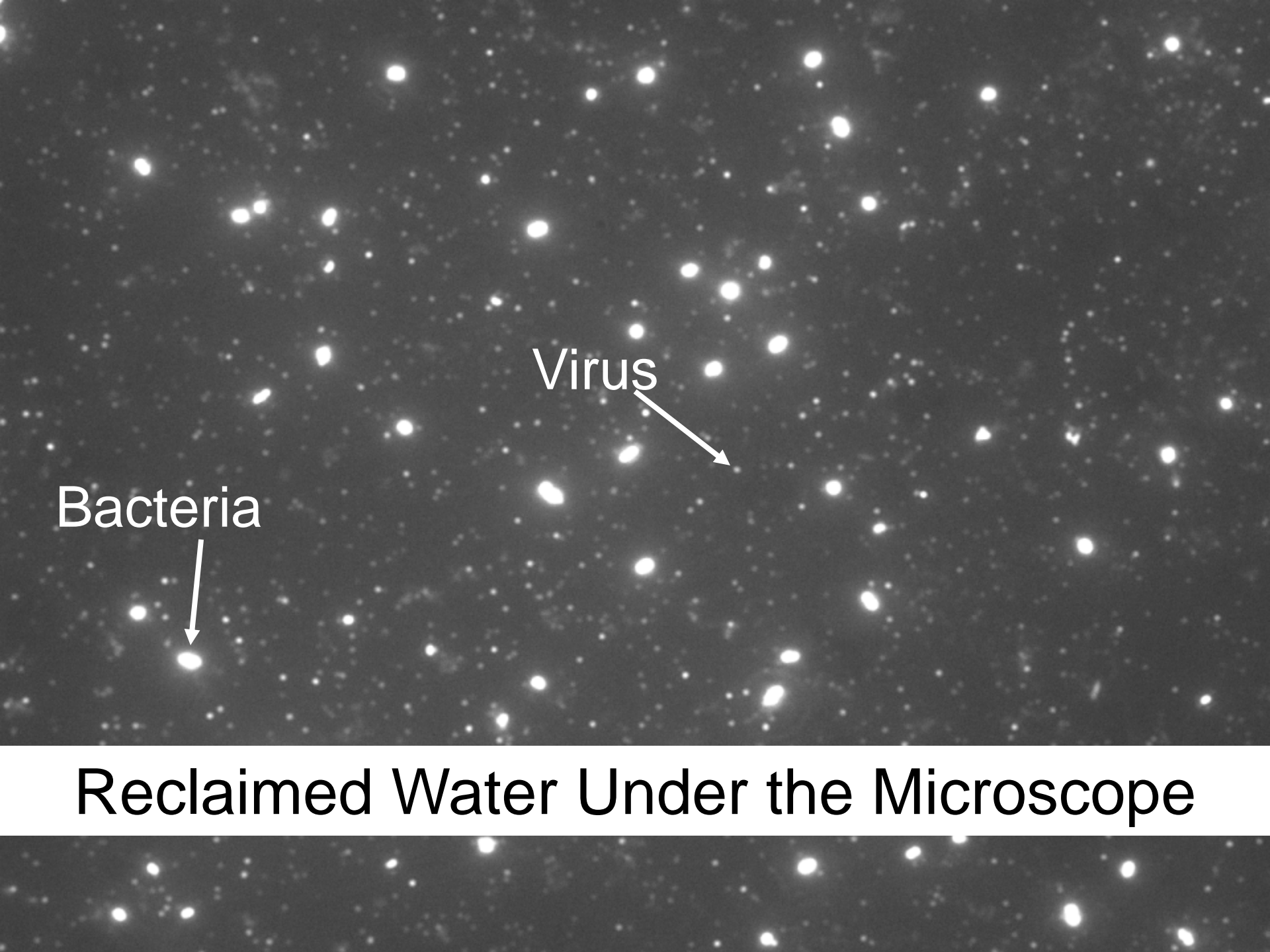


Viruses in Sewage and Reclaimed Water

Mya Breitbart

**University of South Florida
College of Marine Science**

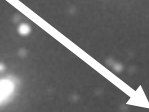




Bacteria



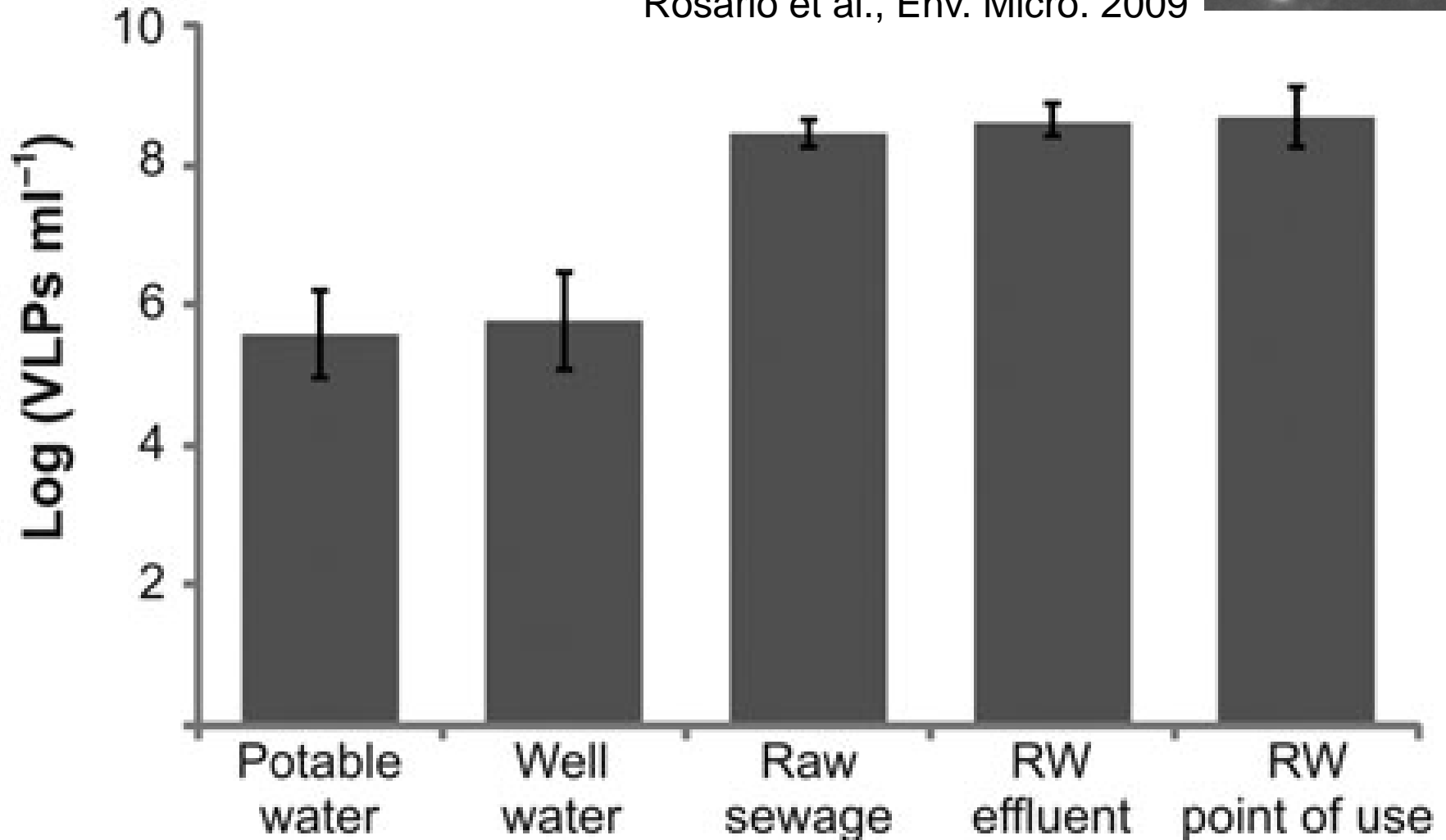
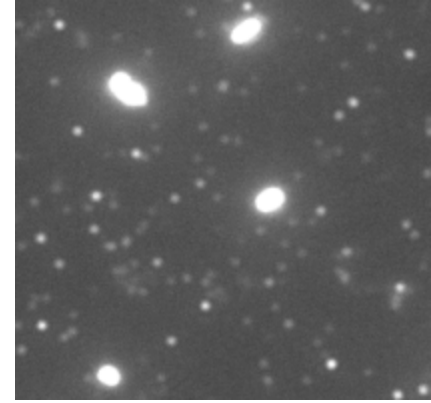
Virus



Reclaimed Water Under the Microscope

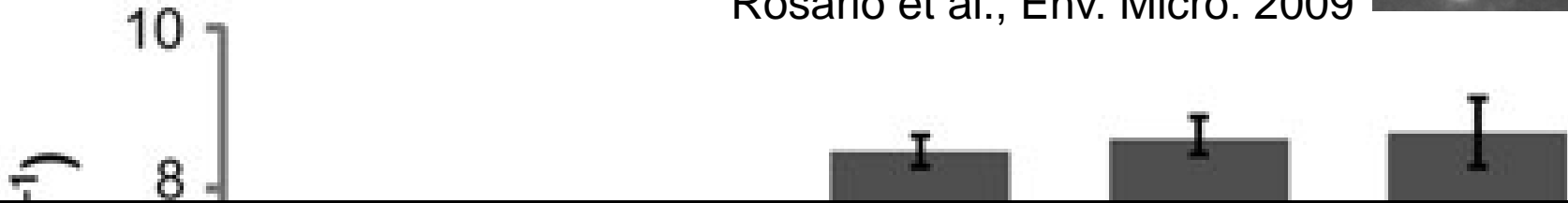
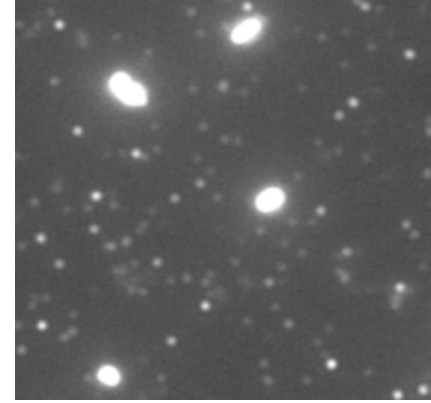
There are ~ 1000 Times More Viruses in Reclaimed Water Compared to Well Water

Rosario et al., Env. Micro. 2009



There are ~ 1000 Times More Viruses in Reclaimed Water Compared to Well Water

Rosario et al., Env. Micro. 2009



Remember...

- Not all viruses are bad (these counts are dominated by bacteriophage)
- These viruses are not necessarily infective
- There is no such thing as a sterile water supply

Why Do We Need to Know What Viruses are in Reclaimed Water?

- To enable us to make educated decisions regarding water reuse
- To protect human health and the health of the environment (prevent problems)
- To develop viral indicators that can be used to assess treatment efficiency
- To identify signatures of wastewater contamination

The Virological Content of Reclaimed Water is Largely Unknown

- The transport/spread of human pathogens is one of the main concerns regarding reclaimed water use
- Classic bacterial indicators of water quality often do not correlate with viral pathogens
- What other types of viruses are in this alternative water supply?

Use **microscopy**, **specific assays**, and **metagenomic sequencing** to analyze the complete viral community

Step 1: Look for known viruses

There is no “universal” assay for all viruses

Viral Group	Percent Detected	
	Raw Sewage	Final Effluent
Adenoviruses	100% (12/12)	25% (3/12)
Enteroviruses	75% (9/12)	8% (1/12)
Hepatitis B	0%	0%
Morbilliviruses	0%	0%
Noroviruses	58% (7/12)	8% (1/12)
Papillomaviruses	0%	0%
Picobirnaviruses	100% (12/12)	33% (4/12)
Reoviruses	0%	0%
Rotaviruses	0%	0%

This narrows the range of viruses to monitor for



Step 1: Look for known viruses

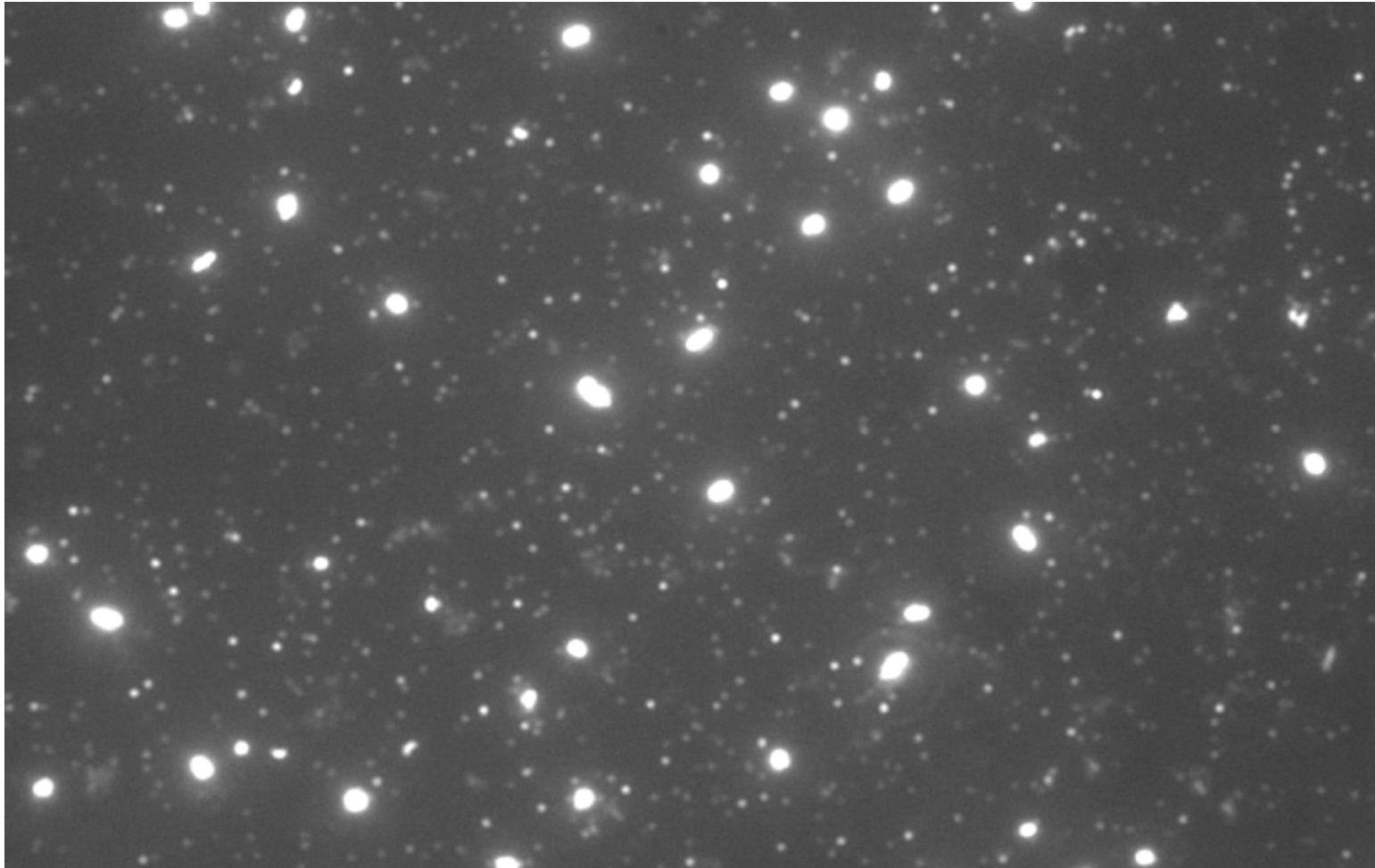
**PCR testing for human pathogens
known to be transmitted via the fecal-
oral route**



Step 2: Look for unknown viruses

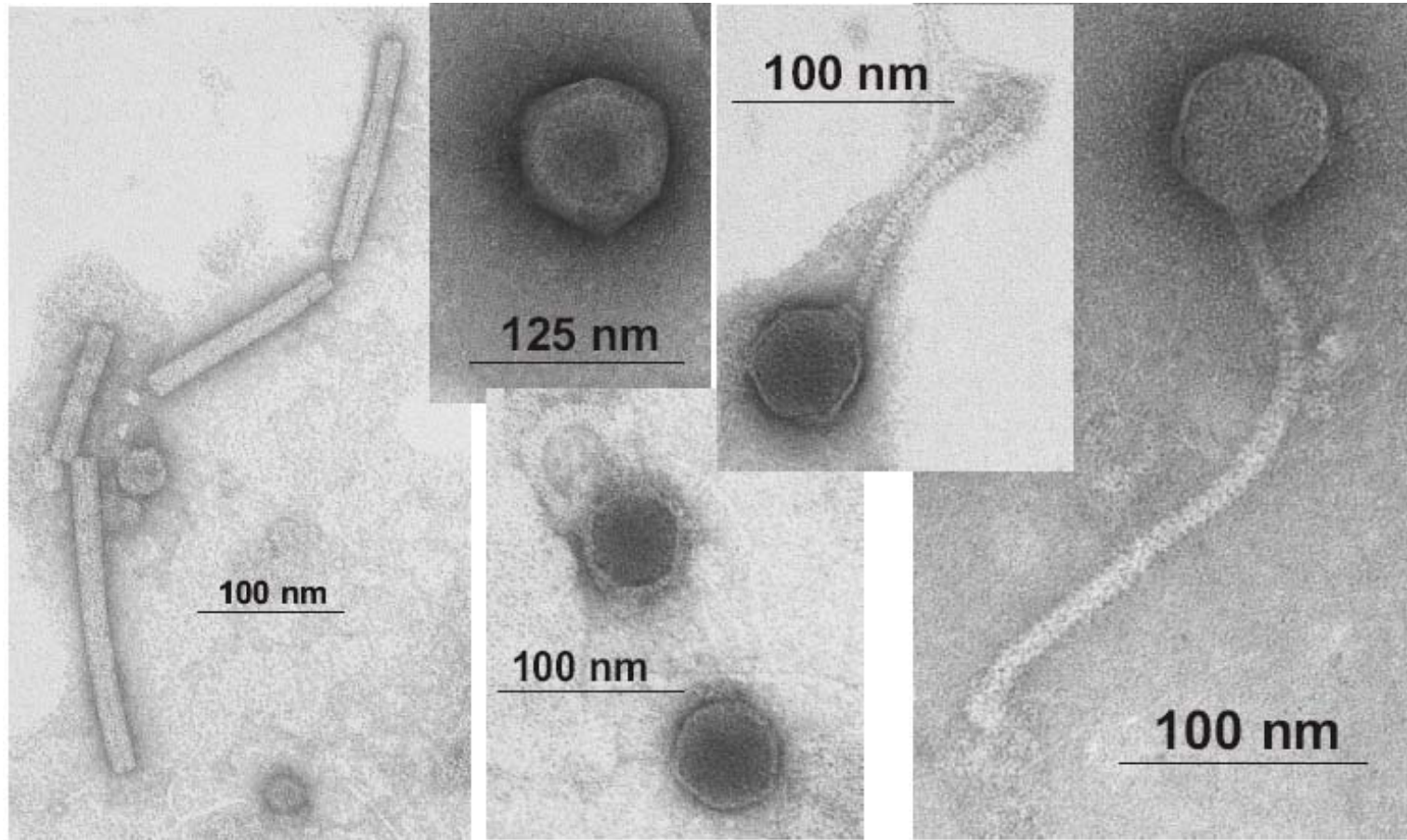
**How can you find something if you
don't know what you're looking for?**

Metagenomics: The sequence-based analysis of the collective genomes contained in a sample



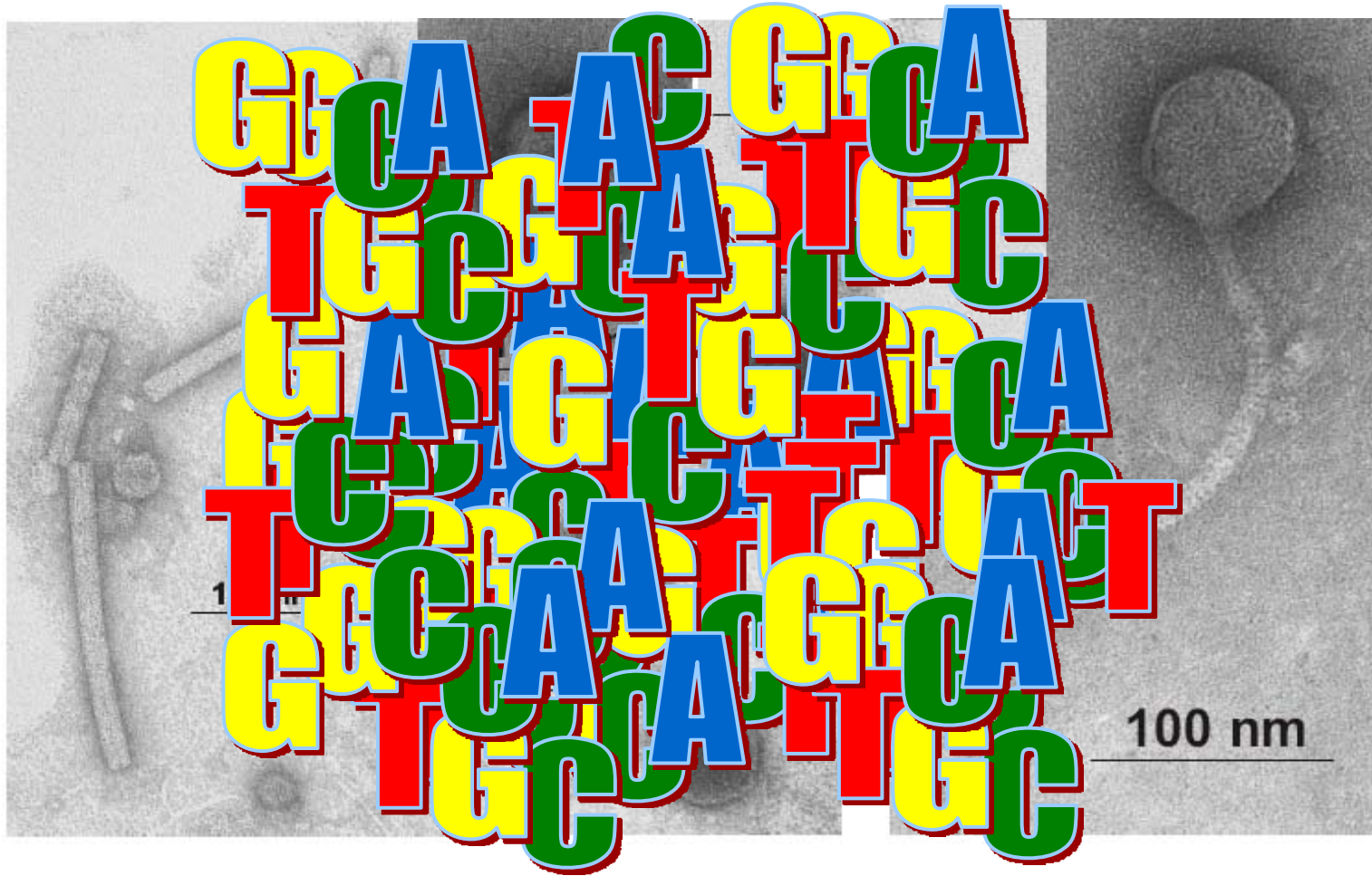
Description of the Complete Viral Community

Metagenomics: The sequence-based analysis of the collective genomes contained in a sample



Description of the Complete Viral Community

Metagenomics: The sequence-based analysis of the collective genomes contained in a sample



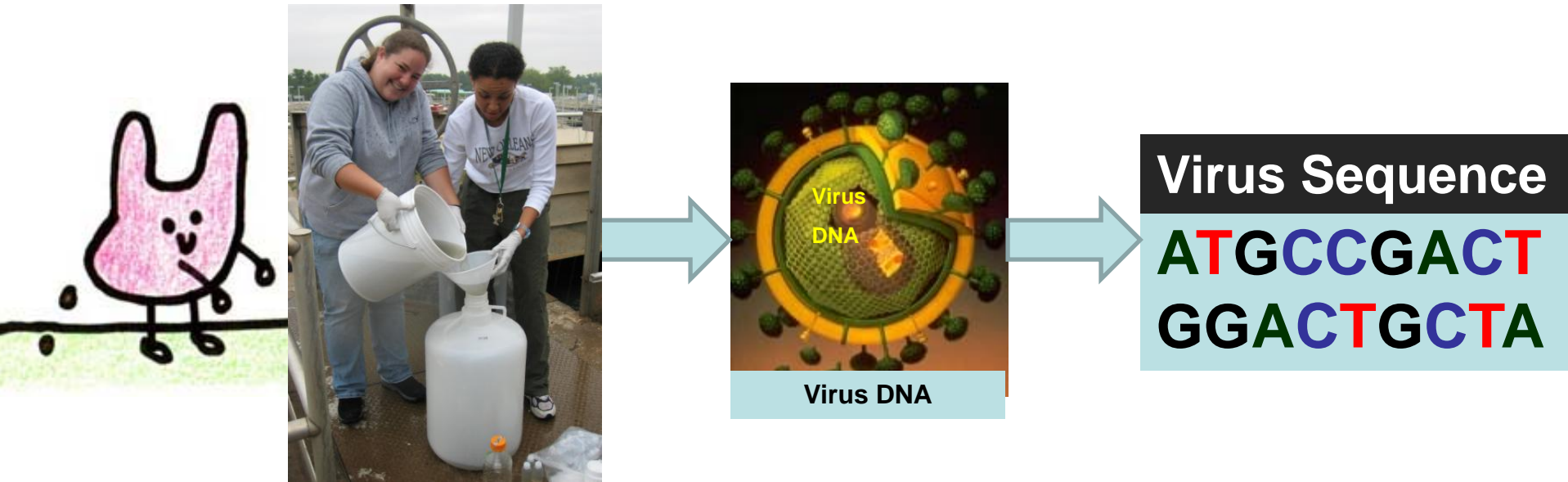
Description of the Complete Viral Community

Viral Metagenomics:

A Novel Method to Discover Novel Viruses

To examine the complete viral community

1. Purify viruses from all other DNA/RNA based on physical properties
2. Extract DNA/RNA from viruses
3. Chop up DNA/RNA and sequence



Metagenomic Sequencing of Reclaimed Water Viruses

Lots of novel sequences (>55% unknown)!

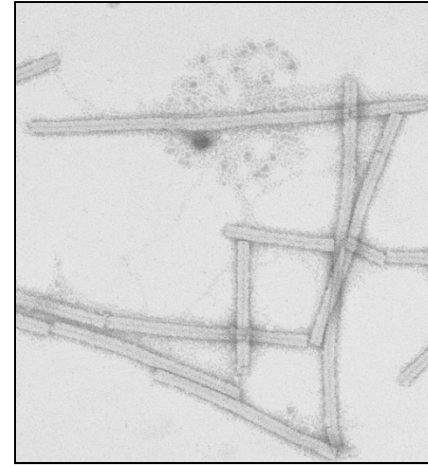
- Most identifiable DNA virus sequences are:
98%
 - Bacteriophage (viruses that infect bacteria)
 - Animal (Non-human) Viruses
 - Plant Viruses
- Most identifiable RNA virus sequences are eukaryotic viruses:
22%
 - Insect Viruses14%
 - Plant Viruses4%
 - Human Viruses

Viruses Found in Reclaimed Water

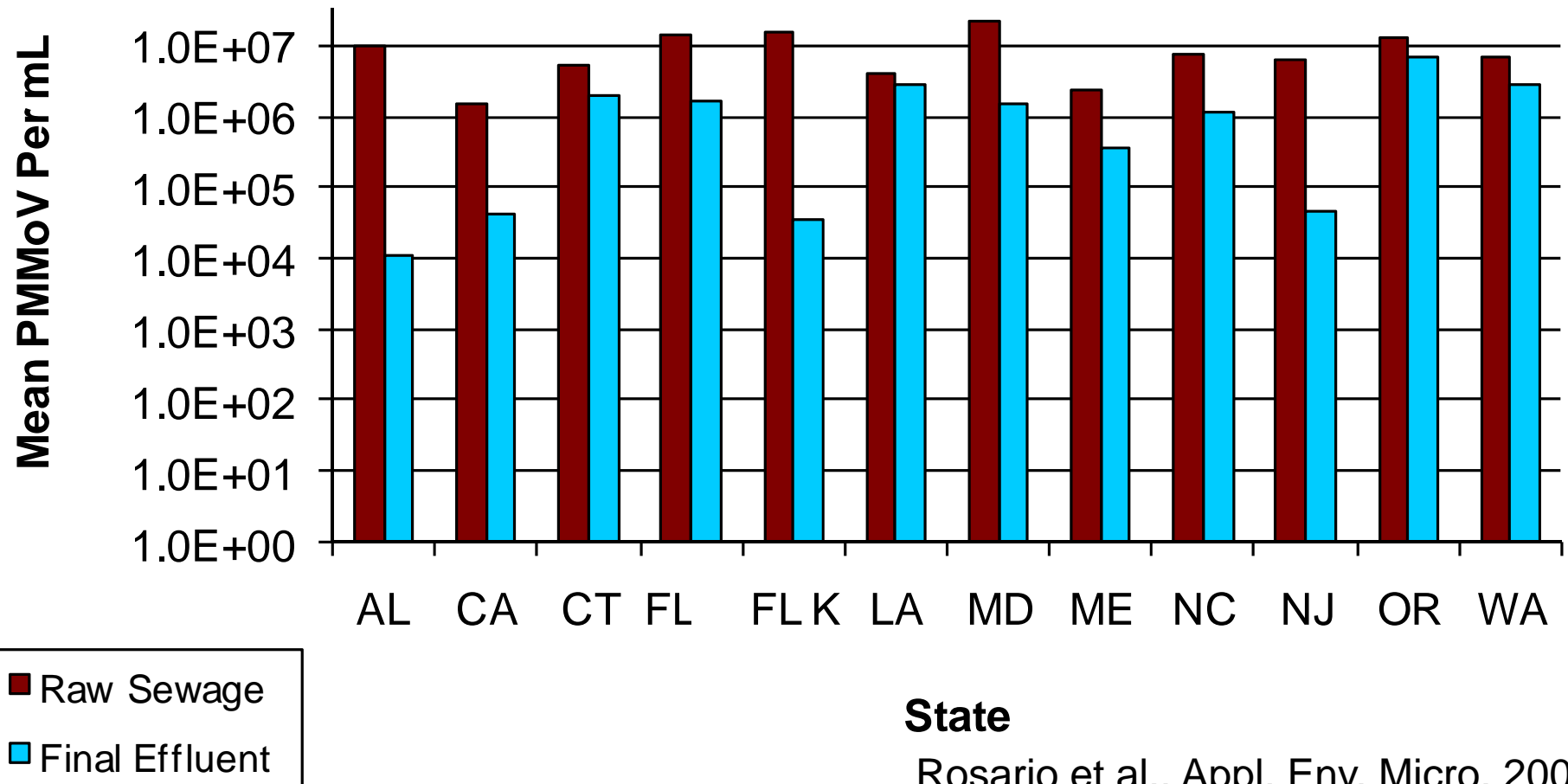
Virus Host	Types of viral homologs	Identity Range (aa)
Humans	Rhinoviruses and enteric viruses (<i>Picornaviridae</i>)	25% - 54%
Animals	Avian and swine circoviruses (<i>Circoviridae</i>), shrimp viruses (<i>Dicistroviridae</i>)	25% - 56%
Plants	Vegetable, grain, and fruit viruses (<i>Geminiviridae</i> , <i>Nanoviridae</i> , <i>Sequiviridae</i> , Tobamovirus genus)	21% - 100%
Insects	Bee, cricket, and aphid viruses (<i>Dicistroviridae</i>)	22% - 74%

Pepper Mild Mottle Virus (PMMoV)

- RNA virus that infect peppers, causes fruit malformation and mottling
- Dominates the RNA viral community in human feces
- 10^6 - 10^9 PMMoV per gram dry weight feces
- Dietary in origin (found in processed pepper foods)
- Still infectious to plants after passage through the human gut

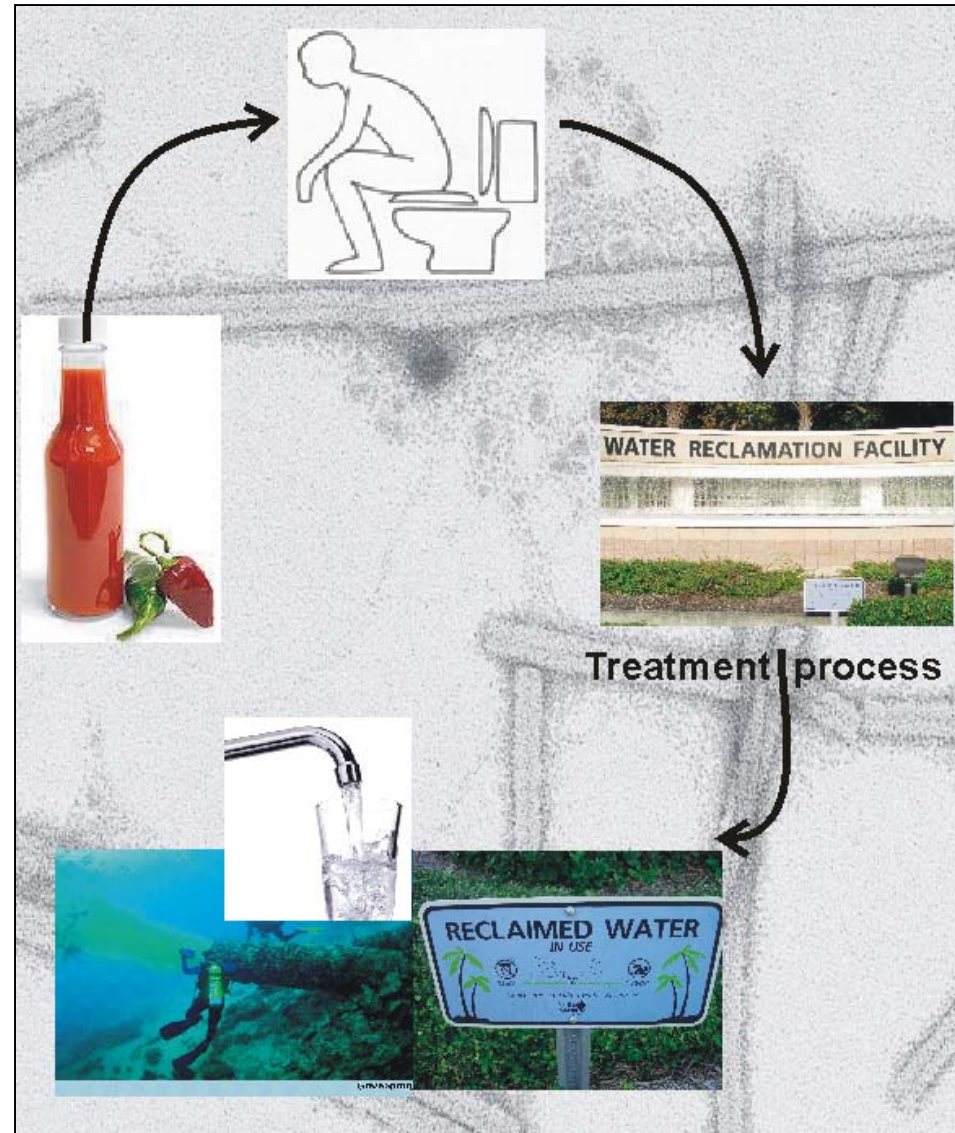


PMMoV is Present at High Concentrations in Raw Sewage and Treated Wastewater From Throughout the United States



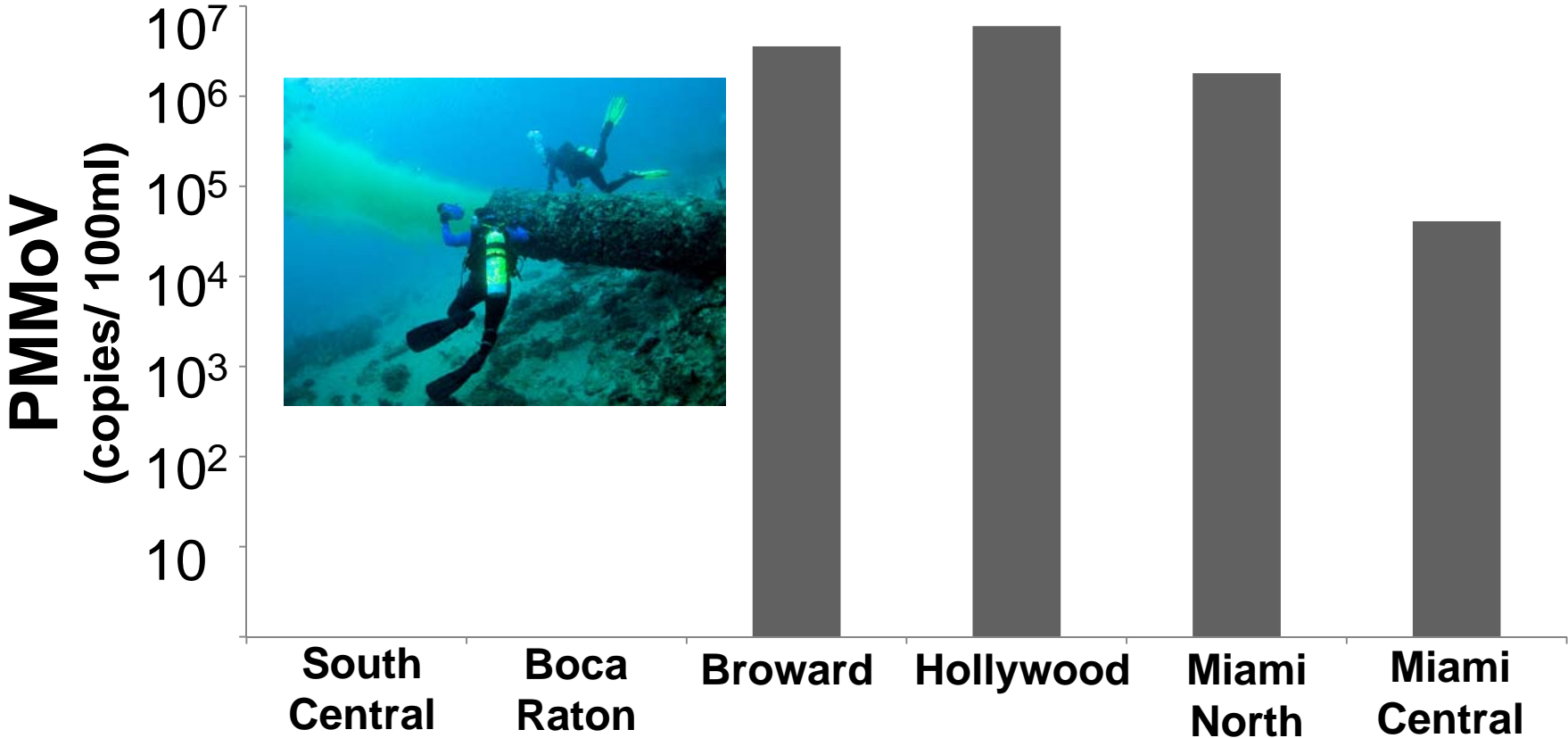
PMMoV as an Indicator of Fecal Pollution

- PMMoV is consistently found at high concentrations in human sewage and treated wastewater
- PMMoV is not dependent on active human infection
- The dietary origin of PMMoV makes it useful for source tracking
(absent in dogs, cows, horses & pigs, but present in chickens/gulls)

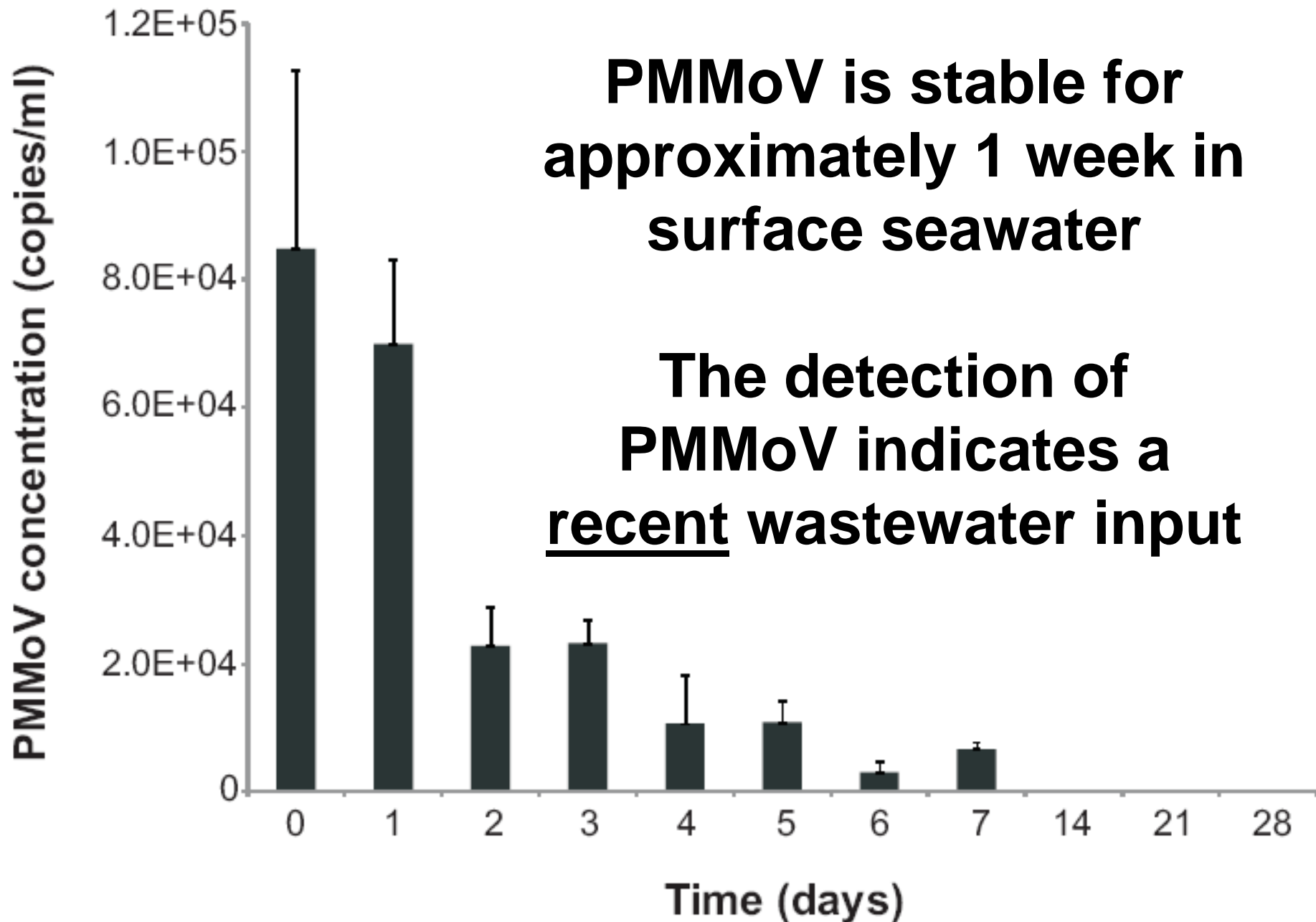


PMMoV Co-occurs with Pathogens & Indicators in Marine Environments Exposed to Wastewater

Rosario et al., Appl. Env. Micro. 2009



Enteroccoci/100ml	-	-	-	+	+	+
Norovirus/100ml	-	2	6	240	350	10
Adenovirus	-	-	-	+	+	-
Cryptosporidium/100L	4	-	10	90	16	210
Giardia/100L	5	-	20	115	120	160



Conclusions

- Viruses are abundant in reclaimed water
 - Lots of novel viruses
 - Most sequences are similar to bacteriophage (different types than are present in potable water)
 - Plant viruses and insect viruses are abundant
- The plant virus PMMoV may be a good indicator of fecal pollution and wastewater treatment efficiency
- It is critical to understand the viral load (identity and infectivity) in reclaimed water – both for public health, and the health of the environment



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<http://www.marine.usf.edu/genomics>

Metagenomics

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References:

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- Zhang, T, M Breitbart, WH Lee, J-Q Run, CL Wei, SWL Soh, ML Hibberd, E Liu, F Rohwer, Y Ruan (2006) RNA viral community in human feces: Prevalence of plant pathogenic viruses. *PLoS Biology*. 4: e3.