Monitoring of *E.coli* and Enterococci in Lake Michigan Beach Sand

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Fecal Indicator Bacteria

- US EPA produced guidelines recommending *Enterococci* and *E. coli* as appropriate bacterial indicators to monitor recreational waters
- Indicator bacteria are used as an alternative to the disease-causing bacteria as there is a positive correlation between these bacteria and the occurrence of gastrointestinal illnesses in human.
- *E. coli* are coliform bacteria found in the feces of humans and warm-blooded animals while *Enterococci* is of the bacterial group *Streptococci*, also occurring in human and animal digestive systems.
- *Enterococci* was found to be better correlated with health outcomes in marine systems, whereas *E. coli* was better correlated with health outcomes in fresh water systems

"U.S. EPA. Recreational water quality criteria.2012" recommended GM and STV Values for 36 and 32 illnesses/1,000 Recreators (NEEAR-GI Illness [NGI]) for Marine and Fresh Waters

Criteria Elements	Estimated illness rate: 36 per 1000 Primary contact		Estimated illness rate: 32 per 1000 Primary contact	
Indicator	Geometric mean (cfu/100mL)	Statistical threshold value (cfu/100mL)	Geometric mean (cfu/100mL)	Statistical threshold value (cfu/100mL)
Enterococci (marine & fresh)	35	130	30	110
E. coli (fresh water)	126	410	100	320

The geometric mean is determined by taking the log of the sample values, averaging those values, and then raising the average to the power of 10. The statistical threshold value is derived by estimating the 90th percentile of the expected water quality distribution around the geometric mean criteria value.

Research Objectives

- Evaluate the concentration and interaction of fecal indicator bacteria *E. coli* and Enterococci in beach sand and water

- Analyze the effects of different types of eluents (DI water and PBS)

- Evaluate the factors affecting bacteria concentration:
  - Presence of algae
  - Rainfall and temperature

Bacteria Analysis

Site Name: BRADFORD BEACH, MILWAUKEE, WI

Sampling location:

- 43°03′41·30″ N, 87°52′20·41″ W on the shore of Lake Michigan, Milwaukee County, Wisconsin
- Three transect location (Transect 1, 2 and 3)

Sampling period:

- Summer and early fall months of 2013 and 2014.
- Sampling frequency: 3 days per week

Sample collection:

- Sand sample from swash zone
- Sand sample from 20 ft inland
- Water sample

Sampling method: IDEXX's method

Bradford beach with the three transect sampling locations (Transect 1, Transect 2 and Transect 3)

Bacteria enumeration using IDEXX's method

1. Homogenize sample on Excella E24 Incubator Shaker Platform at 200 rpm for 5 mins
2. Allow settling of large particles
3. Place samples in two IDEXX bottles (one for *E. coli* and one for ENT)
4. Addition of 200 ml eluent (DI water/PBS) on each bottle
5. Shake sample and allow for particle settling
6. Separation of eluent in 2 separate sterile 120 ml vessel
7. Place 100 ml sample in each vessel (one for *E. coli* and another for ENT)
8. Addition of Colilert and Enterolert indicator and put into Quanti-tray/2000 packet
9. Incubate *E.coli* samples at 35 ^{o}C and Enterococci at 41 ^{o}C for 24 hours

Data Analysis: CANARY Event Detection System

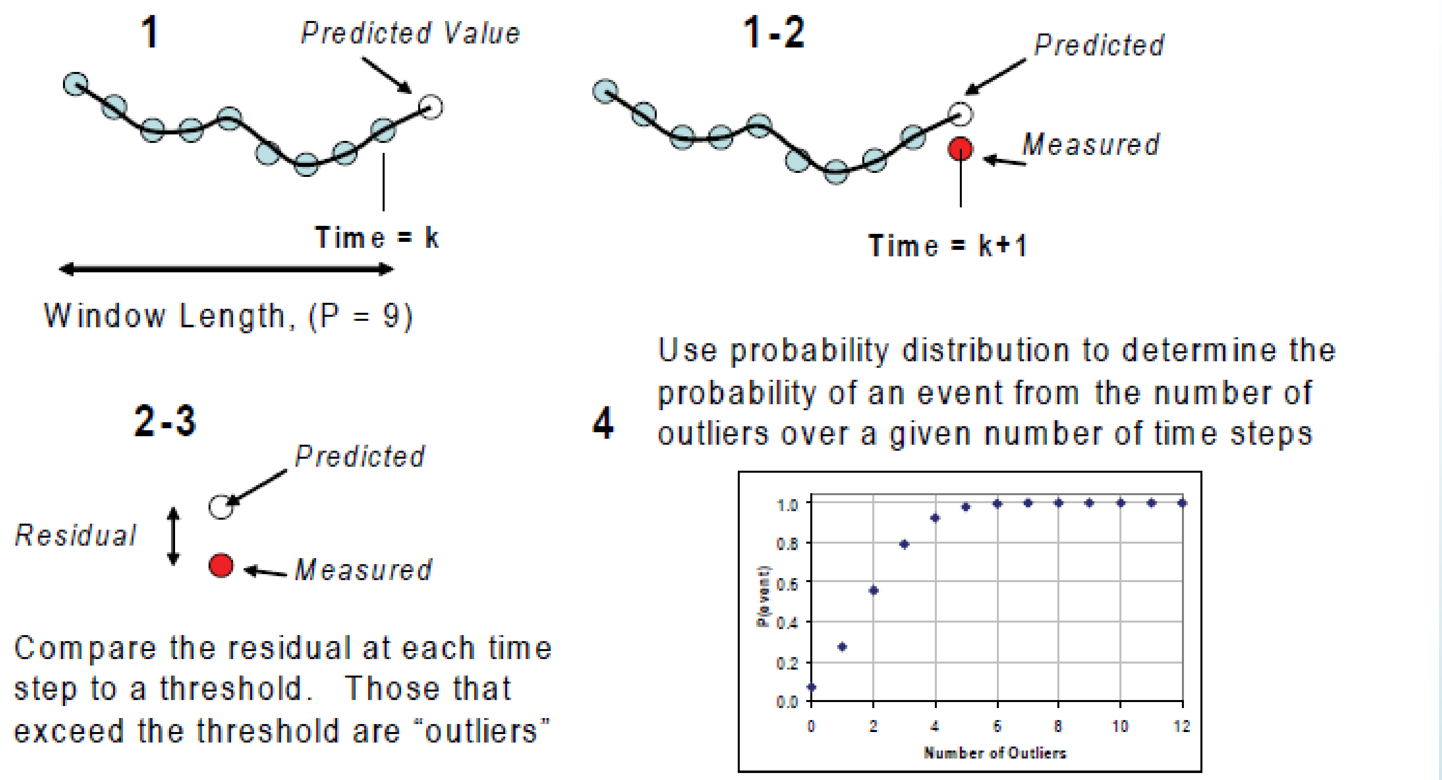
➨The US EPA and Sandia National Laboratories developed an open source software called CANARY, which is based on analysis of receiver operating characteristic (ROC) curves. It is an advanced detection model that enables the use of water quality sensors to detect abnormal water quality event with statistical tool

➨CANARY works by reading real-time or historical data, analyzing the data, and returning the probability of an anomalous water quality event

➨ Three different state estimation models, i.e., Time Series Increments, Linear Prediction Correction Filter (LPCF), and Multivariate Nearest Neighbors (MVNN), are implemented in the prediction algorithms

➨ The event detection algorithms in CANARY continuously adapt to changing water quality values and look for significant deviations from that changing background

CANARY Event detection process



Step 1: Estimation of the future water quality values

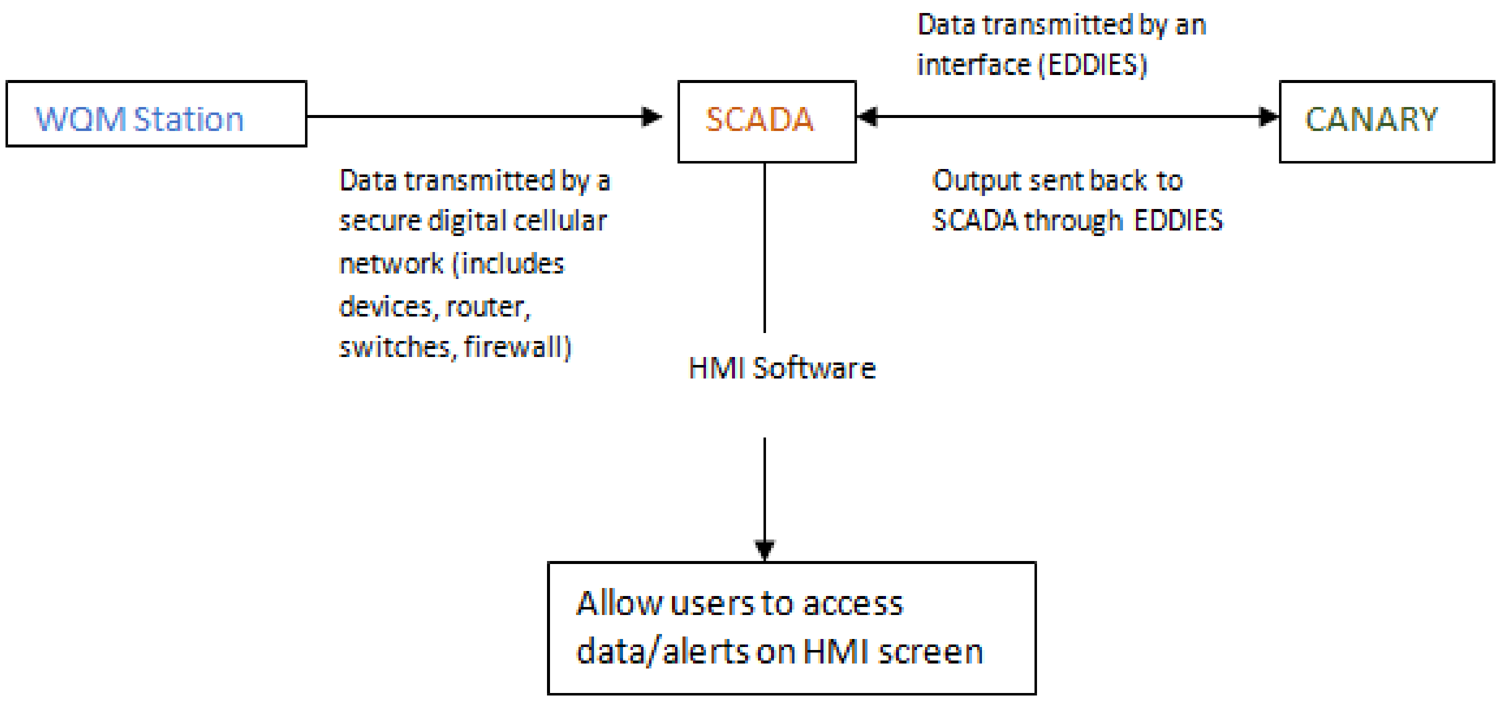
Step 2: Comparison of the estimated values against observed values as they become available and calculate the "residual" as the difference between the estimated and observed values

Step 3: Integration of the residuals across all water quality sensors

Step 4: Calculation of the probability of a water quality event occurring at each measurement time from the residual data using a binomial event discriminator (BED)

Source: Murray, R., Haxton, T., McKenna, S. A., Hart, D. B., Klise, K., Koch, M., Vugrin, E. D., Martin, S., Wilson, M., Cruz, V., and Cutler, L. (2010) *Water quality event detection systems for drinking water contamination warning systems: Development, testing, and application of CANARY*, U.S EPA, Office of Research and Development, National Homeland Security Research Center, Cincinnati, OH. EPA 600/R-10/036

CANARY Data Collection System

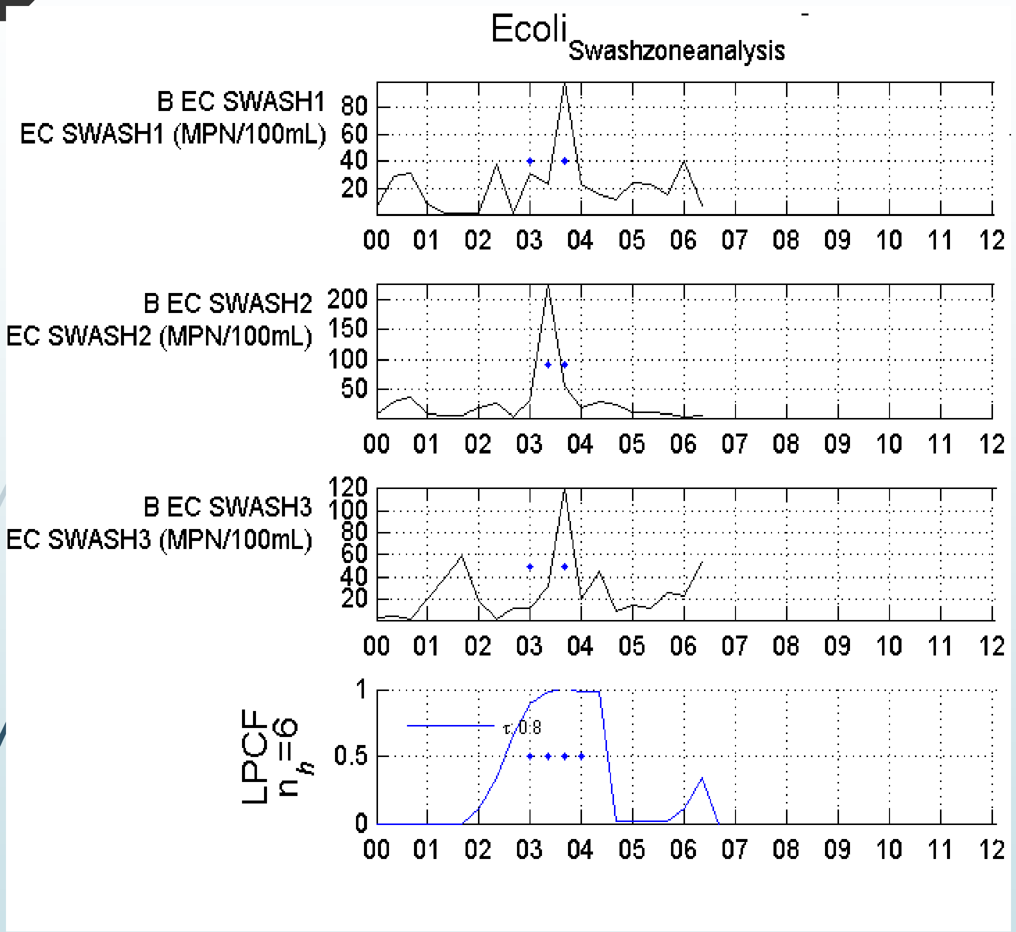


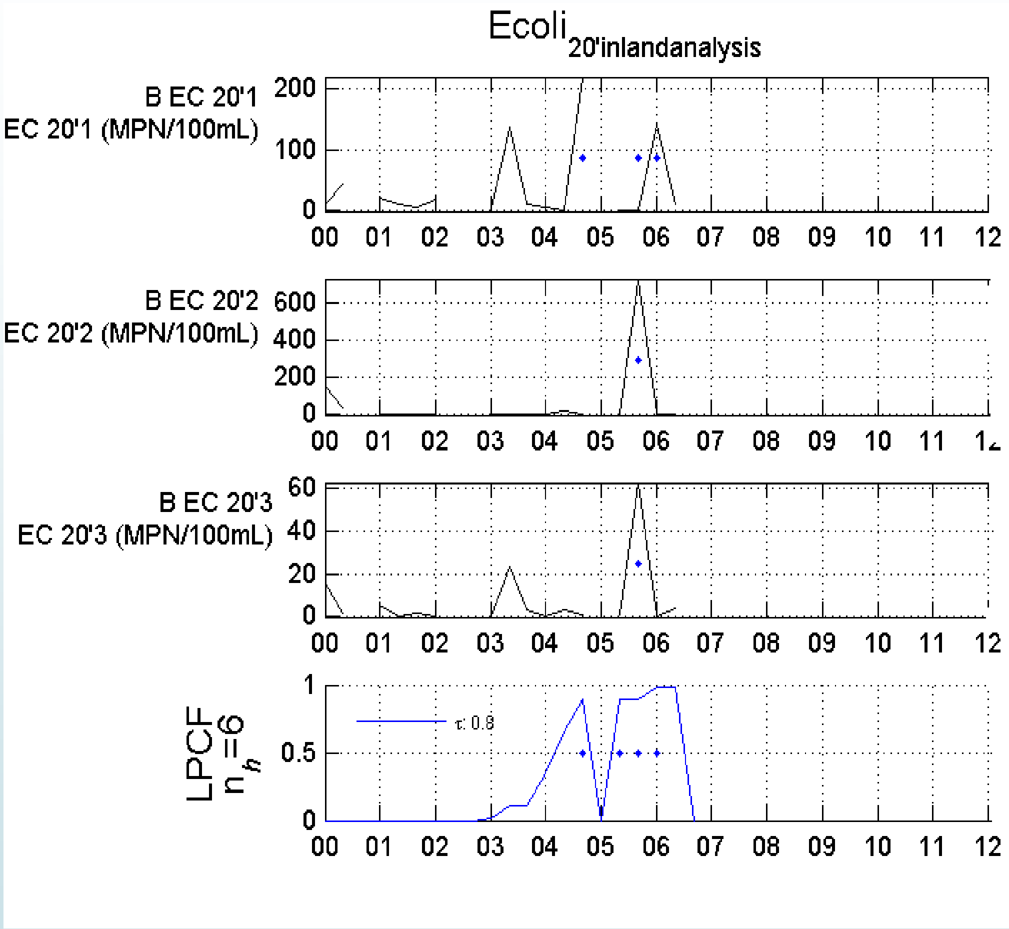
- Data from the WQM stations is transmitted to a central location using a secure digital cellular network.
- SCADA system provides data to and collects results from the CANARY EDS
- Users can access the data on SCADA via Human Machine Interface (HMI) software.
- HMI screens allow users to view a system map detailing the location and status of each monitoring station, and event detection system alerts.

Event Analysis on Bacteria Count

- Event analysis was made on *E. coli* and *Enterococci* count using the EPA CANARY software.
- Each of the bacteria was counted from water sample and from sand sample in swash zone and 20 ft inland at three transect location points designated as transect 1, 2 and 3.
- Each of the bacteria count in sand sample was performed with 200 ml DI water and 200 ml PBS eluent.
- For the purpose of data analysis in CANARY, the overall duration for bacteria count was about 7 hours with a data interval of 20 min in offline mode of CANARY.

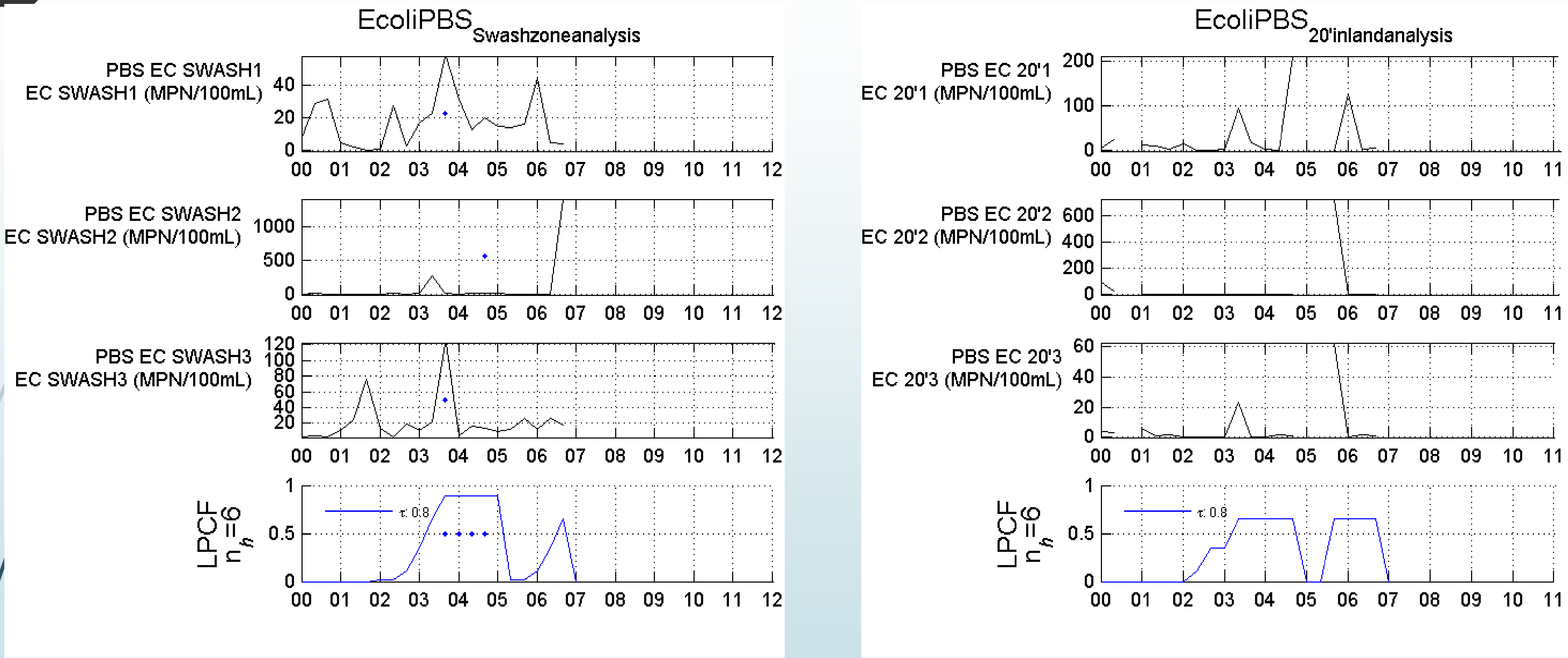
CANARY data analysis (*E.coli* in sand with eluent DI water)





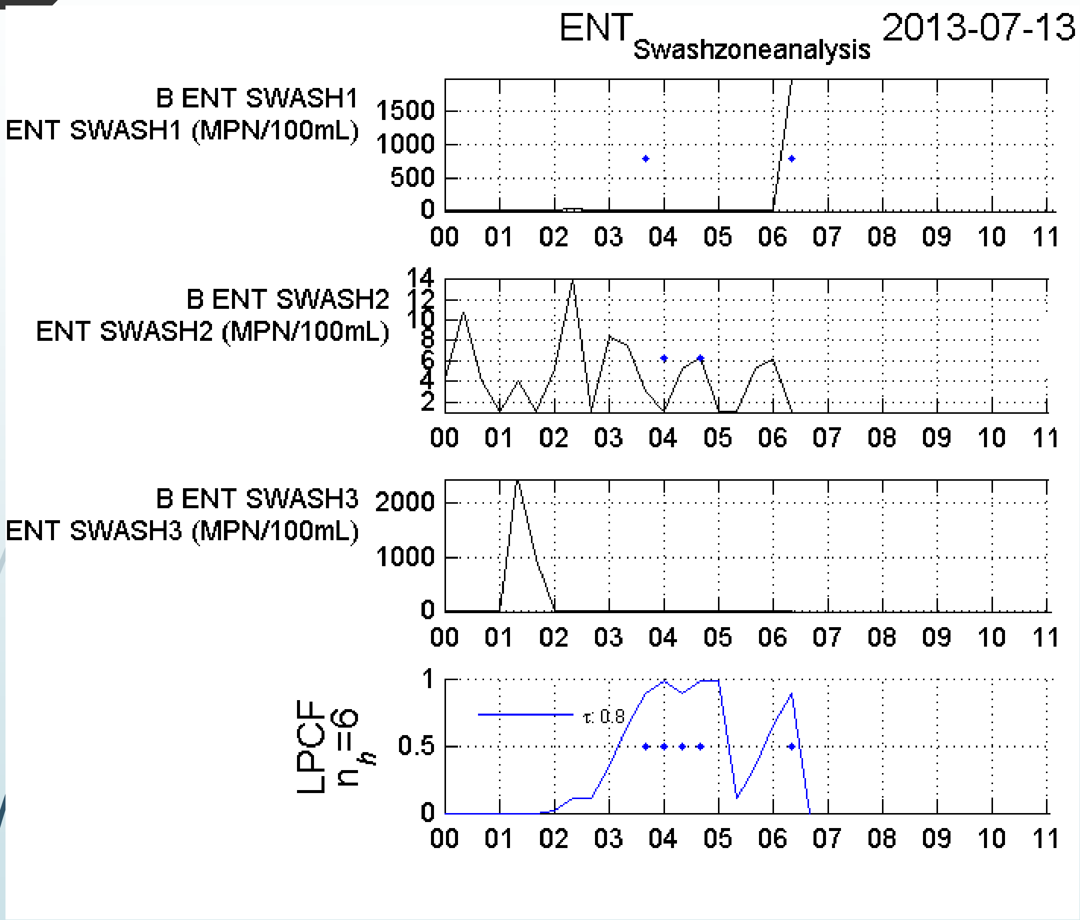
CANARY output for *E. coli* count in sand samples in swash zone (left) and 20 ft inland (right) at each of the three transect with **eluent DI** water during 13th July 2013; Probability of event plot showing total number of detected events 8. (4 detected events for each of the swash zone and 20' inland sample).

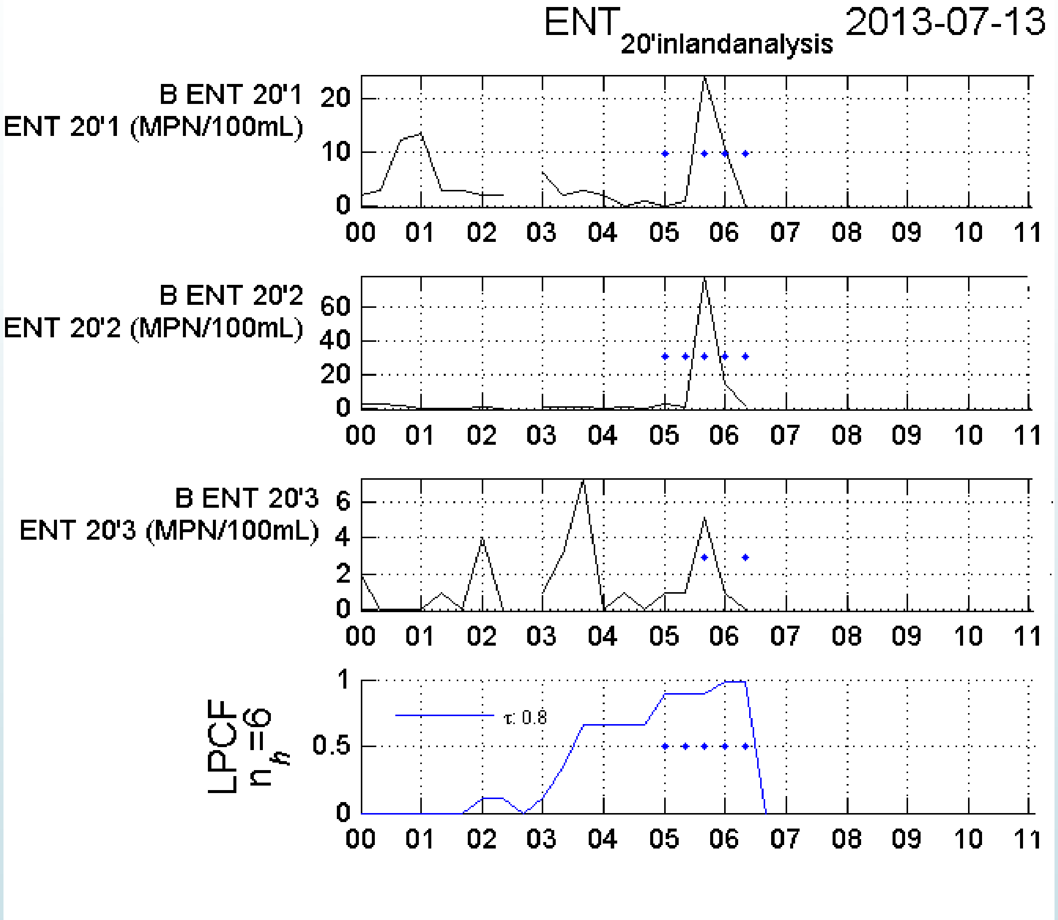
CANARY data analysis (*E.coli* in sand with eluent PBS)



CANARY output for *E. coli* count in sand samples in swash zone (left) and 20 ft inland (right) at each of the three transect with **eluent PBS** during 13th July 2013; Probability of event plot showing total number of detected events 4 (4 detected events in Swash zone and 0 event for 20’ inland)

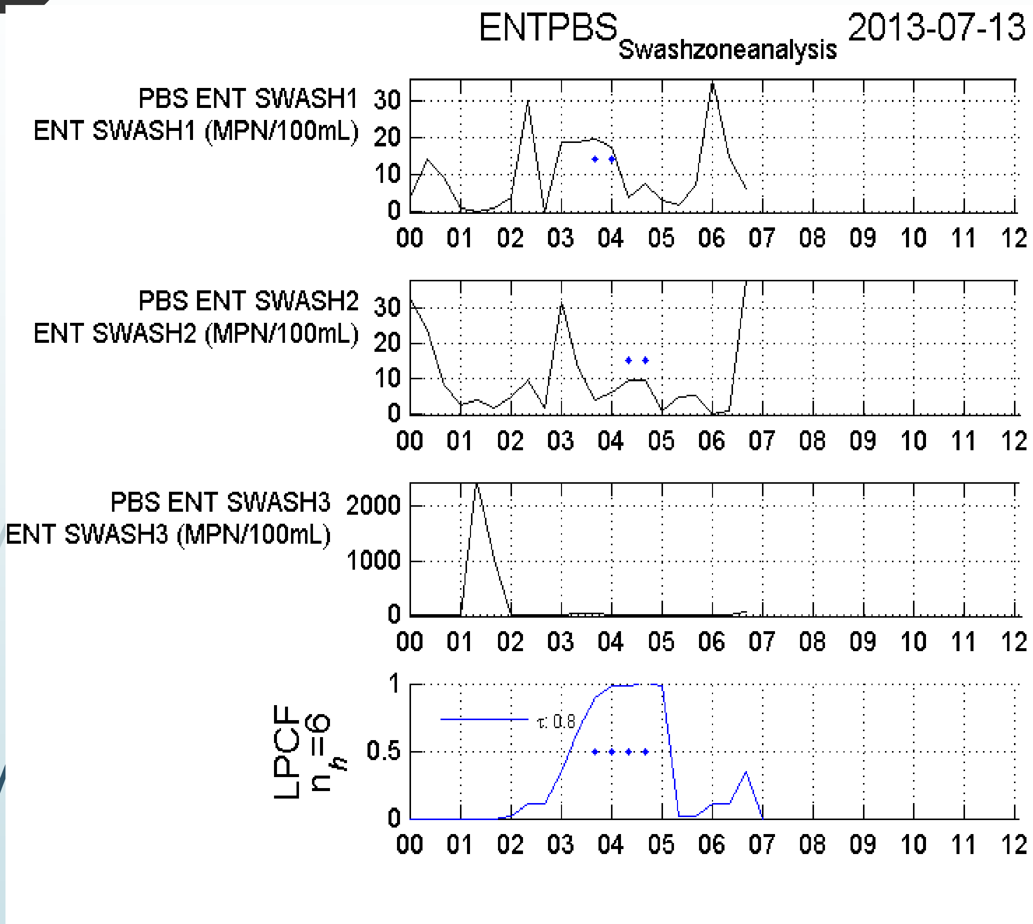
CANARY data analysis (Enterococci in sand with eluent DI water)

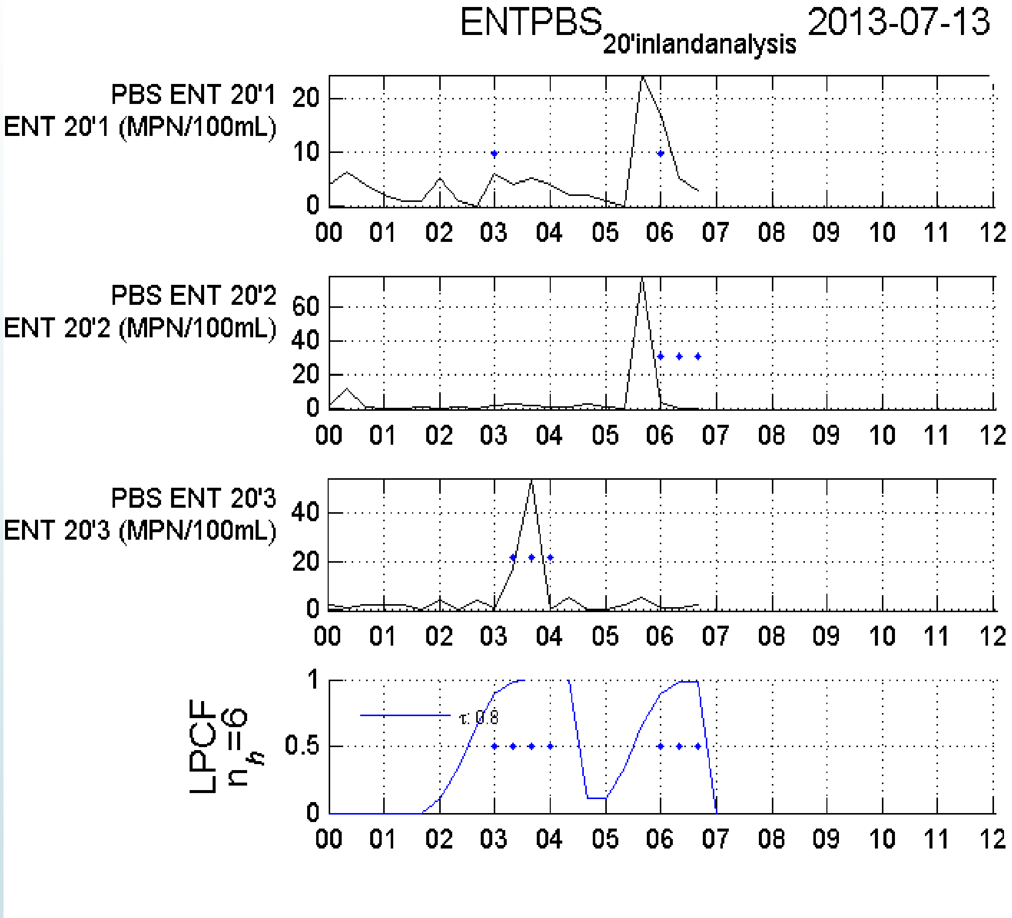




CANARY output for *Enterococci* count in sand samples in swash zone (left) and 20 ft inland (right) at each of the three transect with **eluent DI** water during 13th July 2013; Probability of event plot showing total number of detected events 10 (5 detected events for swash zone and 5 events for 20' inland)

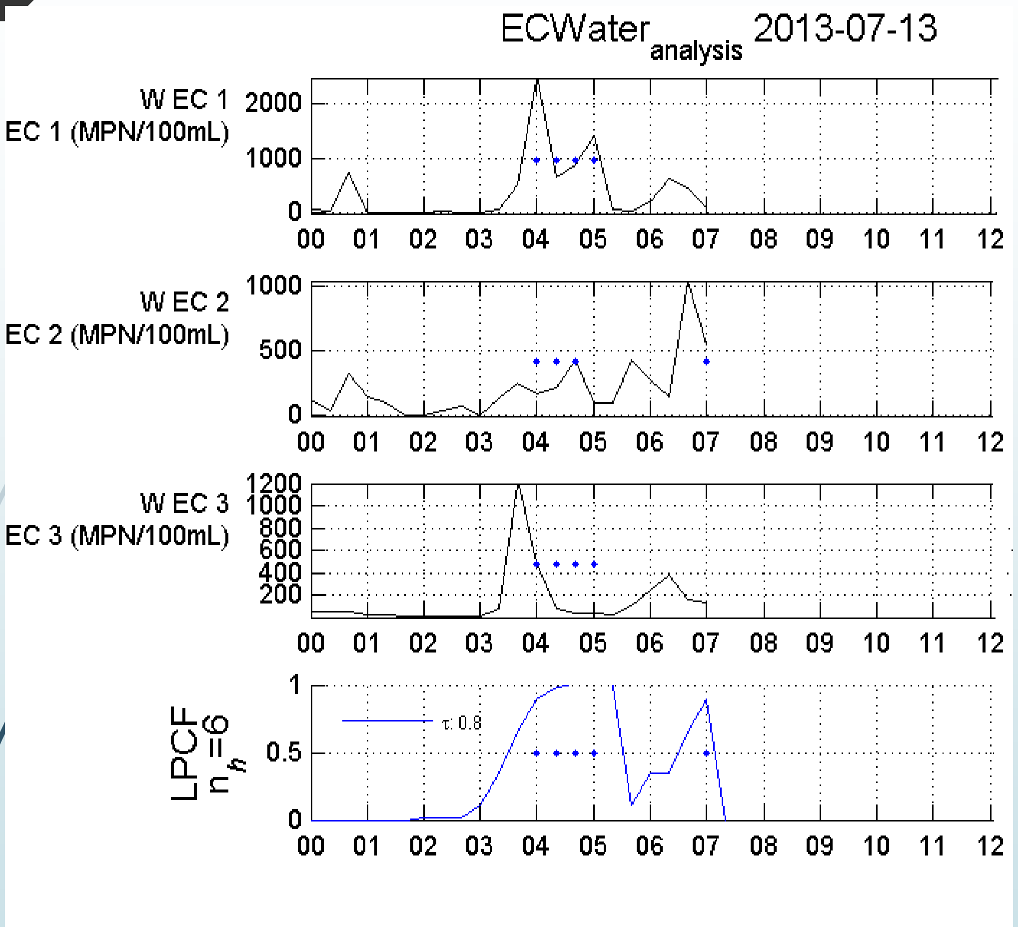
CANARY data analysis (Enterococci in sand with eluent PBS)

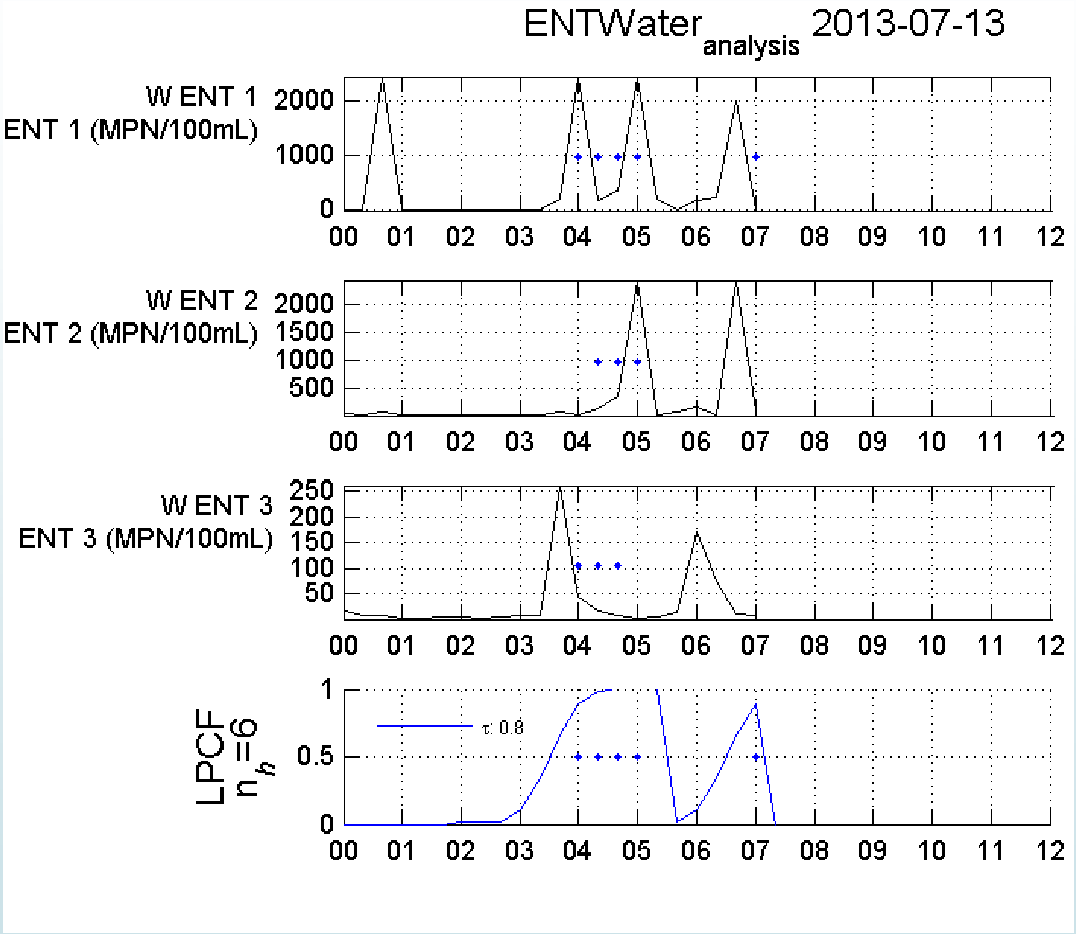




CANARY output for *Enterococci* count in sand samples in swash zone (left) and 20 ft inland (right) at each of the three transect with **eluent PBS** during 13th July 2013; Probability of event plot showing total number of detected events 11 (4 detected events in swash zone and 7 events in 20' inland)

CANARY data analysis (*E.coli* and Enterococci in water sample)





CANARY output for *E.coli* count (left) and Enterococci count (right) in water sample at three transect locations with probability of event plot indicating total number of detected events 5 for each bacteria during 13th July 2013.

Testing on Fecal Indicator Bacteria

Total detected events in sand sample:

FIB	DI water	PBS eluent
E.coli	8	4
Enterococci	10	11

- DI water produced higher number of events than PBS for *E. coli in sand sample.*

Detected events in swash zone:

FIB	DI water	PBS eluent
E.coli	4	4
Enterococci	5	4

Detected events in 20 ft inland:

FIB	DI water	PBS eluent
E.coli	4	0
Enterococci	5	7

- In swash zone, *E.coli* resulted in the same number of events for both the eluents whereas in 20 ft inland no event is found with PBS eluent.
- With DI water, *Enterococci* resulted in higher number of events than *E.coli* in both the swash zone and 20 ft inland sample while with PBS similar number of events were detected in swash zone for both *E.coli* and Enterococci.

Testing on Fecal Indicator Bacteria

Total detected events in sand sample:

FIB	DI water	PBS eluent
E.coli	8	4
Enterococci	10	11

Total detected events in water sample:

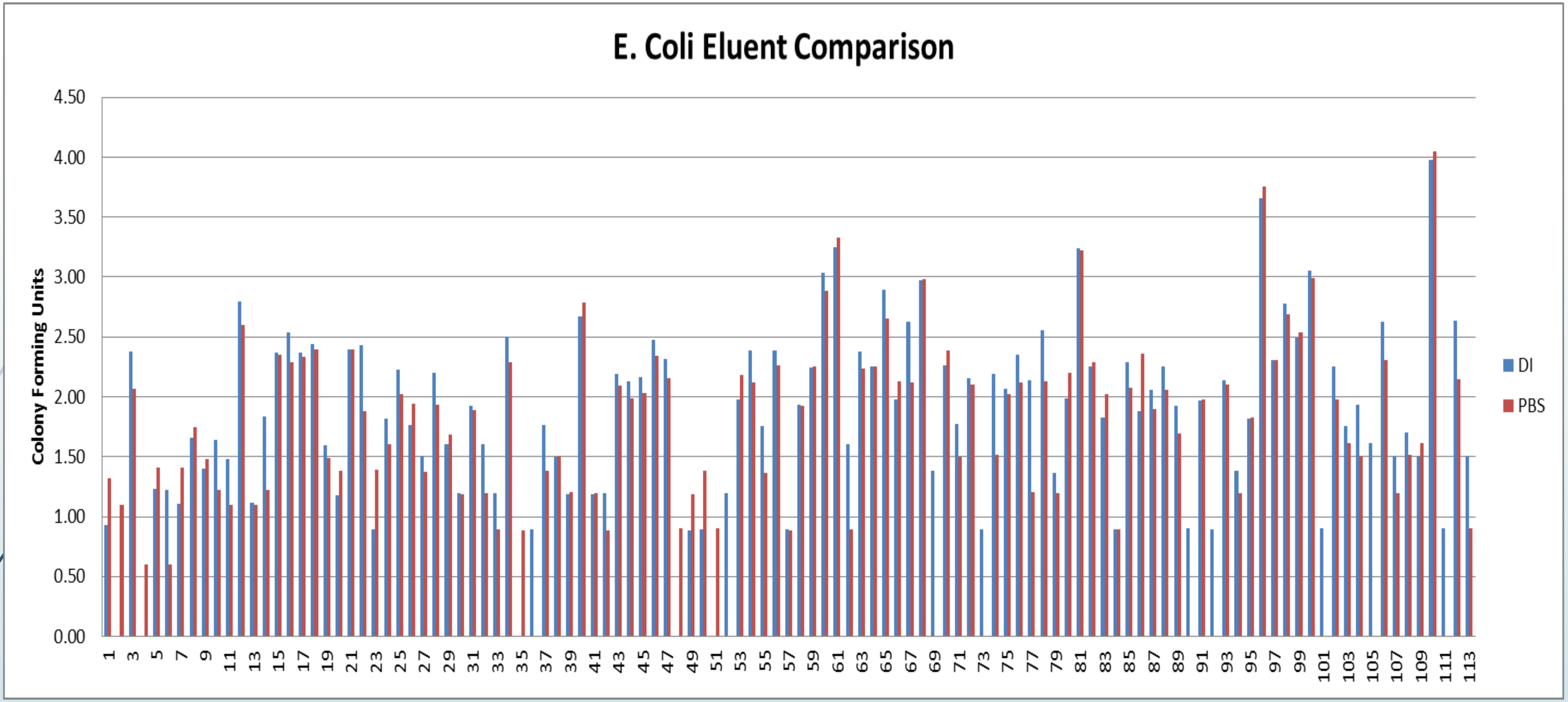
FIB	No. of events
E.coli	5
Enterococci	5

- In sand sample, higher number of events were found for the indicator bacteria than in water sample.
- Number of detected events in water sample was found as 5 for both *E. coli* and ENT bacteria while in sand sample more than 5 events were detected.
- In addition, Enterococci appeared to accumulate in sand to a greater extent than did *E. coli* specially while using PBS.
- These results suggest that freshwater beach sand can be evaluated further for it's potential to serve as a reservoir for indicator bacteria survival.

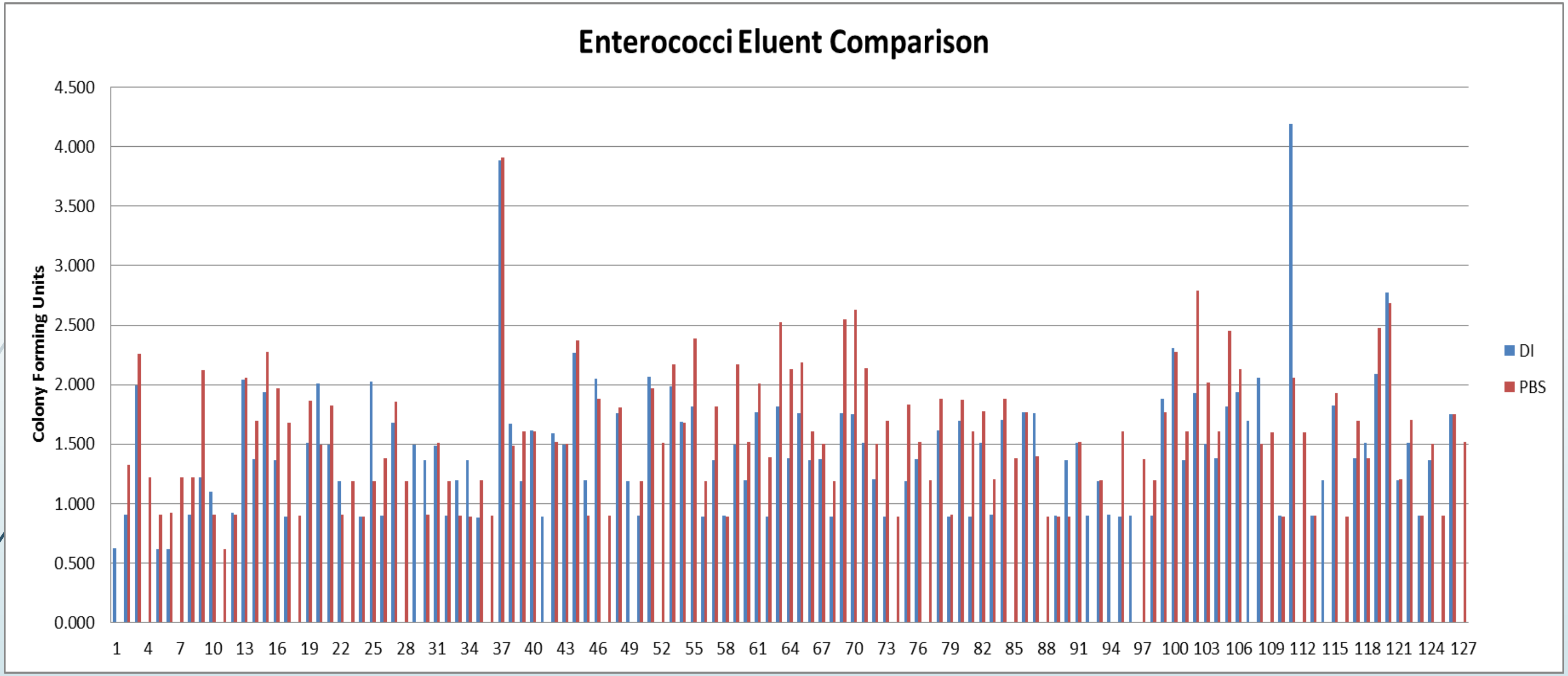
Effect of eluents on bacteria count

Fecal indicator bacteria	Mean value (log MPN/100 g sand) **DI Water**	Mean value (log MPN/100 g sand) **PBS eluent**
E.coli	1.84	1.69
Enterococci	1.24	1.46

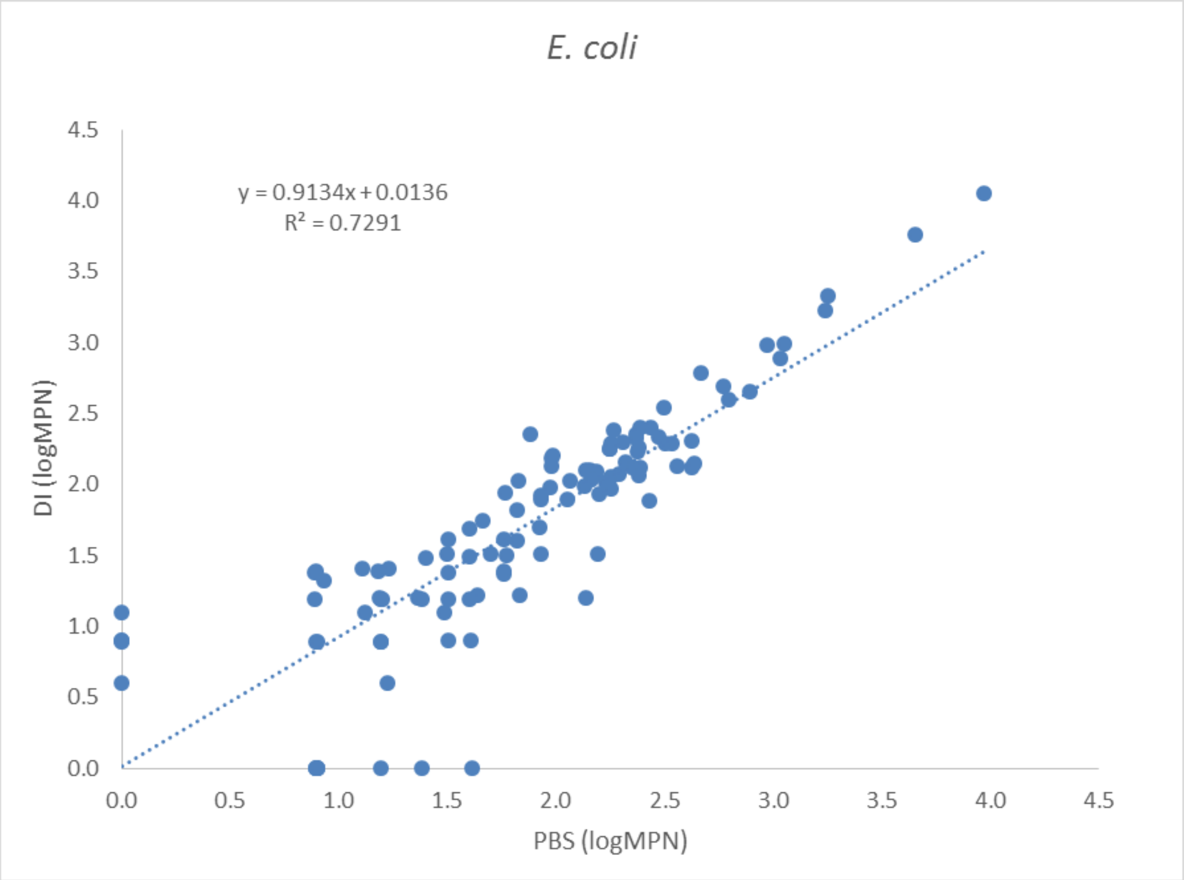
- A paired t-test analysis was done for both bacteria comparing DI water and PBS as eluents.
- For eluent comparison, the total number of samples used for *E.coli* was 113 and for Enterococci 127 during the overall sampling duration
- DI water produced higher *E.coli* counts while PBS produced higher Enterococci counts.

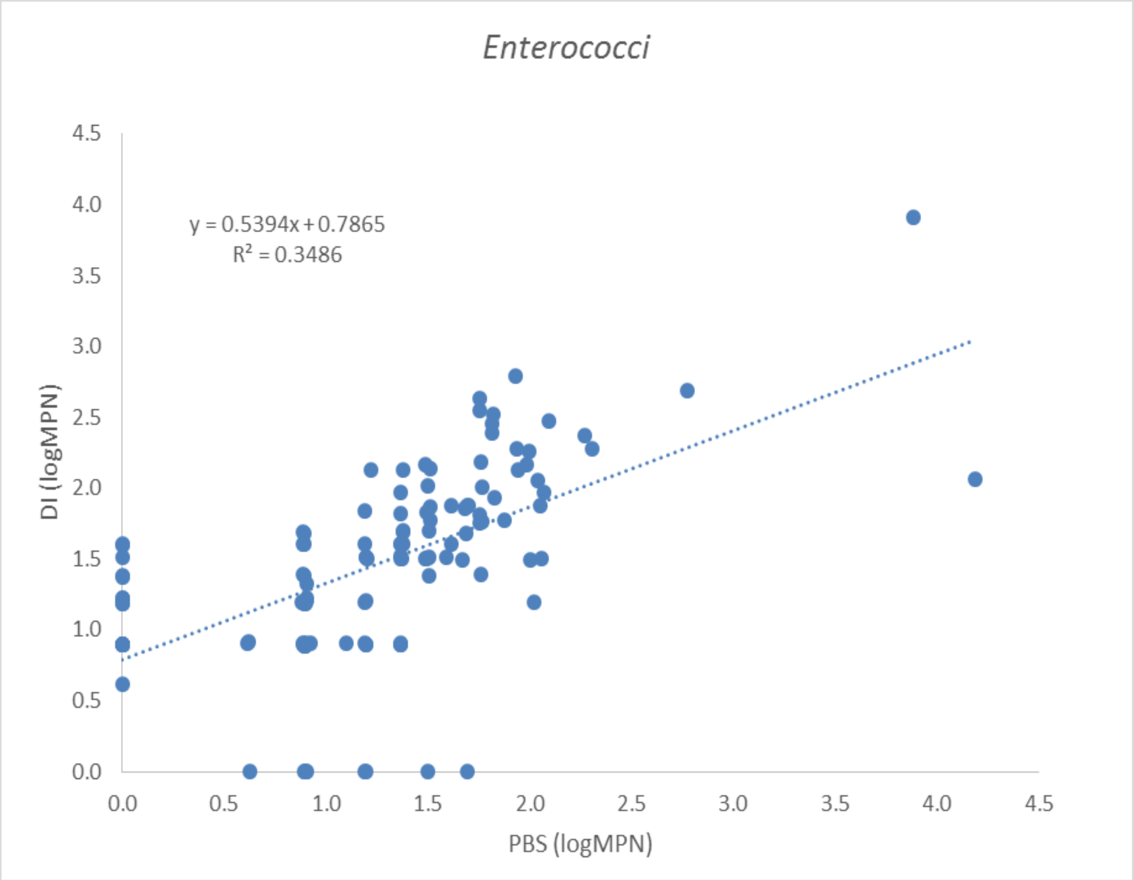


- Eluent comparison for *E.coli* (log MPN values for total number of samples, n = 113)
- DI water produced higher *E.coli* count
- The ratio for each sample between the two eluents was highly variable



- Eluent comparison for Enterococci (log MPN values for total number of samples, n = 127)
- PBS eluent produced higher Enterococci count





- The slope and R-squared value for *E. coli* are 0.913 and 0.73, indicating a fairly strong linear relationship between the MPN generated from DI water and PBS.
- The relationship between the results generated from different eluents for *Enterococci* is less strong with R-squared value 0.35, this could be related to the fact that *Enterococci* is typically used as the indicator bacteria in saltwater whereas *E. coli* is typically used as the indicator bacteria in freshwater.
- **Results indicate that on average, deionized water provided higher MPN than PBS for *E. coli*.**

Impact of presence of algae on bacteria count

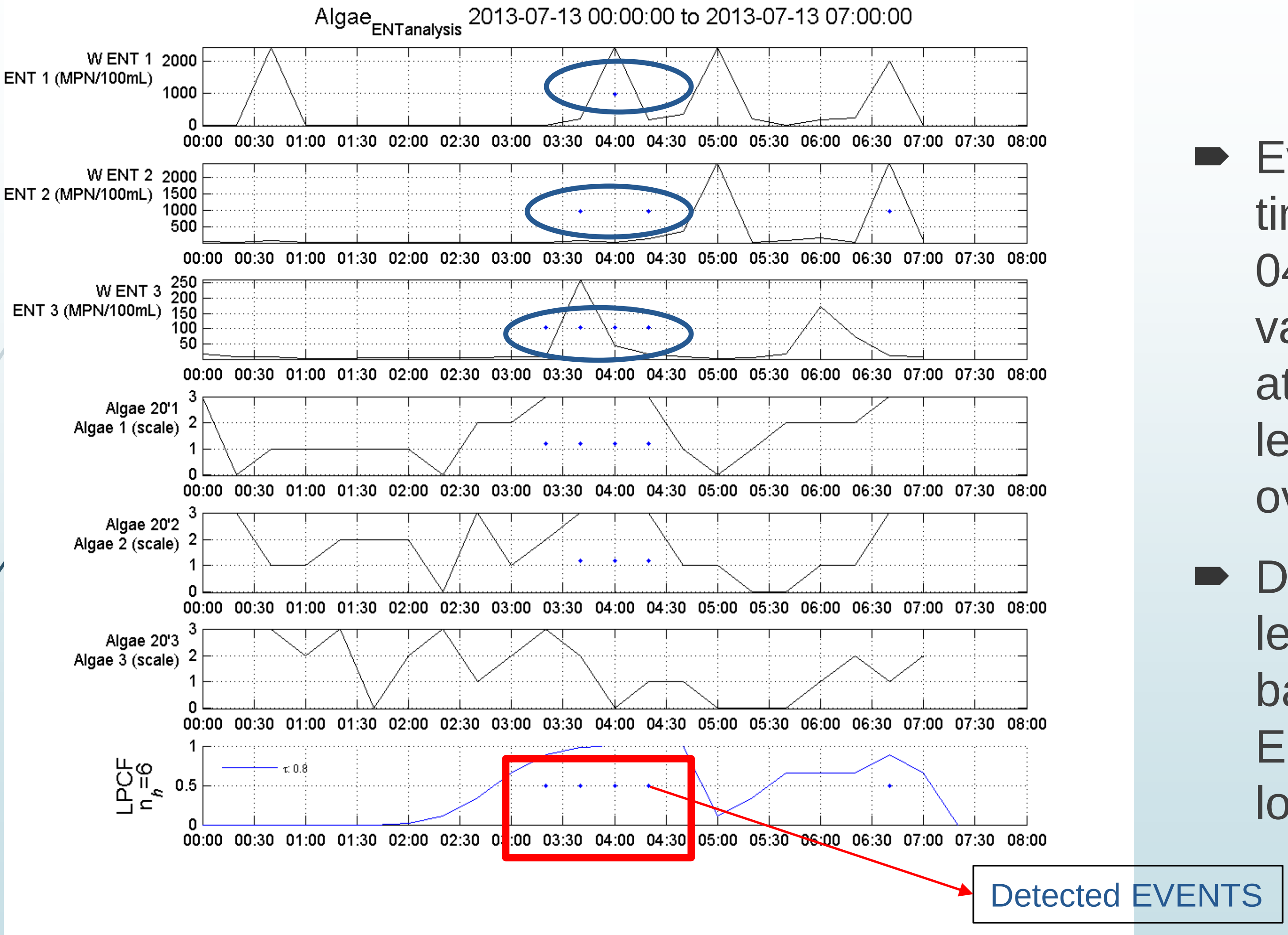
- CANARY was used to analysis the impact of algae on bacteria concentration in water sample.
- Algae level was recorded during sampling duration in different wave action of the beach water near the shore
- A classification system for algae was made to determine the level of algae in beach water:

Level of Algae	Rating scale
No algae present	0
Low presence	1
Moderate presence	2
High presence	3

Impact of presence of algae on bacteria count

- A correlation is established between algae levels and bacterial counts as the events with the highest bacterial counts often occurred on time periods with elevated level of algal presence
- Bacteria concentration deviated from its baseline and detected as anomaly during the time step where the presence of algae was reported as high (scale 3)
- Consequently, events were detected as abnormal water quality

CANARY data analysis: Impact of algae level on *Enterococci*

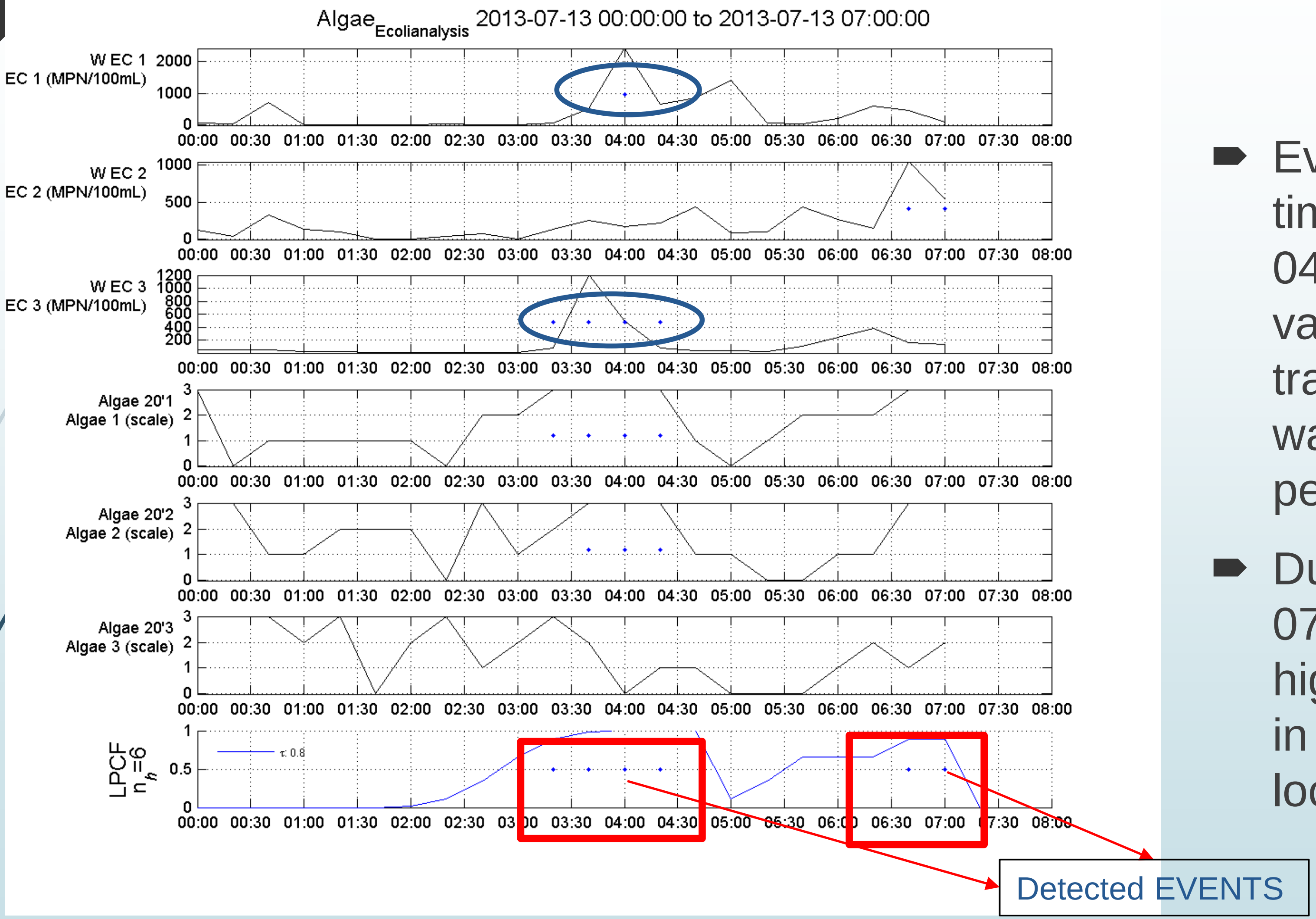


- Events were detected during time step between 03:20 to 04:20 as there were baseline variations of Enterococci count at transect 1,2 and 3. Algae level was also found higher over that period
- During time step 06:40, algae level was found higher with baseline variation in Enterococci count at transect location 2

CANARY output for **Enterococci count** in water sample with impact of algae level

during the sampling period of 13th July 2013 with probability of event plot indicating total 5 events.

CANARY data analysis: Impact of algae level on *E.coli*



CANARY output for *E.coli* count in water sample with impact of algae level during the sampling period of 13th July 2013 with probability of event plot indicating total 6 events

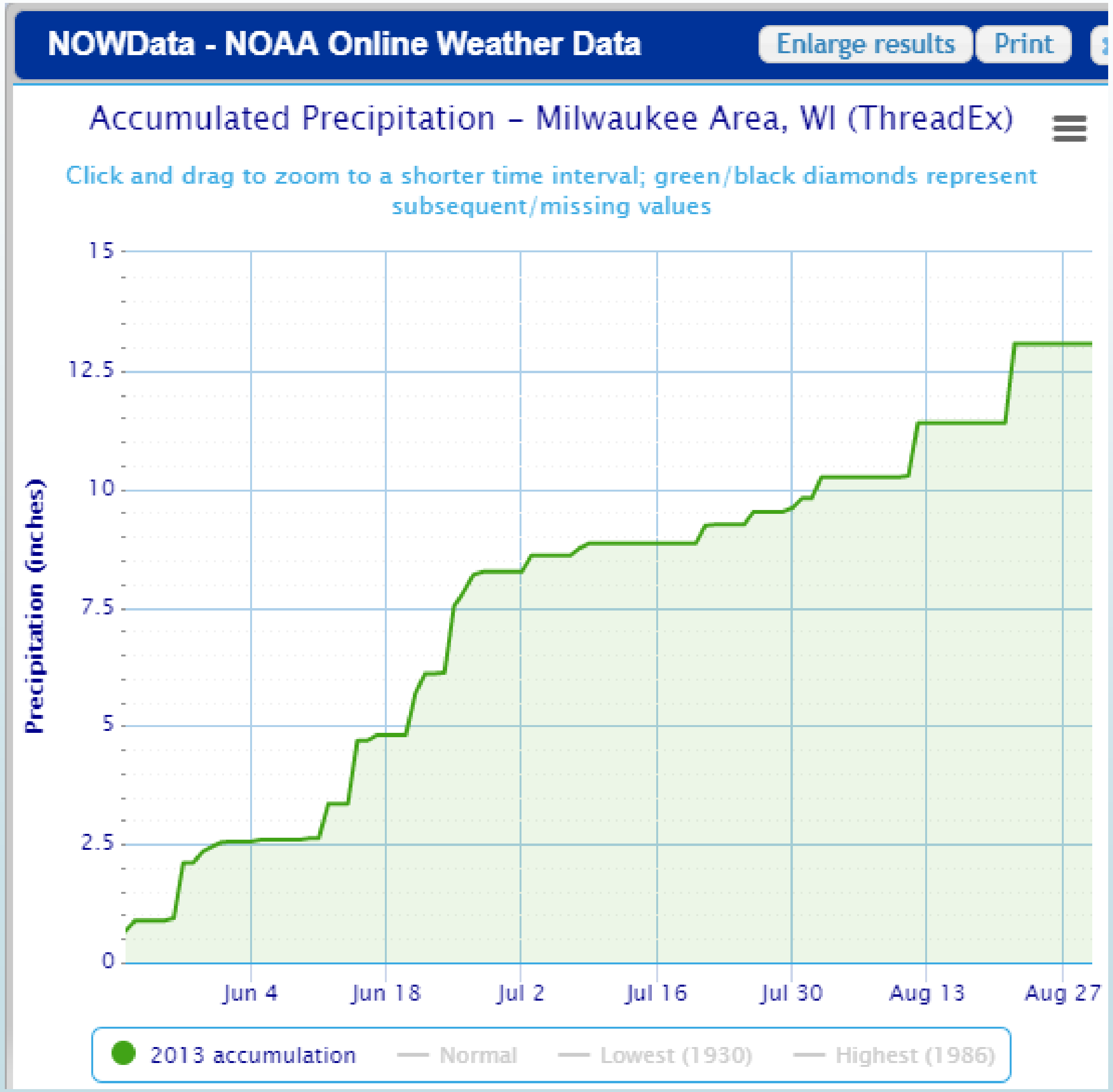
- Events were detected during time step between 03:20 to 04:20 as there were baseline variations of ***E.coli count*** at transect 1 and 3. Algae level was also found higher over that period.
- During time step 06:40 to 07:00, algae level was found higher with significant variation in *E.coli* count at transect location 2.

Correlation between algae level and bacteria count

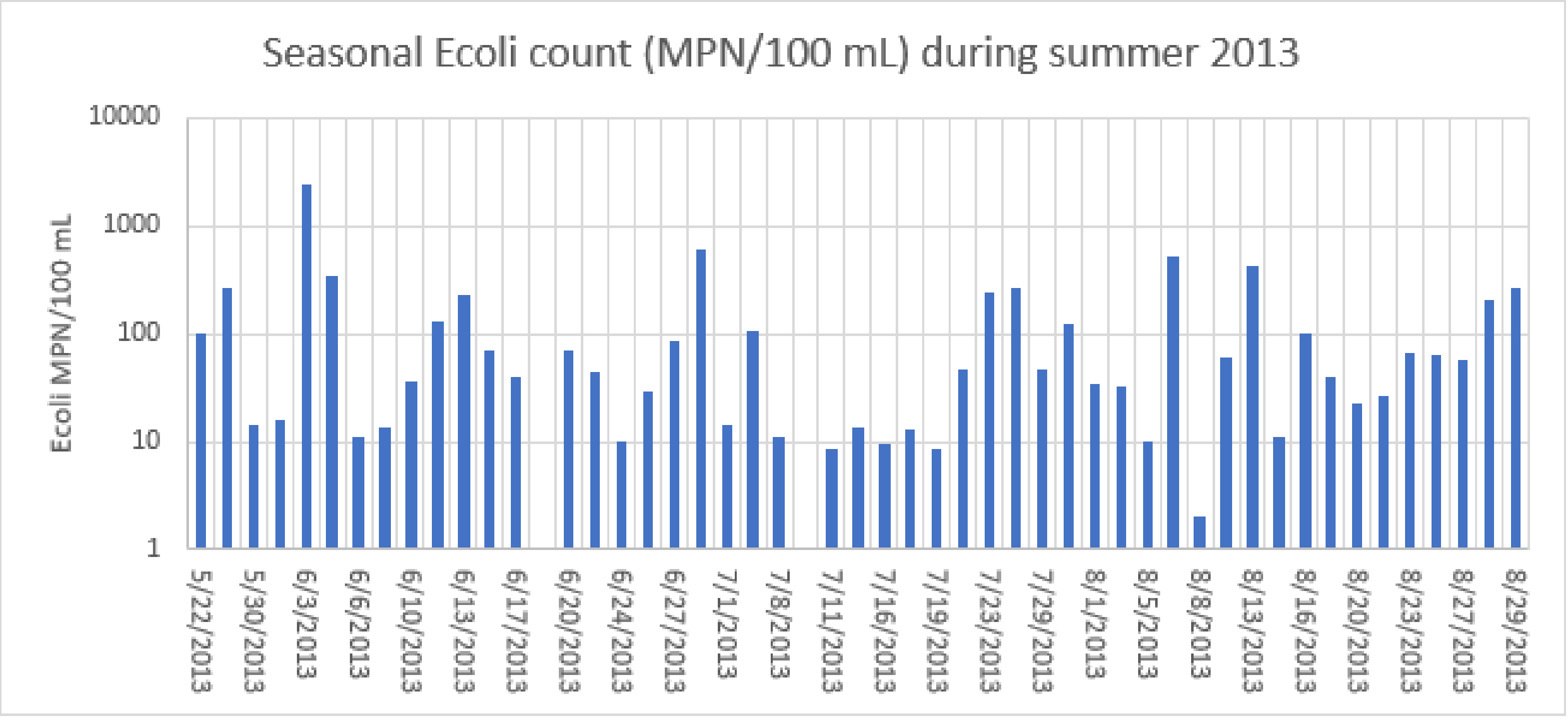
Fecal Indicator bacteria	Sampling location	Algae level (scale)			
		0	1	2	3
E.coli (MPN/100 ml)	Water	25	102	262	612
	Swash Zone	126	232	257	618
Enterococci (MPN/100 ml)	Water	7	115	57	507
	Swash zone	15	50	81	1045

- Average bacteria count during summer from water sample and sand sample in swash zone indicated a positive correlation between the algal presence and bacteria count
- The gradually increasing rating scale of algae at the three transect locations was positively correlated as the bacteria average count increased with the level of algae
- However, there was an exception to that relation for Enterococci count in water sample

Precipitation during Summer (May-August) 2013



Seasonal *E.coli* count (MPN/100 ml) during Summer 2013



Mean value of *E.coli* 145 MPN/100 mL

Source: Water Quality (*E.coli*) monitoring report, Bradford beach, Milwaukee county, Wisconsin Beach Health

Effect of Rainfall on *E.coli* count Statistical Analysis (One sample z test)

One-Sample Z: Ecoli (MPN/100 ml) with DIWater

Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
126	35.8	128.0	11.4	(13.5, 58.2)

μ: mean of Ecoli (MPN/100 ml) with DIWater
Known standard deviation = 127.981

Test

Null hypothesis H_0: $\mu = 145$
Alternative hypothesis H_1: $\mu \neq 145$

Z-Value	P-Value
-9.58	0.000

One-Sample Z: EColi (MPN/100 ml) with PBS

Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
132	32.5	140.8	12.3	(8.5, 56.5)

μ: mean of EColi (MPN/100 ml) with PBS
Known standard deviation = 140.804

Test

Null hypothesis H_0: $\mu = 145$
Alternative hypothesis H_1: $\mu \neq 145$

Z-Value	P-Value
-9.18	0.000

Hypothesis testing:

H0: rainfall does not affect bacteria count

H1: rainfall does affect bacteria count

Reject null hypothesis H_0 : if P-value <0.05

alpha = 0.05 with 95% confidence level

Result: rainfall does affect bacteria count for both DI water and PBS eluents

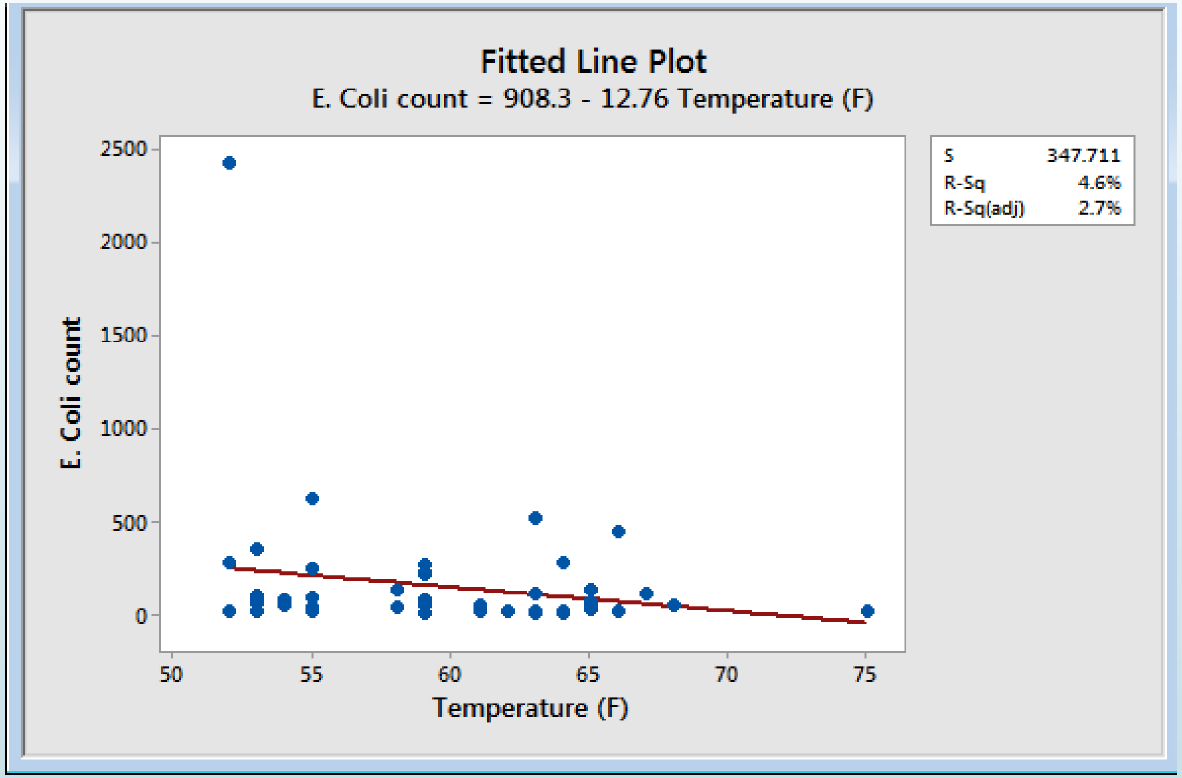
Correlation of *E.coli* with algae level and rainfall

- Correlation of *E.coli* with rainfall event and Algae level was established using the *E.coli* count (MPN/100 mL) during summer 2013 from beach water sample
- The presence of algae level was weakly correlated with *E.coli* count comparatively to the rainfall event
- The effect of rainfall event on *E.coli* count was higher than the impact of Algae level during the sampling period. The reason for this was the previous days rainfall events that occurred during the sampling period
- However, both the factors have significant correlation with the higher concentration of *E.coli*

Correlation of E.coli (R-squared value)	
Alage	Rainfall
0.4867	0.552

Regression analysis of *E.coli* count with water temperature

- The p-value is obtained as 0.130 which is higher than alpha = 0.05 (95% confidence level)
- This is not significant and therefore water temperature is not having much effect on *E.coli* concentration during the sampling period
- The two variables have a weak negative linear correlation with R-squared value 4.6%
- *E.coli* count decreases with higher water temperature over the sampling duration



Summary

- When DI water was used as the eluent, slightly higher *E.coli* counts were reported compared to PBS eluent.
- Water ionic strength can have significant effects on bacterial adhesion to sand due to the impact on electrostatic interactions.
- With an increase in the ionic strength of the background solution, the repulsive force between the two electronegative forces of quartz sand and *E.coli* is lessened. This explains the slightly higher detachment rate for *E.coli* when DI water was used as the eluent compared to PBS.
- Phosphate in PBS, can compete with bacterial cells for binding space on surfaces therefore limiting bacterial adhesion.
- In favorable condition or sandy environment that provides nutrients, protection from sunlight inactivation and protozoan grazing, bacteria may increase in number resulting in higher number of bacteria counts detected as anomalous water quality or EVENT by CANARY.