

Microbial Source Tracking Pilot Projects ~ 2011-12

OVERVIEW

- State freshwater fecal coliform criteria
- KCEL - Microbial Source Tracking (MST) 'tool kit'
- Summary of findings for Juanita Phase II and Phase III



KCEL Microbial Source Tracking “tool kit”

- Fecal coliform (FC) - state water quality standard
- *Escherichia coli* (*E. coli*) - part of the group of fecal coliforms (EPA recommended)
- Sorbitol-fermenting *Bifidobacteria* - human gut bacteria
Reported as presence/absence
- *Rhodococcus coprophilus* - pasture bacteria excreted by grazing animals
Reported as presence/absence
- Human *Bacteroides* qPCR – human gut bacteria
Reported as cells/100 mls
- Ruminant *Bacteroidales* PCR – targets sheep and cattle
Reported as presence/absence



The Usual Suspects

Created by Jonathan Frodge

sorbitol-fermenting *Bifidobacteria*

present/absent

PROS

- constitute large percentage of human gut flora & in higher numbers than fecal coliforms
- human sorbitol fermenting - easily distinguishable from other sources
- do not proliferate in the environment
 - strict anaerobes
- short survival - makes excellent indicators of recent contamination

CONS

- requires media prep, special preservation and collection
- requires 8 days to complete
- negative results should be interpreted with care



human *Bacteroides* qPCR

cells/100 ml

PROS

- comprise ~50 % by weight of the bacterial biomass in the feces of humans.
- do not proliferate in the environment
 - strict anaerobes
 - optimal growth ~ 37°C
- potentially quantitative microbial source tracking tool.

CONS

- DNA persists for up to one week after cell death
- uncertainty around lower threshold for human contamination
- need for relative scale based on known pollution sources



Rhodococcus coprophilus

present/absent

PROS

- found in the manure of domesticated grazing animals
- never been isolated from human feces.



CONS

- culture based – requires media prep in advance
- slow grower – 2- 3 weeks for results
- pasture bacteria – may not be present when domesticated animals are not grazing

ruminant *bacteroidales* PCR

present/absent

PROS

- Does not rely on feeding habits of the animal
- Specific to sheep and cattle
- Positive result indicates fecal contribution from a non-human source



CONS

- Does not detect fecal contamination from other farm associated grazers such as pigs, horses, chickens, alpaca, goat, etc.
- Results to date only represent a small sample set in 2012

MST Lines of Evidence

Fecal Coliform



State Recreational Criteria for Fecal Coliform Bacteria	
<u>Extraordinary</u> single sample >100 CFU/100 ml	<u>Primary</u> single sample >200 CFU/100 ml



Ten State Swimming Beach Criteria	
fecal coliform > 1000 CFU/100 ml	fecs GEOMEAN 200 CFU/100 ml

Other



EPA Recommended	KCEL/literature	KCEL/literature	KCEL/literature
<i>E. coli</i> > 235 CFU/100 ml	<i>Bacteriodes</i> > 300 cells /100 ml	<i>Bidobacteria</i> present	<i>Rhodococcus</i> present

2011 Pilot MST Projects

All are 303(d) listed for fecal coliform bacteria

- Idylwood Creek – creek flows into swimming beach area
- Juanita Creek – swimming beach closures and potential TMDL
- Boise Creek - TMDL
- Issaquah Creek – TMDL
- White Center - Hicks Lake



Juanita Creek Phase I ~ 2008 FC Survey

Intensive FC Bacteria Survey

- Ecology
- City of Kirkland
- King County

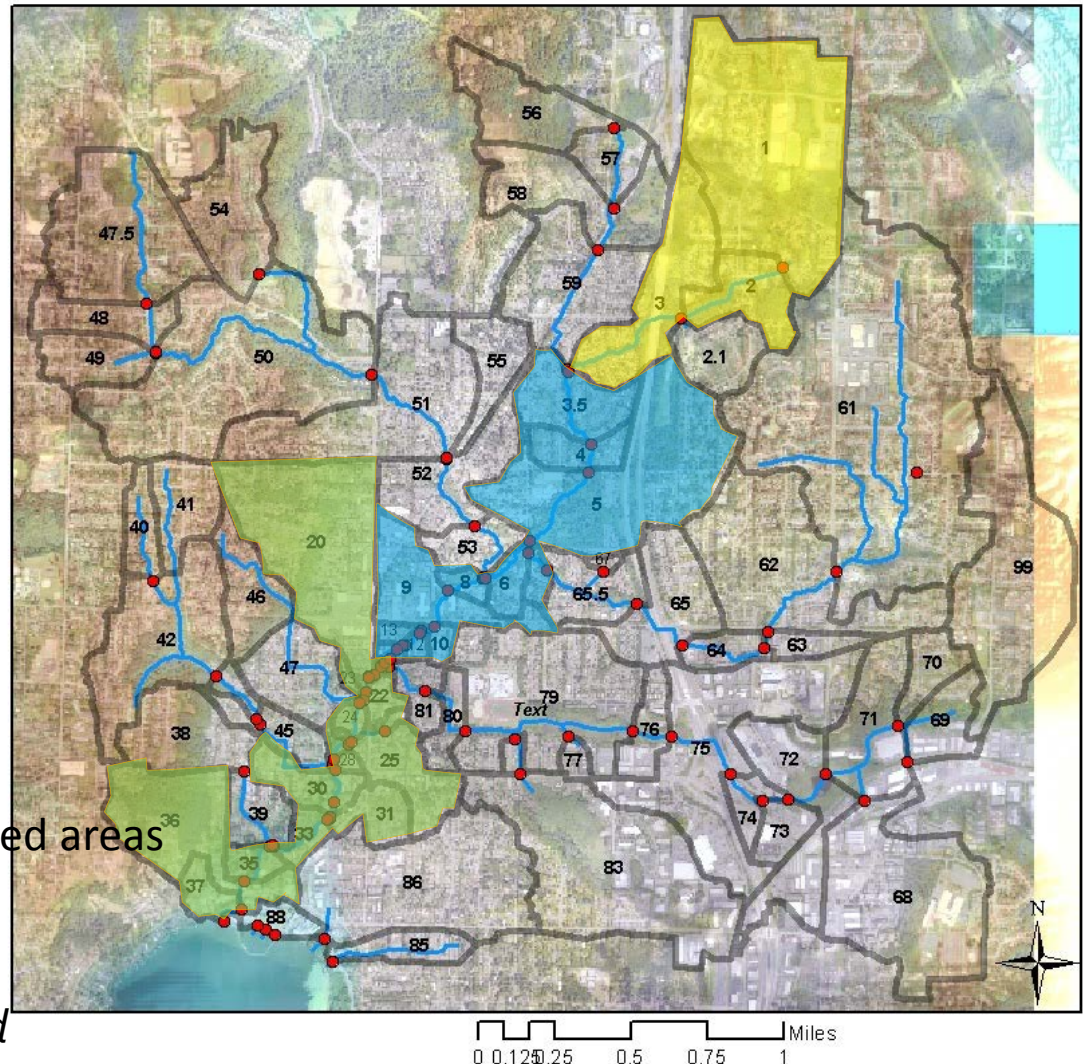
“Direct Implementation”

rather than begin TMDL Process

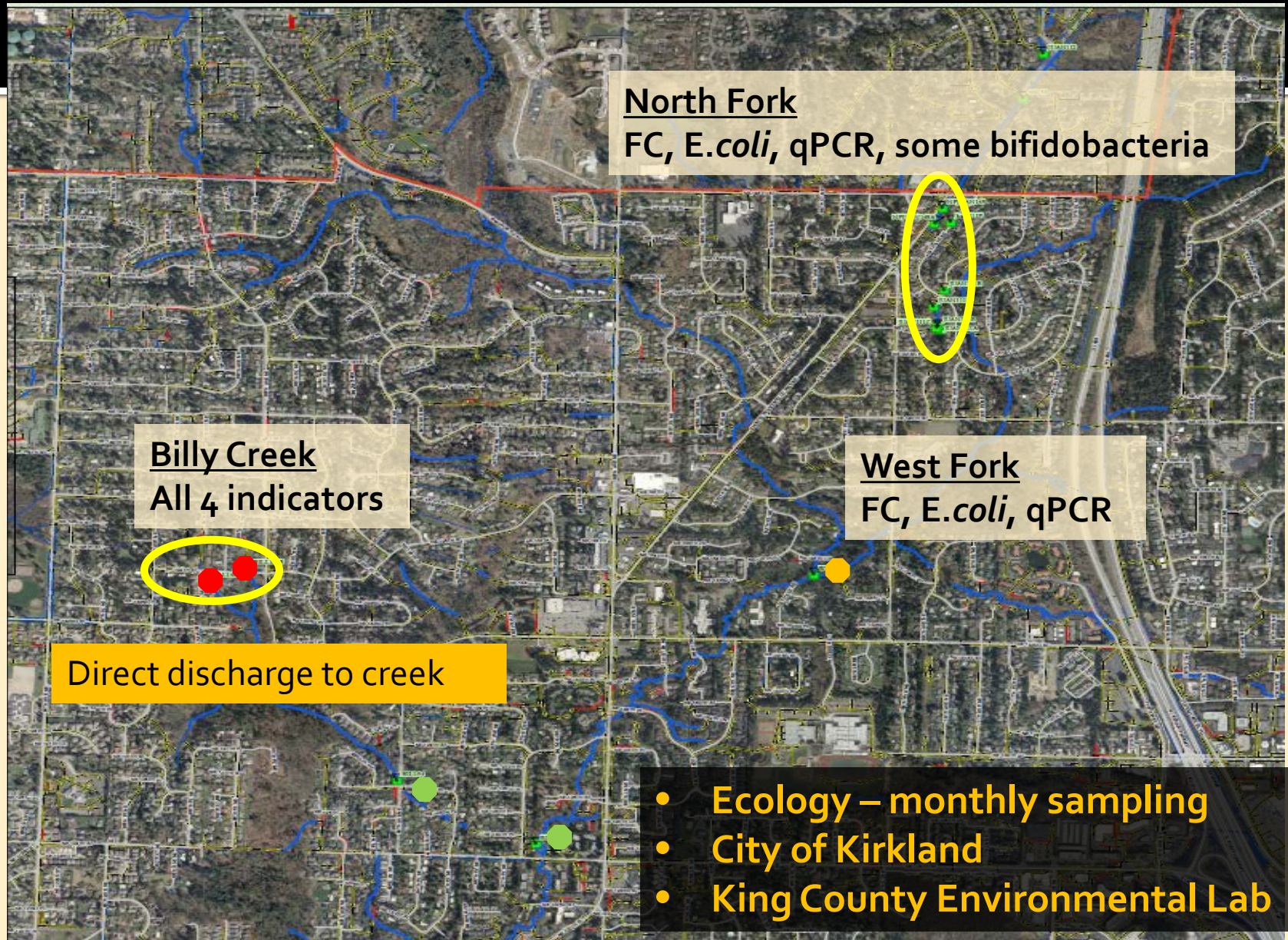
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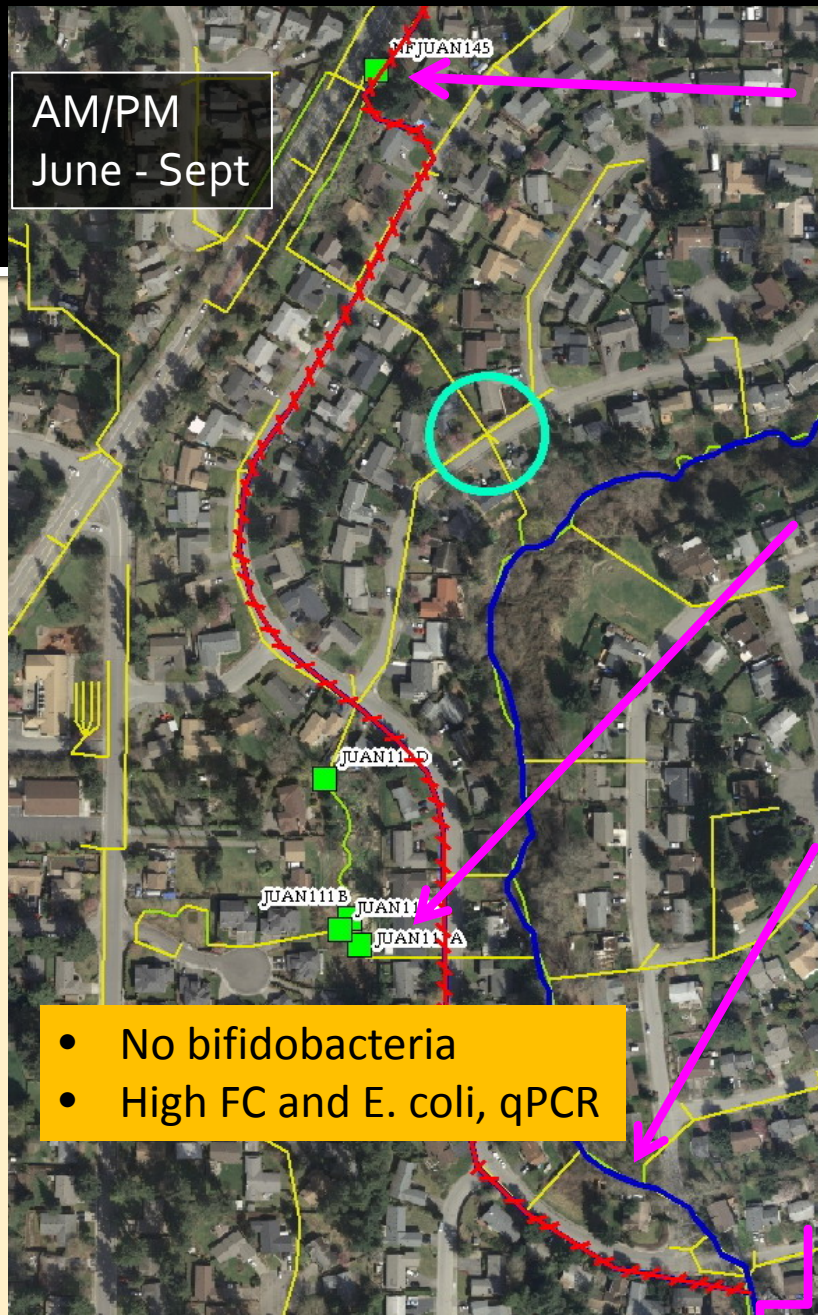
1. Identified areas of concern
2. -Ecology monthly sampling in selected areas
3. -Investigate MST options

Main stem of the creek highlighted

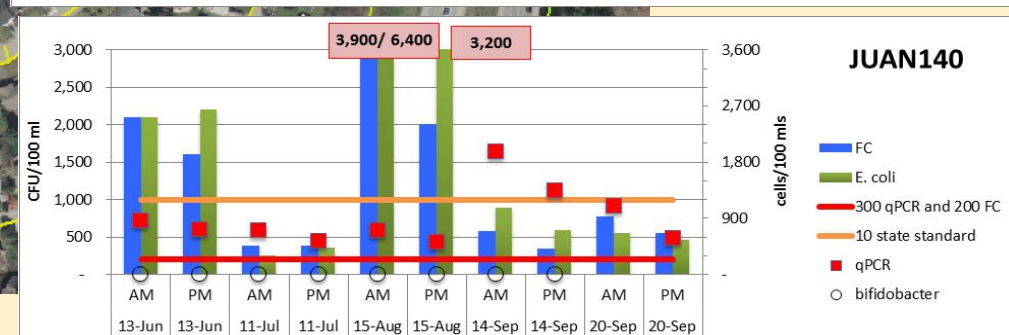
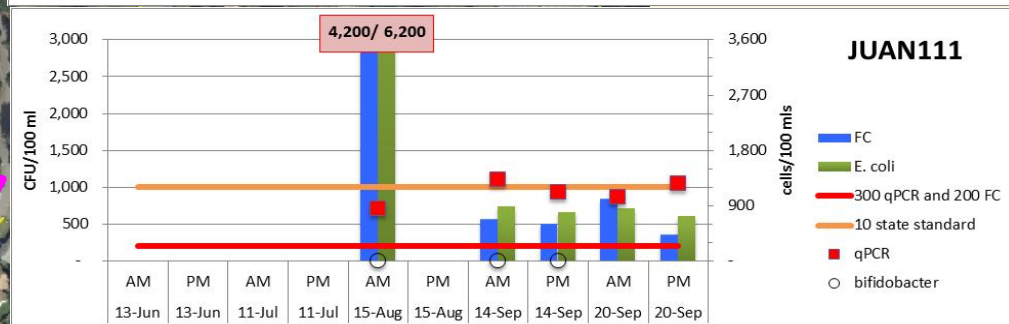
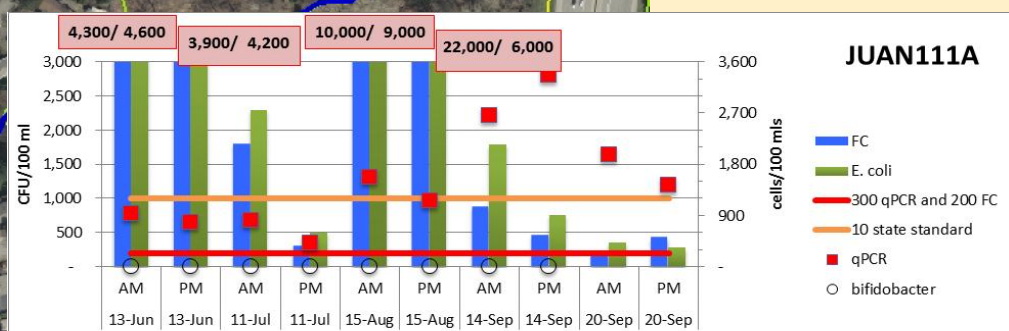
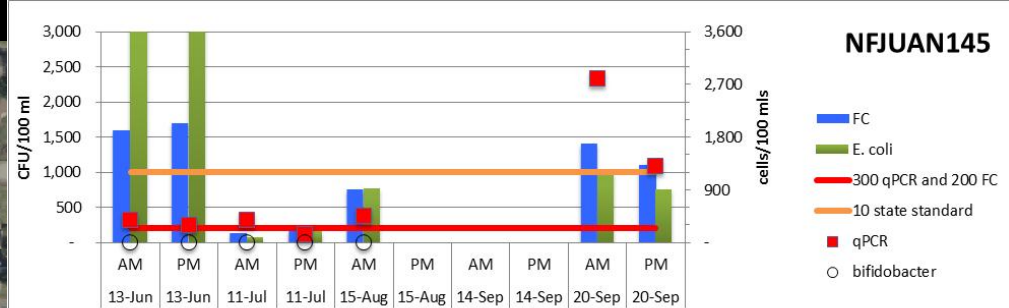


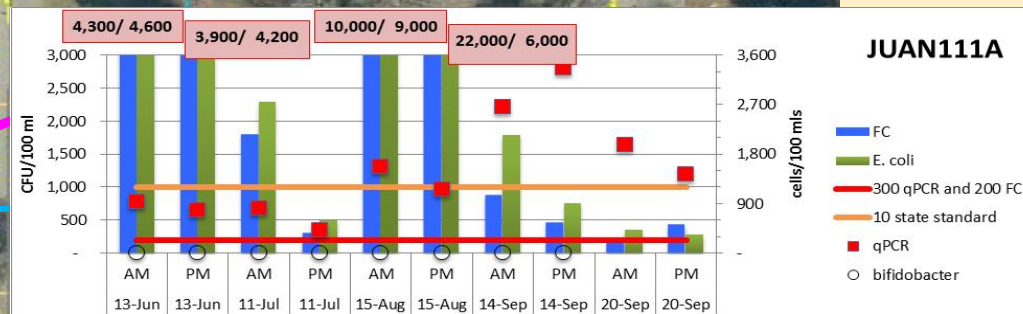
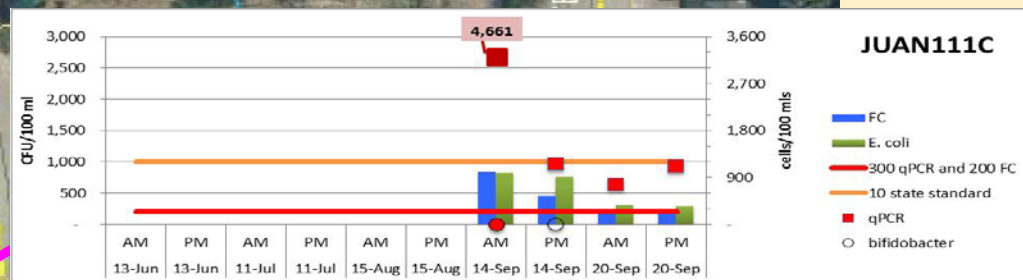
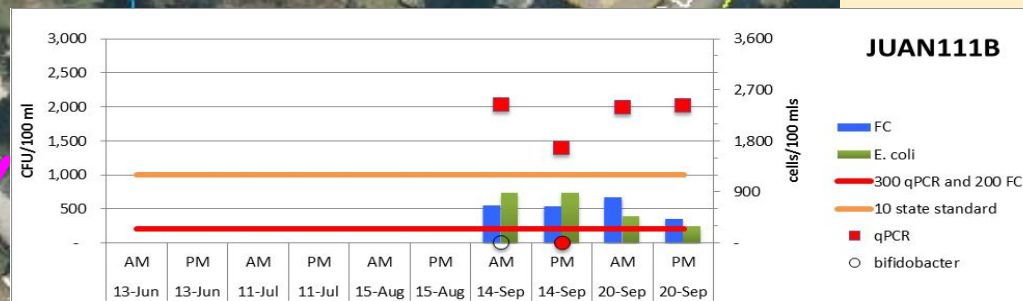
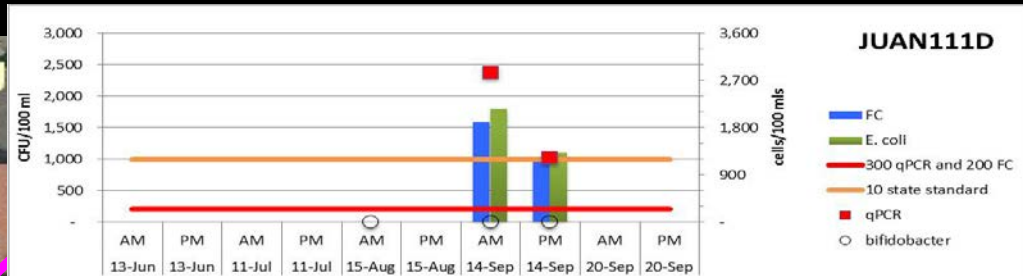
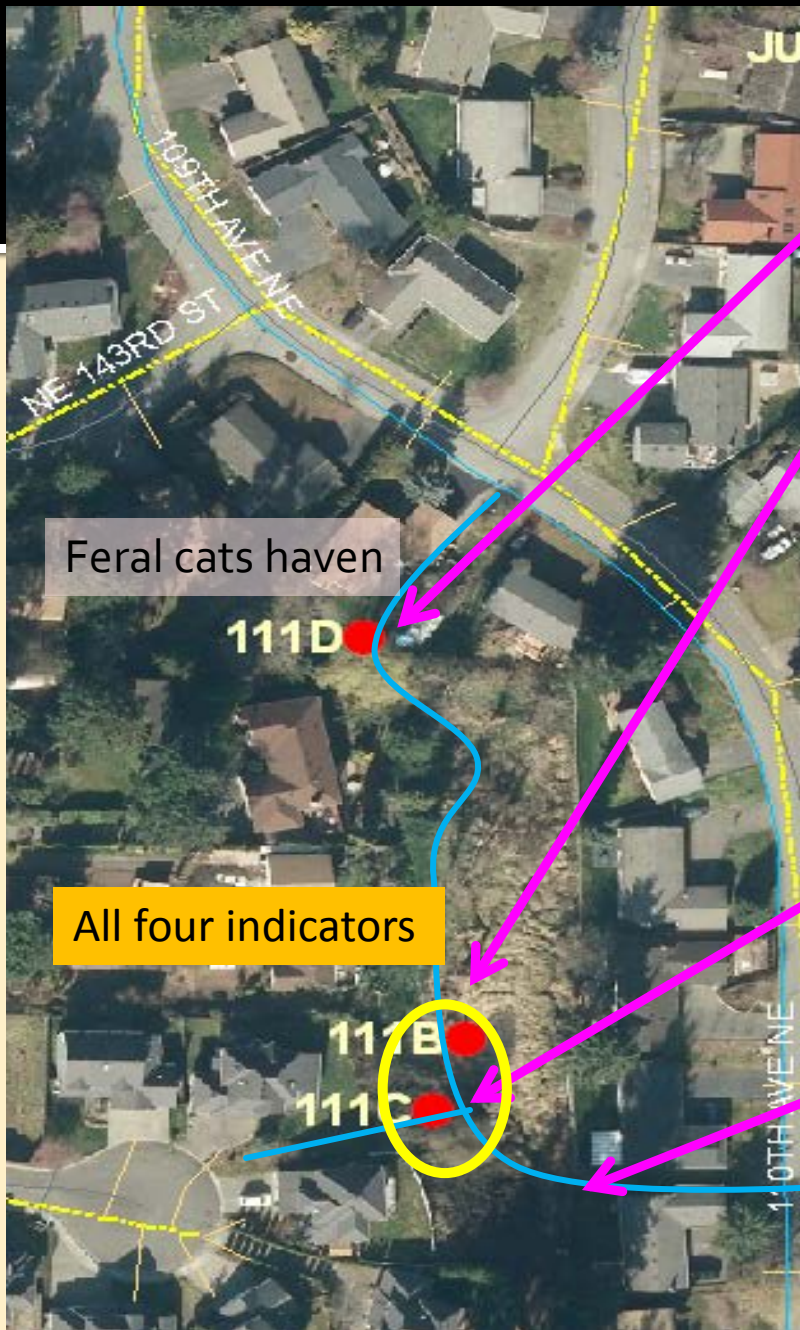
Juanita Creek Phase II ~ 2011 MST



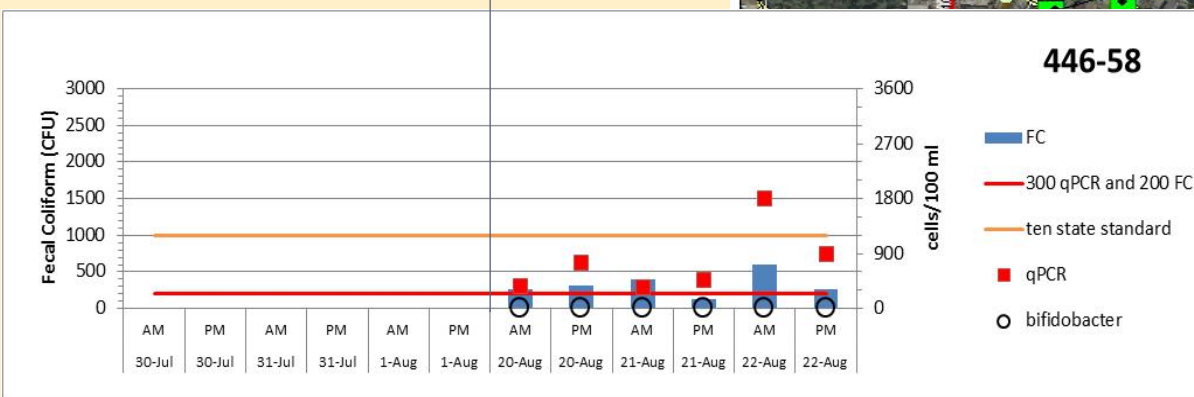
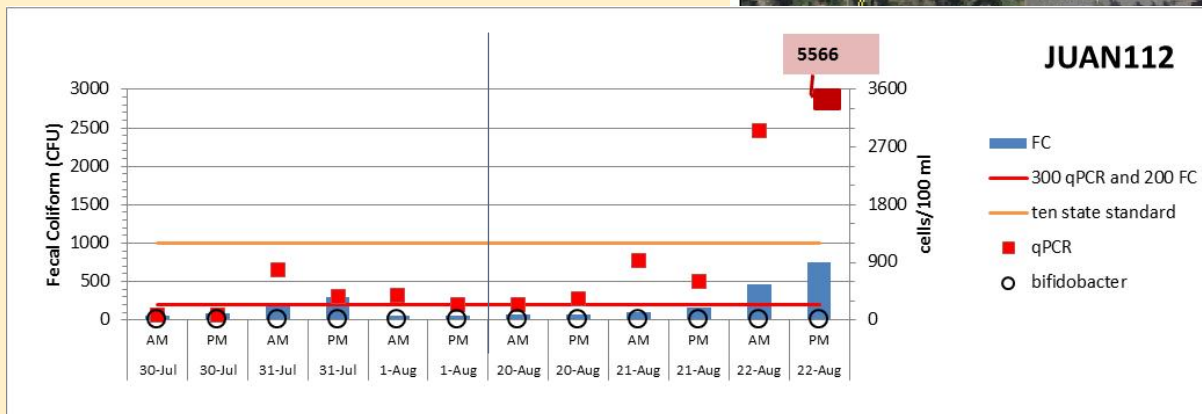
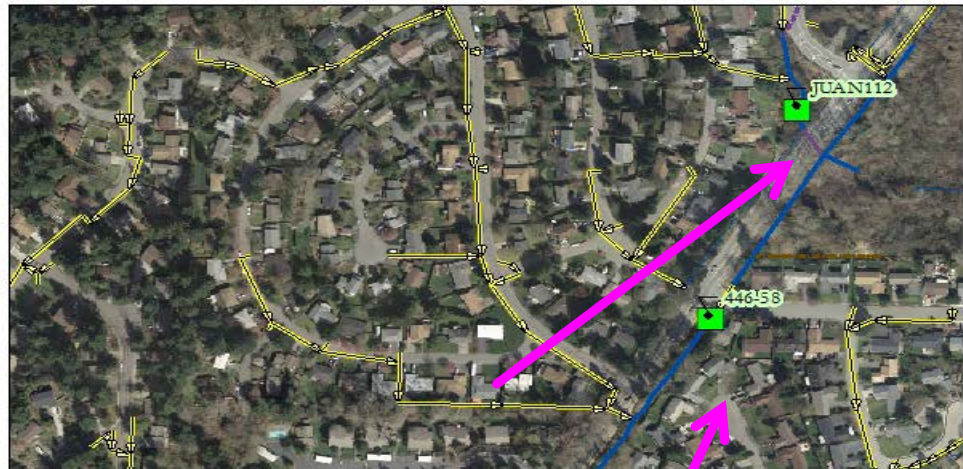


JUANITA PHASE II – North Fork



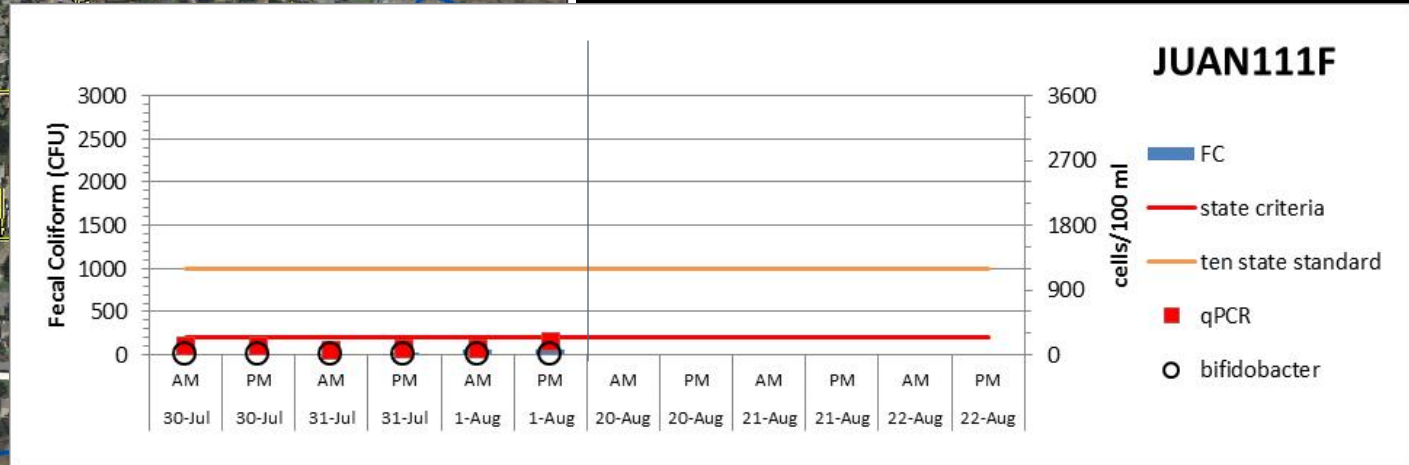
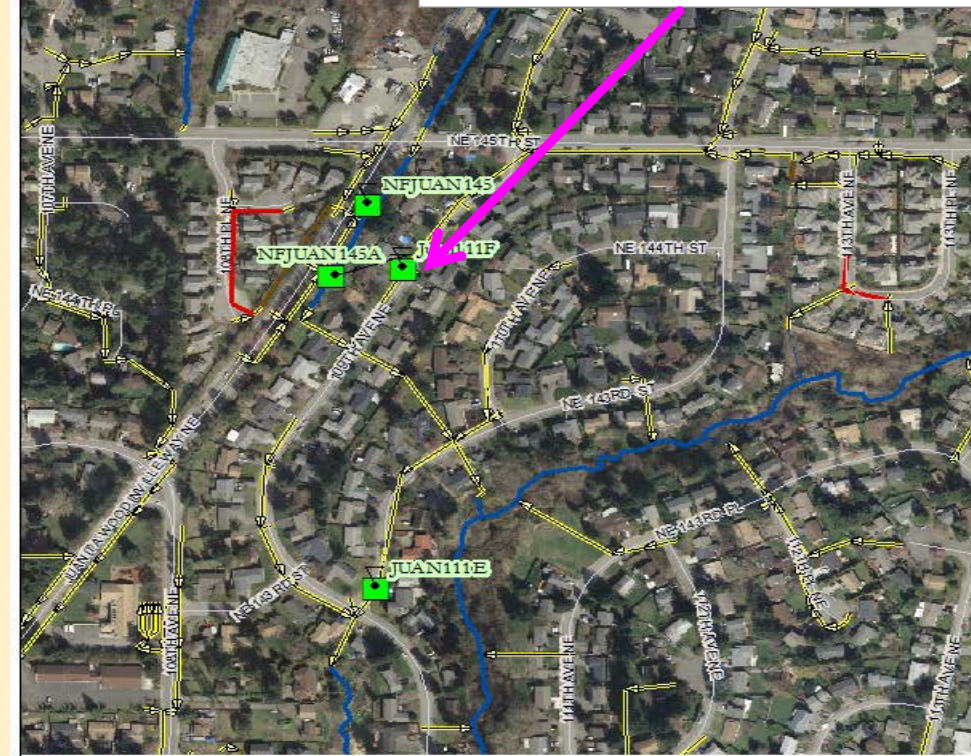


Juanita Creek Phase III ~ 2012



- No bifidobacteria
- FC relatively low

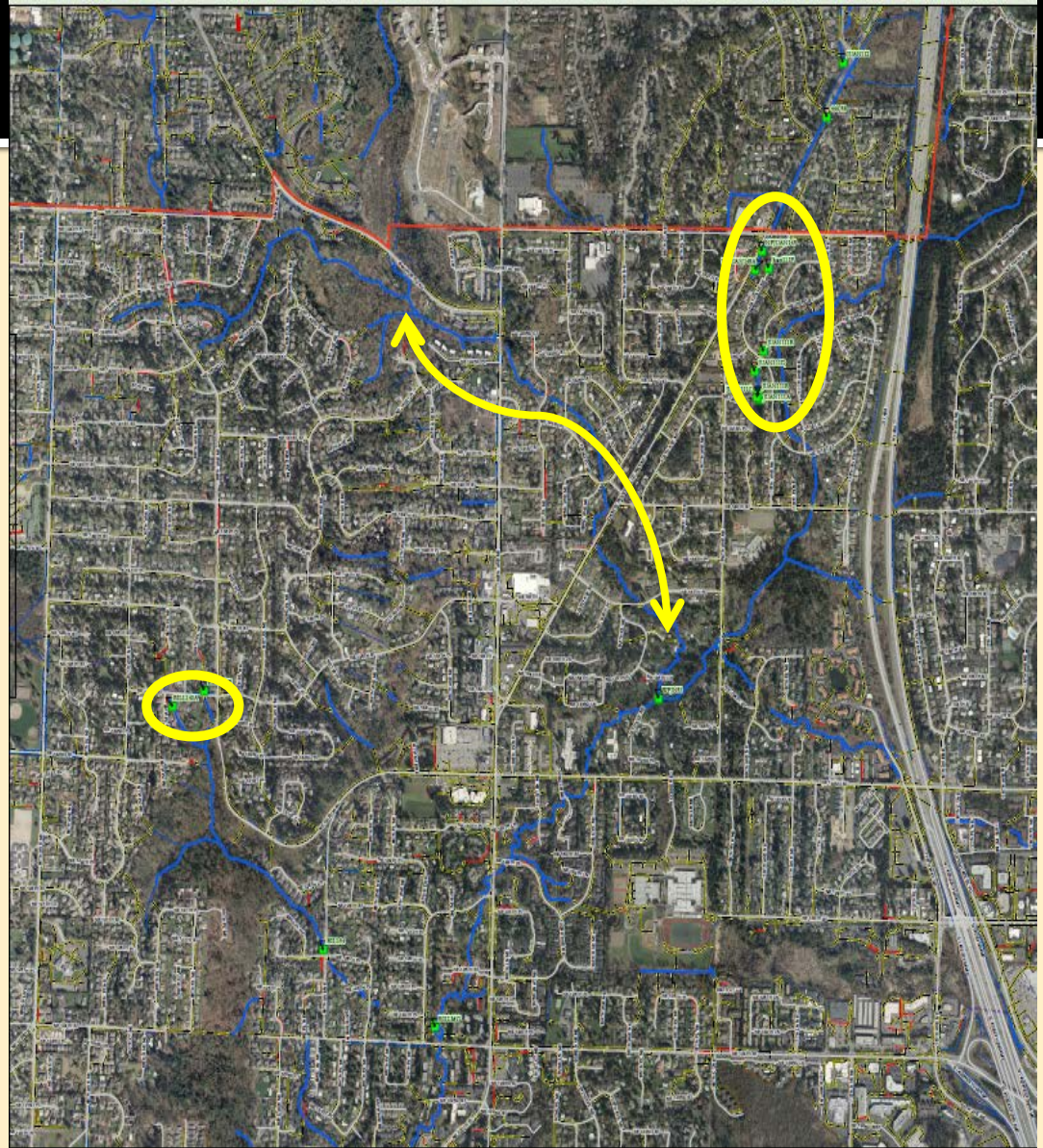
- City of Kirkland
- King County



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Juanita Creek ~ Next Steps

1. Kirkland work with Northshore Utility District to investigate history of sewer hook ups in the areas of concern in North Fork segments
2. Follow up MST testing upper Billy Creek
3. Investigate upstream of West Fork using the MST Tool Kit



MST Tool Kit questions/concerns

1. Are FC and qPCR bacteroides an effective MST Tool Kit alone?
 - As a cost savings is it useful to collect and preserve a qPCR filter with each FC for later analysis dependent upon high FC values?
2. What scale of human bacteroides qPCR values triggers further investigation?
 - Limited literature shows qPCR values in the 100,000 range indicates direct sewage source. Do we put resources towards chasing values that are less than six digits?



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