DEPARTMENT OF HEALTH

Microbes in the Mist

Minnesota's Pathogen Project – Unexpected Encounters in Groundwater

Jim Walsh

MGWA Fall Conference

11/14/2023

Acknowledgements

- Clean Water Council – Clean Water Fund
- Laboratory for Infectious Disease and Environment (USDA-USGS Lab)
- USDA-ARS - Mark Borchardt, Sue Spencer, Joel Stokdyk, Aaron Firnstahl
- USGS Upper Midwest Water Science Center –Randy Hunt, Dave Owens, Andrew Berg, Aliesha Krall, Jared Trost, Mindy Erickson
- MDNR – Paul Putzier, John Barry, Jeff Green, Randy Bradt

MDH Study Team:
IDEPC -Trisha Robinson, Amber Koskey, Stephanie Gretsch
DWP - Anita Anderson, Lih-in Rezania, Jim Walsh, Dane Huber, Jared Schmaedeke, Trisha Sisto, Nathan Gieske, Mike Sutliff, Jane de Lambert, Rich Soule, Ernie Jorgensen, Arianna Giorgi, Tom Alvarez, Hannah Wilson, John Woodside
ESA – Deanna Scher, Nancy Rice
DWP engineers, sanitarians, hydrologists and compliance officers
MDH Drinking Water Protection Section: manager, supervisors, staff
MDH Public Health Lab

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Minnesota wave study

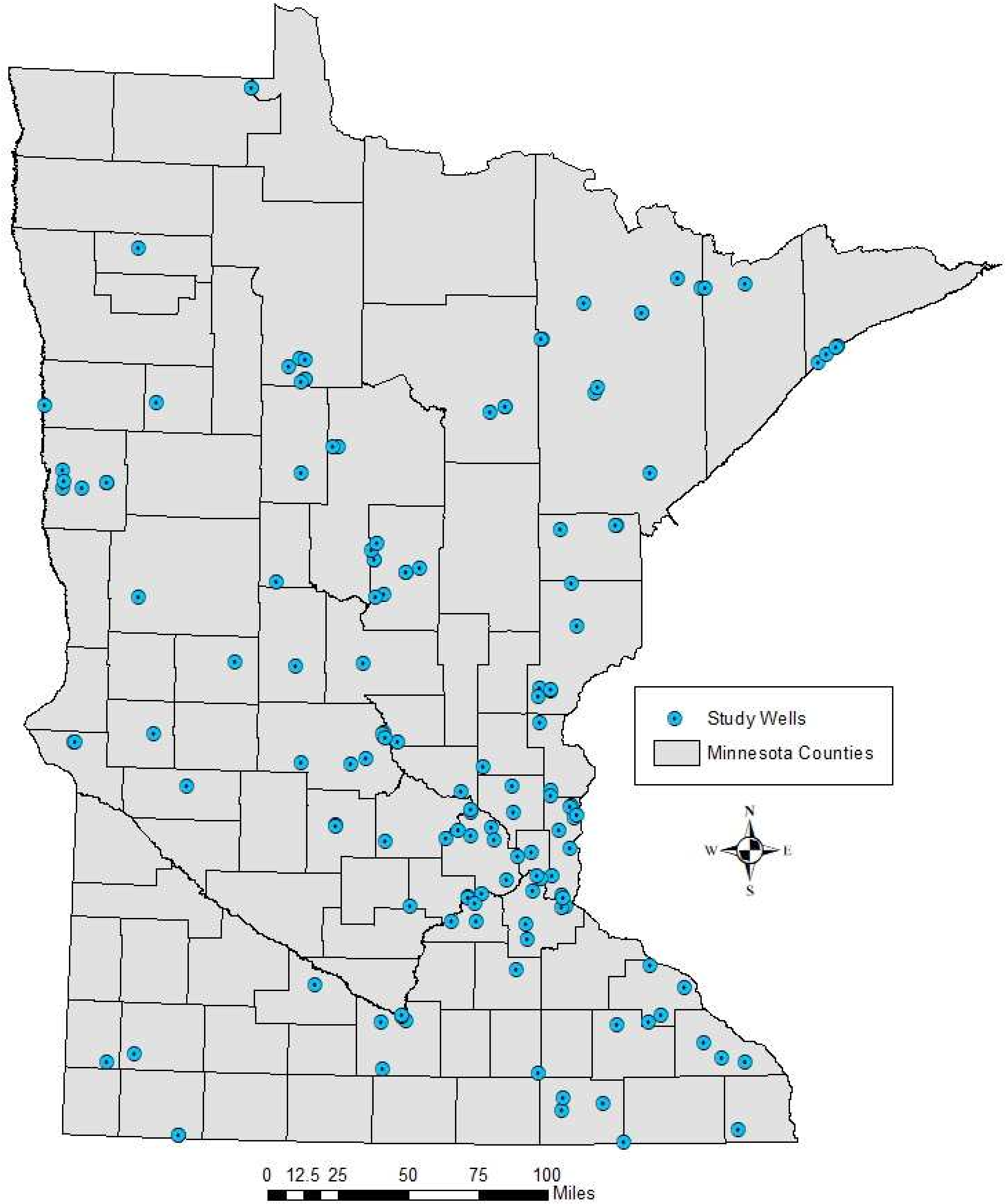
CLEAN WATER LAND & LEGACY AMENDMENT

Impetus for MDH study

Microbial Monitoring 2014-2016 (Virus Study)

- 145 Community & Noncommunity wells
- Bimonthly sampling
  - 117 wells for 1 year
  - 28 wells for 2 years
- Fecal pathogens and indicators
  - Human enteric viruses, others
  - *Salmonella, Bacteroides*, others
  - *Giardia* and *Cryptosporidium*
  - Chemical indicators

qPCR



Community Illness (WAVE) Study

- Weekly sampling at four sites
- Surveyed residents for illness and water use, etc.

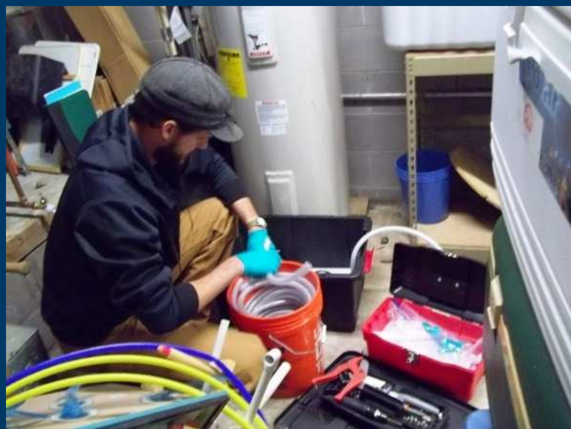
Indicators/water quality

Analytes

- TC/E. coli (MPN-QT)
- Enterococci (MPN-QT)
- Ammonia
- Chloride
- Bromide
- Nitrate (NO2 + NO3)
- TOC
- Boron
- Tritium (^{3}H)
- Stable isotopes: ^{18}O & ^{2}H

Field Parameters

- Temp
- pH
- Conductivity
- D.O.
- ORP

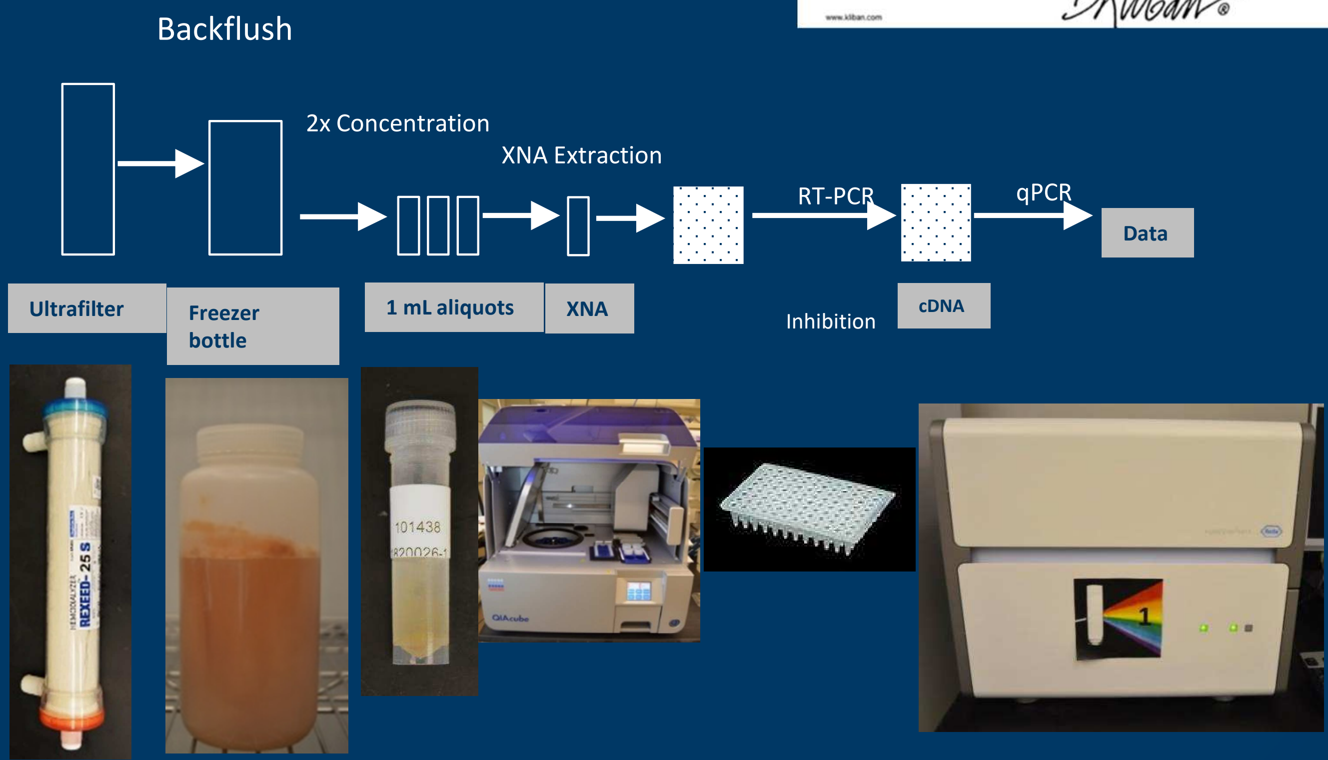


Field Activities

Sampling and analysis

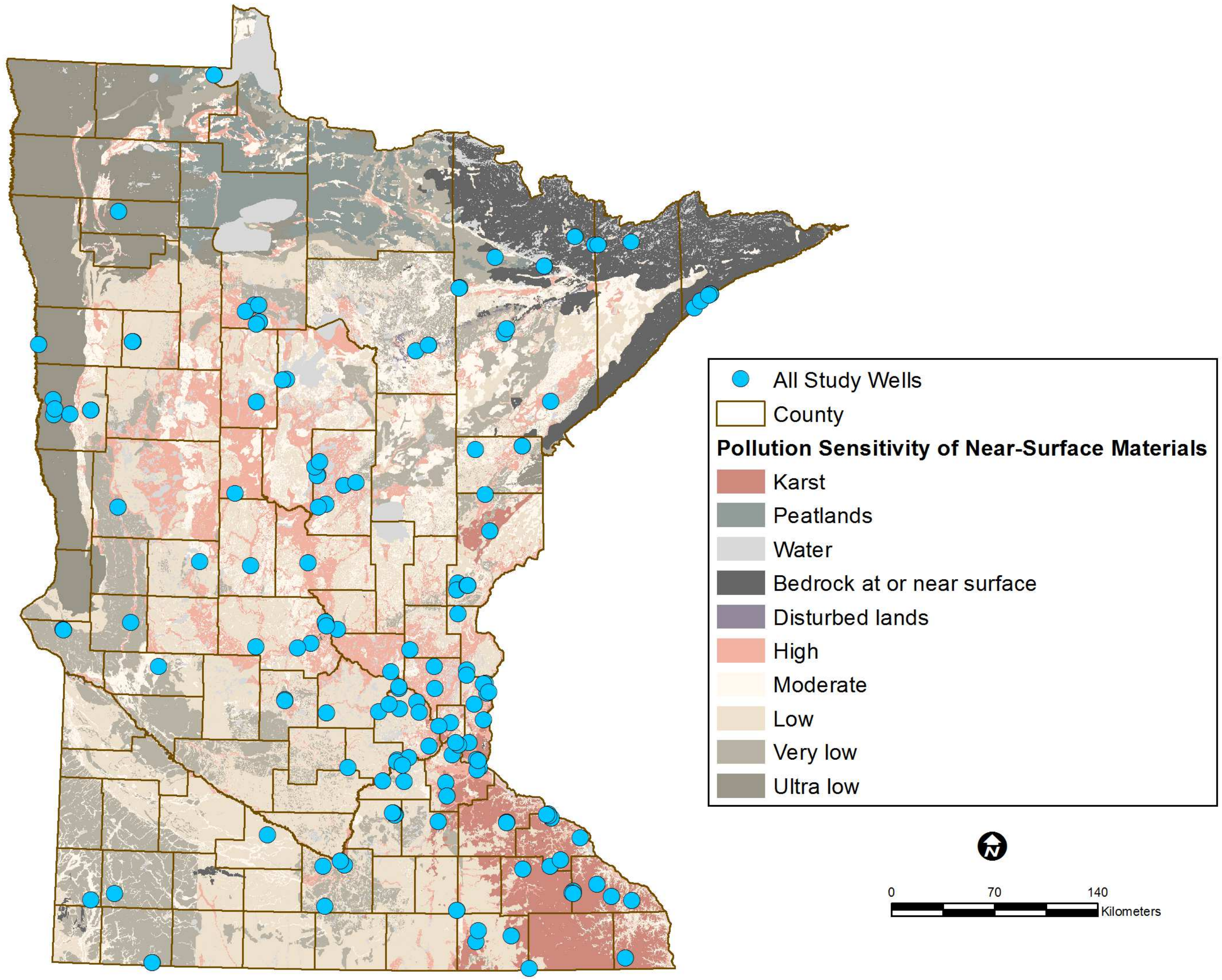
- Laboratory for Infection Disease and Environment (LIDE)
- qPCR: genetic testing
- Culture: *salmonella*, adenovirus, enterovirus
- Microscopy: *Giardia* and *Cryptosporidium*
- Some DNA sequencing human enteric viruses, *salmonella* and *Cryptosporidium*

QPCR analysis work flow – it's a genetic thing



Virus Study Wells – Aquifers Sampled and Relation to Near Surface Pollution Sensitivity

Aquifer Type	# of Wells
Glacial sand	82
Sandstone	33
Fractured Crystalline Rock	13
Limestone	9
Sandstone/ Limestone/ Shale	8
Total	145



Source = Minnesota DNR

Aquifer Type – Virus Study Wells vs. All Minnesota Public Water Supply Wells

Aquifer Type	% Virus Study Wells (n=145)	% MN Public Water Supply Wells* (n=6,640)
Glacial Sand	57	64
Sandstone	23	13
Limestone/ Dolostone	6	3
Sandstone/ Limestone/Shale	5	15
Fractured Crystalline Bedrock	9	5

*Numbers are approximate and contain data only for aquifers with 2 or more wells

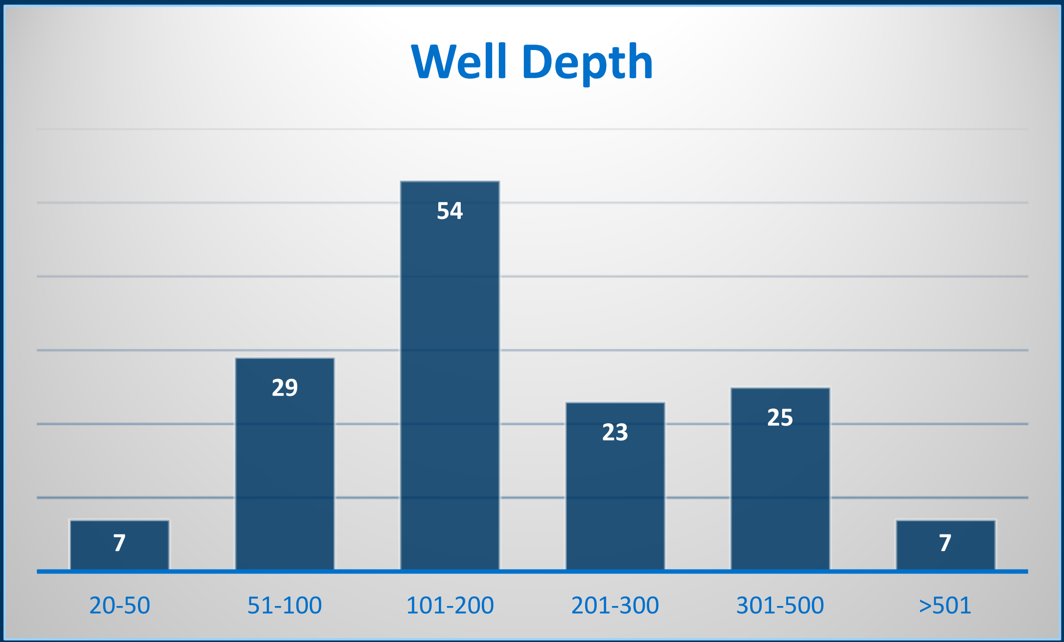
Virus Study Well Characteristics

Public Well Type	# of Wells
Community	88
Noncommunity Nontransient	45
Noncommunity Transient	12

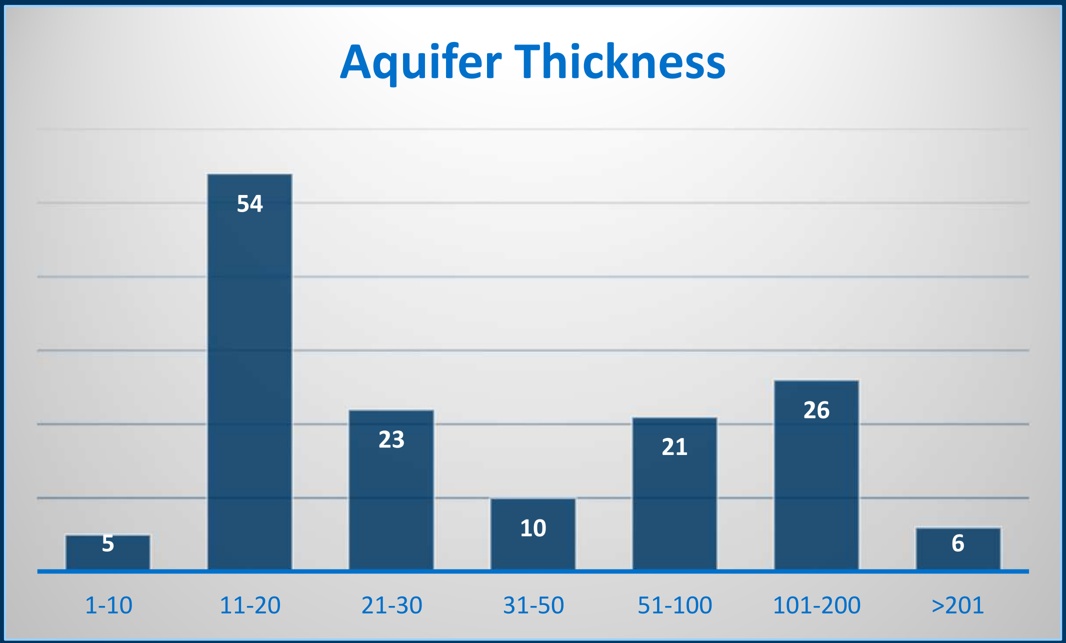
Pumping Rate (gpm)	# of Wells
1-10	77
10-20	14
20-40	9
40-100	12
100-500	29
500-1000	2
>1000	2

Virus Study Well Characteristics

Depth Range	# of Wells	Casing Depth	# of Wells
20-50	7	16-50	19
51-100	29	51-100	33
101-200	54	101-200	43
201-300	23	201-300	19
301-500	25	301-500	15
>501	7	>500	2
Unknown	0	Unknown	13



Virus Study Aquifer Characteristics

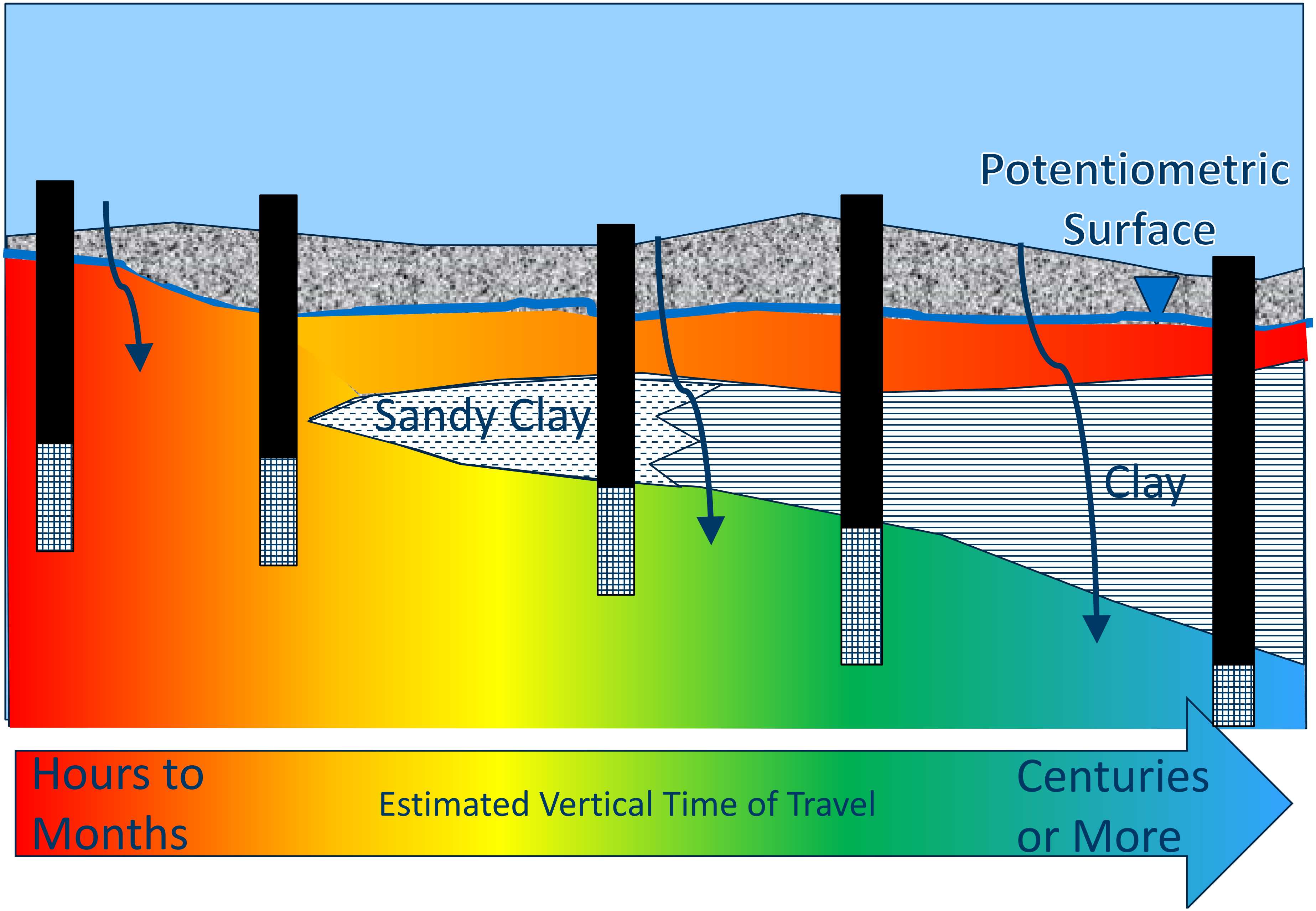


- Most wells finished in thin sand and gravel aquifers
- Thicker aquifers are Paleozoic sandstones and limestones

Hydraulic Conductivity (ft/d)	# of Wells
1-10	35
11-20	28
21-30	15
31-50	28
51-100	18
101-200	8
>201	4
Unknown	9

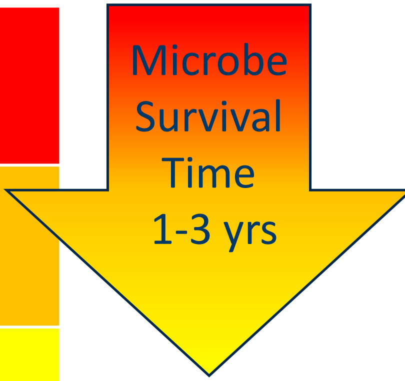
- Hydraulic conductivity was estimated from specific capacity tests
- Most values fall in typical range for fine sands and sandstones

Geologic Sensitivity of Aquifers at Well Locations



Geologic Sensitivity of Aquifers Tapped by Virus Study Wells

Geologic Sensitivity	Estimated Vertical Time of Travel from Land Surface	% Virus Study Wells (n=145)	% MN Public Water Supply Wells* (n=3,632) 30% unknown
Very High	Hours to month or two	22	10
High	Weeks to a year or two	14	9
Moderate	Years to a few decades	22	11
Low	Several decades to a century	27	20
Very Low	More than a century	15	20



Virus Study qPCR Results

	Number	Percent
Wells with any positive results	138	96
Wells positive for more than one microbe	122	84
Wells positive in more than one sampling round	118	81
Wells with at least one result above 10 gc/l	64	44
Wells positive for *Cryptosporidium*	58	40
Wells positive for *Giardia*	6	4

2014-2016 Monitoring Data Summary and Observations

The Bad News

- Microbial detections were widespread
  - 32% of wells had ≥1 human virus detection
  - 70% of wells had ≥1 human pathogen detection

- Some detections were high concentration

- Traditional risk indicators (coliform/e.coli, geologic sensitivity) don't appear to predict pathogen detection

- Larger diameter pathogens are entering groundwater
  - 4% of wells had >1 *Giardia* detection
  - 40% of wells had ≥1 *Cryptosporidium* detection

2014-2016 Monitoring Data Summary and Observations

The Good News

- Intermittent detections
  - 6% of samples had human virus detection
  - 22% of samples had human pathogen detection

- Usually low concentrations

- Not all detections represent infectious organisms

- Not all infectious organisms result in illness

High-Level Summary of 2014-2016 Study Wells

- They are not "exceptional" in terms of risky construction, geologic setting or use (generally good predictors of chemical contamination risk)

- Despite that, many yielded detections of genetic material

<u>Abundance of detections argues for</u>:

- Widespread occurrence of microbial genetic material in subsurface (Why more than chemical contamination? *Very* sensitive analytical method)

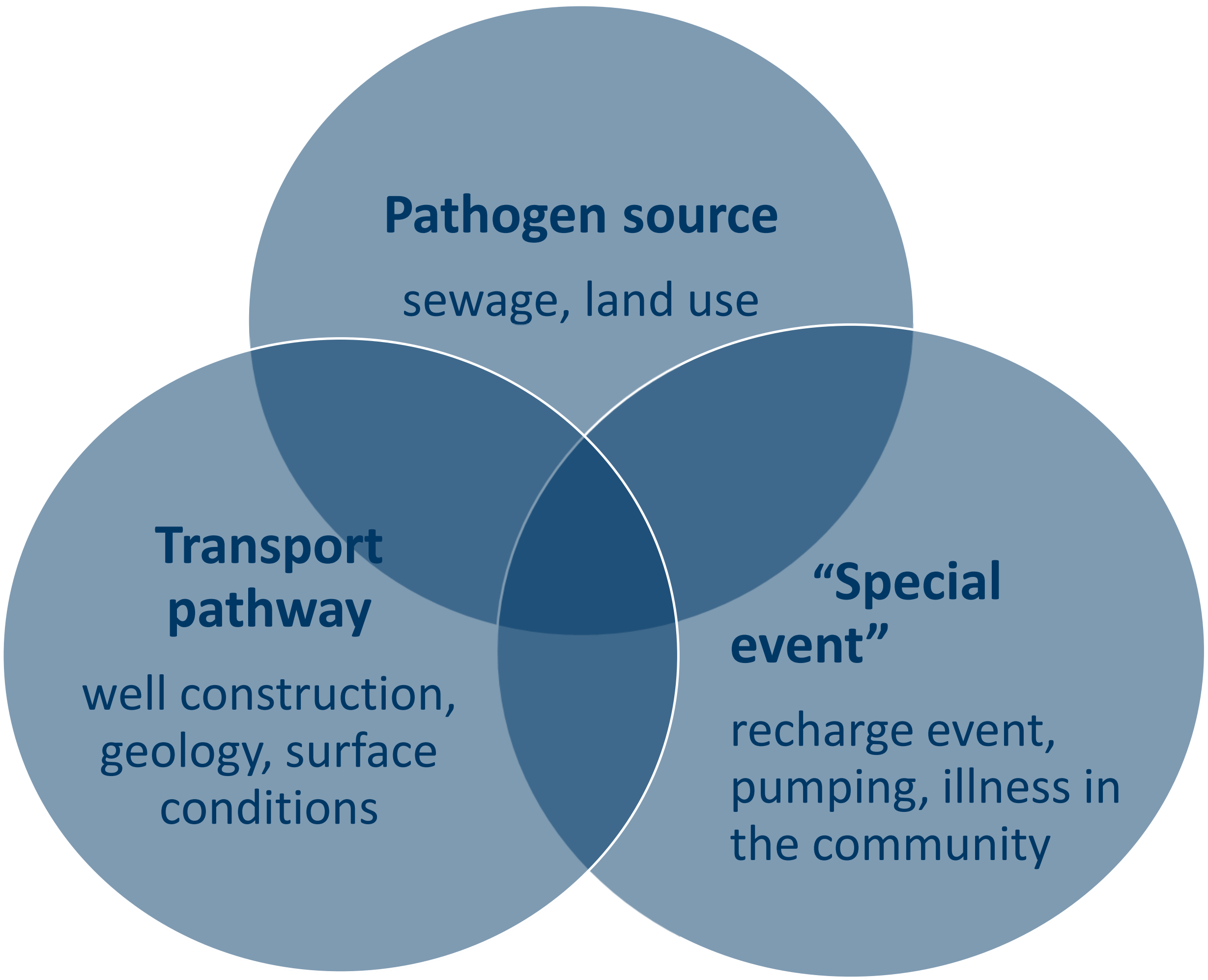
- Likelihood of multiple transport pathways rather than a single "smoking gun" variable

Submultiples		
Value	**SI symbol**	**Name**
10^{-1} g	dg	decigram
10^{-2} g	cg	centigram
10^{-3} g	**mg**	**milligram**
10^{-6} g	**µg**	**microgram**
10^{-9} g	**ng**	**nanogram**
10^{-12} g	**pg**	**picogram**
10^{-15} g	fg	femtogram
10^{-18} g	ag	attogram
10^{-21} g	zg	zeptogram
10^{-24} g	yg	yoctogram

Publications from 2014-2016 study phase

- **Cryptosporidium findings:**
  - Stokdyk et al., 2019 (ES&T) *Cryptosporidium* incidence and surface water influence of groundwater supplying public water systems in Minnesota, USA
- **General occurrence findings:**
  - Stokdyk et al., 2020 (Water Research) Viral, bacterial, and protozoan pathogens and fecal markers in wells supplying groundwater to public water systems in Minnesota, USA
- **Quantitative Health Risk Assessment:**
  - Burch et al., 2022 (ES&T) Statewide Quantitative Microbial Risk Assessment for Waterborne Viruses, Bacteria, and Protozoa in Public Water Supply Wells in Minnesota

Components needed for microbial contamination



Key findings from the WAVE Study

- Higher rates of acute gastrointestinal illness were reported during the weeks viruses were detected in the drinking water source
  - Not statistically significant; can't draw firm conclusions
  - Due to chance? Association is real and study is too small?
- People who had a water filter at home reported higher rates of illness

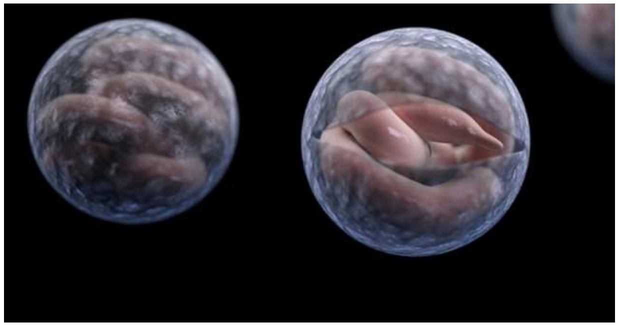
Pathogen Project Wrap Up (2019-2023)

Project Component	Goals Addressed	Resulting Benefit	Status
Statistical Analysis of 2014-2016 Data	Determining risk factors and predicting pathogen occurrence	Better assessing sources and wells by risk	Analysis complete, manuscript in draft form

Statistics:  >80 Factors Evaluated as Controlling Variables

- Well Construction and Use
- Aquifer Characteristics
- Variability in Chemical Indicators of Human Impact
- Land Use/Potential Contaminant Sources in IWMZs and well capture zones

Outcome =
Cryptosporidium Occurrence
(Driver of Health Risk)



- Poor correlation with coliform
- High Infectivity
- Chlorine Tolerance
- Bellwether for smaller organisms

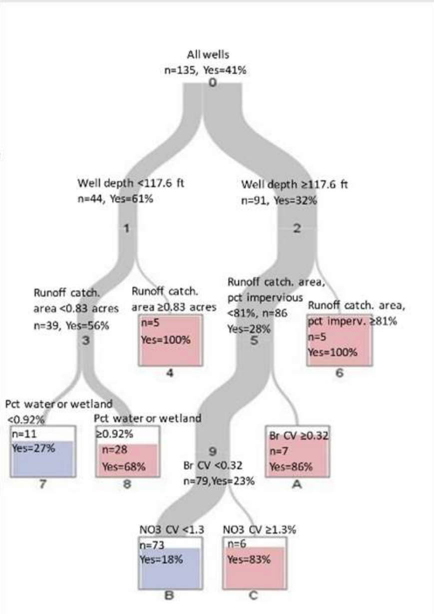
- Precipitation Amount and Timing

Recharge Study Design

Parameters Evaluated and Methods Used for Crypto=Positive Outcome

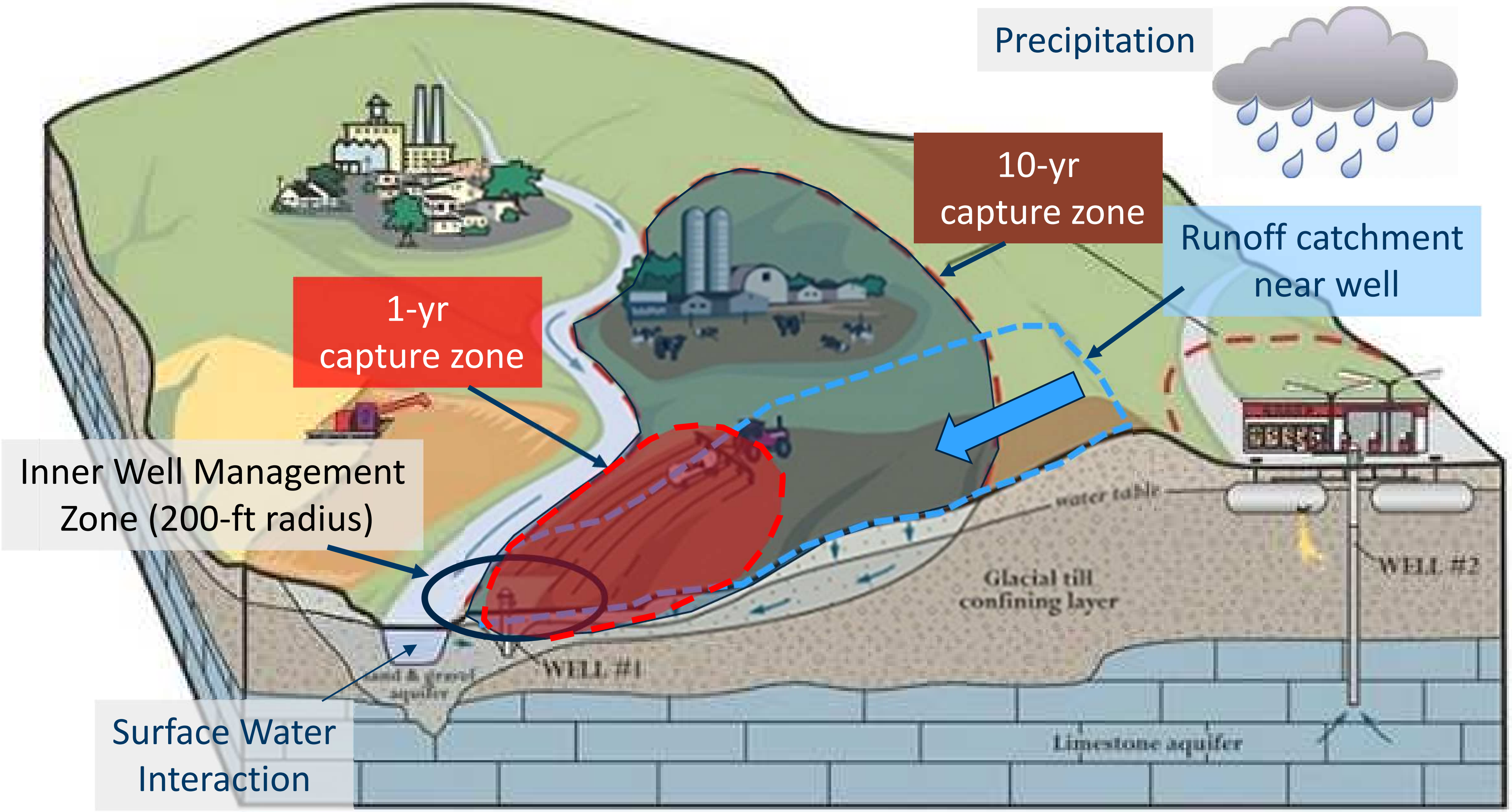
Methods:

1. Univariable
   - Chi-squared test, Cochran-Armitage trend test, Mann-Whitney *U* test
2. Multivariable
   - Only parameters with <20% missing values and p≤0.2 included
   - Variables with p≤0.05 included in final model (Modified Poisson regression model)
   - Classification Trees
   - Sensitivity Analysis



Theme	Potential Predictive Factors	
Well Use and Construction	Well type Year drilled Well depth Depth cased Casing diameter Casing material Drilling method Grouted (yes/no)	Grout material Pct casing grouted Pct grout saturated Annular space Casing jointing method Saturated casing value Discharge rate
Aquifer Characteristics, Connectedness Between Aquifer and Land Surface	Land surface elevation Depth to bedrock Bedrock interface distance Aquifer Type Aquifer porosity type Aquifer porosity Groundwater age from tritium Karst or fractured Geologic sensitivity L score	Near surface pollution sensitivity Vertical hydraulic gradient (mean) Hydraulic conductivity Aquifer thickness Static water level Drawdown Surface water class Surface water subset Primary groundwater class, unbiased
Well Capture Zone, Land Use within Capture Zone	Capture zone area Runoff catchment area Runoff catchment area, pct impervious Pct low intensity development, 1 yr TT Pct medium intensity dev., 1 yr TT Pct high intensity dev., 1 yr TT Pct row crop or pasture, 1 yr TT Dev. mostly agriculture (y/n), 1 yr TT	Pct open water or wetland, 1 yr TT Pct low intensity development, 10 yr TT Pct medium intensity dev., 10 yr TT Pct high intensity dev., 10 yr TT Pct row crop or pasture, 10 yr TT Dev. mostly agriculture (y/n), 10 yr TT Pct open water or wetland, 10 yr TT
Potential Contaminant Sources in the IWMZ	Nbr of pathogen sources Nbr of drainfields Distance to nearest drainfield Nbr of septic/sewage systems Dist. to nearest septic/sewage system Nbr of sewer lines Dist. to nearest sewer line	Nbr of storm sewer lines Dist. to nearest storm sewer line Sewer type Sewer age Design flow Waste treatment type
Chemical and Isotopic Parameters	Variance from average precipitation Temporal variability Nitrate >1 mg/L in past 5 yrs Source total coliform detect ≤5 yrs Distribution total coliform detect ≤5 yrs MDH vulnerability rating Assessment monitoring score Bromide coefficient of variation (CV) Chloride CV Chloride-Bromide CV	Nitrate CV Ammonia CV Boron CV Total organic carbon CV Specific conductance CV Temperature CV d2H CV d18O CV pH CV Dissolved oxygen (DO) CV

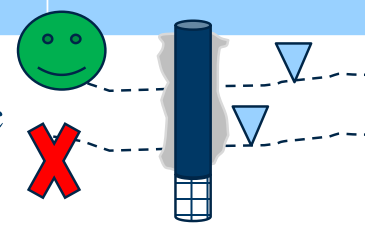
Land Use/Contaminant Sources Evaluated



Modified from Iowa DNR

Important Variables from Univariable/**Multivariable** Analysis

(Winnowed down from 81!)

Theme	***Intuitive and/or human-caused***	***Non-intuitive and/or natural***
Well Use and Construction	• **Shallower well depth** and depth cased • Well casings not extending far beyond static water level • Well casings not fully grouted	

High-level summary of statistical findings

- Some intuitive, others not
- Some indicate human sources, others not (spectrum of risk factors)

Relatively Undeveloped Land
(Animal Sources Dominant?)

Developed Land
(Human Sources Dominant?)

Avoid low areas, open water

Same, plus avoid proximity to wastewater

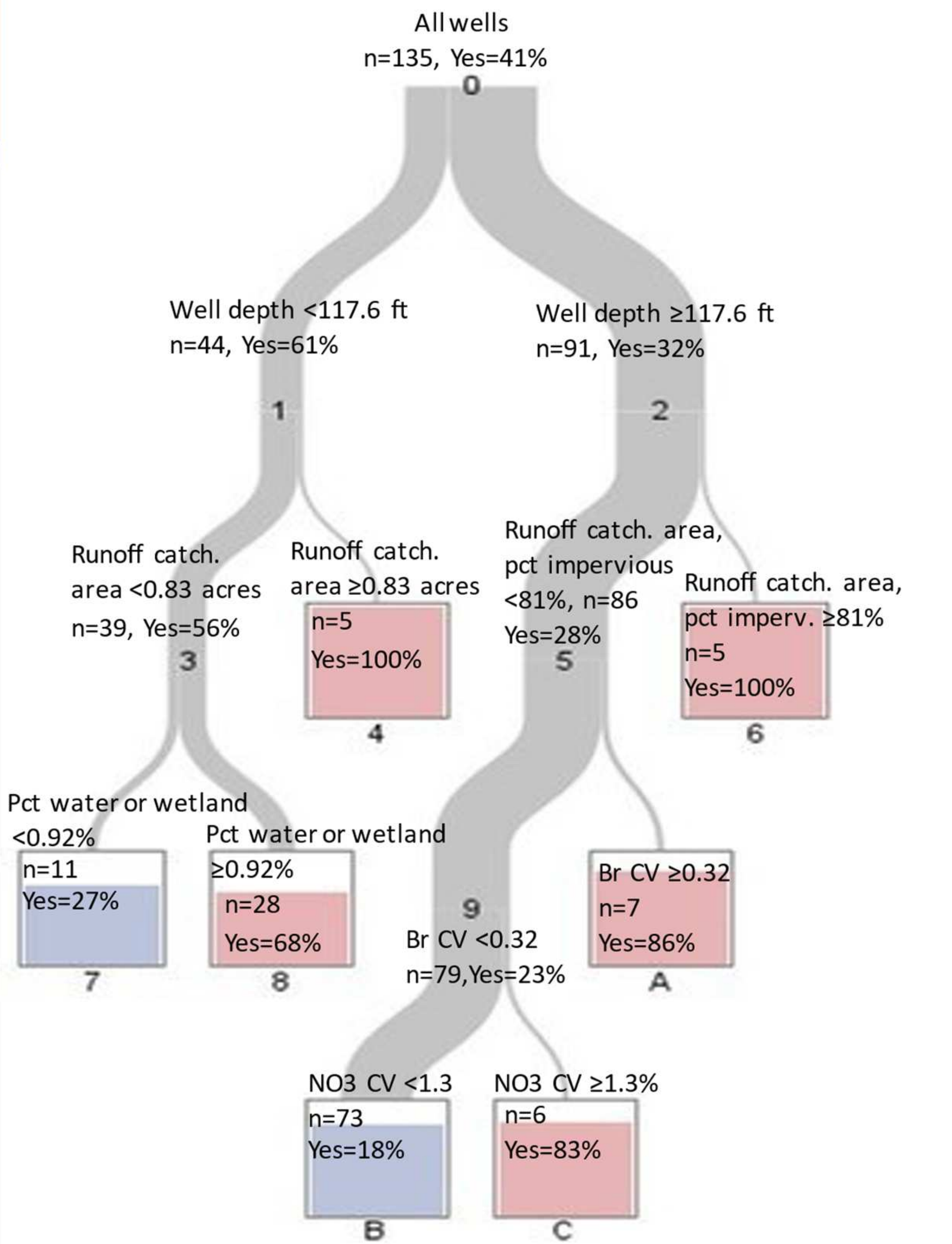
Important Variables from Multivariable Linear Regression Models

Full dataset
Sensitivity Dataset

Variable	Sub-Variable
Bromide CV	
Groundwater age from tritium	Modern
	Mixed
Aquifer porosity type	Secondary
	Primary unconsolidated
Absence of Development	
Runoff catchment area	
Nitrate CV	
Runoff catchment area % impervious	
Well depth	
Ammonia CV	

Important Variables from Classification Tree Models

Variable	Threshold Value
Well Depth	<118 ft.
Runoff Catchment Area	0.83 acres
% Open Water/Wetland (TT1)	1%
Well Depth	>118 ft.
Runoff Catchment Area % Impervious	81%
Bromide CV	32%
Nitrate CV	132% (sensitivity model = 47%)



Results Point to a Spectrum of Risk Factors for Cryptosporidium

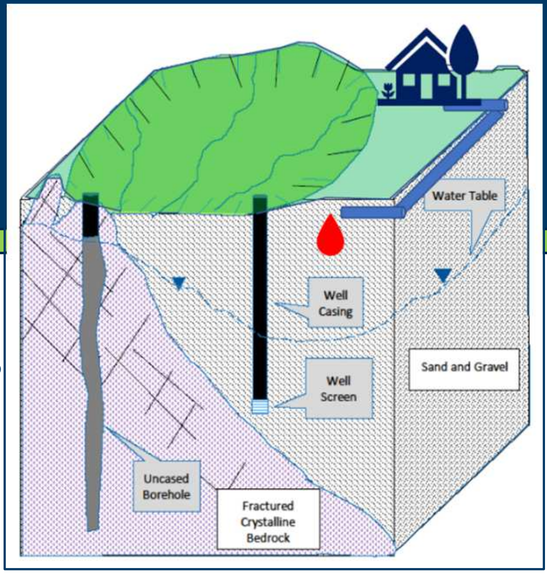
Relatively Undeveloped Land
(Animal Sources Dominant?)

Developed Land
(Human Sources Dominant?)

Avoid low areas, open water

Same, plus avoid proximity to wastewater

Recommendations from Statistical Findings



Well siting: avoid

- Low spots prone to surface water runoff/impermeable surfaces
- Presence of open water/wetlands in 1-yr TOT well capture area
- Locating w/in 70’ (preferably >150’) of septic/sewage sources (esp. 2 or more)

Well construction: avoid

- Shallow wells (< 118 ft) in geologically unprotected fractured bedrock or sand and gravel aquifers with young water and flashy chemical sampling results
- Well casings that aren’t fully grouted and that terminate close to the phreatic surface

Monitoring: promote

- Repeat sampling for parameters like chloride, bromide and nitrate to assess variability and microbial risk

Well Vulnerability Assessments:

- Bolster well vulnerability scoring routines by adding unaccounted for variables and weighting others in accord with these findings, especially for GUDI determinations

Recommendations from Statistical Findings

Well siting: avoid

- Low spots prone to surface water runoff/impermeable surfaces
- Presence of water in 1-yr TOT well capture area
- Proximity (<= 100 ft)/density (3 or more within 200 ft) to septic/sewage sources

Well construction: promote

- Deeper wells (> 118 ft) in geologically protected aquifers with older water, where feasible and not creating exposures to geogenic contaminants (e.g., arsenic)
- Fully-grouted well casings that extend as far below the water table as feasible

Monitoring: promote

- Repeat sampling for parameters like chloride+bromide and nitrate to assess variability and microbial risk

Well Vulnerability Assessments:

- Bolster well vulnerability scoring routines by adding unaccounted for variables and weighting others in accord with these findings, especially for GUDI determinations

Recharge Monitoring Study Basis – Statistical Analysis of 2014-2016 precipitation data

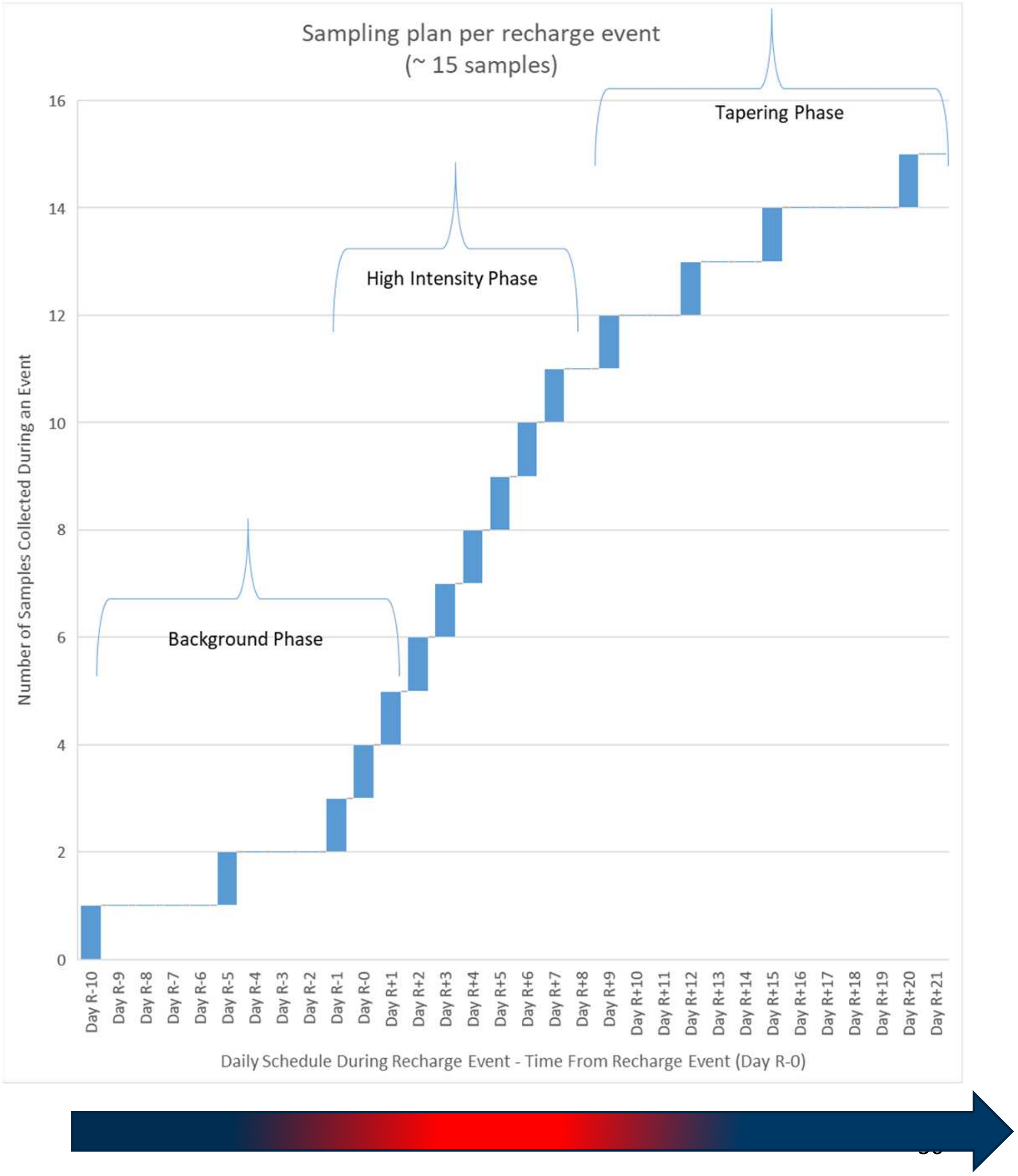
- Greatest total microbial load within 2- and 7-day lag periods from heavy rainfall
- Precipitation occurring in the 24 hours prior to sample collection was most associated with human enteric virus detections

Conclusion:

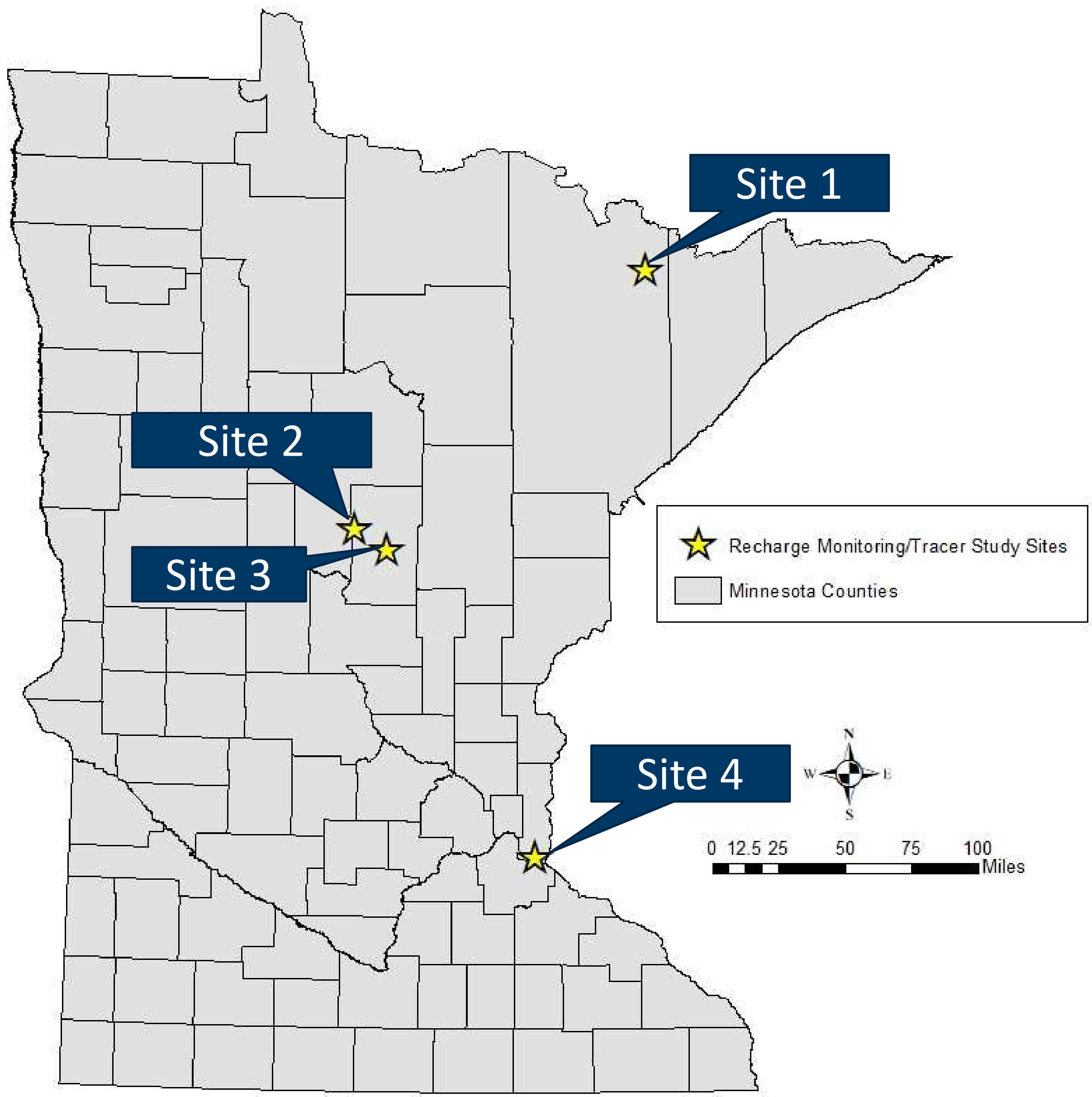
Contamination occurs quickly after precipitation events

Generalized Recharge Monitoring Sampling Plan

- Sampling triggered 10-days from forecast rainfall of 0.5” or greater
- Pre-and post-event samples taper around “burst” of high intensity sampling coinciding with start of precipitation



Map of Recharge Monitoring/Tracer Study Sites



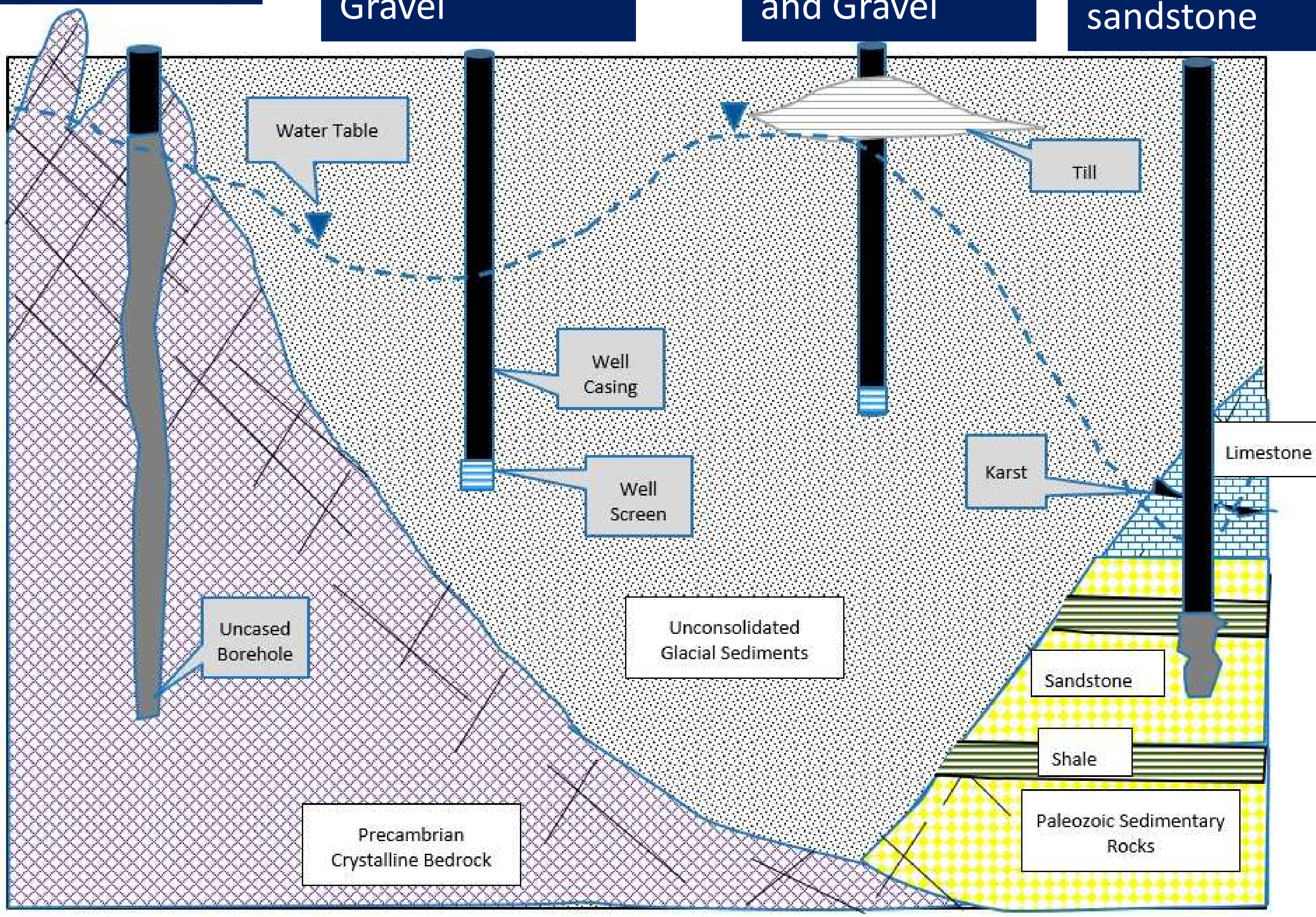
Geologic Settings for Recharge Monitoring/Tracer Study Sites

Site 1
Fractured Crystalline Bedrock

Site 2
Unprotected Glacial Sand and Gravel

Site 3
Protected Glacial Sand and Gravel

Site 4
Deeply Buried Paleozoic sandstone



Recharge-Event Monitoring: Use of Autosamplers

Groundwater

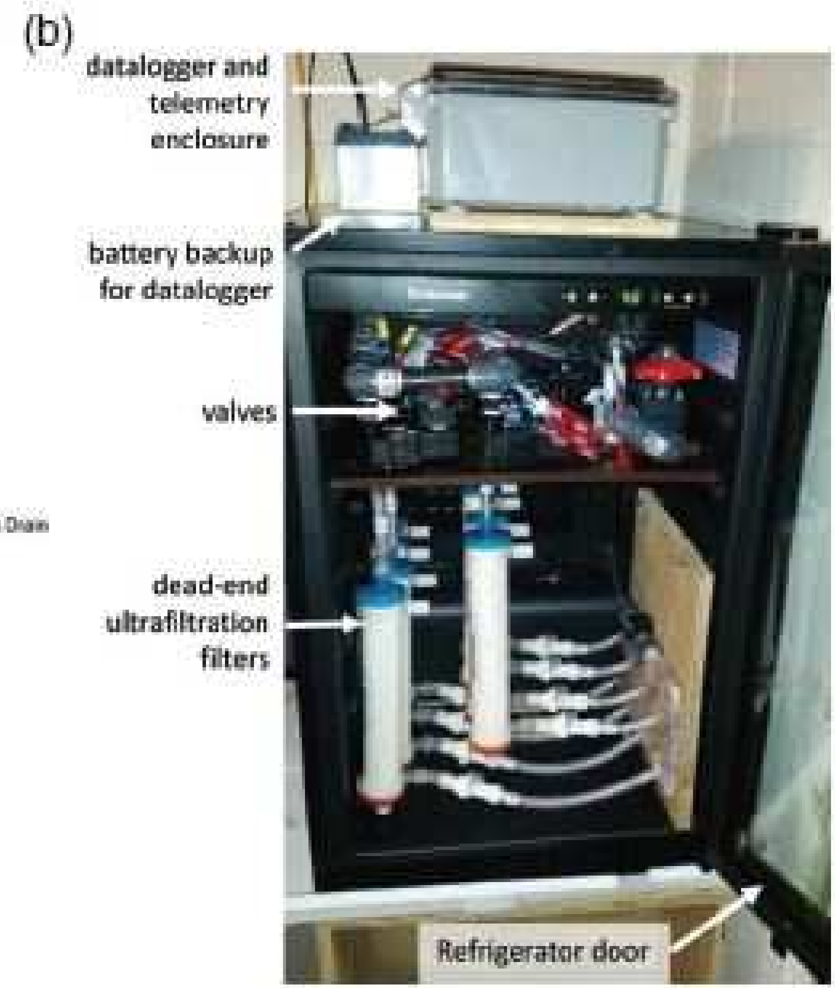
Methods Note/

Automated Time Series Measurement of Microbial Concentrations in Groundwater-Derived Water Supplies

by David W. Owens[1], Randall J. Hunt[1,2], Aaron D. Firnstahl[3], Maureen A. Muldoon[4], and Mark A

Abstract

Fecal contamination by human and animal pathogens, including viruses, bacteria, and protoz
human health hazard, especially with regards to drinking water. Pathogen occurrence in gro
considerably in space and time, which can be difficult to characterize as sampling typically requ
liters of water to be passed through a filter. Here we describe the design and deployment of an au
suited for hydrogeologically and chemically dynamic groundwater systems. Our design focused on
to facilitate transport and quick deployment to municipal and domestic water supplies. We dep



. (b) Photograph looking into automated sampler

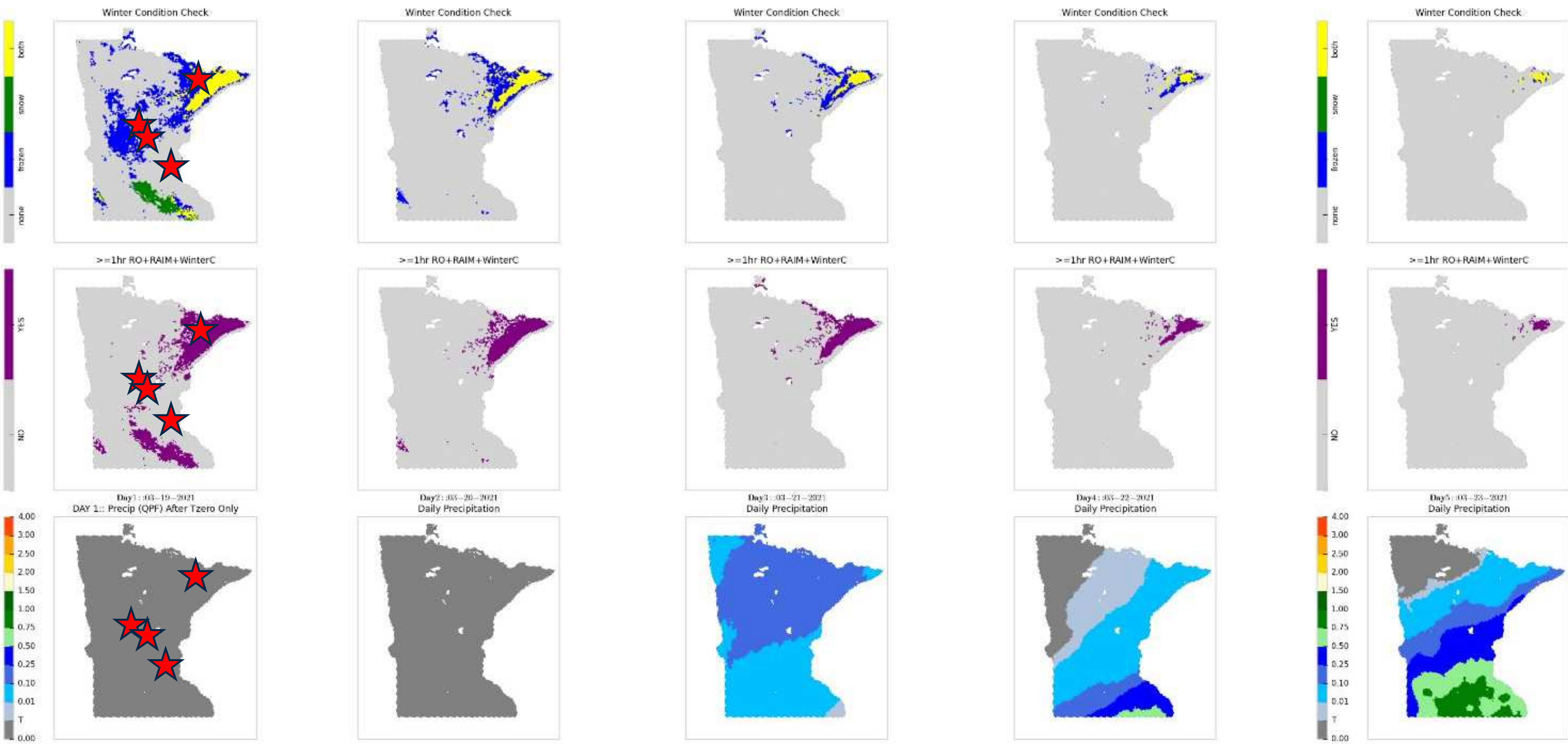
From Owens et al., 2019

Recharge-Event Monitoring

<u>Autosampler Enhancements</u>

- Time-integrated 1L bottle for chemistry and isotopes
- Multiparameter sonde for continuous field parameters

Recharge Monitoring - Use of 10-day Forecasting Tools



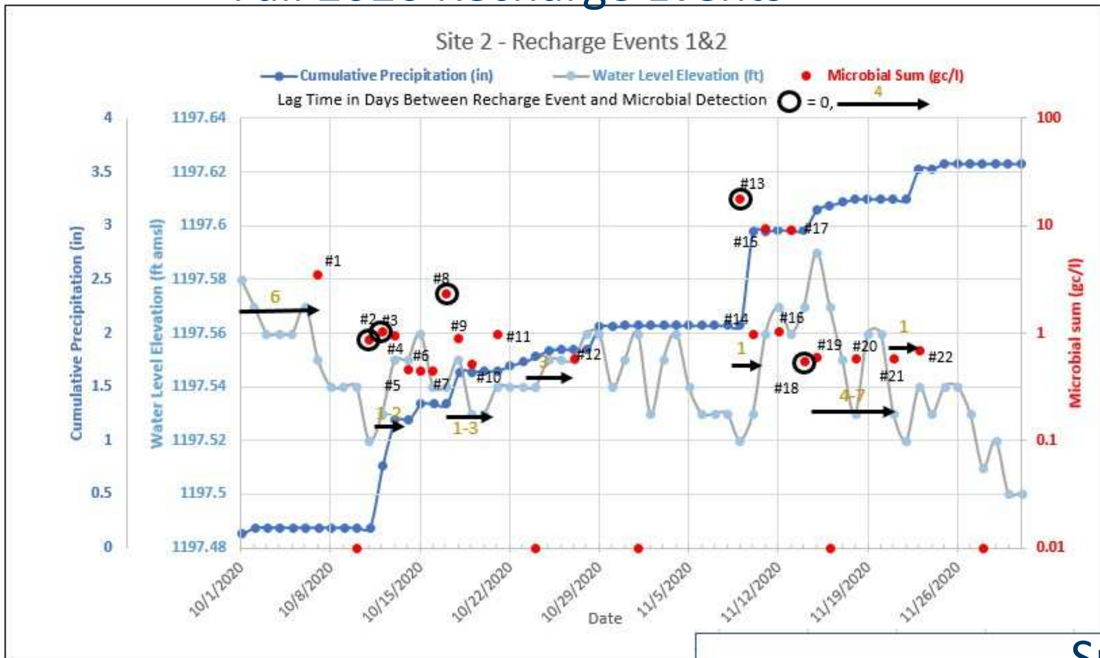
Source = NOAA

Recharge-Event Monitoring – Other Components

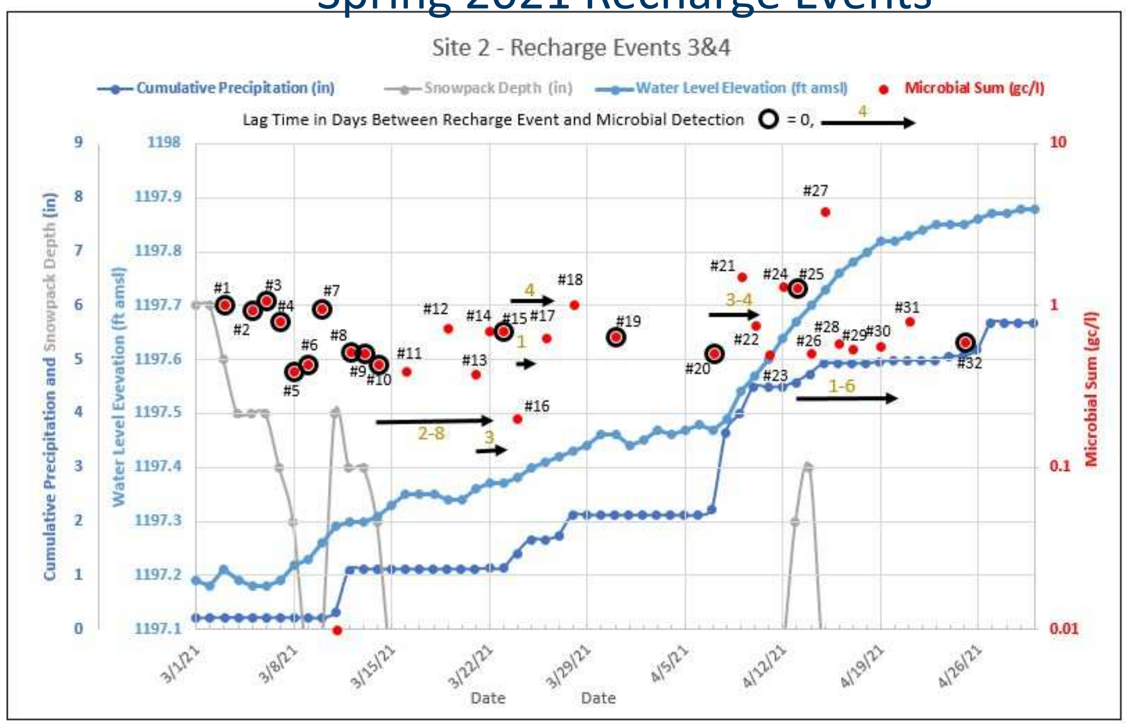
- Paired observation wells and weather stations
- Paired wastewater sampling sites
- Detailed age-dating (tritium-helium and SF6 methods)
- Annular space testing
- Borehole logging at fractured rock site

Lag times at Site 2 in fall and spring

Fall 2020 Recharge Events



Spring 2021 Recharge Events

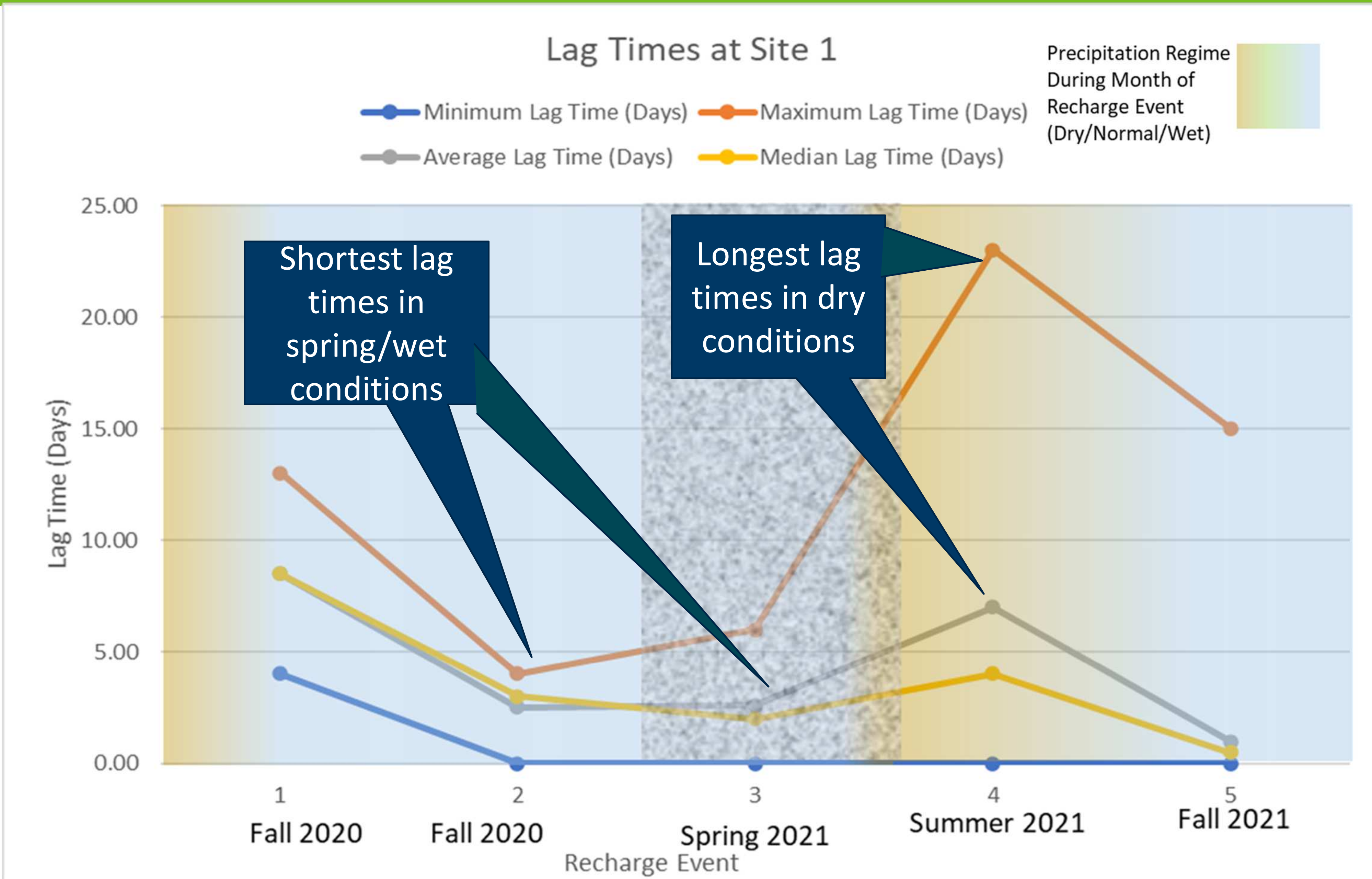


Lag time = time in days between rainfall/snowmelt and microbial detection

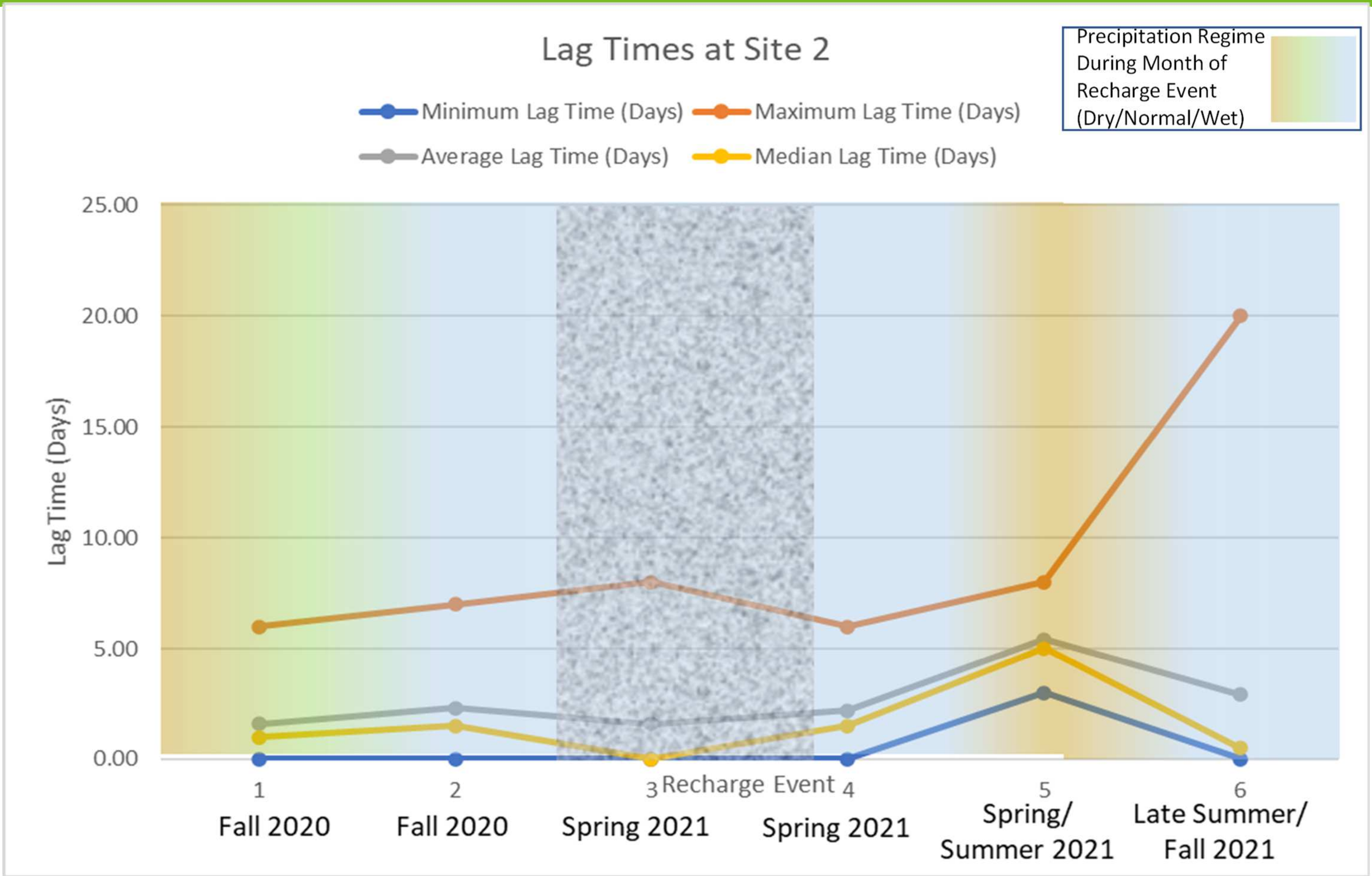
Note extreme variability – especially in the fall

Single or occasional samples could easily miss detections

Lag Time Data

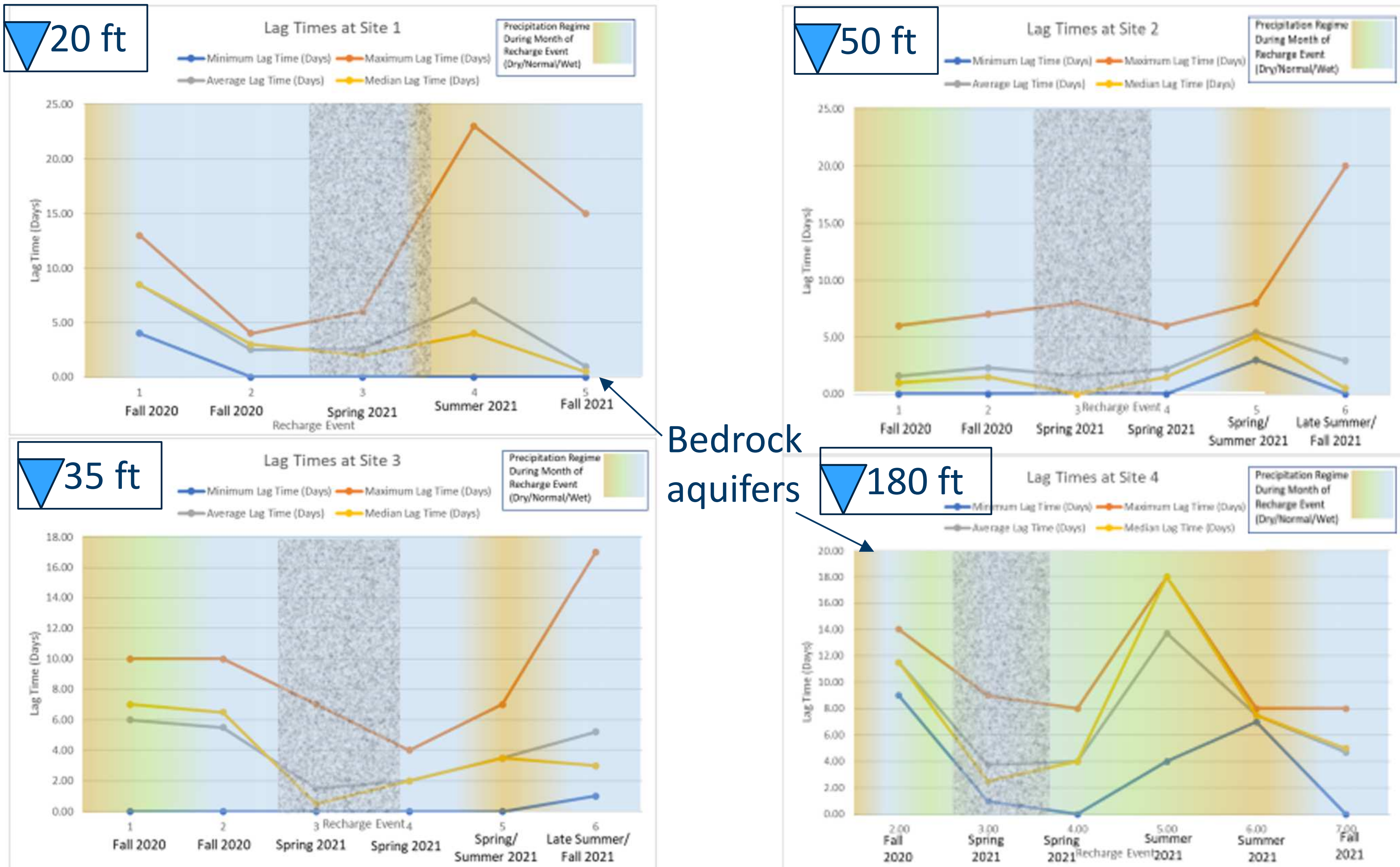


Lag Time Data

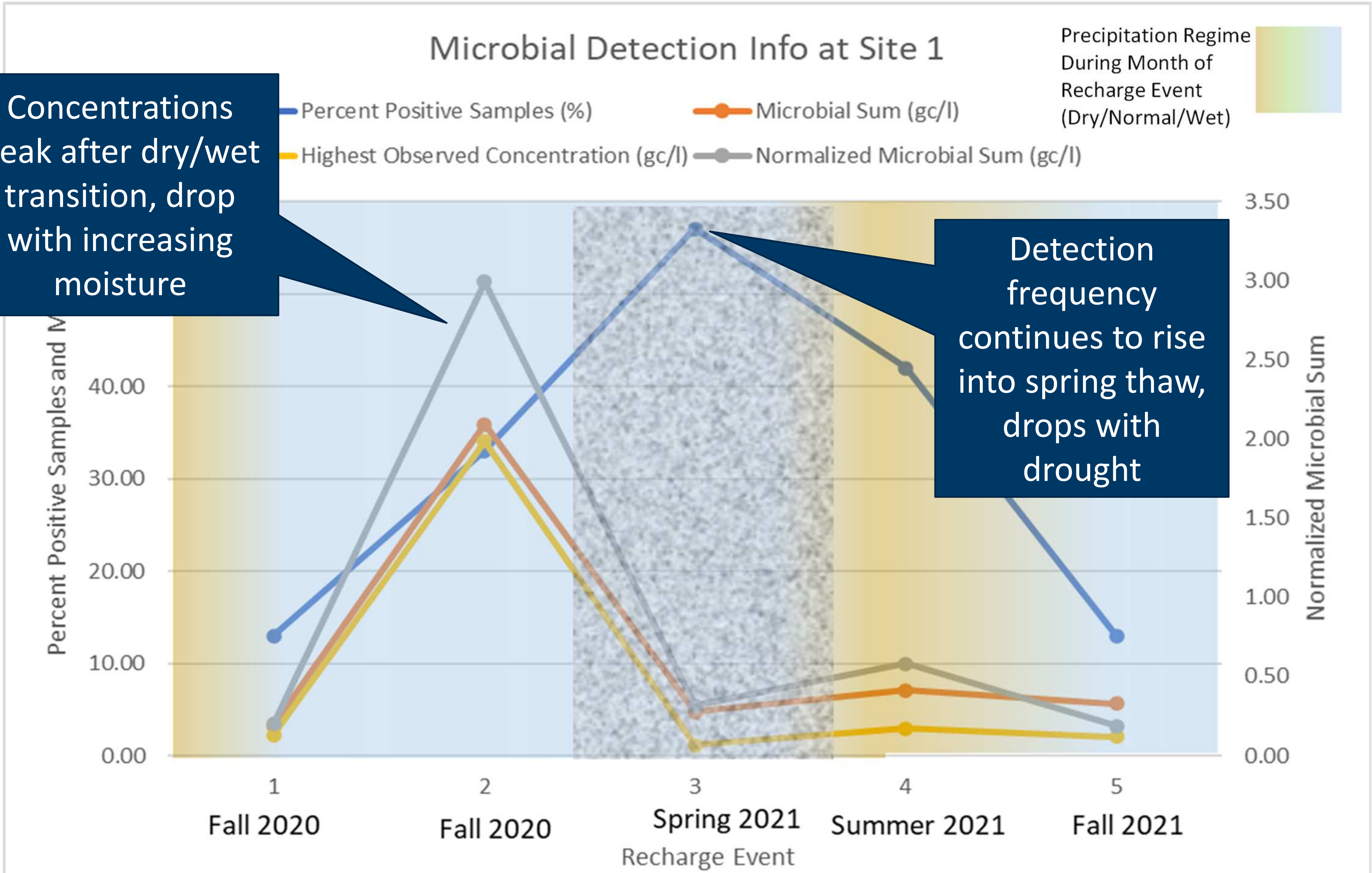


Lag times:

- were shortest in spring, longest during/after drought
- depended on aquifer type and depth to water



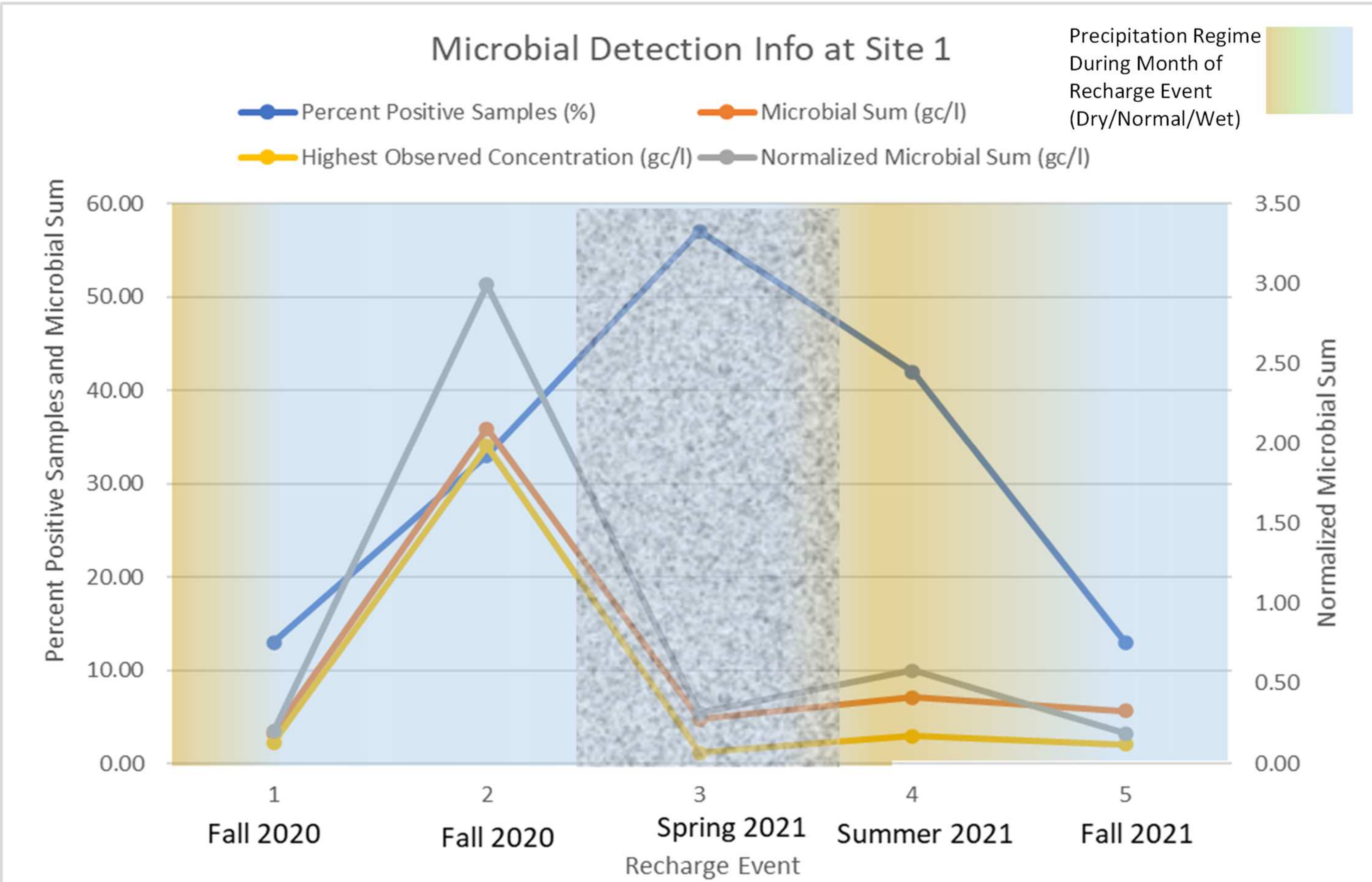
Microbial Detection Frequency and Concentration Data



Concentrations peak after dry/wet transition, drop with increasing moisture

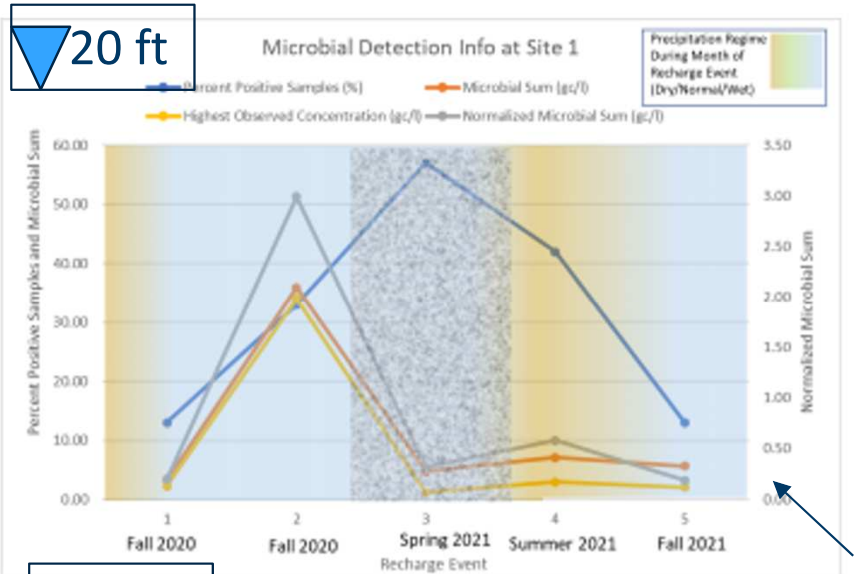
Detection frequency continues to rise into spring thaw, drops with drought

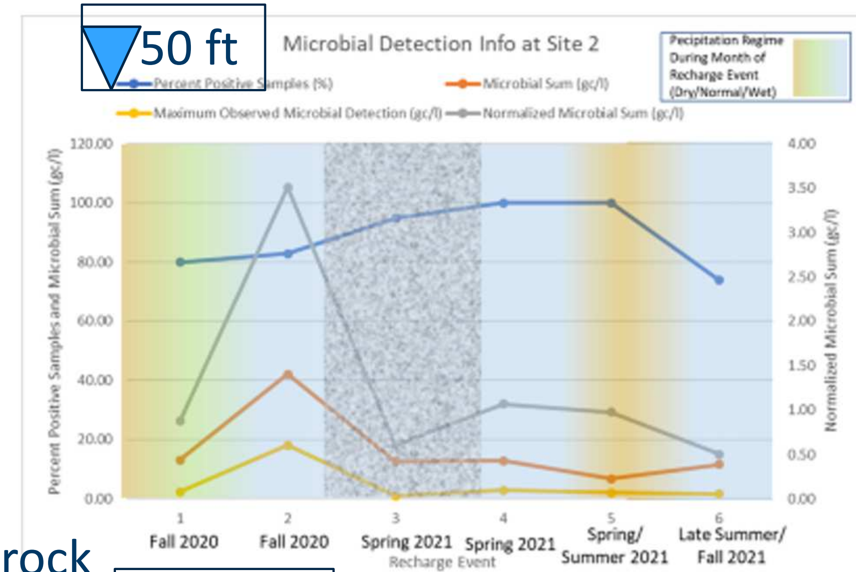
Microbial Detection Frequency and Concentration Data



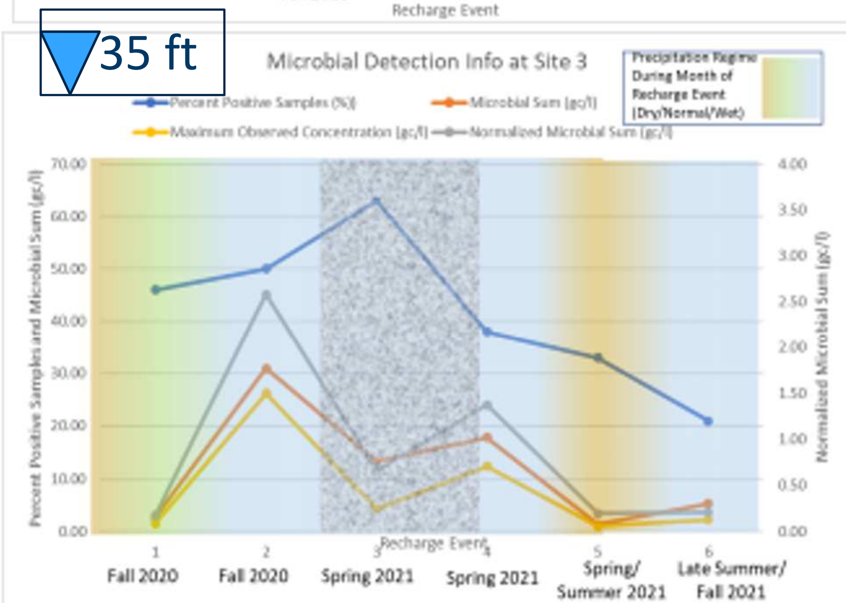
Microbial Detections:

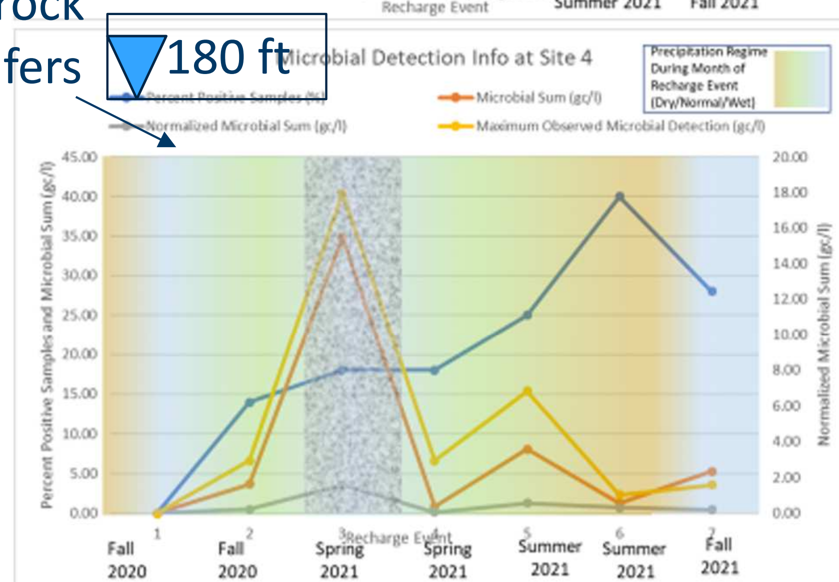
- Highest frequency in spring except for Site 4 (thickest vadose zone)
- Highest concentration in second fall 2020 event (except for Site 4)



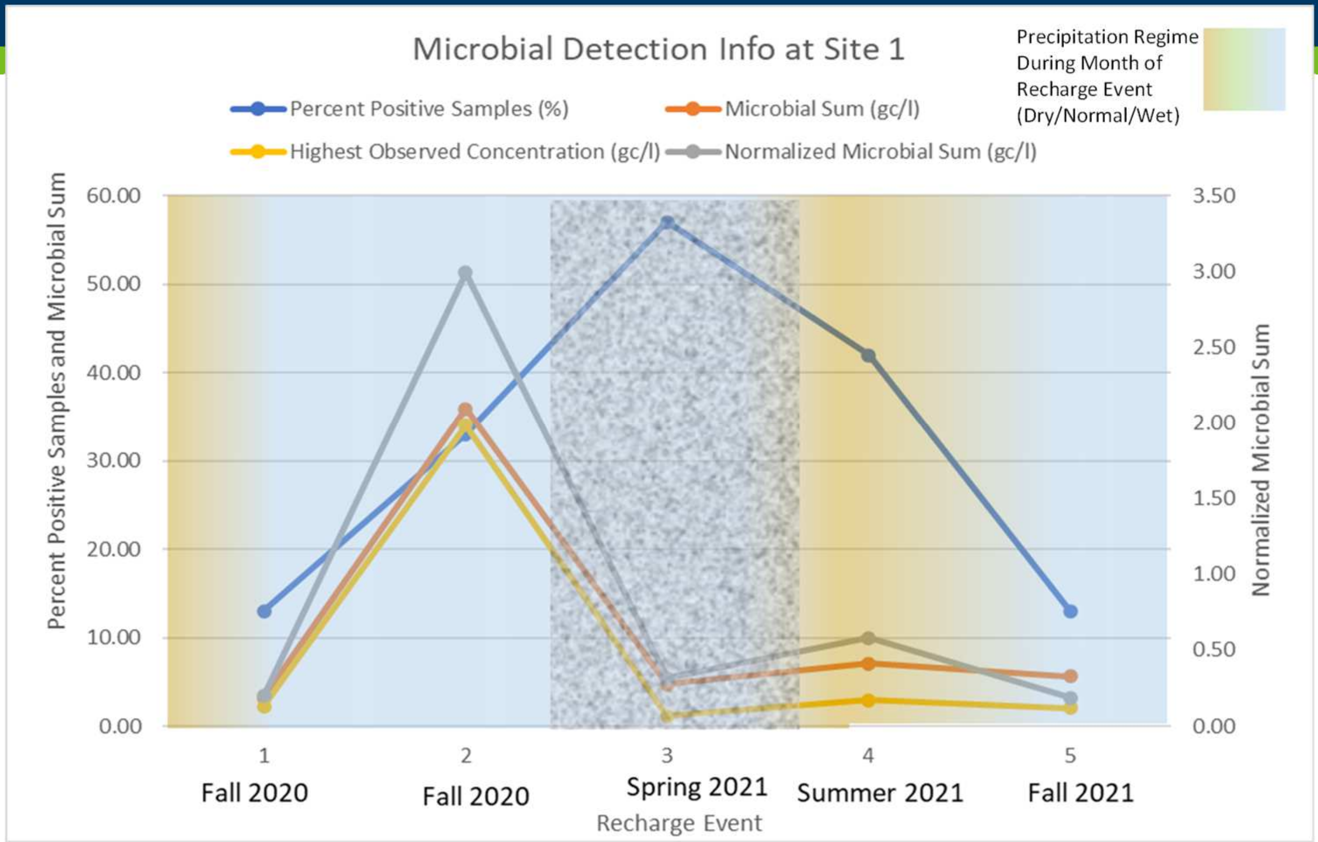


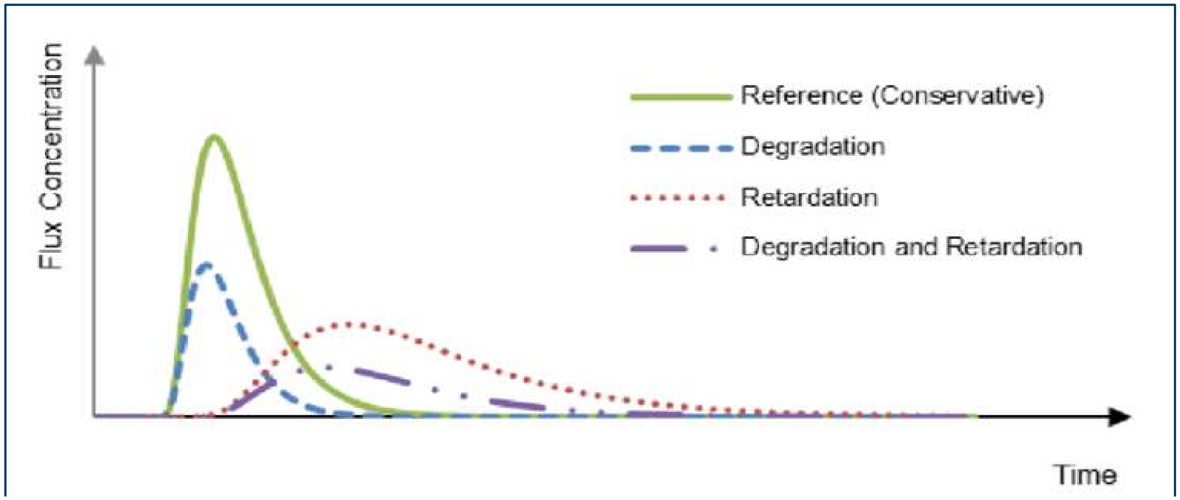
Bedrock aquifers





Microbial Detections:
- Similarities with tracer breakthrough over the 1-year timescale

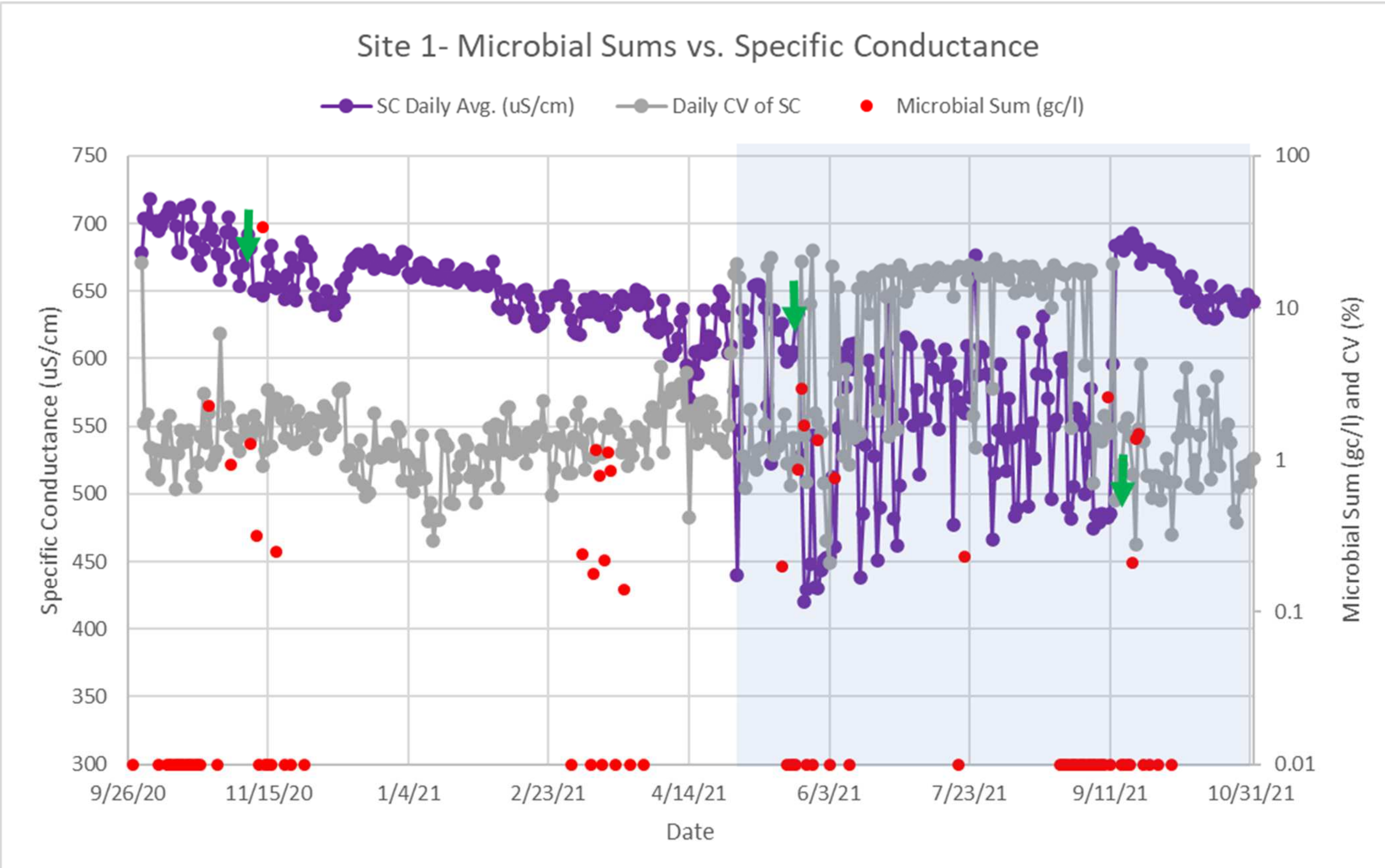




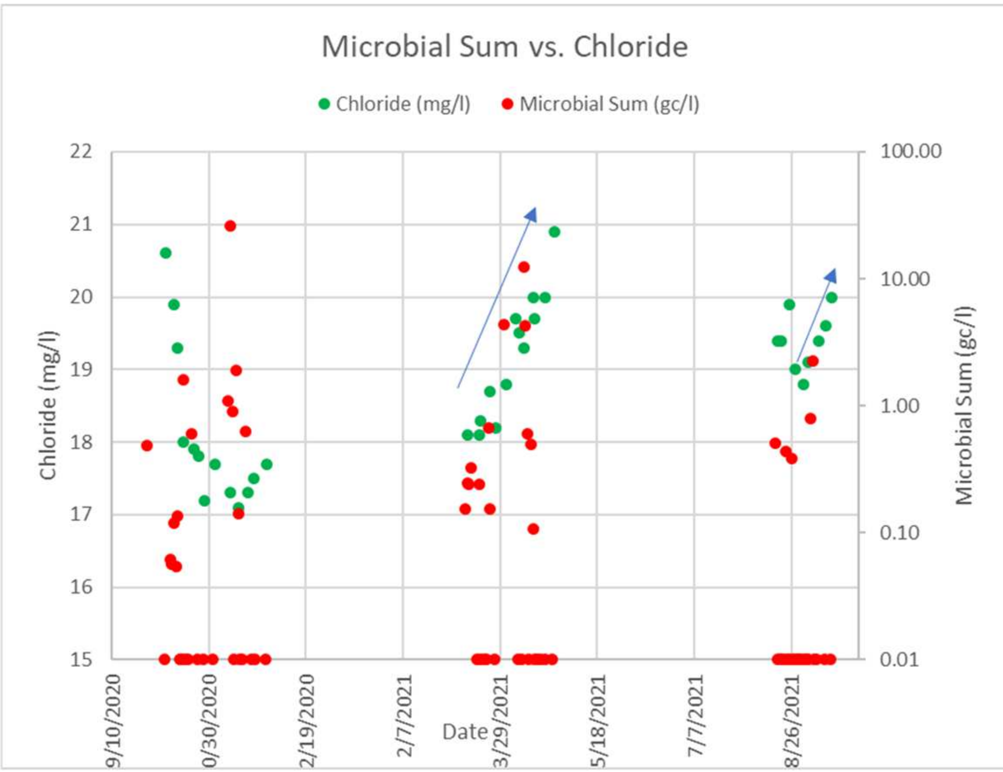
Age Dating vs. Microbial Lag Times

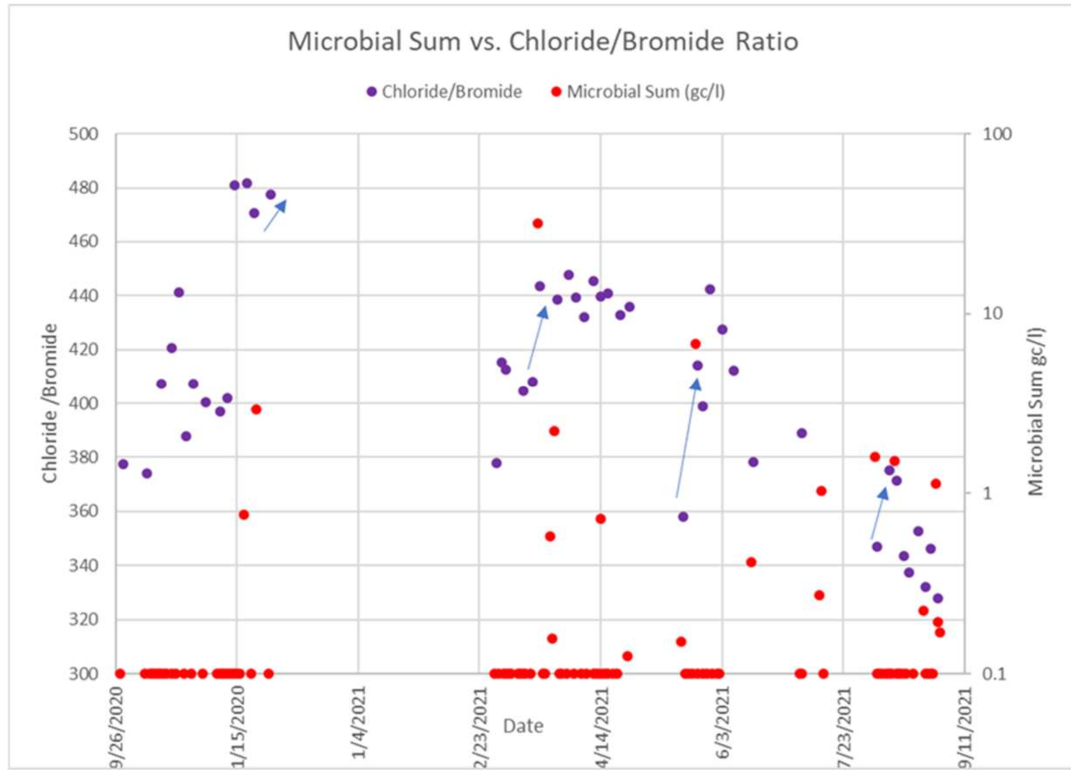
Site	Bulk GW Age (years)	Max. Lag Time (days)	% Young Recharge in the Mix
1	Mix of young and ancient	23	<=20%
2	30	20	<=10%
3	15	17	<=1%
4	31	18	<=2%

Correlation with specific conductance decreases



Correlation with chloride and Cl/Br ratio



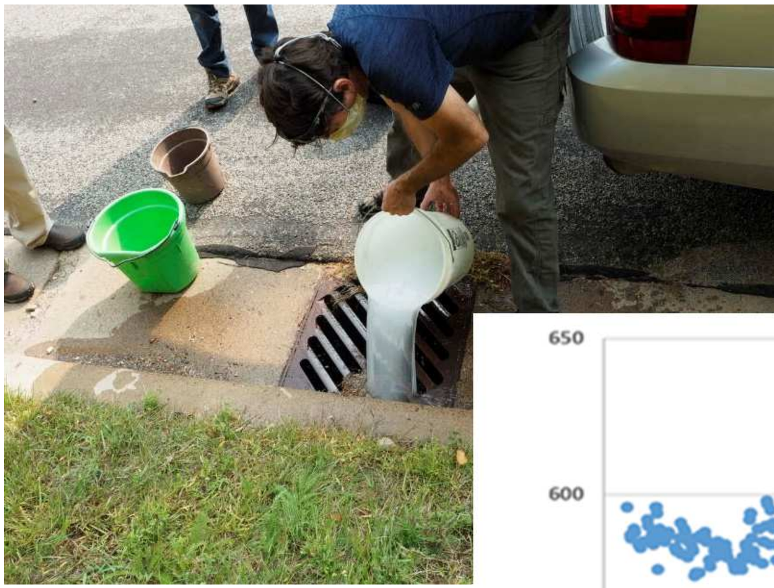


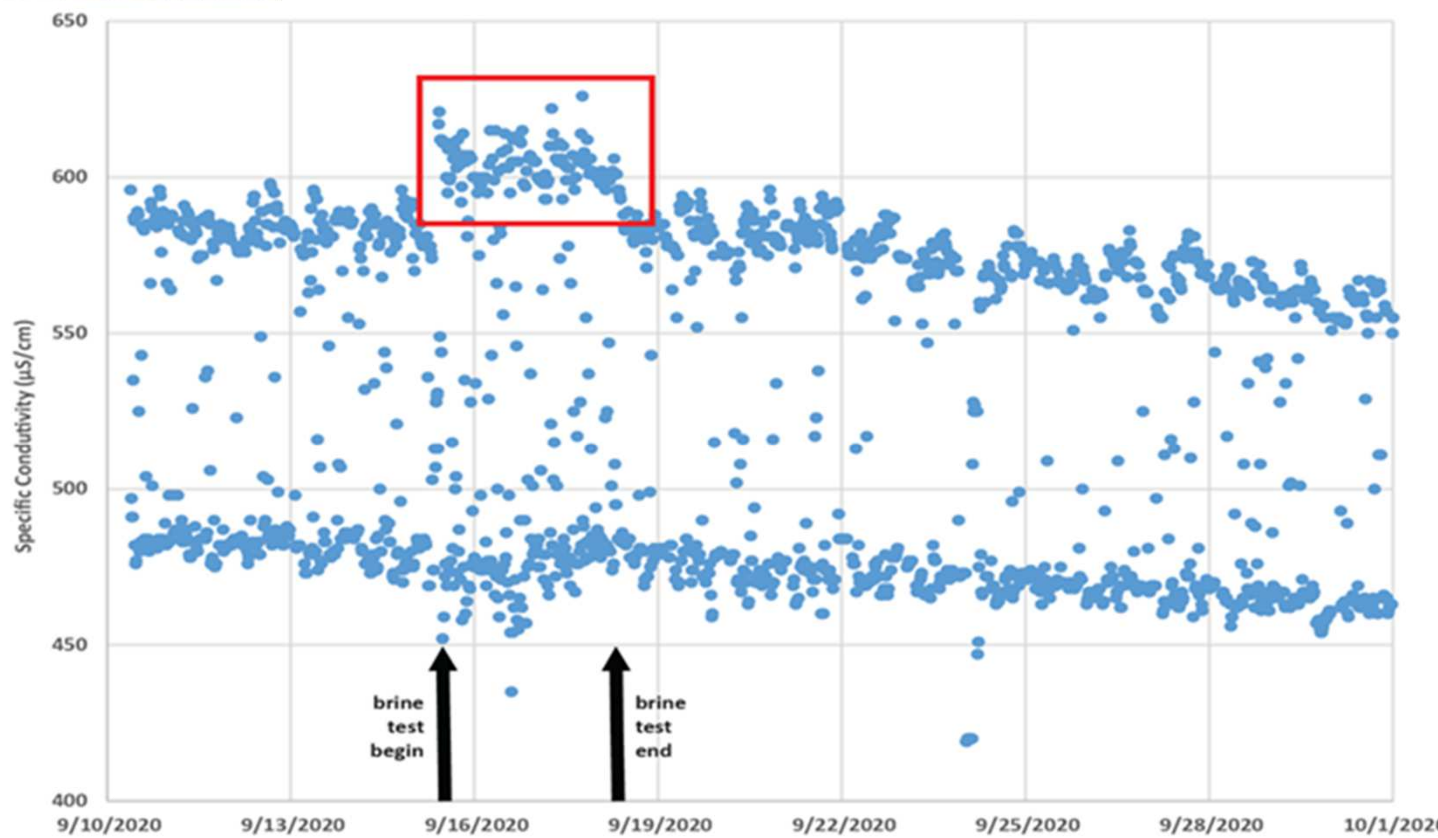
Tracer Studies

Summary of findings at each site and suspected sources

Site	Most Frequently Detected Organism in Well Water	Suspected Microbial Source(s)	Distance to Source(s) (ft) Within ERA	Basis for Suspected Source Identification
1	Human Bacteroides	sewage lift station and/or associated piping	55, 70 and 180	Coincidence with wastewater samples
2	Giardia	septic systems	88, 140	Coincidence with wastewater samples
3	Cryptosporidium	stormwater piping	26	Tracer test
4	Human Bacteroides	septic system	56	Coincidence with wastewater samples

Tracer Studies – storm sewer connection





Recharge Study - Key Findings

- Greatest chance of microbial detections, and shortest lag times between rainfall/snowmelt and detections, in wet periods (spring thaw)

- Lowest detection frequency/longest lag times during and immediately after dry conditions

- Greatest concentrations follow dry-wet transitions (lag set by vadose zone thickness)
- Recharge may be occurring despite other indicators of frozen ground
- Chemical and isotopic indicators may reflect recharge and help assess risk (but not as sensitive as qPCR so not direct surrogate)
- Porous-media vadose zones/aquifers may still behave like “pipes” at localized scales

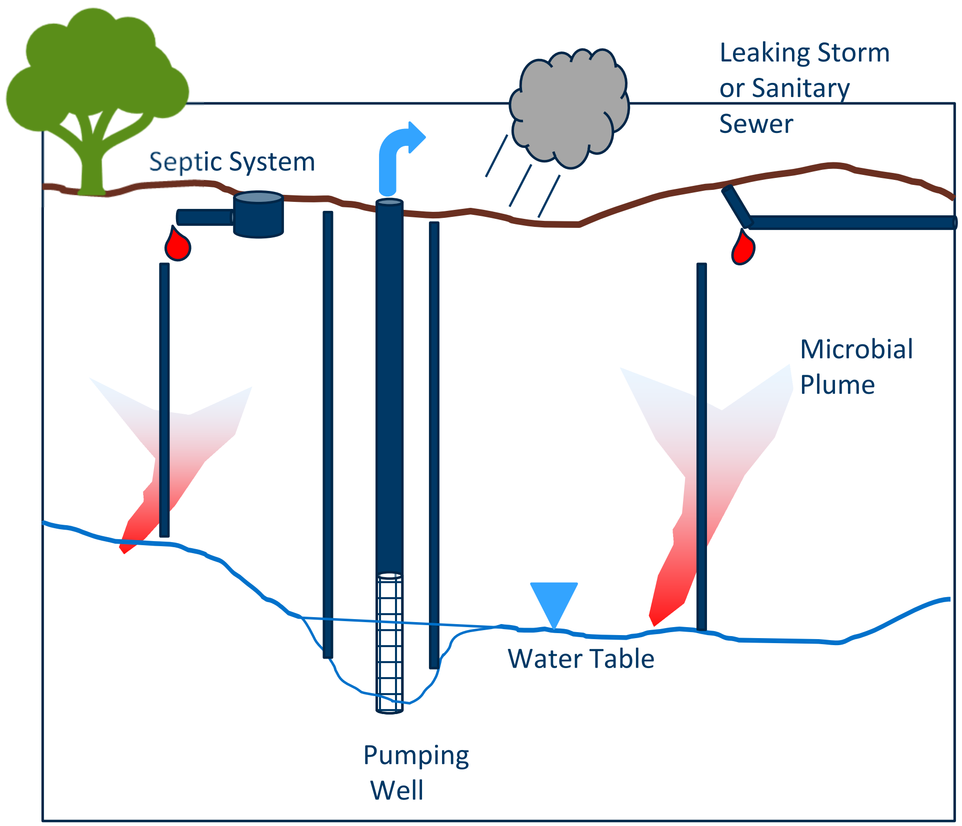
Conceptual Model for Rapid Microbial Transport

- Year-round discharge from septic systems and wastewater/stormwater leakage below the frost zone

- Microbes accumulate in the shallow subsurface during dry periods, but are pushed down during wet ones

- Rapid movement made possible by small, high-permeability features in the subsurface (gravel zones, fractures, macropores)

- Downward movement is accentuated by well pumping – small volumes of fast, pipe-like flow



Recharge Study - Key Findings

- Microbial “pulses” reflect volumetrically small contributions to aquifers, but at time scales much shorter than bulk aquifer water age. Implications for well vulnerability assessments, use of enriched tritium vs. ultra low-level tritium.
- High variability means single or infrequent sampling are unlikely to adequately characterize risk.
- This reinforces the importance of disinfection as a barrier, where disinfection byproducts are not a likely problem.

Preliminary Well Characteristic Variables from Univariate Statistical Methods

- Depth cased
- Casing diameter and Discharge rate
- Drilling method
- Grouted (Y/N) and percent of grout that's saturated
- Year drilled (age of well)
- Depth to bedrock
- Aquifer type (karst/fractured or not)
- Geologic sensitivity
- Vertical hydraulic gradient
- Groundwater age from tritium

Recommendations from Recharge Monitoring Study

Water Quality Monitoring and Well Vulnerability

- Monitor wells for microbial risk (GUDI, etc.) in the spring or during other wet periods for increased chance of detection and sample repeatedly if possible.
- To catch maximum concentrations, sample after transition from dry to wet.
- Incorporate information on antecedent and prevailing moisture conditions when evaluating past monitoring data or planning future studies.
- Incorporate repeat sampling/continuous monitoring for parameters such as chloride, bromide and specific conductance as analogs for risk.
- Transition to use of ultra low-level tritium for well vulnerability determinations and factor other parameters such as chloride/bromide (weight of evidence approach).
- Evaluate comparability of qPCR microbial results with other high-sensitivity chemical methods (PFBA?) for analogs.
- Incorporate tracer and borehole logging studies where appropriate.

Recommendations from Recharge Monitoring Study

Water System Operation and Risk Management

- Use hydrogeologic information when siting wells and contaminant sources (keep wells upgradient and outside 1-yr TOT capture zone of sources, see other from stats analysis).
- Consider use of storage and/or increasing disinfection residuals during peak risk periods (spring thaw, dry-wet transitions).
- Promote disinfection where feasible, given extreme variability of microbial occurrence. Note that UV or filtration may be needed for Crypto removal.