L	SM4500PG	0.003 mg/L
Phosphorus, total	Water	25	0.005-0.2 mg/L	SM4500PF	0.005 mg/L
Solids, total suspended	Water	25	1 – 20 mg/L	SM2540D	1 mg/L
NO_3^- ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	Water	25	n/a	Appendix B	n/a
Periphyton (Plant Tissue)					
Chlorophyll <u>a</u>	Plant tissue	25	0.05 – 100 ug/L	SM10300C(5)	0.05 ug/L
Ash Free Dry Weight	Plant tissue	25	0.05-5 mg	SM10300C	0.05 mg
Percent Total Solids	Plant tissue	25	1-20%	EPA2540	1-100%
Metals[1]	Plant tissue	25	0.05 – 2000 mg/Kg	EPA200.2 EPA200.7	0.05 – 5 mg/Kg
Percent Total Organic Carbon	Plant tissue	25	1-30 %	SM5310B	0.1% carbon
Total Carbon & Nitrogen	Plant tissue	25	0.1-1% of DW	EPA440	0.01% of DW
Total Phosphorus	Plant tissue	25	0.01-1% of DW	EPA200.7	0.01% of DW
NO_3^- ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	Plant tissue	25	Variable	Appendix D	n/a
Bioassessment					
Periphyton Taxonomy	Stream riffles	25	Variable	Barbour 1999	n/a
Macroinvertebrate Taxonomy	Stream riffles	12	Variable	Barbour 1999	n/a

[1]Metals: As, Al, Cd, Cu, Fe, Mn, Ni, P, Pb, Zn
[2] SM: Standard Methods (APHA 1998); EPA: Environmental Protection Agency

9.3 Sample preparation method(s)

Periphyton will be sampled by removing rocks from sampling point. Before staff processing, they will lightly rinse rock surfaces with reverse osmosis/de-ionized (RO/DI) water to remove loosely bound sediment and macroinvertebrates. The surfaces of the rocks will then be scraped with a stiff plastic brush to remove the loosely attached periphyton matrix. This material will be composited in a plastic tray rinsed into a 1-L acid-washed bottle, using RO/DI water, and placed on ice. A minimum of 125 square cm will be sampled at each sampling point.

Periphyton samples will then be prepared for chlorophyll-a and ash-free dry weight analysis by filtering 10 mL sub-sample through a 0.45 micron filter. Remaining composite samples will then be split, centrifuged, and analyzed for percent total solids, total metals, and %TOC. See Table 14 in Section 8.2 for appropriate sample containers and holding times.

9.4 Special method requirements

There are no special methods that will be used for this study.

9.5 Lab(s) accredited for method(s)

All chemical analysis, except for isotope and periphyton nitrogen and carbon, will be performed at MEL, which is accredited for all methods (Table 10). University of Washington Seattle will perform nitrogen isotope and periphyton nitrogen and carbon analysis. Rhithron Associates, Inc. in Missoula, Montana will process and analyze macroinvertebrate and periphyton samples.

10.0 Quality Control (QC) Procedures

10.1 Table of field and laboratory QC required

Manchester Environmental Laboratory (MEL) conducts our laboratory analyses and laboratory procedures following Standard Operating Procedures and other guidance documents. Analytical methods and lower reporting limits are listed in Tables 15 and 16. The University of Washington Seattle will conduct the nitrogen isotope analyses and laboratory procedures following methods provided in Appendix B.

Table 16. Laboratory analytical methods and reporting limits for lab-analyzed synoptic samples.

Parameter	Field		Laboratory			
	Blanks	Replicates	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spikes
Ammonia-N	10%	10%	1/batch	1/batch	1/batch	1/batch
Alkalinity	10%	10%	1/batch	1/batch	1/batch	1/batch
Chloride	10%	10%	1/batch	1/batch	1/batch	1/batch
Hardness	10%	10%	1/batch	1/batch	1/batch	1/batch
Nitrate + nitrite-N	10%	10%	1/batch	1/batch	1/batch	1/batch
Persulfate Nitrogen, Total	10%	10%	1/batch	1/batch	1/batch	1/batch
Phosphorus, soluble reactive	10%	10%	1/batch	1/batch	1/batch	1/batch
Phosphorus, total	10%	10%	1/batch	1/batch	1/batch	1/batch
Solids, total suspended	10%	10%	1/batch	1/batch	1/batch	1/batch
$NO3^-$ ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	10%	10%	1/batch	1/batch	1/batch	n/a
Periphyton Tissue						
Chlorophyll <u>a</u>	n/a	10%	n/a	1/batch	1/batch	n/a
Ash Free Dry Weight	n/a	10%	n/a	1/batch	1/batch	n/a
Percent Total Solids	n/a	10%	n/a	1/batch	1/batch	n/a
Metals[1]	n/a	10%	n/a	1/batch	1/batch	n/a
Percent Total Organic Carbon	n/a	10%	n/a	1/batch	1/batch	n/a
Total Carbon & Nitrogen	n/a	10%	n/a	1/batch	1/batch	n/a
Phosphorus	n/a	10%	n/a	1/batch	1/batch	n/a
$NO3^-$ ($\delta^{15}N$, $\delta^{17}O$, $\delta^{18}O$)	n/a	10%	n/a	1/batch	1/batch	n/a
Periphyton Taxonomy	n/a	10%	n/a	1/batch	1/batch	n/a
Macroinvertebrate Taxonomy	n/a	10%	n/a	1/batch	1/batch	n/a

SM: Standard Methods (APHA 1998); EPA: Environmental Protection Agency.
[1]Metals: As, Al, Cd, Cu, Fe, Mn, Ni, P, Pb, Zn

Field sampling and measurement protocols will follow Ecology's SOPs (Table 13).

10.2 Corrective action processes

QC results may indicate problems with data during the course of the project. The lab will follow prescribed procedures to resolve the problems. Options for corrective actions might include:

- Retrieving missing information.
- Re-calibrating the measurement system.
- Re-analyzing samples within holding time requirements.
- Modifying the analytical procedures.
- Requesting additional sample collection or additional field measurements.
- Qualifying results.

11.0   Data Management Procedures

11.1 Data recording/reporting requirements

Staff will record all field data in a field notebook or an equivalent electronic collection platform. Before leaving each site, staff will check field notebooks or electronic data forms for missing or improbable measurements. Staff will enter field-generated data into Microsoft (MS) Excel® spreadsheets as soon as practical after they return from the field. If data were collected electronically, data will be backed up on Ecology servers when staff return from the field. The field assistant will check data entry against the field notebook data for errors and omissions. The field assistant will notify the field lead or project manager of missing or unusual data.

Lab results will be checked for missing and/or improbable data. MEL will send data through Ecology's Laboratory Information Management System (LIMS). The field lead will check MEL's data for omissions against the "Request for Analysis" forms. The project manager will review data requiring additional qualifiers.

In addition, data will be provided either on Ecology's Effective Monitoring web page (http://www.ecy.wa.gov/programs/eap/tem/index.html), Ecology's Stream Monitoring web page (www.ecy.wa.gov/programs/eap/fw_riv/rv_main.html) or on Ecology's Flow Monitoring web page (www.ecy.wa.gov/programs/eap/flow/shu_main.html).

Field and laboratory data will be tested for trends, using a Seasonal Kendall trend test in SYSTAT® version 13. Summary statistics for all data will be generated using MS Excel®.

11.2 Laboratory data package requirements

Laboratory-generated data reduction, review, and reporting will follow the procedures outlined in the MEL Users Manual (MEL, 2008). Variability in lab duplicates will be quantified, using the procedures outlined in the MEL Users 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.

11.3 Electronic transfer requirements

MEL will provide all data electronically to the project manager through the LIMS to EIM data feed. There is already a protocol in place for how and what MEL transfers to EIM through LIMS.

11.4 Acceptance criteria for existing data

Not applicable. No special criteria are necessary to assess the usability of existing data.

11.5 EIM/STORET data upload procedures

All water quality data will be entered into EIM, following all existing Ecology business rules and the EIM User's Manual for loading, data quality checks, and editing.

12.0   Audits and Reports

12.1 Number, frequency, type, and schedule of audits

12.1 Number, frequency, type, and schedule of audits

No audits will be conducted during this study because of practical constraints. However, there could be a field consistency review by another experienced EAP field staff during the period of this project. The aim of this review is to improve field work consistency, improve adherence to SOPs, provide a forum for sharing innovations, and strengthen our data QA program.

12.2 Responsible personnel

Not applicable.

12.3 Frequency and distribution of report

See Section 5.4.

12.4 Responsibility for reports

See Section 5.4.

13.0   Data Verification

13.1 Field data verification, requirements, and responsibilities

The field lead will verify initial field data before leaving each site. This process involves checking the data sheet for omissions or outliers. If measurement data are missing or a measurement is determined to be an outlier, the measurement will be repeated.

After each sampling week, the field assistant will compare all field data to determine compliance with MQOs. The field assistant will note values that are out of compliance with the MQOs and will notify the field lead. At the conclusion of the study, the field lead will compile a summary of all out of compliance values (if any) and provide it to the project manager for a decision on usability.

13.2 Lab data verification

MEL staff will perform the laboratory verification following standard laboratory practices. After the laboratory verification, the field lead will perform a secondary verification of each data package. This secondary verification will entail a detailed review of all parts of the laboratory data package with special attention to laboratory QC results. The field lead will bring any discovered issues to the project manager for resolution.

13.3 Validation requirements

All laboratory data that have been verified by MEL staff will be validated by a project staff member. Field measurement data that was verified by a project staff member will be validated by a different staff member.

After data entry and data validation tasks are completed, all field, laboratory, and flow data will be entered into the EIM system. EIM data will be independently reviewed by another field assistant for errors at an initial 10% frequency. If significant entry errors are discovered, a more intensive review will be undertaken.

14.0   Data Quality (Usability) Assessment

14.1 Process for determining whether project objectives have been met

After all laboratory and field data are verified and validated, the field lead or project manager will thoroughly examine the data package, using statistics and professional judgment, to determine if MQOs have been met. The project manager will examine the entire data package to determine if all the criteria for MQOs, completeness, representativeness, and comparability have been met. If the criteria have not been met, the field lead and project manager will decide if affected data should be qualified or rejected based upon the decision criteria in the QAPP. The project manager will decide how any qualified data will be used in the technical analysis.

14.2 Data analysis and presentation methods

Data analysis consists of comparing results to water quality standards and detecting changes in monitoring parameters over time. Procedures comparing results to water quality standards are defined in Ecology's Water Quality Program Policy 1-11 (www.ecy.wa.gov/programs/wq/303d/policy1-11Rev.html), in Ecology's Guidance for Effectiveness Monitoring of Total Maximum Daily Loads in Surface Waters (Collyard and Onwumere, 2013).

The sampling design will be considered successful if project objectives are met.

14.3 Treatment of non-detects

Any non-detects will be included in the study analysis. To do this, the non-detect will be replaced by half the detection limit.

14.4 Sampling design evaluation

The project manager will decide whether the data package meets the MQOs, criteria for completeness, representativeness, and comparability, and whether meaningful conclusions (with enough statistical power) can be drawn from the Seasonal Kendall and summary statistics. If so, the sampling design will be considered effective.

14.5 Documentation of assessment

In the technical report, the project manager will include a summary of the data quality assessment findings. This summary is usually included in the data quality section of reports.

15.0   References

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16.0 Appendices

Appendix A. Technical Specification for Continuous Monitoring Stations

Sondes used for continuous monitoring will be deployed in a 2½ inch pipe married to pre-existing stage recording infrastructure (Figures A-1, A-2, A-3, and A-4). A basket at the end of the pipe secures the sonde but allows free exchange of water to the sensors. A standard sonde cable runs through the pipe and connects the sonde to an SDI-12 cable, which connects to a data logger.

Sensor-derived water quality parameters (i.e., nitrate + nitrite, oxygen, temperature, pH, Conductivity and turbidity) will be sampled continuously every 15 minutes in Bertrand Creek at Rathbone Road, and on at the on the Canadian side of the border with the United States(Bertrand Creek @ Ave 0). Both stations will have telemetry capabilities (GOES equipped stations/Data Collection Platforms (DCP)) that will transmit data in three hour blocks to the Department of Ecology Headquarters in Olympia, Washington via a satellite transmitter. These transmissions are received at Ecology Headquarters by an LRGS (Local Readout Ground Station) system. This receiver is located on the roof of the Ecology Headquarters building (Figure A-1). Transmitted data is automatically imported into the Hystra®, a commercial database designed for the management and analysis of hydrologic/water quality data and published to Ecology's website.

Manual calibration readings and handheld field meter readings are entered into the database by the sampler. These readings are entered into the database by the investigator and validated by senior level staff. GOES transmissions are processed at Ecology using DECODES, a commercial software package designed by Ilex Engineering™, to filter and archive raw satellite transmission data. Decoded transmissions are routed to a raw transmission file for the Hydstra® system to processes every 60 minutes. The real-time web reports are generated using the processed transmission files.
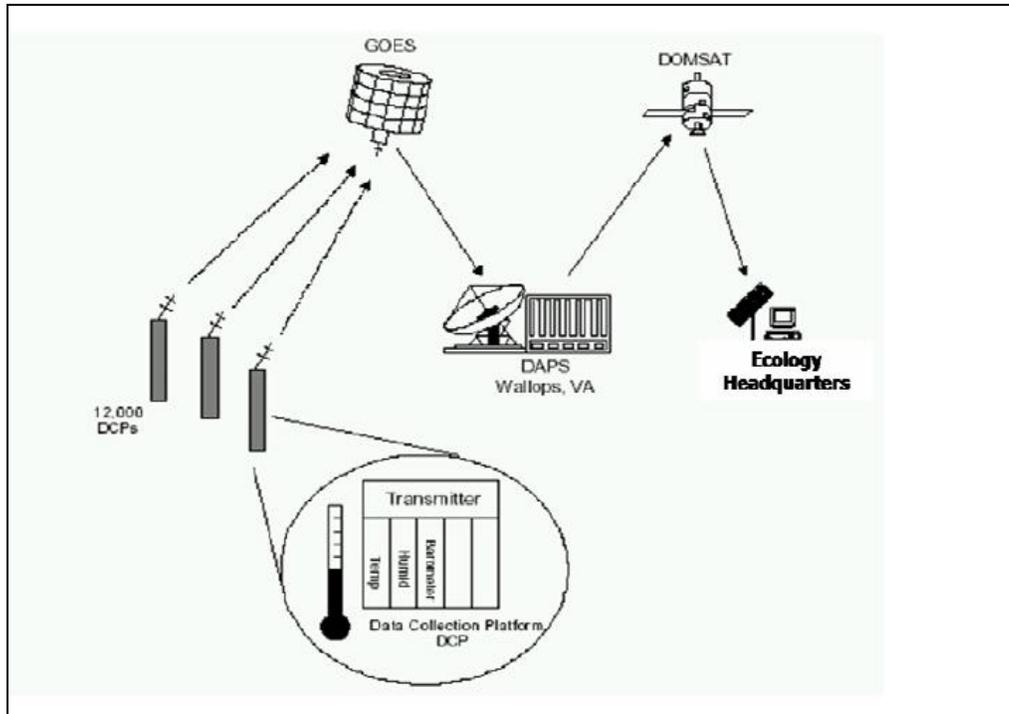
Figure A-1. GOES/DOMSAT diagram describing data flow from field sensors to Ecology Headquarters.

Type 2. GAGE HOUSE

Front View

Side View

SLANT PIPE
AS BUILT

5'

3'

A) GOES Antenna	I) 2" Galvanized Pipe 90
B) GOES Mast	J) 2" Galvanized Pipe Nipple-2"
C) Pressure Treated 4X4 -8ft	K) 2" Galvanized Pipe 90
D) Electrical Panel	L) 2" Galvanized Pipe (Length as Built)
E) Pressure Treated 2X6-3ft	M) Post Backfill
F) 2" Galvanized Pipe "T"	N) 2" Galvanized Pipe Couple
G) Solar Panel	O) 2" Galvanized Pipe Nipple-12"
H) 2" Galvanized Pipe Nipple-12"	P) 2" Brass End Cap

Figure A-2. Technical specifications for gage house structure.
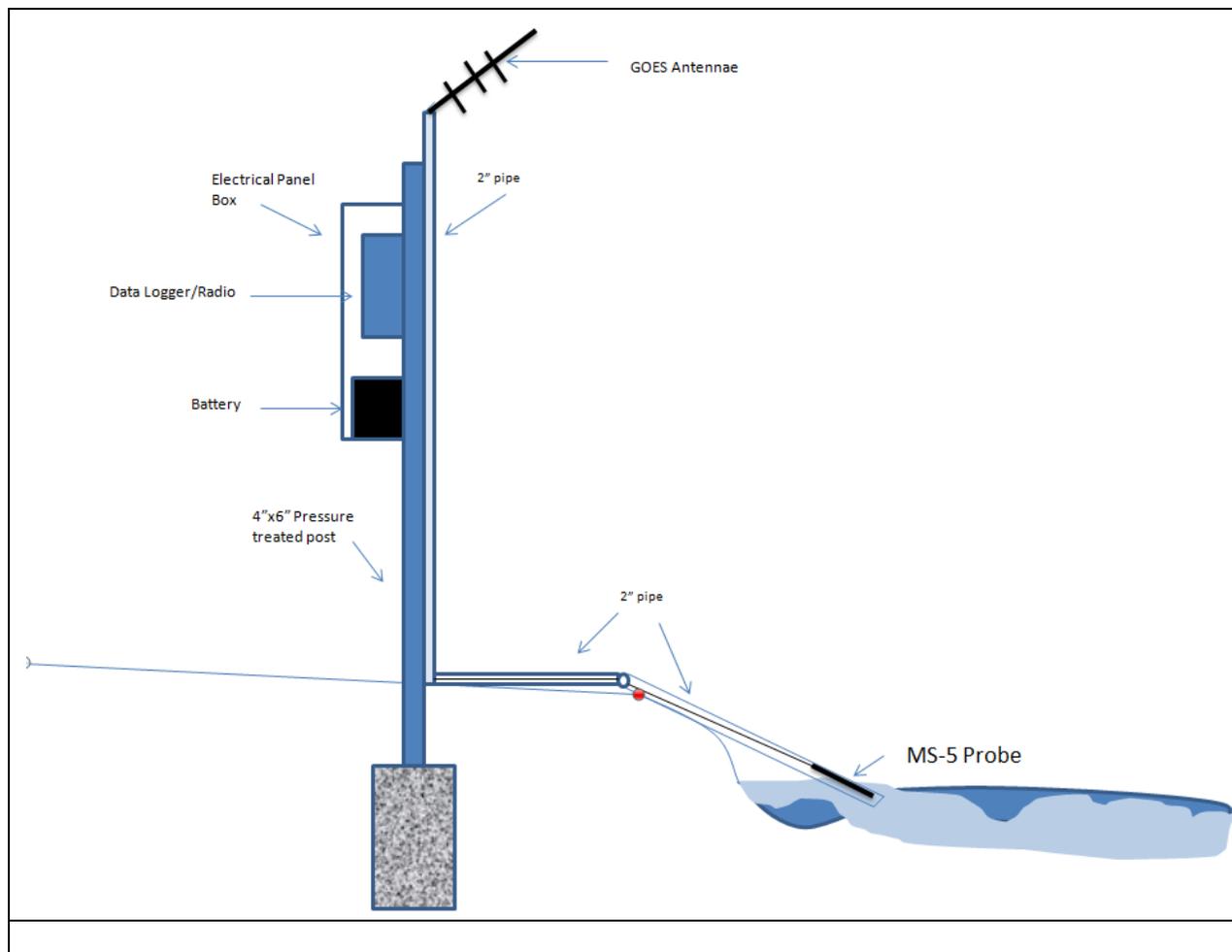
Figure A-3. Technical specifications for gage house structure with MS-5 Probe.
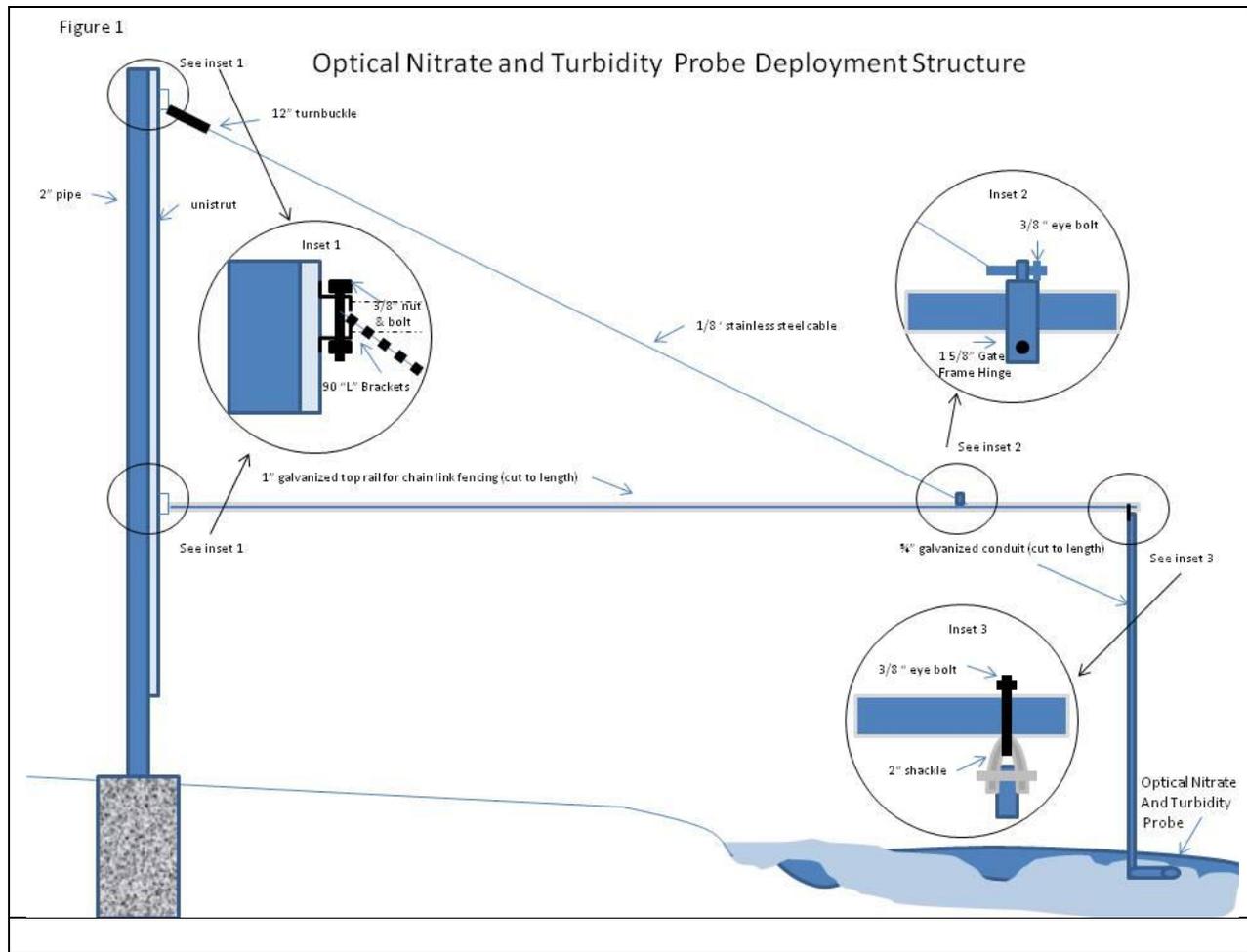
Figure A-4. Technical specifications for optical nitrate and turbidity probe deployment structure.

Appendix B. Quality Control for Procedures for Ambient Monitoring Lab-analyzed Parameters

Field

The accuracy and instrument bias of each sensor will be verified through independent field meter measurements (except nitrate-N) made before and after monthly servicing. Furthermore, accuracy and instrument bias will also be evaluated in field (i.e., conductivity, pH, and dissolved oxygen) and post-deployment calibration checks following the procedures described in Swanson (2010) and with deployment, retrieval, and monthly grab check samples collected as described in Ward (2007). Quality control associated with grab samples is described in Hallock and Ehinger and evaluated annually (Hallock, 2009).

Continuous multiple-parameter monitoring

Procedures outlined in Hallock (2009) will be used to assess the quality of data collected by multi-parameter probes.

Comparisons of grab sample results and field meter measurements to continuous results are determined by linear interpolation between the recorded results preceding and following the grab sample time. All times were first adjusted to Pacific Standard Time. The following QC checks will be performed:

- Examination of a plot of continuous data overlaid with grab sample data for signs of outliers (caused, for example, by signal noise) in the continuous data, or drift in the continuous data compared to the grab data.
- Calculation of the mean difference between continuous and grab sample results. If >2%, continuous results were adjusted for offset and drift, where such adjustment was appropriate as indicated by a plot of the data. This adjustment was made prior to conducting additional QC evaluations.
- Comparison of the average relative standard deviation (RSD) of continuous and grab sample data pairs to the precision requirements (Hallock, 2009).
- Comparison of individual differences between continuous and grab sample results to the accuracy requirements (Hallock, 2009).

If check samples and the post-deployment calibration are not consistent, or if data do not meet quality objectives after adjustments, then the data will be qualified or rejected. Pre-deployment, post-deployment, and anomalous data will be qualified as "REJ" from the raw data set and not used.

Continuous nitrate monitoring

Biofouling and matrix effects such as absorbance by dissolved constituents (i.e., increasing dissolved organic carbon concentrations) and suspended particles (increased turbidity) during the course of deployment can introduce a bias in nitrate-nitrite measurements.

In order to reduce the measurement error associated with biofouling and matrix effects, field servicing of the SUNA sensors are required to ensure the probes are working properly. In order to meet QC criteria, general maintenance and field operations will be conducted during the duration of the deployment period. Standard protocols will include general instrument maintenance, sensor inspection, calibration checks, field cleaning and troubleshooting. Furthermore, quality control protocols described in "USGS: Optical Techniques for the Determination of Nitrate in Environmental Waters: Guidelines for Instrument Selection, Operation, Deployment, Maintenance, Quality Assurance and Data Reporting (Pellerin et al., 2013) will be integrated into the quality assurance process.

Sensor Accuracy Ratings (Table B-1) for Nitrate-N defined by Pellerin et al. (2013) will be used as QC criterion to flag data ($\pm$1-3 times sensor accuracy) during the monthly QC evaluation process and assist with maintenance operations and troubleshooting logistics (Tables B-2 and B-3).

Table B-1. Accuracy ratings based on the absolute sums of the combined fouling, drift, and bias corrections to discrete samples for continuous ultraviolet nitrate measurements.

Analyte	Sensor Accuracy (deviation)	Accuracy Rating	Specifications
Nitrate-N (Satlantic SUNA)	$\pm$0.028 mg/L or $\pm$10% of reading, whichever is greater	Excellent	Within sensor accuracy
		Good	$\pm$1-3 times sensor accuracy
		Fair	$\pm$3-4 times sensor accuracy
		Poor	$\pm$4-6 times sensor accuracy

Table B-2. Daily Maintenance Operations Maintenance and troubleshooting logistics for the Satlantic UV Nitrate-N Sensors.

Maintenance Operation	Method of Determination	Frequency	Location	Troubleshooting Logistics
Review nitrate-N data, assign QA codes (see QC codes for continuous measurements Hallock, 2009)	Inspection of real time remote access telemetry data	Weekly	Ecology HQ	If data are assigned a "Fair Accuracy Rating", flag the data and dispatch field crew to site for servicing within 1-5 days of the initial assessment.
Review Sensor performance metrics (lamp hours, instrument noise, and instrument temperature)	Inspection of Real time Remote access telemetry data	Daily	Ecology HQ	If lamp hours are 50% of total expected life range, determine if nitrate-N data are returning a "Fair Accuracy Rating" and showing signs of positive drift (i.e., >2% RSD), flag the data and dispatch a field crew to site for lamp replacement within a 1-5 days of initial assessment. If within calibration criteria, apply drift corrections if needed.
Review system performance metrics (power, data logger, and data transmission)	Inspection of Real time Remote access telemetry data	Daily	Ecology HQ	If power is >%50, schedule battery replacement during next scheduled servicing and flag data period. Consult the SHU unit regarding data logging and transmission issues.

Table B-3. Field Maintenance Operations for the Satlantic UV Nitrate-N Sensors.

Maintenance Operation	Method of Determination	Frequency	Location
Inspection of station components and field equipment of signs of damage caused by environmental or human disturbance	Visual Inspection	Monthly	Bertrand Creek
Inspection and cleaning of sensors for fouling and corrosion	Visual Inspection Cleaning according to Manufacturer's guidelines	Monthly or when needed	Bertrand Creek
Battery check and replacement	FMU and SHU SOPs	Monthly or when needed	Bertrand Creek
Routine sensor cleaning	Manufacturer's guidelines	Monthly or when needed	Bertrand Creek
Sensor baseline check (Return to lab for calibration if necessary)	Manufacturer's guidelines	Monthly or when needed	Bertrand Creek
Data download	EAP080	Monthly	Bertrand Creek
Collection of discrete water samples; before and after servicing	Ward 2007	Monthly	Bertrand Creek

Discrete Grab Sample Collection

The QA program for discrete grab sample collection will consist of three parts:

- Adherence to standard operating procedures for sample/data collection and periodic evaluation of sampling personnel.
- Consistent instrument calibration methods and schedules.
- The collection of field QC samples according to set times defined by the monitoring schedules.

Field QA protocols are described in detail in Hallock and Ehinger.

Three types of field QC samples will be collected once annually for each station:

- *Duplicate (Sequential) Field Samples.* These will consist of an additional sample collection made approximately 15-20 minutes after the initial collection at a station. These samples will represent the total variability due to short-term, instream dynamics; sample collection and processing; and laboratory analysis.

- *Duplicate (Split) Field Samples.* These will consist of one sample (usually the duplicate sequential sample) split into two containers that are processed as individual samples. We will do this to eliminate instream and sample collection variability so we can assess the remaining variability attributable to field processing and laboratory analysis.

- *Field Blank Samples.* These will consist of the submission and analysis of de-ionized water and are true field process blanks. The blank de-ionized water will be poured into cleaned sample collection equipment, and the sampler will simulate collecting a water sample, including lowering the sampling device to the water surface. The expected value for each analysis will be the reporting limit for that analysis. Significantly higher results will indicate that sample contamination occurred during field processing or during laboratory analysis.

Semi-blind QC samples will be submitted to the laboratory. Samples will be identified as QC samples, but sample type (duplicate, split, or blank) and station will not be identified. Pre-deployment, post-deployment, and anomalous data will be deleted from the raw data set.

Laboratory

MEL will analyze all ambient water quality samples for this study. The MEL Quality Assurance Manual (MEL, 2012) documents the laboratory's quality control procedures in detail. If any of these quality control procedures are not met, the associated results will be qualified and used with caution, or not used at all. Table B-4 outlines the quality objectives associated with MEL's quality control procedures. If check samples and post-deployment calibrations indicate an offset or a linear drift, continuous data may be adjusted as necessary prior to evaluating against data quality objectives. If check samples and the post-deployment calibration are not consistent, or if data do not meet quality objectives after adjustments, then the data will be qualified or rejected.

Pre-deployment, post-deployment, and anomalous data will be qualified as "REJ" from the raw data set and not used.

Table B-4. Measurement quality objectives for lab-analyzed samples.

Parameter	Accuracy/Bias	Precision (% relative standard deviation)	Sensitivity (detection limits)
Ammonia-N	80-120	10 %	0.01 mg/L
Fecal coliform (>20 cfu/100 mL)	NA	50% of pairs <20%; 90% of pairs <50%	1 colony per 100 mg/L
Nitrate + nitrite-N	80-120	10 %	0.01 mg/L
Nitrogen, total	80-120	10 %	0.003 mg/L
Persulfate Nitrogen, total	80-120	10%	0.01 mg/L
Phosphorus, soluble reactive	80-120	10 %	1 ug/L
Phosphorus, total	80-120	10 %	0.005 mg/L
Solids, total suspended	80-120	15 %	0.5 mg/L
Hardness	80-120	10 %	0.3 mg/L
Metals, Total	80-120	10%	0.05 – 5 ug/L
Metals, Dissolved	80-120	10%	0.05 – 1 ug/L

Appendix C. University of Washington Nitrogen Isotope Method

Bacterial Denitrifier Method
Last Modified: 140512 by Schauer

INTRODUCTION

The denitrifier method uses *Pseudomonas aureofaciens* or *Pseudomonas chlororaphis* bacteria to denitrify nitrate (NO_3) to nitrous oxide (N_2O). *P. aureofaciens* is used when the user would like both $\delta^{15}N$ and $\delta^{18}O$ data while *P. chlororaphis* is used when the user is only interested in $\delta^{15}N$. *P. aureofaciens* has markedly reduced exchange rates of the oxygen in water with that of NO_3 during denitrification in contrast to *P. chlororaphis* and thus, $\delta^{18}O$ is more reliable when using *P. aureofaciens*. However, *P.aureofaciens* can yield reduced precision for $\delta^{15}N$ relative to *P. chlororaphis* and thus, if the user is interested in $\delta^{18}O$ and $\delta^{15}N$, consider using both stains for analysis.

Currently the isotope ratio mass spectrometer (Brave Irene) Finnigan DeltaPlus can be configured to run N_2O as N_2O (mass 44, 45, and 46) or as O_2 and N_2 (32, 33, 34, and 28, 29) via hot gold tube pyrolysis of N_2O. This document describes everything from bacteria maintenance to running the mass spectrometer.

SUPPLIES NEEDED

See below within each section for supplies needed

ROUTINE PROCEDURE

GROWTH MEDIA PREPARATION:

1. Weigh 60 g TSB (Trypic Soy Broth) into a 2.5 L bottle.
2. Add 2 L DI water (within 3-4 inches of bottle neck) and stir or shake until completely dissolved.
3. Distribute media into 500 mL bottles by first pouring in 400 mL each then use a graduated cylinder to top off the last 80 mL. There should be a total of 480 mL of media in each 500 mL bottle.
4. Put on pour rings. Keep caps loose on media bottles. Use purple media cap. *Purple caps are used for nitrate media. Orange caps are used for nitrate-free media.*
5. Autoclave (See section on Autoclaving) w/ autoclave tape on bottles.
6. Tighten all caps very soon after autoclave run is complete.
7. When cool, label with ingredients and prep date and store in bacteria cabinet in 303B.

Nutrient Buffer Stock Preparation:

1. Weigh ingredients (2 g KNO_3(Potassium Nitrate) + 10 g K_2HPO_4 (Potassium phosphate) + 4 g $(NH_4)_2SO_4$ (ammonium sulfate)) into a 2.5 L Bottle.
2. Add 2 L DI water (within 3-4 inches of bottle neck) and stir or shake until completely dissolved.

3. Distribute 500 mL into 500 mL bottles.
4. Put on pour rings. Keep caps loose on bottles.
5. Autoclave (See section on Autoclaving) w/ autoclave tape on bottles.
6. Tighten all caps very soon after autoclave run is complete.
7. When cool, label with ingredients and prep date and store in bacteria cabinet in 303B.

Nitrate-Free Media:

1. Weigh ingredients (60 g TSB (Trypic Soy Broth) + 10 g K_2HPO_4 (Potassium phosphate) + 4 g $(NH_4)_2SO_4$ (ammonium sulfate)) into a 2.5 L bottle.
2. Add 2 L DI water (within 3-4 inches of bottle neck) and stir or shake until completely dissolved.
3. Distribute into the smallest possible bottles.
4. Put on pour rings. Keep caps loose on media bottles.
5. Autoclave (See section on Autoclaving) w/ autoclave tape on bottles.
6. Tighten all caps soon after autoclave run is complete.
7. When cool, label with ingredients and prep date and store in bacteria cabinet in 303B.

AUTOCLAVING:

a. Autoclave as part of larger batch for efficiency.
b. Use autoclave tape to determine successful Autoclave run.
c. The autoclave is located in Johnson Hall room 227.
d. Select the appropriate program for your autoclaving purposes. (Specifics can be found under Clean Up)
    a. Any liquid autoclaving
        i. Keep caps on bottles loose for venting purposes.
        ii. The autoclave should be set to "Liquid 60" 121 ºC for 1 hour 30 minutes
    b. Dry items (such as pipette tips, centrifuge tubes, used bacterial agar plates)
        i. Used bacterial agar plates should be placed in autoclave bag which can be found in the cabinets beneath the fume hoods in room 303B.
        ii. The autoclave should be set to "Grav 20" 121°C for ~45 minutes
e. Sign in on autoclave log with name, room number, budget number, cycle used, and whether or not a test strip is present

STARTER CREATION:

1. Add 20 mL of the Buffer Stock Solution into 480 mL of media and inoculate with a centrifuge tube of starter or other leftover harvest media**.** Refer to section on Inoculation of Media Bottles for inoculation steps.
2. When ready, it is time to harvest the bacteria. Equally distribute media to 16 centrifuge tubes and centrifuge (18 ºC, 10 min, 7500 g).

     a. Turn on centrifuge in 302B, use SS-34 rotor, use arrows to find rotor type, set "rcf" to 7500, set temperature to 18 ºC, adjust time to 10 minutes...you can also use "recent" settings if this spin was used recently.
     b. The bacteria plug should be dime-sized and pink when finished.
         i. It is very important that the plug be pink and dime-sized as anything otherwise it will produce poor results.
3. Pour off supernatant into media bottle.
4. Add new media bottle with 20 mL of buffer stock solution to centrifuge tubes to re-suspend dime-sized plug remaining in the centrifuge tubes and transfer to fresh 500 mL media bottle to re-suspend media.
5. Distribute media evenly among x-number of VWR centrifuge, conical bottom tubes with blue caps.
6. Label starter solution with starter label and date. Store in the fridge until needed for inoculating other media.

INOCULATION OF MEDIA BOTTLES:

P. aureofaciens
1. Add 20 mL of the Buffer Stock Solution into the 480 mL of media. Shake well.
2. Remove one centrifuge starter tube from the fridge and re-suspend using the vortex.
3. Once re-suspended and mixed, pour starter into 500 mL media bottle to inoculate the 480 mL of media and 20 mL Buffer Stock Solution mixed earlier.
4. Label bottle(s) with species, and inoculation date.
5. Loosely cap (set cap on bottle) and put on orbital shaker overnight in room 303B. Keep the orbital shaker set to 4. This allows the bacteria to grow in an aerobic environment at a fast rate.
6. The following day, tighten the media bottle cap and leave on orbital shaker an additional night. Tightening the cap reduces oxygen within the media bottle and forces the bacteria to consume nitrite/nitrate.
7. Test for nitrite and nitrate using the fish tank test kits in the bacteria lab drawer. Nitrate and nitrite should test 0 ppm. If this is not the case, leave on the orbital shaker for additional time and retest later.
8. Store in the fridge until you are ready to harvest.
9. Bacteria are ready for harvest 2-4 days after inoculation.

HARVESTING BACTERIA:

1. Equally distribute media to 16 centrifuge tubes and centrifuge (18 ºC, 10 min, 7500 g).
     a. Turn on centrifuge in 302B, use SS-34 rotor, use arrows to find rotor type, set "rcf" to 7500, set temperature to 18 ºC, adjust time to 10 minutes...you can also use "recent" settings if this spin was used recently.
     b. The bacteria plug should be dime-sized and pink when finished.
         i. It is very important that the plug be pink and dime-sized as anything otherwise it will produce poor results.
2. Pour supernatant back into media bottle.
3. Using an autoclaved pipette tip, reconstitute the bacteria plug in 2.8 mL of nitrate free media and vortex until plug is completely dissolved.

4. Add the 2.8 mL aliquots together into 50 mL bottle, then add a few drops of Antifoam B, mix well.
5. Repeat steps 1-4 for a second 500 mL inoculated media bottle.
6. Using an autoclave pipette tip, add 2 ml of media to each of 40 sample vials and put on caps using the crimper. Put a long blue needle in each vial for venting.
   a. For supply efficiency, you can use the same autoclave pipette tip throughout steps 5 - 8 as long as the tip touches nothing to prevent contamination.
7. Invert vials and purge through brown needles, for 3-4 hours (*P. aureofaciens*) or 2-3 hours (*P. chlororaphis*) on manifold located in room 303B.
8. Record details into the Bacteria Notebook (or personal notebook).
   a. Date of harvest, who is harvesting, the samples to be run, inoculation dates, helium pressure used on the manifold, start and end purge times and any other information you feel is necessary.
9. When sufficient time has passed, remove blue needle first, then vial from brown needle.
   a. If you poke yourself with a needle immediately wipe the punctured area with Iodine wipes found in the First Aid Kits in a Blue Box.
10. Label vials 1-40 on the bottom with sharpie.
11. Add designated sample to each vial, taking care to rinse syringe and needle thoroughly with 18 MΩ water between each sample. Using three beakers of 18 MΩ, rinse syringes 3 times and 1 full mL through needle. NEVER touch needle to sample. Always pull up sample and 18 MΩ with syringe only.
12. The injection amount can be calculated using the spreadsheet BacterialDenitrifier_NO3units.xls located in the Methods folder on the lab computers.
    a. The Target NO_3 amount for N_2O method is 20 nmoles
    b. The Target NO_3 amount for O_2N_2 method is 200 nmoles (or can sometimes be 100 nmoles)
13. If possible, rinse needle and syringe with a tiny amount of sample water before injecting each sample into their vial.
14. Put sample vials in Styrofoam containers **upside-down** and put on shaker set to low overnight.

CLEAN UP:

1. Waste media: autoclave as part of normal Liquid 60 batch (see above in Autoclaving) (500 mL bottles and live bacteria rinse bottle)
2. Neutralize used sample media with Hydrochloric acid (HCl) and flush down drain with water.
3. Waste solids: autoclave waste solids in autoclave bag with indicator tape as a "Grav 20" cycle (gravity, ~45 min), then put in garbage.
4. Used pipette tips are autoclaved with waste solids under "Grav 20" cycle and put on shelf for reuse. Use autoclave tap.
5. Vials and media/serum bottles: Caps are taken off and thrown away, waste is poured into designated waste bottle and vials are soaked in soapy water to clean, rinse with tap water, acid bath overnight, rinse well with DI water (or water bath overnight). Dry. Wrap in foil; muffle 500º for 4 hours.
6. Centrifuge tubes: Rinse with ethanol or methanol. Rinse 3x with DI water and air dry them on the counter.

7. All syringe needles go in a labeled plastic sharps container.

MAKING CONCENTRATED SALT SOLUTION FOR STANDARDS:

1. Supplies Needed:
   a. 18 MΩ water located in room 303B
   b. Clean plastic nalgene bottle(s)
   c. Salt for desired solution(s)
        i. IAEA-NO-3 salt and USGS35 are stored in room 303 B in the desiccant chamber.
        ii. USGS34 salt is currently stored in Becky Alexander's lab in Atmospheric Sciences somewhere on the 4th floor.
   d. 1000 mL graduated cylinder
   e. mg scale
   f. Pencil and Pen or Excel
2. Using the pencil and pen or excel, calculate the amount of the salt to weigh out to make 500 mL of 4mM salt solution.
   a. EX: For USGS35

   $$\frac{4\text{mmoles NO3}}{1\text{ L}} \times \frac{1\text{mmol NaNO3}}{1\text{ mmol NO3}} \times \frac{84.9939\text{ mg NaNO3}}{1\text{ mmol NaNO3}} \times \frac{1\text{ L}}{1000\text{ mL}} \times 500\text{ mL} =$$

   = 169.9878 mg NaNO3 salt is needed to make 500 mL of salt solution at 4mM

   b. USGS35 – sodium nitrate
   c. USGS34 and IAEA-NO-3 -- potassium nitrate

   Refer to BacterialDenitrifier_SaltSolutions.xls located in the Methods folder of the lab computer for more help if needed.
3. Rinse out the plastic nalgene bottle thoroughly with 18 MΩ water.
4. Use the graduated cylinder to measure out 500 mL of 18 MΩ water and pour into the thoroughly cleaned plastic nalgene bottle.
5. Measure out the needed amount of salt on the scale and add it the clean nalgene bottle.
6. Cap the bottle and shake until the salt has dissolved.
7. Label the bottle with standard name, concentration, date created, and name of the creator.
8. If the standard is not going to put to immediate use, wrap with parafilm and store in the freezer, otherwise put in the refrigerator

MAKING STANDARDS:

1. Supplies needed:
   a. 18 MΩ water located in room 303B
   b. Clean plastic nalgene bottle
   c. Concentrated salt solution (4mM) of desired standard stored in the freezer (needs to be thawed completely)
   d. 1000 mL graduated cylinder
   e. Pencil and Pen or excel

2. Using the pencil and pen or excel, calculate the amount of 18 MΩ water and concentrated standard mixture you need to make the desired standard.
    ex: For 500 mL of 100 nM of desired standard
        Use 12.5 mL of 4mM desired standard
        Use 487.5 mL of 18 MΩ water
    (Desired Concentration / (4mM of desired standard * 1000)) * total volume of desired standard
    (100uM / (4mM*1000)) * 500 mL = 12.5 mL of desired standard is needed

    500 mL – 12.5 mL = 487.5 mL of 18 MΩ water is needed

    Refer to BacterialDenitrifier_SaltSolutions.xls located in the Methods folder of the lab computer for more help if needed.
3. Rinse out the plastic nalgene bottle thoroughly with 18 MΩ water.
4. Use the graduated cylinder to measure out the correct amount of 18 MΩ water and desired standard water and pour into the thoroughly rinsed nalgene bottles.
5. Label the bottle with standard name, concentration, date created, and name of the creator.
6. If the standard is not going to put to immediate use, wrap with parafilm and store in the freezer, otherwise put in the refrigerator.

ISOTOPE ANALYSIS ON BRAVE IRENE:

1. Make sure the -60 *C trap is on AND cold.
2. Fill the small helium cleanup liquid nitrogen trap.
3. Make sure the GasBench GC is set to 30 *C
4. Fill the liquid nitrogen dewar on the PreCon.
5. Also make sure ethanol trap has thawed and "reslushed" for the next run.
    a. Maintenance on the ethanol trap should occur weekly
    b. Remove trap from the ethanol bath and heat to remove any liquid from the trap.
    c. Check and make sure the ethanol bath has not evaporated. If not, replace it with new ethanol. (A frozen bath indicates ethanol has evaporated away leaving only water from condensation)
6. Reagents should be checked weekly and changed when necessary.
    a. Supplies needed:
        i. Gloves
        ii. Glass wool
        iii. Magnesium Perchlorate
        iv. Ascarite
        v. Glass tube (**3/8" OD, 17.5 cm long**)
        vi. Waste container
        vii. Kim wipes
        viii. Wrenches
    b. Wearing gloves fill the glass tube with the reagents in the order of: Glass wool, Ascarite, Glass wool, Magnesium Perchlorate, Glass wool.
        i. Pack down the glass wool using metal rod.
        ii. Use the vortex to pack down the ascarite and magnesium perchlorate before moving on to the quartz wool.

        iii. When finished, use a Kim wipe to wipe off excess material. Used Kim wipe(s) go into waste container.
- c. Using the wrenches, remove the current reagent tube from Irene and place it into the waste container.
- d. Place the fresh reagent tube with ascarite on the left and tighten using the wrenches.
- e. Check for leaks using the helium leak detector.

7. Lyse (kill) bacteria with 0.2 mL 10M NaOH and shake well
8. Fill rack with vials. The first position vial should be an N2OinHe.
   - a. if you must make an N2OinHe using pure N_2O, inject at most 0.5 uL into a 20 mL vial

MEASURING N$_2$O:

1. On the computer, in the program IsoDat Acquisition, make sure the GasBench Valco valve is in "Load" position. If it is in "Inject" click on it once.
2. Make sure the Helium gauge on the Gas Bench is set to 1.0.
3. Make sure everything associated with the baking out procedure (refer to section on Baking Out GC, IRMS, and the VOC trap) is ready for samples.
4. Fill out Daily Log by:
   - a. Turn off N_2O reference, fill in date, name, source settings and gas pressures
   - b. Right click on mass 45, click "jump to mass", type in 18 (water), once it has jumped, right click, again, on the middle cup, now mass 18 and click "peak center" once it has finished enter the value for mass 18 on the daily log
   - c. For mass 40, right click on mass 18, jump to mass 40, peak center and enter value for mass 40 (argon) in the daily log
     - i. See troubleshooting if values are high
   - d. At the bottom left of the screen, click on the upside down triangle next to N_2O and reselect N_2O. Once it has moved, enter the values for mass 44,45,and 46 into the background section of the daily log for those masses
   - e. Turn on N_2O reference gas and once it is stable, enter these values into the daily log
   - f. enter the number of samples (excluding standards) and the species of bacteria used
   - g. enter notes related to the dataset itself
5. If the system has sat idle or was running a different analysis (i.e. gold tube) or for peace of mind, change over to GasBench mode by clicking on GasBench in bottom left box..
6. Open "Precon_N2O_samples.seq" sequence, enter your sample and standard information
   - a. Identifier 1 – Sample/Standard name
   - b. Identifier 2 – Concentration of the sample/standard
     - i. Usually the only info entered in this column are the standards
   - c. Comment – Injection volume
     - i. For blanks – the volume of 18 MΩ injected
     - ii. For samples and standards - the volume injected before topped off with 18 MΩ
7. Click start; enter your dataset name, making sure that the dominant folder starts with "yymmdd".

MEASURING O₂ AND N₂:

1. In Isodat Acquisition, make sure the Gas Bench Valco valve is in 'Inject' position
2. Make sure the Helium gauge on the Gas Bench is set to 0.7
3. Set the Goldtube temperature to 800°C
   a. Press 'p' button then up or down arrow to desired temperature.
4. Fill out the Daily Log by:
   a. Turn off reference gas #3, fill in date, name, source settings, and gas pressures
   b. At the bottom of the left of the screen, click on the upside down triangle next to N2O and select O2 Once it has moved, enter the values for mass 32, 33, and 34 into the background sections of the daily log for those masses
   c. Repeat step b but select N2
   d. Select N2O. Right click on mass 45, click 'jump to mass', type 18 (water), once it has jumped, right click again, on the middle cup, now mass 18 and click 'peak center.' Once it has finished enter the value for mass 18 on the daily log.
   e. For mass 40 (argon), right click on mass 18, jump to mass 40, peak center and enter value for mass 40 (argon) in the daily log.
   f. Enter the number of samples (excluding standards) and the species of the bacteria used
   g. Enter notes related to the dataset itself.
5. Open "Precon_O2_N2_samples.seq" sequence; enter your sample and standard information.
   a. Identifier 1 – Sample/Standard name
   b. Identifier 2 – Concentration of the sample/standard
      i. Usually the only info entered in this column are the standards
   c. Comment – Injection volume
      i. For blanks – the volume of 18 MΩ injected
      ii. For samples and standards - the volume injected before topped off with 18 MΩ
6. Click Start, enter your dataset name, making sure that the dominant folder starts with the date in "yymmdd" format.
7. Click 'OK.'

BAKING OUT THE GC, IRMS, AND THE VOC TRAP:

1. GC columns: at the north side of the GasBench, press the "P" button on the "Jumo iTron 16". Hold the up arrow down until the set point temperature reads 200 ºC, press the "P" button again to set the temperature.
2. IRMS: Within Acquisition, under the "MS State" window, click the bottom 4 gray buttons to turn them green.
3. VOC Trap: Turn the Thermolyne controller located between the Precon and the autosampler to 4.
4. NOTE, to run samples, the GC should be at 35 ºC, the IRMS heaters should be off, and the VOC trap should be at room temperature.

STANDARDS USED IN ISOTOPE ANALYSIS:

IAEA-NO-3	+4.76-0.2+25.60	potassium nitrate
USGS34	-1.8-0.1-27.9	potassium nitrate
USGS35	+2.7+21.6+57.5	sodium nitrate
USGS34/35mix	+0.45+10.75	nitrate mixture
USGS32	+180.00	potassium nitrate

TROUBLESHOOTING:

1. This method has proven to be very unpredictable due to the ever changing environment which the bacteria are grown. There have been many improvements to the method in the last year but nothing has remained consistently successful. There is a lot of work that needs to be done.
2. If the computer seems sluggish or the IsoDat software has crashed, reboot the computer. Once you have rebooted and opened Acquisition, loud hissing will be coming from the GasBench. Do the following to stop the hissing:
   a. Click Trap 1 and Trap 2 to make the image look as though those traps are in the down position
   b. Click the Split so that the tubing is darkened in the image.
   c. These actions don't do anything functional. They are tied to air valves that are no longer functional and thus don't actually move the traps or alter the split.
3. No sample peaks during a run
   a. Make sure the liquid nitrogen dewar has liquid nitrogen in it otherwise the mass spectrometer cannot produce sample peaks and the sample cannot be analyzed.
4. Low Peaks
   a. Look to see that the vials are bubbling when the needle is injected, if it is not, the needle is clogged
   b. Check for leaks using the helium leak detector
   c. Does N2OinHE produce peaks.
5. Plating
   a. If the plates are growing as lawns instead of streaks, the agar is too diluted. Either there is too much water or not enough Tryptic Soy Agar in the solution.
6. High Backgrounds
   a. May indicate there is a leak. Use the helium leak detector and check for leaks.
   b. Pay particular attention to the valves as they have a history of leaking.

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Appendix B. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

Ambient: Background or away from point sources of contamination.

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

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

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

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 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.

Nonpoint source: Pollution that enters any waters of the state from any dispersed land-based or water-based activities. This includes, but is not limited to, atmospheric deposition, surface-water 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, this includes any unconfined and diffuse source of contamination. Legally, this includes any source of water pollution that does not meet the legal definition of "point source" in section 502(14) of the Clean Water Act.

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

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

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

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

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: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any 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, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Reach: A specific portion or segment of a stream.

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

Salmonid: Fish that belong to the family *Salmonidae*, including any species of salmon, trout, or char.

Stream flow: Discharge of water in a surface stream (river or creek).

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

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

Total Maximum Daily Load (TMDL): A distribution of a substance in a water body designed to protect it from not meeting (exceeding) water quality standards. A TMDL is equal to the sum of all of the following: (1) individual 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. A reserve for future growth is also generally provided.

Total suspended solids (TSS): Portion of solids retained by a filter.

Turbidity: A measure of water clarity. High turbidity can negatively impact on aquatic life.

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.

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. These are water quality-limited estuaries, lakes, and streams that fall short of state surface water quality standard and are not expected to improve within the next two years.

Acronyms and Abbreviations

BMP	Best management practices
DO	(See Glossary above)
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
FC	(See Glossary above)
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
QA	Quality assurance
RM	River mile
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials
TMDL	(See Glossary above)
TOC	Total organic carbon
TSS	(See Glossary above)
USGS	U.S. Geological Survey
WAC	Washington Administrative Code
WRIA	Water Resource Inventory Area

Units of Measurement

°C	degrees centigrade
dw	dry weight
g	gram, a unit of mass
kg	kilograms, a unit of mass equal to 1,000 grams
km	kilometer, a unit of length equal to 1,000 meters
m	meter
mg	milligram
mg/Kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
NTU	nephelometric turbidity units
ug/g	micrograms per gram (parts per million)
umhos/cm	micromhos per centimeter

uS/cm   microsiemens per centimeter, a unit of conductivity
ww   wet weight

Quality Assurance Glossary

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

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

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

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

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

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

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

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

Completeness: The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator. (USEPA, 1997)

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

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

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

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

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

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

Data set: A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010)

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

Examples of data types commonly validated would be:
- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:
- No qualifier, data is usable for intended purposes.
- J (or a J variant), data is estimated, may be usable, may be biased high or low.
- REJ, data is rejected, cannot be used for intended purposes (Kammin, 2010; Ecology, 2004).

Data verification: Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs). Verification is a detailed quality review of a data set. (Ecology, 2004)

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

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

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

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

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

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

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

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

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

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

Method Detection Limit (MDL): This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of

an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero.

Percent Relative Standard Deviation (%RSD): A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:
$$\%RSD = (100 * s)/x$$
where s is the sample standard deviation and x is the mean of results from more than two replicate samples (Kammin, 2010)

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

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

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

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

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

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

Relative Percent Difference (RPD): RPD is commonly used to evaluate precision. The following formula is used:
$$[Abs(a-b)/((a + b)/2)] * 100$$
where "Abs()" is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples (Ecology, 2004).

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

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

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

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

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

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

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

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

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

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

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

References for QA Glossary

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