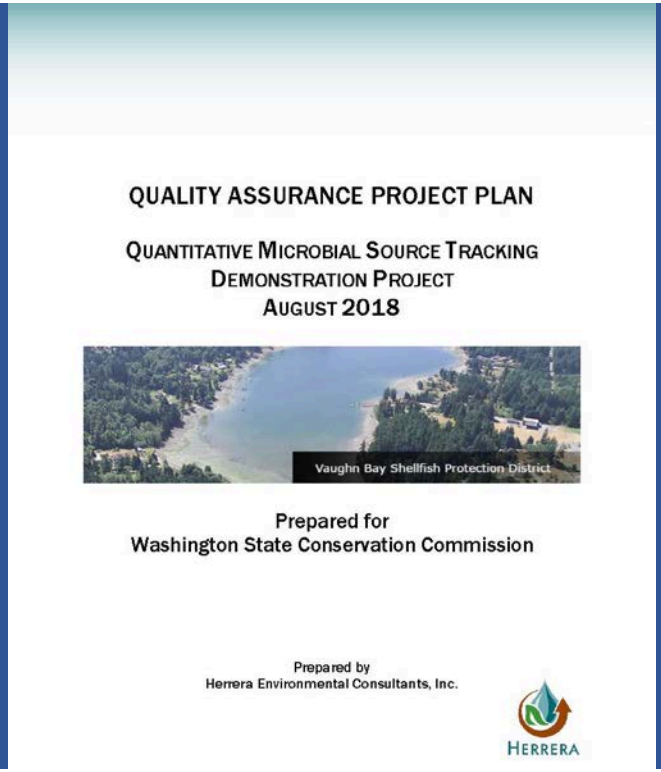
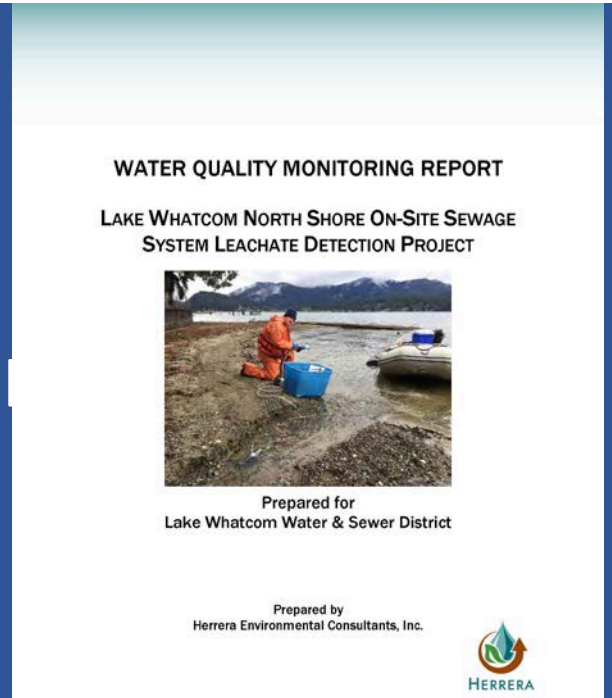
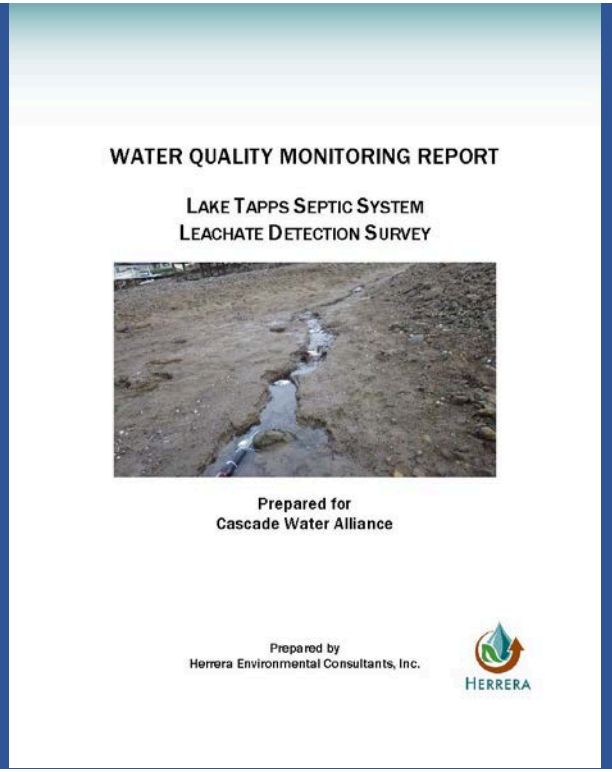
Using MST to Quantify Fecal Sources of Shellfish Harvesting Closures in Puget Sound and Detect Septic System Contamination of Lakes







Rob Zisette, Herrera Environmental Consultants

Source Molecular
Leader in Microbial Source Tracking

MST Demonstration Project

Project Team Responsibilities

Washington State Conservation Commission – Funding, document review, technical committee oversight

Herrera Environmental Consultants – Lead investigator, project management, QAPP preparation, sampling/laboratory coordination, data validation/analysis, stakeholder involvement, and report preparation.

Tacoma Pierce County Health Department – QAPP/report review, fecal source sampling, freshwater sampling

Washington State Department of Health – Marine water sampling

Squaxin Island Tribe – Fecal source and marine water sampling

Source Molecular – Study design, QA/QC, and qPCR analysis

University of Minnesota BioTechnology Institute – Fecal community DNA analysis

Centric Analytical Labs – Fecal bacteria analysis, MST sample filtration

Pierce Conservation District – Farm source control

Pierce County Planning and Public Works – Municipal stormwater source control

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Technical Committee Members:

WSCC – Karla Heintz, Kirk Robinson

EPA – Orin Shanks

Ecology – Tom Gries

WSDA – Gary Barr

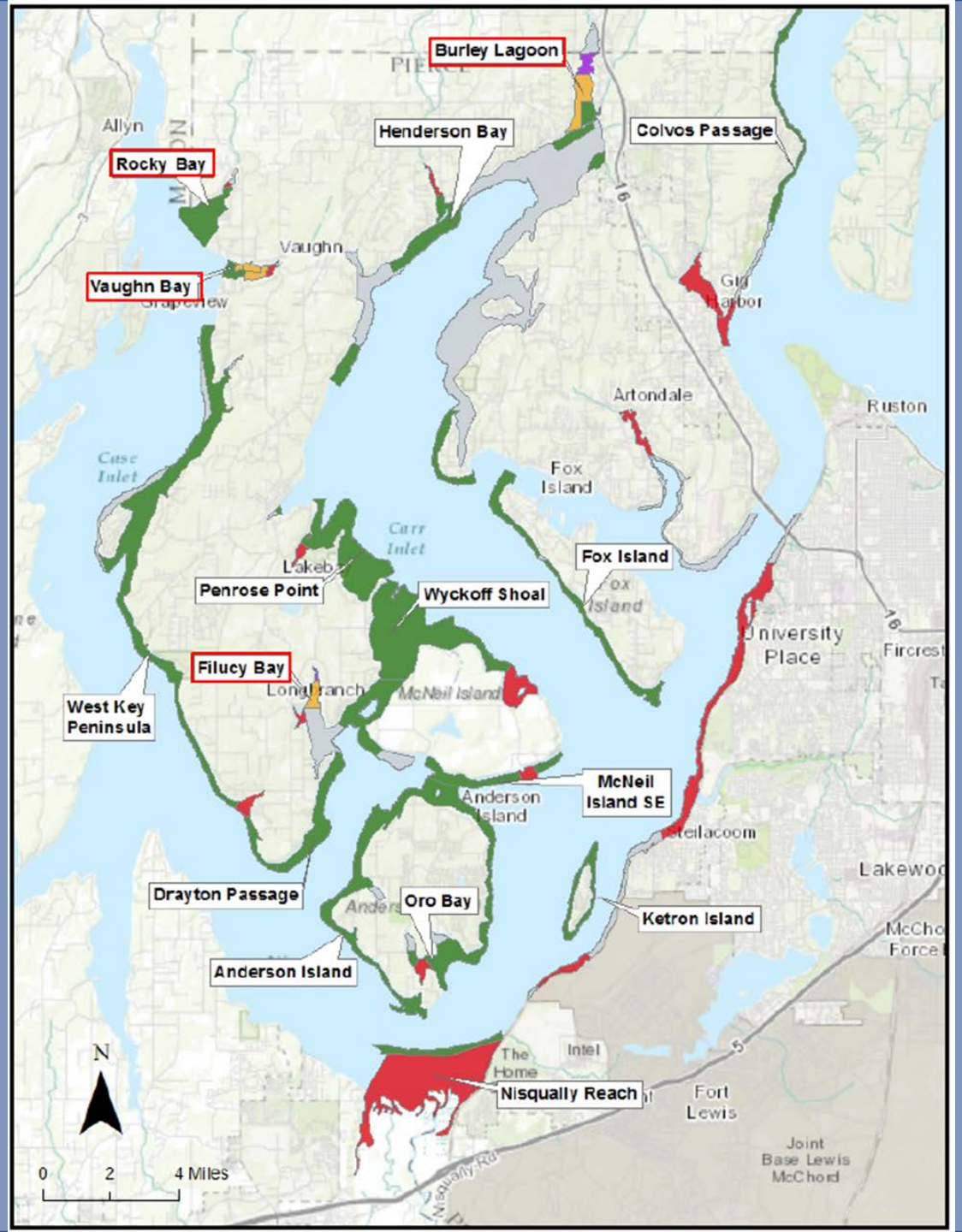
Herrera – Rob Zisette, Gina Catarra

TPCHD – Ray Hanowell, Cindy Callahan

MST Demonstration Project

QAPP and Study Design Process

- Site Description
- Historical Data Analysis
- Project Hypotheses
- Watershed Selection
- Sample Site Selection
- MST Method Selection
- Biomarker Selection
- Sampling and Analysis Plan
- Sampling and Analysis Status
- Fecal Source Validation Results



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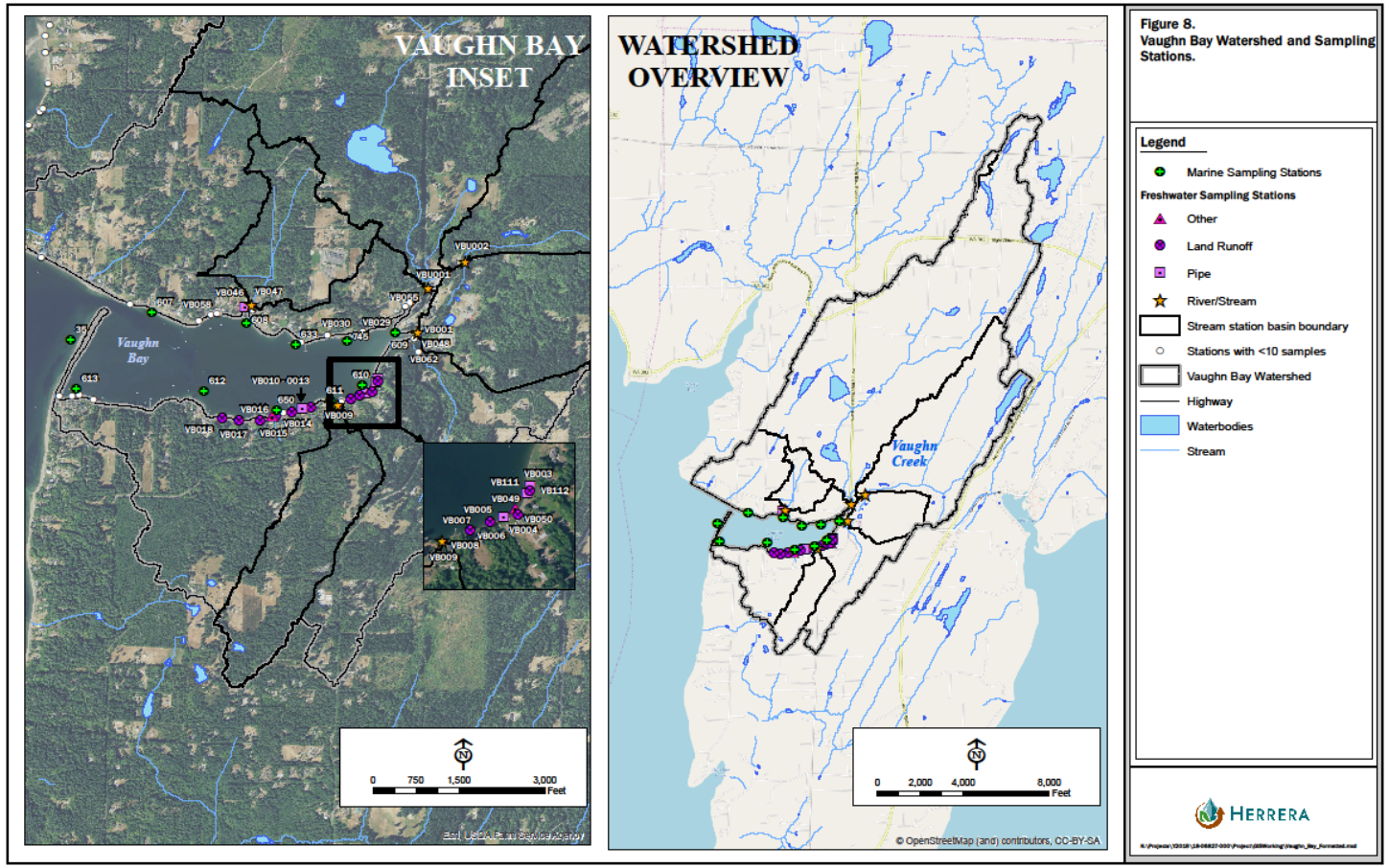
MST Demonstration Project

Historical Data Analysis

Freshwater and marine fecal coliform bacteria data analysis for Rocky, Vaughn, and Filucy Bay watersheds:

- Spatial trends
- Hydrologic trends
- Long-term trends
- Seasonal trends
- Loading analysis



Vaughn Bay Monitoring Stations:

- 19 Freshwater Stations:
  - 483 samples 2015-17
- 10 Marine Stations:
  - 664 samples 2010-17

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MST Demonstration Project
Historical Data Analysis

Freshwater fecal coliform bacteria concentrations in 3 watersheds:

- Geometric Mean Criterion Exceeded:
  - 17 percent (11/63 stations) in Base Flow
  - 35 percent (17/49 stations) in Storm Flow
- 90th Percentile Criterion Exceeded:
  - 51 percent (32/63 stations) in Base Flow
  - 51 percent (25/49 stations) in Storm Flow

		Geomean (CFU/100 mL)			90th Percentile (CFU/100 mL)		
Bay	Site ID	All	Base	Storm	All	Base	Storm
Vaughn Bay	VB001	**80**	**80**	(no data)	**219**	**219**	(no data)
	VB003	22	11	**152**	**796**	**217**	**1,120**
	VB004	26	15	**131**	**1,000**	**146**	**1,500**
	VB005	48	47	**52**	**1,019**	**1,082**	**184**
	VB007	**63**	**76**	32	**570**	**544**	**582**
	VB009	**51**	**50**	**54**	**590**	**520**	**380**
	VB010	39	33	**63**	**348**	**200**	**562**
	VB011	3	2	7	34	17	39
	VB012	3	2	5	37	**184**	37
	VB013	**66**	46	**199**	**648**	**541**	**1,146**
	VB014	22	17	50	92	78	**325**
	VB015	38	9	**95**	**676**	**774**	**338**
	VB016	14	16	8	88	70	97
	VB017	11	13	7	**104**	**100**	**121**
	VB018	**225**	**225**	(no data)	**680**	**680**	(no data)
	VB046	29	20	**114**	**460**	**199**	**1,016**
	VB047	31	20	**130**	**284**	**188**	**963**
	VB049	6	6	7	62	38	**755**
	VB050	10	7	23	**130**	77	**1,125**
	VB111	1	1	3	88	**276**	40
	VB112	39	31	**69**	**729**	**525**	**905**
	VBU001	36	38	25	**160**	**126**	**215**
	VBU002	24	24	20	94	96	69

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MST Demonstration Project
Historical Data Analysis

Freshwater fecal coliform bacteria loading rates show:

- Storm Flow > Base Flow except Vaughn Bay streams
- Drains > Streams except Rocky Bay

Watershed	Base Flow Loading (Million CFU/Day)		Storm Flow Loading (Million CFU/Day)	
	Streams	Drains[a]	Streams	Drains[a]
Rocky Bay	296	289	8,158	2,073
Vaughn Bay	578	598	488	1,296
Filucy Bay	312	462	549	1,263
Total	1,187	1,348	9,196	4,631

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MST Demonstration Project
Historical Data Analysis

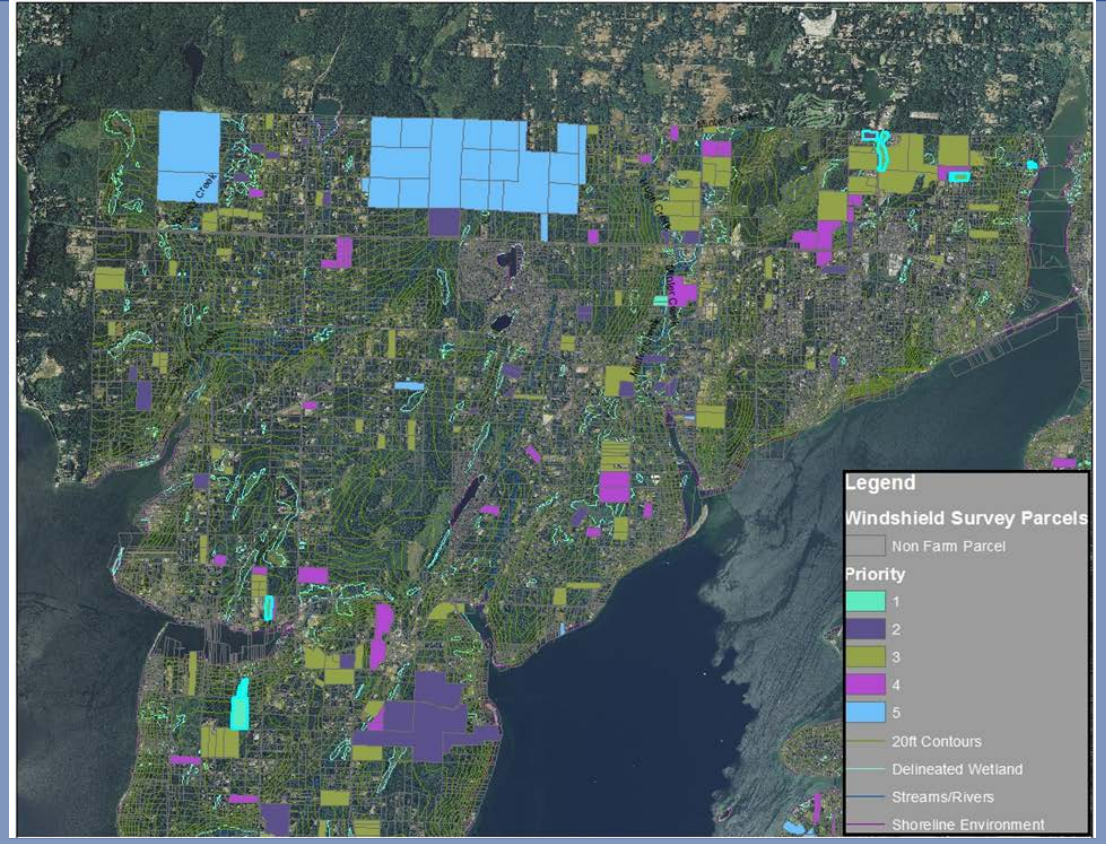
Windshield Farm Survey Data:

Animal counts and fecal coliform production rates based in 1,000-pound animal units (au):

- Cow = 101 billion CFU/day/au
- Horse = 0.42 billion CFU/day/au
- Sheep = 200 billion CFU/day/au
- Chicken = 34 billion CFU/day/au

Farm condition ratings:

- GIS analysis pending data digitization



1. High: Pasture in poor condition. Livestock have access to surface water and/or there is a higher probability of runoff due to topography sloping toward water body. Visual evidence of contamination problem.
2. Medium-High: Pasture in poor condition. Some reason to believe degraded conditions are seasonal or could get worse seasonally. Some areas on property reflect higher levels of management.
3. Medium: Pasture is in fair condition. Open water in vicinity of the property but with limited access or little evidence of use. A moderate probability of runoff.
4. Medium-Low: Pasture in good condition. No open water in vicinity and/or a low probability of contaminated runoff reaching surface water.
5. Low: Visual inspection from roadside indicates historic or recent past farming activity. Pastures not utilized by livestock. No livestock currently on site. Old barns and/or farm equipment evident.

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MST Demonstration Project

Historical Data Analysis

Stream fecal bacteria - watershed attribute correlation analysis:

- Horse fecal production significantly correlated with fecal coliform loading rate

Stream Fecal Coliform Metric for 11 Streams in 3 Watersheds	Septic System Density (#/square mile)	Hydric Soil (%)	Poultry FC (10^9 FC/day)	Cows FC (10^9 FC/day)	Horses/Donkey/Ponies FC (10^9 FC/day)	Sheep/Goats/Llamas FC (10^9 FC/day)	Total Animals FC (10^9 FC/day)	Barren Land (%)	Cropland (%)	Developed (%)	Forest (%)	Grassland/Pasture (%)	Herbaceous Wetlands (%)	Shrubland (%)	Water (%)	Woody Wetlands (%)	Impervious (%)
FC Conc. Geomean All Samples (CFU/100 mL)	0.147	0.325	-0.408	0.335	-0.427	-0.115	0.184	-0.061	-0.151	-0.429	-0.030	0.134	0.010	0.087	-0.097	0.363	-0.198
FC Conc. Geomean Base Flow Only (CFU/100 mL)	0.116	0.380	-0.451	0.404	-0.473	0.036	0.308	-0.082	-0.134	-0.413	-0.136	0.205	0.115	0.138	-0.155	0.439	-0.192
FC Load Geomean All Samples (10^6 CFU/Day)	0.061	-0.210	0.575	-0.120	0.603	-0.085	-0.123	0.296	-0.267	0.351	0.383	-0.446	-0.435	-0.225	0.478	-0.417	0.273
FC Load Geomean Base Flow Only (10^6 CFU/Day)	0.048	-0.195	0.515	-0.119	0.535	-0.061	-0.111	0.250	-0.249	0.324	0.323	-0.388	-0.373	-0.190	0.424	-0.376	0.243

Significant Correlation at alpha = 0.05

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MST Demonstration Project

Project Hypotheses

H_1 – Fecal coliform bacteria concentrations in downgraded shellfish protection areas are primarily affected by loadings in freshwater discharges to those areas.

H_2 – Fecal coliform bacteria concentrations in downgraded shellfish protection areas and freshwater drainages to those areas are higher during storm events than base flow events.

H_3 – Fecal coliform bacteria concentrations in downgraded shellfish protection areas and freshwater drainages to those areas are highest during the seasonal first flush conditions in the fall and in the wettest years.

H_4 – Sources of fecal coliform bacteria present in downgraded shellfish protection areas and watershed drainage will vary spatially, temporally, and hydrologically.

H_5 – Sources of fecal coliform bacteria present in shellfish protection areas and watershed drainage may include humans from onsite septic systems and/or multiple types of farm animals, pets, and wildlife located in the watershed draining to those areas.

H_6 – The qPCR and community-based MST methods will identify fecal sources present in the collected marine and freshwater samples.

H_7 – Characterization of fecal sources in the collected samples will increase the ability to identify effective corrective actions for upgrading shellfish protection areas.

MST Demonstration Project

Watershed Selection

- Vaughn Bay selected because:
  - Largest downgraded area (96 acres)
  - Restricted tidal flushing increases freshwater influence
  - Simple watershed drainage (one main stream)
- Rocky Bay small downgrade area and large forested watershed
- Filucy Bay complicated by several streams, two embayments, and one marina
- Burley Lagoon in Kitsap County with current MST study

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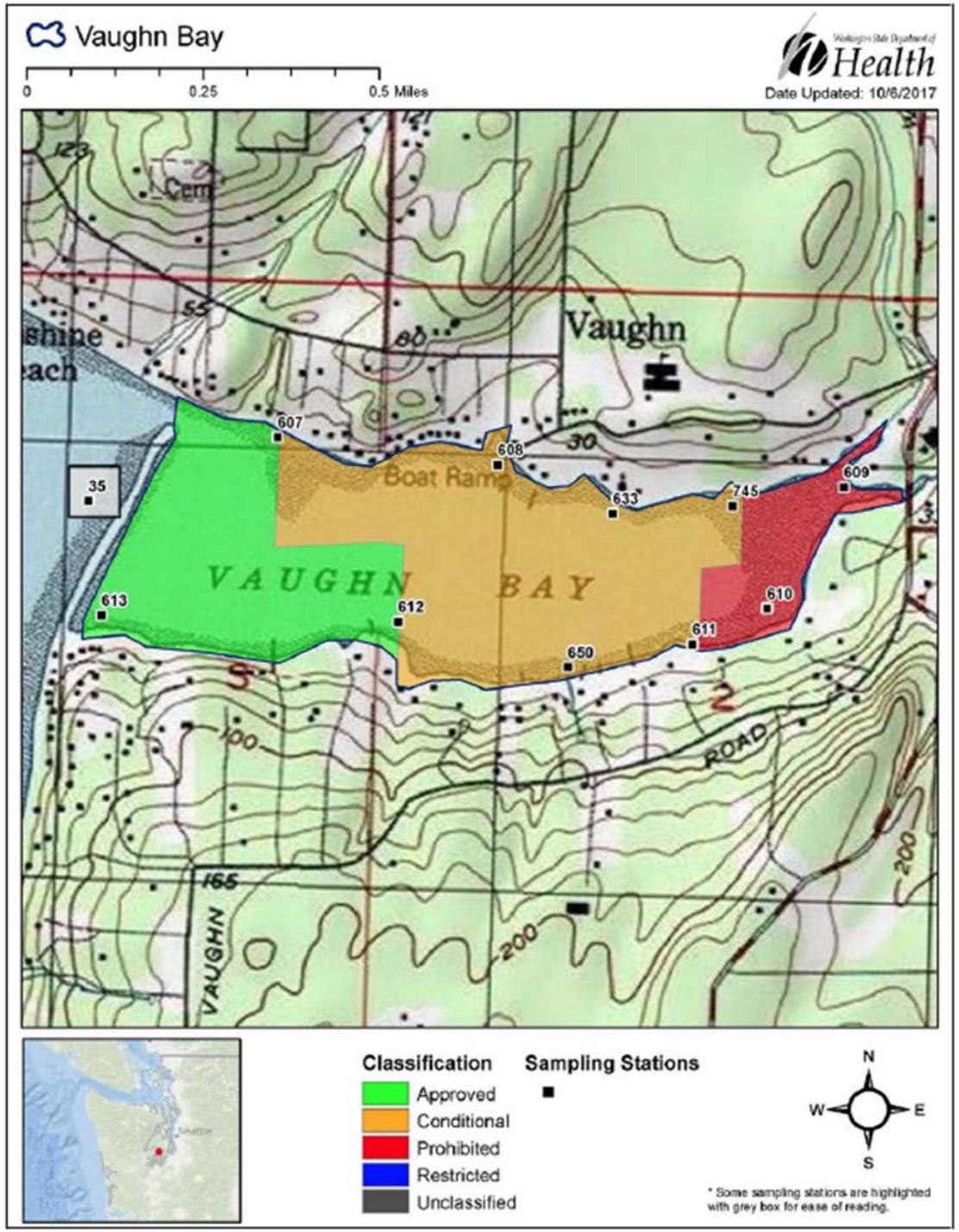


MST Demonstration Project

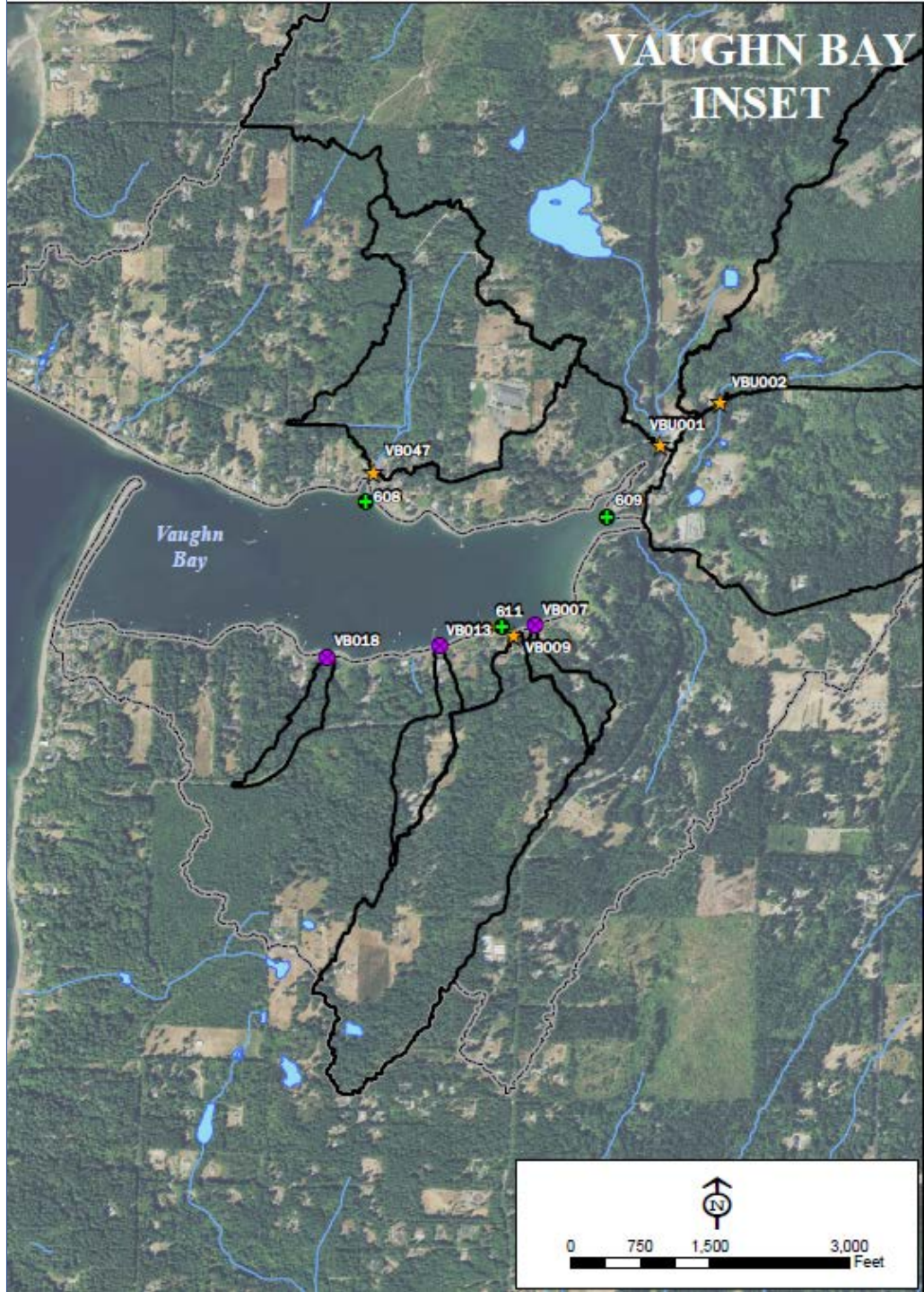
Sample Site Selection

3 marine stations:

- 1 in prohibited area
- 2 in restricted area

7 freshwater stations:

- 2 on each fork of Vaughn Creek
- 2 on small streams
- 3 on problem drains



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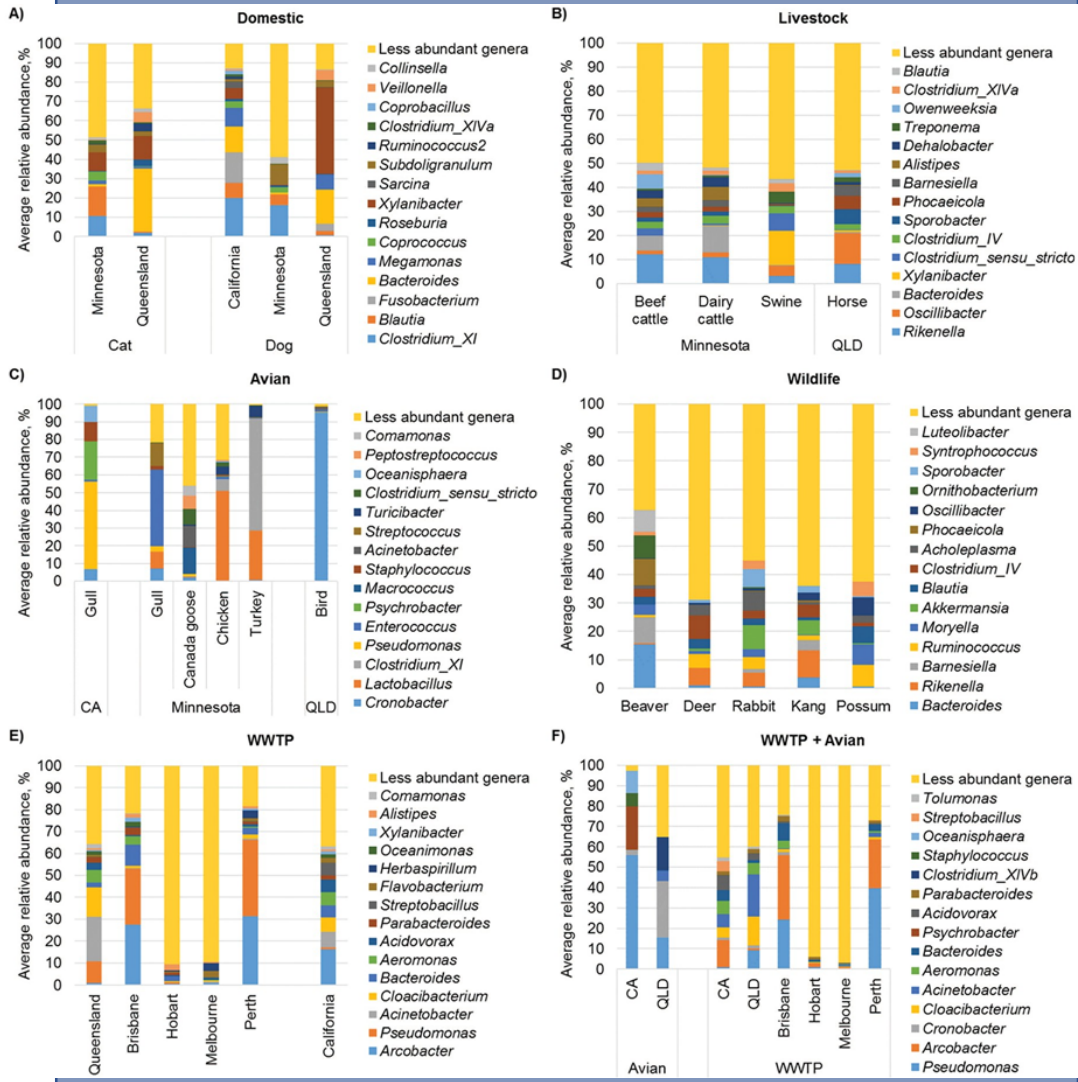
MST Demonstration Project

MST Method Selection

1. Single-host Bacteroidetes quantitative PCR by Source Molecular:
   - High performance without source library
   - Most common, mature, and effective
   - Standardized protocols for many sources
   - Published QC data for sensitivity, selectivity, and detection limits
   - Commercial lab available to all, EPA coordination, and quick turnaround
2. Community-based Next Generation Sequencing by U. of Minnesota BioTechnology Institute:
   - Source library augmented with local sources
   - Wildlife sources without qPCR biomarkers
   - Sewage-septage differentiation



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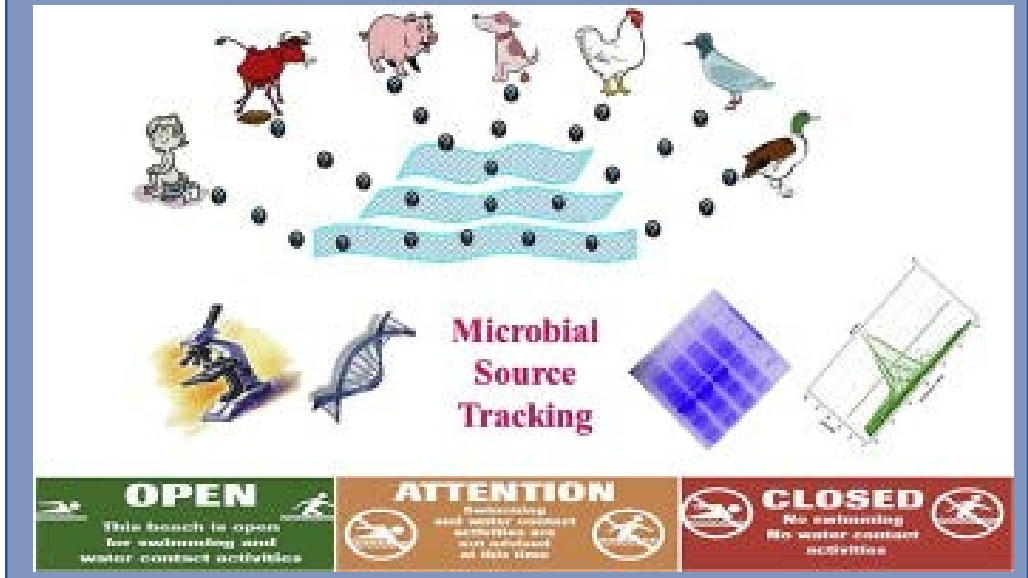
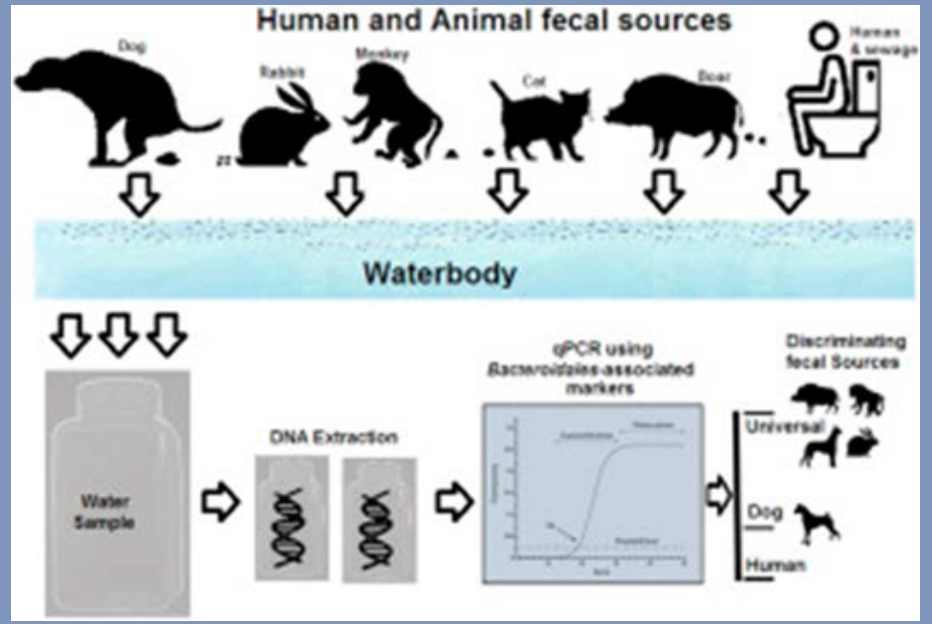
MST Demonstration Project

qPCR Biomarker Selection

1. Human marker HF183 EPA - high sensitivity (low false negatives)
2. Cow marker CowM2 - EPA for range cattle
3. Horse marker HoF597F - only horse marker, validated
4. Ruminant marker Rum2Bac - highest sensitivity, includes cow, sheep, goat, llama, alpaca, and deer
5. Pig marker Pig2Bac - only pig marker, validated
6. Dog marker DG3 - successfully used locally
7. Bird marker GFD – general marker without differentiating specific markers for gulls, geese, chickens, or poultry litter





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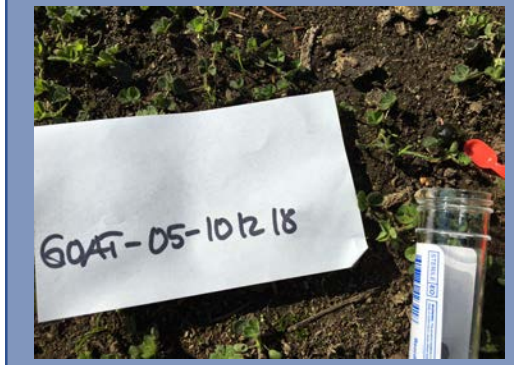
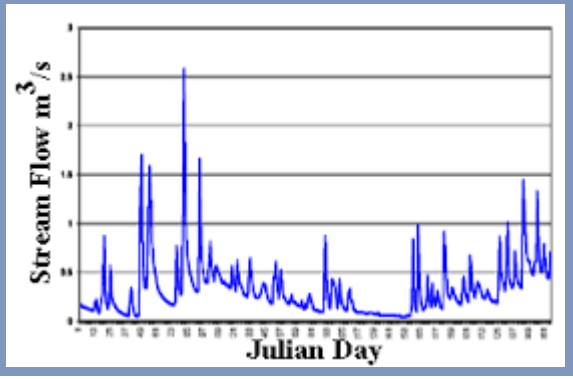
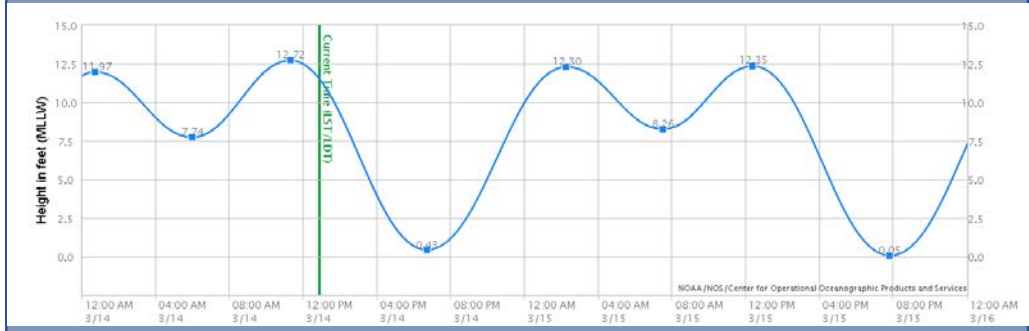
MST Demonstration Project

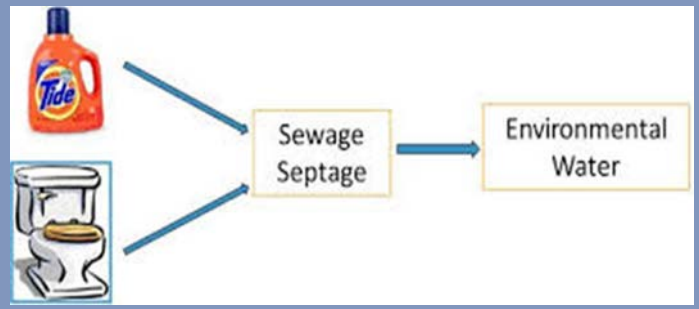
Sampling and Analysis Plan

- Fecal source sampling of ~10 samples for each of 7 human/animal sources (Aug – Sept 2018)
- 8 water grab samples at 7 stations:
  - 5 monthly routine events (Aug - Dec 2018)
  - 3 storm events (Oct - Dec 2018)
- Flow and optical brightener fluorescence
- Fecal coliform, E. coli, and MST sample filtration at local lab, ship frozen filters to SM
- MST sample filter extraction and qPCR analysis by Source Molecular
- Source and water sample extracts to UMBI
- Geospatial statistical analysis of fecal bacteria, MST, climate, and watershed attribute data
- Final report by June 30, 2019









MST Demonstration Project

Sampling and Analysis Status

- Lost first 4 monthly MST samples due to laboratory filtration error
- QAPP addendum and lab training
- Added 3 monthly events in Jan – March
- Missed Feb event due to snow, added April monthly event
- Completed all 7 events (4 base and 3 storm)
- Completed fecal source validation
- qPCR results by May 6, 2019
- Community-based NGS results by June 16, 2019
- Final report by June 30, 2019 to include upland source control actions and MST study protocols for Puget Sound shellfish protection areas

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Membrane Filtration (Concentration of bacteria)

Materials:
- -20°C freezer or dry ice to freeze filters
- Pre-sterilized filter funnels (Pall MicroFunnels cat# FMFNL3020 or equivalent)
-Sidearm flask or waste bottle
-Vacuum manifold that fits the Pall MicroFunnels
-Vacuum source with tubing
-Stainless steel forceps (Millipore cat# XX6200006P)
-Flame source for sterilizing forceps
-Beaker to hold forceps
-Sterile bead tubes (contact Source Molecular to order: info@sourcemolecular.com)
-Fine point permanent marker
-Micro-tube rack (optional)
-Micro-tube box
-Disposable gloves
-100%, 200 proof ethanol for sterilizing forceps
-70% ethanol for cleaning counter tops
-10% bleach solution in spray bottle

Instructions:

Fill out the table on last page and submit with your samples. Without the information requested on the last page, Source Molecular's lab will not be able to provide quantification results.

Wear disposable gloves at all times. Gloves must be changed between samples to prevent cross-contamination. If gloves are soiled, doff the soiled gloves and don a fresh pair immediately. Ensure work surfaces are sterilized by wiping down with a 10% bleach solution followed by 70% ethanol.

1. Assemble the vacuum manifold system. Vacuum manifold should be connected to the waste sidearm flask/bottle which is connected to the vacuum source by a hose.

2. Obtain a sterile Pall MicroFunnel filter funnel (300mL capacity) for each sample, remove from packaging and position on top of the vacuum manifold, press down firmly so the funnel is snug. The MicroFunnels already include the membrane filter and are ready for water to be poured in.

3. Shake water sample to obtain a homogenous mixture. If the water appears to be very turbid, shake and let sit for 10 minutes for the larger suspended particles to

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settle.

4. With the manifold stopcocks in the **closed** position, pour water 100 mL of sample into funnel and make note of the volume added.

5. Open the stopcocks and allow the entire sample to filter through.

6. **Before adding more water to the funnel, turn off the vacuum and close the stopcock in order to accurately measure the volume added.** If the water filtered through fairly quickly (in less than ~20 minutes), add more water. 200 mL of water should be filtered per sample.

   - If the membrane becomes clogged before filtering 200mL and after about 20-30 minutes with stopcock open, continue filtering through a second membrane by doing the following
     - A. Record the volume of water that passed through the filter.
     - B. Pour water remaining in filter funnel back into sample container.
     - C. Transfer the filter membrane to a provided bead tube by following **steps 7- 9.**
     - D. Label the bead tube (with Fine point permanent marker) as membrane "1 of 2" along with the unique sample ID and volume filtered
     - E. Obtain a new filter funnel and continue filtering that sample. Aim to filter a combined total volume of 200mL. Use a maximum of 2 membranes, even if a total volume of 200mL cannot be obtained.
     - F. Transfer the second filter membrane to a bead tube by following **steps 7-9**.
   - Waters too turbid to be filtered should be spun down with an ultracentrifuge at 2500-3000 rpm for a few minutes to pelletize the solid particles. This is **not recommended** however and should only be used as a last resort since it may result in a loss of bacteria adhering to the suspended particles.

7. Turn off vacuum, turn the stopcock to closed position, remove the funnel cylinder from the base by gently squeezing the funnel cylinder and lifting up. The base and funnel should detach. Place the funnel along with lid upside down on the counter so to not contaminate the bottom that comes into contact with the base.

8. Open the pre-labelled bead tube and place the cap upside down on a clean area of the counter. Keep the open bead tube on the tube rack or in a microtube box while you perform the next step.

9. Flame-sterilize two forceps (see below instructions). While the membrane is on the

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filter funnel base, use the sterile forceps to fold it in half and then into a cylinder with the top side facing inward, being careful to handle the membrane only on the edges, where it has not been exposed to the sample. Insert the rolled membrane into the labeled bead tube, place the forceps back into the ethanol and close the tube.

As an alternative to using the flame-sterilization method, pre-sterile, individually packaged, disposable forceps may be used. Use one per sample and throw away.

Flame-sterilizing forceps
Keep the forceps upright in a beaker with roughly 2 inches of 100% ethanol so that the lower stainless steel portion is covered. Remove both forceps and swipe over a flame to burn the ethanol. The forceps are now sterile and ready for use.

Notes:
* DO NOT place forceps down on any surfaces in order to keep them sterile.

* DO NOT place forceps back into the ethanol beaker immediately after flaming since the hot forceps may ignite the ethanol. Wait at least 20 sec.

* Keep ethanol beaker an arms length away from the flame source at all times while working and other ethanol stock materials in a separate room if possible.

* Execute caution when using fire around flammable materials like ethanol. A fire is always a possibility. The nearest fire-hydrant should be located before beginning

10. Ensure all tubes are labeled with the Client's name, sample ID, the date and volume filtered. Document this in the table below.

11. Samples must be shipped to our laboratory in a frozen state. Place them in microtube box and pre-freeze them in the freezer or on dry ice in a Styrofoam cooler. If using dry ice, ensure dry ice is placed all around and on top of the microtube box. Ship on dry ice following the "Filter Packing Instructions" guidelines provided to you.

Clean Up:
- Left over samples and filtrate in the waste flask may be poured down the drain and bottles disposed of in the trash
- 100% ethanol may be poured down the drain with running water
- Squirt a little bit of 70% ethanol down the manifold cups
- Rinse the waste bottle well with water and 70% ethanol, store in a separate bag/box from other materials
- Wipe down counter with 10% bleach followed by 70% ethanol

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Company Name:
Company Address:
Company Phone:

Contact Name:
Filterer's name:
Filterer's Signature:

Filters submitted to Source Molecular

Sample ID	Total Volume Filtered (mL)	# of filters for this sample	Date Filtered	Time Filtered

MST Demonstration Project
Fecal Validation Results

- qPCR analysis of 82 fecal source samples for target and non-target sources
- Objectives for at least 80% sensitivity (true positive) and 80% selectivity (true negative)
- Selectivity low for Horse and Bird, increasing if include DNQ as positive
- Sensitivity high for all markers, indicating low false positives

Marker	Sensitivity (% true positive)			Selectivity (% true negative)		
	n	Quantify	Present	n	Quantify	Present
Human	11	91%	91%	71	100%	100%
Cow M2	10	90%	100%	61	100%	100%
Cow M3	10	80%	80%	61	100%	100%
Horse	10	**50%**	90%	61	100%	96%
Ruminant	31	87%	94%	40	100%	100%
Pig	5	100%	100%	66	100%	100%
Dog	11	91%	91%	60	100%	100%
Bird	14	**57%**	**71%**	57	96%	89%

Human = 11 septage
Ruminant = 10 cow + 5 sheep + 5 goat + 4 llama + 1 alpaca + 6 deer
Bird = 6 goose + 8 gull

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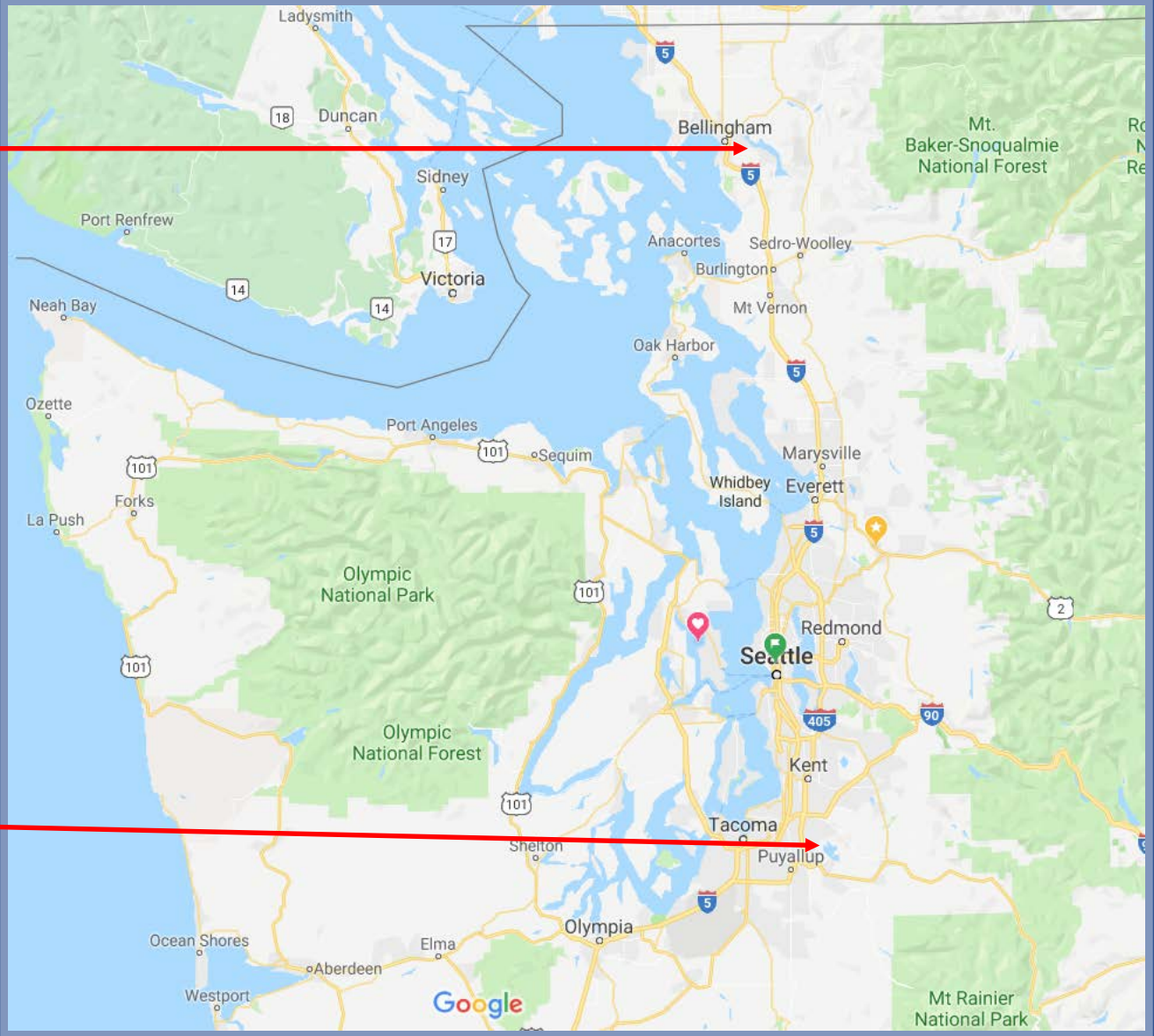
Lake Septic Detection Projects

- Lake Whatcom in 2017
- Lake Tapps in 2018 and 2019

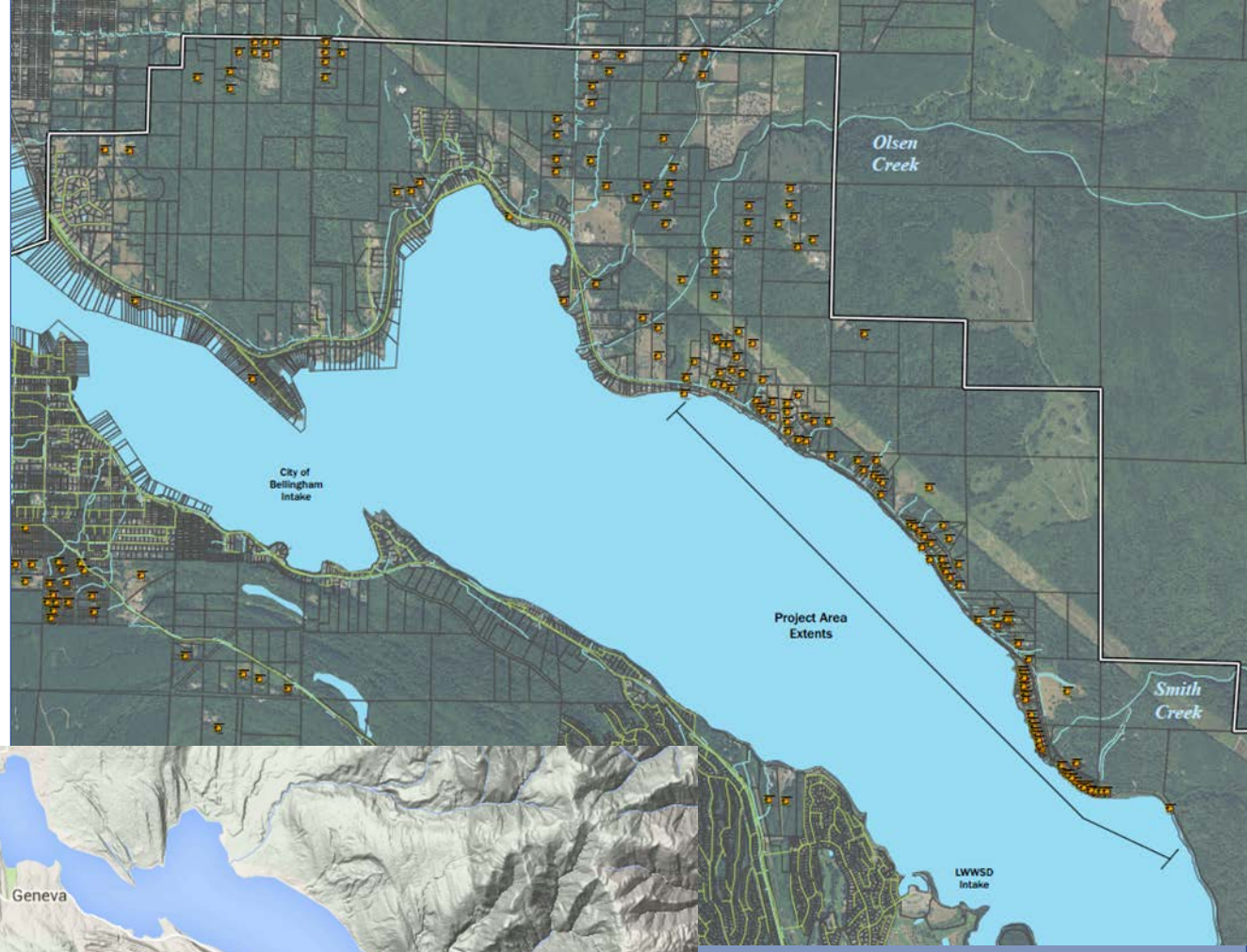


Lake Septic Detection Projects

Lake Whatcom Conditions

- Bellingham water supply and TMDLs
- North Shore Subbasin (3 shore miles)
- Shallow soil over bedrock
- Steep slopes/high rainfall
- 96 OSS with 50% built before 1990 and 40% failed inspections

- Sewer extension requires proof that OSS present environmental and public health risk
- TMDL requires 86% reduction of stormwater TP load ($millions) and only OSS maintenance (status quo)



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Lake Septic Detection Projects

Lake Whatcom Study Parameters

Field Equipment:

- Optical brightener fluorometer (Turner Designs Cyclops/Databank)
- Conductivity/multimeter (YSI)
- Peristaltic pump for lab samples

Lab Parameters:

- Fecal coliform/E. coli bacteria
- Total phosphorus
- Chloride/bromide
- Microbial Source Tracking (MST) using two human Bacteroidetes methods by digital quantitative polymerase chain reaction (qPCR)

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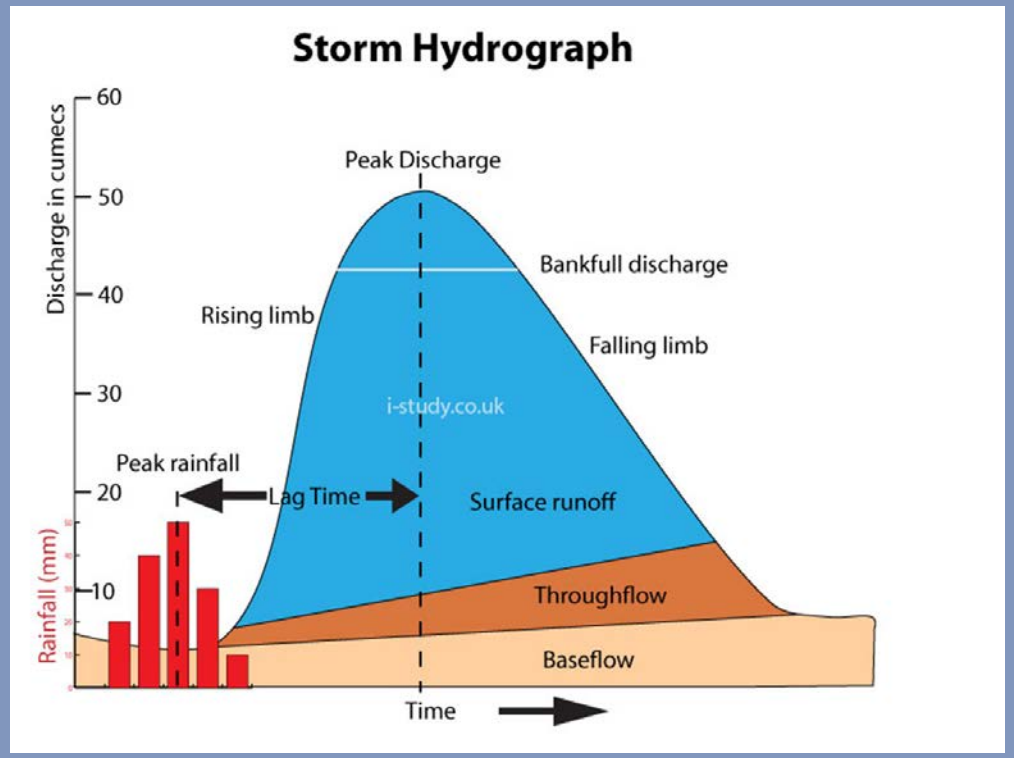
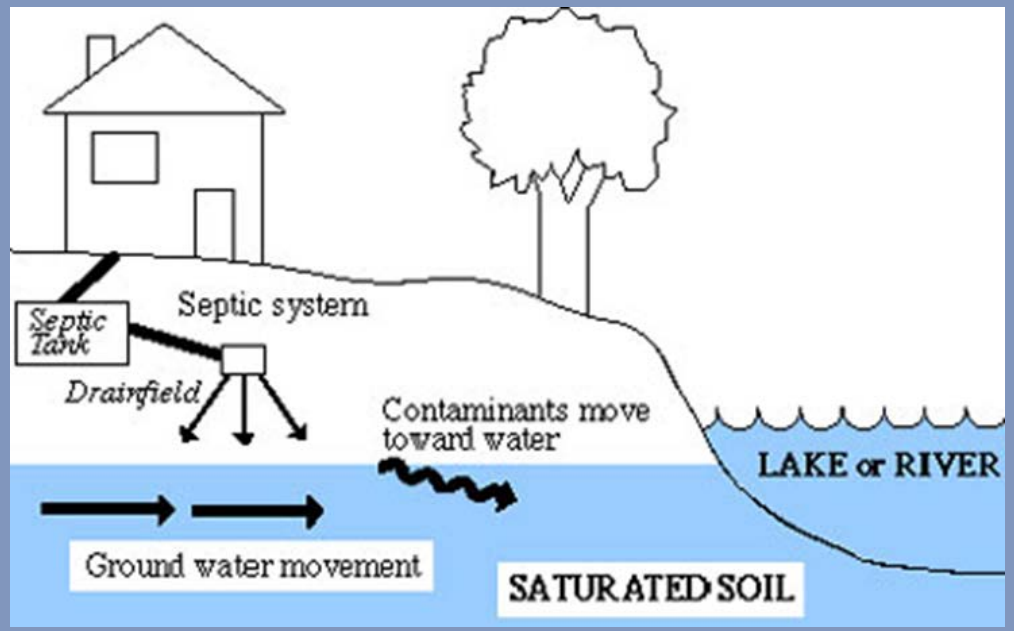
Lake Whatcom Study Design

- Target winter wet weather with high infiltration and high water table for maximum transport potential
- Survey shoreline by boat to access groundwater seeps and outfalls without needing public access
- Conducted 3 boat shoreline surveys during winter wet weather:
  1. 1/19/17 (2.2 inch 48-hr rain)
  2. 3/15/17 (0.9 inch 48-hr rain)
  3. 3/29/17 (1.9 inch 48-hr rain)





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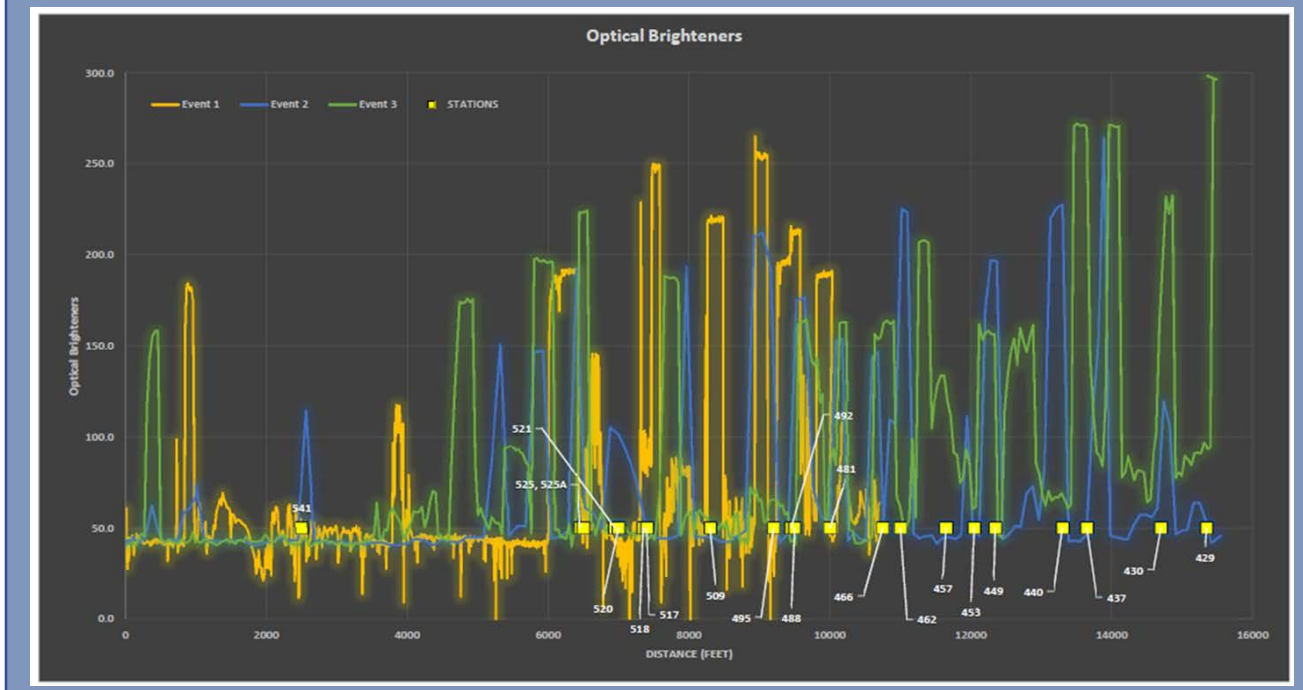
Lake Septic Detection Projects

Lake Whatcom Study Design

- Continuously log OB/YSI/GPS in lake and observed discharges
- Use OB to detect hot spots in lake and ~20 discharges to lake
- Pump sample at lake background sites first and OSS site last
- Survey 1 collected 23 fecal bacteria samples at hot spots
- Surveys 2 and 3 collected 18 fecal bacteria sample at hot spots and test 15 samples for TP, Cl/Br, and 2 human qPCR markers



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Lake Septic Detection Projects

Lake Whatcom MST Method

- Method comparison study using 27 labs:
  - Human dorei marker has high sensitivity, low false negatives
  - Human EPA marker has high selectivity, low false positives
- Source Molecular analysis of data showed 85% of samples had B. dorei detected at or above B. EPA
- Digital qPCR increases sensitivity by amplifying multiple droplets versus one aliquot by standard qPCR
- Each sample analyzed in duplicate and re-analyzed if COV exceeds 30%

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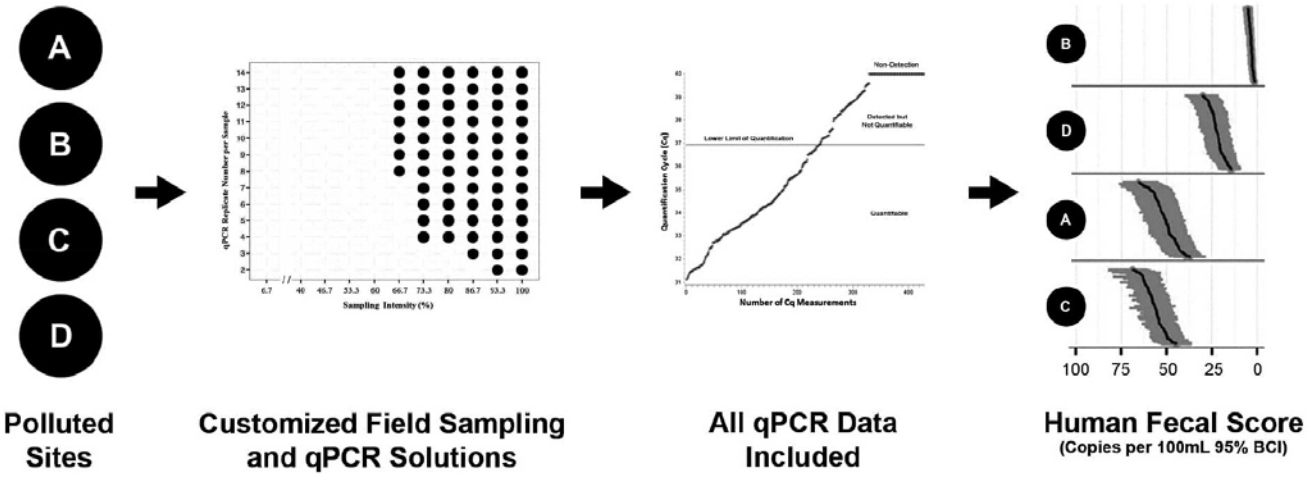
HUMAN FECAL SCORE FOR SITE RANKING



Polluted Sites → Customized Field Sampling and qPCR Solutions → All qPCR Data Included → Human Fecal Score (Copies per 100mL 95% BCI)

STANDARDIZED PROCEDURE

Lake Septic Detection Projects

Lake Whatcom Water Quality Results

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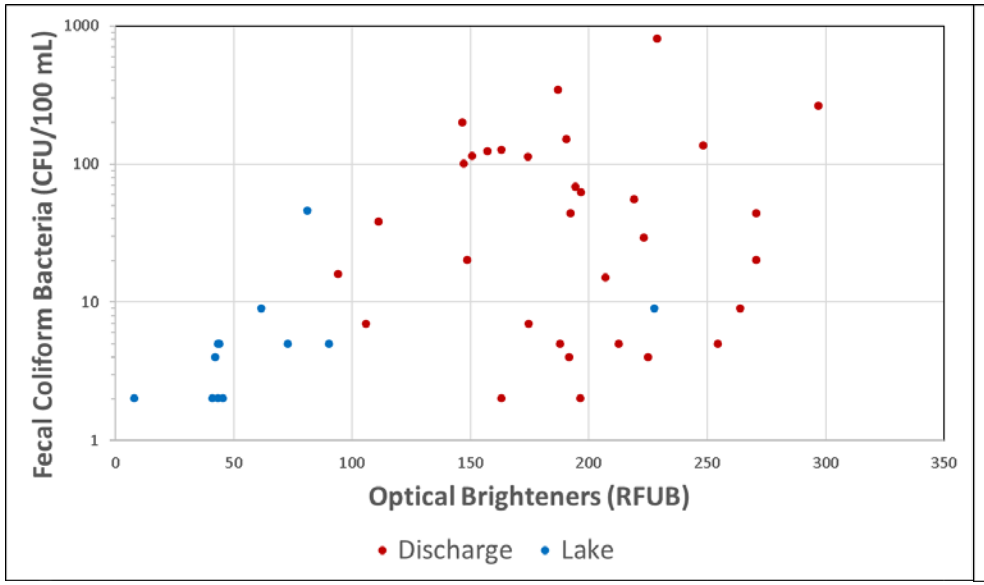
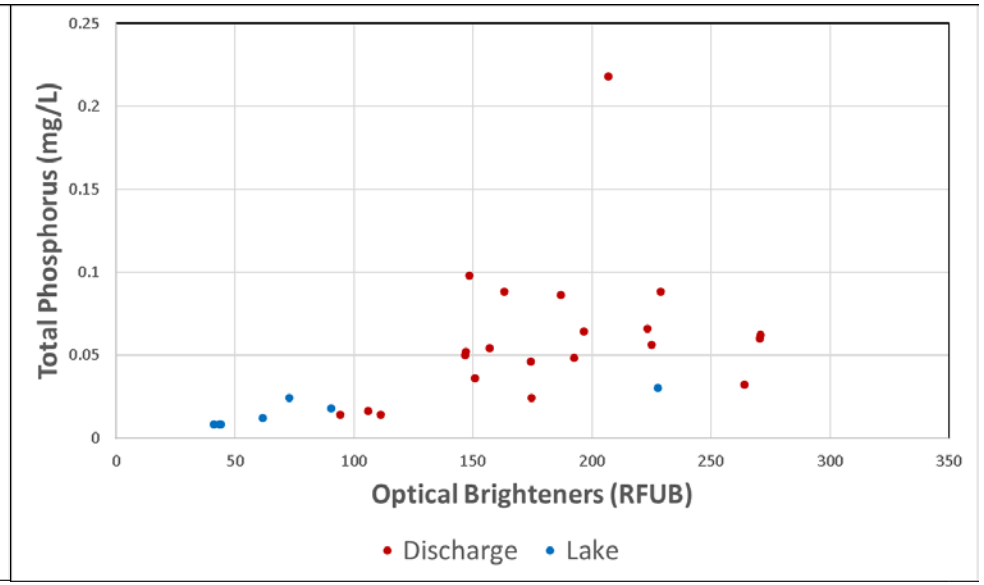
Lake Background < Lake Impact Sites < Discharge <<< OSS

Parameter	Lake Control	Lake Impact	Discharge	OSS
Optical brighteners (RFUB median)	43	81	189	660*
Total phosphorus (mg/L median)	<0.008	0.021	0.054	10.3
Fecal coliform (CFU/100 mL geomean)	3	10	36	2,470,000
Human dorei (copies/100 mL geomean)	1.4	3.7	8.4	1,230
Human EPA (copies/100 mL geomean)	0	0	4.6	88,100

OB correlated with FC and TP

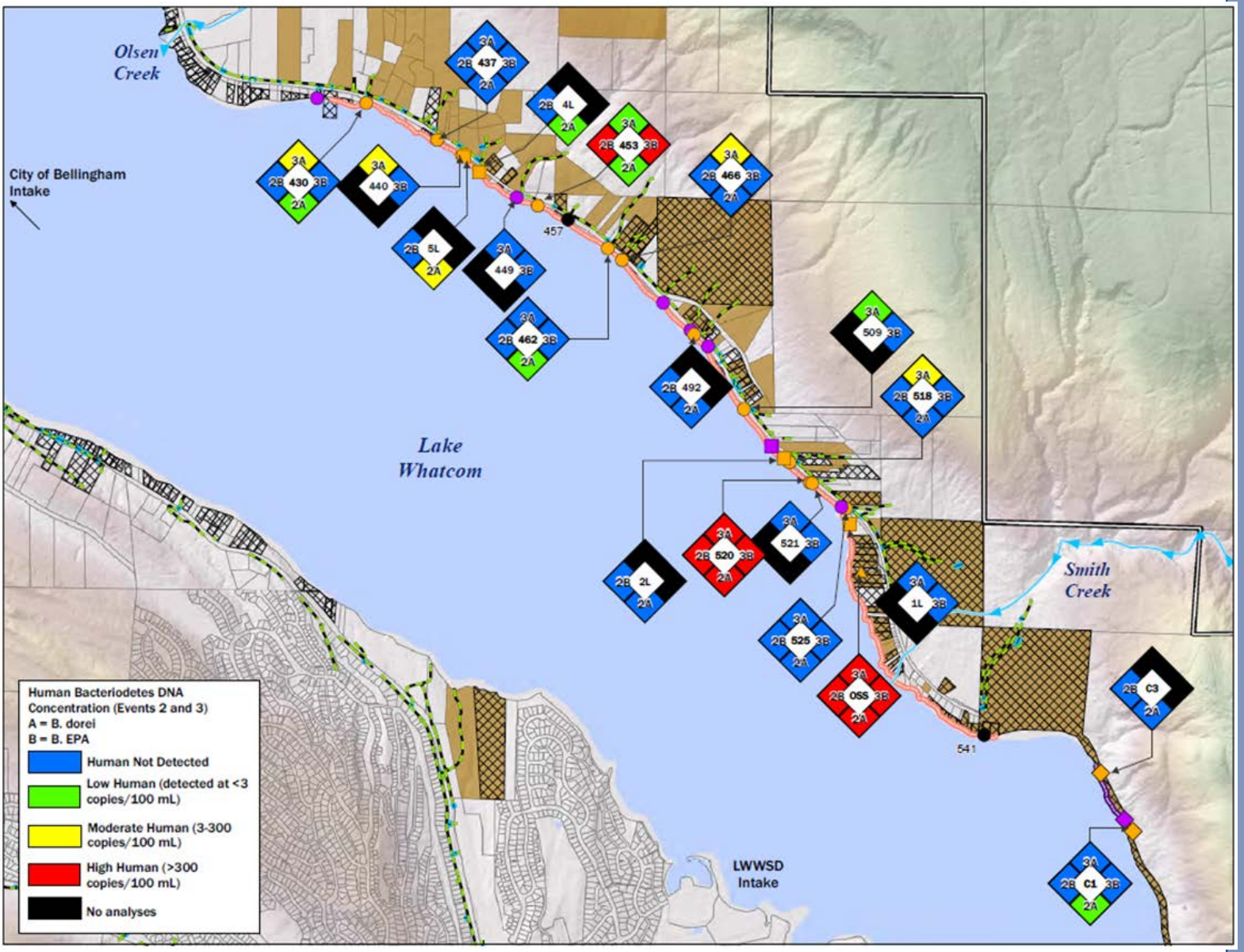
FC not correlated with human DNA





Lake Septic Detection Projects

Lake Whatcom OSS Detections



Human DNA concentrations:

- **Not Detected** at most sites
- **Low** (< 3 copies/100 mL) in one lake background sample
- **Moderate** (detected to 300 copies/100 mL) at 4 discharges and 1 lake station (only dorei marker)
- **High** (>100 x DL) at 2 discharges (1 at OSS level but with moderate fecals)

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Lake Whatcom Study Follow-up Actions

- Health Department inspected high human locations in June 2017 and found no problems
- Preliminary TP loading analysis indicates OSS are low source of phosphorus to lake
- Plans for study redesign and replication next wet season

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Lake Septic Detection Projects

Lake Tapps Conditions

- Cascade Water Alliance from PGE hydropower for future water supply
- Reduced White River turbid inflows and winter drawdowns
- Occasional toxic algae blooms
- Highest density and oldest OSS in northwest area
- Northwest shoreline survey by Health Dept. in March 2005 found 22 of 23 outfalls were dry
- 9 foot drawdown for 18 days in January 2018 for dam repair prompted investigation

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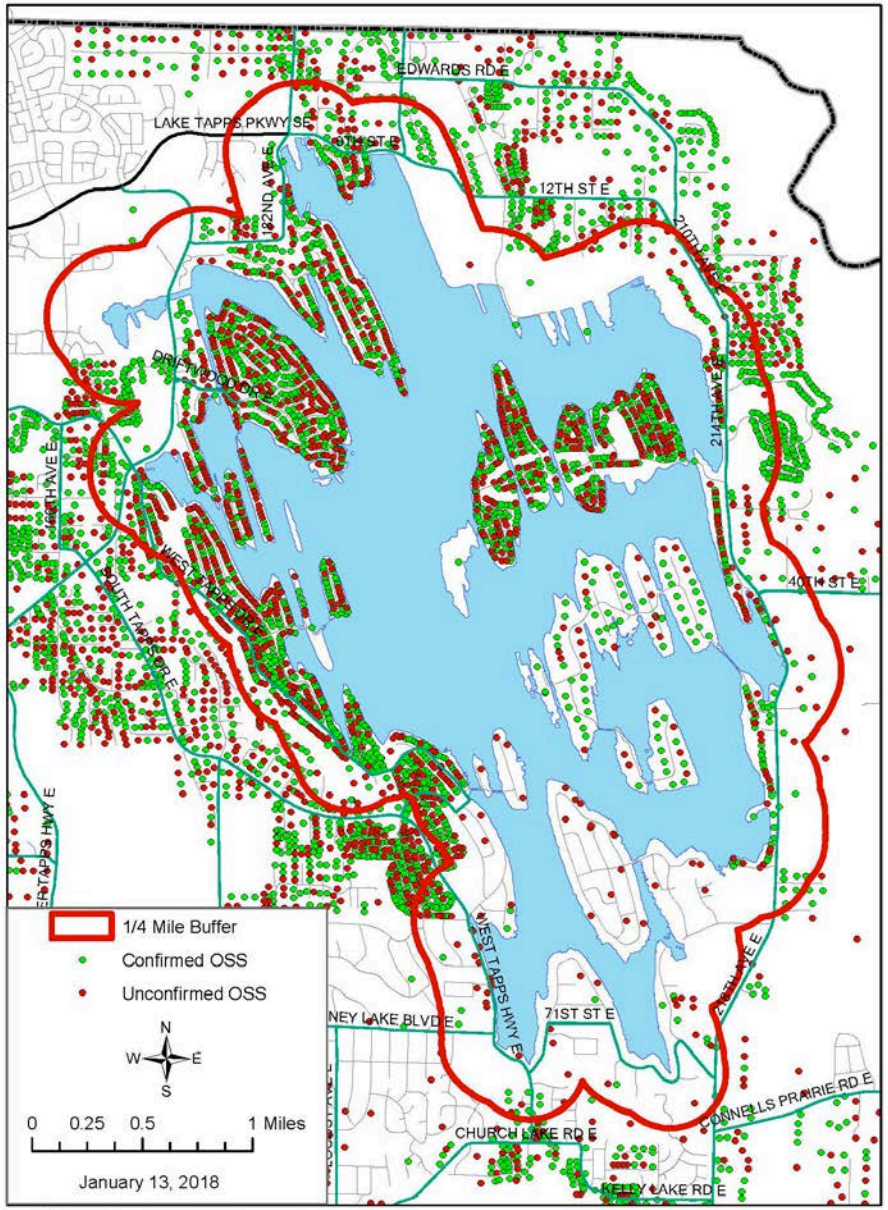
OSS Vicinity of Lake Tapps



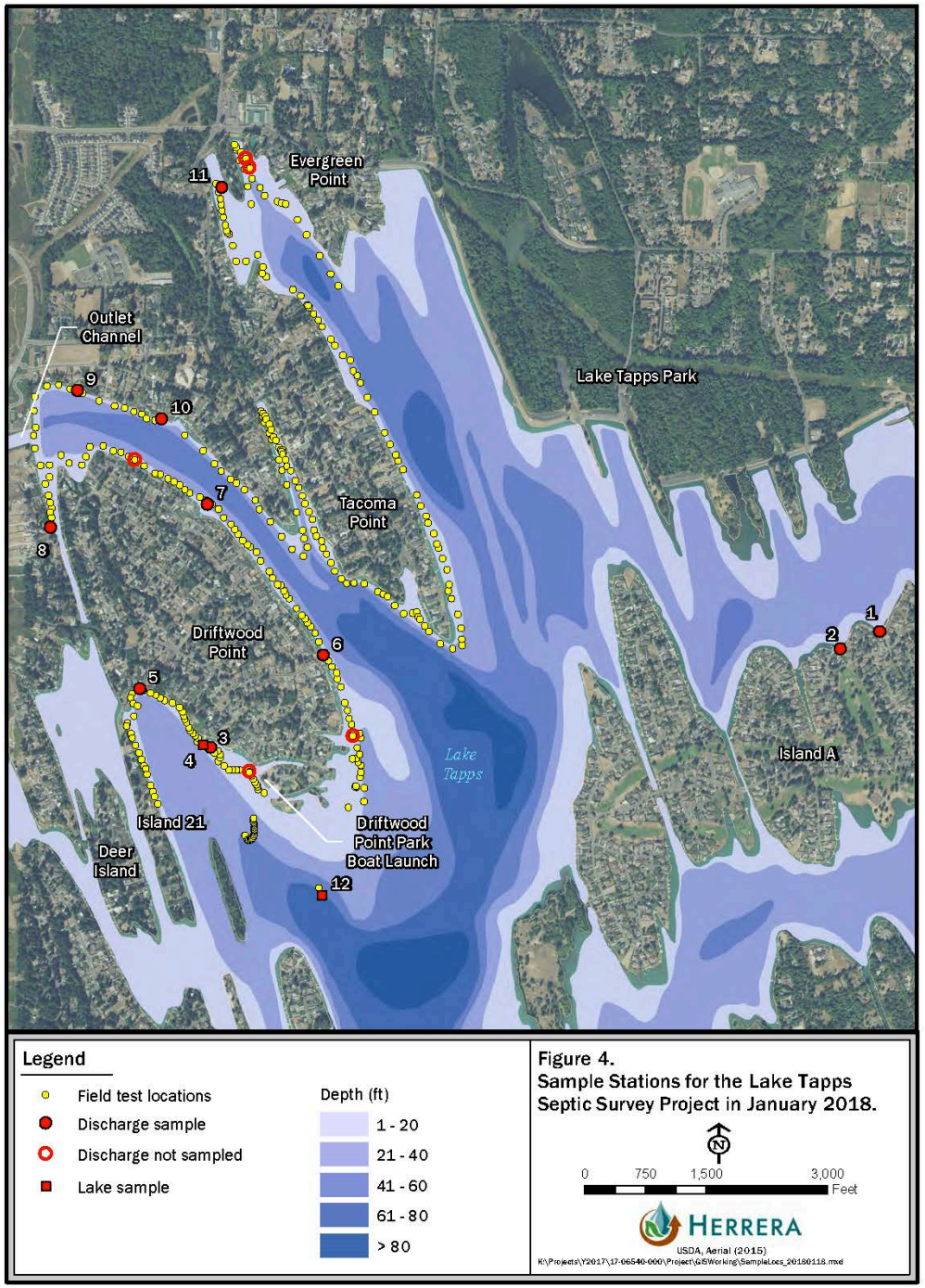
Tacoma - Pierce County Health Department
Healthy People in Healthy Communities

Figure 3.
On-Site Septic Systems in the Vicinity of Lake Tapps.

Lake Septic Detection Projects

Lake Tapps Study Methods

- 1 boat survey along 8 miles shore in winter wet weather (Jan 18, 2018)
- Continuously log OB and positions at shore (○) and in discharges (●)
- Sample 2 outfalls of concern on Island A and 1 lake background site
- Use OB to detect 1 hot spot in lake and 8 of 13 drainages to NW lake
- Analyze samples for EC, FC, TP, and 2 human biomarkers (dorei/EPA)



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Lake Tapps OSS Detections

2018 Human 1+2 Biomarker Totals:

- 5 Not Detected (including lake bkgd)
- 3 Low (detected 3 - 10,000 copies/100 mL)
- 2 Moderate (10,000 – 100,000)
- 1 High (> 100,000 copies/100 mL)

Repeat sampling on January 23, 2019 at:

- 8 moderate-high human or FC discharges
- 2 locations upstream of moderate-high
- 1 OSS

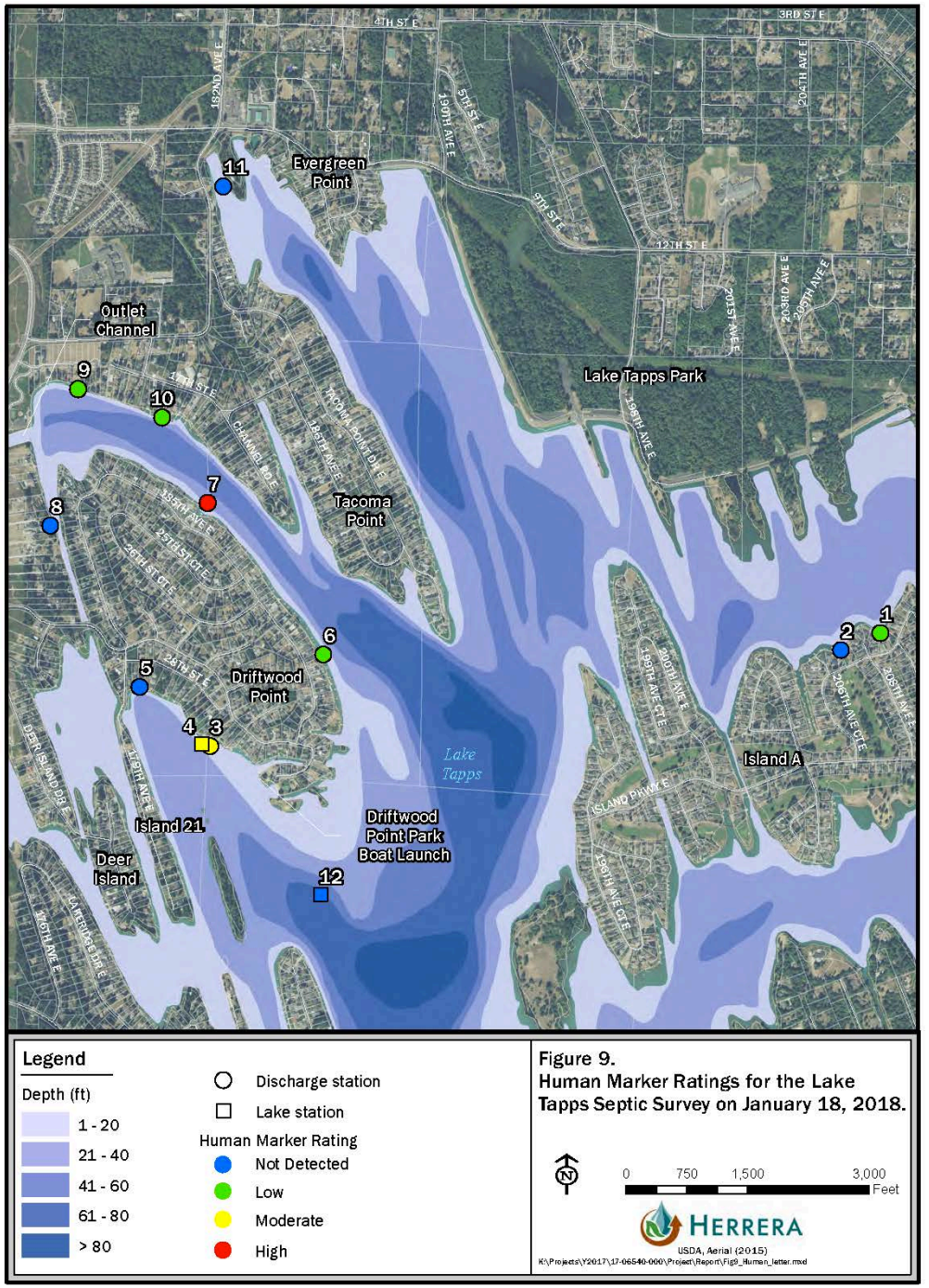

Figure 9.
Human Marker Ratings for the Lake Tapps Septic Survey on January 18, 2018.

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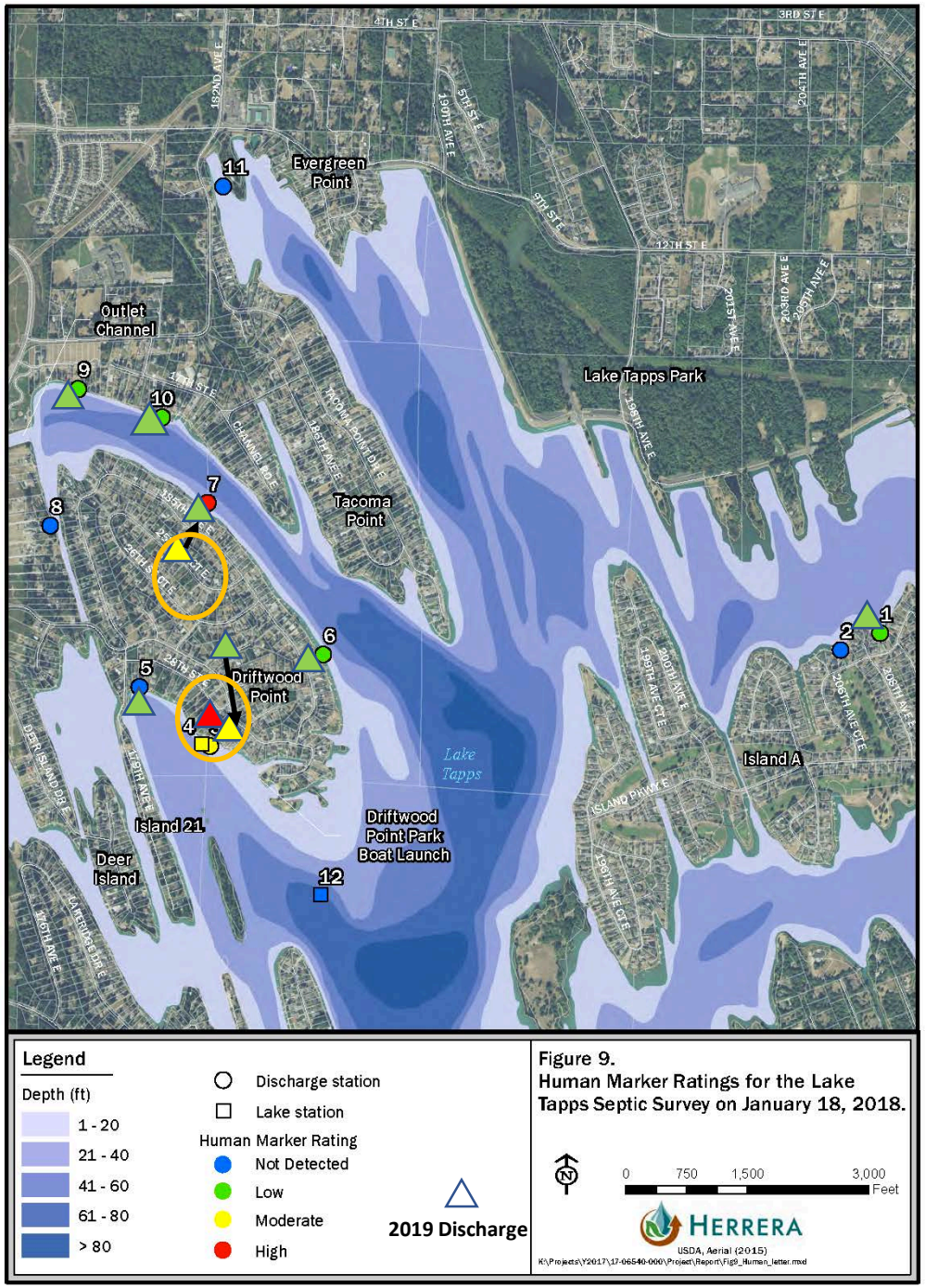
Lake Septic Detection Projects

Lake Tapps OSS Verification

2019 (Δ) Human 1 Biomarker (dorei):

- Detected in all 11 samples
- 7 Low (detected <10,000)
- 3 Moderate (10,000 – 100,000 includes OSS)
- 1 High (> 100,000 copies/100 mL)

Preparing 2019 report recommending OSS source investigation by Health Dept. in two areas (◯) of Driftwood Point draining to Sites 3 and 7



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Questions?

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