

National Environmental Monitoring Conference
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Using qPCR as a Rapid
Detection Method for
Determining Nitrification and
Data Incorporation into a
Nitrification Potential Index
(NPI) in a South Texas
Distribution System

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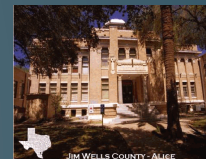
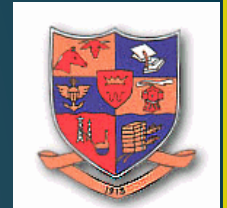
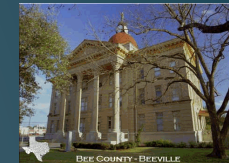
Key Points

- Background
- Monitoring Program
- Nitrification
- DNA Lab
- PCR Data
- NPI



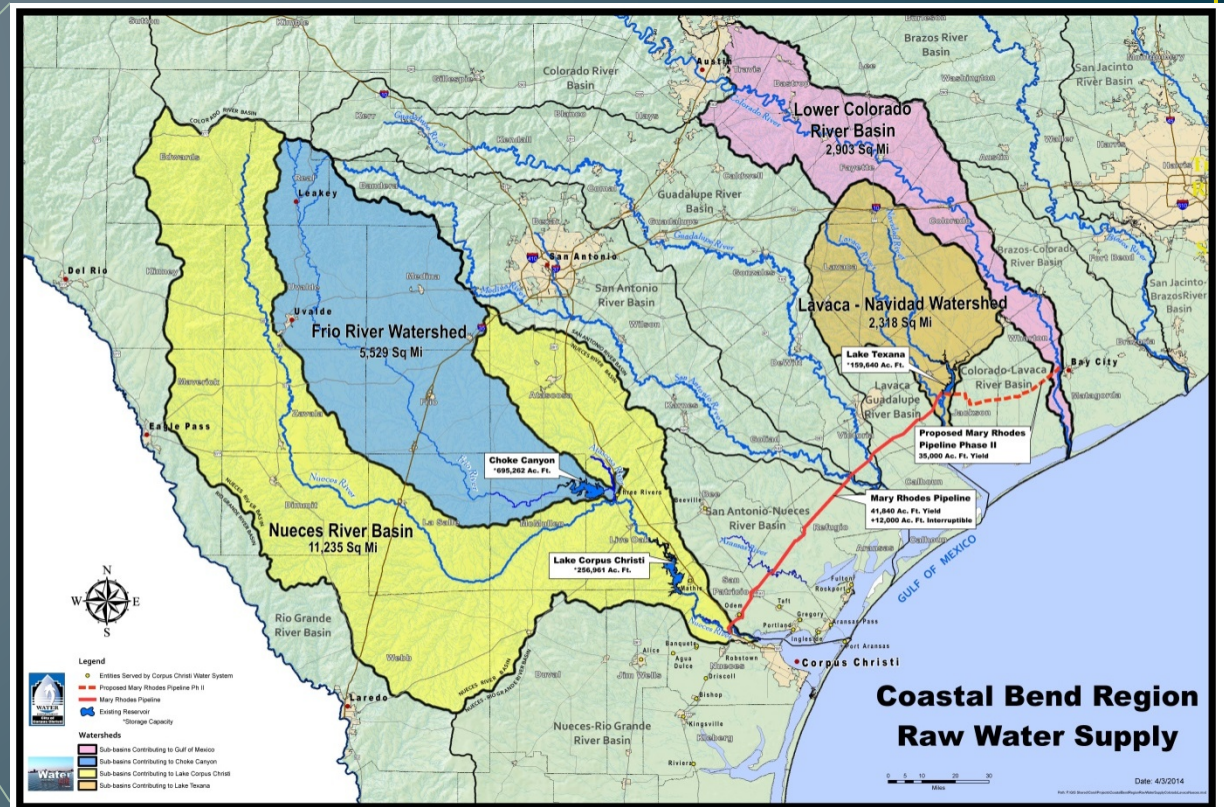
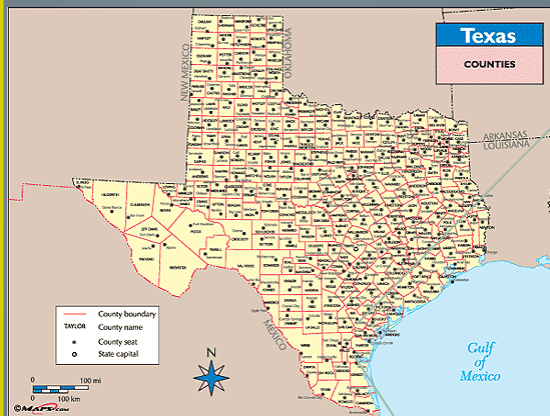
City of Corpus Christi, Texas

- Serves over 85,000 service connections and 300,000 residents.
- Supplies water to 7 counties (Aransas, Bee, Jim Wells, Kleberg, Live Oak, Nueces and San Patricio).
- Some industrial customers include:



Introduction

- Water Resources
 - Nueces River through Choke Canyon and Lake Corpus Christi
 - Lake Texana through 101 mile long Mary Rhodes Phase I Pipeline
 - Colorado River through Mary Rhodes Phase II Pipeline (in construction)



Background

- During the summer of 2007, City issued a boil water notice as a result of an acute MCL violation.

Local

City issues boil water notice for all areas

From staff reports

Originally published 03:12 p.m., August 14, 2007

Updated 03:12 p.m., August 14, 2007

CORPUS CHRISTI — The City of Corpus Christi has issued a boil water advisory for all areas of the city as a precautionary measure until further notice.

Three of four sampling areas which indicated bacteria have been cleared of bacteria. Results from one sampling area that has been retested are not ready for evaluation.

Citizens can receive on-going information about the water boil advisory on the city website at www.cctexas.com or 826-INFO or 826-CITY during normal business hours.

In response to the boil-water notice issued by the city, CCISD officials will provide bottled water to all student athletes, band members and drill team members who are practicing for the upcoming school year.

Superintendent Scott Elliff and district officials met Tuesday afternoon to discuss how to respond to the citywide water boil notice issued about 1:20 p.m.

Teachers, faculty and staff participating in in-service training at schools are encouraged to bring their own bottled water.

Background

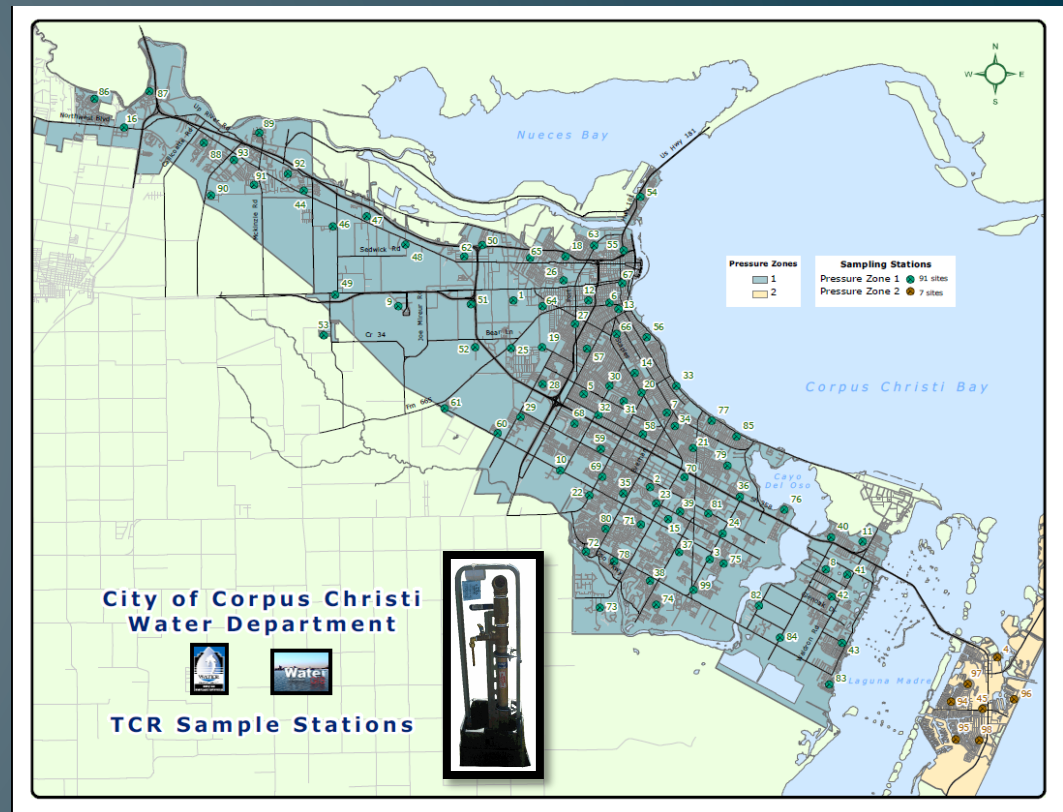
- Nitrification was the main culprit of the “water issue”.
- Nitrification was linked to prolonged residence time in water mains.



TCR Sites for Nitrification Monitoring

Total Coliform Rule Program

- Required by EPA and TCEQ
 - City to collect at least 150 samples per month.
- The City has 98 dedicated samples stations (for bacterial and chemistry samples) throughout 2 pressure zones.



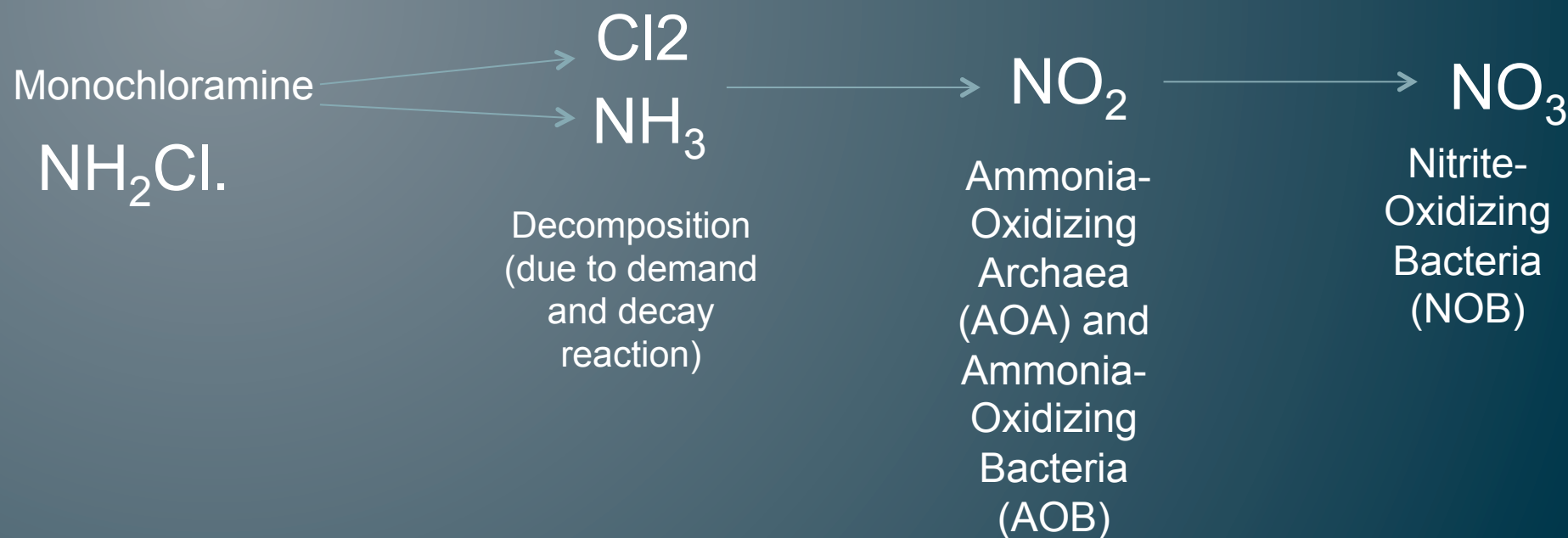
Background

- Total Coliform Rule Program includes additional chemistry monitoring of:
 - Total Residual Chlorine (TRC)
 - Monochloramine
 - Total Ammonia
 - Free Available Ammonia (FAA)
 - Nitrite
 - Nitrate
 - pH
 - Temperature



Nitrification

Nitrification is a natural microbiological process that occurs in ammonia-containing aquatic systems, such as chlororaminated drinking water distribution and storage systems



Nitrifying Bacteria (AOB)

- Part of Beta and Gamma Proteobacteria phylogeny of the domain Bacteria
- Oxidize ammonia to nitrite
- Gram negative chemolithoautotrophic aerobes
 - Use oxygen as terminal electron acceptor
 - Use NH_3 as electron donor
 - Carbon dioxide is the carbon source reduced during the Calvin Cycle
- Genera *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, and *Nitrosolobus*.
- Some marine and freshwater species
- Culture based method of AOB is incubation in the dark at room temperature for 21 to 28 days!

Nitrifying Archaea (AOA)

- Archaea are one of three domains of life
- Prokaryotic like bacteria, but have similarities to eukaryotes
- Not a lot of studies done on Archaea, let alone AOA
- Recently found in aquarium environments
- As more studies are done, AOA has been found to be in freshwater distribution systems as well
- It's worth looking for if it is causing nitrification in our system!

Nitrification

- Nitrifying bacteria are not a direct threat to public health
- Nitrification episodes are marked by a change in several water quality parameters, some of which have public health implications
 - ↓ chloramine residual
 - ↓ dissolved oxygen
 - ↓ pH
 - ↑ Heterotrophic Plate Count (HPC) bacterial growth
 - ↑ nitrite
 - ↑ nitrate
- These could result in water quality violations under the Total Coliform Rule and the Surface Water Treatment Rule, regulated by EPA and TCEQ

Nitrification

- Because AOB thrive on ammonia, limiting the residual free ammonia available for these bacteria can be one of the major control strategies for preventing nitrification
- Nitrite often persist in drinking water distribution systems because NOB grow so slowly
 - When AOB produce nitrite from oxidation of ammonia, then NOB consume the nitrite and produce nitrate.
- When nitrification is present, chlorine residual decreases, and the possibility of having total coliforms and *E. coli* may increase.

Detecting Biology Before Chemistry

- To actually detect the biology before the chemistry happens in a nitrification event would be ideal
- Traditional way of detecting AOB by culture presently takes 3-4 weeks of growing samples to quantify by Most Probable Number (MPN)
 - Recovery rate is not very good
 - The water the original sample came from is long gone by then!
 - AOB are slow growers
- Rapid detection method to quantify AOA/AOB done by PCR

PCR as EDS for Nitrification

- Utilizing PCR method with our conventional environmental parameters will give us a better profile of water quality in the distribution system and allow us to project future water quality
- PCR can be used as an early detection system (EDS) for a nitrification event
- We will actually be detecting the organisms the root cause of nitrification, whereas before we were catching the metabolic byproducts of these same organisms AFTER the nitrification has already happened!



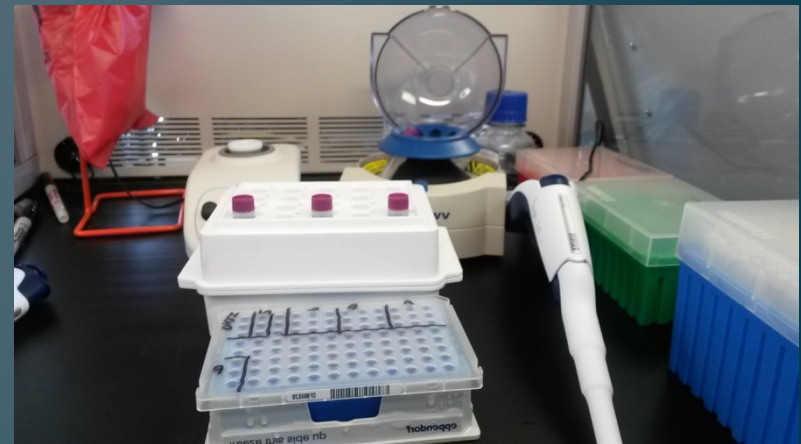
DNA Lab Startup

- Dr. Sungwoo Bae and Dr. Mary Jo Kirisits of University of Texas-Austin did applied research background for the PCR program
- They performed a microbial community analysis in the Corpus Christi chloraminated distribution system to identify key nitrifying microorganisms, as well as pyrosequencing
 - Varied sampling done geographically over a variety of temperatures
- They optimized three probes, with primers:
 - AOA: ammonia-oxidizing archaea
 - AOB: ammonia-oxidizing bacteria
 - AOC: ammonia-oxidizing *Nitrosomonas oligotropha*-like bacteria



Basic Procedure

- Water Quality Field Staff collects 20 liters of water from site
- Water Utilities Laboratory staff filters water in the DNA lab
- DNA is isolated from water filters using a DNA extraction kit
- PCR is performed on extracted DNA
- Data is analyzed to get a quantifiable number



- Sampling done morning of day 1, filtered afternoon of day 1, and PCR performed morning of day 2 with results within 24 hours of collection
- Full suite of analysis is also be performed on each sample for a better water quality profile



Correlation with AOA

$$\text{AOA} = B_1 + B_2 \text{TRC} + B_3 \text{NH}_3 + B_4 \text{AOB} + B_5 \text{AOC} + B_6 \text{FAA} + B_7 \text{MONO} + B_8 \text{Nitrite} + B_9 \text{Nitrate} + B_{10} \text{WaterTemperature} + B_{11} \text{pH}$$

Variable	P Value
TRC	.724774
NH3	.757714
AOB	<2.2E-16
AOC	.009951
FAA	.371855
MONO	.391629
NITRITE	.742117
NITRATE	.750164
WT	.804200
pH	.197251

Correlation with AOB

$AOB = B_1 + B_2TRC + B_3NH_3 + B_4AOA + B_5AOC + B_6FAA + B_7MONO + B_8Nitrite + B_9Nitrate + B_{10}WaterTemperature + B_{11}pH$

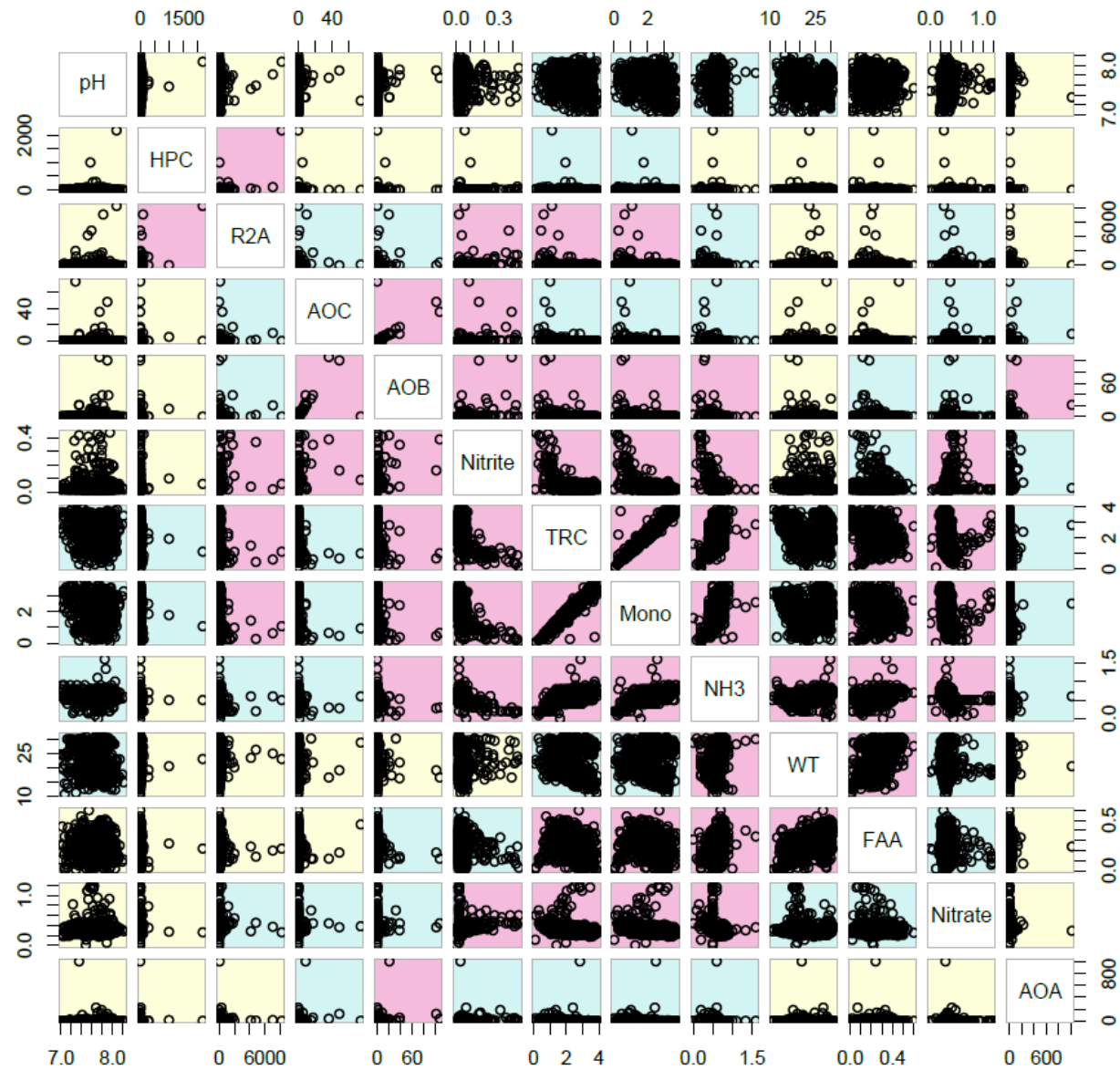
Variable	P Value
TRC	0.4738982
NH3	. 0.0002037
AOA	< 2.2e-16
AOC	< 2.2e-16
FAA	0.0104482
MONO	0.7923724
NITRITE	2.959e-13
NITRATE	0.5969537
WT	0.5395790
pH	0.4400738

Correlation with AOC

$$\text{AOC} = B_1 + B_2\text{TRC} + B_3\text{NH}_3 + B_4\text{AOA} + B_5\text{AOB} + B_6\text{FAA} + B_7\text{MONO} + B_8\text{Nitrite} + B_9\text{Nitrate} + B_{10}\text{WaterTemperature} + B_{11}\text{pH}$$

Variable	P Value
TRC	0.660612
NH3	0.007727
AOA	8.63e-13
AOC	< 2.2e-16
FAA	0.115761
MONO	0.891224
NITRITE	0.001496
NITRATE	0.561776
WT	0.926425
pH	0.279686

Variables Ordered and Colored by Correlation



Nitrification and Response

- A PCR data threshold will be established once we get a baseline of data from all of the sites after a year
- PCR data will be combined with surrogate parameter data to get a true idea of a nitrification event
- Incorporation of DNA data and surrogate parameters into a Nitrification Potential Index (NPI)
 - Data does not produce practical information to support decision making, but NPI will be a tool to turn water quality data into information

Definition of Nitrification events for the Nitrification Potential Index

Variables for NPI:

- Chlorine residuals (mg/L)
- Monochloramine (mg/L)
- Free available ammonia-N (mg/L)
- Nitrite-N (mg/L)
- Nitrate-N (mg/L)
- pH (s.u.)
- Temperature (°C)

Chemical and physical
variables

- HPC (cfu/mL)
- R2A (cfu/mL)

Biological variables

- DNA Analysis (from September 2014):
- Identification of Nitrification Archaea and Bacteria
- DNA Quantification (gc/mL) (from September 2014):

Genetic
variables

Canadian Council of Ministers of Environment Water Quality Index

- The index we are using to determine nitrification is the CCME-WQI.
- Uses 3 factors to develop a score from 0-100 grading the water quality.
 - Scope.
 - reports the number of variables not meeting current water quality objectives.
 - Frequency.
 - measures the number of times the thresholds are not met.
 - Amplitude.
 - measures the extent to which the objectives are exceeded.

Index definition for the Nitrification Potential Index

The Canadian Council of Ministers of Environment Water Quality Index (CCME-WQI)- *Water quality index based on Physico-Chemical Characteristics.*

$$\text{CCME WQI} = 100 - (\sqrt{F_1^2 + F_2^2 + F_3^2} / 1.732)$$

F_1 = (number of failed variables / total number of variables) x 100 (**Scope**)

F_2 = (number of failed test / total number of test) x 100 (**Frequency**)

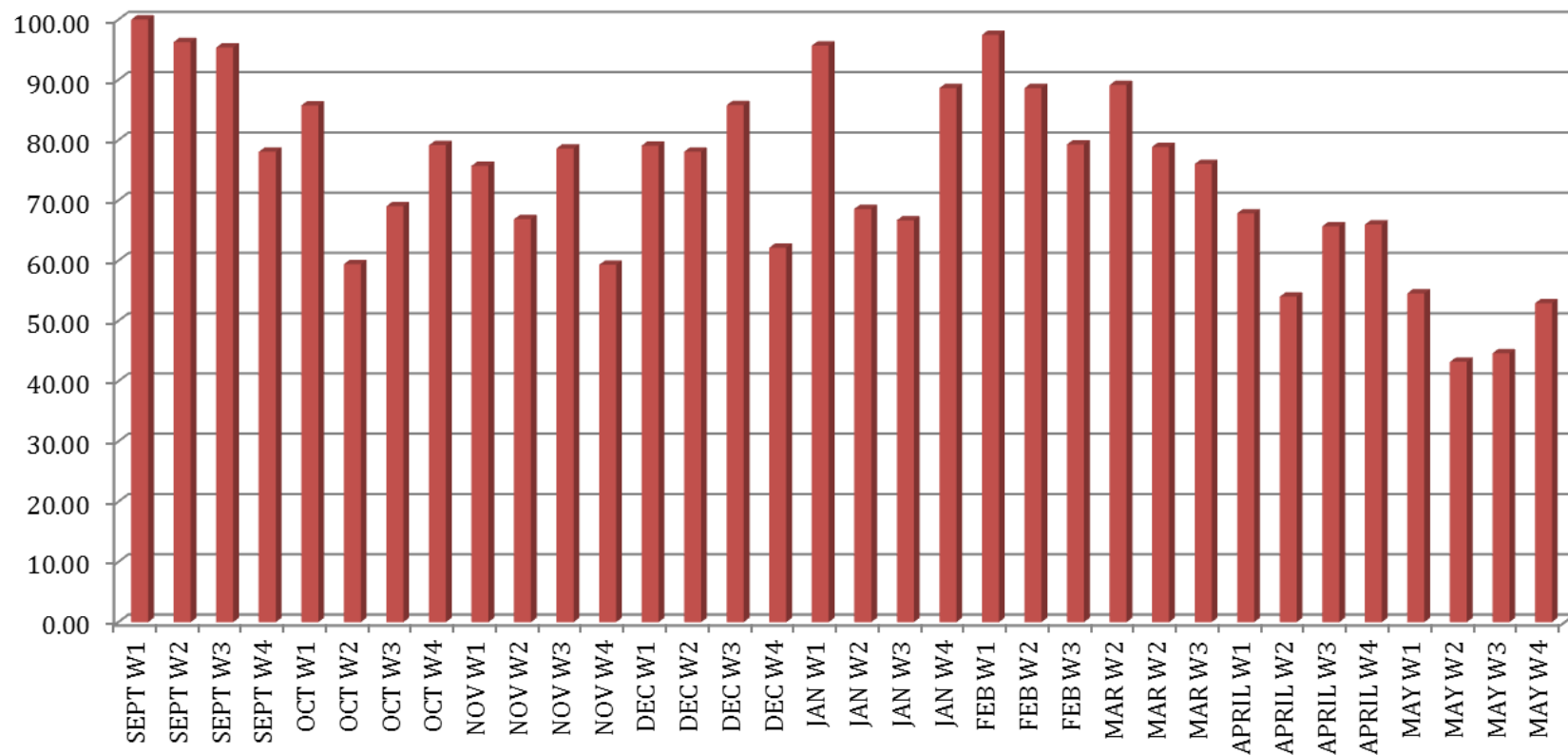
F_3 = (nse / (0.01nse + 0.01)) (**Amplitude**)

Characteristics:

- Combination of parameters
- Practicality-Equation could be utilized in mobile field operating solution

Weekly NPI

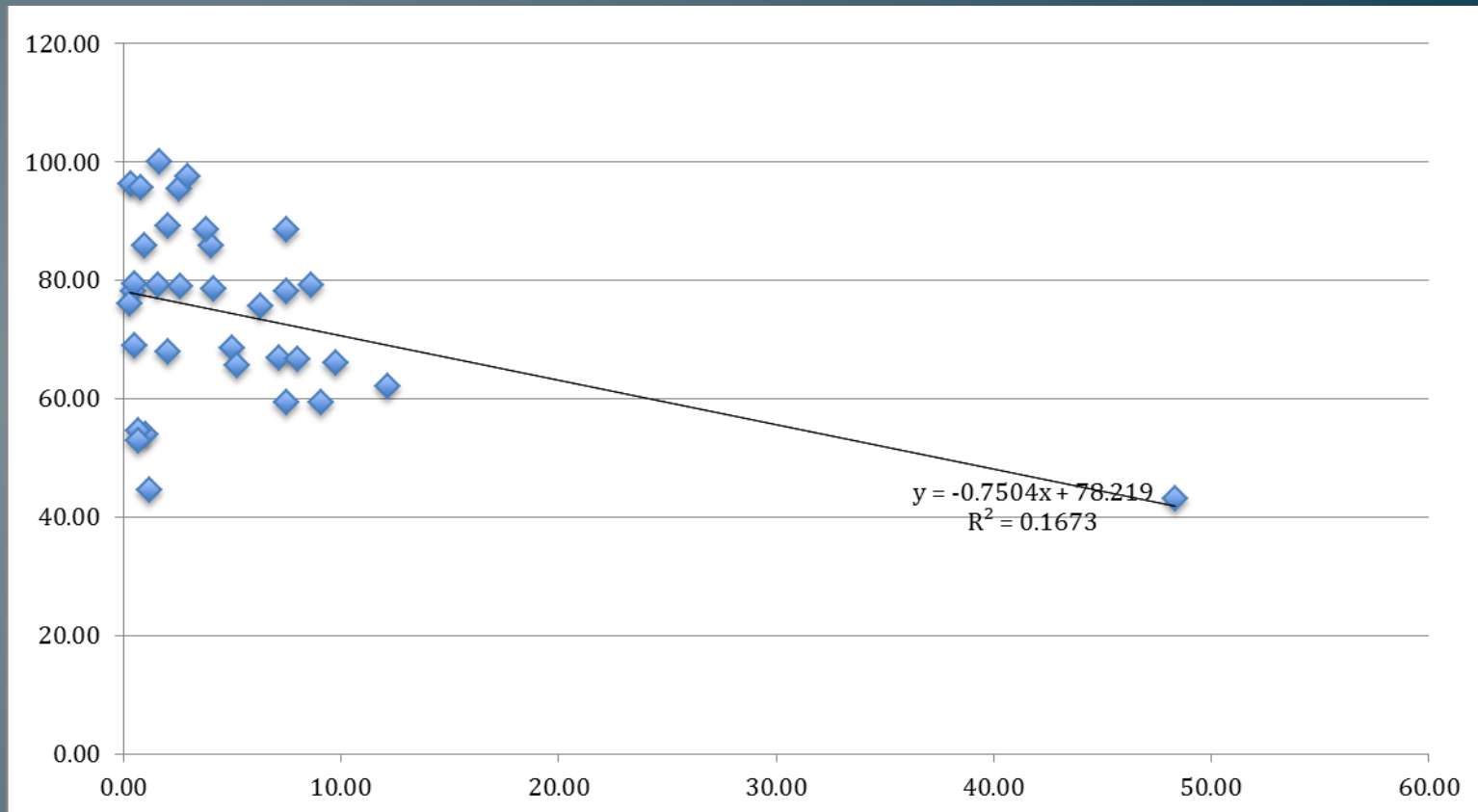
CCME-WQI WEEKLY



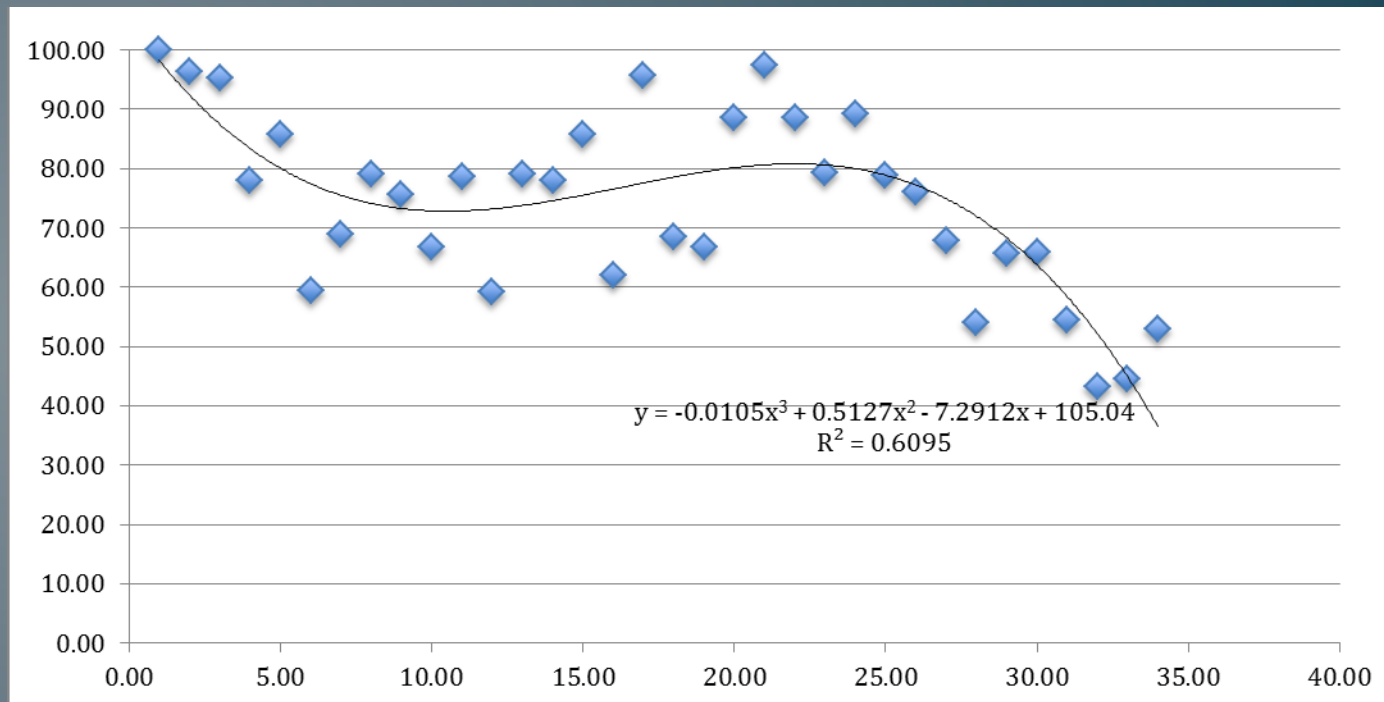
Correlation of Probes with NPI

AOA INDEX CORRELATION	-0.40901277
AOB INDEX CORRELATION	-0.352566851
AOC INDEX CORRELATION	-0.682632976

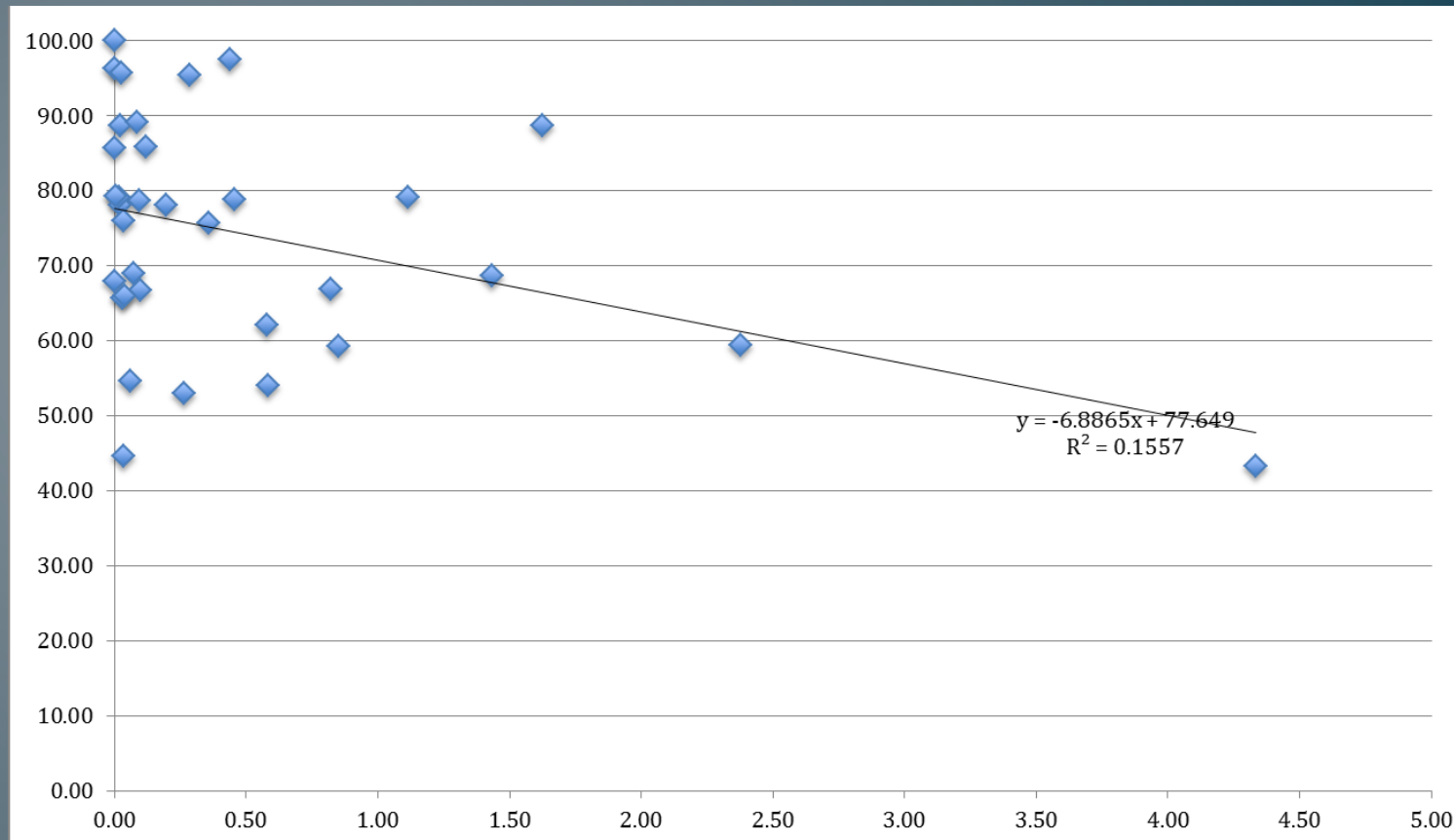
Mean Weekly AOA vs Weekly NPI values



Mean Weekly AOB vs Weekly NPI values

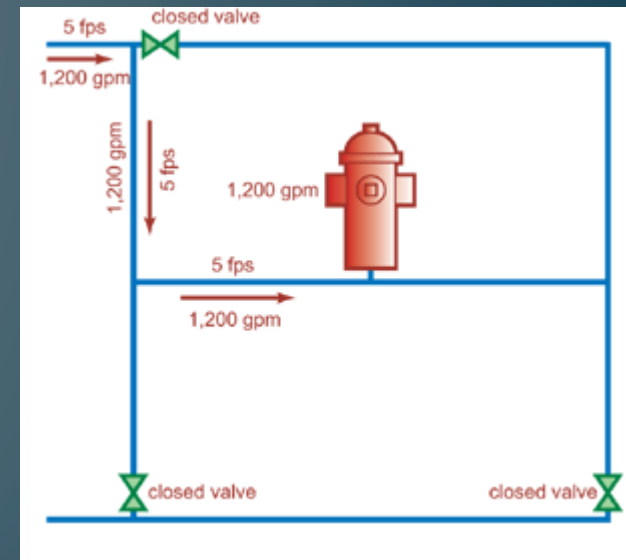


Mean Weekly AOC vs Weekly NPI values



Mitigation

- Unidirectional Flushing (UDF)
- Mechanical Cleaning
 - Pigging
- Chemical cleaning
 - Chlorine Burnout
- Pipe Replacements
 - CIP



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