Viral Metagenomic Analysis Workflow for Characterization of Particle-Associated Virus Community in Wastewater

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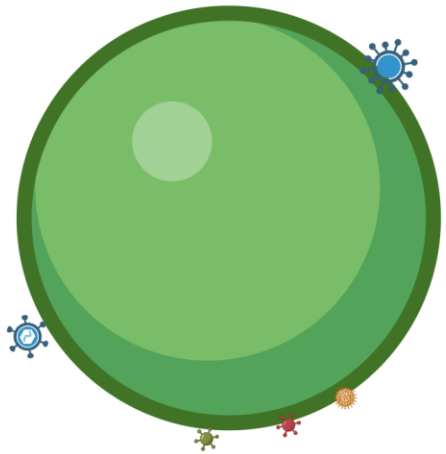
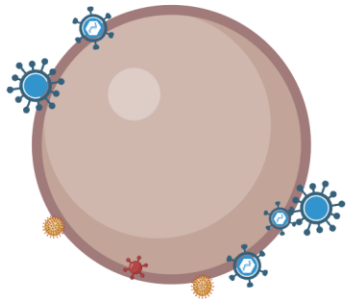
Why study particle associated viruses

- Interact with a board range of inorganic and organic particles
- Enhance their transmission, survival, and infection
- Wastewater acts as a significant reservoir of human enteric viruses
- Treated wastewater has a profound impact on natural aquatic environments and human health
- Especially when reused

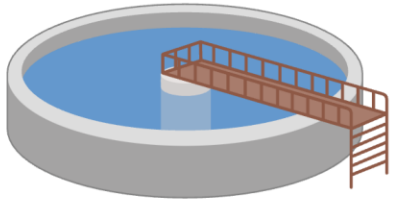
crAssphage
Pepper mild mottle virus (PMMoV)
MS2
adenovirus
enterovirus
norovirus



large settable particles

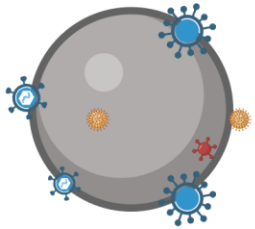


large suspensible particles



Secondary effluents for water reuse

large to small particle sizes



small suspensible particles

colloid particles

small particles, vesicle virus and free viruses

Previously

- particle-associated viruses (PAVs) studies were predominantly conducted in the lab scale
- often employed spiked virus surrogates
- detect with culture method, qPCR, or dPCR

Aims

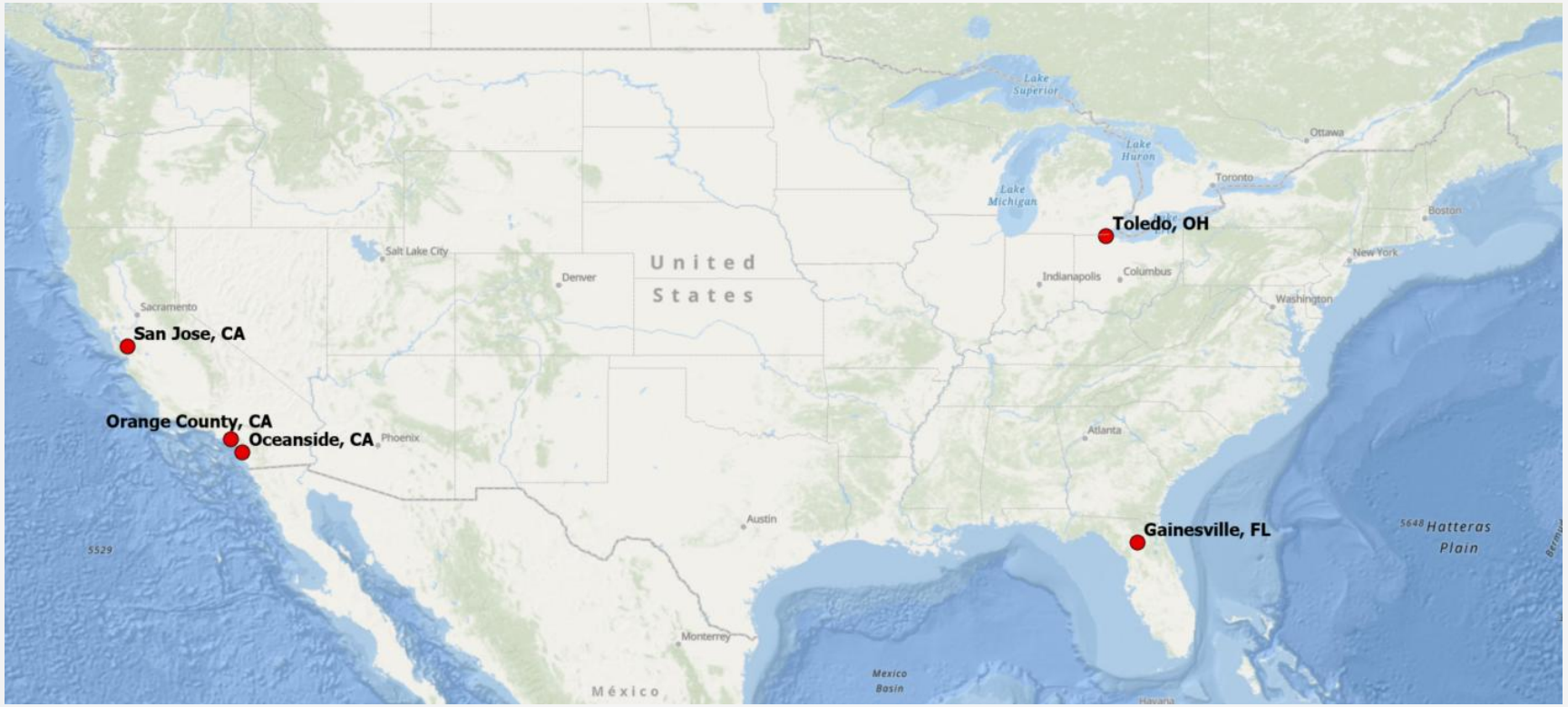
1. develop a sequencing and bioinformatic workflow to streamline viral metagenomic analysis
2. characterize the communities of particle-associated viruses in wastewater using metagenomics.
3. investigate novel viral indicator from treated wastewater for water reuse.

Methods

WSU

Sample collection

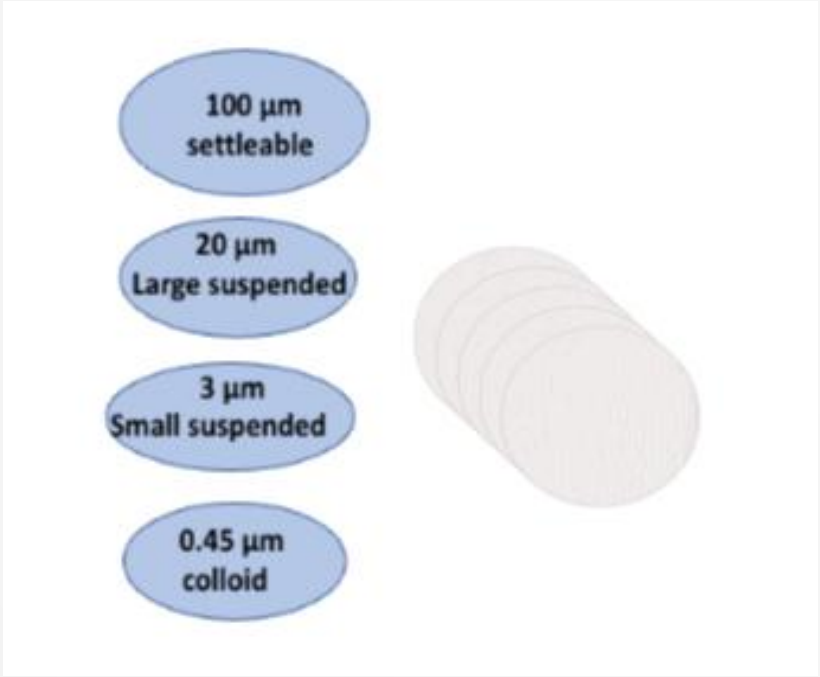
Secondary treated wastewater effluent samples (n = 25) from five full-scale water reuse facilities were collected (CA, FL, OH).



Methods

Sample processing

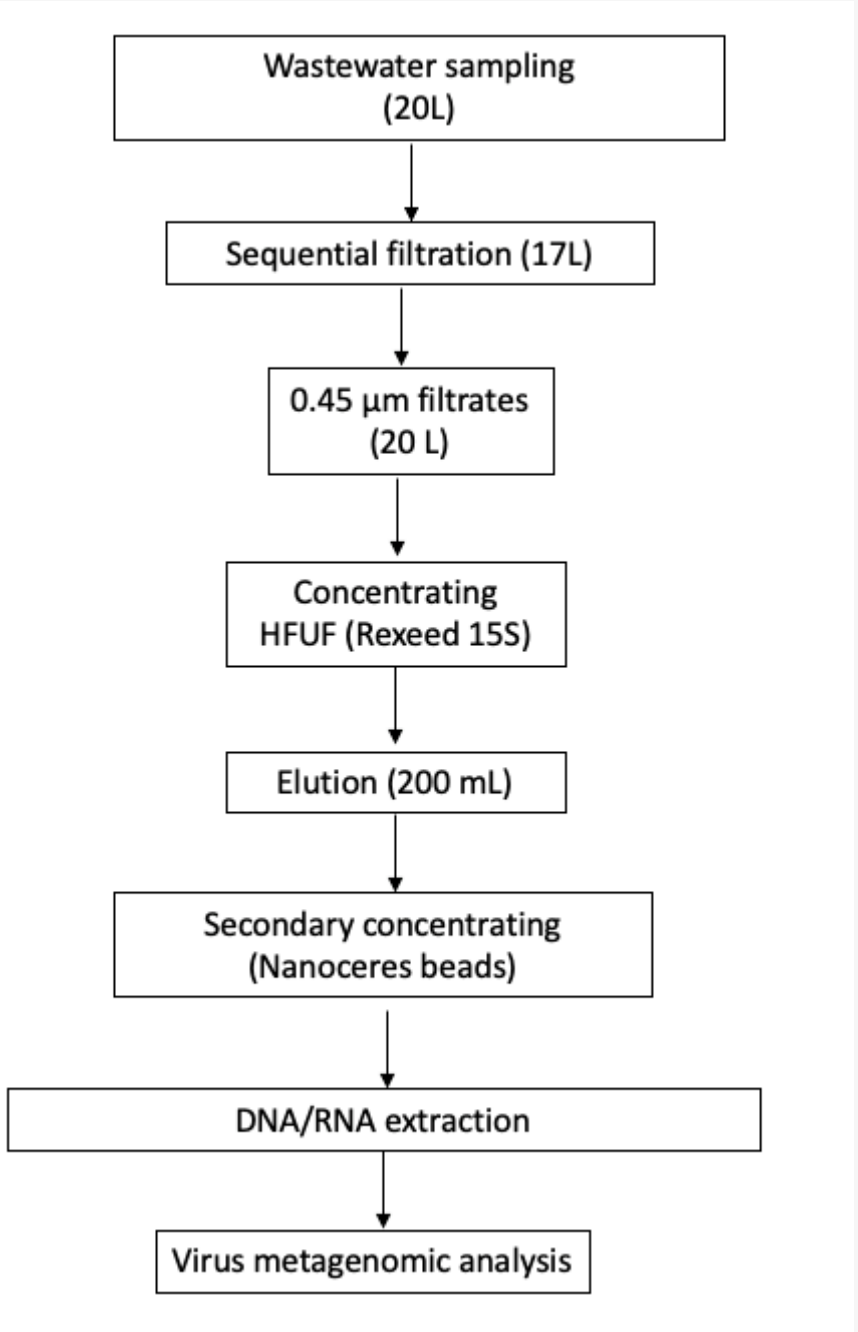
Sequential filtration treatments to enrich the viruses with intact capsid, and extraction using NanoCeres Beads and QIAGEN Allprep Viral kit



Methods

Sample processing flowchart

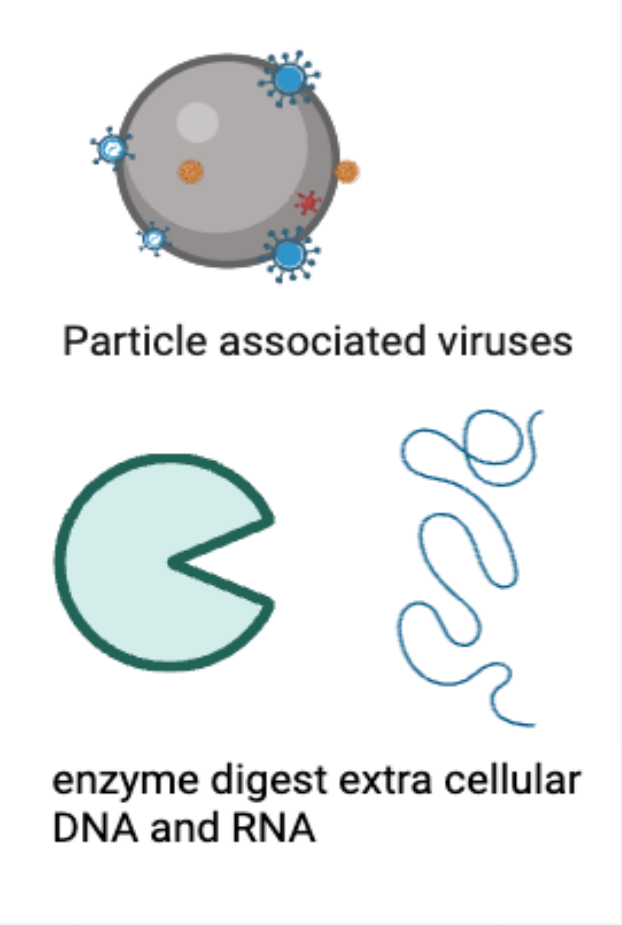
- 5 sampling events were included in this study and in total 25 samples were studied. Technical duplicates include filter pore sizes 100 µm, 20 µm, 3 µm, 0.45 µm, and filtrate for each sampling events.

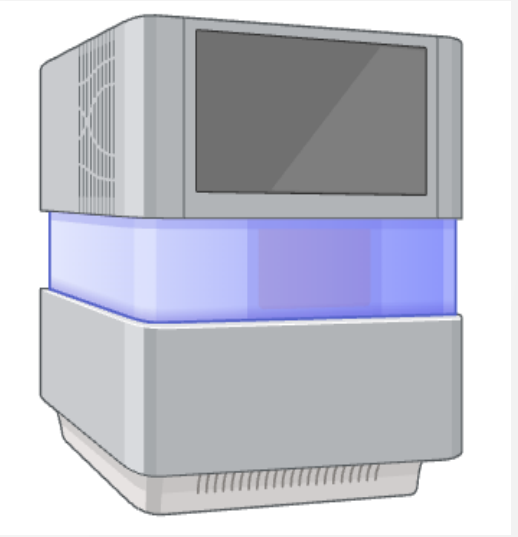


Methods

Library preparation

For RNA library, a DNase treatment was performed on the extracted nucleic, using KAPA Hyperplus kit and sequenced on Nextseq 2000.

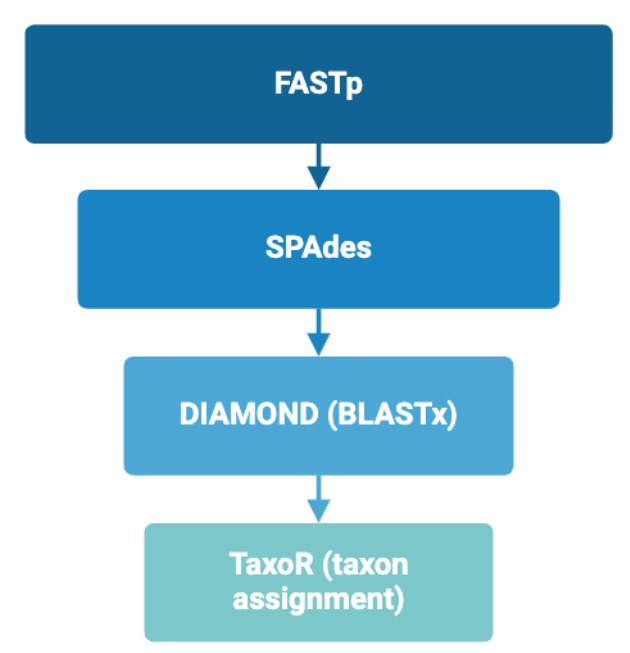




Methods

Metagenomic analysis

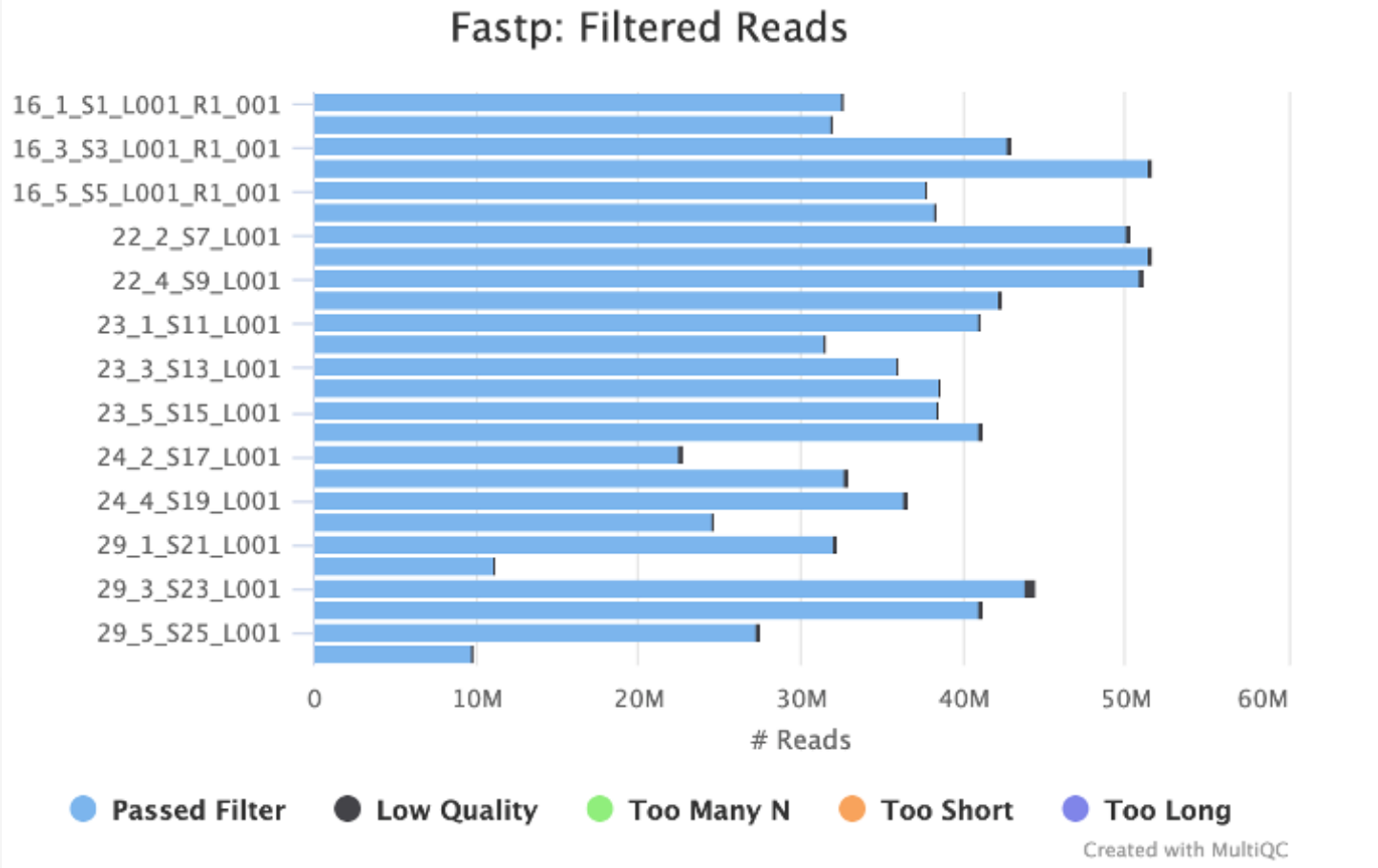
Sequencing reads were processed by a metagenomic workflow managed by Snakmake, including trimming and quality control (fastp), de-novo assembly (SPAdes and QUAst), virus classification and binning (Virsorter2 and CheckV), reference genome alignment (DIAMOND and BLASTx), and annotation (R-taxon package).



Results

Sequencing reads and quality

Reads after quality control. On average 40 million reads were generated and over 98% of the reads passed quality control for each sample.



Results

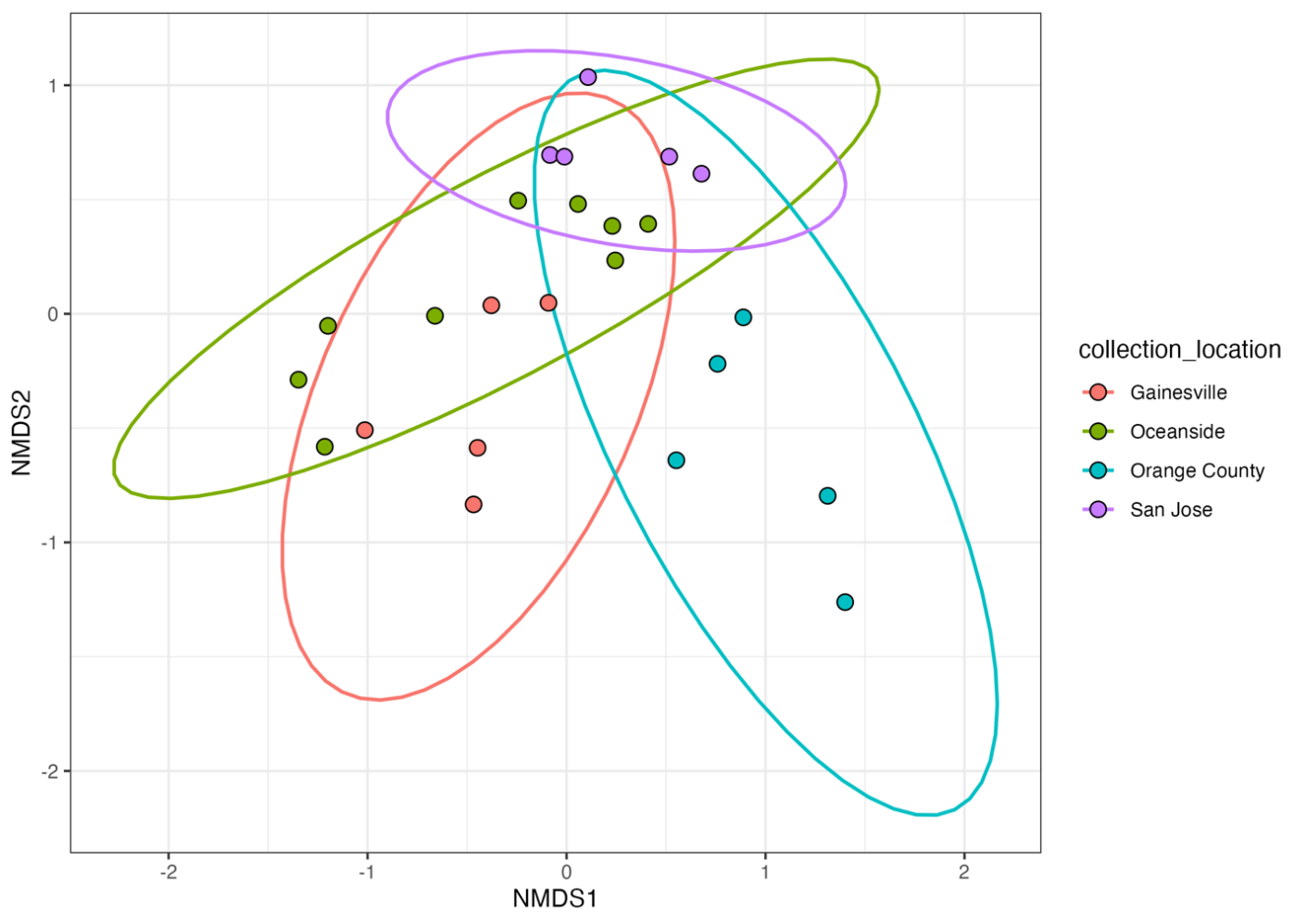
Sequence assembly and annotation

Reads after quality control. Virus contigs were filtered by DIAMOND blastx, VirSorter2, and CheckV. Results sorted by family level

Results

virus community analysis

Non-Metric Dimensional Scaling (NMDS) diagram for samples (family level) from different locations. Ellipse size 0.75 was used in the plot to represent the clusters and groups. Samples from San Jose indicate less variability and more homogeneous virus family, which is significantly different from Orange County samples by PERMANOVA analysis.



Conclusion

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This study provides insights on particles-associated viruses in secondary treated wastewater which is the source water for reclamation.

- **Enzyme Treatment and RNA Virus Yield:**
  - The treatement of samples with a cocktail of enzymes to degrade extracellular nucleic acids prior to extraction, and then used a targeted enzyme treatment to remove contaminating DNA after extraction. This meticulous enzymatic approach significantly improved the yield of RNA viruses from the wastewater samples, allowing for a more comprehensive analysis of the viral community present.
- **Bioinformatics Pipeline for Viral Indicator Genome Assessment**:
  - Leveraging this pipeline, we able to analyze a larger sample size efficiently, providing insights into the diversity and abundance of viruses present in the wastewater. This scalable approach enabled the identification of specific viral indicators associated with wastewater treatment processes, aiding in the evaluation of water reclamation systems for potential reuse.

Conclusion

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- **Novel viral indicators for water reuse**
  - A robust metagenomic analysis can guide the development of novel viral indicators and surrogates for assessing the performance of wastewater treatment and water reuse processes.
- **Prediction of novel viruses based on artificial intelligence**
- **Plant viruses abundant** in wastewater
  - their implication on water reuse, especially for irrigation
- **Open science**:
  - Metagenomic tools and bioinformatic pipelines

Questions

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