



# Water Environment Federation<sup>®</sup>

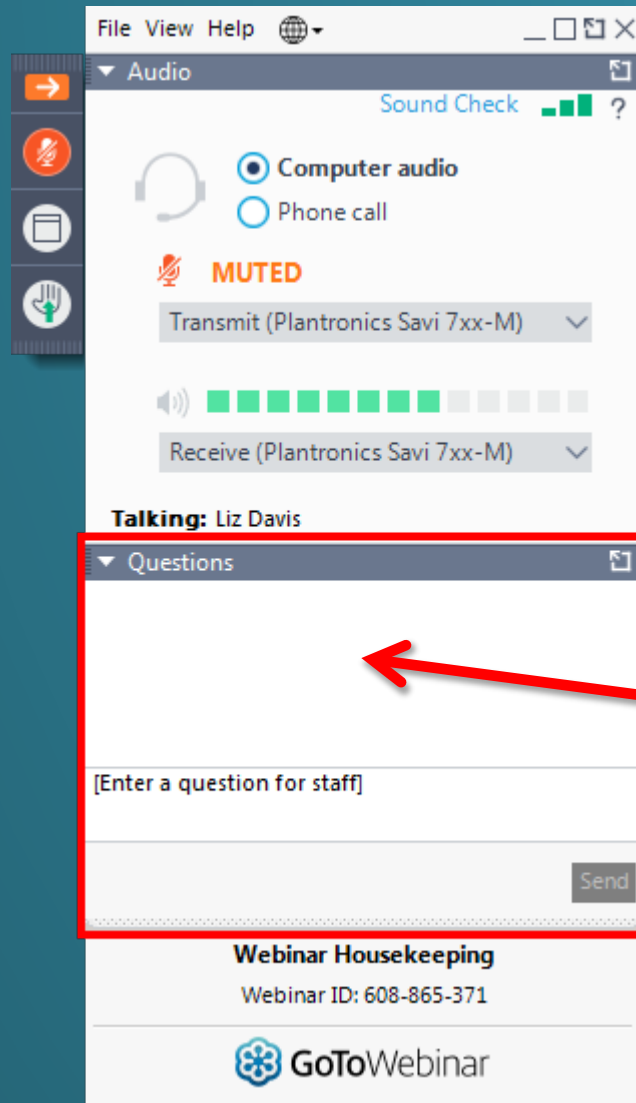
the water quality people<sup>®</sup>

# Going Viral: Working Towards Virus Risk Management In Wastewater Treatment Systems

Thursday May 7, 2019

1:00 – 2:30 PM ET

# How to Participate Today



- **Audio Modes**

- Listen using Mic & Speakers
- Or, select “Use Telephone” and dial the conference (please remember long distance phone charges apply).

- **Submit your questions using the Questions pane.**

- **A recording will be available for replay shortly after this webcast.**



# Water Environment Federation Coronavirus Resources

- [Coronavirus Resource Page](#)
- [The Water Professional's Guide to the 2019 Novel Coronavirus](#)
- [Webcast: Updates on Novel Coronavirus For Water Professionals \(Feb 25\)](#)
- [Words On Water #128: Coronavirus and Water Treatment \(March 2\)](#)
- [Webcast: Pandemic Continuity of Operations \(COOP\) Essential Personnel \(March 19\)](#)
- [Webcast: Pandemic Continuity of Operations \(COOP\) Essential Personnel \(March 19\)](#)
- [Webcast: Clean Water Act Regulatory Issues in a Pandemic \(March 20\)](#)
- [Blog: What We Know About Coronavirus and Water Treatment \(March 26\)](#)
- [Words On Water #134: Des Moines Water Works Shelters-In-Place to Respond to Coronavirus \(March 27\)](#)
- [WEF Webcast Discusses Regulatory Concerns During Coronavirus Crisis \(March 27\)](#)
- [Experts Share Advice on Continuity of Operations During Coronavirus Pandemic \(March 27\)](#)
- [Pulse Check – WEF Poll Finds Utilities Confident in Operations, Changing Work Arrangements \(March 31\)](#)
- [Accommodating Essential Water Services \(March 31\)](#)
- [Webcast: Shelter-in-Place in Response to Coronavirus: Approaches from Two Facilities \(April 9\)](#)
- [Webcast: Key Considerations in Responding to Coronavirus \(April 13\)](#)
- [Pulse Check - Coronavirus and Supply Chain Disruption \(April 16\)](#)
- [Webcast: Wastewater Epidemiology Webcast \(Public Service Announcement\) \(April 24\)](#)

# Water Environment Federation

## Forthcoming Coronavirus Resources

- **Coronavirus and Water Systems (update & expansion on the Water Professional's Guide to COVID-19)**
- **Residuals and Biosolids Issues Concerning COVID-19 Virus**
- **Words On Water: Evaluating Coronavirus Risks in Residuals (working title)**

**Additionally: WEF has convened a Blue-Ribbon Panel to Evaluate Biological Hazards and Precautions for Wastewater Workers**

# Today's Moderator



**Naoko Munakata**

Supervising Environmental Engineer



**LOS ANGELES COUNTY  
SANITATION DISTRICTS**

*Converting Waste Into Resources*

# Key Questions

- How do we know our disinfection processes are working?
- How much disinfection is “enough”?



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# Indicator Organisms

- Proxy for pathogens
- Historically bacteria
  - Non-pathogenic, safe to handle
  - Relatively easy, fast, and cheap to measure
- Viruses?





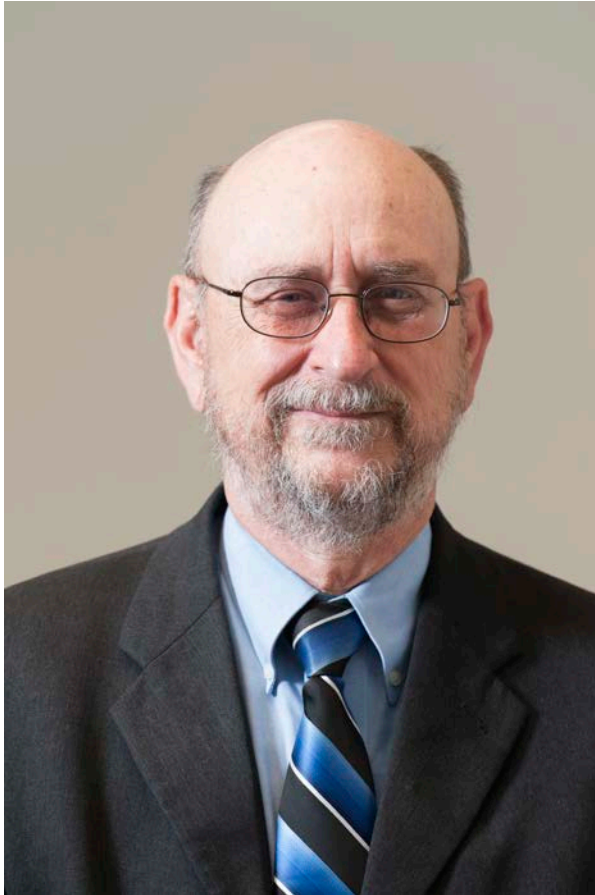
# Questions about Viral Indicators

- Would plants that are in compliance with bacterial objectives suddenly be out of compliance with viral objectives?
- Would they improve protection of public health and the environment?
- What is a “good” indicator? Many pathogens, all behave differently.

# Today's Speakers

- Charles Gerba, *University of Arizona*
  - Waterborne Viruses: Biology, Indicators and Distribution in the Aquatic Environment
- Kyle Bibby, *University of Notre Dame*
  - High-Consequence Viral Pathogen Disinfection
- Thomas Worley-Morse, *Hazen and Sawyer*
  - Potential Impacts of US EPA Coliphage Criteria on Wastewater Treatment and Reuse

# Our Next Speaker



## Charles Gerba, PhD

Professor, Epidemiology and Biostatistics  
Department of Environmental Science



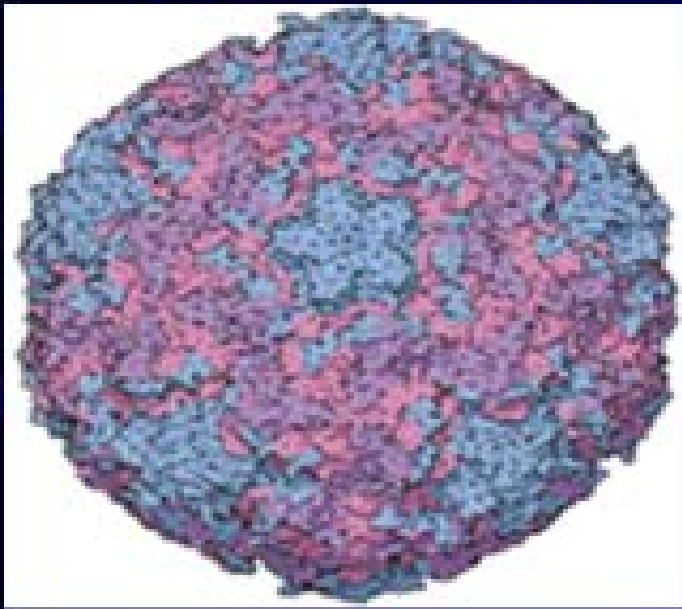
# Waterborne Viruses

Biology, indicators and distribution in  
the aquatic environment



# Types of Water borne/ based Pathogens

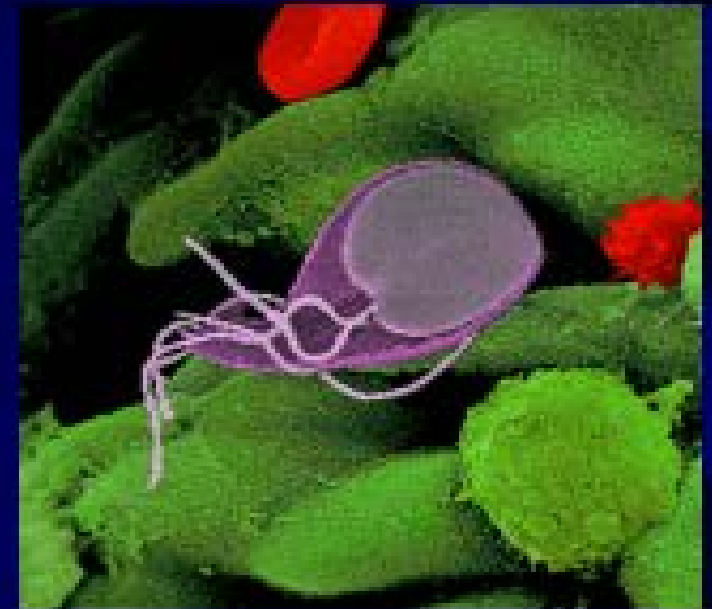
Viruses



Bacteria

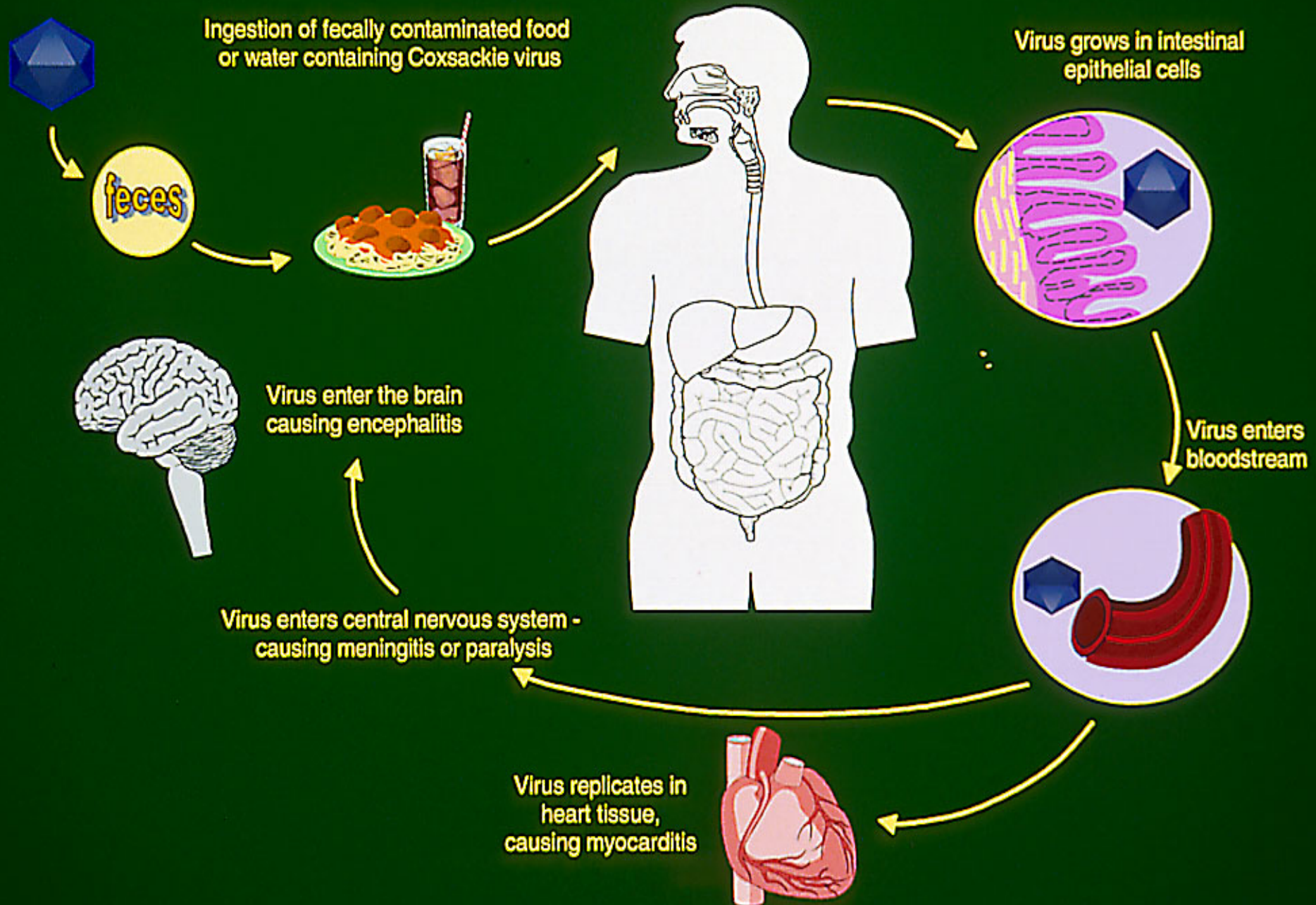


Parasites



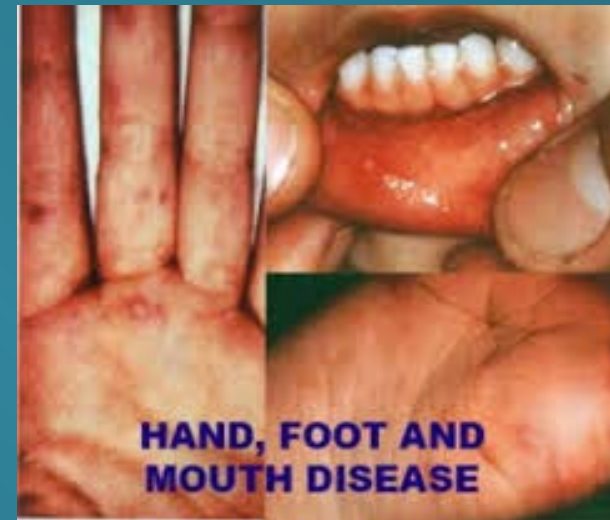
Human Enteric Viruses of Concern	Number of Types
Enteroviruses	
Poliovirus Coxsackievirus Echovirus Enteroviruses	124
Hepatitis A virus	1
Reoviruses	8
Rotaviruses	3
Adenoviruses	61
Astroviruses	7
Circoviruses	1
Hepatitis E virus	1
Calciviruses	2
Picobirnaviruses	2

# Phases in a Coxsackievirus Infection



# Illnesses Associated with Enteroviruses

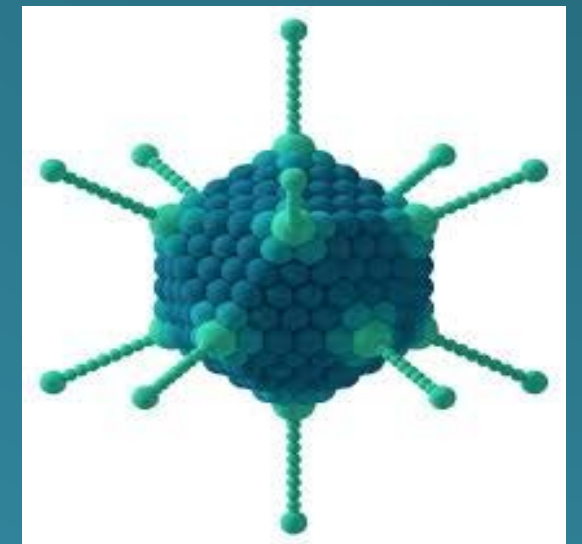
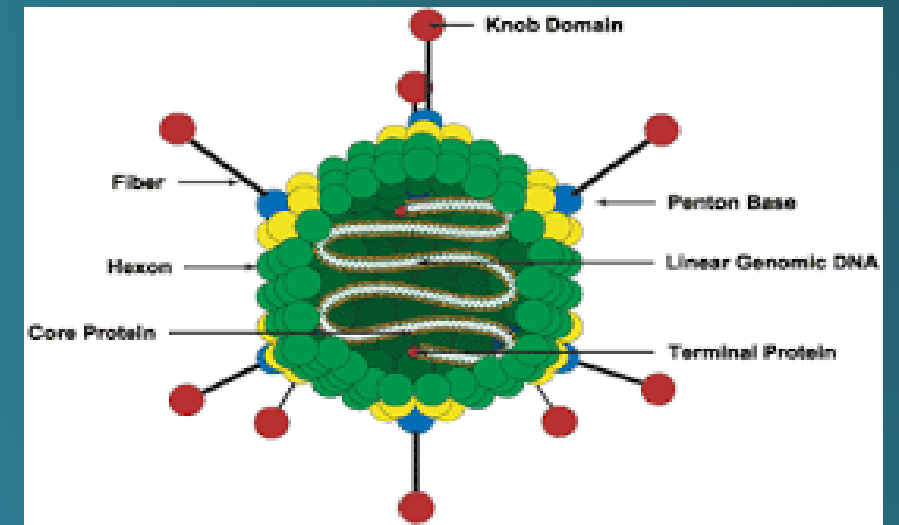
- Respiratory
- Fever and rash
- Meningitis
- Hand, foot and mouth disease
- Myocarditis
- Paralysis
- Mental disorders





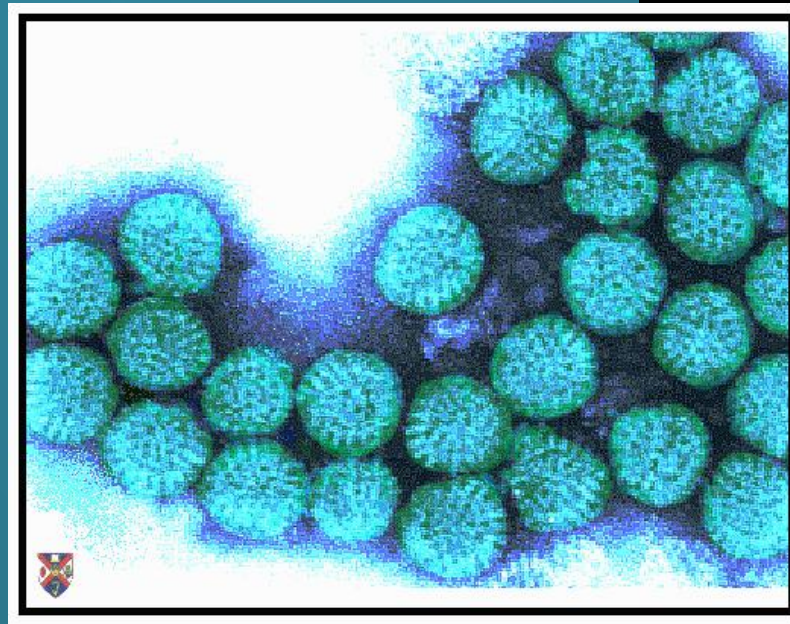
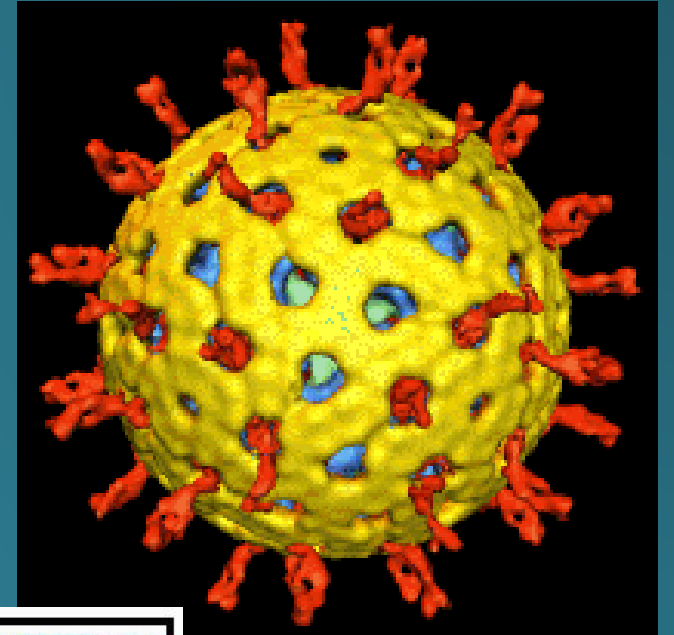
# Adenovirus

- ds DNA
- Second most common cause of childhood gastroenteritis
- Also a cause of eye, throat, and respiratory infections
- Outbreaks associated with swimming and drinking water
- Most common enteric virus in sewage and high levels year around
- Longest surviving enteric virus in water?
- Enteric virus most resistant to UV light disinfection



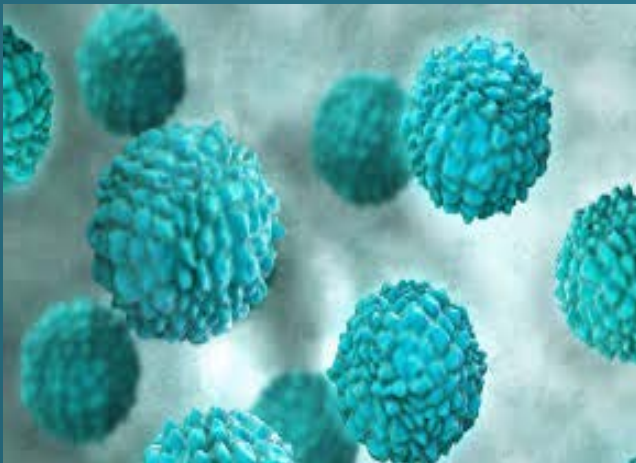
# Rotavirus

- dsRNA
- Causes diarrhea in both children and adults
- Causes both water and food borne outbreaks



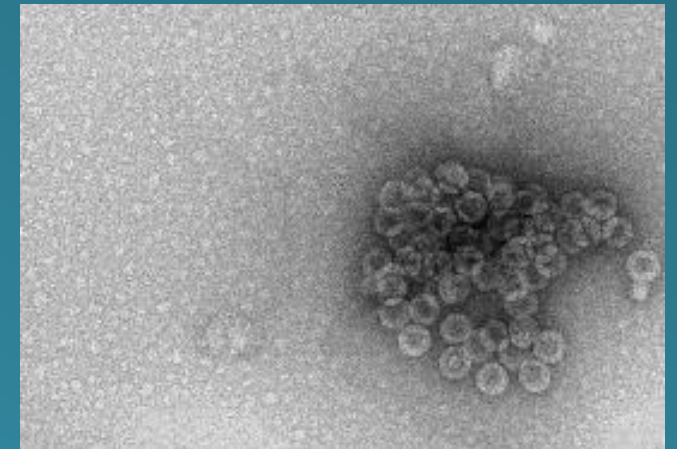
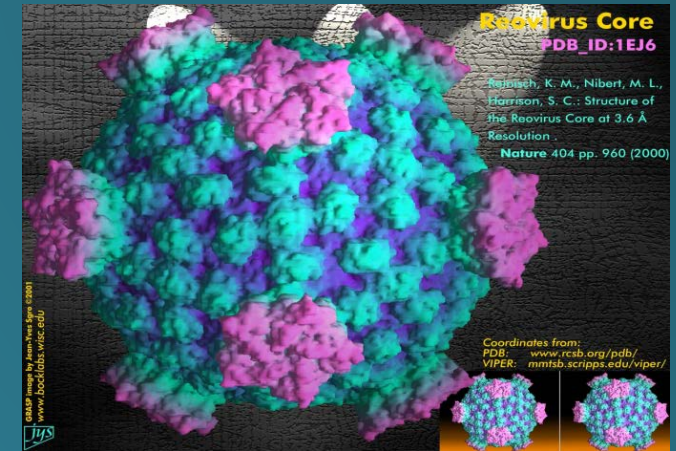
# Norovirus

- **Most common cause of viral diarrhea**
- **No long-lasting immunity**
- **New more virulent strain may have evolved over the last several years**
- **Outbreaks by food, drinking water and recreational waters**



# The Curious Case of Reoviruses

- Most common infectious virus detected in raw sewage, activated sludge, and after chlorine/ozone disinfection
- Forms aggregates with other types of viruses (i.e. poliovirus)
- Forms super sized aggregates (100 to 3,000 virions)
- Exhibits complement reactivation within and between types (because it has a segmented genome) i.e. a dead couple dead = one alive





# Indicators of Pathogenic Viruses

## Bacteriophages

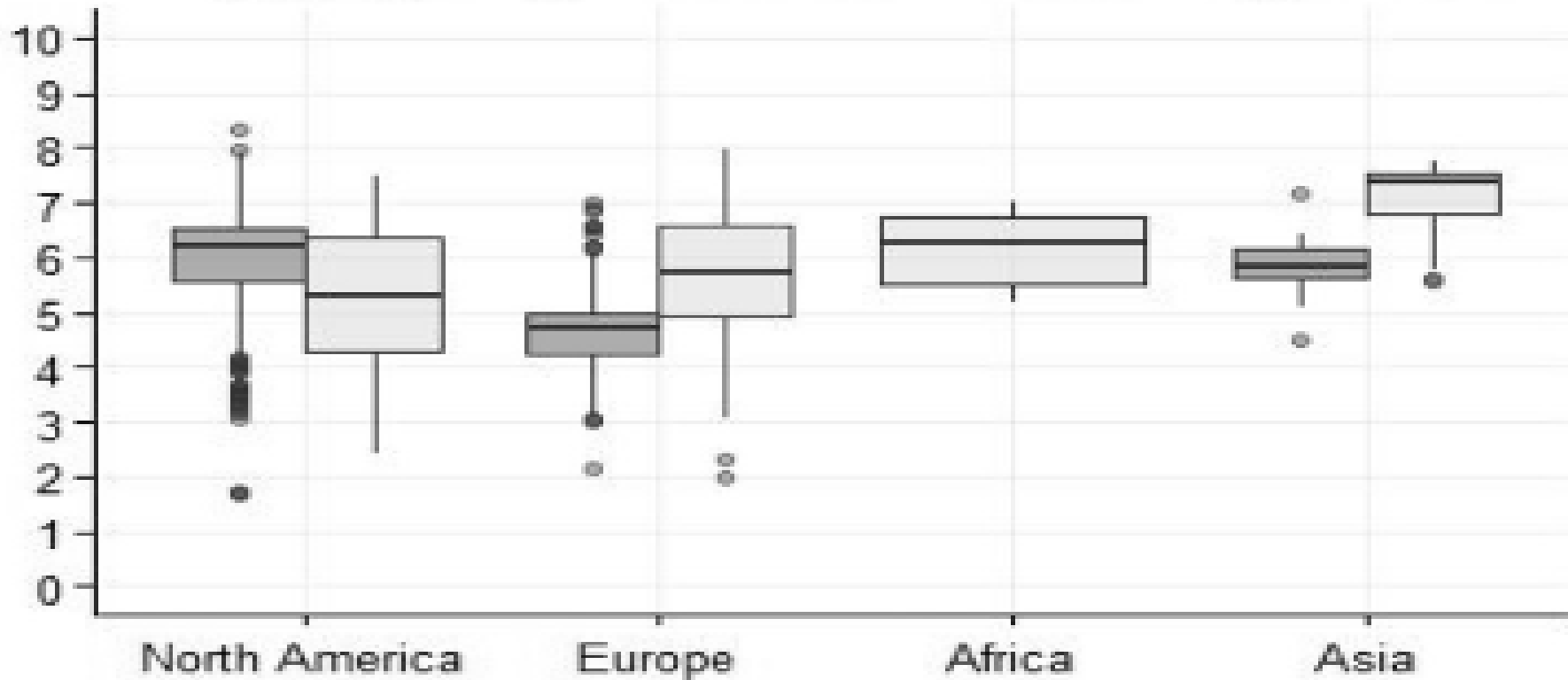
- High Concentrations in Wastewater year around
- Low cost simple and rapid methods for detection available for detection of infectious virus
- Structure, shape and resistance to disinfectants similar to many enteric viruses

## Plant viruses

- High concentrations in wastewater year around
- Only detected by qPCR
- Infectious virus detected after advanced treatment and disaffection
- Detected after RO, Managed aquifer recharge

# Coliphages in Raw Wastewater by Region

Coliphage Density (log<sub>10</sub> PFU/L)

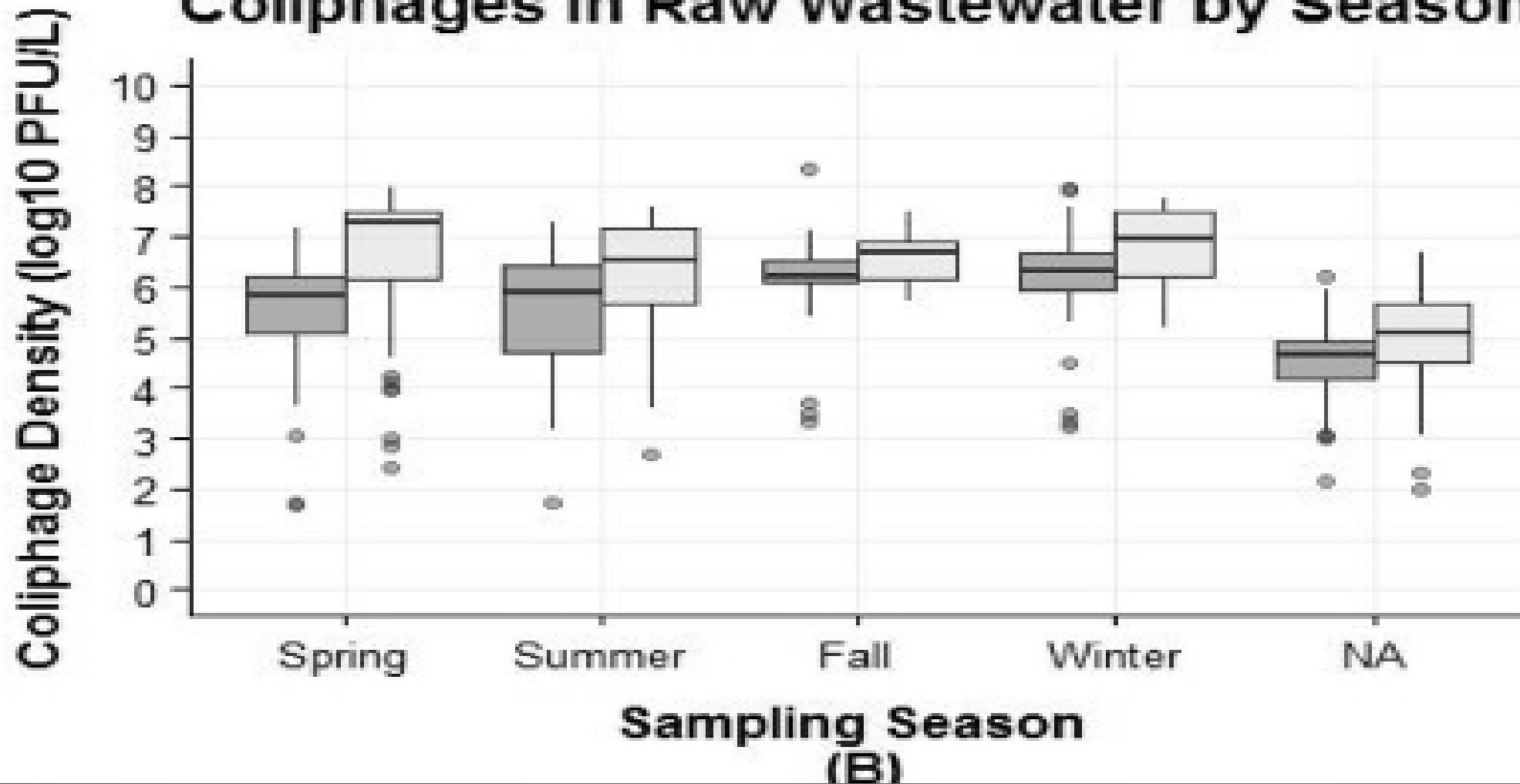


Sampling Location  
(A)

Nappier et al 2019 Water Research

Male-specific Somatic

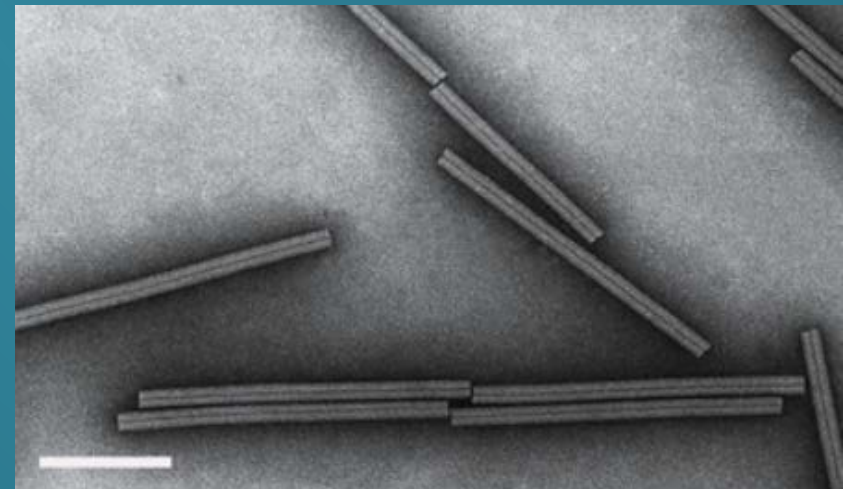
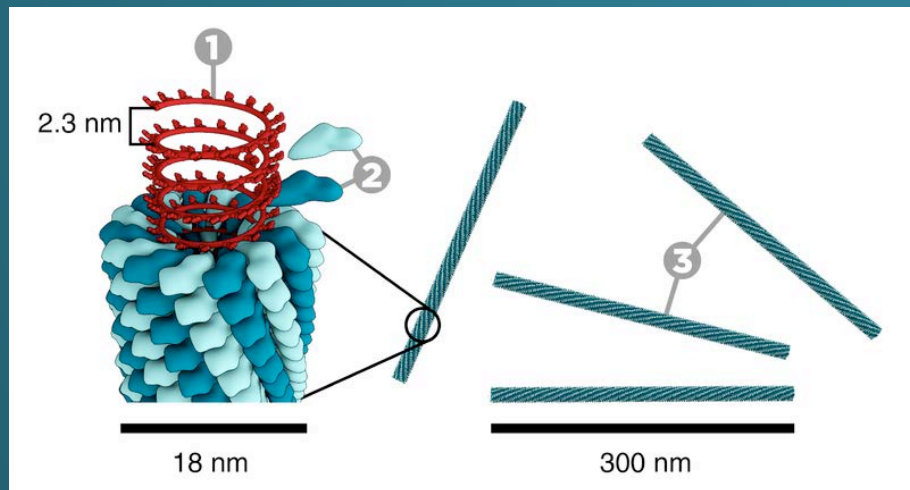
# Coliphages in Raw Wastewater by Season



Nappier et al 2019 Water Research

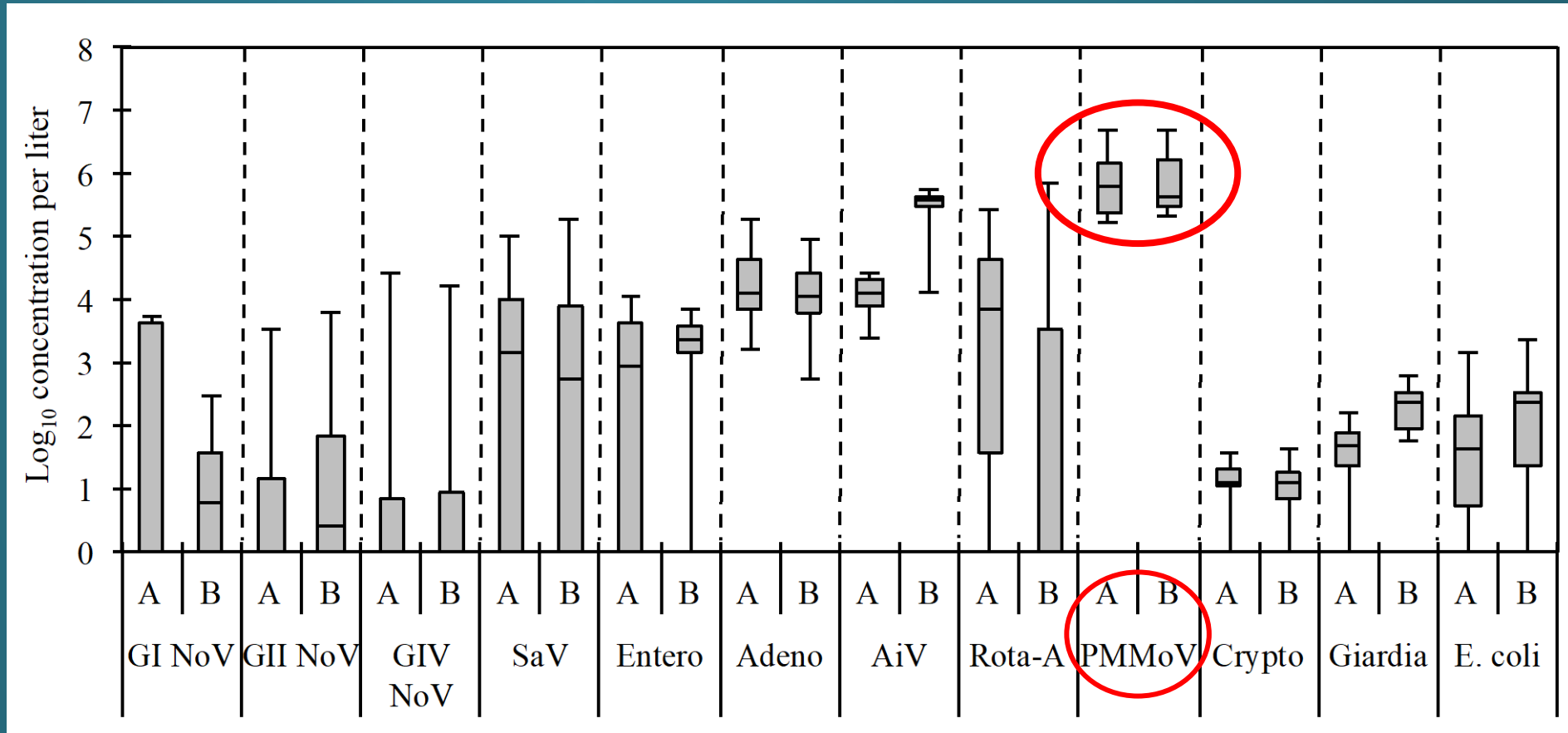
# PMMoV virion properties

- Non-enveloped, rod shape (~18 nm diameter, predominant length ~300 - 310 nm)
- Coat protein self-assembles into helical structure around RNA
- Highly resistant to chemical and physical agents.
- Thermal inactivation point: 90° C, pI: 3.38~3.71





# PMMoV most common virus found in effluent



Viruses

Protozoa

PMMoV showed the highest mean concentration

$6.4 \times 10^5$  copies/L at Ina WWTP

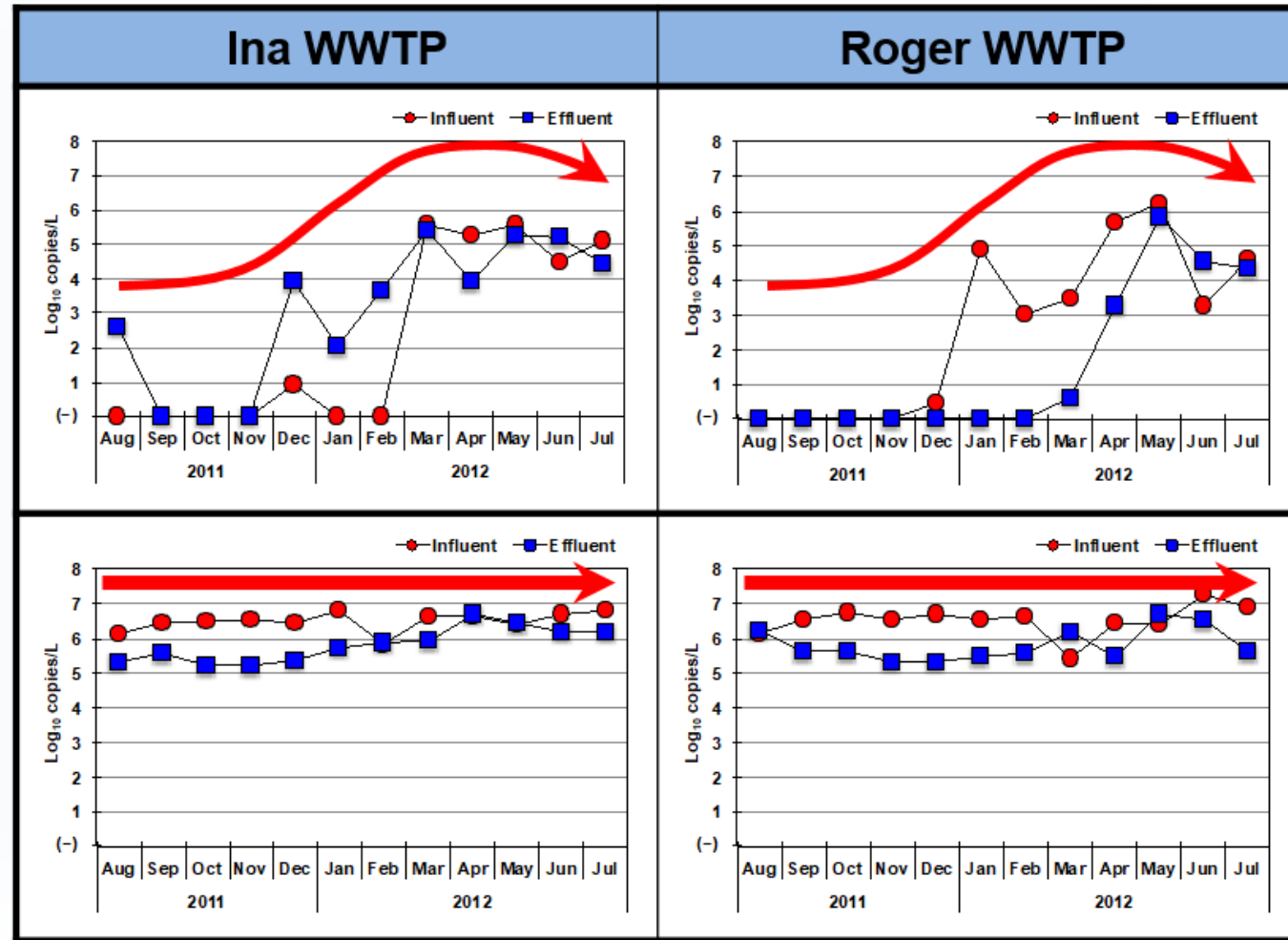
$6.3 \times 10^5$  copies/L at Roger WWTP

(\*A: Ina, B: Roger, box: 50% of the data (25-75 percentile), “whiskers”, max-min)

Schmitz *et al.* 2016

# No seasonal variation of PMMoV

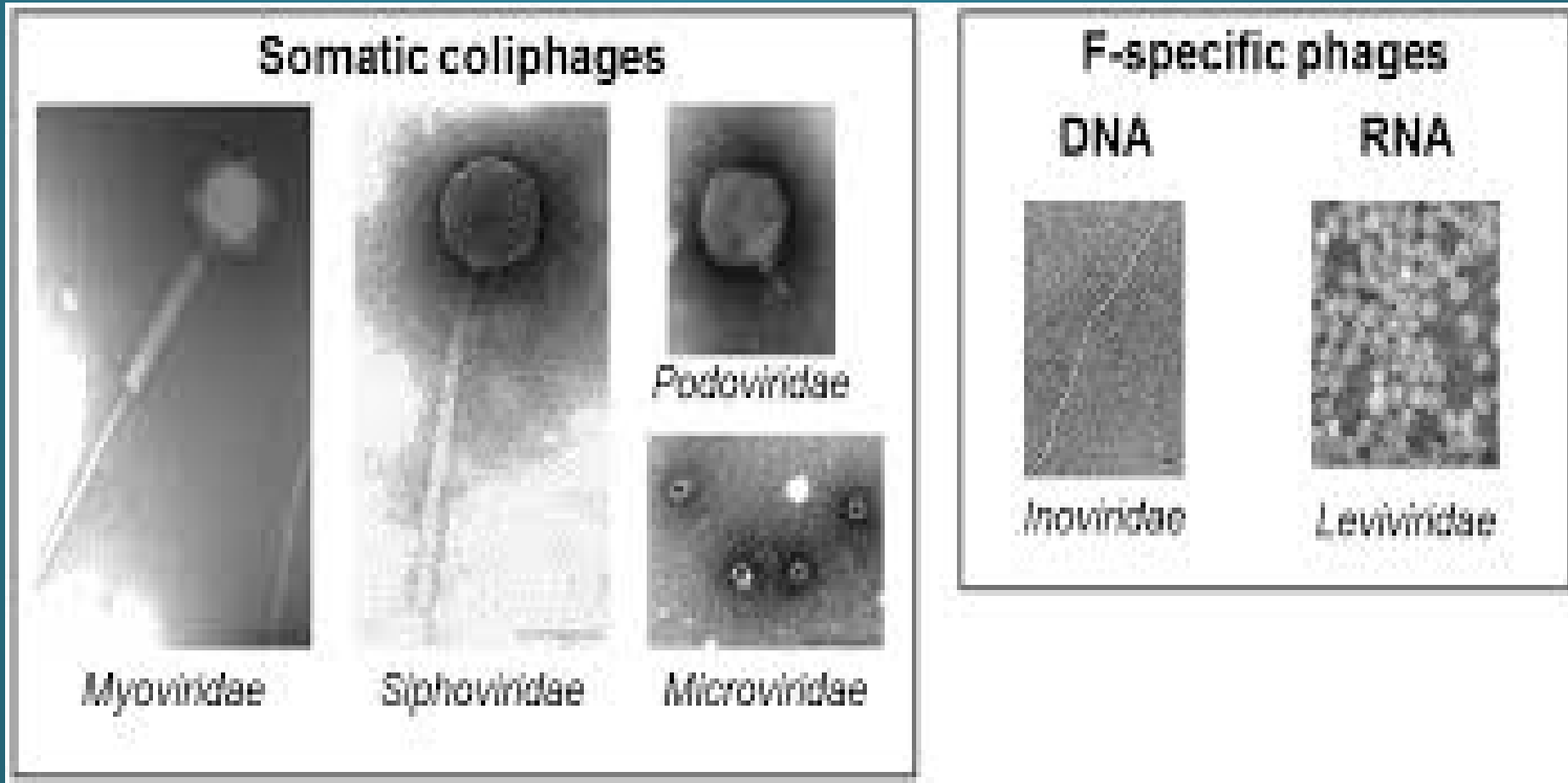
Group A rotavirus



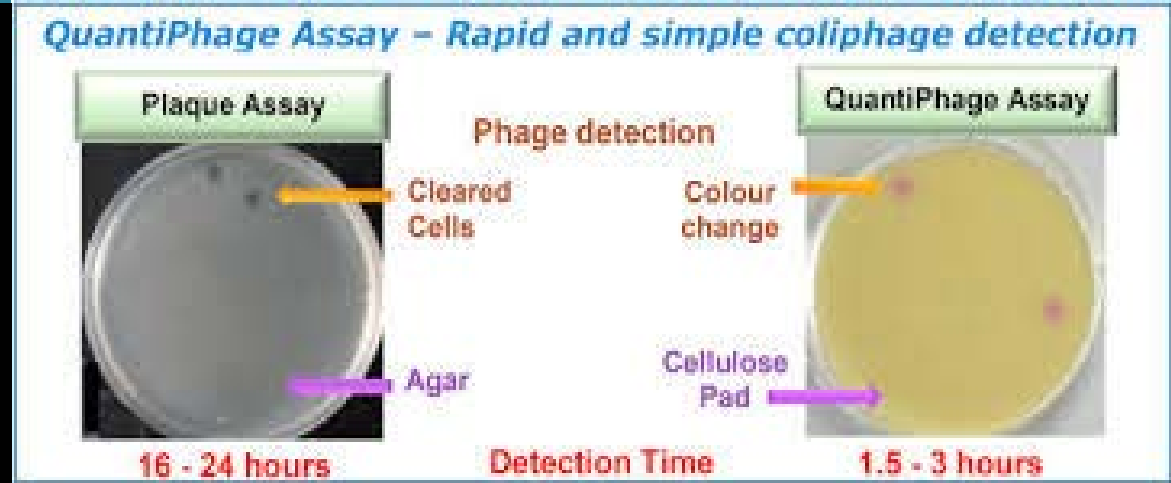
Pepper Mild Mottle virus

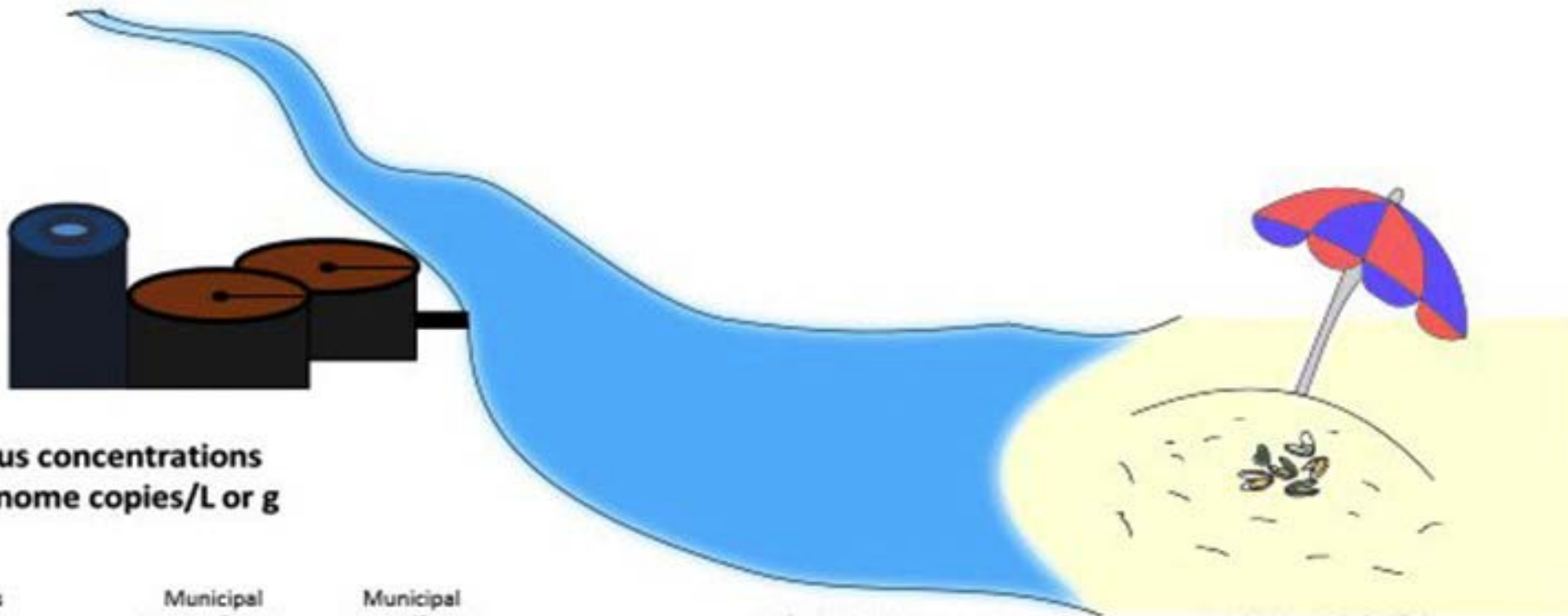
Kitajima *et al.* 2012

# Two Types of Coliphage – Bacteriophage which infect coliforms



# Test kits available for Rapid Detection





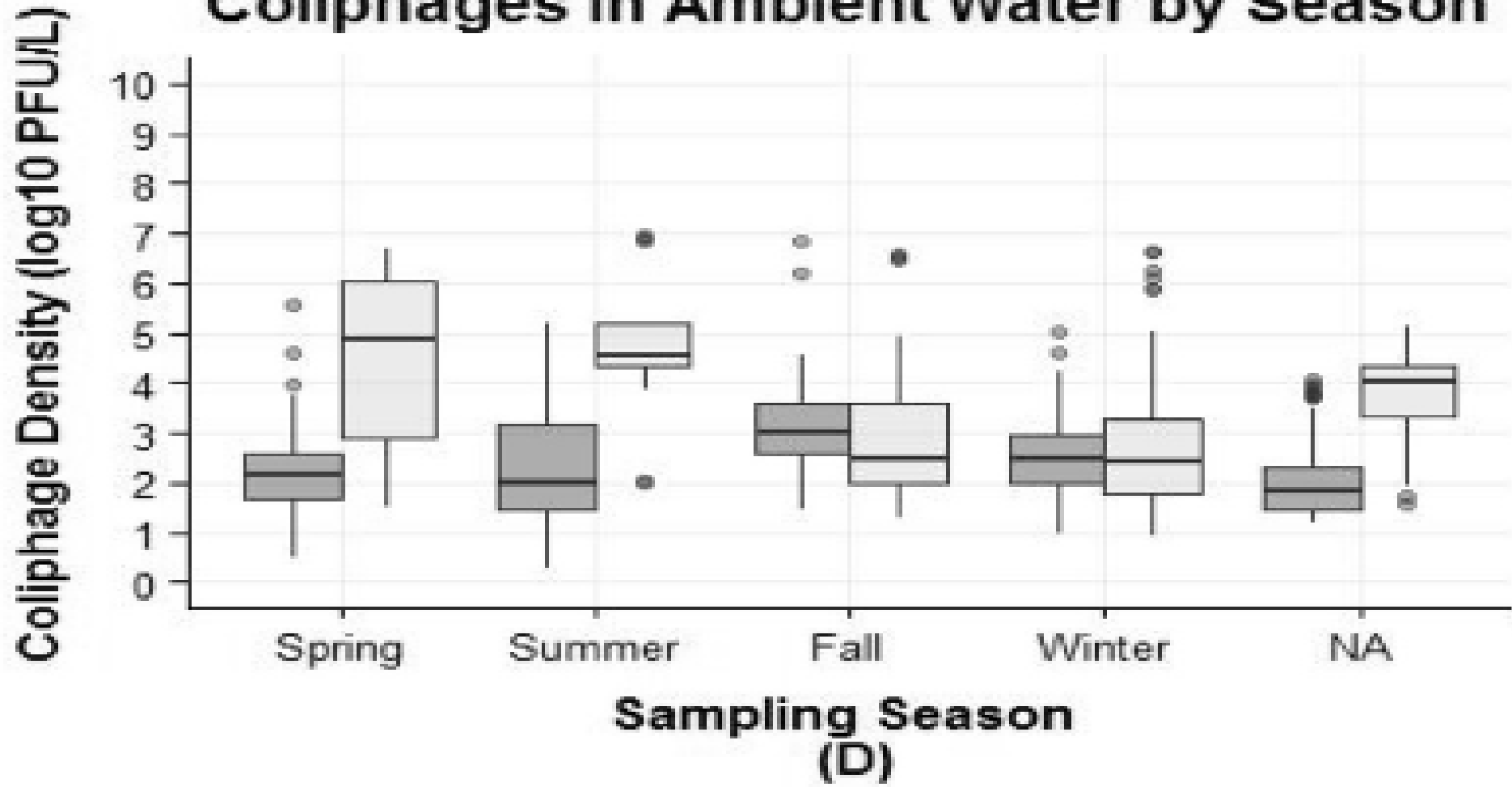
**Virus concentrations**  
Genome copies/L or g

Virus	Municipal WW influent	Municipal WW effluent	Surface water	Sediment and shellfish
Adenovirus	$10^5 - 2 \times 10^6$	$5 \times 10^5 - 10^6$	$7 \times 10^3 - 10^4$	$10^1 - 2 \times 10^3$
JC polyomavirus	$10^5 - 6 \times 10^5$	$3 \times 10^3 - 2 \times 10^4$	$3 \times 10^2 - 10^3$	$10^2 - 7 \times 10^2$
Sapovirus	$10^2 - 3 \times 10^6$	$0 - 4 \times 10^2$	$0 - 10^2$	0
Norovirus	$10^2 - 7 \times 10^8$	$5 \times 10^2 - 4 \times 10^4$	$0 - 10^2$	$0 - 10^2$





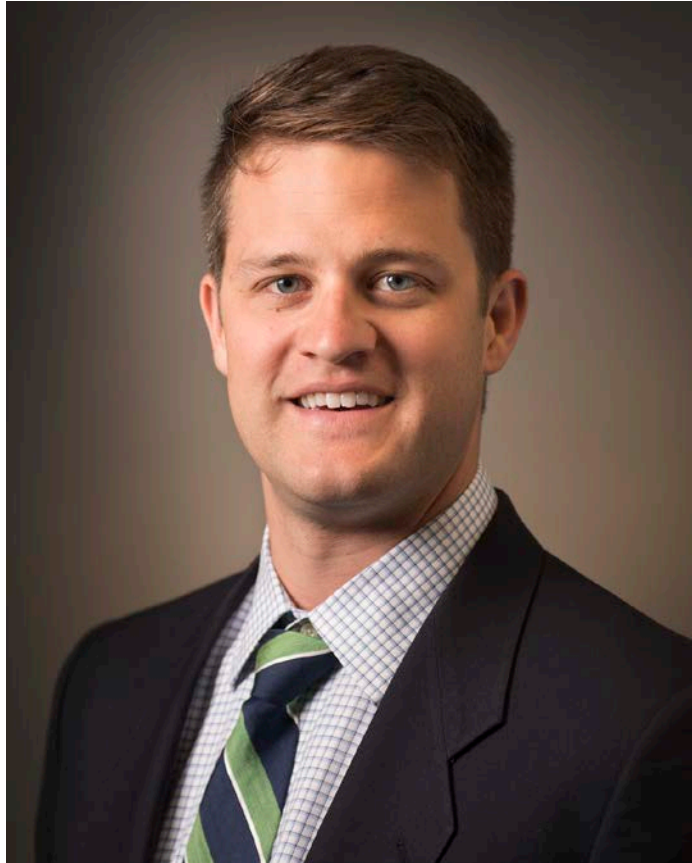
# Coliphages in Ambient Water by Season



Nappier et al 2019  
Water Research

 Male-specific  Somatic

# Our Next Speaker



**Kyle Bibby PhD, PE**

Associate Professor and Wanzek  
Collegiate Chair

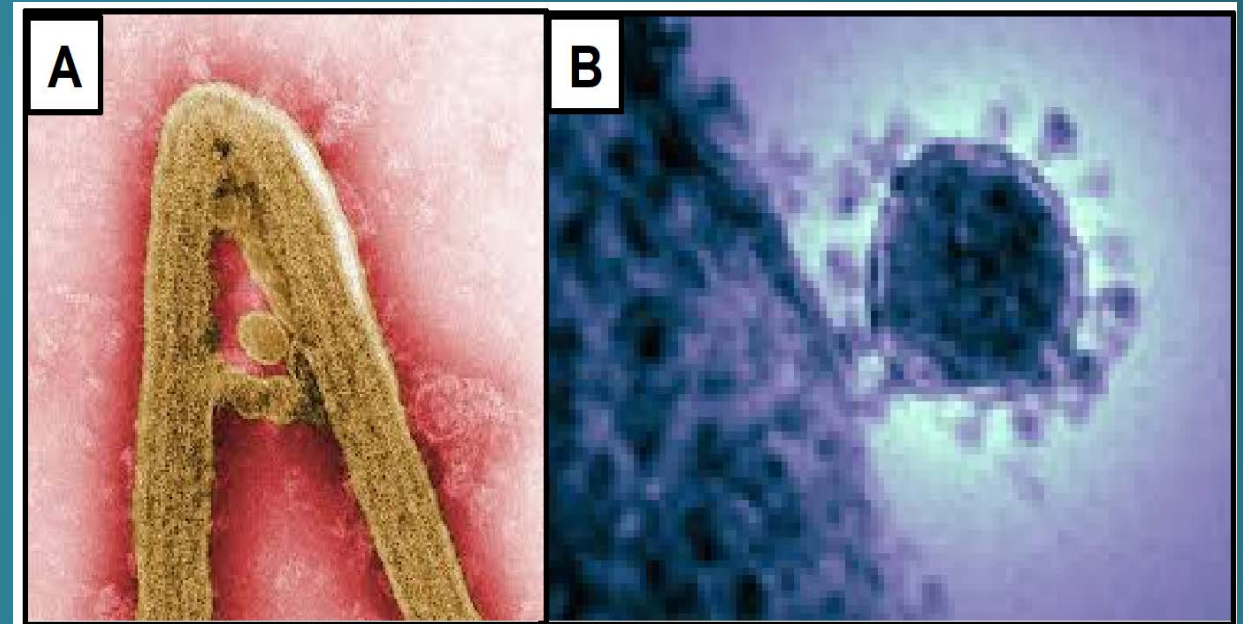
Civil and Environmental Engineering  
and Earth Sciences



# High-Consequence Viral Pathogen Disinfection

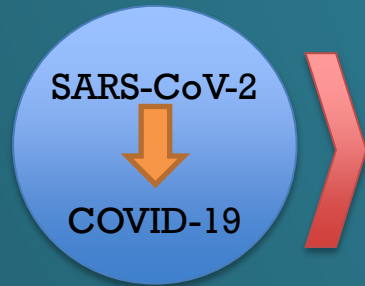
# Examples of High Consequence Viruses

- Ebola virus
- Lassa Virus
- *Coronaviruses, including SARS-CoV-2*



A. Lassa virus; B. MERS CoV  
Source: cdc.gov

# High-Consequence Viruses 'Tough'?



Prions (e.g., Creutzfeldt-Jacob Disease)

Bacterial Spores (e.g., *Bacillus anthracis*)

Coccidia (e.g., *Cryptosporidium*)

Mycobacteria (e.g., *Mycobacterium avium* complex)

Non-enveloped viruses (e.g., Poliovirus, Norovirus, Adenovirus)

Fungi (e.g., *Aspergillus*)

Vegetative Bacteria (e.g., *E. coli*, *Pseudomonas*, etc.)

Enveloped viruses (e.g., COVID-19, SARS, Ebola, Hepatitis B)

Most resistant to environmental conditions and disinfection



Most susceptible

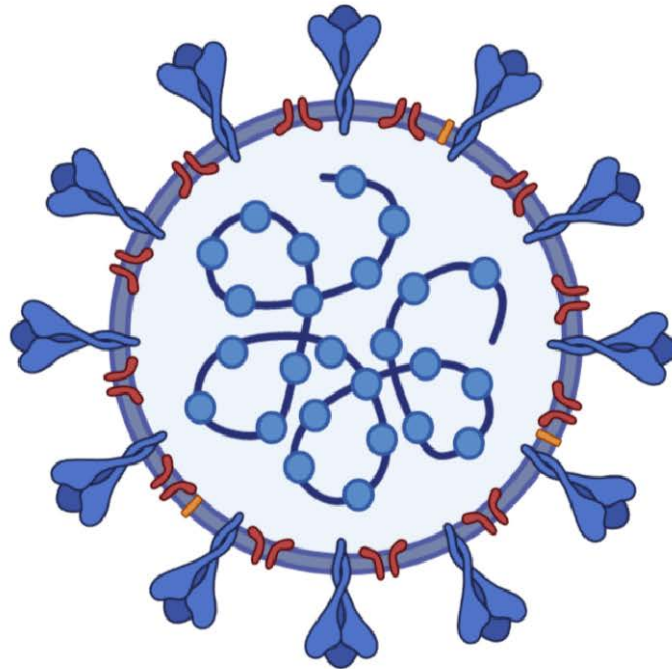
CDC. Guideline for Disinfection and Sterilization in Healthcare Facilities (2017). Adapted from: Favero & Bond (2001); Russel (1998).

Slide credit – Dr. Rasha Maal-Bared



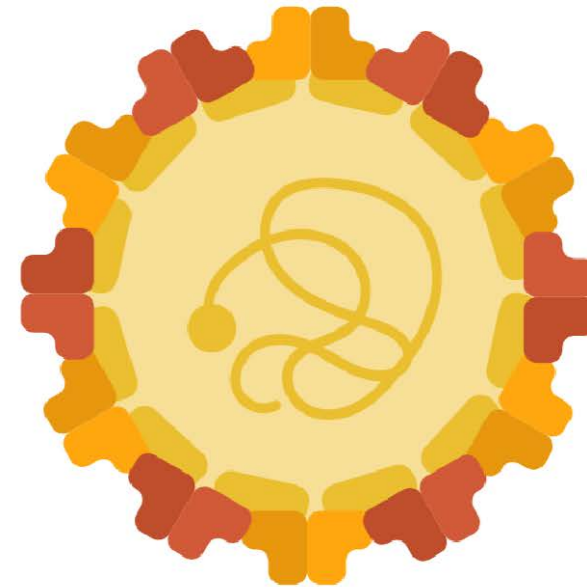
# What is an Enveloped Virus?

Enveloped RNA



SARS-CoV-2

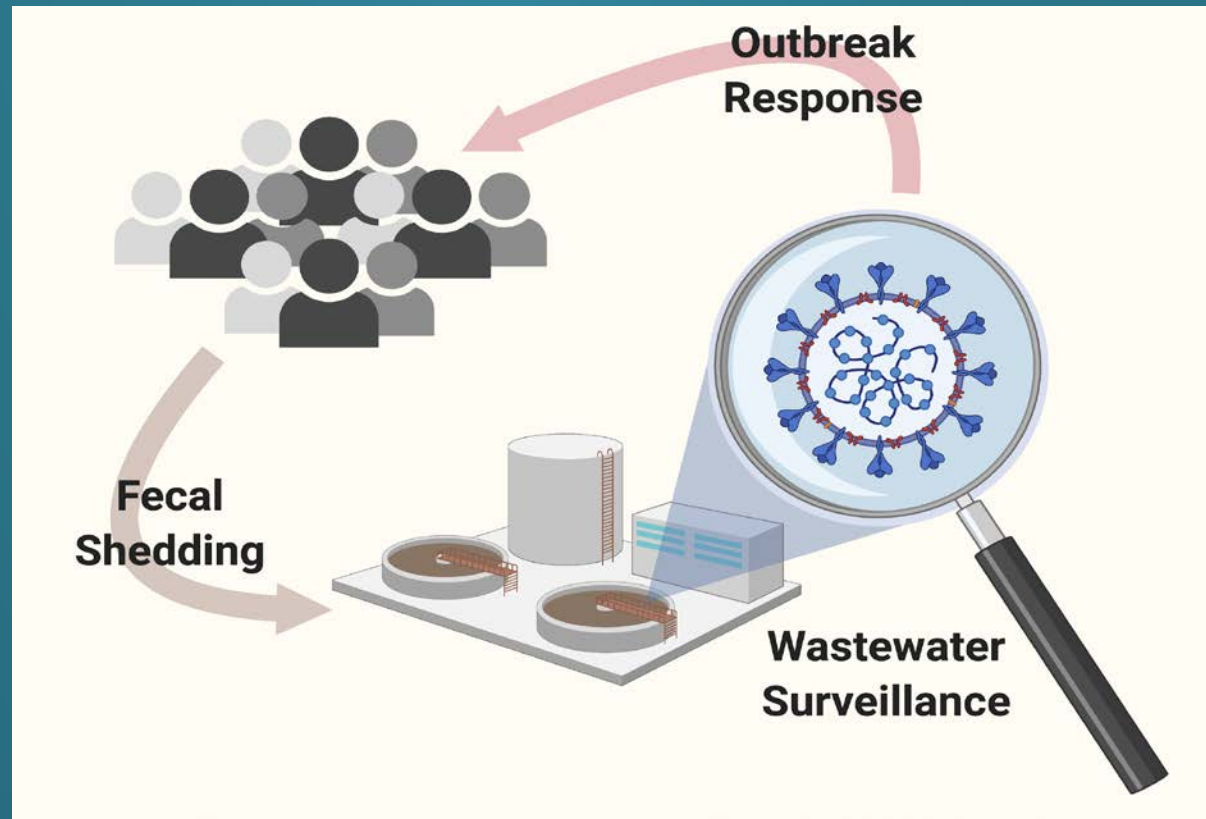
Nonenveloped RNA



Picornavirus

# SARS-CoV-2 RNA in Wastewater

- Based on RNA – does not identify an infectious virus



# Is Infectious SARS-CoV-2 in Wastewater?

- Not identified yet.
- More evidence is needed.
- Initial reports found infectious virus in stool<sup>1</sup>, but this has not been replicated<sup>2</sup>.

1. Zhang, Yong, et al. "Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19)." *China CDC Weekly* 2.8 (2020): 123-4.

2. Wölfel, Roman, et al. "Virological assessment of hospitalized patients with COVID-2019." *Nature* (2020): 1-10.

# Is Infectious SARS-CoV-2 in Wastewater?

- May require improved cell lines<sup>1</sup>, and SARS-CoV-2 replicates in human gut enterocytes<sup>2</sup>.
- Infectious virus may enter the wastewater stream from other sources, e.g. sputum

1. Matsuyama, Shutoku, et al. "Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells." *Proceedings of the National Academy of Sciences* 117.13 (2020): 7001-7003.

2. Lamers, Mart M., et al. "SARS-CoV-2 productively infects human gut enterocytes." *Science* (2020).

# Is Drinking Water Treatment Effective?

- Yes!
- Multiple barriers designed to effectively remove virus

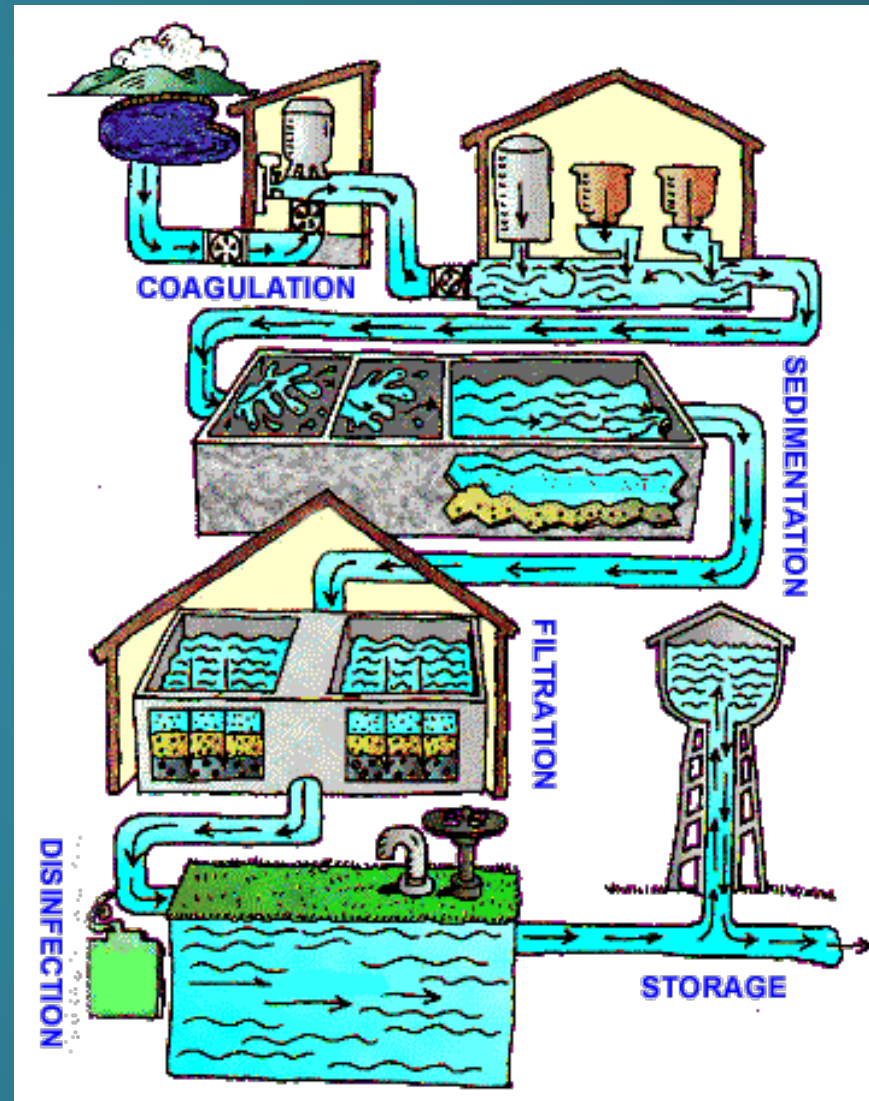
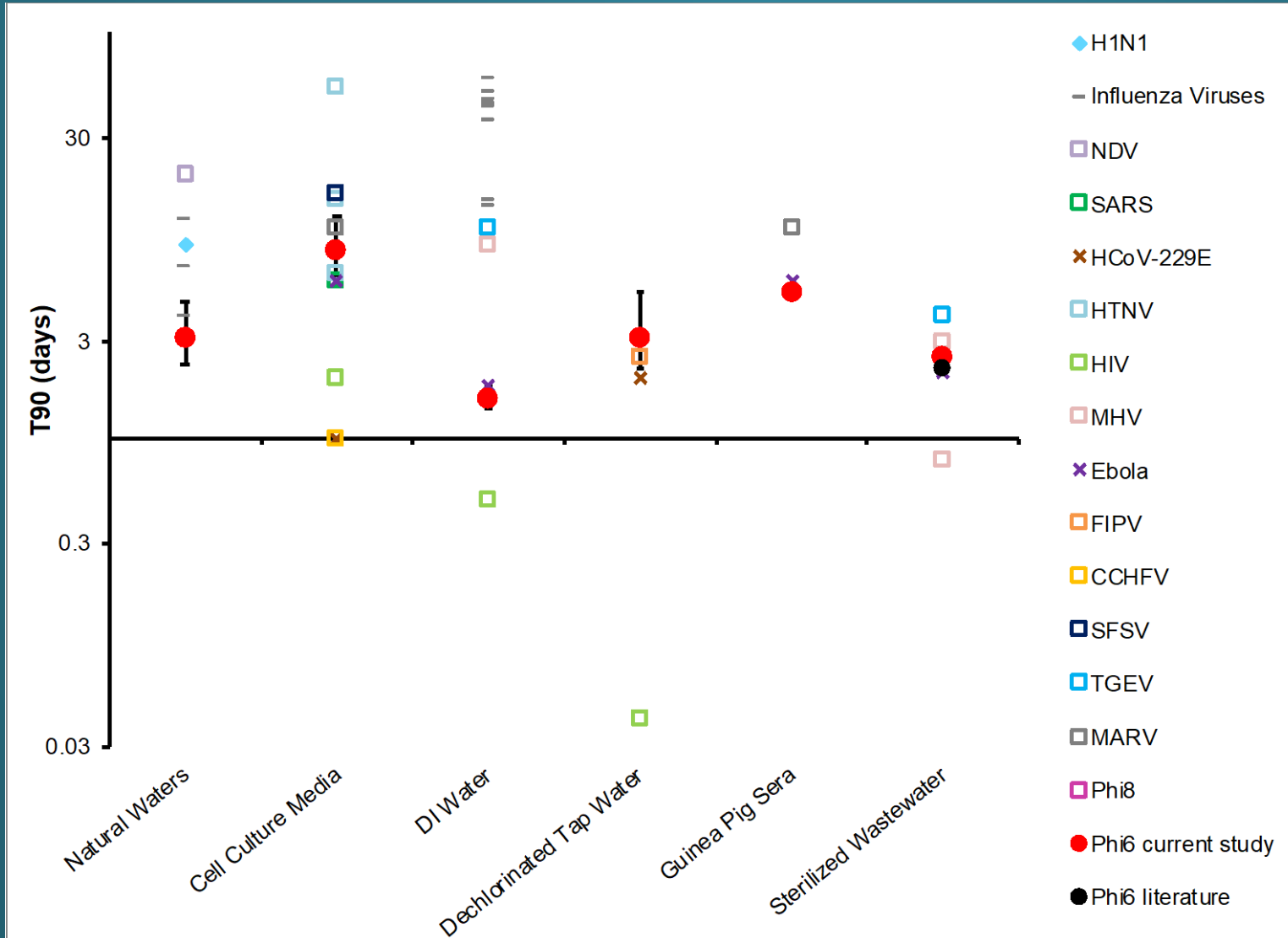


Figure Source:  
CDC/EPA

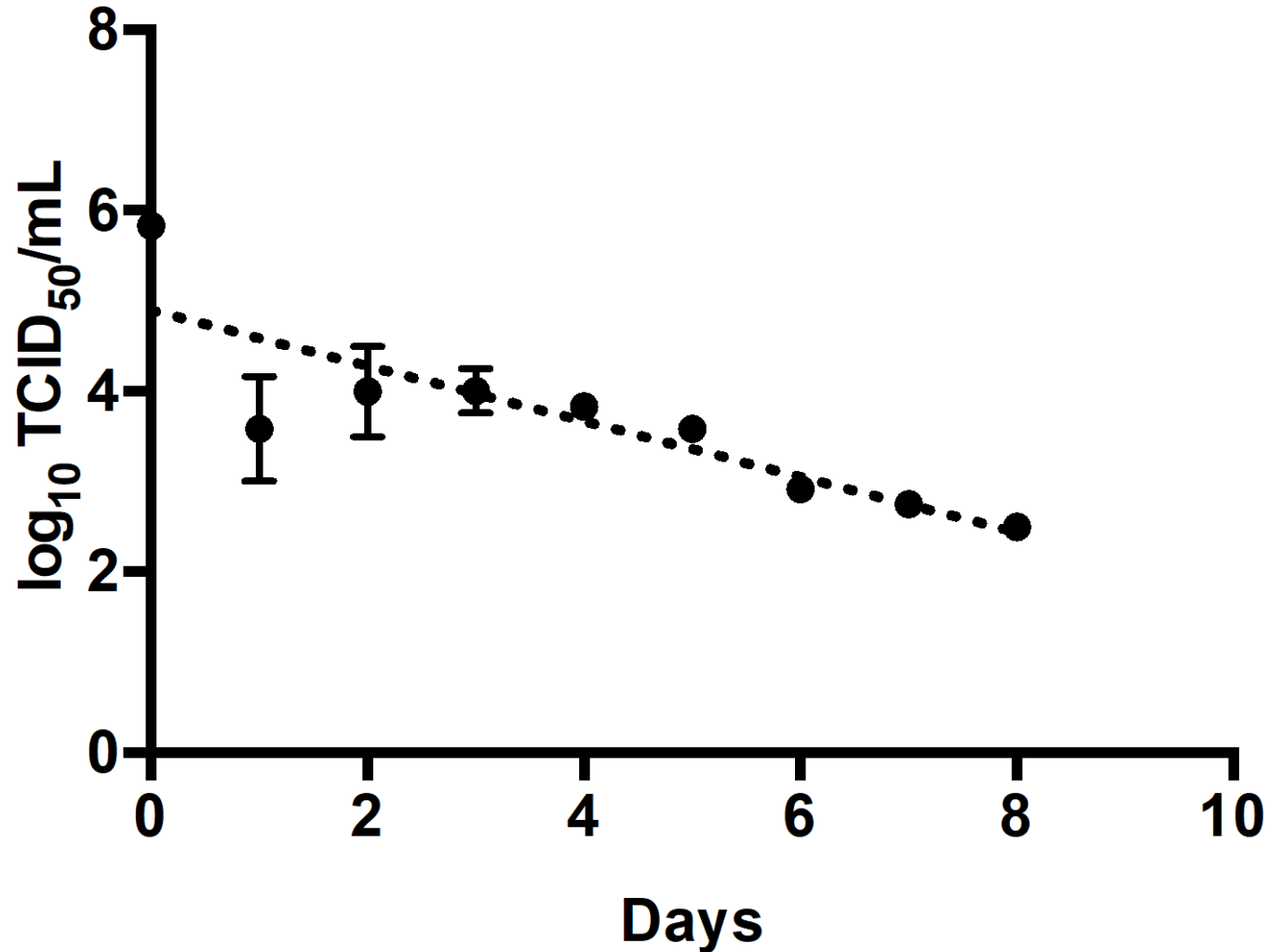


# Enveloped Virus Persistence in Water



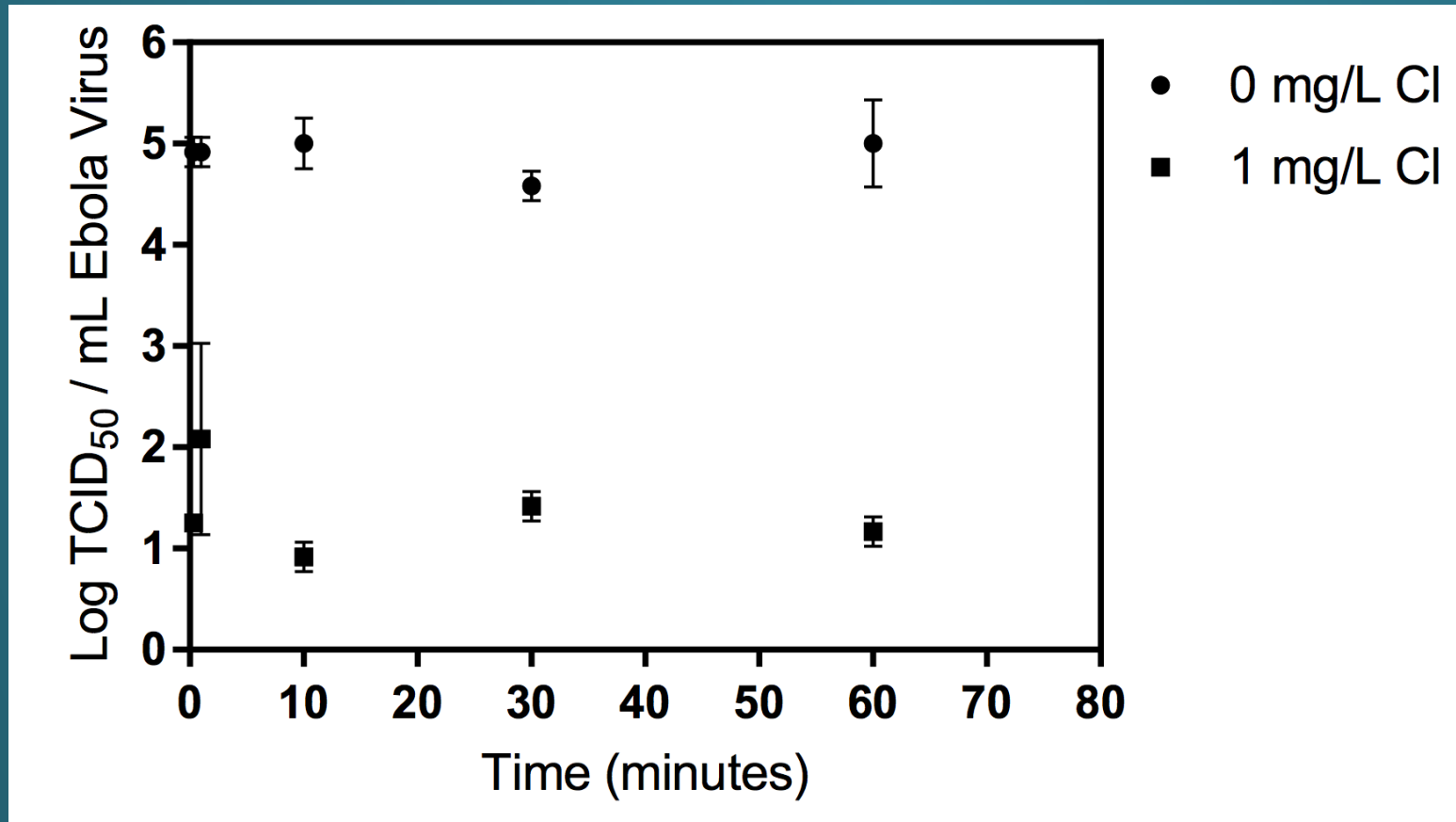
Aquino de Carvalho et al.  
 "Evaluation of Phi6 persistence and suitability as an enveloped virus surrogate." *Environmental science & technology* 51.15 (2017): 8692-8700.

# Persistence - Ebola



Bibby, K., Fischer, R. J., Casson, L. W., Stachler, E., Haas, C. N., & Munster, V. J. (2015). Persistence of Ebola virus in sterilized wastewater. *Environmental science & technology letters*, 2(9), 245-249.

# Disinfection - Ebola



Bibby, Kyle, Robert J. Fischer, Leonard W. Casson, Nathalia Aquino de Carvalho, Charles N. Haas, and Vincent J. Munster. "Disinfection of Ebola virus in sterilized municipal wastewater." *PLoS neglected tropical diseases* 11, no. 2 (2017).

- 5 mg/L and 10 mg/L removed virus to below detection limit

Virus	Water type	Temperature (°C)	Days persisted	Reduction time			Reference
				T <sub>90</sub>	T <sub>99</sub>	T <sub>99.999</sub>	
Human coronavirus	Filtered tap water	23		6.76			(Gundy et al., 2009)
				day			
	Unfiltered tap water	23		8.09			
				day			
	Filtered tap water	4		392			
				day			
SARS-CoV <sup>c</sup>	Filtered primary effluent	23		1.57			(Xin Wei Wang et al., 2005b)
				day			
	Unfiltered primary effluent	23		2.36			
				day			
	Unfiltered secondary effluent	23		1.85			
				day			
	Hospital wastewater	20	3				
	Domestic sewage	20	3				
	Dechlorinated tap water	20	3				
	Phosphate buffer saline	20	14				
	Hospital wastewater	4	14				
	Domestic sewage	4	14				
Dechlorinated tap water	4	14					
Phosphate buffer saline	4	14					
Stool	20	3					
Urine	20	17					

Kitajima, Masaaki, et al.  
 "SARS-CoV-2 in wastewater: State of the knowledge and research needs." *Science of The Total Environment* (2020): 139076.

# Coronavirus Disinfection

- Experiments on SARS-CoV-2 disinfection ongoing
- Expectation is that current disinfection practice is sufficient, but research and data is needed to confirm!



**KBibby@nd.edu**

**@kylejbibby**

# Our Next Speaker



**Thomas Worley-Morse, PhD, PE**  
Principal Engineer

**Hazen**



# Potential Impacts of US EPA Coliphage Criteria on Wastewater Treatment and Reuse

Thomas O. Worley-Morse PhD, PE, Melanie A. Mann, PE,  
Diane Roher, Raul Gonzalez, PhD





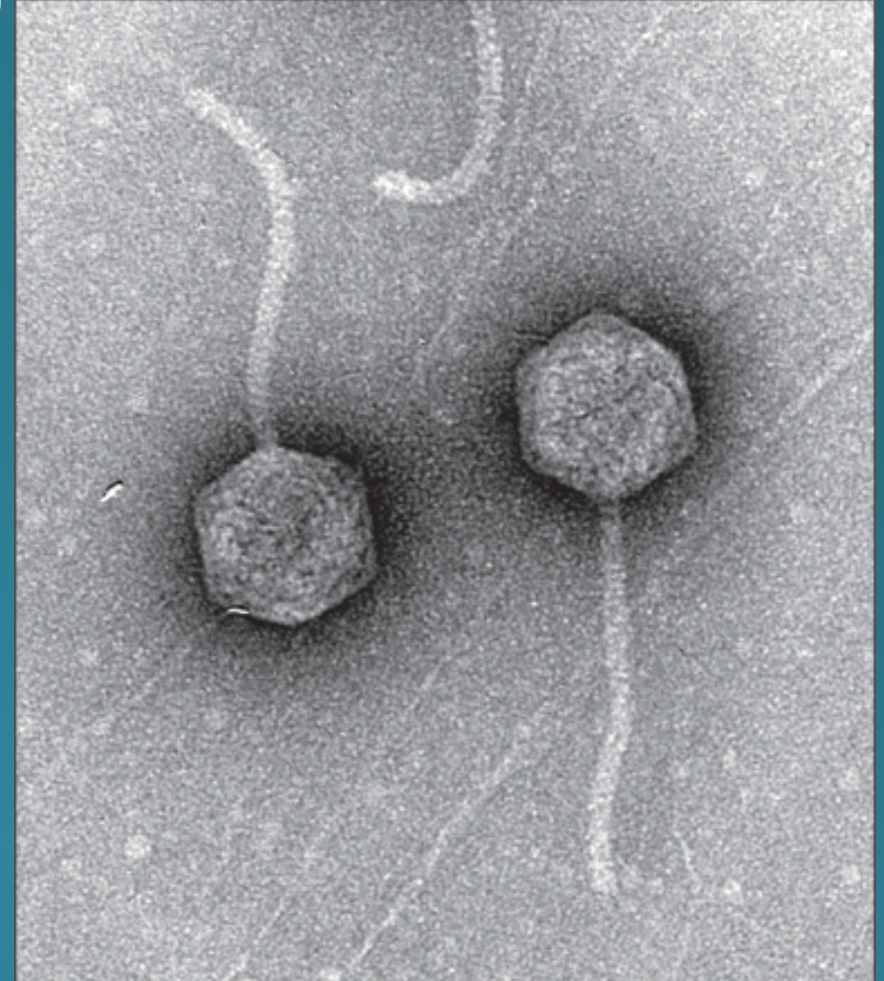
# Talk Outline

- US EPA's coliphage efforts
  - Coliphages
  - RWQC
  - Drivers
- WRF coliphage project
  - Industry concerns
  - Indicator removal
  - Cost Impacts
- Implications for wastewater treatment and reuse



# US EPA Is Evaluating Coliphage Recreational Water Quality Criteria (RWQC)

- Bacteriophages
  - Viruses that infect bacteria
  - Not pathogens
  - Outnumber bacteria 10 to 1
- Coliphages are specific to *E. coli*
  - Somatic
  - Male-specific (F<sup>+</sup> type) (like MS2)
  - Likely conservative indicator regarding COVID-19
- Recreational Water Quality Criteria
  - Recommendations/guidance for states to protect users of recreational water
  - Not regulations
  - Indirectly impacts NPDES discharge permits for WRRFs



<http://aem.asm.org/content/77/8/F1.medium.gif>

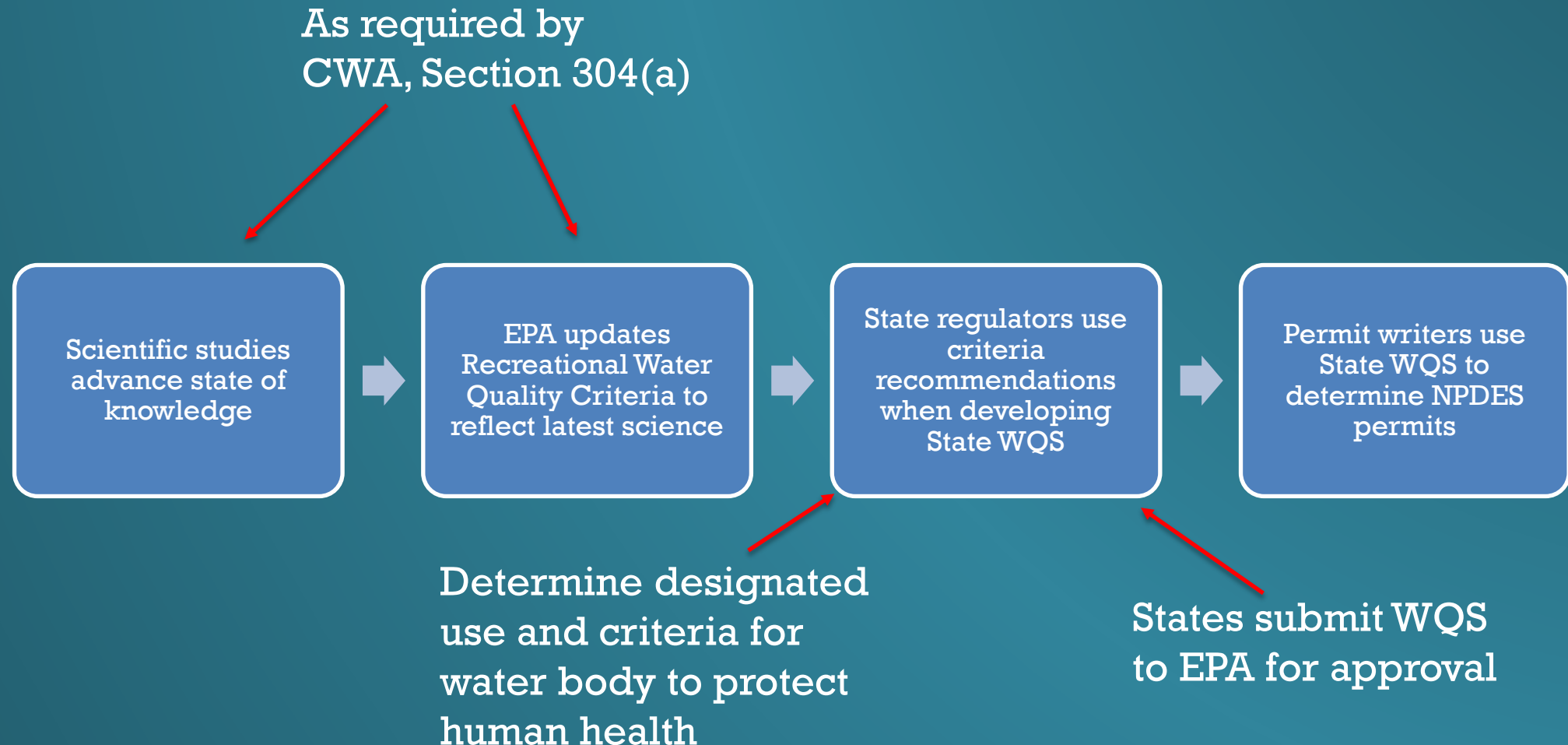


# 2012 Recreational Water Quality Criteria

- Bacterial indicators only
- Recommendations provide two options
- Statistical Threshold Value
  - 90% percentile value shall not be exceeded by 10% of the samples

2012 EPA RWQC	Recommendation 1 Estimated Illness Rate 36/1,000		Recommendation 2 Estimated Illness Rate 32/1,000	
	GM (CFU/100 mL)	STV (CFU/100 mL)	GM (CFU/100 mL)	STV (CFU/100 mL)
Enterococci (marine & fresh)	35	130	30	110
<i>E. coli</i> (fresh)	126	410	100	320

# Relationship Between Recreational Water Quality Criteria and NPDES Permits



# Drivers for the Coliphage RWQC



- Viruses contribute to disease burden in recreational waters
  - Actual health impact is underreported/unknown
- Fate of bacterial indicators is different than viruses in WRRFs
- US FDA use of coliphage as indicator for shellfish contamination
- Increased prevalence of de-facto reuse
- Opportunity to use coliphages as an indicator organism for viruses
  - Non-pathogenic
  - Viable/non-viable testing
  - Robust methods
  - Common in raw sewage

# WRF 4880

# Coliphage Study



# Industry Concerns of Potential Impacts

- Industry gaps on



- Limited fate and treatability data on indigenous coliphages
- Operational changes versus equipment and process upgrades?
- Cost impacts and long-term planning considerations?



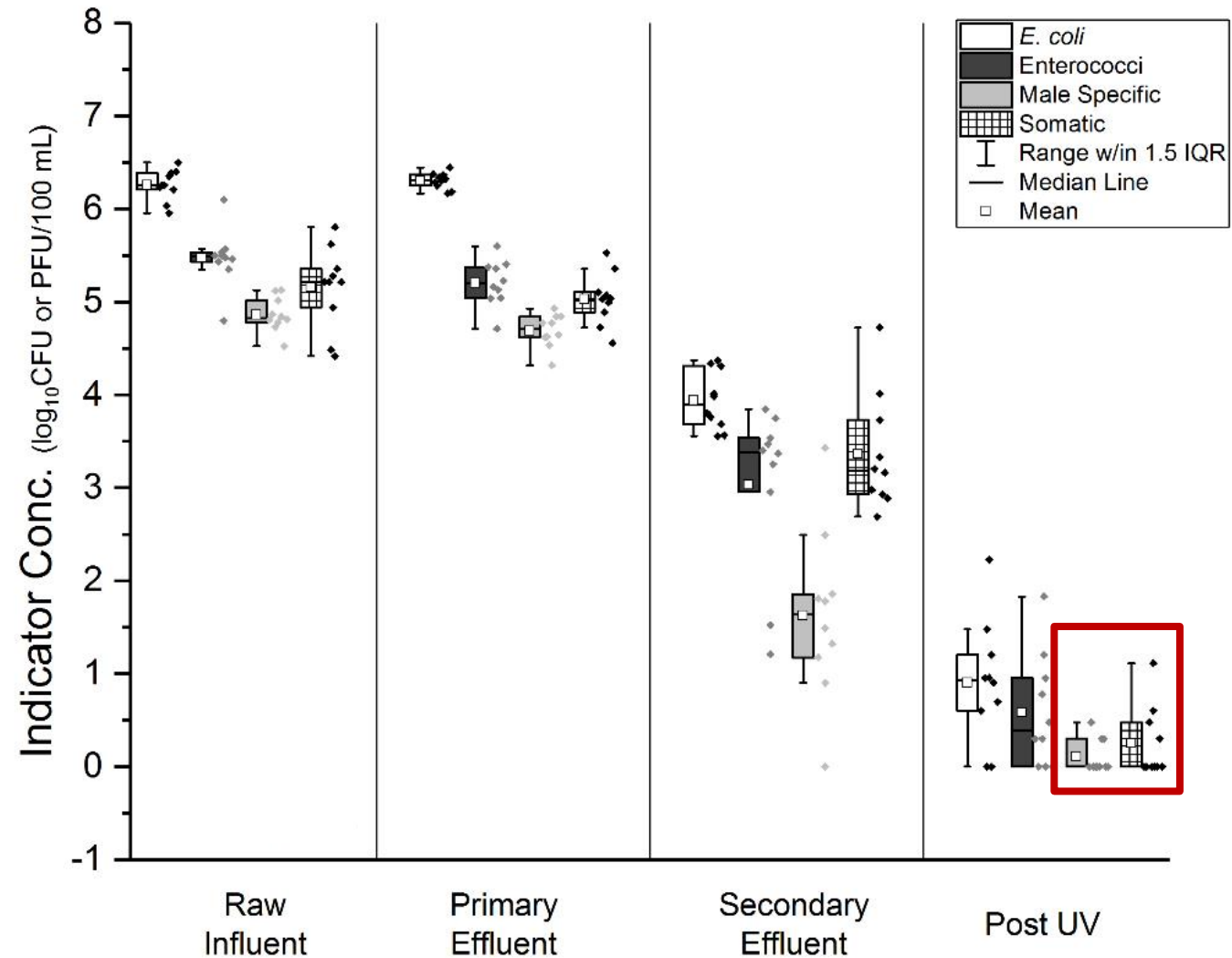
# WRF 4880: Treatability of Coliphages and Costs of Reducing Coliphages at WRRFs

- In response to the EPA's plans for coliphage (somatic and/or male specific) RWQC
- Need for research and guidance to estimate impacts of coliphage criteria from a treatability perspective
- Seven utilities and nine facilities
- Four components: gaps analysis, year-long sampling campaign, disinfectant treatability study, cost estimates

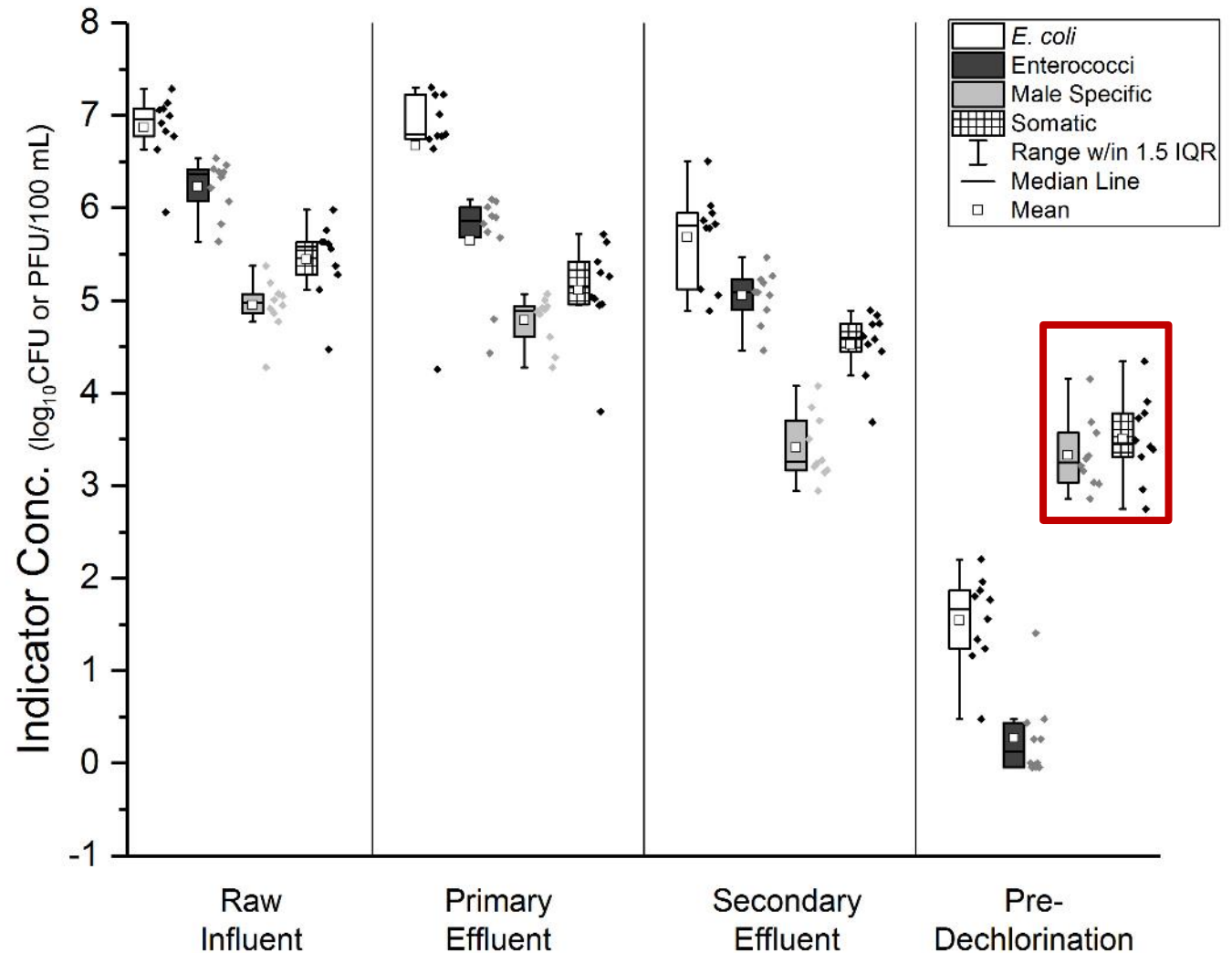




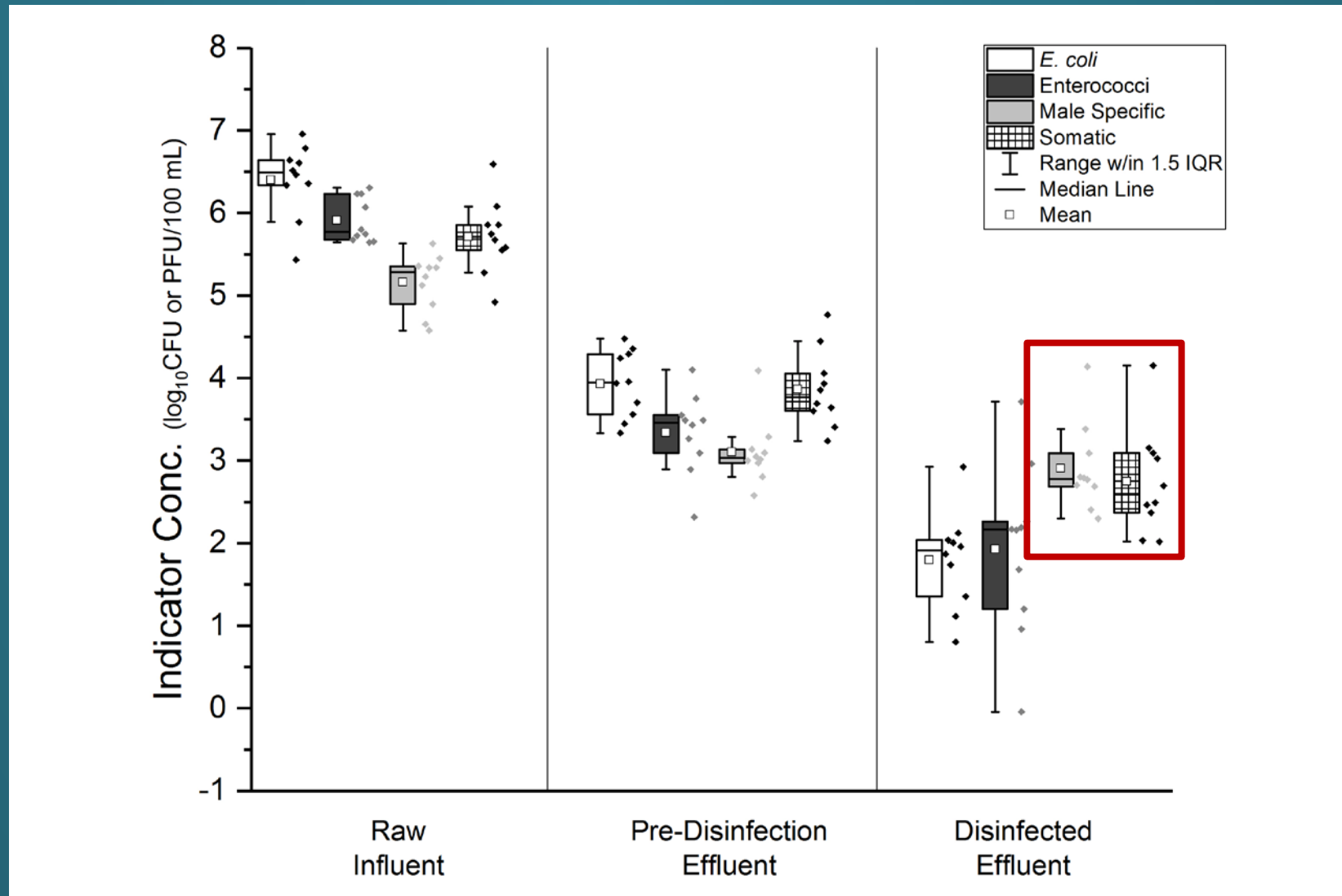
# BNR Facility with UV Disinfection



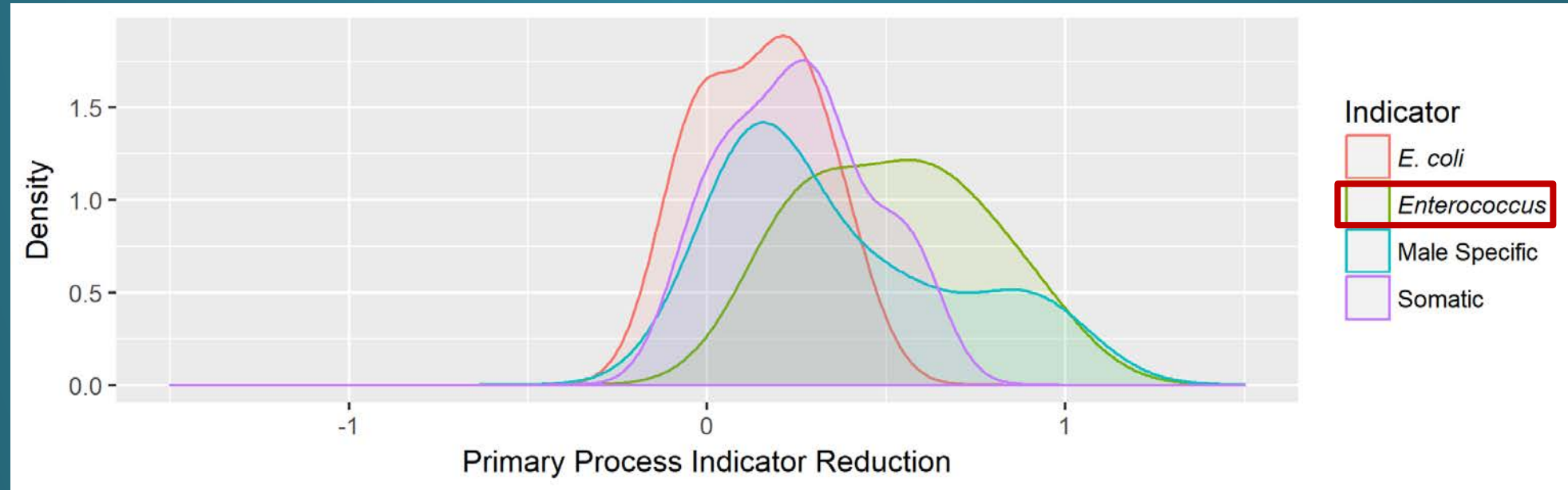
# Non-BNR Facility with Chlorine Disinfection (Chloramines)



# BNR Facility with Peracetic Acid Disinfection



# Primary Process Indicator Removal



- Primary process removal was typically less than 1 log
- Greater primary removal observed for *Enterococcus*

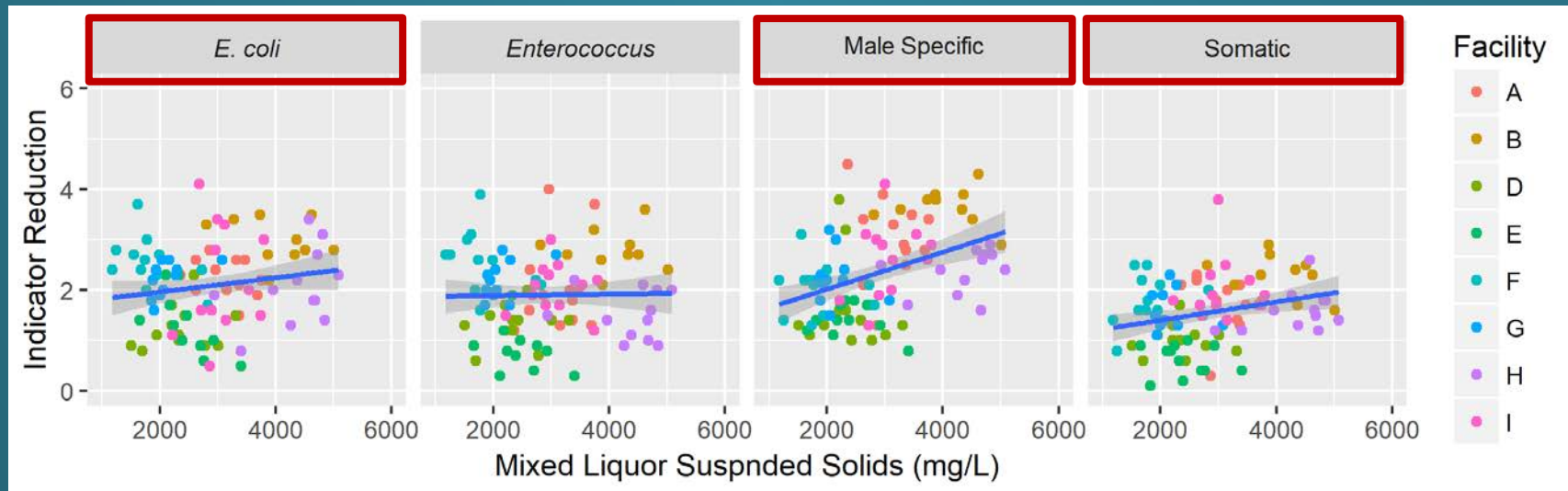
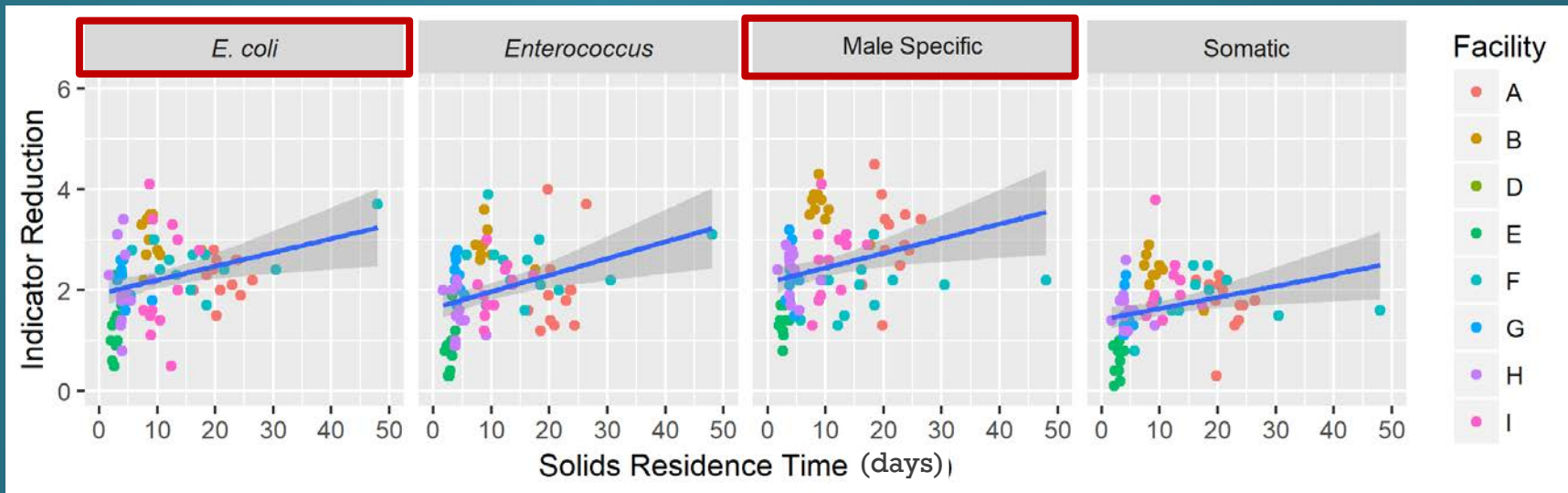
# Indicator Reduction in Secondary Treatment

\* Primary Plus Secondary Reduction

Facility	Secondary Process	<i>E. coli</i>	Enterococci	Male Specific	Somatic
Facility A	5-stage BNR	2.4	2.2	3.1	1.7
Facility B*	5-stage BNR	3.0	2.8	3.7	2.3
Facility D*	Step Aeration AS	1.2	1.3	1.5	1.0
Facility E*	Pure O <sub>2</sub> AS	1.0	0.6	1.4	0.6
Facility F	SBR	2.5	2.6	2.1	1.8
Facility G*	IFAS	2.2	2.2	2.2	1.3
Facility H	3 cell BNR	2.1	1.5	2.4	1.6
Facility I	3 cell BNR (A2O)	2.2	2.1	2.6	2.0

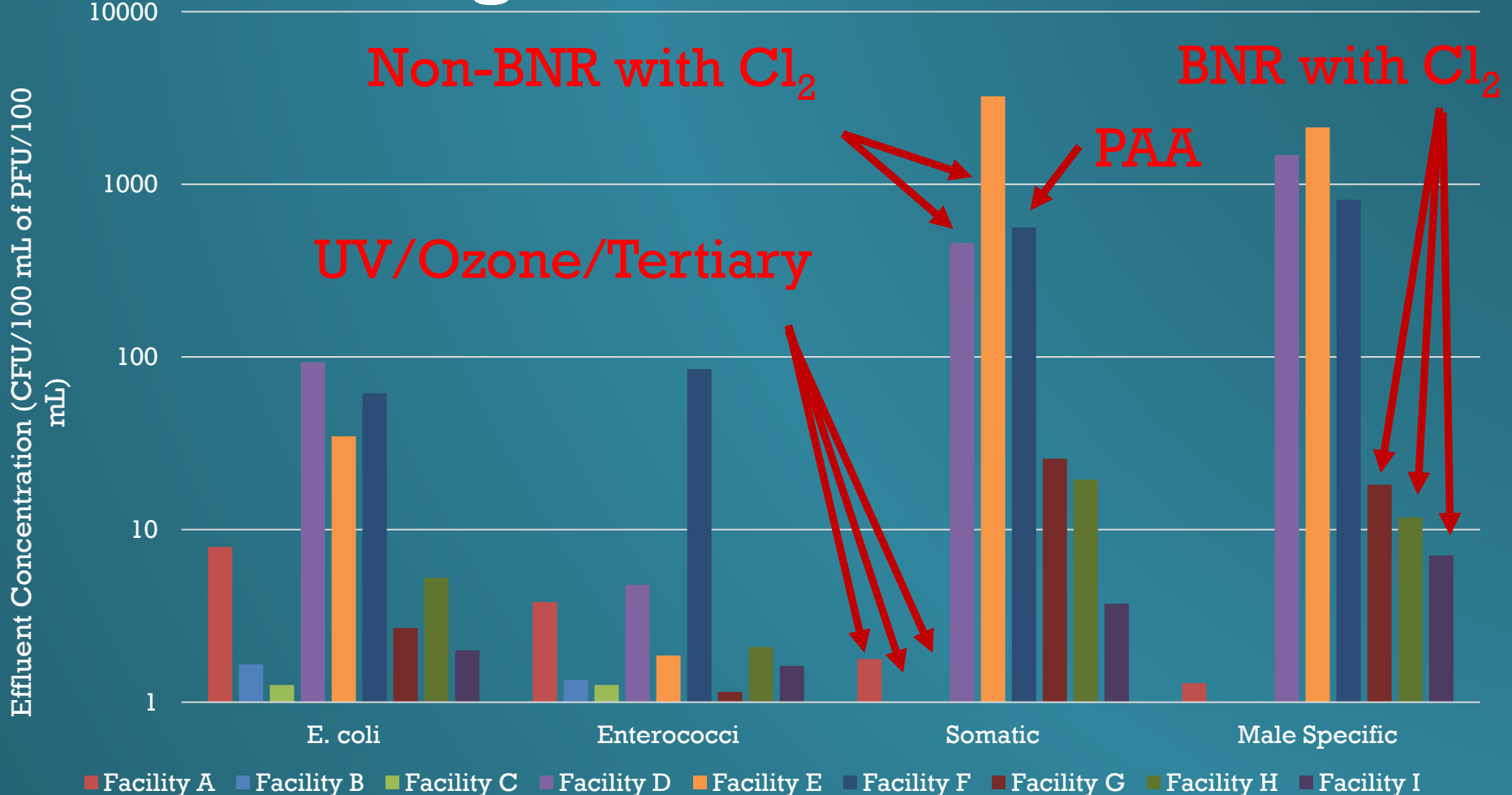
BNR provides and additional 1-log removal

# Secondary Indicator Reduction as a Function of SRT and MLSS

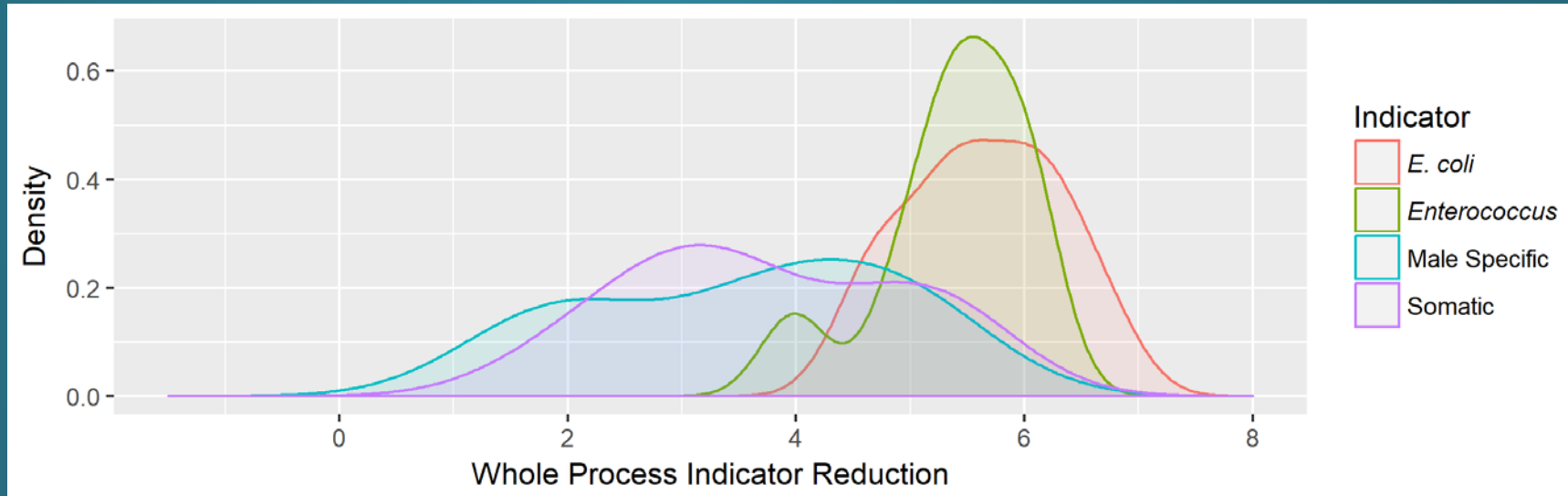




# Mean Effluent Coliphage Varies with Treatment Configuration and Disinfectant



# Whole Process Indicator Reduction



- Average bacterial reduction ~ 5.5 logs
- Average viral indicator reduction ~ 3.5 logs
- Consistent bacterial indicator reduction versus variable coliphage reduction

# Impacts of Phage Criteria Depend on Secondary Treatment and Disinfectant

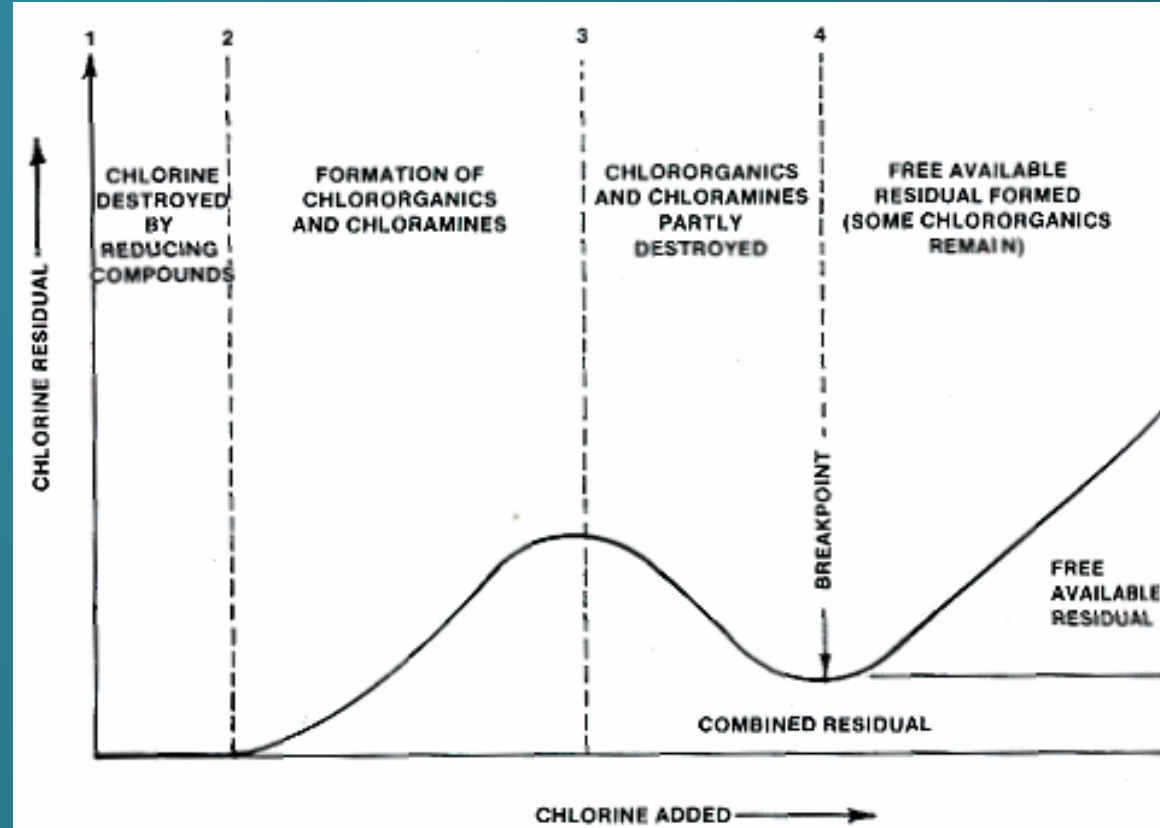
- Observed coliphage reduction
  - Greater with BNR, tertiary treatment, UV and ozone
  - Lower with non-BNR, chlorine, and peracetic acid
- Typical disinfectant performance for coliphages
  - Free chlorine > UV > Monochloramine, PAA
- WERF Study (2008) of 4,450 utilities
  - 75% use chlorine
  - 21% use UV



# Chlorine Disinfection Depends on the Ammonia Concentration

Chlorine to Ammonia Ratio	Chlorine Species
2:1	Monochloramine
4:1	Monochloramine
6:1	Mono with Di and Tri
7.6:1	Theoretical Breakpoint
10+:1	Typical WW Breakpoint

4 mg/L of  $\text{NH}_3$   
as N requires  
~ 40 mg/L  $\text{Cl}_2$



<http://water.me.vccs.edu/concepts/chlorchemistry.html>

# Treatment Alternatives for Additional Coliphage Reduction

Treatment Alternatives for Coliphage Inactivation	
Conventional	Higher disinfectant dose and/or time
	Convert to BNR
	Convert to UV
	Breakpoint Chlorination
Non-Conventional	Enhanced Chemical Coagulation
	Chlorine Dioxide
	Ozone
	Dual Disinfectant

# Cost Example: BNR Conversion

- Non-BNR 10-mgd facility with chlorine disinfection

Option	1	2
Configuration	Nitrification Only	Biological Nutrient Removal
Constituent Removed	BOD and Ammonia	BOD and Nitrogen
MLSS (mg/L)	2,400	3,200
SRT (days)	8	16
Basin Volume	200%	300%
ROM BNR Tanks Cost	\$4 M	\$8 M
Overflow Rate (gal/sf/day)	250	250
Clarifier Area (sf)	15,000	15,000
ROM Clarifier Costs	\$3 M	\$3 M
Total ROM Cost	\$7 M	\$11 M



# Cost Example: UV Upgrade (10 mgd Facility)

UV LPHO	
UVT	65%
Peak Flow, mgd	30
Dose, mJ/cm <sup>2</sup> (MS2 RED)	35
Redundancy	Standby channel
<b>ROM Cost Estimate</b>	<b>\$ 4 M</b>



# Cost Example: Breakpoint Chlorination

- Non-BNR 10-mgd facility with chlorine disinfection, forming chloramines

Breakpoint Chlorination	
Non-Breakpoint Dose Sodium Hypochlorite	6 mg/L
ROM Cost per <b>year</b> (\$1.00 per lb active Cl <sub>2</sub> )	\$180,000
Ammonia-N Concentration	<b>4 mg/L</b>
Breakpoint Dose (10:1)	40 mg/L
<b>ROM Cost per year</b> (\$1.00 per lb active Cl <sub>2</sub> )	<b>\$1,220,000</b>

~7x Increase in  
Chemical Cost



# Project Conclusions & Implications

# US EPA's Timeline

- US EPA schedule
  - Review of Coliphages April 2015
  - 2016 Experts Review March 2016
  - 2012 5-Year Review May 2018
  - Methods 1642 and 1643 September 2018
  - Draft Criteria Unknown

**Coliphage as an indicator:** Risk assessments, epidemiological studies, and outbreak data indicate that viruses cause most illnesses associated with recreational waters impacted by human sources. The EPA is exploring the development of recreational criteria for such a viral indicator to augment the bacterial indicators in the 2012 RWQC and advance public health protection in recreational waters.



# Implications of Coliphage RWQC for Wastewater Industry

- WRRFs designed and permitted for bacterial removal
- Health risk is underestimated and under reported
- Non-BNR facilities with chlorine or PAA disinfection may see greatest impact
- Extensive costs to improve viral removal





# WRF 4880 Implications for Water Reuse

- Climate change will necessitate water reuse
- Coliphage criteria will improve viral reduction
- Reduce risk for *de facto* reuse
- Where is it more cost effective to treat? At water treatment plant or wastewater treatment plant?
- What about protozoa reduction?  
(<https://www.ncbi.nlm.nih.gov/pubmed/22564037>)



# Questions and Answers

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# Questions?