Appendix D. Temporal Studies, Tryptophan, and Optical Brighteners

PGST coordinated sample collection of EC over a 24-hour period, to test if there are advantages to sampling at certain times of day. PGST collected water samples using a Hach Sigma SD 900 Portable Sampler (autosampler) which collected one sample per hour during the 24-hour sampling periods. Variability in results between samples was enough to warrant a second test, to determine the range of variability between split and replicate samples. A second collection period was coordinated with personnel collecting samples by hand four times a day over 72 hours.

Temporal studies were conducted on the PGST Reservation, and in Jefferson County at Irondale Creek and the Duckabush River. A temporal study was planned at Lofall Creek in Kitsap County during the wet season of 2015-2016 however was eventually canceled after excessive rainfall. Results showed that EC levels did vary significantly temporally, beyond the variability found between split and replicate samples.

PGST recommends that future projects looking to utilize an autosampler should consider a model which can be easily dismantled and autoclaved. Additionally, unless the autosampler is going to be deployed regularly at a set location with proper infrastructure to house the equipment, using personnel to collect samples by hand is likely the more practical approach.

Additionally, PGST used a Turner Designs Cyclops 7 Submersible Fluorometer with tryptophan and optical brightener sensors to determine if *in situ* measurements of tryptophan and optical brighteners are a useful proxy for identifying EC hotspots. Results showed no correlation between optical brighteners and EC at the three time of day study sites where the fluorometer was deployed. At one of the three sites, there was a weak correlation between EC and tryptophan.

24-Hour Sampling- Preliminary Temporal Study

Photo Credit: Devon Hayes, Hans Daubenberger deploying autosampler and optical brightener probe at Irondale Creek.

24-hour sampling was conducted between April 21 and 22nd 2015 on the PGST Reservation at Shoreline Survey Station PGS 24, which was a confirmed hotspot from the wet season survey. 24-hour sampling was also conducted in Jefferson County at the Irondale Creek PIC monitoring station PH028 between August 26 and 27th 2015.

PGST staff used a programmable Hach Sigma SD 900 Portable Sampler (autosampler), set up with a (24) 575mL Bottle Kit for automated sampling. The autosampler was programmed to automatically collect discrete water samples at preset or fixed-interval times over no more than a 24-hour period. These containers were cleaned and decontaminated between 24-hour sampling events. At the end of the 24-hour test, PGST personnel transferred water samples into 110 milliliter bottles

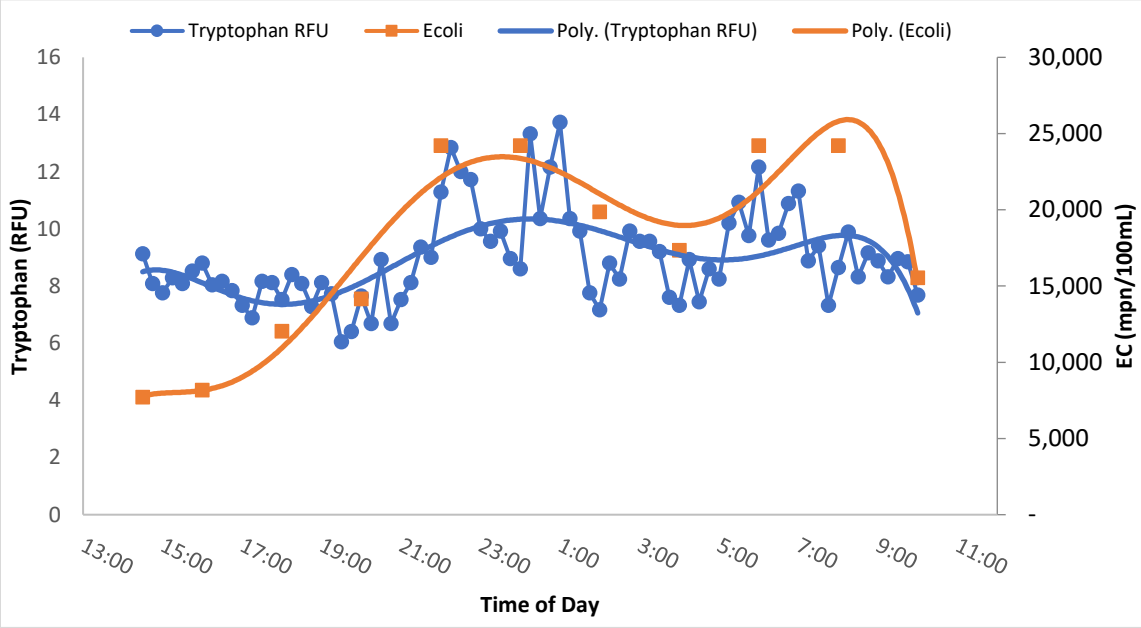
and immediately delivered them to the laboratory for analysis. Two to three blind split replicates were also delivered to the lab to examine variation. PGST staff programmed the autosampler to pump water through the tubing between samples to prevent bacteria growth and potential bias in analytical results.

PGST also collected discrete grab samples at the beginning before installing the autosampler and the end of the sampling period. This was to ensure samples were representative of the stream conditions at the time period and not potentially contaminated by bacteria from previous collection events, or otherwise influenced by the holding time or sampling system.

Results from the Reservation ranged between 1.0 and 248.1 mpn/100mL. The standard deviation was 63.7 and the coefficient of variation was 2.08, showing that results were disperse between samples taken at different times of day at this site. Irondale Creek yielded higher EC levels with a lower variation. Results ranged between 7701 and 24196 mpn/100mL with a standard deviation of 5750.1 and a coefficient of variation of 0.33.

During the 24hr sampling period of Irondale Creek, a Turner Designs Cyclops 7 Submersible Fluorometer was also deployed, to sample tryptophan and optical brightener levels at 15 minute intervals. Results showed a minor correlation between EC levels and tryptophan (r = 0.2453, Figure 1), but much less association with optical brighteners (r = 0.1662).

Figure 1. Tryptophan and EC levels at Irondale Creek



72-Hour Sampling

This sampling design was intended to determine variation between different temporal samples and single events (collected within 20 minutes). Greater variation between single event samples than the variation amongst different temporal samples would suggest there is no benefit to implementing temporal sampling, however if variation between single event samples is

significantly less than variation between temporal samples, then temporal sampling may improve hotspot identification.

72-hour sampling was conducted on the PGST Reservation upstream of Shoreline Survey station PGS 19, near Bud Purser Lane. The other 72-hour test location was at the Duckabush River tributary (also known as Pierce Creek) next to the Brinnon Volunteer Fire Station (42). The PGST Environmental Contractor (Devon Hayes) collected the water samples by hand during the 72hr test according to standard operating procedures for collection and handling EC samples.

Methods

Collection events took place at 8am, 12pm, 3pm, and 8pm on Oct. 31st-Nov. 2nd at the PGST Reservation site and on Nov. 14th-16th at the Duckabush River. Two 100 mL and three 50 mL samples were collected from the stream within 30 seconds of one another. Next, the first 100 mL sample was gently agitated and 50 mL was poured into the sixth sample bottle to produce a split replicate. This process was repeated for the second 100 mL sample to create a second split replicate, for a total of seven samples to be delivered to the lab.

At 3pm, three additional replicates were collected and held overnight, to determine whether longer holding time affected results of sample analysis. Field blank samples consisting of distilled water were submitted blind to the laboratory at a rate of one per sample batch. Salinity analysis was conducted on Duckabush River samples to test for effects of high tide on the site. Sampling on the PGST Reservation was located in a stream well above the high tide line and therefore did not require salinity analysis.

Samples were delivered to the laboratory immediately following collection at 8am, 12pm, and 3pm, excepting the three additional field replicates collected at 3pm. 8pm samples were submitted to the laboratory immediately following the 8am sample collection, with the 8am samples and the select replicates from 3pm the day before.

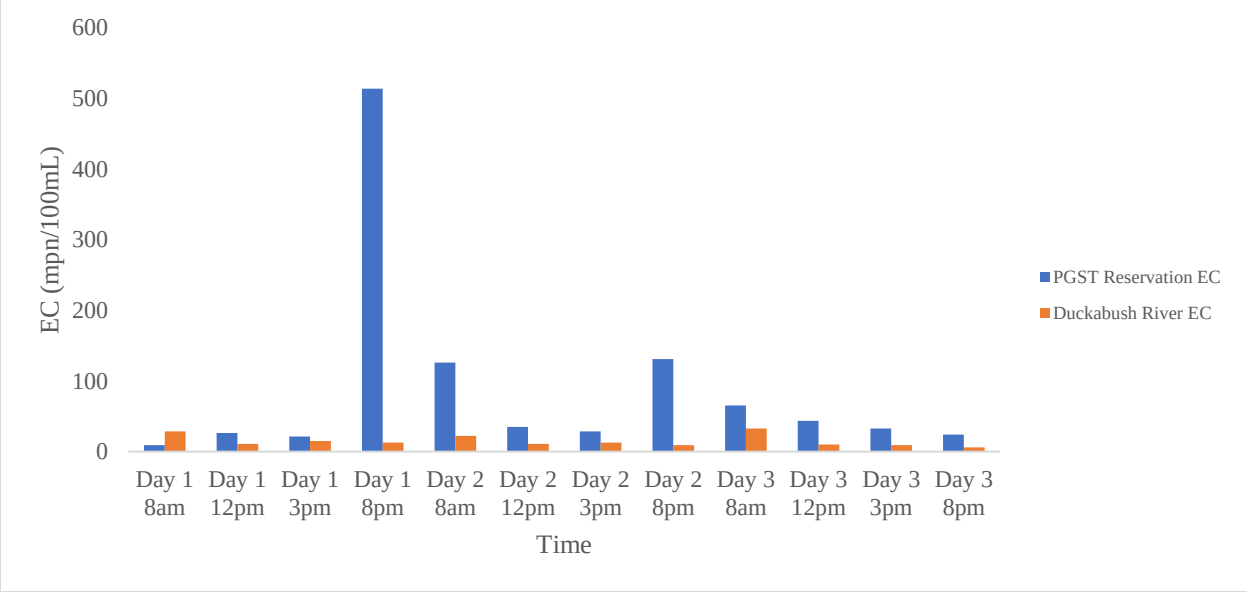
The Turner Designs Cyclops 7 Submersible Fluorometer was deployed at the PGST Reservation site just downstream of the EC sampling location on Oct. 30th and retrieved on Nov. 3rd. The fluorometer was deployed at the Duckabush River just after the 12pm measurement on Nov. 14th, and retrieved on Nov 18th. Optical brighteners and amino acid tryptophan measurements were set to be taken every 15min. The values recorded 15min before, during, and 15min after the EC sampling event times were averaged to give corresponding tryptophan and optical brightener values.

Results

A total of 99 water samples were collected over 72hrs for PGST Reservation EC analysis, including 6 blanks of distilled water. The first 7 samples were collected at the shoreline, and the rest upstream primarily for safe access, and a more controlled environment that adequately represented site conditions. Analytical results for EC ranged between less than 10 mpn/100mL to 783 mpn/100mL, which was detected at 8pm on 10/31/16 (Figure 2).

For the Duckabush River 72-hour test, 104 water samples were collected for EC analysis, including 12 distilled water blanks. 10 water samples were analyzed for salinity after the sample station was found inundated by king tides. EC results ranged from less than 10 mpn/100mL to 63 mpn/100mL, which was detected at 3pm on the third day (Figure 2).

Figure 2. Results of 72-hr E. coli test. Results of primary samples (n = 5 for each sampling event, no splits or blanks included) were averaged for each sampling event.



In the Duckabush River, E. coli values were highest at 8:00am on each of the three days sampled. At 8:00pm on October 31st, E. coli values at Bud Purser were approximately 4x higher than results from any other sampling event at that site during the 3 days studied. EC values varied significantly with time of day, in both systems, for each day sampled (Table 1). Samples did not vary notably within a single sampling event, however variance did increase as EC values increased.

Table 1. P-values for analysis of variance between EC sampling events (TOD) for Bud Purser and Duckabush (Single Factor Anova). *indicates a significant result

Bud Purser		Duckabush	
Date	P-value	Date	P-value
31-Oct	3.38E-15*	14-Nov	0.012226*
1-Nov	1.3E-05*	15-Nov	0.046141*
2-Nov	0.008859*	16-Nov	0.000164*

Split samples were not significantly different from their counterparts in either system (BP p = 0.8178, DB p = 0.7489, Anova: Two-Factor without Replication). Holding samples on ice for 6-17.5hrs before delivering them to the lab did not significantly alter results (BP p = 0.8025, DB p = 0.4770 Anova: Two-Factor without Replication).

At both sites Tryptophan and Optical Brightener values varied significantly between days (Tryptophan: DB p = 0.036616, BP p = 5.13E-07, Optical Brighteners: DB p = 4.62E-09, BP p =1.63E-15). Time of day had varied results (Tables 2 and 3).

Table 2. P-values for analysis of variance between Tryptophan sampling events for Bud Purser and Duckabush (Single Factor Anova, except 14-Nov which was a t-Test paired two sample for means). *indicates a significant result

Bud Purser		Duckabush	
Date	P-value	Date	P-value
31-Oct	5.87E-10*	14-Nov	0.97613
1-Nov	0.081139	15-Nov	0.065428
2-Nov	0.074953	16-Nov	0.177793

Table 3. P-values for analysis of variance between Optical Brighteners sampling events for Bud Purser and Duckabush (Single Factor Anova, except 14-Nov which was a t-Test paired two sample for means). *indicates a significant result

Bud Purser		Duckabush	
Date	P-value	Date	P-value
31-Oct	0.026521*	14-Nov	0.076448
1-Nov	0.001722*	15-Nov	1.01E-05*
2-Nov	0.349408	16-Nov	4.13E-07*

Despite the variability, both Tryptophan, and Optical Brighteners were significantly higher in Bud Purser than in the Duckabush River ($p = 4.49E-05$, $p = 1.38E-27$ respectively). Optical brighteners were highest at Bud Purser on Nov 1st, and Tryptophan was highest on October 31st (Figure 3, Figure 4). There was no correlation between Tryptophan or Optical Brightener values and E. coli levels, suggesting that neither can serve as a sufficient proxy for EC at the two sites tested.

Figure 3. Results of the 72-hr optical brighteners data collection. Values are an average of results collected within a 30-min time window of corresponding EC sampling event times (n=3 for each sampling event).

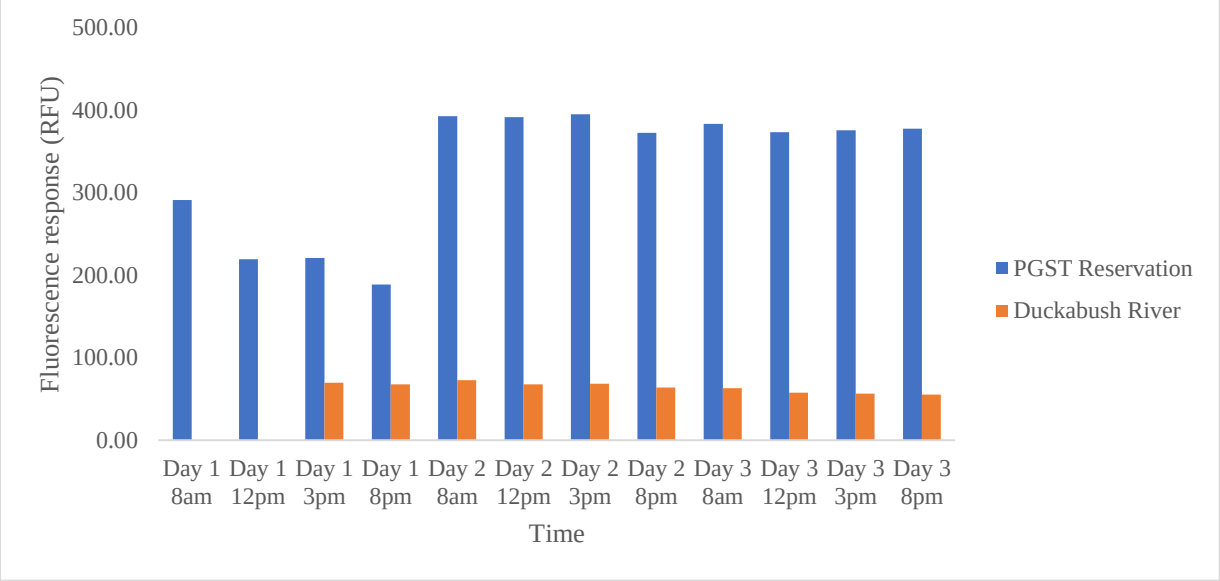
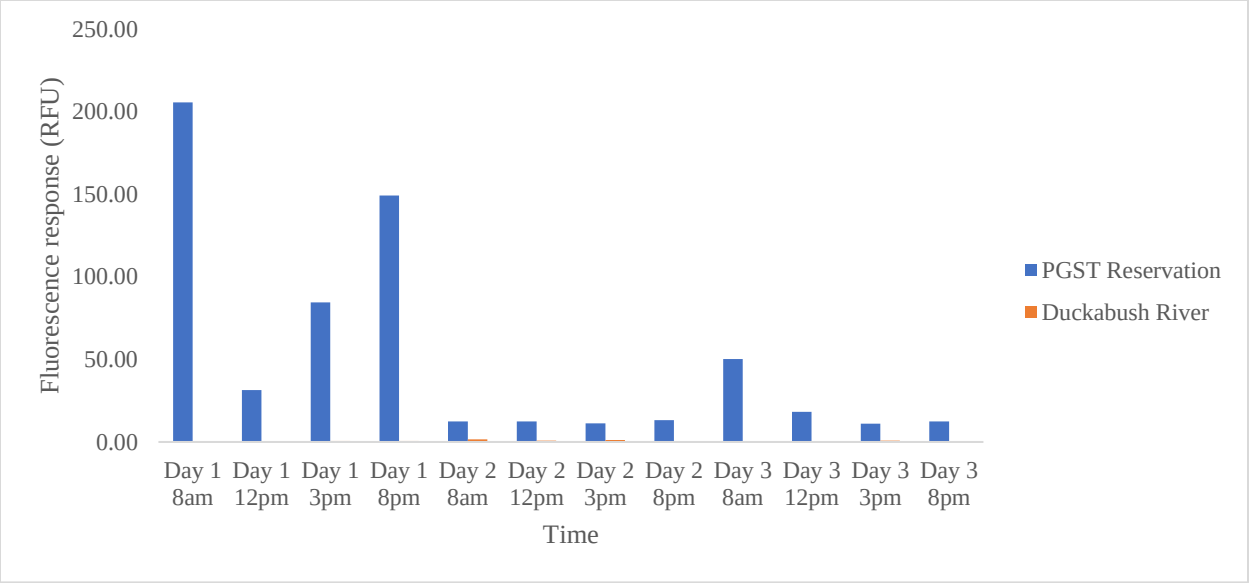


Figure 4. Results of the 72-hr tryptophan data collection. Values are an average of results collected within a 30-min time window of corresponding EC sampling event times (n = 3 for each sampling event).



Recommendations

Temporal sampling[1] in important shellfish harvest and recreational areas would likely reduce human exposure to fecal contamination.

Temporal sampling is valuable for increasing the probability of correctly identifying hotspots and reducing the risk to human health through exposure to fecal bacteria.

The temporal investigation results showed that variation was low between samples taken within a sampling event[2], but samples taken at different times of day produced results above and below threshold criteria for hotspot identification.

In situ tryptophan and optical brightener sampling is unlikely to be a good proxy for EC contamination. Tryptophan and optical brighteners may provide evidence of anthropogenic fecal contamination.

To evaluate the usefulness of tryptophan and optical brighteners for determining anthropogenic fecal contributions, an informative follow-up study could include the analysis of diluted samples from waste treatment facilities relative to environmental samples from systems with varying fecal concentrations.

[1] Temporal sampling in this report refers to collecting a minimum of 4 samples 3hrs apart over a 12hr interval.
[2] A sampling event in this study refers to a set of samples taken within 30 seconds of one another collectively representing one particular time of day.