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Pathogen Monitoring in Untreated Wastewater

CALIFORNIA Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS

THE METROPOLITAN WATER DISTRICT OF SOUTHERN CALIFORNIA

Pathogen Monitoring in Untreated Wastewater

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THE Water Research FOUNDATION

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Abstract and Benefits

Abstract:
The California State Water Resources Control Board (SWB) is currently developing statewide regulations for direct potable reuse (DPR). To support this effort, the SWB is undertaking research projects aimed at addressing six critical knowledge gaps. This report focuses on the second of these DPR research projects (DPR-2), which seeks to develop and implement an optimized pathogen monitoring Standard Operating Protocol (SOP) to better characterize the concentration of human pathogens in raw wastewater. To accomplish these tasks, the DPR-2 Technical Work Group reviewed the literature to inform a quality assurance project plan (QAPP) that builds off of and addresses several shortcomings of past studies. Methods to detect relevant waterborne pathogens in raw wastewater were optimized prior to the full-scale campaign and are described in the QAPP. During the 14-month monitoring campaign, over 120 samples were collected from five wastewater treatment plants in California. The samples were analyzed for two protozoa (*Cryptosporidium* and *Giardia*) using microscopy methods, three enteric viruses (enterovirus, adenovirus, and norovirus) using culture and/or molecular methods, one enveloped virus (SARS-CoV-2) using molecular methods, and male-specific coliphage using culture methods. The method recovery efficiency was measured in either every sample (protozoa and enveloped virus) or every other sample (enteric virus), providing the ability to confirm minimum recoveries were achieved and correct the concentrations for organism losses during sample processing. The results from this study provide the industry with a large, and high-quality dataset, as demonstrated by the high degree of method sensitivity, method recovery, and frequency of recovery measurement. High-quality data on pathogen concentrations in raw wastewater is critical for confirming the level of treatment needed to reduce pathogen concentrations down to acceptable levels for potable water in DPR projects. Recommendations on how these data should be used by the SWB are provided.

Benefits:

- Developed optimized analytical methods for detection of protozoa and enteric virus in raw wastewater
- Provides the industry with a large, high-quality dataset of relevant waterborne pathogen concentrations in raw wastewater
- Dataset will be used by the SWB to confirm the log removal values necessary to adequately protect public health in direct potable reuse projects

Keywords: pathogen monitoring, wastewater, potable reuse, methods, risk

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Acronyms and Abbreviations

AdV	Adenovirus
BCS	Biological Consulting Services
CDC	Centers for Disease Control and Prevention
COVID-19	Disease caused by infection with SARS-CoV-2
DPR	Direct potable reuse
DPR-2	Direct Potable Reuse Research Project #2
EPA	Environmental Protection Agency
EV	Enterovirus
GC	Genome copies
IPR	Indirect potable reuse
LACSD	Los Angeles County Sanitation Districts
LASAN	City of Los Angeles Sanitation and Environment
MPN	Most probably number
ND	Non-detect
NoV	Norovirus
OCSD	Orange County Sanitation District
PATTP	Probabilistic assessment of treatment train performance
PEG	Polyethylene glycol
QAPP	Quality Assurance Project Plan
QA/QC	Quality assurance/quality control
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
RFQ	Request for Qualifications
SD	City of San Diego
SFPUC	San Francisco Public Utilities Commission
SOP	Standard operating procedures
SWB	California State Water Resources Control Board
TWG	Technical work group
WHO	World Health Organization
WWTP	Wastewater treatment plant

CHAPTER 1

Background

The California State Water Resources Control Board (SWB) has recommended research be conducted to address knowledge gaps for developing criteria for Direct Potable Reuse (DPR). Six research project have been identified to address these gaps (Olivieri et al. 2016). The focus of the second of these research projects, DPR-2, is to assess the concentration of relevant pathogens in raw wastewater via a year-long monitoring campaign. The two principal objectives of DPR-2 are:

- To provide the SWB with better empirical data on the concentration and variability of pathogens in raw wastewater for the purpose of verifying log removal values necessary to adequately protect public health in DPR projects
- To develop recommendations for the collection and analysis of pathogen data in raw wastewater that may be used in future monitoring efforts

To accomplish these objectives, four project phases for DPR-2 were identified:

- Phase 1 – Literature and Methods Review
- Phase 2 – Develop Monitoring Plan and RFQ
- Phase 3 – Conduct Pathogen Monitoring
- Phase 4 – Data Analysis and Preparation of Guidance

A Technical Work Group (TWG) was established to develop the project deliverables and provide oversight of the year-long monitoring campaign. The deliverables from these four project phases are summarized in the following chapters of this report:

- Chapter 2. Literature and Methods Review
- Chapter 3. Results from Methods Pre-testing
- Chapter 4. Project Plan
- Chapter 5. Pathogen Monitoring Campaign Results
- Chapter 6. SARS-CoV-2 Monitoring
- Chapter 7. Recommendations

CHAPTER 2

Literature and Method Review

The first phase of this project was to identify which pathogens should be monitored, what data from the literature could be leveraged to inform the project plan, and which methods should be used to detect the pathogens in the monitoring campaign. These three steps and their outcomes are discussed in the Literature and Methods Review (Pecson et al. 2021). The highlights from this Literature and Methods Review are summarized below.

2.1 Selection of Pathogens and Indicator Organisms

The first step of DPR-2 was to identify the target pathogens and/or indicator organisms that should be prioritized for the monitoring study and for potential use in DPR regulatory development. The pathogens included in previous regulations for the Surface Water Treatment Rules (SWTR) and indirect potable reuse (IPR) are enteric virus, *Giardia*, and *Cryptosporidium.* The rationale behind these pathogens in the regulations was to select pathogens that are:

Representative of the different types of pathogens that occur in water

- Known to pose a high health burden in the US (based both on infectivity and occurrence)
- Relatively resistant to treatment such that they correspond to sufficiently conservative treatment requirements

The TWG decided this rationale was appropriate and agreed that these pathogens should be considered for the DPR regulations as well. For the monitoring campaign, the TWG broadened the field to supplement traditional virus monitoring (enterovirus) to also include norovirus and adenovirus. Furthermore, the TWG decided to supplement traditional enumeration methods (culture and microscopy) with molecular enumeration techniques, namely, quantitative polymerase chain reaction (qPCR). Finally, the TWG decided to include male-specific coliphage in the list of organisms to monitor due to its historic importance as an indicator organism and relative ease of measurement. The organisms and quantification approaches included in the DPR-2 pathogen monitoring campaign are summarized in Table 2-1.

Table 2-1. Pathogens and Indicator Organisms Selected for the DPR-2 Pathogen Monitoring Campaign.

Pathogen	Quantification Approach
Cryptosporidium	Microscopy
Giardia	Microscopy
Enterovirus	Culture
Enterovirus	Molecular
Adenovirus	Culture
Adenovirus	Molecular
Norovirus (GIA, GIB, GII)	Molecular
Male-specific coliphage	Culture

2.2 Literature Review

The literature review was used to compile relevant past studies that have monitored for the pathogens of interest (determined in Section 2.1) in raw wastewater. The compiled studies were analyzed to:

- Develop a dataset of past results to compare to the DPR-2 findings
- Identify shortcomings in past studies to inform the project plan for DPR-2

To compile the relevant past studies, the TWG began by evaluating four recent literature reviews that included summaries of pathogen monitoring studies (Trussell et al. 2013, Cooper et al. 2012, Bambic et al. 2011, Haramoto et al. 2018). Through these reviews over 100 studies were identified that measured pathogens in raw wastewater. A set of screening criteria was developed to determine which of these papers were most relevant. The screening criteria included:

- Type of literature: primary literature exclusively
- Study matrix: must be domestic or mostly raw wastewater
- Study location: in the United States or Canada
- Study date: must be later than 2000 for protozoa due to major methods improvements

14 of these 100 papers passed these screening criteria. Two of the papers were a discussion of the same dataset and another one of the studies did not use quantitative methods, narrowing the list down to 12 papers. The list of 12 papers was supplemented with two studies in Norway and Australia that were used for the development of California's indirect potable reuse regulations and several recent pathogen monitoring studies in California, resulting in a total of 17 relevant studies. The pathogen concentrations measured in each of these 17 studies are plotted and compared to the concentrations measured in the DPR-2 monitoring campaign in Chapter 5. Chapter 5 also discusses whether the results from any of these studies should be included to supplement the DPR-2 dataset.

The literature review provided confidence to the TWG that it is feasible to achieve the goals of DPR-2 (i.e., quantify the distribution of concentrations of pathogens in raw wastewater), though adaptations to the existing methods were recommended to improve the method sensitivity. Additionally, one of the keys elements that was missing from most of the studies was method recovery, which was reported in only a fraction of the studies. Knowing the method recovery is critical for both determining the *actual* concentration of pathogens (rather than the relative concentration) and is also important for quality control. Therefore, along with method modifications to improve sensitivity, quantifying method recovery was one of the main improvements that the DPR-2 study used in relation to these past studies.

2.3 Methods Review and Recommendations

The goal of the methods review was to summarize the analytical methods that are available for quantifying pathogen concentrations in water/wastewater and propose method modifications to address the challenges identified in Section 2.1. The recommended methods and proposed method modifications for monitoring the pathogens of interest in raw wastewater are summarized in Table 2-2. The TWG recommended using EPA standard methods when available and methods from the literature when EPA methods were not available. Because the EPA standard methods were not designed for use with raw wastewater, modifications were proposed to increase their suitability and sensitivity with raw wastewater. The proposed modifications were evaluated in the method pre-testing, described in Chapter 3.

Additionally, this method review focused on identifying the quality control provisions that are important for providing high quality data. The TWG agreed that the quality control provisions that are part of the

EPA standard methods (e.g., positive control, negative controls, inhibition controls) should be included in each of the methods for this study. Additionally, appropriate matrix spike surrogates should be included in all or most samples to determine the method recovery (Table 2-2). A matrix spike is generated by adding a known amount (a spike) of a microorganism to a sample, testing the spiked sample, and determining the fraction that was recovered. Matrix spikes help to assess for variability in recovery between samples due to differences in the wastewater matrix itself, sample processing, etc. ColorSeed was selected as the matrix spike surrogate to assess *Cryptosporidium* and *Giardia* recovery. Two phages, MS2 and PhiX174, were selected as the matrix spike surrogates to assess enterovirus, adenovirus, and norovirus recovery.

Table 2-2. Recommended Methods for DPR-2 Pathogen Monitoring.

Pathogen	Quantification Approach	Standard Method	Modifications to Evaluate	Matrix Spike
Cryptosporidium	Microscopy	EPA 1693	Evaluate different sample volumes and pellet volumes; Compare direct centrifugation vs. filtration	ColorSeed
Giardia	Microscopy	EPA 1693	Evaluate different sample volumes and pellet volumes; Compare direct centrifugation vs. filtration	ColorSeed
Enterovirus	Culture	EPA 1615	Try lower sample volume; PEG precipitation instead of filtration & organic flocculation	MS2 and PhiX174[1]
Enterovirus	Molecular	EPA 1615	Try lower sample volume; PEG precipitation instead of filtration & organic flocculation	MS2 and PhiX174[1]
Adenovirus	Culture	No standard method	Use sample concentrate that is produced for EV and NoV analysis; enumerate using A459 (Rigotto 2011)	MS2 and PhiX174[1]
Adenovirus	Molecular	No standard method	Use sample concentrate that is produced for EV and NoV analysis; enumerate using primer/probes from Ko 2005	MS2 and PhiX174[1]
Norovirus (GIA, GIB, GII)	Molecular	EPA 1615	Try lower sample volume; PEG precipitation instead of filtration & organic flocculation	MS2 and PhiX174[1]
Male-specific coliphage	Culture	EPA 1602		MS2 and PhiX174[1]

[1]After sample is concentrated, the matrix spike will be enumerated by culture methods using 1602 and molecular methods using primer/ probe sets from Turgeon et al. (2014)

CHAPTER 3

Results from Methods Pre-testing

Based on the methods review discussed in Chapter 2, the methods proposed in Section 2.3 were tested prior to the full-scale monitoring campaign. The goal of the methods pre-testing was to accomplish the following two objectives:

1. Identify the method modifications that provide sufficiently high method sensitivity to minimize the frequency of non-detect values.
2. Develop initial estimates for the recovery efficiency and the concentration of the pathogens in raw wastewater to help guide the development of the QA/QC acceptance criteria.

The methods pre-testing was accomplished in two phases. The first phase, completed by Biovir Laboratories in Spring 2019, involved optimization of the protozoa method and initial testing of the virus method. Additional optimization of the virus method was completed in the second phase of the pre-testing in Fall 2019 by BCS Laboratories.

3.1 Phase 1: Protozoa Method Optimization

To optimize the protozoa method, one sample was analyzed in triplicate using six modified versions of EPA 1693. The six method versions included testing of three sample volumes (100 mL, 500 mL, and 1000 mL) and two concentration approaches (centrifugation alone and filtration followed by centrifugation). The method recovery was determined in each sample replicate using ColorSeed as a matrix spike. The combination of volume and concentration that produced the highest sensitivity and recovery was then verified on a second sample (analyzed in triplicate) from a different wastewater treatment plant (WWTP). The results from this testing are summarized in Table 3-1 and the method with the optimal combination of conditions is highlighted in green.

The method options were evaluated based on the matrix spike recovery efficiency and their ability to obtain detectable *Cryptosporidium* and *Giardia* concentrations. The only sample volume that gave detectable *Cryptosporidium* concentrations was 1000 mL. Both the direct centrifugation and the filtration method resulted in detectable *Cryptosporidium* concentrations at a sample volume of 1000 mL and had similar *Cryptosporidium* ColorSeed recoveries. However, the direct centrifugation method was considered preferable over the filtration method for the following reasons:

- The direct centrifugation method had better *Giardia* ColorSeed recovery at the 1000 mL sample volume than the filtration method.
- The direct centrifugation method is less labor intensive than the filtration method.
- The pellet volume resulting from the direct centrifugation method scaled linearly with the sample volume while the pellet volume resulting from the filtration method did not scale linearly. Therefore, there was concern that the filtration method may result in sample loss at higher sample volumes.

Additionally, based on the results of this testing, the TWG recommended that a pellet volume of 4 mL (instead of 2 mL described in EPA 1693) be analyzed to ensure consistent detects of *Cryptosporidium* and *Giardia*. **In summary, the TWG recommended the following three key modifications to EPA 1693 for detection of *Cryptosporidium* and *Giardia* in raw wastewater:**

- Sample volume of 1000 mL
- Direct centrifugation to concentrate the sample
- Analysis of at least 4 mL of pellet volume

Table 3-1. Results from Methods Pre-testing Phase 1: Optimization of EPA 1693 for Measuring *Cryptosporidium* and *Giardia* in Raw Wastewater.

Sample	Volume and Method	Vol. of pellet produced [analyzed] (mL)	*Crypto.* Recovery (%)	*Crypto.* Conc. (oocyst/L)	*Giardia* Recovery (%)	*Giardia* Conc. (cyst/L)
SD 3/29/19	100 mL Centrifugation	1.0 [1.0]	82 [81 - 84]	<10 [<10]	40 [22 - 59]	1477 [1380 - 1630]
SD 3/29/19	100 mL Filtration	1.0 [1.0]	78 [70 - 85]	<10 [<10]	43 [38 - 51]	1510 [1370 - 1630]
SD 3/29/19	500 mL Centrifugation	4.0 – 5.0 [2.0]	69 [65 - 73]	<4 [<4 - <5]	7 [4 - 10]	516 [484 - 564]
SD 3/29/19	500 mL Filtration	3.0 – 4.0 [2.0]	74 [67 - 80]	<3 [<3 - <4]	8 [2 - 12]	556 [405 - 660]
SD 3/29/19	1000 mL Centrifugation	9.0 [2.0 – 4.0]	70 [50 - 86]	8 [5 - 9]	26 [9 - 47]	1021 [603 - 1427]
SD 3/29/19	1000 mL Filtration	3.0 - 5.0 [2.0]	73 [35 - 104]	3 [3 - 3]	6 [5 - 8]	392 [320 - 443]
LACSD 5/12/19	1000 mL Centrifugation	6.0 [4.0]	53 [45 - 58]	27 [18 -32]	30 [26 - 35]	11147 [10488 - 11606]

Values reported are the average of triplicate measurements. The brackets show the minimum and maximum. Results shown are not corrected for recovery. Samples were analyzed by Biovir Laboratories. The selected optimal method version is highlighted in green. SD = City of San Diego North City Water Reclamation Plant; LACSD = Sanitation District of Los Angeles County Joint Water Pollution Control Plant.

3.2 Phase 2: Virus Method Optimization

The goal of the phase 2 pre-testing was 1) to verify the performance of the methods at the five WWTPs participating in the full-scale monitoring campaign and 2) to optimize the virus methods. The first round of the phase 2 pre-testing involved analyzing a single sample from each of the five WWTPs using the standard operating procedure (SOPs) developed by the TWG (described in the Request for Qualifications (RFQs) from laboratories). The SOPs included the TWG's proposed modifications to the EPA 1615 for virus quantification in raw wastewater, specifically, a sample volume of 1 L and a PEG/chloroform

concentration procedure. The focus of this testing was on optimizing the method sensitivity by improving recovery efficiency. To evaluate this, a known quantity of poliovirus (an enterovirus) was spiked into the sample in excess of the background concentration. In addition to measuring the recovery of the two phage matrix spikes (MS2 and PhiX174), the recovery of the spiked poliovirus was measured. The focus of this pre-testing was on the optimization of the concentration procedure. Consequently, only one enumeration method – culture enumeration – was used rather than both culture and molecular. The first round of the phase 2 pre-testing showed low recovery of PhiX174, MS2, and poliovirus at all five WWTPs (Table 3-2). BCS Laboratories analyzed fractions at different stages of the sample processing procedure and determined that the majority of the virus loss was occurring during the PEG precipitation step. BCS Laboratories suggested increasing the PEG (120 g/ L instead of 96 g/L) and NaCl concentrations (52.6 g/L instead of 28 g/L) and increasing the stirring time with PEG (2 hr instead of 1 hr). In the second round of the testing, one wastewater sample was analyzed using four modified versions of the EPA 1615-based virus SOP (Table 3-2). In addition to testing the higher PEG and NaCl concentration and longer stir times, other modifications included testing citrate/phosphate buffer instead of TRIS/Glycine buffer and filtration through a 0.22 micron filter instead of chloroform extraction. The higher PEG and NaCl concentrations and longer stir times increased the recovery of PhiX174, MS2, and poliovirus by an order of magnitude (with chloroform extraction). The citrate/phosphate buffer provided similar results to the TRIS/Glycine buffer but required less pH adjustment and was therefore deemed preferable. The optimized method, highlighted in green in Table 3-2 was then verified on a third sample and showed similar recoveries (a longer stirring time was tested and showed similar performance). Additionally, this pre-testing indicated that MS2 and PhiX174 are acceptable surrogates since they demonstrated similar recovery to poliovirus.

In summary, the TWG recommended the following three key modifications to EPA 1615 for concentration of raw wastewater for enumeration of enteric virus:

- Sample volume of 1000 mL
- Concentration using PEG precipitation (12% PEG, 0.9 M NaCl, 2-16 hr stirring) and chloroform extraction
- Addition of MS2 and PhiX174 as surrogates to assess recovery

The samples were also analyzed for *Cryptosporidium* and *Giardia* using the optimized protozoa method (data not shown). The recovery of *Cryptosporidium* and *Giardia* was similarly high (30 – 80%) at all five WWTP and so no further optimizations were deemed necessary.

Table 3-2. Results from Method Pre-testing Phase 2: Optimization of EPA 1615 for Measuring Enterovirus, Adenovirus, and Norovirus in Raw Wastewater.

Round	Sample	SOP Modifications	PhiX174 Recovery %	MS2 Recovery %	Poliovirus Recovery %
1	LACSD 10/28/19	As described in DPR-2 RFP	0.4	0.9	0.8
1	SD 10/28/19	As described in DPR-2 RFP	0.4	1.0	2.5
1	LASAN 10/28/19	As described in DPR-2 RFP	0.2	0.7	2.8
1	OCSD 10/29/19	As described in DPR-2 RFP	1.1	0.4	0.4
1	SFPUC 10/30/19	As described in DPR-2 RFP	1.2	1.5	3.3
2	SD 11/04/19	Increased PEG concentration (12%) and NaCl concentration (0.9 M), 2-hr stir, chloroform extraction, TRIS/glycine buffer	11	31	32
2	SD 11/04/19	Increased PEG concentration (12%) and NaCl concentration (0.9 M), 2-hr stir, 0.22 micron filter, TRIS/glycine buffer	2.4	7	0.7
2	SD 11/04/19	Increased PEG concentration (12%) and NaCl concentration (0.9 M), 2-hr stir, chloroform extraction, citrate/phosphate buffer	11	35	32
2	SD 11/04/19	Increased PEG concentration (12%) and NaCl concentration (0.9 M), 0.22 micron filter, citrate/phosphate buffer	2.4	5	0.7
3	SFPUC 11/11/19	Increased PEG concentration (12%) and NaCl concentration (0.9 M), 16-hr stir, chloroform extraction, citrate/phosphate buffer	5.4	28	N/A

Samples were analyzed by BCS Labs. Recoveries were based on culture enumeration. The optimal method version is highlighted in green. LACSD = Los Angeles County Sanitation Districts' Joint Water Pollution Control Plant; SD = City of San Diego North City Water Reclamation Plant; LASAN = City of Los Angeles Sanitation and Environment Hyperion Water Reclamation Plant; OCSD = Orange County Sanitation District Plant 1; SFPUC = San Francisco Public Utilities Commission Southeast Treatment Plant

CHAPTER 4

Project Plan

The next major step in DPR-2 was the development of the DPR-2 full-scale pathogen monitoring campaign project plan, which is detailed in the Quality Assurance Project Plan (QAPP) (Cel Analytical Inc. 2020). The QAPP describes the project organization, sample collection and analysis plan, and the quality controls established for sample collection, analysis, and data management. Key appendices of the QAPP include the optimized standard operating procedures (SOPs), sampling plan, and online data reporting spreadsheet. The QAPP was developed based on the information gathered from the Literature and Methods Review and Method Pre-Testing. Highlights of the QAPP are described below.

4.1 Sampling Plan

The 14-month monitoring campaign was designed to capture the distribution of pathogen concentrations present in raw wastewater and provide the SWB with a high-quality data set using the optimized analytical methods and quality control criteria established in the QAPP. To provide the SWB with the most relevant data for potable reuse regulations, five California wastewater agencies were selected that have either demonstrated interest in a future direct potable reuse project or have already committed to an indirect potable reuse project. The five wastewater agencies that were selected to participate in this study and the corresponding wastewater treatment plant (WWTP) are shown in Table 4-1.

Table 4-1. Wastewater Treatment Plants Sampled.

Agency	Wastewater Treatment Plant	Average Capacity	Population in Sewershed	Percent of Wastewater that is Municipal
Los Angeles County Sanitation Districts (LACSD)	Joint Water Pollution Control Plant	260 MGD	3.5 million	86%
City of Los Angeles Sanitation and Environment (LASAN)	Hyperion Water Reclamation Plant	275 MGD	4.6 million (includes all LASAN service area)	90%
City of San Diego	North City Water Reclamation Plant	30 MGD (max capacity)	1.4 million	>97%
San Francisco Public Utilities Commission (SFPUC)	Southeast Treatment Plant	48 MGD	750,000 (includes all SFPUC service area)	98%
Orange County Sanitation District	Plant No. 1	120 MGD	2.6 million (includes all OCSD service area)	80%

Each of the five WWTPs collected and shipped 24 raw wastewater grab samples at a frequency of approximately one sample every two weeks (a total of 120 samples from the five WWTPs). Three labs were selected to analyze the samples:

- Cel Analytical
- Biological Consulting Services (BCS)
- Scientific Methods

The sampling schedule was developed with the intent to collect samples on different days of the week and at different times of day to capture as close to the full range of pathogen concentrations as possible. The 14-month monitoring campaign began in December 2019 and will continue through the end of January 2021.

The complete sampling plan can be found in Appendix 3 of the QAPP, which is available on the 4989 project page of the WRF website.

4.2 Standard Operating Procedures (SOPs)

Detailed SOPs for processing samples to quantify the concentration of *Cryptosporidium*, *Giardia*, enterovirus (culture and molecular), adenovirus, (culture and molecular), norovirus (culture and molecular), and male-specific coliphage, along with matrix spike surrogates to assess recovery, were developed based on the results of the Method Pre-Testing (Chapter 3) and TWG recommendations. The complete SOPs can be found in Appendix 1 of the QAPP, which is available on the 4989 project page of the WRF website.

4.3 Quality Assurance Controls and Criteria

All quality controls for the sample collection and analysis are described in the QAPP, including:

- Hold times
- Shipping temperatures
- Matrix spikes
- Negative controls
- Positive controls
- Inhibition controls
- Demonstration of capability
- Ongoing precision and recovery

The frequency of each control and the acceptable limits are detailed in the QAPP. One unique aspect of this study is that the matrix spike recovery was measured in every sample for protozoa and every other sample (from a given WWTP) for virus. The acceptable limit for the matrix spike recovery was determined based on the method pre-testing results and adjusted based on additional results from the first few months of the monitoring campaign. To ensure that the quality control limits were met, the data were subject to several rounds of review, including review by the performing laboratory, the lead laboratory QA/QC officer (Rick Danielson), the Project QA/QC Officer (Walter Jakubowski), and the Technical Work Group.

All data were entered into the project's online reporting spreadsheet where calculations were performed using fixed formulas to minimize calculation errors and to facilitate review of the data quality.

CHAPTER 5

Pathogen Monitoring Campaign Results

The results from the 14-month pathogen monitoring campaign are presented in the following sections. First, the summary statistics for each pathogen and matrix spike is presented. Second, the distribution of each pathogen is shown and is compared to distributions from the literature. Third, an approach for combining the data from DPR-2 and select literature studies in a single distribution is described and the results are presented. Fourth, the pathogen concentrations at the five WWTPs are compared. Fifth, the time series for each pathogen is presented. Lastly, the concentrations of enterovirus and adenovirus measured via molecular methods are compared to the concentrations measured via culture methods.

Tables with the uncorrected concentrations, recovery efficiencies, and recovery-corrected concentrations for the full dataset are provided in Appendix A (Tables A-1, A-2, A-3, A-4, and A-6).

5.1 Summary Statistics

Results for data from the beginning of the campaign (December 2019) through January 2021 (120 samples) are shown in Table 5-1. The low number of non-detects (NDs) and high recovery efficiencies demonstrate that the optimized methods provided a high degree of sensitivity to enumerate pathogens throughout the campaign. The measurement of the recovery efficiency in every sample (protozoa) or every other sample (virus) allowed for the losses of the pathogens during sample processing to be quantified and accounted for to provide a more accurate representation of the concentration in each sample. Both the recovery-corrected and uncorrected concentrations are summarized in Table 5-1. Due to the high recovery efficiency (all average values were above 38%), the corrected and uncorrected concentrations are all of the same order of magnitude.

The distributions of pathogen concentrations were well described by a $\log_{10}$-normal distribution as determined by Shapiro-Wilk normality test (all distributions resulted in p-values greater than 0.05 with the exception of the adenovirus molecular and norovirus GIA, which had a relatively high frequency of NDs and detected-but-non-quantifiables [DNQs])[1]. Therefore, the concentration data was $\log_{10}$-transformed before calculating the descriptive statistics. To account for the values below the limit of detection (LOD) (i.e., NDs) and values between the LOD and limit of quantification (LOQ) (i.e., DNQs), the data were fit using the function “fitdistcens” from the R package “fitdistRplus,” which estimates the mean and standard deviation for a $\log_{10}$-normal distribution using maximum likelihood estimation (Delignette-Muller and Dutang 2015).

The LOD and LOQ for each method is shown in Table 5-1. The LOD and LOQ are a function of the instrument detection (quantification) limit, method concentration factor (CF), and recovery, as shown by the following equation:

[1]Values below the limit of quantification are not considered in the Shapiro-Wilk test. Given the low frequency of NDs and DNQs for most of the pathogens, the Shapiro-Wilk test was considered appropriate. For pathogens with a higher frequency of NDs and DNQs (e.g., adenovirus with the molecular method), the log-normal fit of the data was visually inspected to determine if a log-normal fit was appropriate.

$$LOD\ (LOQ)\ = \frac{Instrument\ detection\ (quantification)\ limit}{CF\ \times\ Recovery}$$

The instrument detection limit and quantification limit for protozoa and culturable virus were both taken to be 1 organism per pellet or inoculum volume analyzed, respectively. For the molecular assays, the instrument detection limit was determined by running 20 replicates of the standards and determining the concentration that resulted in 90% of the replicates showing amplification above the fluorescence threshold. The limit of quantification was the concentration that resulted in the 20 replicates have a coefficient of variation of 35% or less (method described in Forootan et al. 2017).

Table 5-1. Summary of DPR-2 Pathogen Monitoring Campaign Results

Parameter	*Crypto-sporidium*	*Giardia*	EV Culture	AdV Culture	EV Molecular	AdV Molecular	NoV GIA Molecular	NoV GIB Molecular	NoV GII Molecular
Number of samples	120	120	122	122	122	122	122	122	122
Percent positives (%)[1]	98	100	95	83	72	69	34	47	72
Mean (log_{10} organisms/L)[2]	1.7 [1.2]	4.0 [3.5]	3.2 [2.8]	2.8 [2.4]	4.9 [4.6]	4.3 [4.0]	3.8 [3.3]	3.6 [3.2]	4.0 [3.6]
Standard Deviation (log_{10} organisms/L)[2]	0.4 [0.4]	0.4 [0.3]	1.0 [0.9]	1.0 [0.9]	0.8 [0.8]	1.6 [1.5]	1.0 [1.0]	0.9 [1.0]	1.2 [1.3]
Min (log_{10} organisms/L)[3]	0.5 [0.0]	3.1 [2.7]	1.8 [1.6]	2.0 [1.8]	4.1 [3.9]	4.2 [3.7]	4.4 [4.0]	4.0 [3.7]	3.8 [3.6]
Max (log_{10} organisms/L)	2.8 [2.5]	5.0 [4.4]	5.4 [5.1]	5.1 [4.7]	7.3 [6.7]	7.6 [7.1]	6.5 [6.3]	5.9 [5.3]	7.9 [7.3]
Recovery-corrected limit of detection (log_{10} organisms/L)[4]	0.6	0.6	2.0	2.0	4.5	3.8	3.9	3.8	3.8
Recovery-corrected limit of quantification (log_{10} organisms/L)[4]	0.6	0.6	2.0	2.0	4.9	4.3	4.6	4.2	4.3
Recovery (%)	39 [1 – 90]	40 [4 – 80]	MS2: 49 [7 – 146] PhiX174: 55 [6 – 197]	MS2: 49 [7 – 146] PhiX174: 55 [6 – 197]	MS2: 40 [5 – 162] PhiX714: 56 [3 – 180]	MS2: 40 [5 – 162] PhiX714: 56 [3 – 180]	MS2: 40 [5 – 162] PhiX714: 56 [3 – 180]	MS2: 40 [5 – 162] PhiX714: 56 [3 – 180]	MS2: 40 [5 – 162] PhiX714: 56 [3 – 180]

Concentrations are shown as the log-transformed recovery-corrected concentrations [log-transformed uncorrected concentrations]
Recoveries are shown as the mean [minimum – maximum] (not log-transformed)
[1]Percent of samples above the limit of detection.
[2]Mean and standard deviation were estimated using the function fitdistcens from the R package fitdistRplus. Values below the limit of quantification were considered left-censored.
[3]Lowest measured concentration above the limit of quantification
[4]The limit of detection and limit of quantification varied between samples due to varying concentrate (or pellet) volume and recovery; the mean is shown here.

5.2 Distributions

Distributions of the log10-transformed concentrations, both with and without recovery correction, for each pathogen are shown in Figure 5-1 through Figure 5-7. The pathogen distributions measured in the DPR-2 monitoring campaign are compared to the results from relevant literature studies identified in the Literature and Methods review (Chapter 2) as well as the results from a recent pathogen monitoring campaign in San Diego, California (Trussell Technologies 2020).[2] For ease of reading, several of the studies will be referred to by the location and starting date of the study; specifically, Trussell Technologies and Michigan State University 2017 will be referred to as "Oceanside 2015," Trussell Technologies 2017 will be referred to as "San Diego 2016," Trussell Technologies 2018 will be referred to as "Monterey 2013", and Trussell Technologies 2020 will be referred to as "San Diego 2019." With the exception of two studies (Tetra Tech and Melbourne Water 2011 and San Diego 2019), none of these literature studies corrected for recovery.

The NDs and DNQs in each dataset were considered in determining the probability distributions but only the detectable quantities in each dataset are plotted. For example, if 50% of the data were NDs or DNQs then the distribution plot would start at the 50th percentile. The high sensitivity of the methods utilized in the DPR-2 monitoring campaign compared to the literature studies is illustrated by the fact that the DPR-2 dataset has detectable quantities at both the high end and low end of the distribution. Many literature studies only show detectable quantities at the higher end of the distribution leading to left-censored datasets.

Comparing the detectable concentrations from the DPR-2 monitoring campaign to the literature studies, the concentration of *Cryptosporidium* fell within the range of measured concentrations, while the *Giardia* concentrations measured in DPR-2 were higher than what was measured in most previous studies (Figure 5-1 and Figure 5-2). Similarly, the enterovirus and adenovirus concentrations measured using culture methods in DPR-2 were higher than values measured in most previous studies (Figure 5-3 and Figure 5-4), while the molecular concentrations were lower than most previous studies (Figure 5-5 and Figure 5-6). The norovirus GII concentrations measured in DPR-2 were similar to one previous study, which were both lower than the other two previous literature studies (Figure 5-7).

California's indirect potable reuse (IPR) regulations were based on a maximum concentration of 10^5 organism/L in raw wastewater for enteric virus and *Giardia*, and 10^4 organism/L of *Cryptosporidium* (Hultquist 2016). The recovery-corrected *Giardia* concentrations measured in DPR-2 did reach 10^5 cyst/L at the 99th percentile (recovery-corrected), but did not exceed 10^5 cyst/L. The recovery-corrected culturable enterovirus and adenovirus concentrations measured in DPR-2 reached 10^5 MPN/L at the 95th percentile for enterovirus and 99th percentile for adenovirus. The recovery-corrected culturable enterovirus concentration exceeded 10^5 MPN/L in six samples out of 122 with a maximum concentration of $10^{5.44}$ MPN/L. The recovery-corrected culturable adenovirus concentration exceeded 10^5 MPN/L in one sample out of 122 with a maximum of concentration of $10^{5.08}$ MPN/L. The *Cryptosporidium*

[2] Some of the literature studies only reported limited statistics on the concentrations (e.g., the number of samples, percent positive, maximum concentration, and minimum detectable concentration) rather than the concentrations associated with each data point. In these cases, this information was used to plot the distribution assuming a $\log_{10}$-normal distribution. Two of the literature studies (Gennaccaro et al. 2003 and Rose et al. 2001) identified in the Literature and Methods review provided insufficient data to plot the distribution and are therefore not shown in Figure 5-1 through Figure 5-7.

concentrations measured in DPR-2 never reached a concentration of 10^4 oocysts/L (maximum value of $10^{2.8}$ oocyst/L after recovery correction).

The data were fit to a $\log_{10}$ normal distribution, as described in Section 5.1. Using the following equation, the $\log_{10}$ concentration at any given percentile can be modeled:

$$Log_{10}\ concentration\ \left(\frac{organism}{L}\right) = \boldsymbol{Mean} + (\boldsymbol{Standard\ Deviation} \times\ Z\ score)$$

Where the z-score is the number of standard deviations away from the mean. The z-score and percentile are interchangeable using a standard normal distribution table. The mean and standard deviation for each distribution are shown in Table 5-1. The *Cryptosporidium* concentrations measured in DPR-2 never reached a concentration of 10^4 oocysts/L (maximum value of $10^{2.8}$ oocyst/L after recovery correction). One of the advantages of modeling the distribution of data is that the probability of obtaining a value higher or lower than the measured values can be estimated. For example, using the modeled $\log_{10}$ normal distribution of *Cryptosporidium*, the probability of obtaining a value of 10^4 organism/L can be estimated at >99.999%. Using the modeled distributions in a probabilistic assessment of treatment train performance or quantitative microbial risk assessment (such as the DPRisk tool from DPR-1) allows for the full range of potential concentrations to be estimated, rather than being limited to the discrete data points.

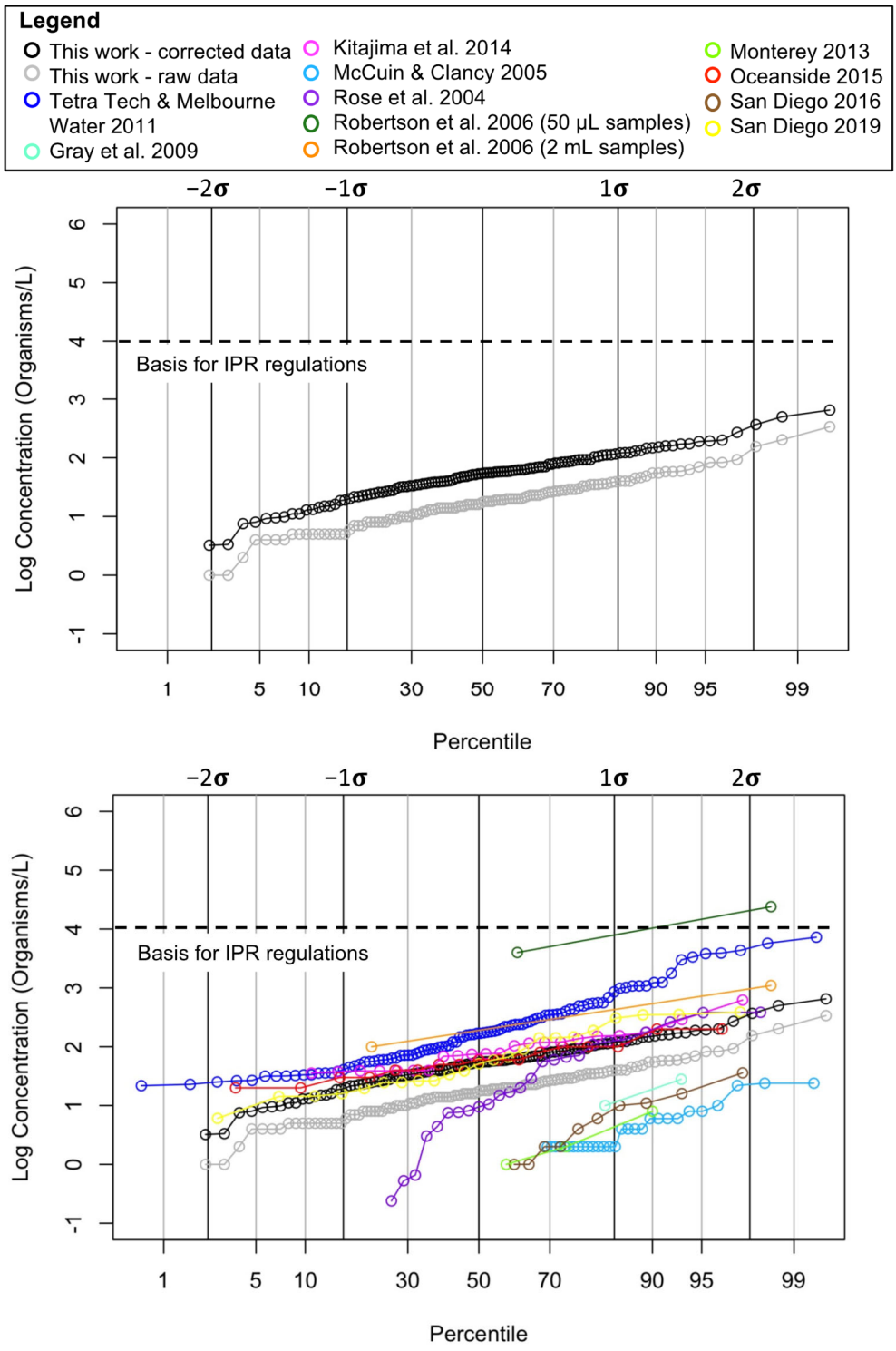

Figure 5-1. Distribution of *Cryptosporidium* Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile. Dashed line indicates the *Cryptosporidium* concentration used to develop California's IPR regulations (10^4 oocyst/L).

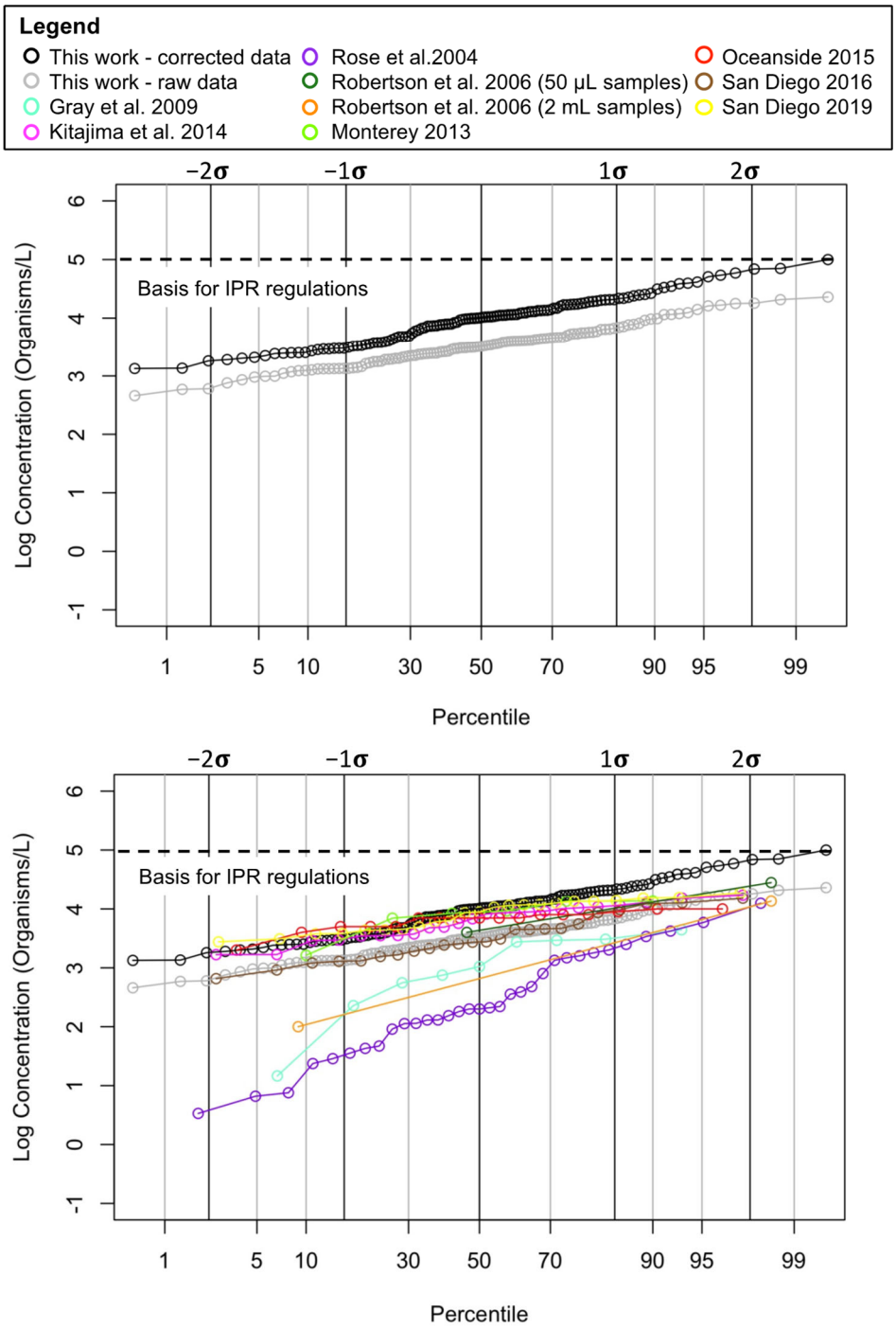

Figure 5-2. Distribution of *Giardia* Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile. Dashed line indicates the *Giardia* concentration used to develop California's IPR regulations (10^5 cyst/L).

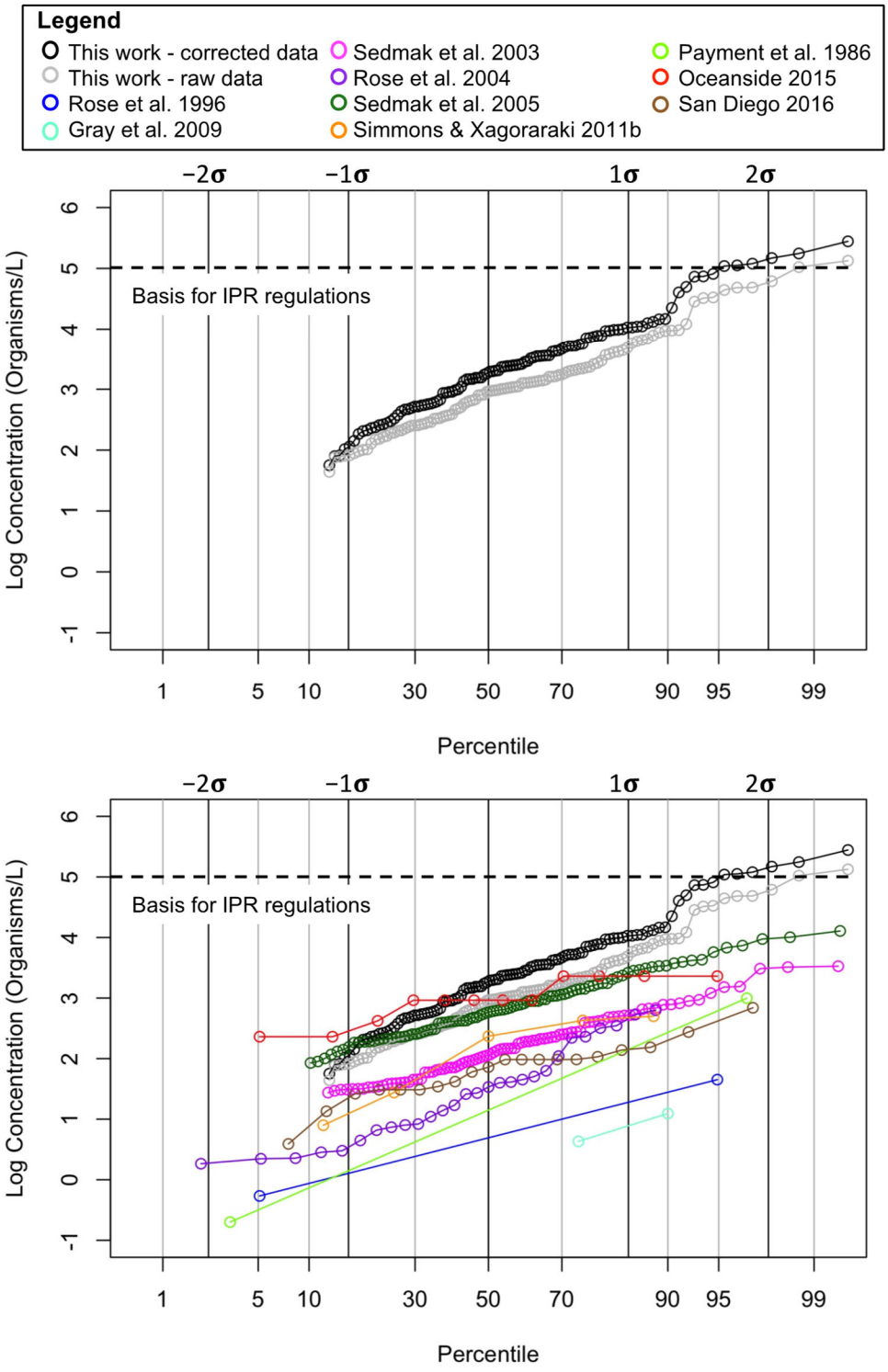

Figure 5-3. Distribution of Culturable Enterovirus Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile. Dashed line indicates the enteric virus concentration used to develop California's IPR regulations (10^5 MPN/L).

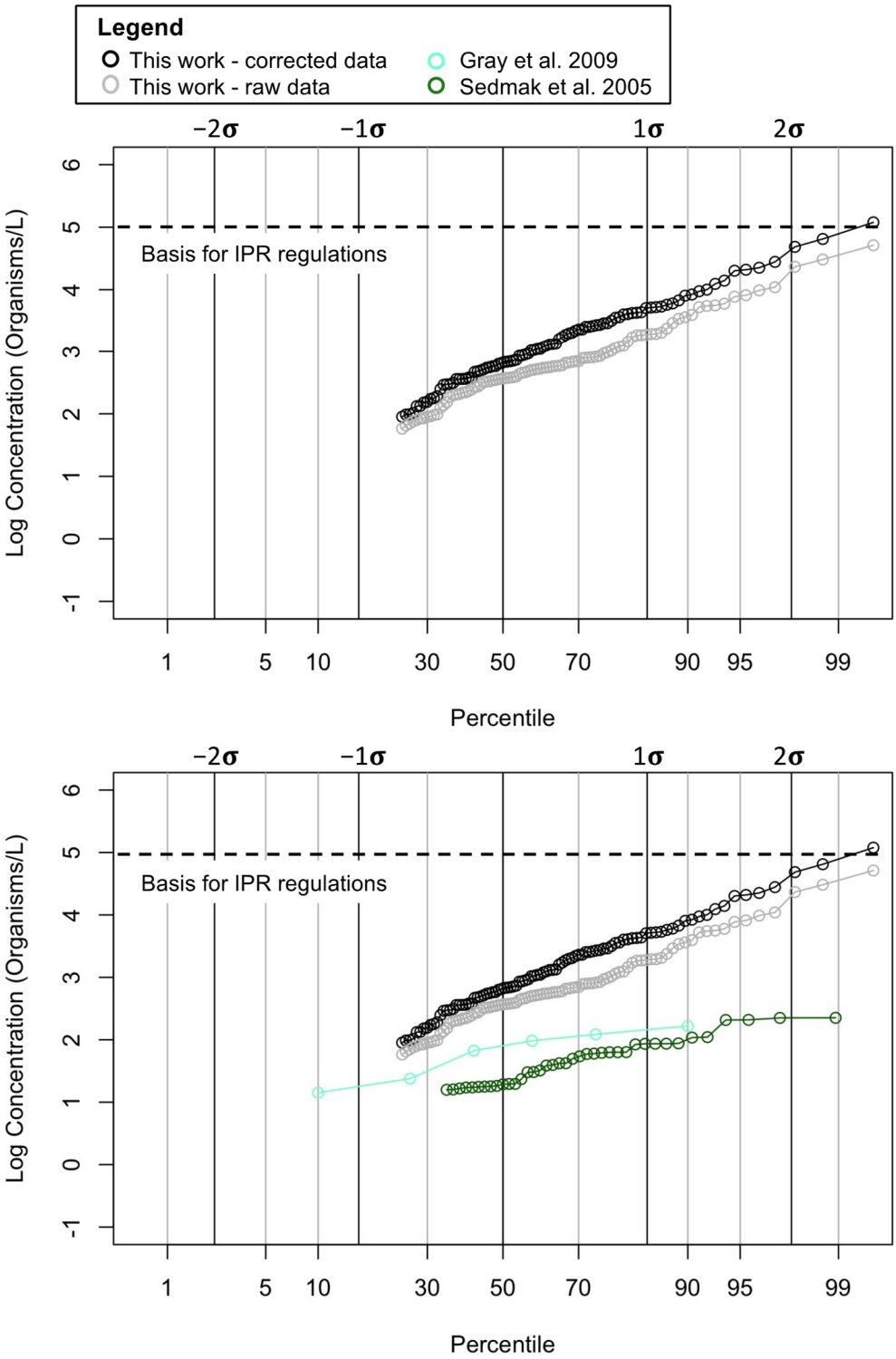

Figure 5-4. Distribution of Culturable Adenovirus Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile. Dashed line indicates the enteric virus concentration used to develop California's IPR regulations (10^5 MPN/L).

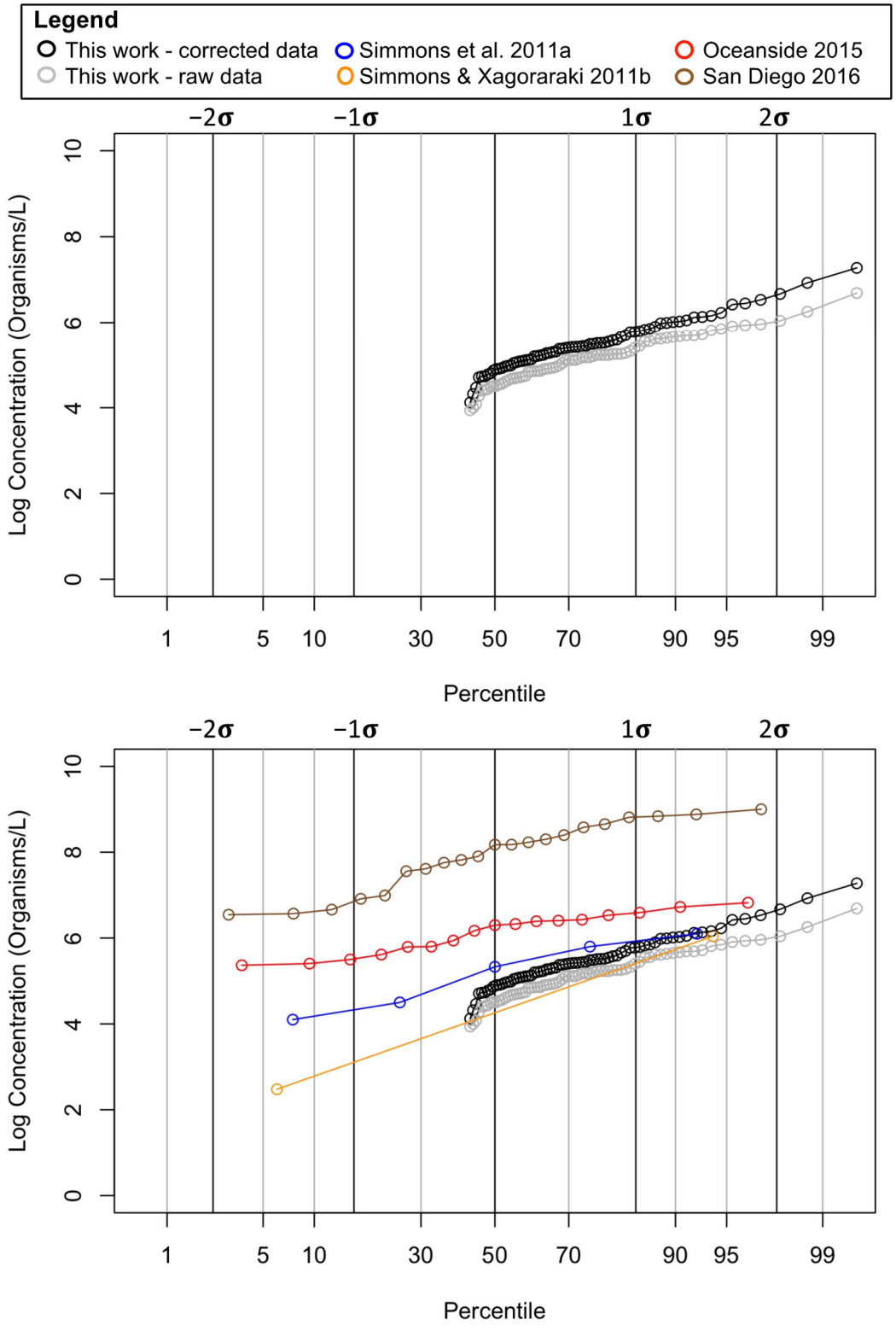

Figure 5-5. Distribution of Enterovirus (Molecular) Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile.

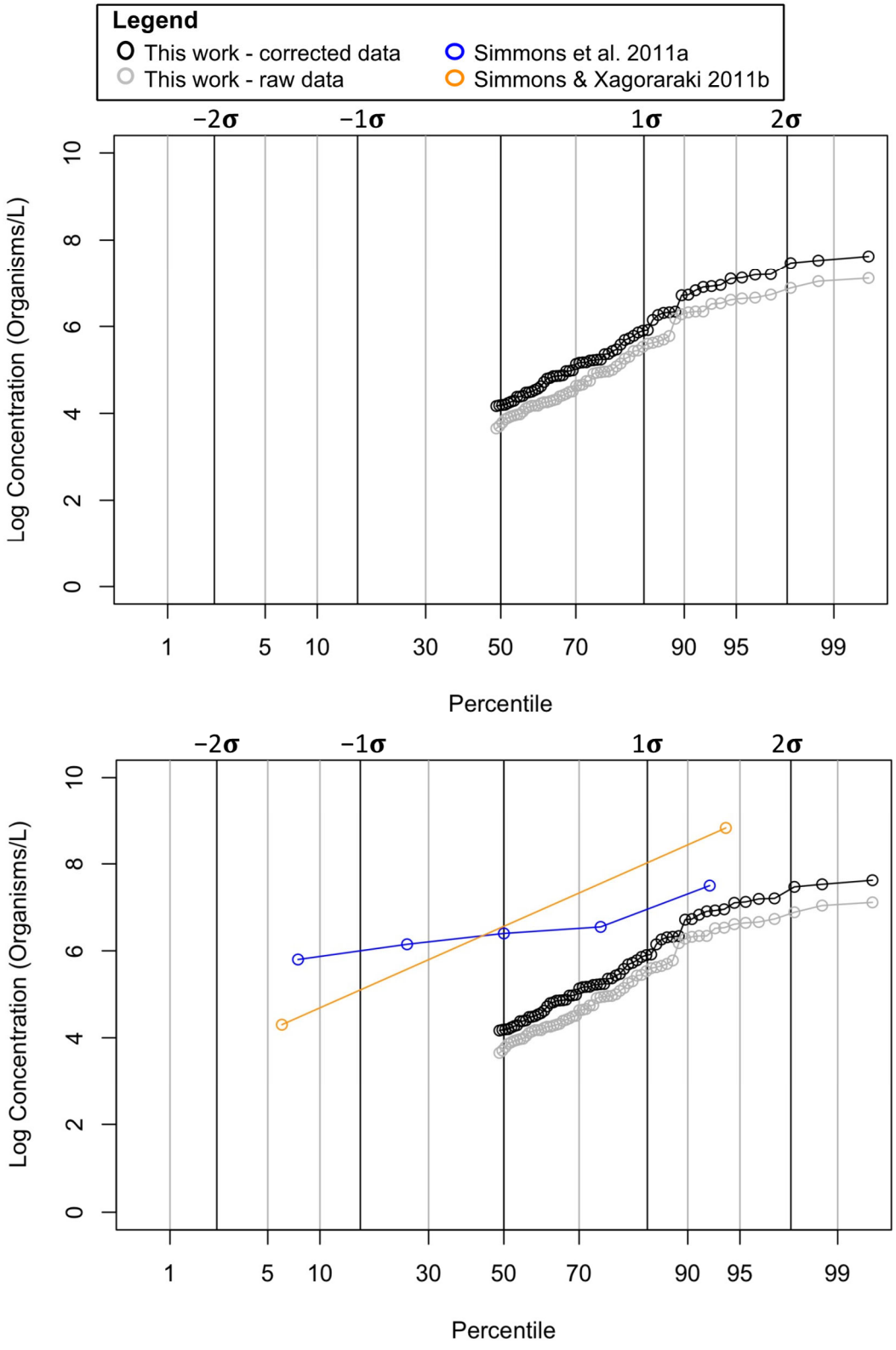

Figure 5-6. Distribution of Adenovirus (Molecular) Concentrations Measured in DPR-2 (Top) and Compared to Relevant Literature Studies (Bottom).

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile.

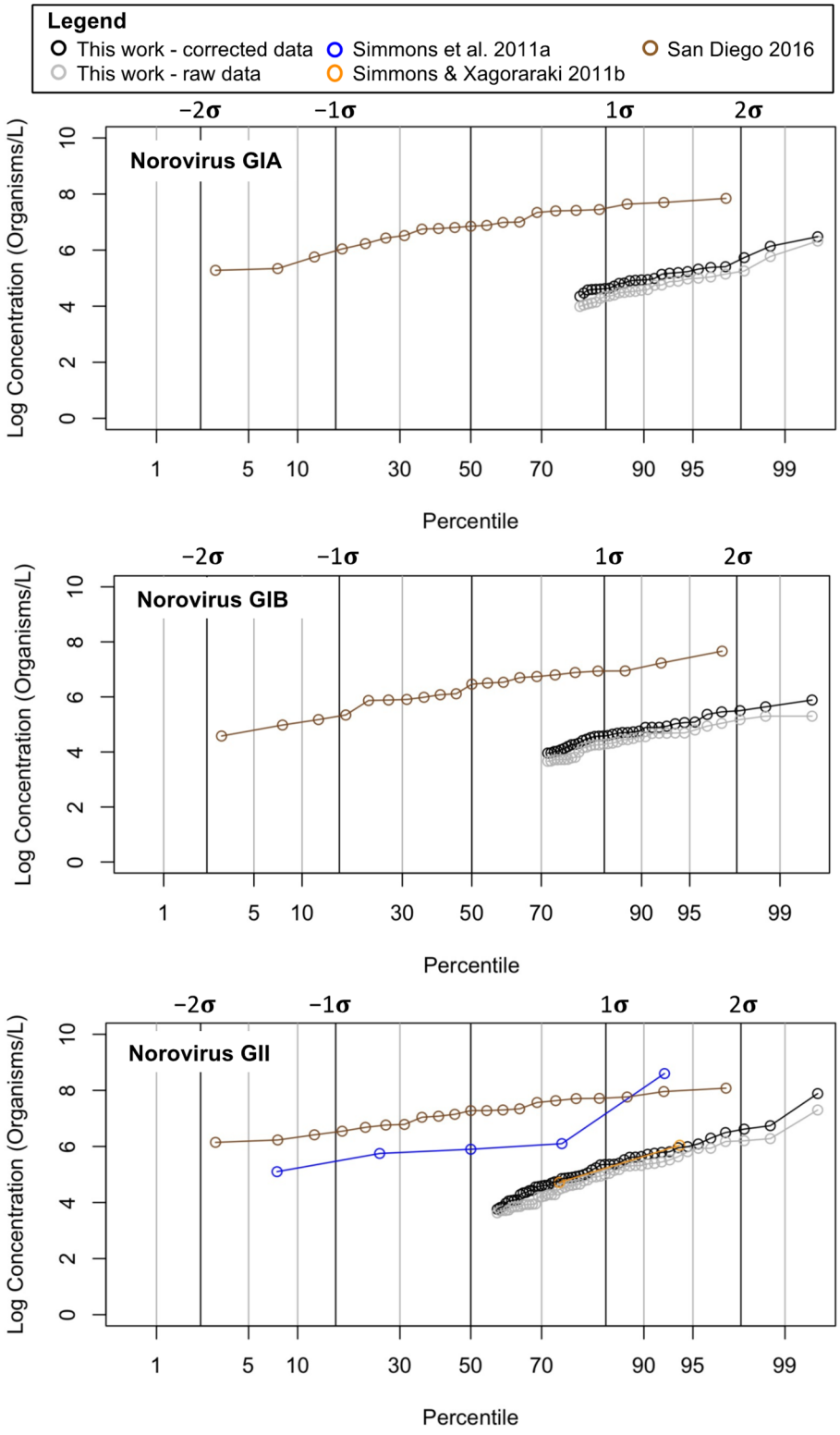

Figure 5-7. Distribution of Norovirus GIA, GIB, and GII (Molecular) Concentrations Measured in DPR-2 Compared to Relevant Literature Studies.

The top x-axis shows the number of standard deviations (**σ**) from the mean, while the bottom x-axis shows the percentile.

5.3 Combined Distribution with Select Datasets

The SWB is interested in a single distribution that combines the DPR-2 dataset with other high quality datasets. The following criteria were established by the TWG for identifying these high quality datasets:

1. Recovery was measured (and reported) for at least one sample of raw wastewater
2. The measured recovery was at least 5%
3. The percent of the samples with concentrations above the method detection limit was at least 50%

The studies that met these quality criteria for each pathogen are summarized in Table 5-2. Only one of the studies that measured virus in raw wastewater also measured recovery. The lack of studies measuring virus recovery could be due to the fact that the matrix spike specified in the EPA method 1615 for virus enumeration is poliovirus. Poliovirus is a challenging organism for many labs to work with for safety reasons and spiking poliovirus requires an additional parallel sample to be processed since poliovirus is detected by the same cell line and primer/probes as native enterovirus. MS2 and PhiX174 were selected as the matrix spikes for virus quantification in DPR-2 to address these issues of safety, cost, and complexity (Cel Analytical Inc. 2020).

Table 5-2. Literature Studies That Meet Quality Criteria for Including in a Combined Dataset.

Pathogen	Number of studies from literature review	Met Criterion 1	Met Criteria 1 and 2	Met Criterion 3	Met Criteria 1, 2, 3
Cryptosporidium	10[a]	7 studies	6 studies	7 studies	Tetra Tech and Melbourne Water 2011 Monterey 2013 Kitajima et al. 2014 Oceanside 2015 San Diego 2019
Giardia	8	5 studies	4 studies	8 studies	Monterey 2013 Kitajima et al. 2014 Oceanside 2015 San Diego 2019
Enterovirus Culture	9[b]	1 studies	1 studies	8 studies	Oceanside 2015
Adenovirus Culture	2	none	none	2 studies	None
Enterovirus Molecular	4	1 study	1 study	4 studies	Oceanside 2015
Adenovirus Molecular	2	none	none	2 studies	None
Norovirus GIA Molecular	1	none	none	1 study	None
Norovirus GIB Molecular	1	none	none	1 study	None
Norovirus GII Molecular	3	none	none	2 studies	None

[a]One of the studies (Gennaccaro et al. 2003) identified in the Literature Review is not counted here since insufficient data were provided in the paper to plot the distribution.
[b]One of the studies (Rose et al. 2001) identified in the Literature Review is not counted here since insufficient data were provided in the paper to plot the distribution.

The following approach was used to combine these datasets with the DPR-2 dataset into a single distribution:

1. Correct the measured concentrations for recovery. If recovery was only measured in a subset of samples, use the average recovery to correct the concentrations for all samples.
2. Log_{10}-transform the recovery-corrected data
3. Plot the distribution:
   a. Use imputation to assign a numerical value to each result that is below the limit of quantification (NDs and DNQs). Imputation can be achieved by fitting each literature distribution using the function "fitdistcens" from the R package "fitdistRplus," assuming a normal distribution for the log_{10}-transformed data (set the limit of quantification as the lowest measured concentration if not reported). The fitted equation along with the z-score for each ND or DNQ is then used to estimate the values for each ND or DNQ.
   b. Combine all log_{10}-transformed recovery-corrected data points from the selected studies (including the imputed data points) and assign a rank to each data point. Determine the percentile corresponding to the rank using the following equation:

$$Percentile = \frac{Rank - 0.375}{Total\ number\ of\ data\ points + 0.25}$$

   Transform the percentile to the z-score using a standard normal distribution table.

   c. Plot the log_{10}-transformed recovery-corrected concentration vs. the z-score
4. Model the distribution:
   a. Combine all log_{10}-transformed recovery-corrected data points, including the NDs and DNQs. Mark both NDs and DNQs as below the LOQ. Set the LOQ for each literature dataset as the lowest measured concentration (if LOQ is not reported). Assume a normal distribution for the log_{10}-transformed data. Use the function "fitdistcens" from the R package "fitdistRplus" to determine the mean and standard deviation. The combined distribution will be described by the following equation:

$$Log\ concentration\left(\frac{organism}{L}\right) = \boldsymbol{Mean} + (\boldsymbol{Standard\ Deviation} \times Z\ score)$$

The resulting distributions for *Cryptosporidium, Giardia*, and enterovirus (culture), and enterovirus (molecular) are shown in Figures 5-8, 5-9, 5-10, 5-11, and 5-12. Distributions for *Cryptosporidium* were developed both with and without the Tetra Tech and Melbourne Water (2011) dataset since this study was conducted in Australia rather than the United States, but it involved a high degree of quality control (e.g., matrix spikes in every sample). The models for each distribution are compared to the models for just the DPR-2 dataset in Table 5-3.

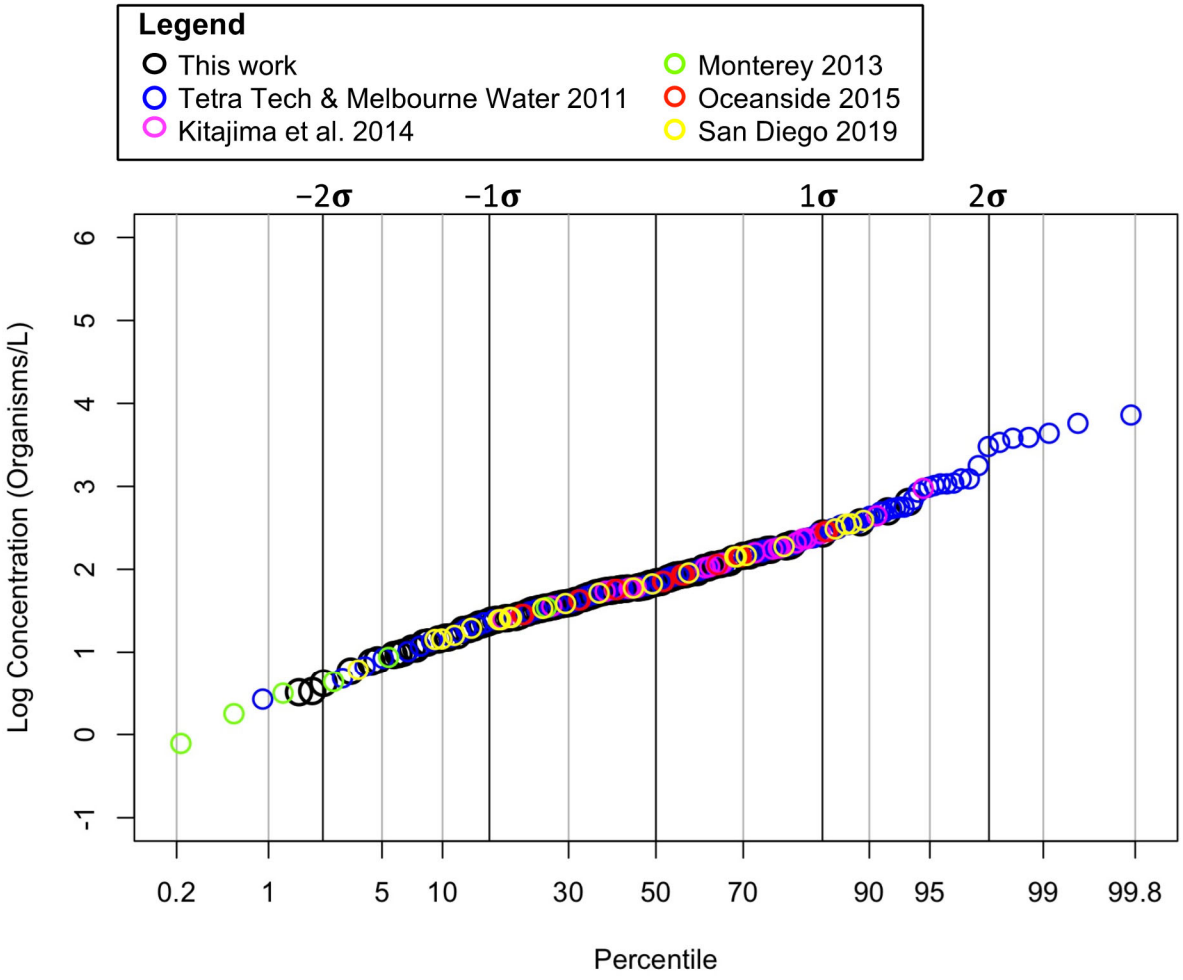

Figure 5-8. Combined Distribution of Recovery-Corrected *Cryptosporidium* Concentrations from the DPR-2 Dataset and Select Literature Datasets (Including Tetra Tech and Melbourne Water 2011).
Imputation was used to estimate values below the limit of quantification.

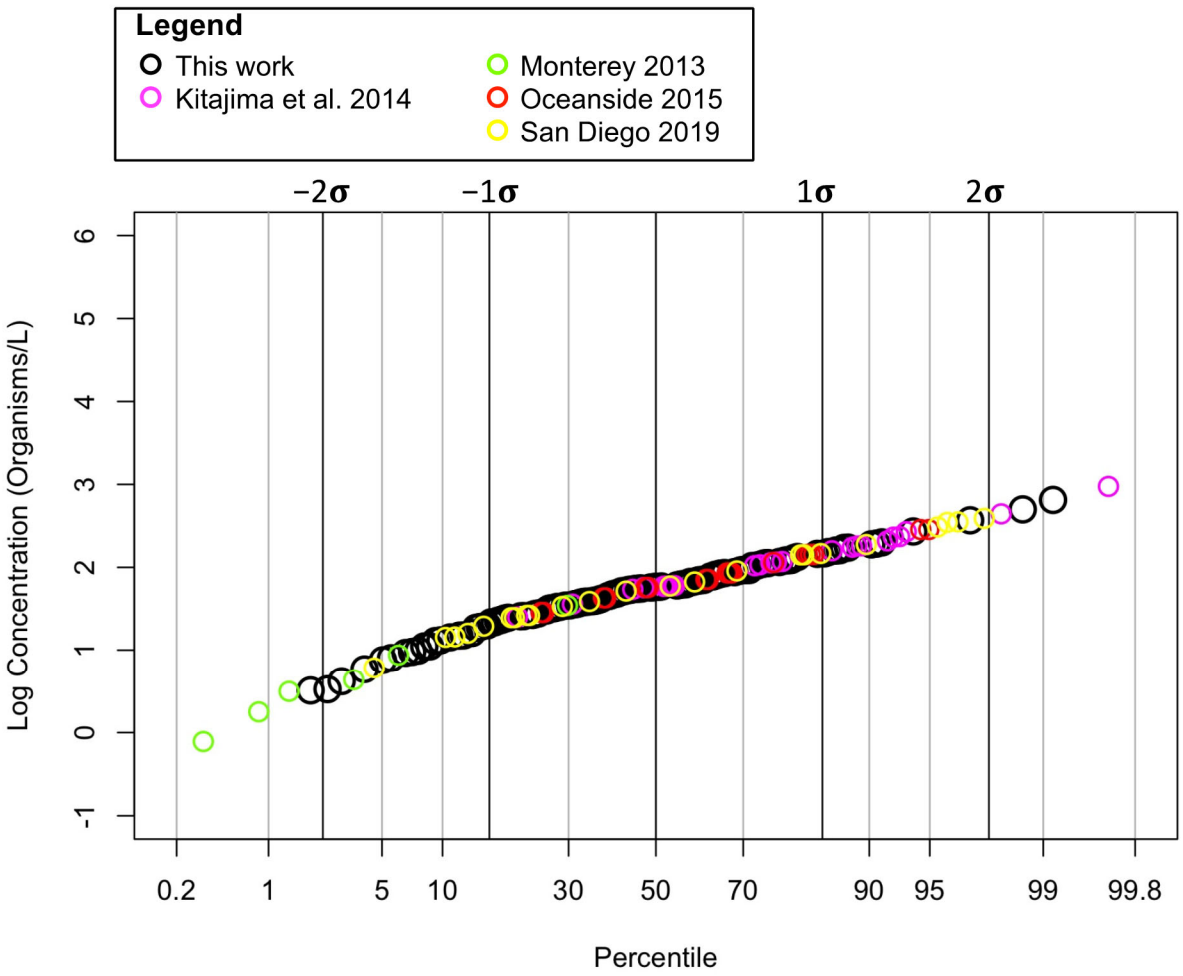

Figure 5-9. Combined Distribution of Recovery-Corrected *Cryptosporidium* Concentrations from the DPR-2 Dataset and Select Literature Datasets (Excluding Tetra Tech and Melbourne Water 2011). Imputation was used to estimate values below the limit of quantification.

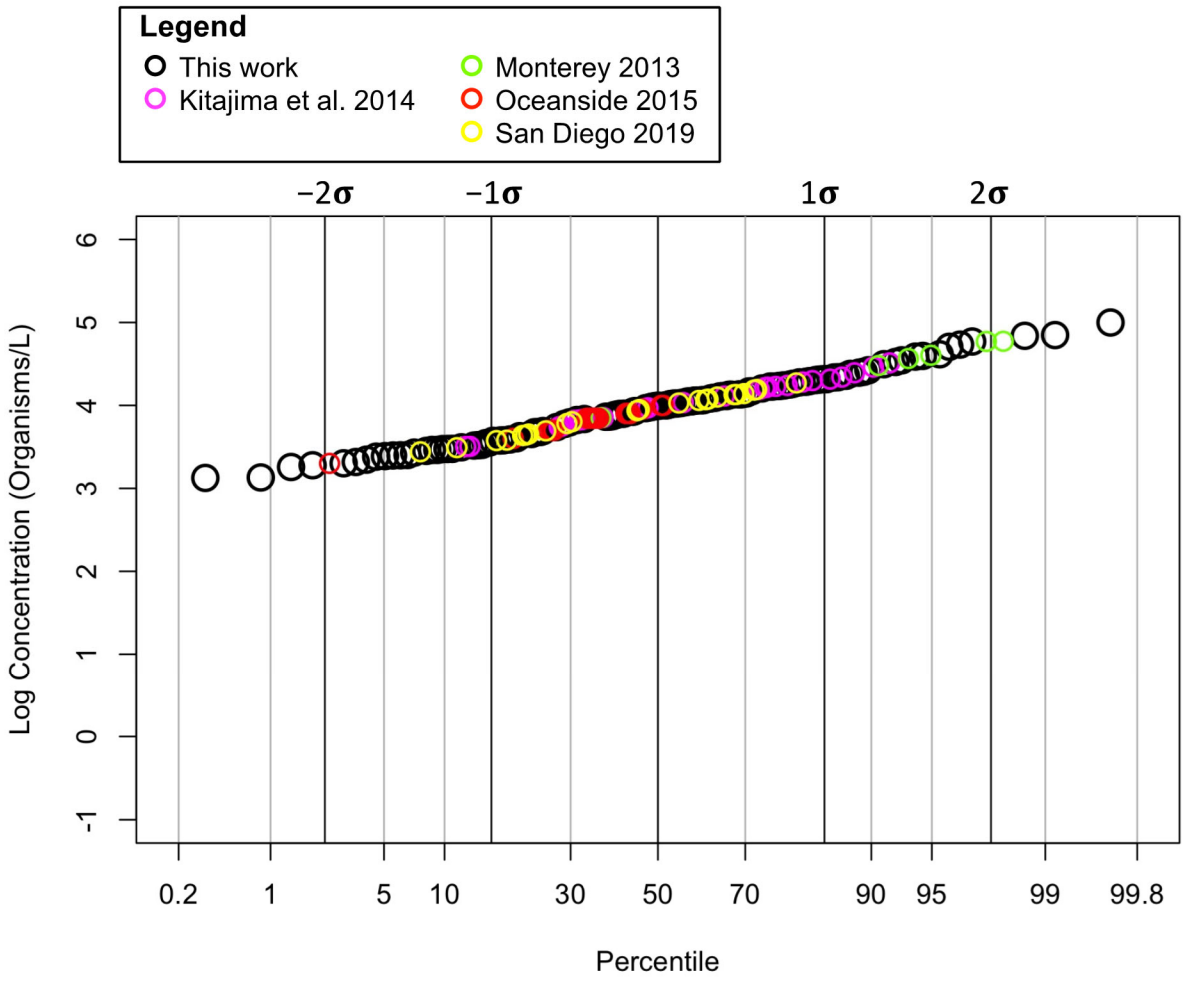

Figure 5-10. Combined Distribution of Recovery-Corrected *Giardia* Concentrations from the DPR-2 Dataset and Select Literature Datasets.

Imputation was used to estimate values below the limit of quantification.

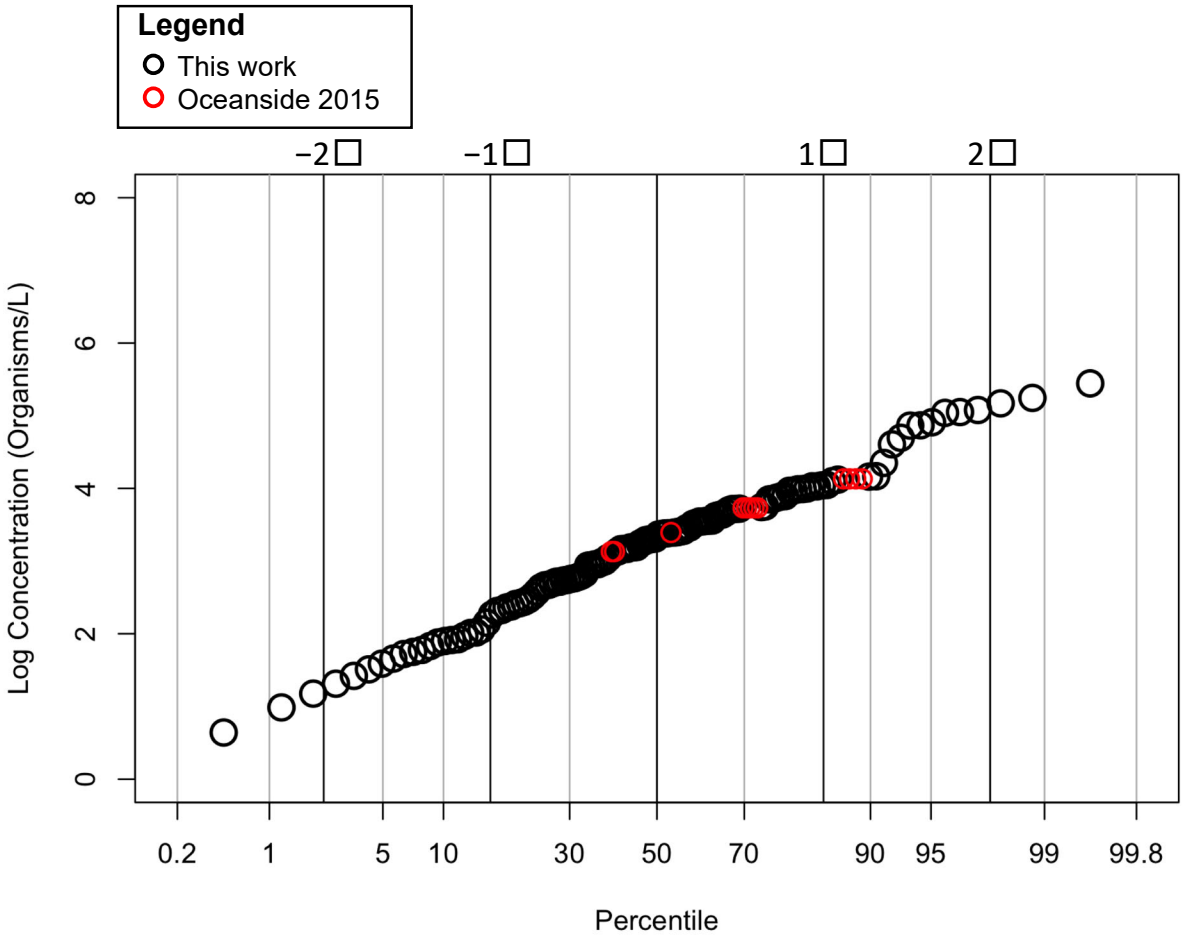

Figure 5-11. Combined Distribution of Recovery-Corrected Culturable Enterovirus Concentrations from the DPR-2 Dataset and Select Literature Datasets.

Imputation was used to estimate values below the limit of quantification.

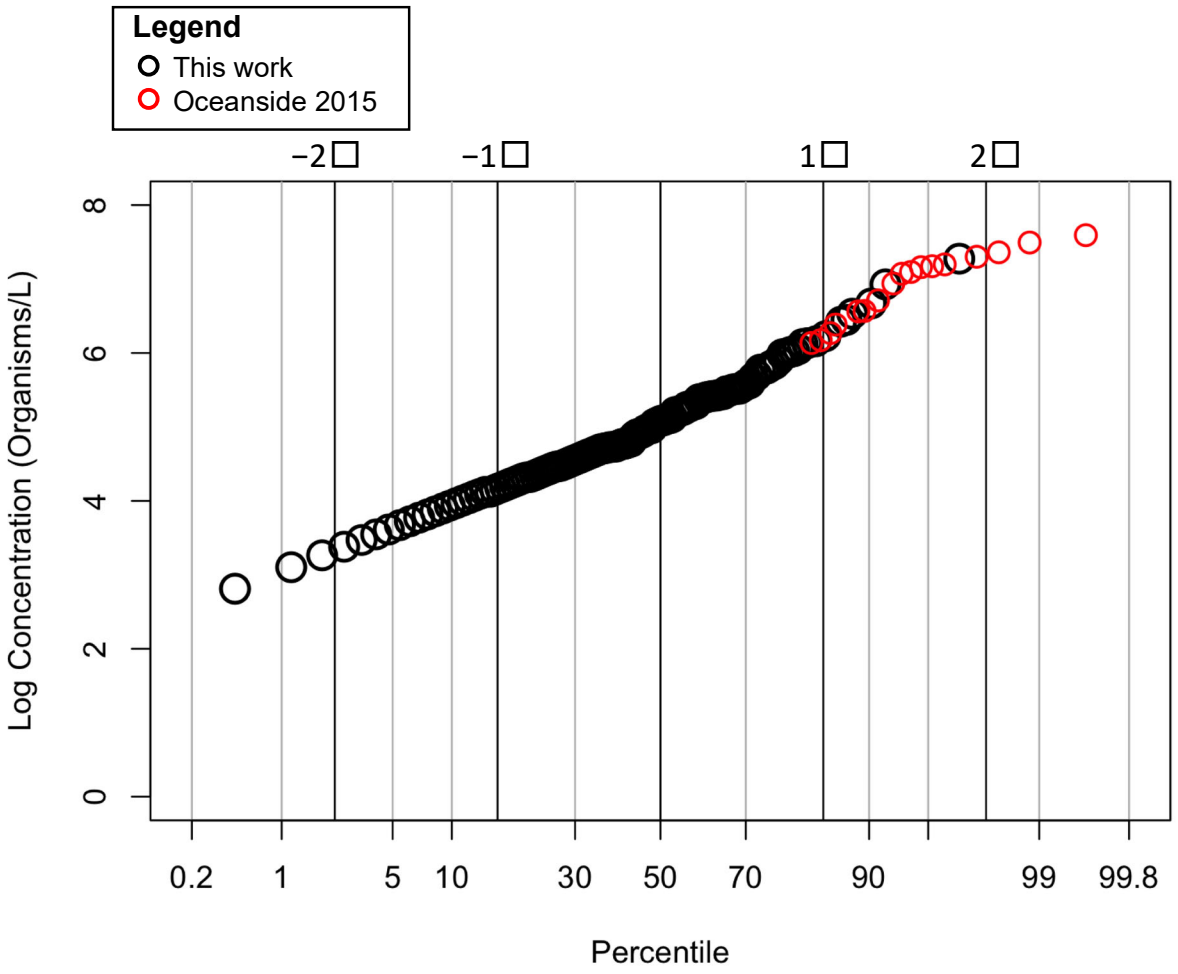

Figure 5-12. Combined Distribution of Recovery-Corrected Enterovirus (Molecular) Concentrations from the DPR-2 Dataset and Select Literature Datasets.
Imputation was used to estimate values below the limit of quantification.

Table 5-3. Model for Combined Distributions and DPR-2 Only Distribution.[1]

Pathogen	Combined distribution mean	Combined distribution standard deviation	DPR-2 mean	DPR-2 standard deviation
Cryptosporidium	1.9 (with Tetra Tech and Melbourne Water 2011) 1.7 (without Tetra Tech and Melbourne Water 2011	0.6 (with Tetra Tech and Melbourne Water 2011) 0.5 (without Tetra Tech and Melbourne Water 2011	1.7	0.4
Giardia	4.0	0.4	4.0	0.4
Enterovirus Culture	3.2	1.0	3.2	1.0
Adenovirus Culture	--	--	2.8	1.0
Enterovirus Molecular	5.1	1.1	4.9	0.8
Adenovirus Molecular	--	--	4.3	1.6
Norovirus GIA Molecular	--	--	3.8	1.0
Norovirus GIB Molecular	--	--	3.6	1.0
Norovirus GII Molecular	--	--	4.0	1.2

[1]The distributions are described by the following equation, using the mean and standard deviation in this table:

$$Log\ concentration\ \left(\frac{organism}{L}\right) = \boldsymbol{Mean} + (\boldsymbol{Standard\ Deviation} \times Z\ score)$$

5.4 Comparison of Facilities

Box plots comparing the recovery-corrected concentrations of each pathogen at the 5 WWTPs are shown in Figure 5-13, 5-14, 5-15, and 5-16 . The frequency of detects, pathogen concentrations, and recoveries at each of the five WWTPs are summarized in Table 5-5. Two approaches were used to evaluate whether there was a statistically significant difference between the recovery-corrected concentrations at the 5 WWTPs. The first approach was to use one-way analysis of variance (ANOVA), excluding all NDs and DNQs. A Tukey post-hoc test was used to perform multiple pair-wise comparisons. The second approach was to use a rank-based ANOVA test—the Kruskal-Wallis Test—and assign all NDs and DNQs the same rank. A post-hoc test with Bonferroni adjustment for multiple comparisons was performed. In both approaches, comparisons with a p-value less than 0.05 were considered significant.

In general, differences between the recovery-corrected pathogen concentrations at the five WWTPs were not statistically different using either approach. Exceptions are shown in Table 5-4.

Table 5-4. WWTPs with Statistically Significant Differences in Pathogen Concentrations.

Pathogen	WWTPs with significant differences	P-value from ANOVA test, Tukey post-hoc	P-value from Kruskal-Wallis test, Bonferroni post-hoc
Cryptosporidium	LASAN 0.45- $\log_{10}$ higher than SD	0.00086	0.0017
Cryptosporidium	LASAN 0.33- $\log_{10}$ higher than LACSD	0.03	0.015
Cryptosporidium	LASAN 0.27- $\log_{10}$ higher than OCSD	Not statistically different ($p > 0.05$)	0.022
Enterovirus (culture)	LASAN 0.78-$\log_{10}$ higher than SD	0.015	Not statistically different ($p > 0.05$)
Enterovirus (culture)	SFPUC 0.69-$\log_{10}$ higher than SD	0.034	0.025
Adenovirus (culture)	LASAN 0.61-$\log_{10}$ higher than SD	Not statistically different ($p > 0.05$)	0.0069
Enterovirus (molecular)	SFPUC 0.65-$\log_{10}$ higher than SD	0.018	Not statistically different ($p > 0.05$)

There was no significant difference in the recoveries used to correct the pathogen concentrations between the five WWTPs with three exceptions:

- SD's molecular virus recovery (average of 68%) was significantly greater than LASAN's molecular virus recovery (average of 39%) with a p-value of 0.0042
- SD's molecular virus recovery (average of 68%) was significantly greater than OCSD's molecular virus recovery (average of 44%) with a p-value of 0.028
- SD's molecular virus recovery (average of 68%) was significantly greater than SFPUC's molecular virus recovery (average of 42%) with a p-value of 0.0082

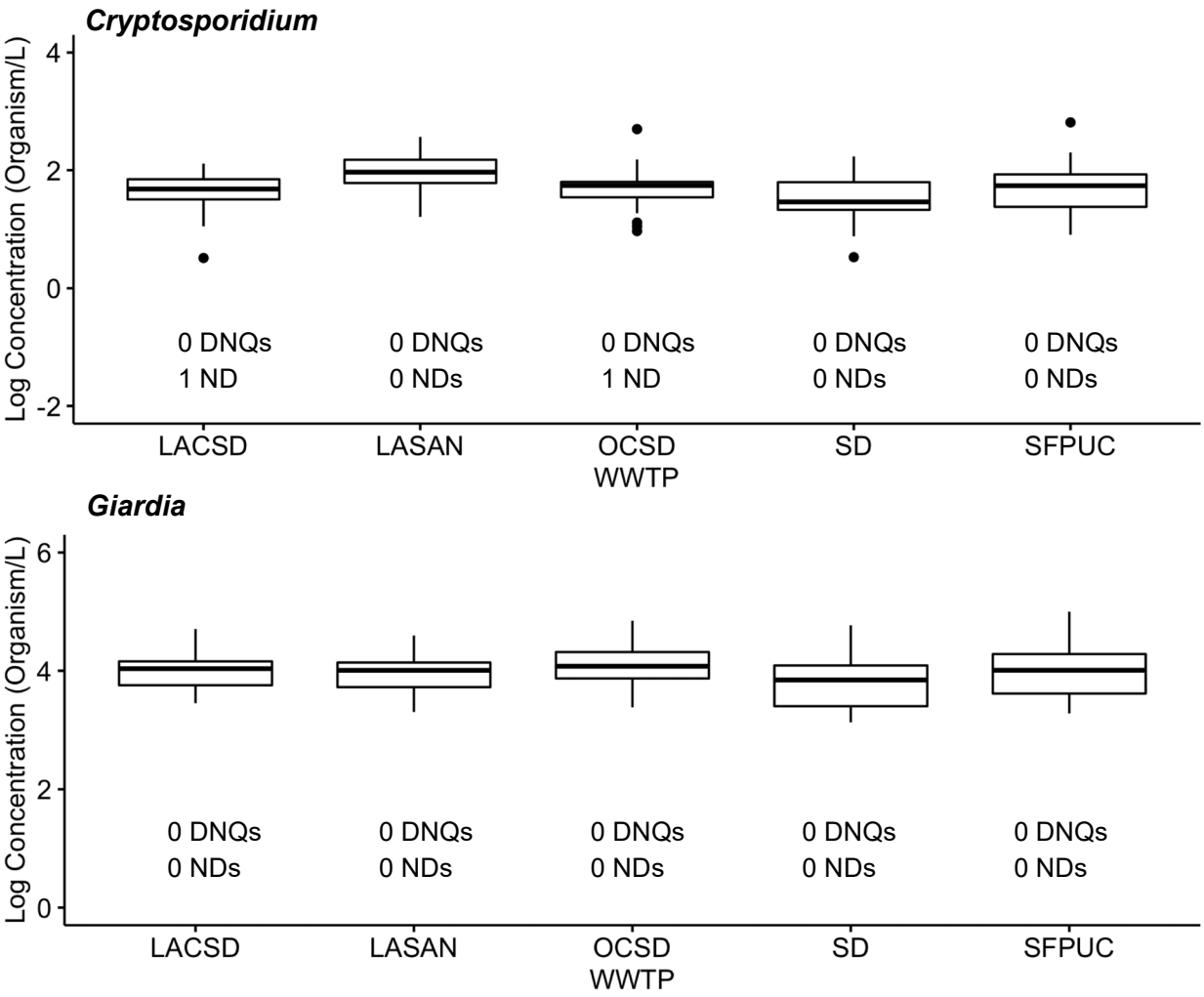

Figure 5-13. Boxplots Comparing the Recovery-Corrected Pathogen Concentrations at the Five WWTPs.

The midline of the box shows the median; the upper and lower hinge of the box show the 75th and 25th percentiles, respectively; the whiskers extend to the furthest data point up to 1.5 times the interquartile range (outliers are data points outside of this range and are plotted as points). The number of NDs and DNQs, which were excluded from the box plot, are shown in text.

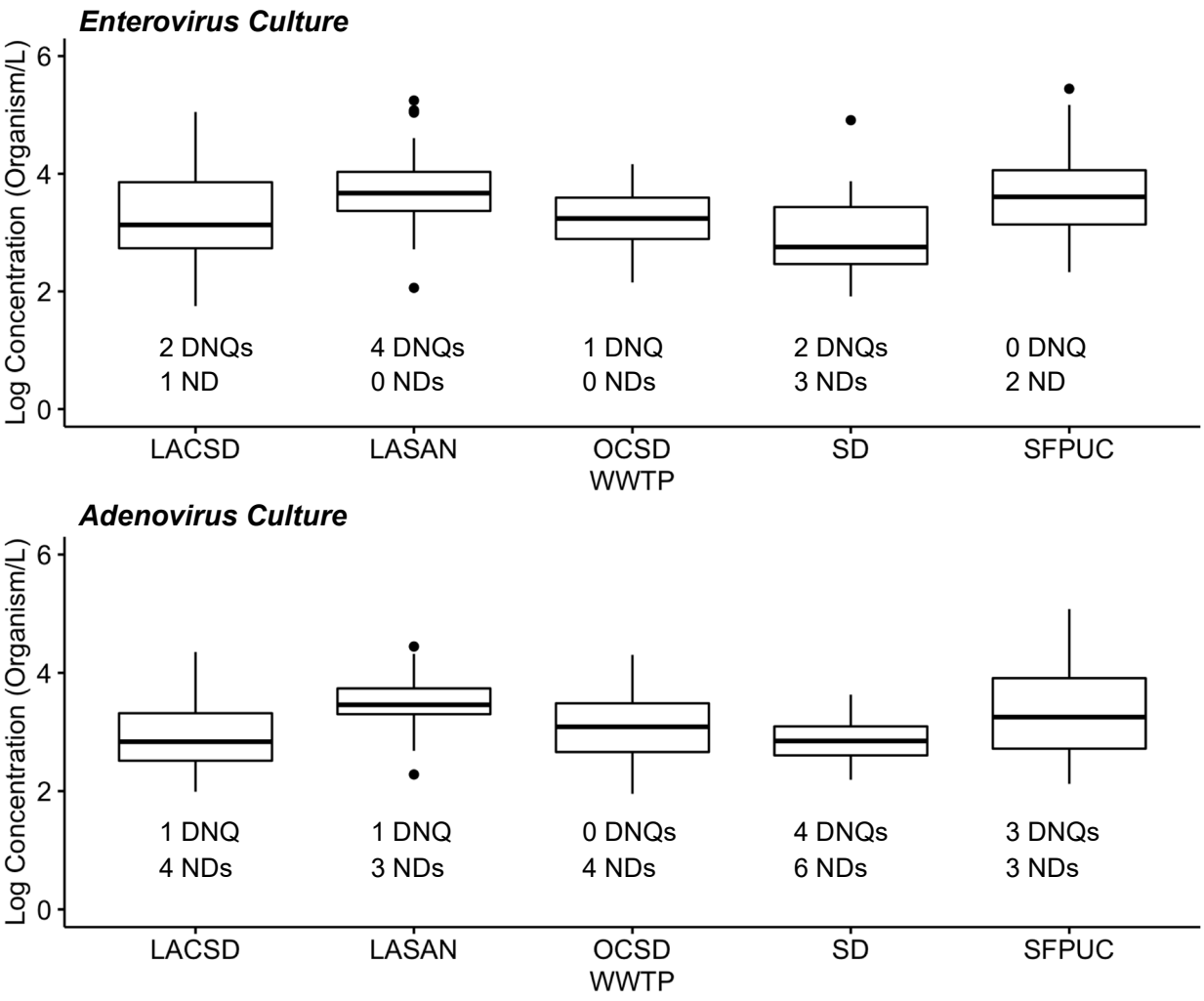

Figure 5-14. Boxplots Comparing the Recovery-Corrected Pathogen Concentrations at the Five WWTPs (Continued).

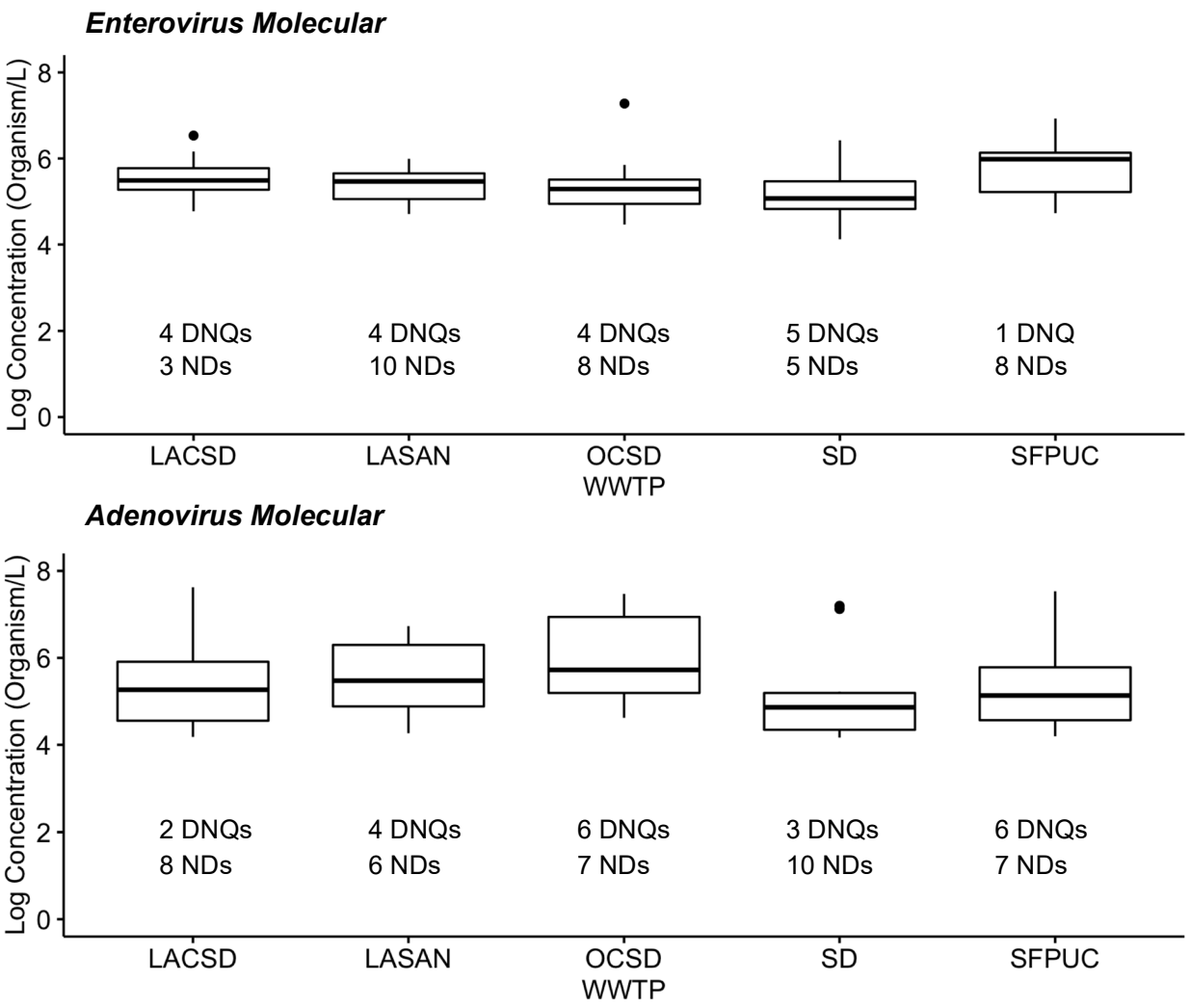

Figure 5-15. Boxplots Comparing the Recovery-Corrected Pathogen Concentrations at the Five WWTPs (Continued).

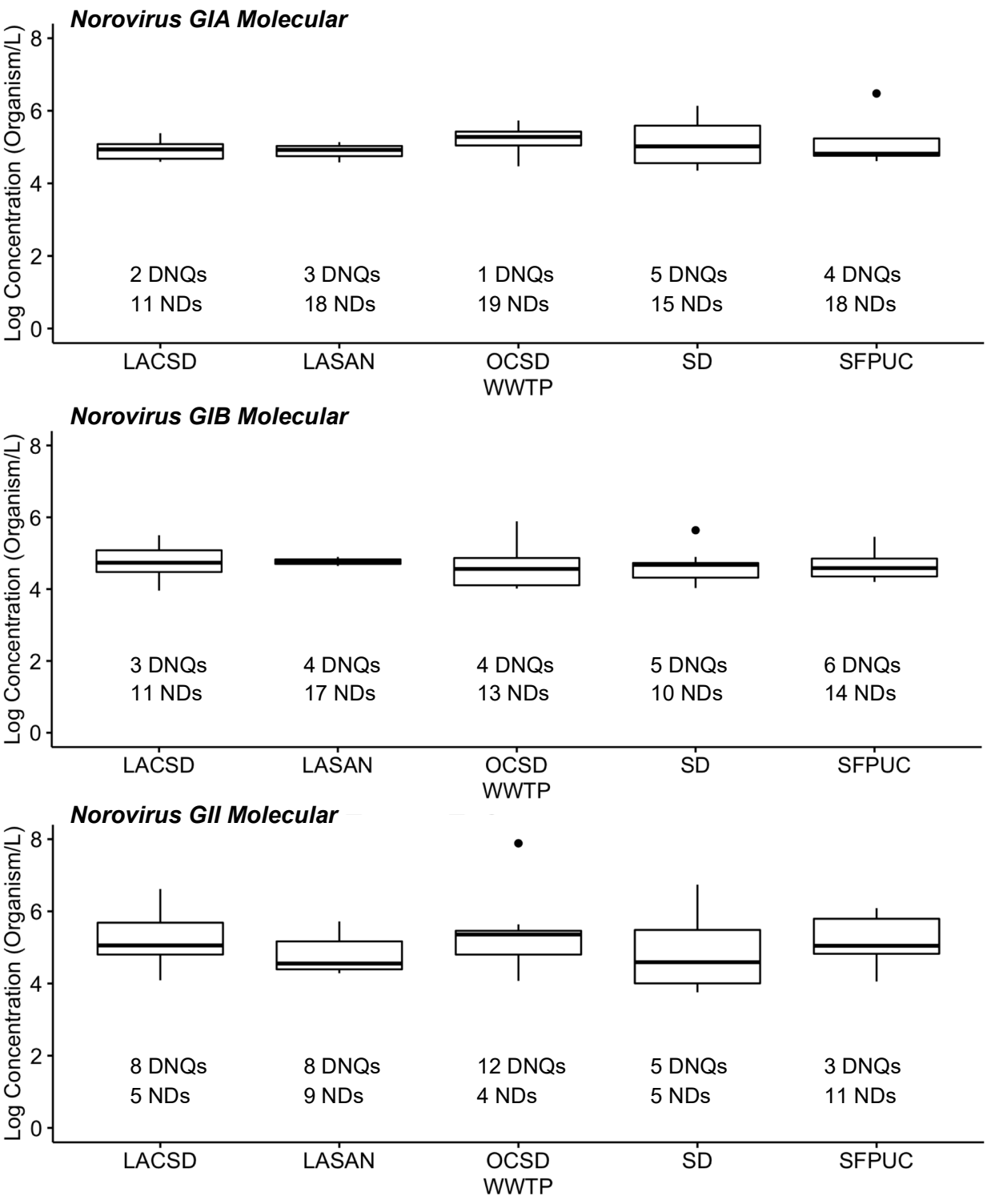

Figure 5-16. Boxplots Comparing the Recovery-Corrected Pathogen Concentrations at the Five WWTPs (Continued).

Table 5-5. Summary of DPR-2 Pathogen Monitoring Campaign Results by WWTP.

WWTP	Parameter	*Crypto*.	*Giardia*	EV Culture	AdV Culture	EV Molecular	AdV Molecular	NoV GIA Molecular	NoV GIB Molecular	NoV GII Molecular
LACSD	# Detects/Total Samples	23/24	24/24	23/24	20/24	21/24	16/24	13/24	13/24	19/24
LACSD	Concentration (log_{10} #/L)	1.6 ±0.4	4.0 ±0.3	3.0 ±1.2	2.6 ±0.9	5.2 ±0.7	4.5 ±1.4	4.5 ±0.5	4.0 ±0.9	4.2 ±1.3
LACSD	Recovery (%)	45 ±18	40 ±19	56 ±29	56 ±29	49 ±20	49 ±20	49 ±20	49 ±20	49 ±20
LASAN	# Detects/Total Samples	24/24	24/24	24/24	21/24	14/24	18/24	6/24	7/24	15/24
LASAN	Concentration (log_{10} #/L)	2.0 ±0.3	4.0 ±0.3	3.4 ±1.1	3.2 ±0.8	4.8 ±0.7	4.7 ±1.3	3.8 ±0.7	3.1 ±1.0	3.9 ±0.8
LASAN	Recovery (%)	33 ±18	39 ±17	47 ±21	47 ±21	39 ±29	39 ±29	39 ±29	39 ±29	39 ±29
OCSD	# Detects/Total Samples	23/24	24/24	24/24	20/24	16/24	17/24	5/24	11/24	20/24
OCSD	Concentration (log_{10} #/L)	1.7 ±0.4	4.1 ±0.4	3.2 ±0.6	2.9 ±0.7	4.8 ±0.9	4.2 ±1.9	3.5 ±1.2	3.7 ±0.9	3.4 ±1.8
OCSD	Recovery (%)	42 ±21	40 ±19	46 ±28	46 ±28	44 ±22	44 ±22	44 ±22	44 ±22	44 ±22
SD	# Detects/Total Samples	24/24	24/24	21/24	18/24	19/24	14/24	9/24	14/24	19/24
SD	Concentration (log_{10} #/L)	1.5 ±0.4	3.8 ±0.4	2.7 ±0.9	2.2 ±0.9	4.7 ±0.7	3.8 ±1.6	2.8 ±1.6	3.6 ±1.0	4.2 ±1.2
SD	Recovery (%)	38 ±21	43 ±21	53 ±27	53 ±27	68 ±29	68 ±29	68 ±29	68 ±29	68 ±29
SFPUC	# Detects/Total Samples	24/24	24/24	24/26	23/26	18/26	19/26	8/26	12/26	15/26
SFPUC	Concentration (log_{10} #/L)	1.7 ±0.4	4.0 ±0.5	3.5 ±0.9	2.9 ±1.1	5.3 ±0.9	4.4 ±1.4	3.4 ±1.3	3.7 ±0.8	4.4 ±1.0
SFPUC	Recovery (%)	36 ±16	37 ±12	59 ±24	59 ±24	42 ±34	42 ±34	42 ±34	42 ±34	42 ±34

Concentrations (mean ± standard deviation) are shown as the log_{10}-transformed recovery-corrected concentrations. Mean and standard deviation were estimated using the function fitdistcens from the R package fitdistRplus. Values below the limit of quantification were considered left-censored. Recovery percentages (mean ± standard deviation) were not log_{10}-transformed. The virus recovery is the average of the MS2 and PhiX174 recovery.

5.5 Concentrations over Time

The recovery-corrected concentrations of each pathogen at the five WWTPs are plotted over time in Figures 5-17 through 5-25. No clear seasonal trends were observed in the concentrations of *Cryptosporidium*, *Giardia*, and enterovirus (molecular). Because the concentration of norovirus (GIA, GIB, and GII) was frequently below the limit of quantification during the duration of this campaign, no seasonal trend is discernable. Both culturable and molecular adenovirus showed a decrease in concentrations beginning in April 2020, as evidenced by the higher frequency of NDs and DNQs. It should be noted that the COVID-19 stay-at-home order also began in March 2020 and restrictions lasted through the end of this study (January 2021). In contrast, the culturable enterovirus concentrations at the LACSD and SFPUC WWTPs appeared to increase in the summer months.

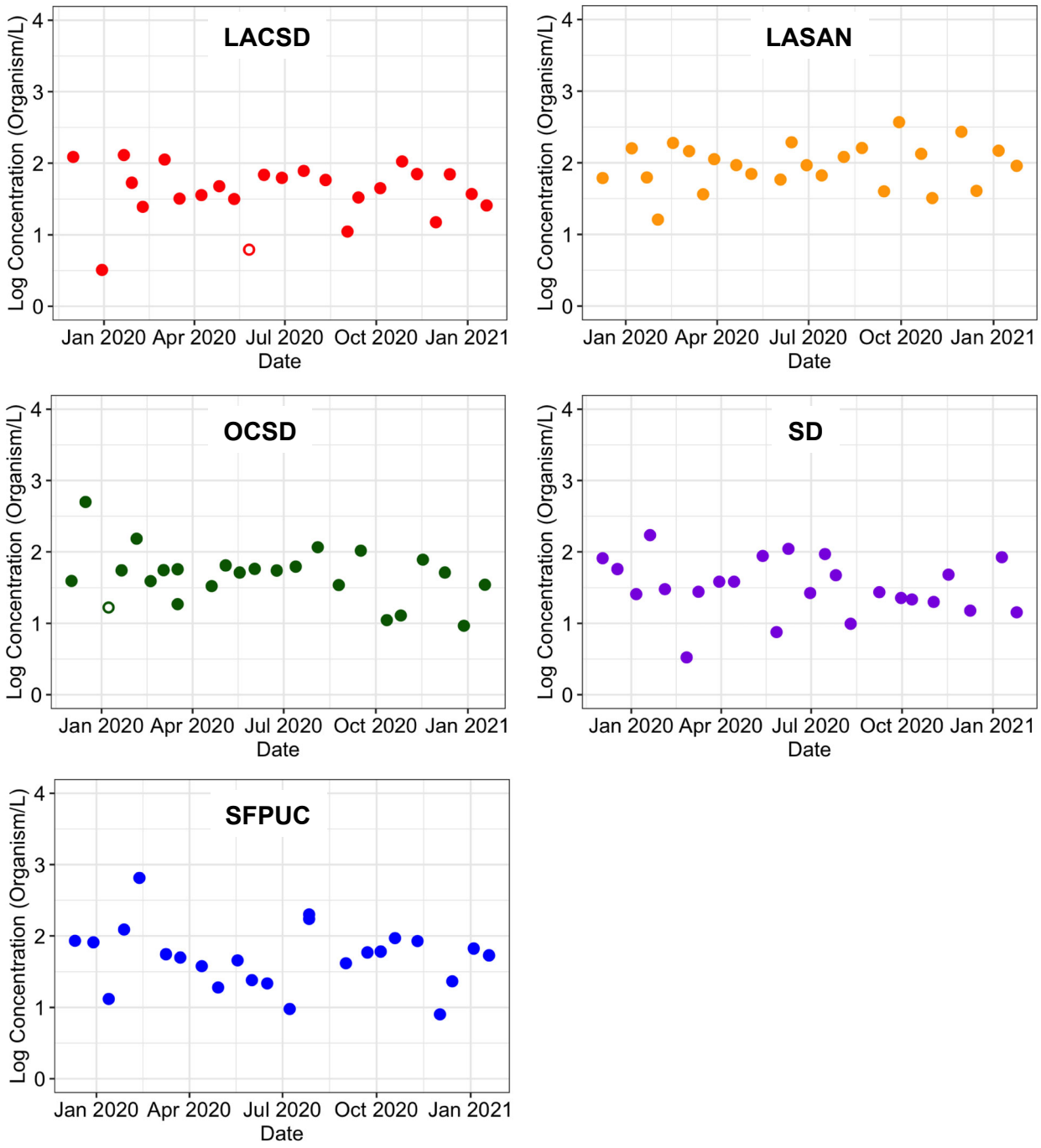

Figure 5-17. Times Series of the Recovery-Corrected *Cryptosporidium* Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

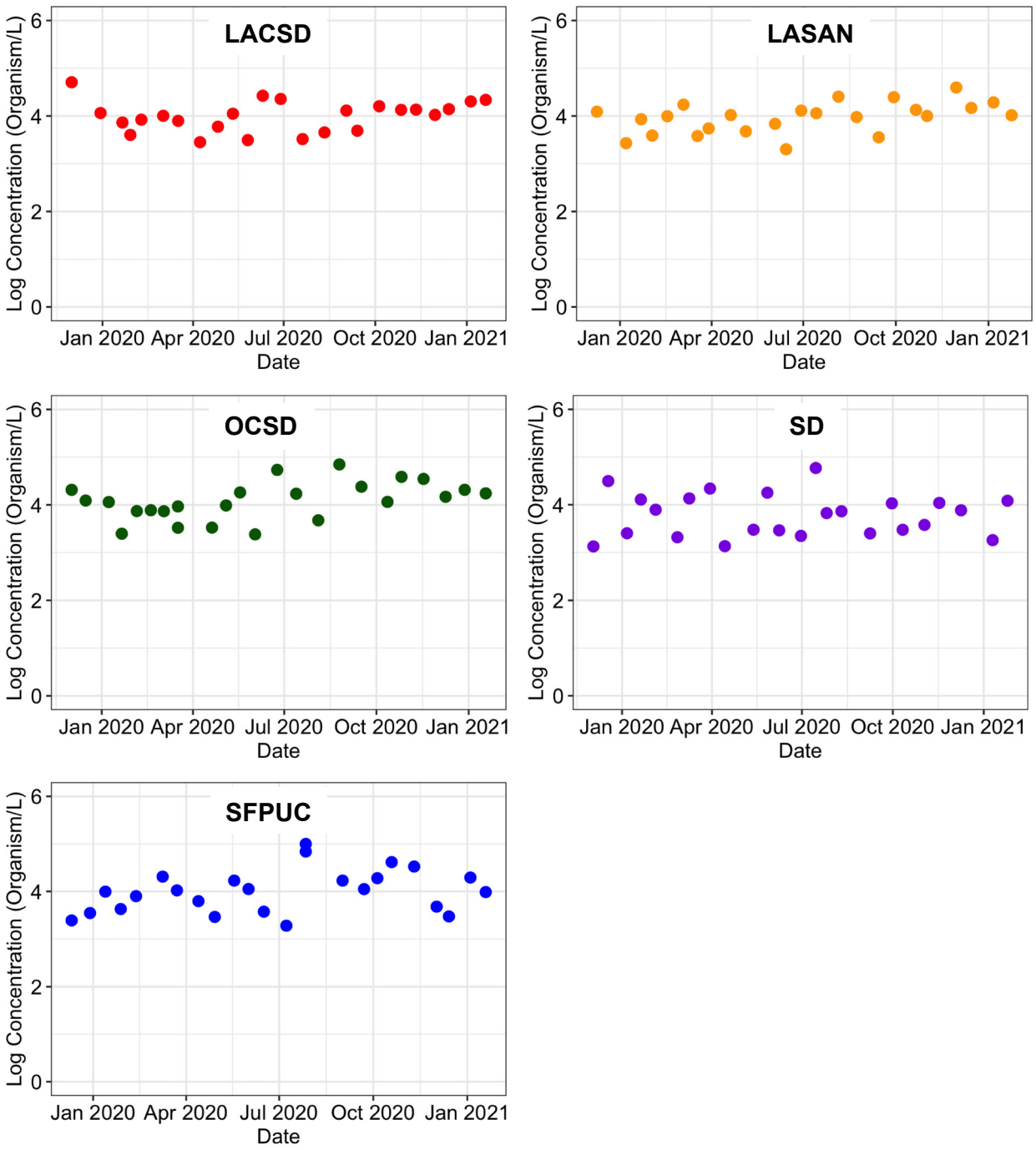

Figure 5-18. Times Series of the Recovery-Corrected *Giardia* Concentrations at the Five WWTPs.
NDs and DNQs are shown as unfilled circles at the limit of quantification.

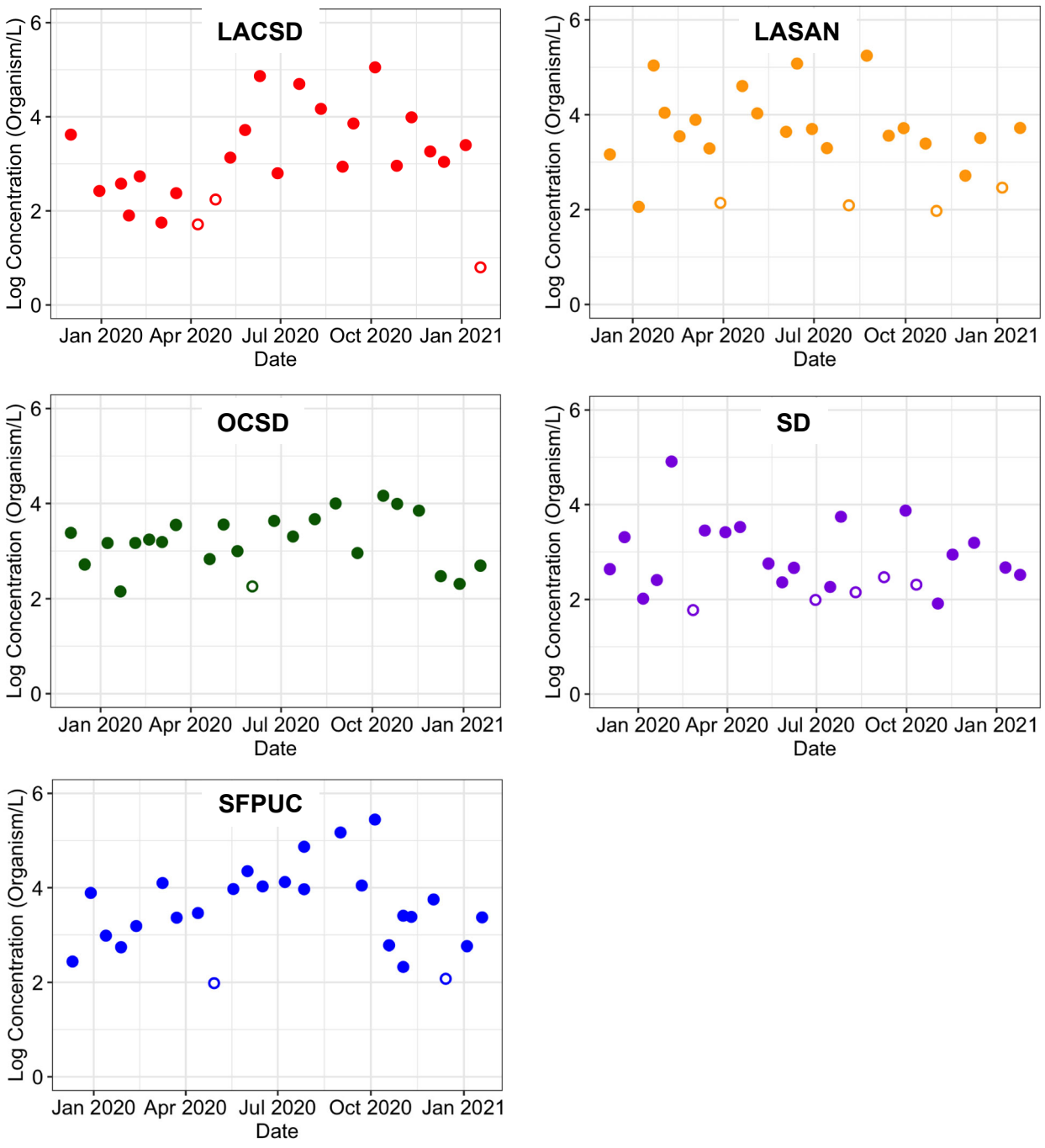

Figure 5-19. Times Series of the Recovery-Corrected Culturable Enterovirus Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

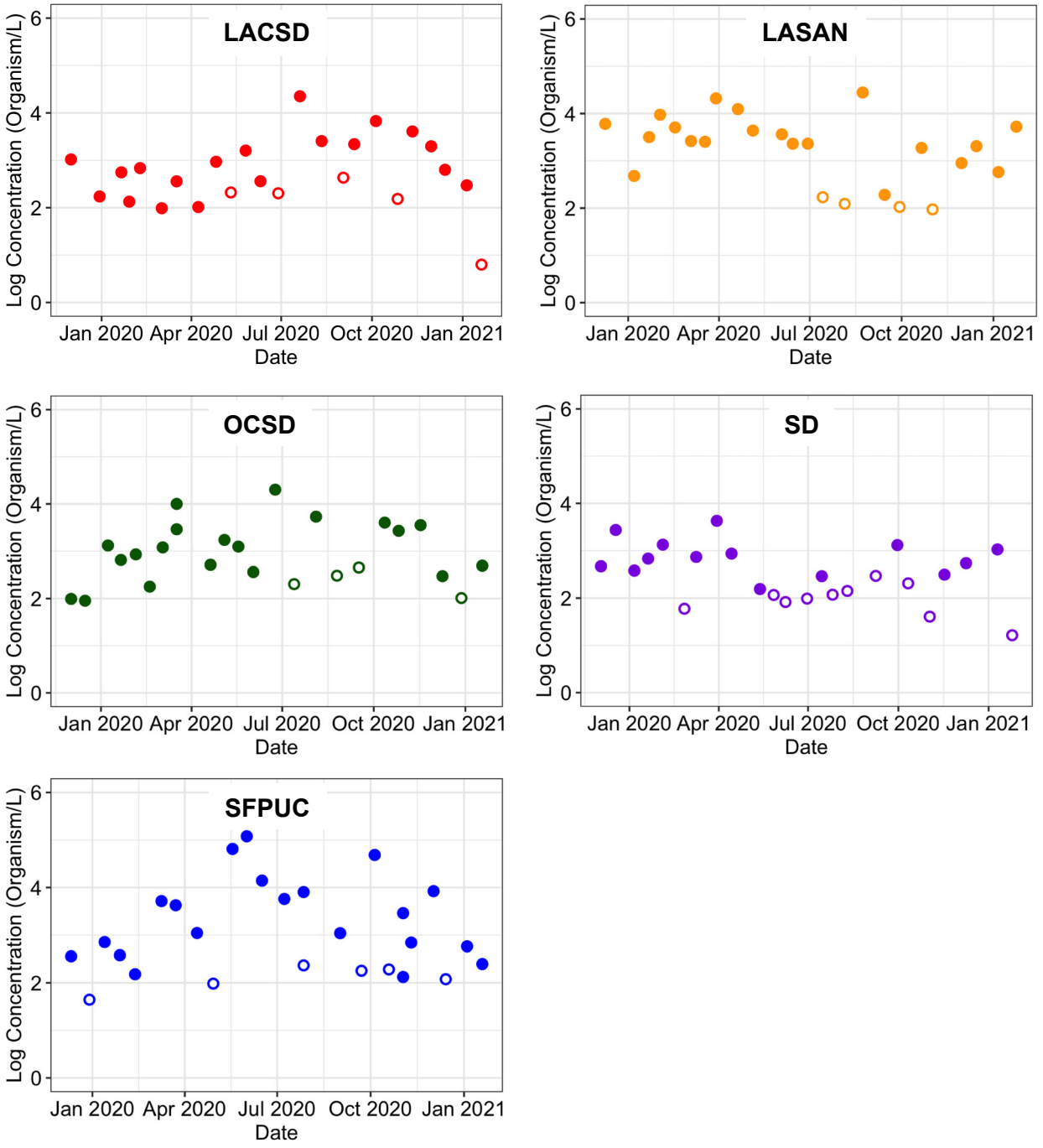

Figure 5-20. Times Series of the Recovery-Corrected Culturable Adenovirus Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

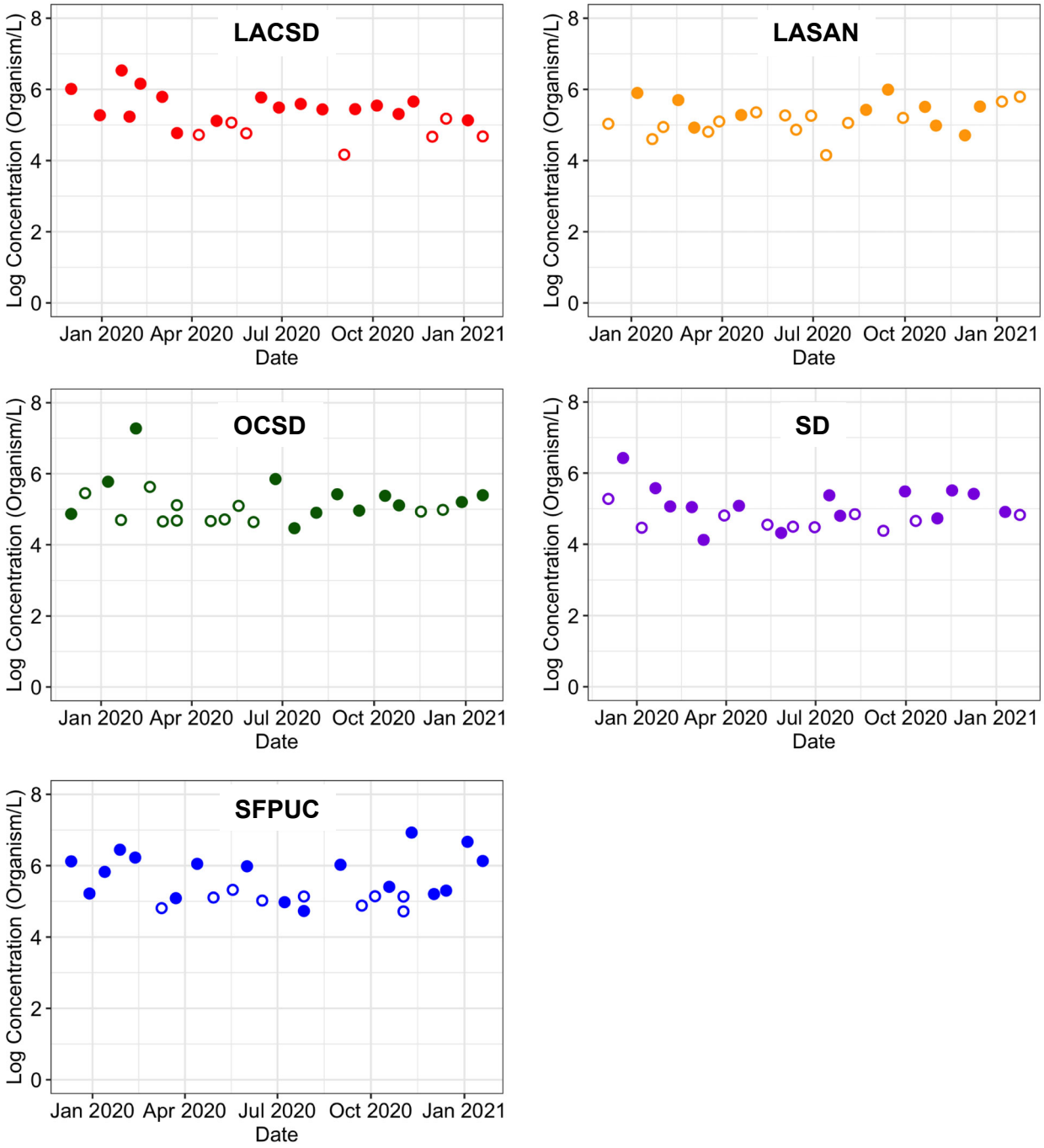

Figure 5-21. Times Series of the Recovery-Corrected Enterovirus Molecular Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

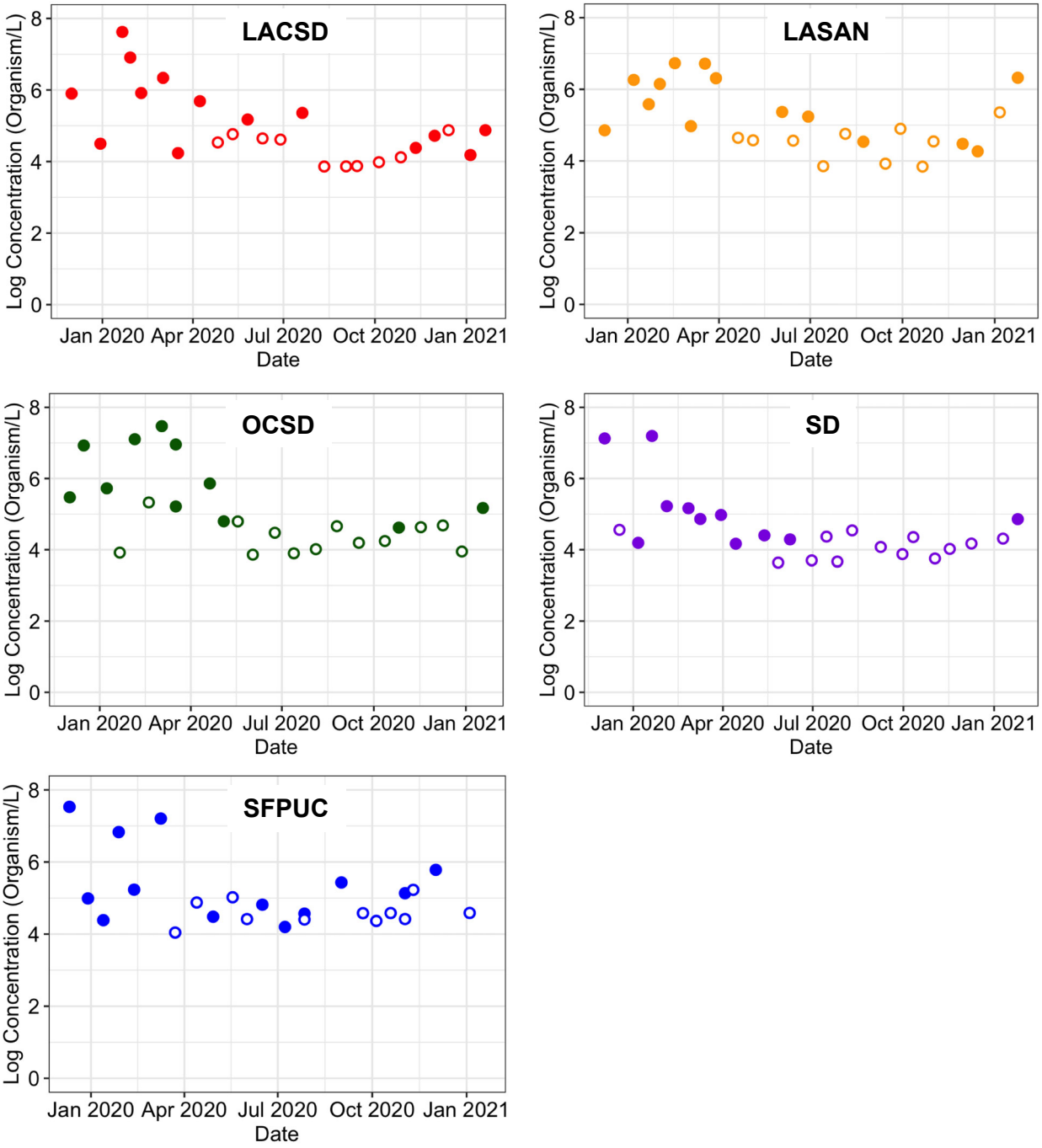

Figure 5-22. Times Series of the Recovery-Corrected Adenovirus Molecular Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

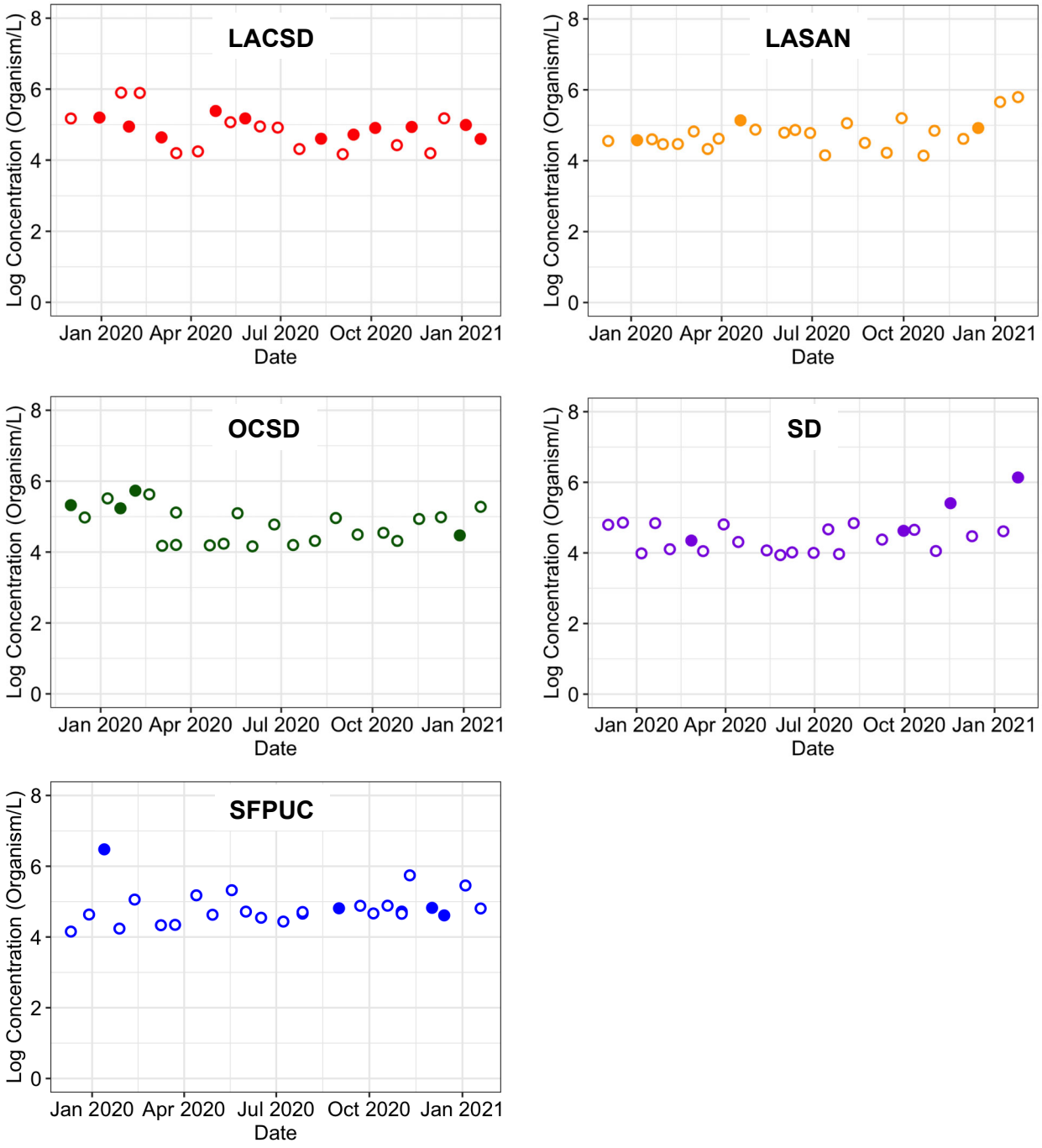

Figure 5-23. Times Series of the Recovery-Corrected Norovirus GIA Molecular Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

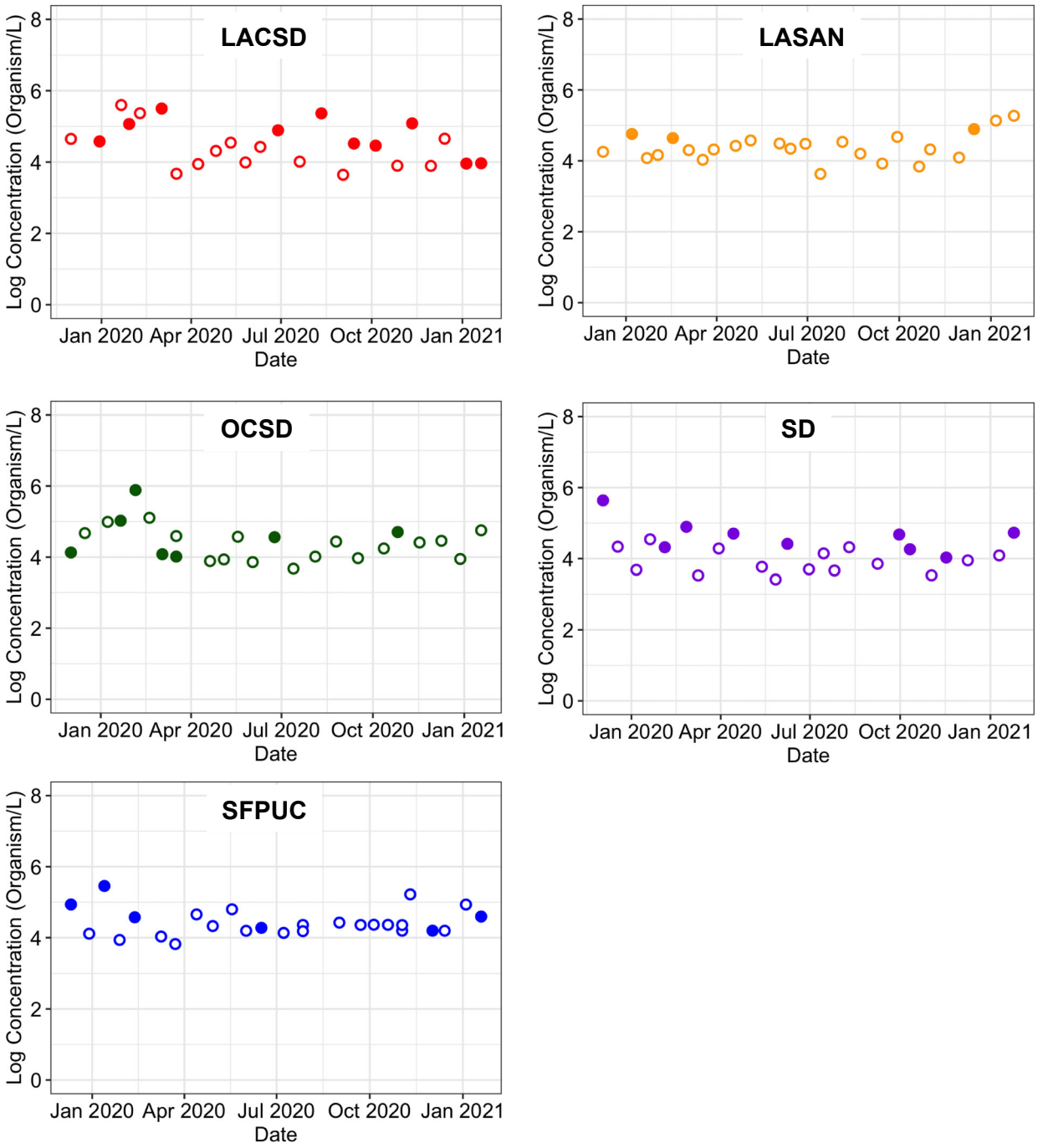

Figure 5-24. Times Series of the Recovery-Corrected Norovirus GIB Molecular Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

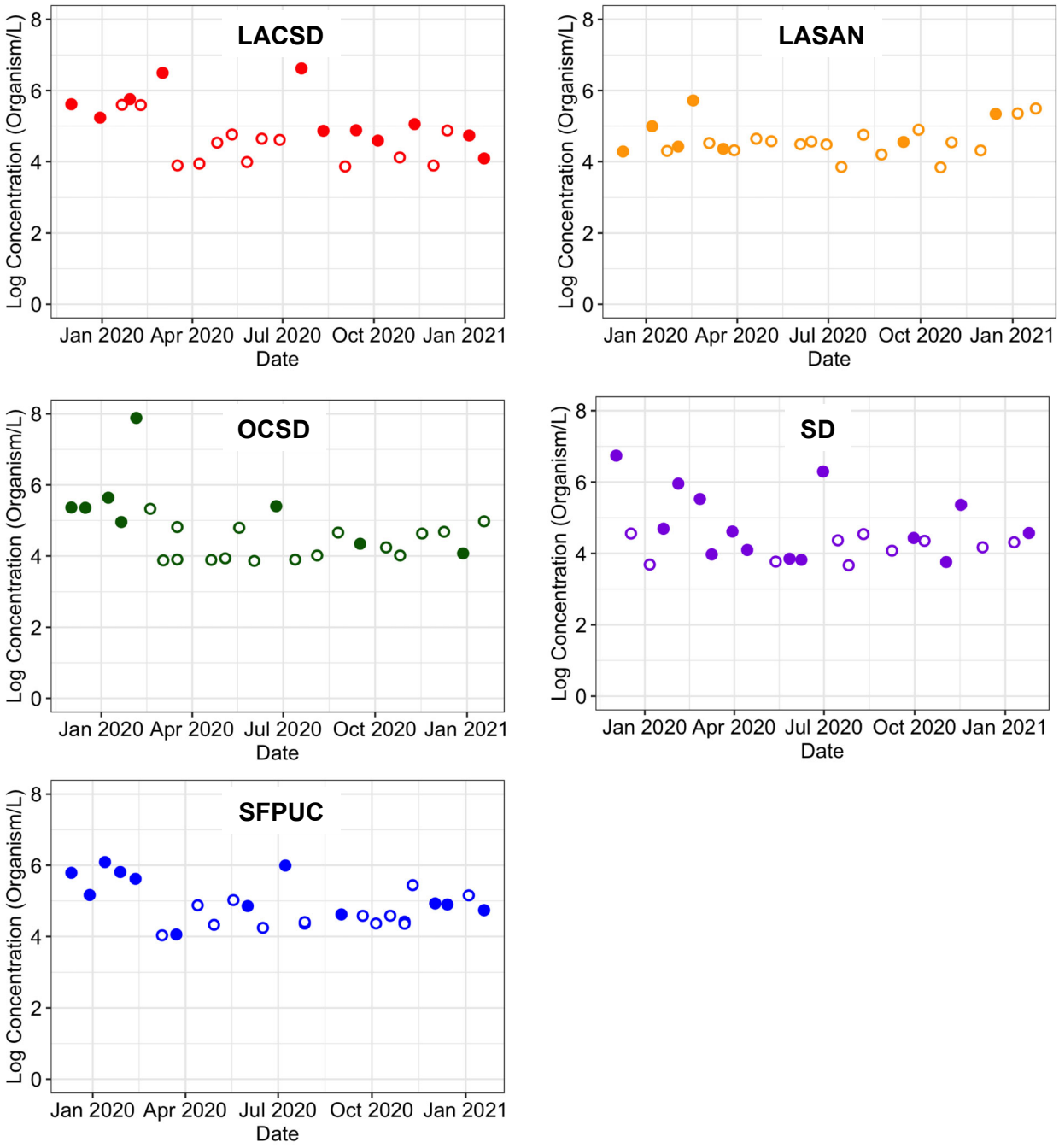

Figure 5-25. Times Series of the Recovery-Corrected Norovirus GII Molecular Concentrations at the Five WWTPs.

NDs and DNQs are shown as unfilled circles at the limit of quantification.

5.6 Comparison of Molecular to Culture Concentrations

The time series of the ratio of the recovery-corrected culturable enterovirus and adenovirus concentrations to the recovery-corrected molecular concentrations is shown in Figure 5-26. The average ratio between the molecular enterovirus concentrations and the culturable enterovirus concentrations was 2.1 $\log_{10}$. The average ratio between the molecular adenovirus concentrations and the culturable adenovirus concentrations was 2.4 $\log_{10}$. Note, there was a large degree of variability in the molecular to culture ratio for both enterovirus and adenovirus, with a range of approximately 0 to 4 $\log_{10}$ for enterovirus and 0 to 5 $\log_{10}$ for adenovirus (probability plot shown in Figure 5-27). Additionally, the molecular to culture ratio measured in this study was lower than the range measured in the San Diego 2016 study, where the ratio ranged from 4.5 to 8-log. The implications of this variability in the molecular to culture ratio are discussed in the Chapter 7.

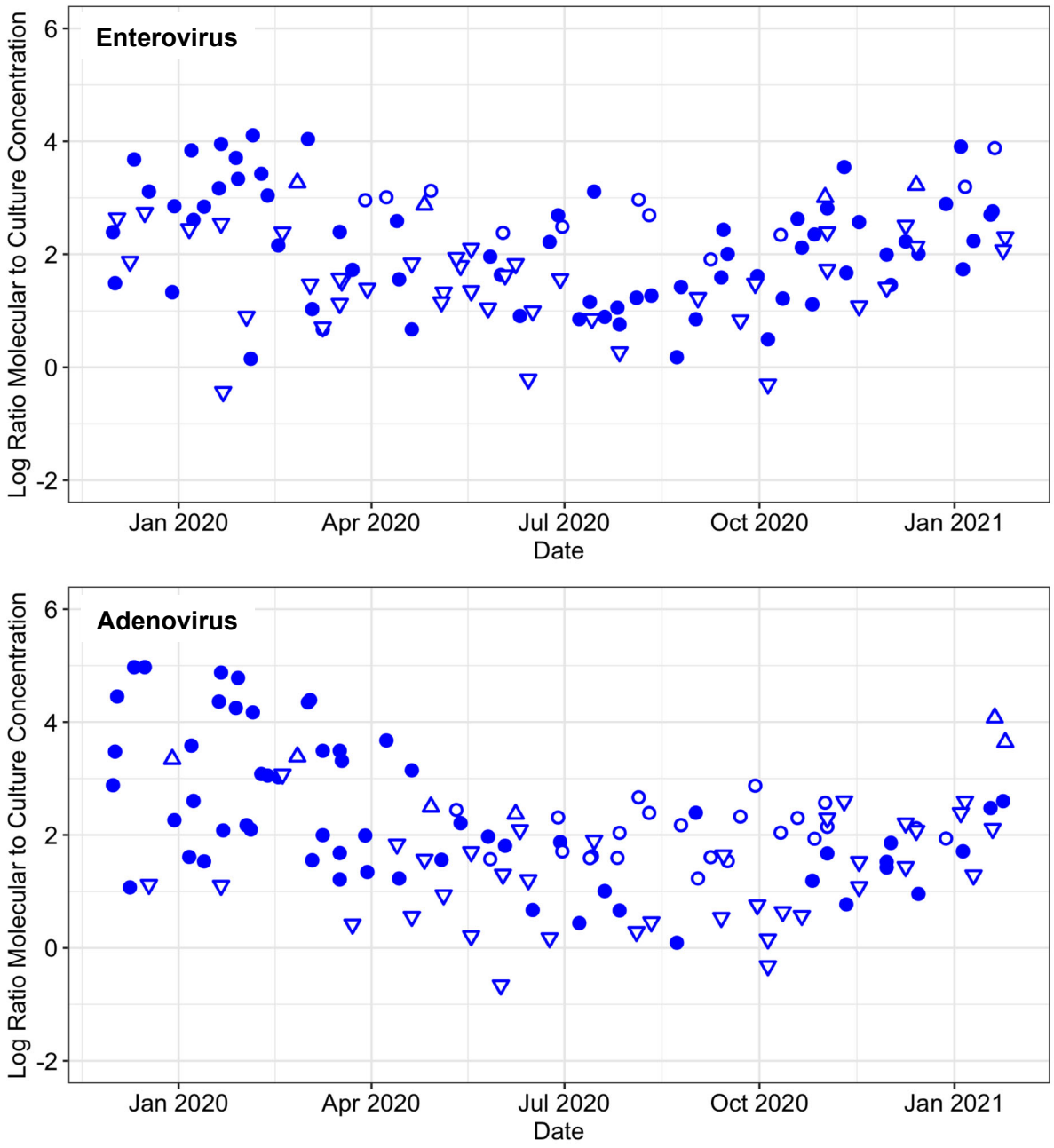

Figure 5-26. Ratio of the Recovery-Corrected Concentrations Measured Using Molecular Methods to Concentrations Measured Using Culture Methods for Enterovirus and Adenovirus.

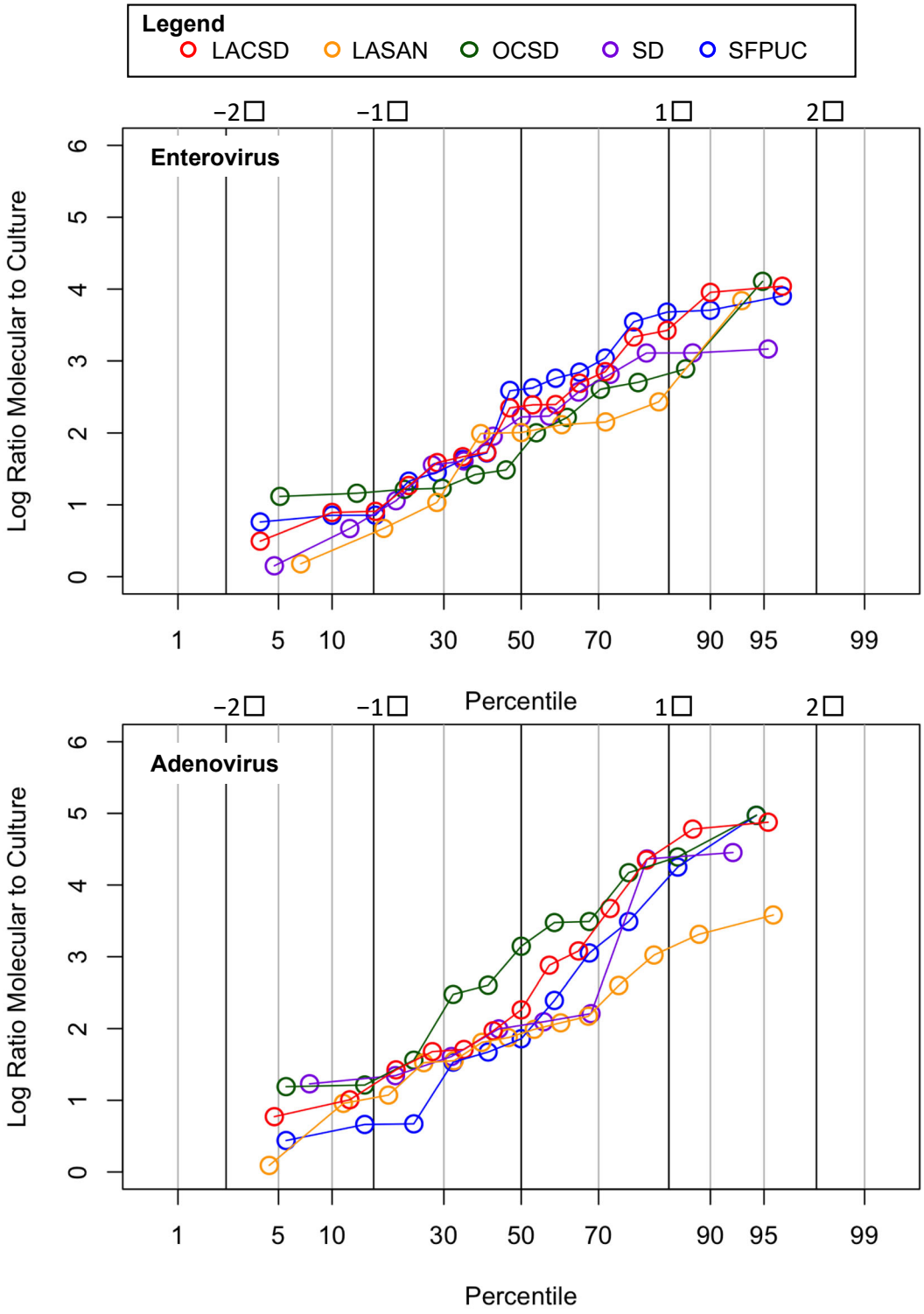

Figure 5-27. Probability Plot of the Ratio of the Recovery-Corrected Concentrations Measured Using Molecular Methods to Concentrations Measured Using Culture Methods for Enterovirus and Adenovirus.

Ratios for each of the five WWTPs are distinguished by color. Only samples where both the culture and molecular concentrations were above the LOQs are shown here.

CHAPTER 6

SARS-CoV-2 Monitoring

As the COVID-19 pandemic spread into California in February 2020, and early studies were reporting the presence of SARS-CoV-2 genetic material in wastewater (Ahmed et al. 2020, Medema et al. 2020, Wu et al. 2020), the TWG began coordinating with the SWB to include SARS-CoV-2 in the DPR-2 monitoring campaign. Beginning in March 2020, SARS-CoV-2 analysis was included for every sample collected during the DPR-2 monitoring campaign. SARS-CoV-2 concentrations were measured using quantitative PCR with three primer/probe sets: CDC N1, CDC N2, and WHO E. Beginning in April 2020, the method recovery was measured by spiking a known quantity of another betacoronavirus, OC43, to each sample.

One benefit of the earlier sampling events was that nucleic acid extracts from the wastewater samples had been archived and could be used retrospectively to quantify SARS-CoV-2 concentrations. Given the timing of the campaign, the archived samples offered an opportunity to evaluate both a pre-COVID-19 "baseline" period (November 2019 to February 2020) as well as the period when the epidemic led to more overt, widespread infection and disease in California (starting in March 2020). The SARS-CoV-2 concentrations were non-detect (ND) in all archived samples through March 2020 (Table 6-1). However, one concern was that the original virus SOP—which was developed for non-enveloped enteric viruses—may not be compatible with the enveloped SARS-CoV-2 virus. In particular, the original virus SOP included a concentration step using PEG followed by a chloroform extraction, and it was hypothesized that the use of an organic solvent may alter or damage the coronavirus's lipid membrane and impact the accuracy of the quantification. In fact, the OC43 recovery for the PEG/chloroform was very low: 0.16%. Therefore, while the NDs in the archived samples could be due to an absence of SARS-CoV-2, it could also be due to the low sensitivity of the PEG/chloroform method for enveloped virus. Beginning in April 2020, some of the samples had detectable quantities of SARS-CoV-2 using the PEG/chloroform method, despite the low method sensitivity (Table 6-1). Note, because the recovery of enveloped viruses is very low with the PEG/chloroform method, the uncertainty associated with the recovery-corrected concentrations using this method is likely high.

Table 6-1. Archived Sample Results and Comparison of Original PEG/Chloroform Method to Optimized Ultrafiltration Method.

	Original PEG/ Chloroform Method (GC/L)			**Optimized Method for Enveloped Virus (Ultrafiltration) (GC/L)**		
	N1	N2	E	N1	N2	E
November 2019 – March 2020 (36 samples)	ND	ND	ND	Method not yet developed		
3/29/20 LASAN	ND	ND	ND	500,000	1,400,000	--
4/8/20 LACSD	ND	ND	ND	7,900	11,000	ND
4/13/20 SFPUC	11,000,000	14,000,000	ND	130,000	410,000	380,000
4/14/20 SD	ND	ND	ND	1,700	14,000	ND
4/20/20 LASAN	3,500,000	ND	ND	1,700,000	2,000,000	130,000

Raw wastewater concentrations corrected for recovery based on OC43 matrix spikes (0.03 to 1.8% for the Original PEG/Chloroform Method and 2 to 7% for the Optimized Ultrafiltration Method).

The TWG worked with the DPR-2 laboratories to develop an optimized method for enveloped virus analysis. After quickly testing a variety of concentration methods in March 2020, the method that proved to have the greatest sensitivity was an ultrafiltration method. The optimized standard operating procedure (SOP) for detection of enveloped viruses is described in the DPR-2 QAPP (Cel Analytical Inc. 2020). All of the initial samples that were analyzed using the optimized method (beginning at the end of March 2020) had detectable quantities of SARS-CoV-2 (Table 6-1). The OC43 recovery efficiency of the optimized method is two orders of magnitude higher than that of the PEG/chloroform method, with an average recovery of 20% (Table 6-2). The full dataset with the uncorrected concentrations, recovery, and recovery-corrected concentrations is provided in Table A-5.

The LOD and LOQ for the optimized method were measured using the same rigorous method that was used for the other pathogens in the campaign (see Section 5.1) (Table 6-2). However, the majority of the samples had SARS-CoV-2 concentrations below the LOQ and LOD of the optimized method. Despite not meeting the same quality standard as the rest of the DPR-2 dataset, there was still value to be gained from the SARS-CoV-2 results below the LOD and LOQ. Therefore, a SARS-CoV-2 result was only considered non-detect if amplification was below the fluorescence threshold at a cycle number of 40. The LOQ shown in Figures 6-1 through 6-5 is the lowest detectable concentration reported by the laboratories (not the LOQ measured using the Forootan method shown in Table 6-2).

Table 6-2. Summary Statistics on Number of Detects and Recovery Efficiency with the Optimized Enveloped Virus Method.

	SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 E
Number of samples	97	97	85
Percent positives (%)[1]	77%	81%	61%
Recovery (%)	20 [1 – 158]	20 [1 – 158]	20 [1 – 158]
Recovery-corrected LOD determined using the Forootan method (log_{10} organisms/L)[2]	5.6	5.6	5.6
Recovery-corrected LOQ determined using the Forootan method (log_{10} organisms/L)[2]	5.8	5.9	6.2

[1]A sample was considered positive if the amplification was above the fluorescence threshold at a cycle number less than or equal to 40.
[2]The limit of detection and limit of quantification varied between samples due to varying recovery; the mean is shown here.

The recovery-corrected concentrations over time at the five WWTPs measured using the optimized method are plotted in Figure 6-1 through 6-5. The recovery-corrected concentrations are compared California's public health data on the number of new confirmed cases of COVID-19 infections in the county served by the WWTP (California Open Data Portal, n.d.), the total COVID-19 hospitalizations in the county served by the WWTP (California Open Data Portal, n.d.), and the testing positivity rate in California (The COVID Tracking Project, n.d.). The SARS-CoV-2 generally increased between November 2020 and January 2021 when California experienced a large increase in confirmed COVID-19 infections and hospitalizations (California Open Data Portal, n.d.). The SARS-CoV-2 concentrations also spiked in isolated instances when an increase in confirmed COVID-19 infections and hospitalizations was not

observed. Note, the DPR-2 sampling plan was developed to capture the full distribution of concentrations over time, including instantaneous minimum and maximum values. To obtain this information, grab samples (rather than composite samples) were purposefully collected at different times of day and different days of the week. Consequently, these factors may lead to greater variability than studies using composite samples. Additionally, samples were measured by two laboratories (BCS Labs and Cel Analytical); despite following the same method and taking considerable measures to align results between the two labs, some lab-to-lab variability may occur. Such an outcome would be in line with the findings from a recent large-scale, interlaboratory comparison of SARS-CoV-2 methods for raw wastewater (Pecson et al. 2021).

To better track trends in SARS-CoV-2 concentrations over time, samples from a given WWTP should be analyzed by the same laboratory using the exact same SOP each time (Pecson et al. 2021). Additionally, increasing the frequency of sample collection would allow the changes in concentration to be more easily distinguished from the variability associated with the analysis of biological samples. Collecting composite samples instead of grab samples may also help to minimize the variability associated with sampling. Lastly, a high sensitivity method will be important for tracking trends across a range of concentrations.

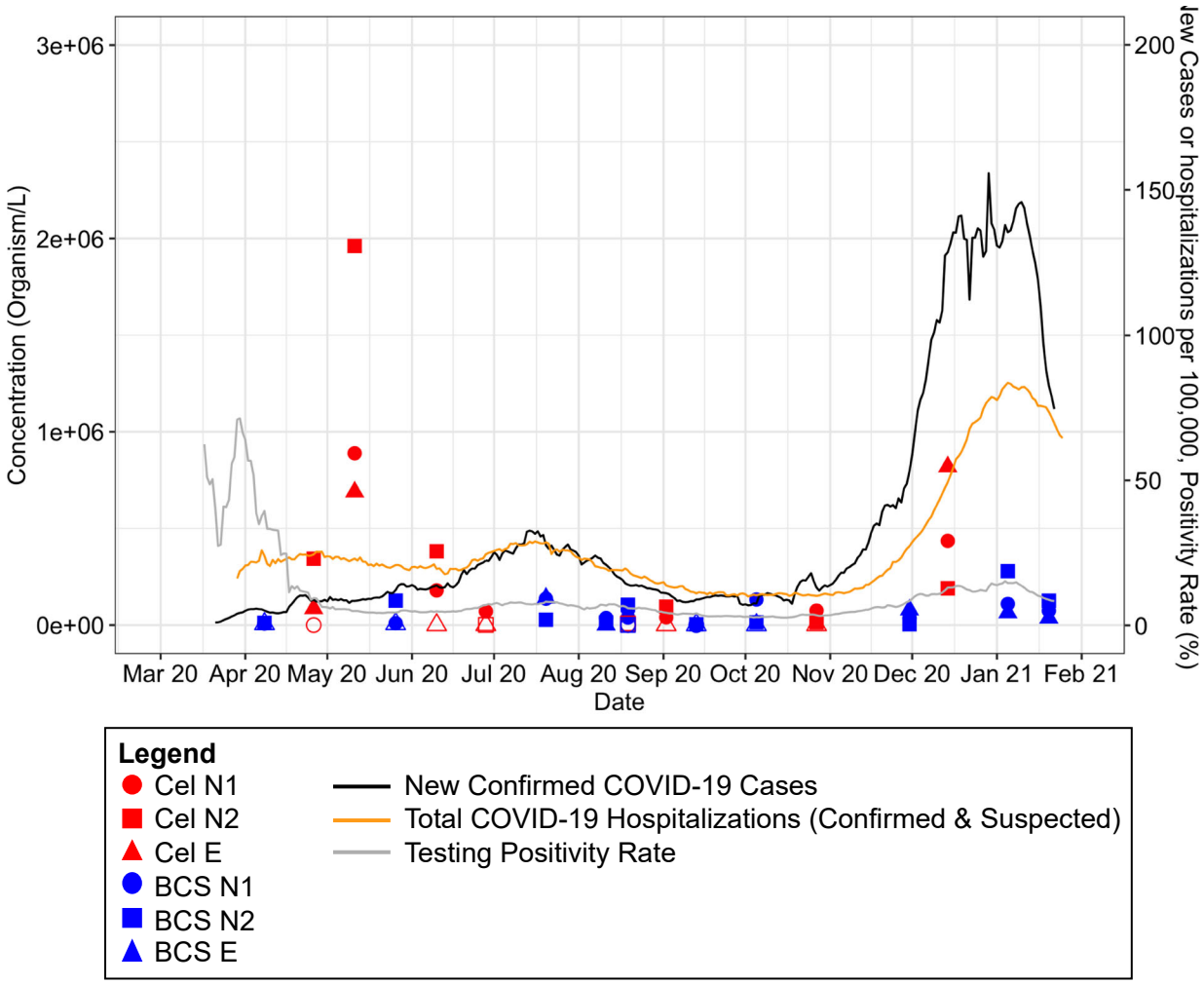

Figure 6-1. LACSD Recovery-Corrected SARS-CoV-2 Concentrations (N1, N2, and E Targets) Measured Using the Optimized Ultrafiltration Method.

Unfilled icons represent samples where the fluorescence was below the threshold at a cycle number of 40; the value associated with the unfilled icon is the lowest detectable concentration reported by the laboratories. The data are compared to public data on the number of new confirmed cases of COVID-19 infections in Los Angeles County (7-day rolling average) (California Open Data Portal, n.d.), COVID-19 hospitalizations in Los Angeles County (California Open Data Portal, n.d.), and testing positivity rate in California (7-day rolling average) (California Open Data Portal, n.d.).

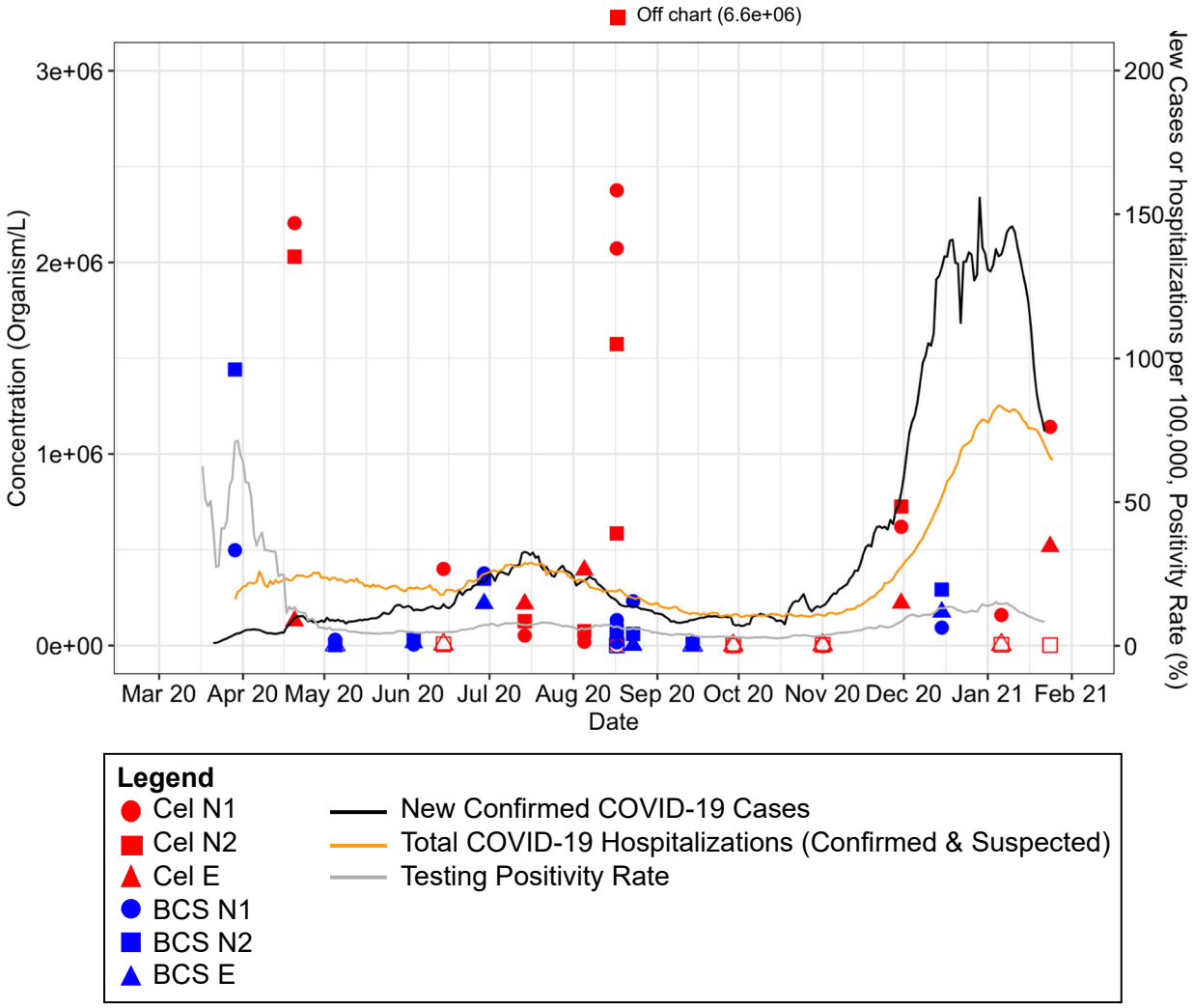

Figure 6-2. LASAN Recovery-Corrected SARS-CoV-2 Concentrations (N1, N2, and E Targets) Measured Using the Optimized Ultrafiltration Method.

Unfilled icons represent samples where the amplification was below the fluorescence threshold at a cycle number of 40; the value associated with the unfilled icon is the lowest detectable concentration reported by the laboratories. The data are compared to public data on the number of new confirmed cases of COVID-19 infections in Los Angeles County (7-day rolling average) (California Open Data Portal, n.d.), COVID-19 hospitalizations in Los Angeles County (California Open Data Portal, n.d.), and testing positivity rate in California (7-day rolling average) (California Open Data Portal, n.d.).

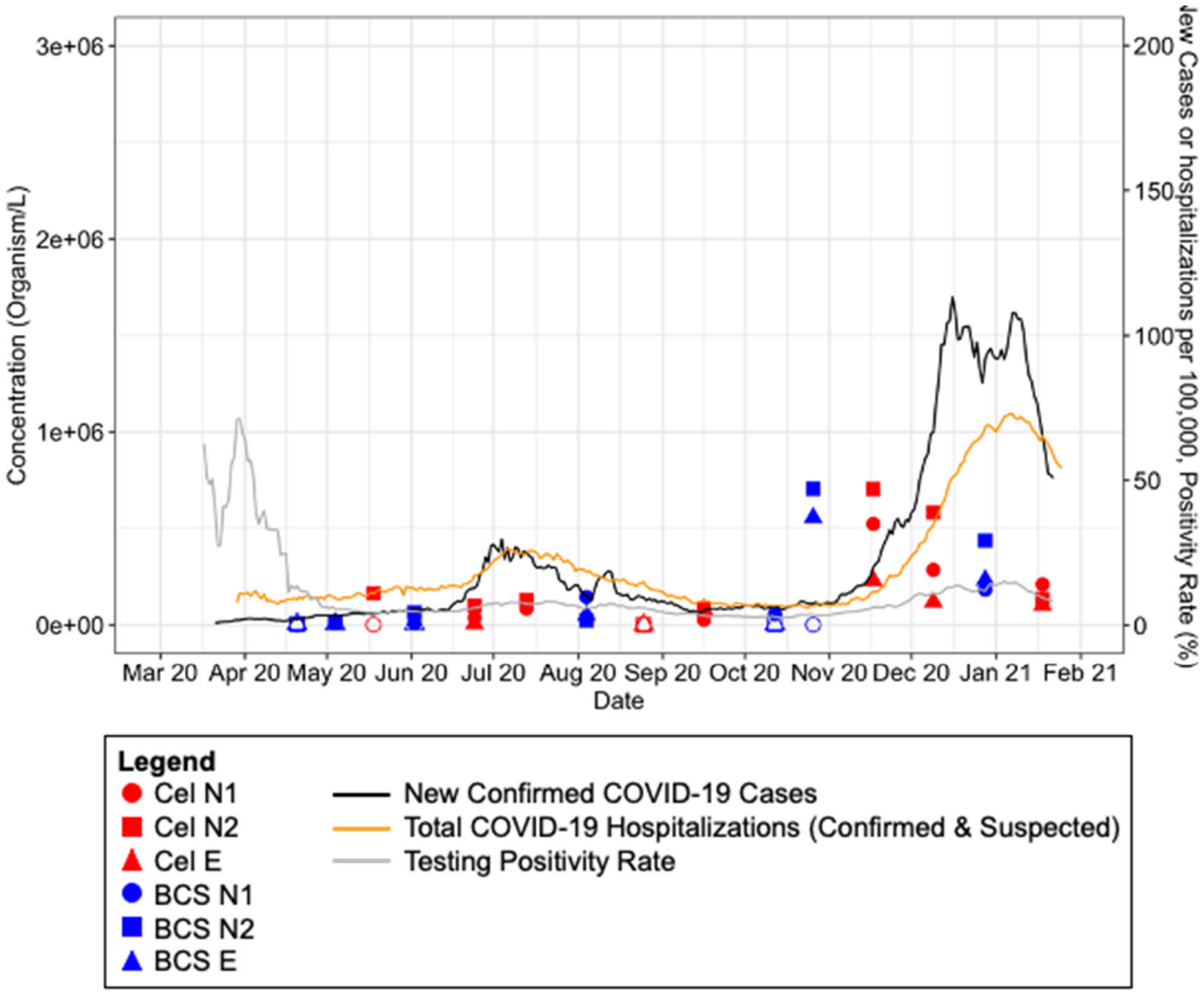

Figure 6-3. OCSD Recovery-Corrected SARS-CoV-2 Concentrations (N1, N2, and E Targets) Measured Using the Optimized Ultrafiltration Method.

Unfilled icons represent samples where the amplification was below the fluorescence threshold at a cycle number of 40; the value associated with the unfilled icon is the lowest detectable concentration reported by the laboratories. Two data points with the E primer/probe set were outliers (>1-log higher than the N1 and N2 primer/probe sets) and are not plotted: Cel's 5/18/20 sample with a concentration of $10^{7.8}$ GC/L and Cel's 7/13/20 sample with a concentration of $10^{6.9}$ GC/L. The data are compared to public data on the number of new confirmed cases of COVID-19 infections in Orange County (7-day rolling average) (California Open Data Portal, n.d.), COVID-19 hospitalizations in Orange County (California Open Data Portal, n.d.), and testing positivity rate in California (7-day rolling average) (California Open Data Portal, n.d.).

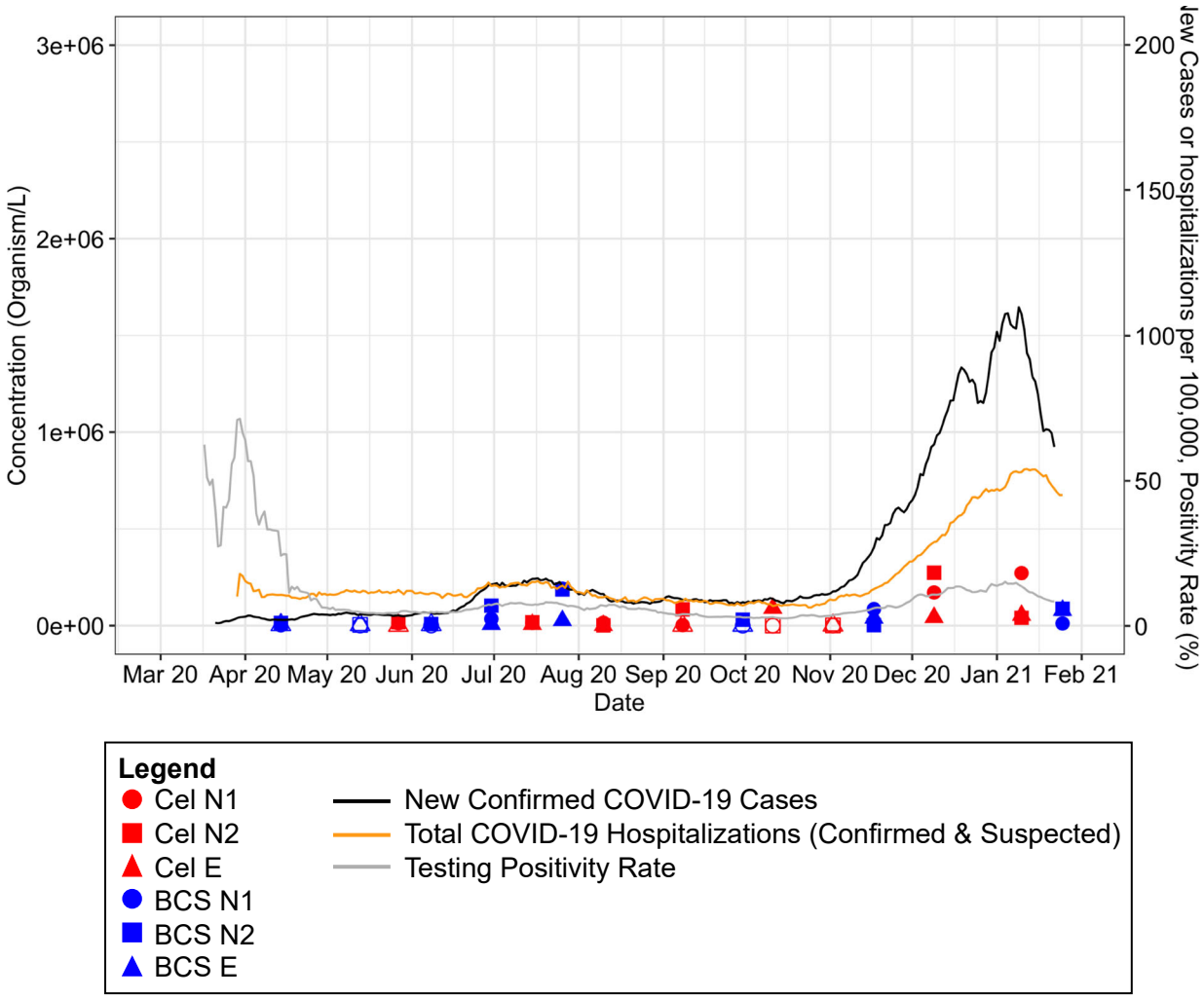

Figure 6-4. SD Recovery-Corrected SARS-CoV-2 Concentrations (N1, N2, and E Targets) Measured Using the Optimized Ultrafiltration Method.

Unfilled icons represent samples where the amplification was below the fluorescence threshold at a cycle number of 40; the value associated with the unfilled icon is the lowest detectable concentration reported by the laboratories. The data are compared to public data on the number of new confirmed cases of COVID-19 infections in San Diego County (7-day rolling average) (California Open Data Portal, n.d.), COVID-19 hospitalizations in San Diego County (California Open Data Portal, n.d.), and testing positivity rate in California (7-day rolling average) (California Open Data Portal, n.d.).

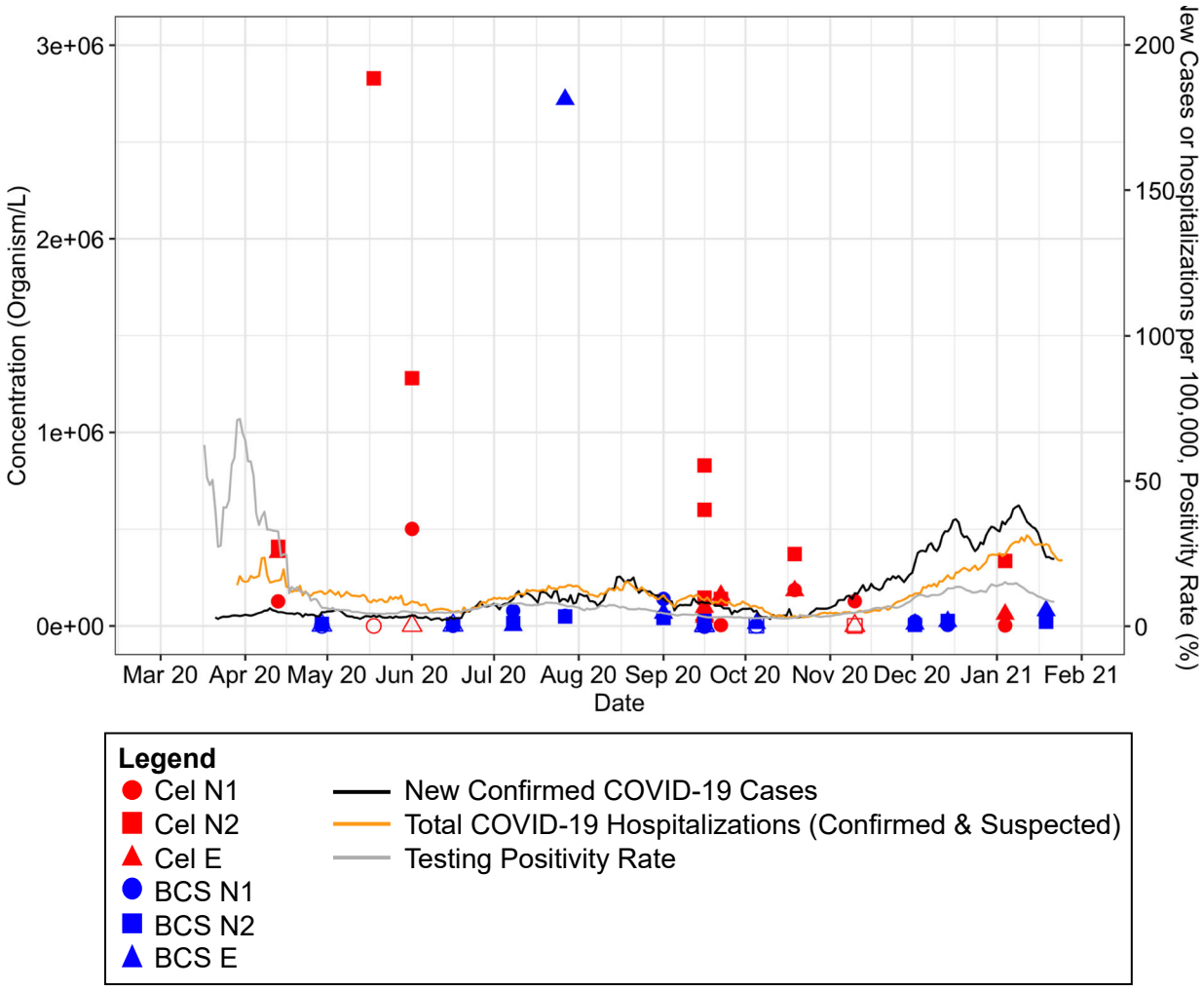

Figure 6-5. SFPUC Recovery-Corrected SARS-CoV-2 Concentrations (N1, N2, and E Targets) Measured Using the Optimized Ultrafiltration Method.

Unfilled icons represent samples where the amplification was below the fluorescence threshold at a cycle number of 40; the value associated with the unfilled icon is the lowest detectable concentration reported by the laboratories. One data point with the E primer/probe set was an outlier (1-log higher than the N1 and N2 primer/probe sets) and is not plotted: Cel's 5/18/20 sample with a concentration of $10^{7.3}$ GC/L. Additionally, the data from Cel's 7/27/20 sample (concentrations of $10^{7.6}$, $10^{9.1}$, and $10^{8.3}$ for N1, N2, and E, respectively) were considered outliers and are not plotted since they were 2-log higher than BCS's analysis of the same sample The data are compared to public data on the number of new confirmed cases of COVID-19 infections in San Francisco County (7-day rolling average) (California Open Data Portal, n.d.), COVID-19 hospitalizations in San Francisco County (California Open Data Portal, n.d.), and testing positivity rate in California (7-day rolling average) (California Open Data Portal, n.d.).

While the optimized method used for the DPR-2 monitoring campaign was more sensitive for SARS-CoV-2 than the original PEG/chloroform method, future optimizations could be explored to reduce the frequency of non-detects. A recent large-scale, interlaboratory comparison of SARS-CoV-2 methods revealed that the method developed by the research group of one of the members of the TWG, Dr. Nelson at UC Berkeley, could provide greater sensitivity (Pecson et al. 2021). BCS labs conducted a side-by-side comparison, analyzing a single split sample with both the DPR-2 ultrafiltration method and Dr. Nelson's direct extraction method (Whitney et al. 2020). The recovery-corrected concentrations from

the two methods were in good agreement (Table 6-3). Based on the results of this single split sample, Dr. Nelson's method had a LOD that was three times lower than the DPR-2 ultrafiltration method (i.e., three times greater sensitivity), as calculated using the concentration factor (CF) and matrix spike recovery (same matrix spike, OC43, for both methods):

$$LOD\ (\frac{GC}{L}) = \frac{Instrument\ detection\ limit\ (\frac{GC}{L})}{CF\ \times\ Recovery}$$

The sensitivity of Dr. Nelson's method may improve even further with practice by the lab. Additionally, fewer non-detects were obtained using Dr. Nelson's method. Other considerations for these method include:

- **Sample Volume:** Both methods require a similar sample volume (30 mL for the DPR-2 ultrafiltration method and 40 mL for the Dr. Nelson's direct extraction method)
- **Cost:** Both methods are anticipated to have a similar cost from a commercial lab
- **Ease of processing:** Dr. Nelson's method would require less effort than the DPR-2 ultrafiltration method if a large number of samples are being processed
- **Laboratory equipment requirements**: Dr. Nelson's method requires less high-tech instrumentation than the DPR-2 ultrafiltration method, which requires a high-speed centrifuge

Table 6-3. Comparison of the DPR-2 Ultrafiltration Method and Dr. Nelson's Direct Extraction Method for Quantification of SARS-CoV-2 in Raw Wastewater.

Method	Primer/ Probe Set	Recovery-Corrected Concentration (Log_{10} GC/L)	Matrix Spike Recovery (%)	Concentration Factor	Comments[1]
DPR-2 Ultrafiltration	CDC N1	ND	2.0	60	Both replicates were ND
DPR-2 Ultrafiltration	CDC N2	5.8	2.0	60	One replicate was ND
DPR-2 Ultrafiltration	WHO E	5.7	2.0	60	
Nelson Direct Extraction	CDC N1	5.1	5.0	80	
Nelson Direct Extraction	CDC N2	5.5	5.0	80	
Nelson Direct Extraction	WHO E	5.4	5.0	80	One replicate was ND

[1]RNA extracts were run in duplicates

CHAPTER 7

Recommendations

In this final section, the DPR-2 TWG provides recommendations for the use of the data in the development of DPR regulations, recommendations for future pathogen monitoring studies, and additional topics for research.

7.1 Use of DPR-2 Data for Regulatory Development

The TWG recommends that the DPR-2 dataset be used as inputs for DPR regulatory development related to probabilistic assessments of treatment train performance (PATTP) and quantitative microbial risk assessment (QMRA). Based on a review of the recent literature, the TWG finds the DPR-2 dataset to be the best available, high quality data based on the following criteria:

- **Large dataset:** the 120-sample datasets for the pathogens and indicators are as large and frequently larger than many of the previously reported studies. The large datasets allow the probability distributions to be extended below the 1st percentile and above the 99th percentile. This range provides greater clarity on the shape of the tails of the distribution where rare but important concentrations fall. As described in DPR-1, these rare, high values in a dataset may lead to important impacts on risk.
- **High frequency of quantifiable values:** over the course of the study, approximately 94% of all of the culture and microscopy assays yielded quantifiable values. This rate of non-detects ranks this study among the top studies in terms of percent detections. Optimization of the methods to improve sensitivity, such as the decision to evaluate larger volumes of pellet for cysts and oocysts, contributed to this outcome.
- **Rigorous QA/QC:** the strict requirements for QA/QC in this study provide further confidence in the quality of the results. The use of positive and negative controls, matrix spikes, extraction controls, frequent communication between the laboratories and the TWG, and other requirements supports the findings that the distribution of concentrations closely represents true variability in the numbers and not artifacts of the enumeration itself.
- **Geographic distribution:** samples were collected at multiple locations across Northern and Southern California, which allows the aggregated dataset to provide a better estimate of concentrations across the state.
- **Impact of seasonality:** by sampling over 14 months (including two winters and a summer), the data include variability associated with seasonal differences in concentrations.

While the data have been plotted as discrete data points in Chapter 5, the TWG recommends that the data be fit and used as modeled distributions. This recommendation applies if the data can be well described by such distributions. Per the discussion in Chapter 5, the data for all the pathogens were well described by $\log_{10}$ normal distributions. One benefit of this approach is that the modeled distributions allow for estimates outside of the range of discrete values obtained through the study. For example, a 100-point dataset only allows observations of values to the 99th percentile (i.e., 1 – (1/100)), meaning that the discrete values would not be able to predict less frequent occurrences, such as the 99.9th percentile. By using a modeled distribution, the full range of values can be included in the PATTP and QMRA. The DPRisk tool developed in DPR-1 allows for the input of both discrete values and modeled distributions. Because there were no systematic differences in the concentrations across the different

wastewater treatment plants, the TWG recommends using the entire aggregated dataset rather than tailoring the data to a specific facility.

Beyond the DPR-2 data, the TWG identified a set of literature studies that also met strict requirements for QA/QC (Section 5.3). The TWG developed a method to incorporate the literature values into the DPR-2 pathogen distributions. The TWG recommends that the DPR-2 dataset be augmented with these studies in order to leverage additional high-quality data. The consistency of the distribution parameters with and without the additional studies provides evidence that the DPR-2 dataset aligns well with the previous studies.

7.2 Quality Control

Throughout the project, the TWG emphasized the importance of rigorous QA/QC requirements for the study. All pathogen monitoring studies will show variability in pathogen concentrations in wastewater. The two main sources of variability include a) changes in the concentration of pathogens present in wastewater (i.e., true variability), as well as b) variability due to the sampling, processing, and enumeration steps (i.e., apparent variability). QA/QC requirements can help to minimize or eliminate many sources of apparent variability so that the data better reflect the true distribution of pathogens in wastewater. By requiring the labs to run and document the results for the various QA/QC controls, this study provides a high level of confidence for future users of the data. The TWG recommends that all future studies document all of the QA/QC steps and the results of these steps in their reports. The DPR-2 QAPP should serve as a template for the level of QA/QC rigor for future studies.

One difficulty that arose when attempting to incorporate historical data into the DPR-2 dataset was the variations in the methods that were employed at different times. Even with the DPR-2 study, modifications were made initially to improve the quality of the enteric virus assays, and two additional rounds of methods modifications were made during the campaign to further optimize SARS-CoV-2 enumeration as well. By documenting method performance—including matrix spikes and recovery efficiencies—differences between methods can be quantified and normalized. With such data in hand, it opens the door to integrating historical data with new data. For these reasons, the TWG recommends the use of the DPR-2 QAPP (or equivalent) to help integrate new studies with both past and future efforts.

One of the key quality control steps was the use of matrix spikes. In this study, the TWG required a matrix spike be processed in every sample for protozoa assays, and in every other sample for enteric virus assays. The matrix spikes provide insight into the reproducibility of the methods between labs, between locations, and over time at a given location. Matrix spikes can give insight into the variability of the wastewater matrix and help identify events that might lead to better or worse recovery of the pathogens from the matrix (e.g., wet weather events). In this study, the acceptable range of recovery efficiencies was specified in the QAPP, meaning that the labs were required to flag and/or repeat samples that did not fall within this range. In this study, the average recovery efficiencies across all pathogens (with the exception of SARS-CoV-2) ranged from 39 to 56%, meaning that the recovery-corrected values were approximately 2-fold higher than the uncorrected data. These recovery efficiencies were much higher and less variable than those reported in a recent interlaboratory methods comparison for SARS-CoV-2 (Pecson et al. 2021). In that study, the recovery efficiencies spanned seven orders of magnitude compared to the approximate two orders of magnitude differences observed here. The TWG recommends the use of matrix spikes to quantify recovery and that both the uncorrected and corrected values be included in future reporting along with recovery efficiency. The TWG recommends correcting data for recovery efficiency and using the recovery-corrected data as inputs into quantitative

microbial risk assessments (QMRA). Therefore, the DPR-2 data and literature data were corrected for recovery efficiency when developing the combined-distributions provided in Section 5.3.

7.3 Impact of COVID-19 on DPR-2 Dataset

The impact of COVID-19 on the DPR-2 dataset is not clear. Based on a comparison of the DPR-2 data with historical pathogen distributions, there was not an observable, systematic shift in the concentrations. One hypothesis is that the COVID-related restrictions (e.g., shelter-in-place, social distancing) would decrease the incidence of acute gastrointestinal and other illnesses by reducing transmission. Nevertheless, multiple pathogens were present at concentrations equivalent to or higher than the highest concentrations observed in the literature review, e.g., *Giardia*, culturable enterovirus, and culturable adenovirus. These findings raise doubts on this hypothesis. On the other hand, the molecular results for all of the measured viruses (enterovirus, adenovirus, and norovirus) were toward the low end of the historical distributions with multiple samples below the LOQ and LOD. Finally, the *Cryptosporidium* concentrations fell in the middle of those reported previously.

To provide further insight into this question, the TWG recommends that the SWB continue to collect high-quality pathogen data in raw wastewater both during and after the COVID-19 pandemic. On the drinking water side, surface water treatment plants are required to perform a watershed sanitary survey every five years that includes—among other requirements—a summary of source water quality monitoring data and a description of activities and sources of contamination (CCR Section 64665). The Long Term 2 Enhanced Surface Water Treatment Rule (LT2) supplemented existing regulations to address *Cryptosporidium* in systems with higher risk. LT2 requires two rounds of source water monitoring to characterize the risk from *Cryptosporidium* (categorized into four risk "bins") and to determine the appropriate level of treatment to control that risk. These 24-month campaigns require monthly monitoring of *Cryptosporidium*, *E. coli*, and turbidity for most treatment plants (40 CFR § 141.701). The logic of characterizing source waters and confirming treatment requirements would seem to apply to all potable applications regardless of the source water. This "source to tap" view of protection is a part of the Safe Drinking Water Act and was recommended as an appropriate strategy for potable reuse as well. Given the relative lack of pathogen information in wastewater compared to surface waters, requiring periodic monitoring campaigns would provide additional data with which to characterize pathogen concentrations and further assess the impact of COVID-19. If the State Water Board includes requirements for monitoring, the TWG recommends that agencies utilize the QAPP and SOPs developed by the DPR-2 project (Cel Analytical Inc. 2020).

While additional data would be useful, the TWG agrees that sufficient data have been collected to date (both through DPR-2 and historical studies) to inform the development of DPR regulations.

7.4 Use of Molecular Data

Collecting both culture and molecular data for enterovirus and adenovirus provided important insights into the use of molecular data for pathogen monitoring campaigns. One of the key knowledge gaps associated with pathogen monitoring data is how to interpret and relate the concentrations of genome copies (GC) (i.e., molecular data) to the concentration of infectious pathogens (IU). It remains poorly understood what ratio (or ratios) should be used to interrelate these two values. Through this study, we observed significant variability in these ratios across the two viruses for which we had both molecular and culture data. The range of GC:IU ratios spanned 4-5 orders of magnitude from as low as 1:1 to as high as 100,000:1. The factors leading to this wide variability cannot be determined from this study, but merit additional study.

One conclusion from these findings is that it is not straightforward to translate from GC to IU in that a single value cannot appropriately characterize this relationship. Instead, future efforts using molecular data should evaluate a wide range of ratios when attempting to translate from GC to IU. When available, the SWB should select culture-based methods over molecular methods in order to minimize the variability associated with this translation. The culture-based methods produce data that are easier to incorporate into QMRA than the molecular data due to the fact that the dose-response relationships are often based on quantification through culturing.

Nevertheless, the TWG sees a number of benefits in continuing to collect molecular data along with culture data. For many important public health pathogens, culture methods do not exist meaning that molecular assays provide our only insight into their concentrations. Insight into the GC:IU ratios of non-culturable viruses can be gained by evaluating these ratios in culturable viruses. For example, the distribution of GC:IU ratio for adenovirus and enterovirus could be used to develop estimates for other non-culturable viruses like norovirus. The DPR-1 TWG recommended that a wide range of ratios be used in the interpretation of norovirus data (i.e., from 1:1 to 10,000:1). The findings from DPR-2 support this recommendation by providing further evidence of the appropriateness of such ratios.

While DPR-1 focused exclusively on quantifying the concentration of pathogens in raw wastewater, future pathogen monitoring efforts may also evaluate the removal of pathogens through treatment processes. For such studies, it will be important to collect robust, large datasets characterizing the removal or inactivation of the pathogens through different unit processes. In this application, molecular methods may be able to quantify pathogen removal as well as the culture-based methods. Some advantages of the molecular methods would be their lower cost and higher throughput compared to culture. For these reasons, the TWG believes that molecular methods will continue to offer important insights for pathogen studies, and recommends that molecular data continue to be collected.

7.5 Future Efforts

Advancements continue to be made in the field of pathogen monitoring and detection. These advancements should continue to be assessed by the SWB and incorporated (when applicable) into future monitoring efforts. For example, the DPR-2 QAPP and SOPs led to an advancement in the quality and sensitivity of pathogen monitoring in raw wastewater, as evidenced by the low rates of non-detects, ability to detect low concentrations, and the reproducibility of the findings between the labs using the methods. Additional modifications that further enhance recovery and lead to greater sensitivity or reproducibility should be evaluated for inclusion. Ideally, any method modifications would be evaluated head-to-head with the current best methods to clearly document their benefits and challenges.

This study emphasized the use of matrix spikes as a key quality control step. One assumption that is needed is that the matrix spike behaves similarly to the pathogen of interest. For the matrix spike to accurately reflect the recovery of the target pathogen, it should behave similarly to the pathogen through each step of the enumeration method. Additional studies that compare the behavior of the matrix spike to the target should be included in the SWB's ongoing review of the scientific literature. The need for such comparisons depends on the organism and the matrix spike utilized. For the protozoa, the ColorSeed matrix spike is essentially equivalent to the native *Giardia* cysts and *Cryptosporidium* oocysts with the exception that they have been enumerated in known quantities and uniquely stained to distinguish them from the native protozoa. In the case of the viruses, two phages were used to assess the recovery of enterovirus, adenovirus, and norovirus. Based on results from the pre-testing, MS2 and PhiX174 demonstrated similar recoveries to poliovirus, providing evidence that they are acceptable surrogates for assessing the fate of pathogenic enteric viruses.

Finally, one additional area of study that the TWG would encourage is the quantification of pathogen removal through wastewater treatment plants. DPR-2 demonstrates that pathogen monitoring in raw wastewater can be effectively accomplished, opening the door to evaluate other locations in the treatment train to evaluate process performance. While the DPR-2 QAPP identified optimum SOPs for pathogen enumeration in raw wastewater, the methods will likely need to be further adapted for use in primary, secondary, or tertiary effluents. Assuming that pathogen removal and inactivation is occurring through these processes, methods will need to ensure that they have the appropriate sensitivity to quantify effluent concentrations with minimal non-detect values. Lower concentrations will require higher concentration factors, though the removal of solids and other matrix components will likely make it easier to do these steps in treated effluents.

APPENDIX A

Uncorrected Concentrations, Recovery Efficiencies, and Recovery-Corrected Concentrations

The uncorrected concentrations, recoveries, and recovery-corrected concentrations of *Cryptosporidium* and *Giardia* are shown in Table A-1, culturable enterovirus and adenovirus in Table A-2, molecular enterovirus and adenovirus in Table A-3, molecular norovirus (GIA, GIB, GII) in Table A-4, and molecular SARS-CoV-2 (N1, N2, and E primer/probe sets) in Table A-5. The native male-specific phage concentrations are shown in Table A-6.

Table A-1. *Cryptosporidium* and *Giardia* Uncorrected Concentrations, Recoveries, and Corrected Concentrations.

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
LACSD	12/1/19	8.3E+01	9.2E+03	68%	18%	1.2E+02	5.1E+04
LACSD	12/30/19	1.0E+00	3.1E+03	31%	27%	3.2E+00	1.2E+04
LACSD	1/21/20	3.5E+01	3.4E+03	27%	47%	1.3E+02	7.3E+03
LACSD	1/29/20	1.6E+01	3.1E+03	30%	76%	5.3E+01	4.0E+03
LACSD	2/9/20	1.8E+01	4.6E+03	73%	55%	2.5E+01	8.4E+03
LACSD	3/2/20	4.6E+01	4.0E+03	41%	40%	1.1E+02	1.0E+04
LACSD	3/17/20	1.8E+01	3.6E+03	56%	45%	3.2E+01	7.9E+03
LACSD	4/8/20	9.0E+00	1.8E+03	25%	62%	3.6E+01	2.8E+03
LACSD	4/26/20	2.3E+01	3.0E+03	48%	51%	4.8E+01	6.0E+03
LACSD	5/11/20	2.0E+01	2.6E+03	63%	23%	3.2E+01	1.1E+04
LACSD	5/26/20	ND	1.3E+03	16%	42%	ND	3.1E+03
LACSD	6/10/20	3.3E+01	5.3E+03	48%	20%	6.9E+01	2.7E+04
LACSD	6/28/20	2.0E+01	5.0E+03	32%	22%	6.3E+01	2.3E+04
LACSD	7/20/20	2.1E+01	2.0E+03	27%	61%	7.8E+01	3.3E+03
LACSD	8/11/20	1.4E+01	1.3E+03	24%	28%	5.8E+01	4.5E+03
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	9/2/20	1.0E+01	8.2E+03	90%	63%	1.1E+01	1.3E+04
LACSD	9/13/20	1.5E+01	3.8E+03	45%	78%	3.3E+01	4.9E+03
LACSD	10/5/20	2.7E+01	2.4E+03	60%	15%	4.5E+01	1.6E+04
LACSD	10/27/20	5.8E+01	7.8E+03	55%	58%	1.1E+02	1.3E+04
LACSD	11/11/20	3.6E+01	3.4E+03	51%	25%	7.1E+01	1.4E+04
LACSD	11/30/20	6.0E+00	2.4E+03	40%	23%	1.5E+01	1.1E+04
LACSD	12/14/20	2.8E+01	5.6E+03	40%	40%	7.0E+01	1.4E+04
LACSD	1/5/21	1.9E+01	6.5E+03	51%	32%	3.7E+01	2.0E+04
LACSD	1/20/21	8.0E+00	2.6E+03	31%	12%	2.6E+01	2.2E+04
LASAN	12/9/19	1.9E+01	4.1E+03	31%	33%	6.1E+01	1.2E+04
LASAN	1/7/20	5.9E+01	1.3E+03	37%	49%	1.6E+02	2.7E+03
LASAN	1/22/20	1.0E+01	2.1E+03	16%	25%	6.3E+01	8.5E+03
LASAN	2/2/20	5.0E+00	3.1E+03	31%	80%	1.6E+01	3.9E+03
LASAN	2/17/20	7.0E+01	6.9E+03	37%	70%	1.9E+02	9.9E+03
LASAN	3/4/20	1.6E+01	5.7E+03	11%	33%	1.5E+02	1.7E+04
LASAN	3/18/20	8.0E+00	1.2E+03	22%	31%	3.6E+01	3.8E+03
LASAN	3/29/20	1.8E+01	2.5E+03	16%	45%	1.1E+02	5.5E+03
LASAN	4/20/20	3.9E+01	3.0E+03	42%	29%	9.3E+01	1.0E+04
LASAN	5/5/20	1.4E+01	3.0E+03	20%	62%	7.0E+01	4.8E+03
LASAN	6/3/20	7.0E+00	2.5E+03	12%	36%	5.8E+01	6.9E+03
LASAN	6/14/20	2.9E+01	6.0E+02	15%	30%	1.9E+02	2.0E+03

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
LASAN	6/29/20	2.5E+01	4.5E+03	27%	35%	9.3E+01	1.3E+04
LASAN	7/14/20	3.0E+01	3.2E+03	45%	28%	6.7E+01	1.1E+04
LASAN	8/5/20	5.8E+01	9.7E+03	48%	38%	1.2E+02	2.5E+04
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/23/20	2.9E+01	3.2E+03	18%	34%	1.6E+02	9.5E+03
LASAN	9/14/20	1.2E+01	1.7E+03	30%	47%	4.0E+01	3.6E+03
LASAN	9/29/20	1.6E+02	6.7E+03	42%	27%	3.7E+02	2.5E+04
LASAN	10/21/20	2.0E+01	1.3E+03	15%	10%	1.3E+02	1.3E+04
LASAN	11/1/20	1.9E+01	4.4E+03	59%	44%	3.2E+01	1.0E+04
LASAN	11/30/20	2.0E+02	1.8E+04	75%	45%	2.7E+02	4.0E+04
LASAN	12/15/20	1.3E+01	2.4E+03	32%	16%	4.1E+01	1.5E+04
LASAN	1/6/21	9.3E+01	5.4E+03	63%	28%	1.5E+02	1.9E+04
LASAN	1/24/21	4.9E+01	7.1E+03	54%	69%	9.1E+01	1.0E+04
OCSD	12/2/19	2.4E+01	1.2E+04	61%	56%	3.9E+01	2.1E+04
OCSD	12/16/19	5.0E+00	7.6E+03	1%	62%	5.0E+02	1.2E+04
OCSD	1/8/20	ND	4.6E+02	12%	4%	ND	1.1E+04
OCSD	1/21/20	1.6E+01	1.3E+03	29%	54%	5.5E+01	2.5E+03
OCSD	2/5/20	5.5E+01	4.0E+03	36%	54%	1.5E+02	7.4E+03
OCSD	2/19/20	1.8E+01	2.9E+03	46%	38%	3.9E+01	7.7E+03
OCSD	3/3/20	1.5E+01	5.4E+03	27%	73%	5.6E+01	7.4E+03

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
OCSD	3/17/20	1.2E+01	1.3E+03	21%	40%	5.7E+01	3.3E+03
OCSD	3/17/20	8.0E+00	4.5E+03	43%	48%	1.9E+01	9.3E+03
OCSD	4/20/20	7.0E+00	1.2E+03	21%	37%	3.3E+01	3.3E+03
OCSD	5/4/20	1.1E+01	4.0E+03	17%	41%	6.5E+01	9.7E+03
OCSD	5/18/20	3.5E+01	6.4E+03	68%	35%	5.1E+01	1.8E+04
OCSD	6/2/20	1.1E+01	1.1E+03	19%	46%	5.8E+01	2.4E+03
OCSD	6/24/20	2.8E+01	1.6E+04	51%	30%	5.5E+01	5.4E+04
OCSD	7/13/20	3.3E+01	3.9E+03	53%	23%	6.2E+01	1.7E+04
OCSD	8/4/20	2.9E+01	2.2E+03	25%	47%	1.2E+02	4.8E+03
OCSD	8/25/20	2.0E+01	1.4E+04	58%	20%	3.4E+01	7.0E+04
OCSD	9/16/20	8.3E+01	1.8E+04	80%	75%	1.0E+02	2.4E+04
OCSD	10/12/20	7.0E+00	2.2E+03	63%	19%	1.1E+01	1.2E+04
OCSD	10/26/20	8.0E+00	4.6E+03	62%	12%	1.3E+01	3.9E+04
OCSD	11/17/20	4.5E+01	9.8E+03	58%	28%	7.8E+01	3.5E+04
OCSD	12/9/20	1.7E+01	4.3E+03	33%	29%	5.2E+01	1.5E+04
OCSD	12/28/20	5.0E+00	4.6E+03	54%	22%	9.3E+00	2.1E+04
OCSD	1/18/21	2.3E+01	1.1E+04	66%	66%	3.5E+01	1.7E+04
SD	12/3/19	2.6E+01	5.9E+02	32%	44%	8.1E+01	1.3E+03
SD	12/18/19	2.3E+01	1.2E+04	40%	38%	5.8E+01	3.1E+04
SD	1/6/20	9.0E+00	1.3E+03	35%	53%	2.6E+01	2.5E+03
SD	1/20/20	3.6E+01	4.2E+03	21%	33%	1.7E+02	1.3E+04
SD	2/4/20	1.6E+01	4.0E+03	53%	51%	3.0E+01	7.9E+03
SD	2/26/20	1.0E+00	1.3E+03	30%	64%	3.3E+00	2.1E+03
SD	3/9/20	5.0E+00	4.6E+03	18%	34%	2.8E+01	1.4E+04
SD	3/30/20	5.0E+00	4.4E+03	13%	20%	3.8E+01	2.2E+04

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
SD	4/14/20	5.0E+00	7.6E+02	13%	56%	3.8E+01	1.4E+03
SD	5/13/20	1.4E+01	2.2E+03	16%	75%	8.8E+01	3.0E+03
SD	5/27/20	4.0E+00	4.1E+03	53%	23%	7.5E+00	1.8E+04
SD	6/8/20	2.2E+01	2.1E+03	20%	72%	1.1E+02	2.9E+03
SD	6/30/20	4.0E+00	9.5E+02	15%	43%	2.7E+01	2.2E+03
SD	7/15/20	4.0E+01	5.9E+03	43%	10%	9.3E+01	5.9E+04
SD	7/26/20	9.0E+00	2.3E+03	19%	35%	4.7E+01	6.7E+03
SD	8/10/20	8.0E+00	3.1E+03	81%	43%	9.9E+00	7.3E+03
SD	9/8/20	2.0E+01	1.4E+03	73%	55%	2.7E+01	2.5E+03
SD	9/30/20	5.0E+00	1.9E+03	22%	18%	2.3E+01	1.1E+04
SD	10/11/20	1.3E+01	9.9E+02	60%	33%	2.2E+01	3.0E+03
SD	11/2/20	1.3E+01	2.5E+03	65%	65%	2.0E+01	3.8E+03
SD	11/17/20	1.4E+01	2.6E+03	29%	24%	4.8E+01	1.1E+04
SD	12/9/20	8.0E+00	1.8E+03	53%	23%	1.5E+01	7.7E+03
SD	1/10/21	6.3E+01	1.6E+03	75%	90%	8.4E+01	1.8E+03
SD	1/25/21	5.0E+00	2.7E+03	35%	22%	1.4E+01	1.2E+04
SFPUC	12/11/19	3.5E+01	8.6E+02	41%	35%	8.5E+01	2.4E+03
SFPUC	12/29/19	2.6E+01	1.2E+03	32%	35%	8.1E+01	3.5E+03
SFPUC	1/13/20	5.0E+00	3.9E+03	38%	39%	1.3E+01	9.9E+03
SFPUC	1/28/20	2.7E+01	1.4E+03	22%	34%	1.2E+02	4.3E+03
SFPUC	2/12/20	3.4E+02	4.0E+03	52%	50%	6.5E+02	7.9E+03
SFPUC	3/9/20	1.0E+01	4.1E+03	18%	20%	5.6E+01	2.0E+04
SFPUC	3/23/20	1.4E+01	3.3E+03	28%	31%	5.0E+01	1.1E+04
SFPUC	4/13/20	1.1E+01	2.0E+03	29%	32%	3.8E+01	6.2E+03
SFPUC	4/29/20	4.0E+00	1.4E+03	21%	48%	1.9E+01	2.9E+03

WWTP	Date	*Cryptosporidium* Uncorrected Concentration (oocyst/L)	*Giardia* Uncorrected Concentration (oocyst/L)	*Cryptosporidium* Recovery	*Giardia* Recovery	*Cryptosporidium* Recovery-Corrected Concentration (cyst/L)	*Giardia* Recovery-Corrected Concentration (cyst/L)
SFPUC	5/18/20	1.6E+01	5.7E+03	35%	34%	4.6E+01	1.7E+04
SFPUC	6/1/20	1.4E+01	3.6E+03	58%	32%	2.4E+01	1.1E+04
SFPUC	6/16/20	5.0E+00	1.9E+03	23%	52%	2.2E+01	3.7E+03
SFPUC	7/8/20	2.0E+00	9.9E+02	21%	52%	9.5E+00	1.9E+03
SFPUC	7/27/20	3.8E+01	2.1E+04	19%	30%	2.0E+02	6.9E+04
SFPUC	7/27/20	1.9E+01	2.3E+04	11%	23%	1.7E+02	1.0E+05
SFPUC	9/1/20	2.0E+01	6.4E+03	48%	38%	4.2E+01	1.7E+04
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/22/20	4.0E+01	5.6E+03	68%	50%	5.9E+01	1.1E+04
SFPUC	10/5/20	2.3E+01	2.8E+03	38%	15%	6.1E+01	1.9E+04
SFPUC	10/19/20	4.0E+01	1.2E+04	43%	30%	9.3E+01	4.1E+04
SFPUC	11/2/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	11/2/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	11/10/20	5.5E+01	1.7E+04	65%	50%	8.5E+01	3.3E+04
SFPUC	12/2/20	4.0E+00	1.9E+03	50%	40%	8.0E+00	4.8E+03
SFPUC	12/14/20	1.0E+01	1.8E+03	43%	60%	2.3E+01	3.0E+03
SFPUC	1/4/21	1.4E+01	3.3E+03	21%	17%	6.7E+01	2.0E+04
SFPUC	1/19/21	1.5E+01	3.7E+03	28%	38%	5.4E+01	9.7E+03

N/A = Not analyzed for this parameter in this sample
ND = Not detected (concentration below the limit of detection)

Table A-2. Culturable Enterovirus and Adenovirus Uncorrected Concentrations, Recoveries, and Corrected Concentrations.

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
LACSD	12/1/19	2.1E+03	5.2E+02	8%	92%	4.2E+03	1.0E+03
LACSD	12/30/19	3.8E+02	2.5E+02	88%	197%	2.6E+02	1.7E+02
LACSD	1/21/20	3.3E+02	4.8E+02	72%	101%	3.8E+02	5.6E+02
LACSD	1/29/20	8.2E+01	1.4E+02	87%	119%	8.0E+01	1.3E+02
LACSD	2/9/20	2.7E+02	3.4E+02	8%	92%	5.4E+02	6.8E+02
LACSD	3/2/20	4.4E+01	7.6E+01	73%	83%	5.6E+01	9.7E+01
LACSD	3/17/20	1.0E+02	1.5E+02	13%	72%	2.4E+02	3.6E+02
LACSD	4/8/20	3.1E+01 (DNQ)	6.5E+01	85%	41%	4.9E+01 (DNQ)	1.0E+02
LACSD	4/26/20	5.5E+01 (DNQ)	3.3E+02	18%	52%	1.6E+02 (DNQ)	9.3E+02
LACSD	5/11/20	6.4E+02	ND	26%	69%	1.4E+03	ND
LACSD	5/26/20	2.3E+03	7.0E+02	51%	36%	5.2E+03	1.6E+03
LACSD	6/10/20	4.4E+04	2.2E+02	35%	86%	7.3E+04	3.6E+02
LACSD	6/28/20	2.5E+02	ND	30%	50%	6.3E+02	ND
LACSD	7/20/20	1.2E+04	5.5E+03	17%	32%	5.0E+04	2.2E+04
LACSD	8/11/20	3.8E+03	6.6E+02	20%	32%	1.5E+04	2.5E+03
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	9/2/20	1.7E+02	ND	25%	13%	8.7E+02	ND
LACSD	9/13/20	2.0E+03	6.0E+02	24%	31%	7.2E+03	2.2E+03
LACSD	10/5/20	3.3E+04	2.0E+03	32%	26%	1.1E+05	6.7E+03

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
LACSD	10/27/20	5.1E+02	8.0E+01 (DNQ)	75%	37%	9.1E+02	1.4E+02 (DNQ)
LACSD	11/11/20	3.0E+03	1.2E+03	40%	21%	9.7E+03	4.1E+03
LACSD	11/30/20	9.6E+02	1.0E+03	75%	30%	1.8E+03	2.0E+03
LACSD	12/14/20	1.0E+03	5.8E+02	124%	60%	1.1E+03	6.3E+02
LACSD	1/5/21	1.9E+03	2.2E+02	110%	39%	2.5E+03	3.0E+02
LACSD	1/20/21	ND	ND	110%	39%	ND	ND
LASAN	12/9/19	1.4E+03	6.0E+03	113%	85%	1.5E+03	6.0E+03
LASAN	1/7/20	8.9E+01	3.7E+02	83%	72%	1.1E+02	4.8E+02
LASAN	1/22/20	2.8E+04	8.3E+02	7%	45%	1.1E+05	3.2E+03
LASAN	2/2/20	6.6E+03	5.6E+03	69%	50%	1.1E+04	9.4E+03
LASAN	2/17/20	2.7E+03	3.9E+03	83%	72%	3.5E+03	5.1E+03
LASAN	3/4/20	2.0E+03	6.8E+02	7%	45%	7.8E+03	2.6E+03
LASAN	3/18/20	3.9E+02	5.1E+02	24%	16%	2.0E+03	2.5E+03
LASAN	3/29/20	4.8E+01 (DNQ)	7.7E+03	32%	42%	1.3E+02 (DNQ)	2.1E+04
LASAN	4/20/20	9.5E+03	2.9E+03	17%	30%	4.0E+04	1.2E+04
LASAN	5/5/20	5.8E+03	2.4E+03	40%	69%	1.1E+04	4.4E+03
LASAN	6/3/20	1.7E+03	1.5E+03	31%	49%	4.4E+03	3.6E+03
LASAN	6/14/20	4.9E+04	9.3E+02	31%	50%	1.2E+05	2.3E+03
LASAN	6/29/20	1.3E+03	5.8E+02	22%	28%	5.0E+03	2.3E+03
LASAN	7/14/20	1.1E+03	ND	44%	69%	2.0E+03	ND
LASAN	8/5/20	7.9E+01 (DNQ)	ND	46%	89%	1.2E+02 (DNQ)	ND
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/23/20	6.2E+04	9.7E+03	29%	41%	1.8E+05	2.8E+04
LASAN	9/14/20	1.6E+03	8.5E+01	35%	54%	3.6E+03	1.9E+02
LASAN	9/29/20	4.1E+03	ND	49%	108%	5.2E+03	ND
LASAN	10/21/20	1.1E+03	8.1E+02	37%	49%	2.5E+03	1.9E+03
LASAN	11/1/20	5.1E+01 (DNQ)	5.1E+01 (DNQ)	49%	66%	8.9E+01 (DNQ)	8.9E+01 (DNQ)
LASAN	11/30/20	1.9E+02	3.3E+02	50%	23%	5.2E+02	9.0E+02
LASAN	12/15/20	1.3E+03	8.5E+02	38%	45%	3.2E+03	2.0E+03
LASAN	1/6/21	9.4E+01 (DNQ)	2.0E+02	40%	29%	2.7E+02 (DNQ)	5.8E+02
LASAN	1/24/21	1.7E+03	1.7E+03	30%	35%	5.3E+03	5.3E+03
OCSD	12/2/19	2.2E+03	9.0E+01	105%	78%	2.4E+03	9.8E+01
OCSD	12/16/19	3.4E+02	5.8E+01	80%	49%	5.2E+02	9.0E+01
OCSD	1/8/20	1.1E+03	9.8E+02	68%	80%	1.5E+03	1.3E+03
OCSD	1/21/20	7.6E+01	3.5E+02	64%	43%	1.4E+02	6.5E+02
OCSD	2/5/20	9.5E+02	5.5E+02	80%	49%	1.5E+03	8.5E+02
OCSD	2/19/20	8.2E+02	8.4E+01	38%	56%	1.7E+03	1.8E+02
OCSD	3/3/20	2.4E+02	1.9E+02	23%	8%	1.5E+03	1.2E+03
OCSD	3/17/20	9.8E+02	8.0E+02	33%	22%	3.6E+03	2.9E+03
OCSD	3/17/20	6.9E+02	2.0E+03	7%	32%	3.5E+03	1.0E+04
OCSD	4/20/20	2.7E+02	2.0E+02	43%	36%	6.7E+02	5.2E+02
OCSD	5/4/20	1.1E+03	5.3E+02	33%	28%	3.6E+03	1.7E+03
OCSD	5/18/20	3.7E+02	4.6E+02	28%	46%	9.9E+02	1.2E+03

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
OCSD	6/2/20	3.8E+01 (DNQ)	8.0E+01	23%	21%	1.7E+02 (DNQ)	3.6E+02
OCSD	6/24/20	2.3E+03	1.1E+04	48%	61%	4.3E+03	2.0E+04
OCSD	7/13/20	8.1E+02	ND	46%	34%	2.0E+03	ND
OCSD	8/4/20	1.6E+03	1.9E+03	34%	35%	4.7E+03	5.4E+03
OCSD	8/25/20	2.6E+03	ND	45%	7%	1.0E+04	ND
OCSD	9/16/20	1.5E+02	ND	26%	7%	9.0E+02	ND
OCSD	10/12/20	6.8E+03	1.9E+03	45%	49%	1.5E+04	4.0E+03
OCSD	10/26/20	4.2E+03	1.1E+03	42%	43%	9.8E+03	2.7E+03
OCSD	11/17/20	4.6E+02	2.3E+02	7%	6%	7.1E+03	3.6E+03
OCSD	12/9/20	2.1E+02	2.1E+02	75%	64%	3.0E+02	3.0E+02
OCSD	12/28/20	7.8E+01	ND	39%	37%	2.1E+02	ND
OCSD	1/18/21	6.6E+02	6.6E+02	143%	123%	4.9E+02	4.9E+02
SD	12/3/19	3.6E+02	3.9E+02	83%	81%	4.3E+02	4.7E+02
SD	12/18/19	9.1E+02	1.2E+03	42%	47%	2.0E+03	2.7E+03
SD	1/6/20	8.1E+01	3.0E+02	83%	73%	1.0E+02	3.8E+02
SD	1/20/20	2.1E+02	5.6E+02	83%	81%	2.6E+02	6.8E+02
SD	2/4/20	3.3E+04	5.5E+02	35%	47%	8.1E+04	1.3E+03
SD	2/26/20	ND	3.3E+01 (DNQ)	52%	67%	ND	5.5E+01 (DNQ)
SD	3/9/20	1.0E+03	2.7E+02	28%	46%	2.8E+03	7.4E+02
SD	3/30/20	4.8E+02	7.9E+02	11%	26%	2.6E+03	4.3E+03
SD	4/14/20	1.4E+03	3.6E+02	21%	62%	3.4E+03	8.7E+02
SD	5/13/20	2.6E+02	7.0E+01	38%	52%	5.7E+02	1.6E+02
SD	5/27/20	1.3E+02	6.1E+01 (DNQ)	79%	33%	2.3E+02	1.1E+02 (DNQ)
SD	6/8/20	2.3E+02	ND	55%	42%	4.6E+02	ND

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
SD	6/30/20	3.4E+01 (DNQ)	3.4E+01 (DNQ)	42%	36%	8.7E+01 (DNQ)	8.7E+01 (DNQ)
SD	7/15/20	1.7E+02	2.7E+02	146%	40%	1.8E+02	2.9E+02
SD	7/26/20	1.6E+03	3.3E+01 (DNQ)	29%	30%	5.5E+03	1.1E+02 (DNQ)
SD	8/10/20	ND	ND	85%	24%	ND	ND
SD	9/8/20	ND	ND	24%	8%	ND	ND
SD	9/30/20	2.2E+03	3.8E+02	35%	23%	7.5E+03	1.3E+03
SD	10/11/20	1.4E+02 (DNQ)	ND	71%	70%	2.0E+02 (DNQ)	ND
SD	11/2/20	1.0E+02	ND	118%	133%	8.2E+01	ND
SD	11/17/20	2.5E+02	9.1E+01	42%	16%	8.8E+02	3.1E+02
SD	12/9/20	1.3E+03	4.6E+02	81%	86%	1.6E+03	5.5E+02
SD	1/10/21	1.9E+02	4.3E+02	43%	39%	4.7E+02	1.1E+03
SD	1/25/21	9.6E+01	ND	42%	16%	3.3E+02	ND
SFPUC	12/11/19	2.9E+02	3.8E+02	107%	101%	2.8E+02	3.6E+02
SFPUC	12/29/19	9.3E+03	5.0E+01 (DNQ)	110%	130%	7.8E+03	4.2E+01 (DNQ)
SFPUC	1/13/20	8.0E+02	5.9E+02	73%	92%	9.6E+02	7.1E+02
SFPUC	1/28/20	5.7E+02	4.0E+02	107%	101%	5.5E+02	3.8E+02
SFPUC	2/12/20	1.3E+03	1.3E+02	15%	150%	1.5E+03	1.5E+02
SFPUC	3/9/20	8.8E+03	3.6E+03	73%	67%	1.3E+04	5.2E+03
SFPUC	3/23/20	1.0E+03	1.9E+03	10%	78%	2.3E+03	4.2E+03
SFPUC	4/13/20	1.3E+03	5.0E+02	27%	64%	2.9E+03	1.1E+03
SFPUC	4/29/20	ND	ND	73%	42%	ND	ND
SFPUC	5/18/20	4.4E+03	3.0E+04	44%	50%	9.4E+03	6.5E+04
SFPUC	6/1/20	9.7E+03	5.1E+04	28%	58%	2.2E+04	1.2E+05
SFPUC	6/16/20	6.2E+03	8.2E+03	76%	41%	1.1E+04	1.4E+04

WWTP	Date	Culturable Enterovirus Uncorrected Concentration (MPN/L)	Culturable Adenovirus Uncorrected Concentration (MPN/L)	MS2 Recovery	PhiX174 Recovery	Culturable Enterovirus Recovery-Corrected Concentration (MPN/L)	Culturable Adenovirus Recovery-Corrected Concentration (MPN/L)
SFPUC	7/8/20	7.9E+03	3.4E+03	79%	40%	1.3E+04	5.7E+03
SFPUC	7/27/20	4.8E+04	5.3E+03	52%	79%	7.4E+04	8.0E+03
SFPUC	7/27/20	3.7E+03	8.7E+01 (DNQ)	13%	66%	9.3E+03	2.2E+02 (DNQ)
SFPUC	9/1/20	1.1E+05	7.8E+02	24%	118%	1.5E+05	1.1E+03
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/22/20	4.8E+03	ND	31%	56%	1.1E+04	ND
SFPUC	10/5/20	1.3E+05	2.3E+04	28%	68%	2.8E+05	4.8E+04
SFPUC	10/19/20	2.9E+02	8.6E+01 (DNQ)	49%	46%	6.0E+02	1.8E+02 (DNQ)
SFPUC	11/2/20	1.5E+02	9.6E+01	19%	125%	2.1E+02	1.3E+02
SFPUC	11/2/20	6.1E+02	6.9E+02	22%	26%	2.6E+03	2.9E+03
SFPUC	11/10/20	1.3E+03	3.7E+02	53%	52%	2.4E+03	7.0E+02
SFPUC	12/2/20	1.4E+03	2.1E+03	31%	18%	5.6E+03	8.4E+03
SFPUC	12/14/20	ND	ND	24%	40%	ND	ND
SFPUC	1/4/21	3.3E+02	3.3E+02	56%	58%	5.8E+02	5.8E+02
SFPUC	1/19/21	9.3E+02	9.8E+01	17%	62%	2.4E+03	2.5E+02

N/A = Not analyzed for this parameter in this sample

ND = Not detected (concentration below the limit of detection)

DNQ = Detected but not quantifiable (concentration below the limit of quantification); value shown is an approximation.

Recovery of was measured using two surrogates, MS2 and PhiX174, in every other sample from a given WWTP. The recovery was interpolated for samples where recovery of MS2 and PhiX174 was not directly measured. To determine the recovery corrected concentrations, the average of the MS2 and PhiX174 recovery for a given sample was used.

Table A-3. Molecular Enterovirus and Adenovirus Uncorrected Concentrations, Recoveries, and Corrected Concentrations.

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
LACSD	12/1/19	3.5E+05	2.7E+05	43%	25%	1.0E+06	7.9E+05
LACSD	12/30/19	8.9E+04	1.5E+04	15%	80%	1.9E+05	3.2E+04
LACSD	1/21/20	1.7E+05	2.1E+06	5%	5%	3.4E+06	4.2E+07
LACSD	1/29/20	7.2E+04	3.4E+06	11%	73%	1.7E+05	8.1E+06
LACSD	2/9/20	4.9E+05	2.8E+05	43%	25%	1.4E+06	8.2E+05
LACSD	3/2/20	1.7E+05	6.0E+05	32%	23%	6.2E+05	2.2E+06
LACSD	3/17/20	3.2E+04	9.3E+03	93%	15%	5.9E+04	1.7E+04
LACSD	4/8/20	ND	1.8E+05	7%	67%	ND	4.9E+05
LACSD	4/26/20	9.7E+04	1.3E+04 (DNQ)	143%	6%	1.3E+05	1.7E+04 (DNQ)
LACSD	5/11/20	ND	ND	133%	10%	ND	ND
LACSD	5/26/20	1.1E+04 (DNQ)	5.6E+04	7%	68%	2.9E+04 (DNQ)	1.5E+05
LACSD	6/10/20	4.1E+05	ND	124%	14%	5.9E+05	ND
LACSD	6/28/20	2.5E+05	ND	96%	66%	3.1E+05	ND
LACSD	7/20/20	1.5E+05	8.8E+04	8%	69%	3.9E+05	2.3E+05
LACSD	8/11/20	1.3E+05	ND	38%	57%	2.7E+05	ND
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	9/2/20	6.8E+03 (DNQ)	ND	68%	119%	7.3E+03 (DNQ)	ND
LACSD	9/13/20	1.6E+05	ND	69%	46%	2.8E+05	ND
LACSD	10/5/20	1.7E+05	2.3E+03 (DNQ)	49%	48%	3.5E+05	4.7E+03 (DNQ)

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
LACSD	10/27/20	1.1E+05	ND	37%	71%	2.0E+05	ND
LACSD	11/11/20	1.8E+05	9.5E+03	30%	49%	4.6E+05	2.4E+04
LACSD	11/30/20	ND	2.6E+04	27%	72%	ND	5.3E+04
LACSD	12/14/20	1.1E+04 (DNQ)	ND	7%	22%	7.6E+04 (DNQ)	ND
LACSD	1/5/21	7.9E+04	8.9E+03	23%	94%	1.4E+05	1.5E+04
LACSD	1/20/21	1.4E+04 (DNQ)	4.4E+04	23%	94%	2.4E+04 (DNQ)	7.5E+04
LASAN	12/9/19	ND	2.0E+04	24%	32%	ND	7.1E+04
LASAN	1/7/20	6.5E+05	1.5E+06	107%	57%	7.9E+05	1.8E+06
LASAN	1/22/20	ND	8.6E+04	23%	22%	ND	3.8E+05
LASAN	2/2/20	ND	4.5E+05	18%	46%	ND	1.4E+06
LASAN	2/17/20	4.1E+05	4.4E+06	107%	57%	5.0E+05	5.4E+06
LASAN	3/4/20	1.9E+04	2.1E+04	23%	22%	8.4E+04	9.3E+04
LASAN	3/18/20	ND	1.9E+06	13%	60%	ND	5.2E+06
LASAN	3/29/20	ND	5.0E+05	11%	38%	ND	2.0E+06
LASAN	4/20/20	1.3E+05	ND	92%	45%	1.9E+05	ND
LASAN	5/5/20	ND	2.4E+03 (DNQ)	9%	17%	ND	1.8E+04 (DNQ)
LASAN	6/3/20	ND	2.8E+04	10%	14%	ND	2.3E+05
LASAN	6/14/20	3.3E+04 (DNQ)	ND	101%	80%	3.6E+04 (DNQ)	ND
LASAN	6/29/20	ND	1.9E+04	11%	11%	ND	1.7E+05
LASAN	7/14/20	8.0E+03 (DNQ)	ND	110%	115%	7.1E+03 (DNQ)	ND
LASAN	8/5/20	ND	ND	60%	61%	ND	ND
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/23/20	8.5E+04	1.1E+04	45%	19%	2.7E+05	3.4E+04
LASAN	9/14/20	5.2E+05	ND	79%	27%	9.8E+05	ND
LASAN	9/29/20	1.2E+04 (DNQ)	ND	11%	7%	1.3E+05 (DNQ)	ND
LASAN	10/21/20	1.5E+05	2.6E+03 (DNQ)	49%	44%	3.2E+05 (DNQ)	5.6E+03
LASAN	11/1/20	1.2E+04	4.0E+03 (DNQ)	8%	17%	9.6E+04 (DNQ)	3.2E+04
LASAN	11/30/20	8.7E+03	5.1E+03	6%	28%	5.1E+04	3.0E+04
LASAN	12/15/20	1.3E+05	7.3E+03	19%	60%	3.3E+05	1.8E+04
LASAN	1/6/21	ND	3.8E+04 (DNQ)	11%	26%	ND	2.1E+05 (NDQ)
LASAN	1/24/21	6.2E+04 (DNQ)	4.2E+05	16%	24%	3.1E+05 (DNQ)	2.1E+06
OCSD	12/2/19	3.5E+04	1.4E+05	14%	81%	7.4E+04	2.9E+05
OCSD	12/16/19	3.6E+04 (DNQ)	2.2E+06	30%	22%	1.4E+05 (DNQ)	8.5E+06
OCSD	1/8/20	4.5E+05	4.0E+05	120%	31%	6.0E+05	5.3E+05
OCSD	1/21/20	ND	1.9E+03 (DNQ)	19%	72%	ND	4.2E+03 (DNQ)
OCSD	2/5/20	4.9E+06	3.3E+06	30%	22%	1.9E+07	1.3E+07
OCSD	2/19/20	9.2E+04 (DNQ)	4.6E+04 (DNQ)	65%	21%	2.1E+05 (DNQ)	1.1E+05 (DNQ)
OCSD	3/3/20	ND	1.3E+07	24%	64%	ND	3.0E+07
OCSD	3/17/20	ND	4.1E+06	35%	56%	ND	9.0E+06
OCSD	3/17/20	ND	1.8E+04	10%	12%	ND	1.6E+05
OCSD	4/20/20	1.1E+04	3.4E+05	45%	49%	2.3E+04	7.2E+05
OCSD	5/4/20	ND	3.2E+04	30%	72%	ND	6.3E+04
OCSD	5/18/20	5.0E+04 (DNQ)	ND	19%	91%	9.1E+04 (DNQ)	ND

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
OCSD	6/2/20	ND	2.0E+03 (DNQ)	15%	95%	ND	3.6E+03 (DNQ)
OCSD	6/24/20	7.0E+05	ND	28%	170%	7.1E+05	ND
OCSD	7/13/20	2.5E+04	ND	57%	114%	2.9E+04	ND
OCSD	8/4/20	3.1E+04	2.0E+03 (DNQ)	10%	68%	7.9E+04	5.1E+03 (DNQ)
OCSD	8/25/20	1.9E+05	ND	86%	58%	2.6E+05	ND
OCSD	9/16/20	3.6E+04	ND	48%	31%	9.1E+04	ND
OCSD	10/12/20	5.5E+04	2.0E+03 (DNQ)	6%	40%	2.4E+05	8.7E+03 (DNQ)
OCSD	10/26/20	4.3E+04	1.4E+04	6%	61%	1.3E+05	4.2E+04
OCSD	11/17/20	ND	ND	9%	5%	ND	ND
OCSD	12/9/20	ND	ND	19%	17%	ND	ND
OCSD	12/28/20	7.0E+04	2.0E+03 (DNQ)	6%	82%	1.6E+05	4.5E+03 (DNQ)
OCSD	1/18/21	7.2E+04	4.3E+04	28%	30%	2.5E+05	1.5E+05
SD	12/3/19	3.3E+04 (DNQ)	4.6E+06	37%	32%	9.6E+04 (DNQ)	1.3E+07
SD	12/18/19	1.8E+06	2.2E+04 (DNQ)	65%	71%	2.6E+06	3.2E+04 (DNQ)
SD	1/6/20	ND	1.3E+04	14%	153%	ND	1.6E+04
SD	1/20/20	1.3E+05	5.4E+06	37%	32%	3.8E+05	1.6E+07
SD	2/4/20	8.3E+04	1.2E+05	74%	70%	1.2E+05	1.7E+05
SD	2/26/20	6.9E+04	9.1E+04	14%	111%	1.1E+05	1.5E+05
SD	3/9/20	1.0E+04	5.5E+04	82%	69%	1.3E+04	7.3E+04
SD	3/30/20	6.0E+04 (DNQ)	9.2E+04	162%	33%	6.2E+04 (DNQ)	9.4E+04
SD	4/14/20	5.0E+04	6.1E+03	13%	70%	1.2E+05	1.5E+04
SD	5/13/20	ND	1.5E+04	18%	101%	ND	2.5E+04
SD	5/27/20	2.6E+04	ND	143%	107%	2.1E+04	ND
SD	6/8/20	ND	1.5E+04	23%	131%	ND	1.9E+04

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
SD	6/30/20	1.1E+04 (DNQ)	ND	16%	136%	1.4E+04 (DNQ)	ND
SD	7/15/20	3.6E+05	ND	124%	180%	2.4E+05	ND
SD	7/26/20	4.7E+04	ND	8%	141%	6.3E+04	ND
SD	8/10/20	ND	ND	68%	116%	ND	ND
SD	9/8/20	ND	ND	13%	51%	ND	ND
SD	9/30/20	1.8E+05	2.2E+03 (DNQ)	25%	93%	3.1E+05	3.7E+03 (DNQ)
SD	10/11/20	1.2E+04 (DNQ)	ND	41%	66%	2.2E+04 (DNQ)	ND
SD	11/2/20	4.0E+04	ND	69%	81%	5.3E+04	ND
SD	11/17/20	1.4E+05	2.3E+03 (DNQ)	41%	45%	3.3E+05	5.3E+03 (DNQ)
SD	12/9/20	1.5E+05	ND	68%	47%	2.6E+05	0.0E+00
SD	1/10/21	3.2E+04	ND	66%	13%	8.1E+04	0.0E+00
SD	1/25/21	2.4E+04 (DNQ)	3.1E+04	41%	45%	5.6E+04	7.2E+04
SFPUC	12/11/19	4.3E+05	1.1E+07	12%	53%	1.3E+06	3.4E+07
SFPUC	12/29/19	1.7E+05	1.0E+05	102%	103%	1.7E+05	9.8E+04
SFPUC	1/13/20	4.7E+05	1.7E+04	10%	130%	6.7E+05	2.4E+04
SFPUC	1/28/20	9.1E+05	2.2E+06	12%	53%	2.8E+06	6.8E+06
SFPUC	2/12/20	8.1E+05	8.2E+04	64%	32%	1.7E+06	1.7E+05
SFPUC	3/9/20	ND	7.7E+06	15%	81%	ND	1.6E+07
SFPUC	3/23/20	7.1E+04	4.9E+03 (DNQ)	42%	74%	1.2E+05	8.4E+03 (DNQ)
SFPUC	4/13/20	4.9E+05	ND	31%	56%	1.1E+06	ND
SFPUC	4/29/20	ND	7.9E+03	21%	31%	ND	3.0E+04
SFPUC	5/18/20	ND	ND	19%	37%	ND	ND
SFPUC	6/1/20	8.6E+05	1.2E+04 (DNQ)	80%	99%	9.6E+05	1.3E+04 (DNQ)
SFPUC	6/16/20	ND	1.8E+04	20%	35%	ND	6.5E+04

WWTP	Date	Molecular Enterovirus Uncorrected Concentration (GC/L)	Molecular Adenovirus Uncorrected Concentration (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Enterovirus Recovery-Corrected Concentration (GC/L)	Molecular Adenovirus Recovery-Corrected Concentration (GC/L)
SFPUC	7/8/20	2.7E+04	4.5E+03	18%	39%	9.5E+04	1.6E+04
SFPUC	7/27/20	ND	8.5E+03	14%	32%	ND	3.7E+04
SFPUC	7/27/20	8.1E+04	ND	141%	161%	5.4E+04	ND
SFPUC	9/1/20	1.8E+05	4.6E+04	10%	24%	1.1E+06	2.7E+05
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/22/20	ND	ND	73%	98%	ND	ND
SFPUC	10/5/20	ND	2.9E+03 (DNQ)	12%	38%	ND	1.2E+04 (DNQ)
SFPUC	10/19/20	5.1E+04	3.8E+03 (DNQ)	5%	35%	2.6E+05	1.9E+04 (DNQ)
SFPUC	11/2/20	ND	2.0E+03 (DNQ)	9%	22%	ND	1.3E+04 (DNQ)
SFPUC	11/2/20	4.1E+03 (DNQ)	2.5E+04	7%	29%	2.3E+04 (DNQ)	1.4E+05
SFPUC	11/10/20	1.1E+06	ND	7%	19%	8.5E+06	ND
SFPUC	12/2/20	5.3E+04	2.0E+05	14%	52%	1.6E+05	6.1E+05
SFPUC	12/14/20	4.8E+04	ND	14%	34%	2.0E+05	ND
SFPUC	1/4/21	2.8E+05	ND	9%	3%	4.7E+06	ND
SFPUC	1/19/21	2.1E+05	2.5E+03 (DNQ)	15%	16%	1.4E+06	1.6E+04 (DNQ)

N/A = Not analyzed for this parameter in this sample

ND = Not detected (concentration below the limit of detection)

DNQ = Detected but not quantifiable (concentration below the limit of quantification); value shown is an approximation.

Recovery of was measured using two surrogates, MS2 and PhiX174, in every other sample from a given WWTP. The recovery was interpolated for samples where recovery of MS2 and PhiX174 was not directly measured. To determine the recovery corrected concentrations, the average of the MS2 and PhiX174 recovery for a given sample was used.

Table A-4. Molecular Norovirus (GIA, GIB, and GII) Uncorrected Concentrations, Recoveries, and Corrected Concentrations.

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
LACSD	12/1/19	2.5E+04 (DNQ)	7.6E+03 (DNQ)	1.4E+05	43%	25%	7.4E+04	2.2E+04 (DNQ)	4.1E+05 (DNQ)
LACSD	12/30/19	7.5E+04	1.8E+04	8.2E+04	15%	80%	1.6E+05	3.8E+04	1.7E+05
LACSD	1/21/20	ND	ND	ND	5%	5%	ND	ND	ND
LACSD	1/29/20	3.7E+04	4.9E+04	2.4E+05	11%	73%	8.8E+04	1.2E+05	5.7E+05
LACSD	2/9/20	ND	ND	6.7E+04 (DNQ)	43%	25%	ND	ND	2.0E+05 (DNQ)
LACSD	3/2/20	1.2E+04	8.7E+04	8.6E+05	32%	23%	4.4E+04	3.2E+05	3.1E+06
LACSD	3/17/20	ND	ND	2.1E+03 (DNQ)	93%	15%	ND	ND	3.9E+03 (DNQ)
LACSD	4/8/20	ND	ND	1.6E+03 (DNQ)	7%	67%	ND	ND	4.3E+03 (DNQ)
LACSD	4/26/20	1.8E+05	ND	ND	143%	6%	2.4E+05	ND	ND
LACSD	5/11/20	ND	ND	ND	133%	10%	ND	ND	ND
LACSD	5/26/20	5.6E+04	2.9E+03 (DNQ)	1.8E+03 (DNQ)	7%	68%	1.5E+05	7.7E+03 (DNQ)	4.8E+03 (DNQ)
LACSD	6/10/20	ND	9.1E+03 (DNQ)	1.5E+04 (DNQ)	124%	14%	ND	1.3E+04 (DNQ)	2.2E+04 (DNQ)
LACSD	6/28/20	3.3E+04 (DNQ)	6.3E+04	2.8E+04 (DNQ)	96%	66%	4.1E+04 (DNQ)	7.8E+04	3.5E+04 (DNQ)
LACSD	7/20/20	ND	ND	1.6E+06	8%	69%	ND	ND	4.2E+06
LACSD	8/11/20	1.9E+04	1.1E+05	3.5E+04	38%	57%	4.0E+04	2.3E+05	7.4E+04
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	8/19/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	9/2/20	ND	ND	ND	68%	119%	ND	ND	ND
LACSD	9/13/20	3.0E+04	1.9E+04	4.4E+04	69%	46%	5.2E+04	3.3E+04	7.7E+04
LACSD	10/5/20	3.9E+04	1.4E+04	1.9E+04	49%	48%	8.0E+04	2.9E+04	3.9E+04

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
LACSD	10/27/20	ND	ND	3.5E+03 (DNQ)	37%	71%	ND	ND	6.5E+03 (DNQ)
LACSD	11/11/20	3.4E+04	4.8E+04	4.5E+04	30%	49%	8.6E+04	1.2E+05	1.1E+05
LACSD	11/30/20	ND	ND	1.9E+03 (DNQ)	27%	72%	ND	ND	3.8E+03 (DNQ)
LACSD	12/14/20	ND	ND	ND	7%	22%	ND	ND	ND
LACSD	1/5/21	5.7E+04	5.3E+03	3.2E+04	23%	94%	9.7E+04	9.1E+03	5.5E+04
LACSD	1/20/21	2.3E+04	5.4E+03	7.2E+03	23%	94%	3.9E+04	9.2E+03	1.2E+04
LASAN	12/9/19	4.6E+03 (DNQ)	ND	5.4E+03	24%	32%	1.6E+04 (DNQ)	ND	1.9E+04
LASAN	1/7/20	3.1E+04	4.7E+04	8.1E+04	107%	57%	3.8E+04	5.7E+04	9.9E+04
LASAN	1/22/20	ND	ND	3.9E+03 (DNQ)	23%	22%	ND	ND	1.7E+04 (DNQ)
LASAN	2/2/20	ND	3.2E+03 (DNQ)	8.5E+03	18%	46%	ND	1.0E+04 (DNQ)	2.7E+04
LASAN	2/17/20	2.3E+04 (DNQ)	3.6E+04	4.3E+05	107%	57%	2.8E+04 (DNQ)	4.4E+04	5.2E+05
LASAN	3/4/20	ND	2.3E+03 (DNQ)	3.8E+03 (DNQ)	23%	22%	ND	1.0E+04 (DNQ)	1.7E+04 (DNQ)
LASAN	3/18/20	ND	2.0E+03 (DNQ)	8.4E+03	13%	60%	ND	5.5E+03 (DNQ)	2.3E+04
LASAN	3/29/20	ND	ND	4.5E+03 (DNQ)	11%	38%	ND	ND	1.8E+04 (DNQ)
LASAN	4/20/20	9.4E+04	ND	1.5E+04 (DNQ)	92%	45%	1.4E+05	ND	2.2E+04 (DNQ)
LASAN	5/5/20	ND	ND	ND	9%	17%	ND	ND	ND
LASAN	6/3/20	ND	ND	ND	10%	14%	ND	ND	ND
LASAN	6/14/20	ND	ND	1.7E+04 (DNQ)	101%	80%	ND	ND	1.9E+04 (DNQ)
LASAN	6/29/20	ND	ND	ND	11%	11%	ND	ND	ND
LASAN	7/14/20	ND	ND	ND	110%	115%	ND	ND	ND
LASAN	8/5/20	ND	ND	ND	60%	61%	ND	ND	ND
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/17/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	8/23/20	ND	ND	2.6E+03 (DNQ)	45%	19%	ND	ND	8.1E+03 (DNQ)
LASAN	9/14/20	4.5E+03 (DNQ)	ND	1.9E+04	79%	27%	8.5E+03 (DNQ)	ND	3.6E+04
LASAN	9/29/20	ND	ND	ND	11%	7%	ND	ND	ND
LASAN	10/21/20	ND	1.6E+03 (DNQ)	2.6E+03 (DNQ)	49%	44%	ND	3.4E+03 (DNQ)	5.6E+03 (DNQ)
LASAN	11/1/20	ND	ND	2.3E+03 (DNQ)	8%	17%	ND	ND	1.8E+04 (DNQ)
LASAN	11/30/20	ND	ND	ND	6%	28%	ND	ND	ND
LASAN	12/15/20	3.3E+04	3.1E+04	8.7E+04	19%	60%	8.4E+04	7.8E+04	2.2E+05
LASAN	1/6/21	ND	ND	ND	11%	26%	ND	ND	ND
LASAN	1/24/21	ND	ND	ND	16%	24%	ND	ND	ND
OCSD	12/2/19	1.0E+05	6.4E+03	1.1E+05	14%	81%	2.1E+05	1.3E+04	2.3E+05
OCSD	12/16/19	ND	ND	5.9E+04	30%	22%	ND	ND	2.3E+05
OCSD	1/8/20	1.2E+05	3.7E+04 (DNQ)	3.3E+05	120%	31%	1.6E+05	4.9E+04 (DNQ)	4.4E+05
OCSD	1/21/20	7.8E+04	4.8E+04	4.1E+04	19%	72%	1.7E+05	1.1E+05	9.0E+04
OCSD	2/5/20	1.4E+05	2.0E+05	2.0E+07	30%	22%	5.4E+05	7.7E+05	7.7E+07
OCSD	2/19/20	ND	2.8E+04 (DNQ)	4.6E+04 (DNQ)	65%	21%	ND	6.5E+04 (DNQ)	1.1E+05 (DNQ)
OCSD	3/3/20	ND	5.3E+03	2.3E+03 (DNQ)	24%	64%	ND	1.2E+04	5.2E+03 (DNQ)
OCSD	3/17/20	ND	4.7E+03	1.8E+03 (DNQ)	35%	56%	ND	1.0E+04	4.0E+03 (DNQ)
OCSD	3/17/20	ND	ND	3.5E+03 (DNQ)	10%	12%	ND	ND	3.2E+04 (DNQ)
OCSD	4/20/20	ND	ND	1.8E+03 (DNQ)	45%	49%	ND	ND	3.8E+03 (DNQ)

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
OCSD	5/4/20	ND	ND	ND	30%	72%	ND	ND	ND
OCSD	5/18/20	ND	ND	1.7E+04 (DNQ)	19%	91%	ND	ND	3.1E+04 (DNQ)
OCSD	6/2/20	ND	ND	2.0E+03 (DNQ)	15%	95%	ND	ND	3.6E+03 (DNQ)
OCSD	6/24/20	ND	3.6E+04	2.5E+05	28%	170%	ND	3.6E+04	2.5E+05
OCSD	7/13/20	ND	ND	3.4E+03 (DNQ)	57%	114%	ND	ND	4.0E+03 (DNQ)
OCSD	8/4/20	ND	ND	2.8E+03 (DNQ)	10%	68%	ND	ND	7.2E+03 (DNQ)
OCSD	8/25/20	ND	ND	1.6E+04 (DNQ)	86%	58%	ND	ND	2.2E+04 (DNQ)
OCSD	9/16/20	ND	ND	8.7E+03	48%	31%	ND	ND	2.2E+04
OCSD	10/12/20	ND	ND	ND	6%	40%	ND	ND	ND
OCSD	10/26/20	ND	1.7E+04	1.7E+03 (DNQ)	6%	61%	ND	5.1E+04	5.1E+03 (DNQ)
OCSD	11/17/20	ND	ND	ND	9%	5%	ND	ND	ND
OCSD	12/9/20	ND	2.6E+03 (DNQ)	ND	19%	17%	ND	1.4E+04 (DNQ)	ND
OCSD	12/28/20	1.3E+04	2.0E+03 (DNQ)	5.2E+03	6%	82%	3.0E+04	4.5E+03 (DNQ)	1.2E+04
OCSD	1/18/21	ND	ND	1.4E+04 (DNQ)	28%	30%	ND	ND	4.8E+04 (DNQ)
SD	12/3/19	1.1E+04 (DNQ)	1.5E+05	1.9E+06	37%	32%	3.2E+04 (DNQ)	4.3E+05	5.5E+06
SD	12/18/19	2.5E+04 (DNQ)	ND	1.2E+04 (DNQ)	65%	71%	3.7E+04 (DNQ)	ND	1.8E+04 (DNQ)
SD	1/6/20	7.9E+03 (DNQ)	ND	3.0E+03 (DNQ)	14%	153%	9.5E+03 (DNQ)	ND	3.6E+03 (DNQ)
SD	1/20/20	ND	ND	1.7E+04	37%	32%	ND	ND	4.9E+04
SD	2/4/20	ND	1.5E+04	6.5E+05	74%	70%	ND	2.1E+04	9.0E+05
SD	2/26/20	1.4E+04	4.9E+04	2.1E+05	14%	111%	2.2E+04	7.8E+04	3.4E+05
SD	3/9/20	ND	ND	7.1E+03	82%	69%	ND	ND	9.4E+03

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
SD	3/30/20	3.1E+04 (DNQ)	9.4E+03 (DNQ)	4.0E+04	162%	33%	3.2E+04 (DNQ)	9.6E+03 (DNQ)	4.1E+04
SD	4/14/20	ND	2.1E+04	5.2E+03	13%	70%	ND	5.1E+04	1.3E+04
SD	5/13/20	ND	ND	3.0E+03 (DNQ)	18%	101%	ND	ND	5.0E+03 (DNQ)
SD	5/27/20	ND	1.6E+03 (DNQ)	8.9E+03	143%	107%	ND	1.3E+03 (DNQ)	7.1E+03
SD	6/8/20	ND	2.0E+04	5.1E+03	23%	131%	ND	2.6E+04	6.6E+03
SD	6/30/20	ND	ND	1.5E+06	16%	136%	ND	ND	2.0E+06
SD	7/15/20	ND	ND	ND	124%	180%	ND	ND	ND
SD	7/26/20	ND	1.7E+03 (DNQ)	ND	8%	141%	ND	2.3E+03 (DNQ)	ND
SD	8/10/20	ND	ND	ND	68%	116%	ND	ND	ND
SD	9/8/20	ND	ND	6.4E+02 (DNQ)	13%	51%	ND	ND	2.0E+03 (DNQ)
SD	9/30/20	2.5E+04	2.8E+04	1.6E+04	25%	93%	4.2E+04	4.7E+04	2.7E+04
SD	10/11/20	1.2E+04 (DNQ)	9.8E+03	ND	41%	66%	2.2E+04 (DNQ)	1.8E+04	ND
SD	11/2/20	ND	1.3E+03 (DNQ)	4.3E+03	69%	81%	ND	1.7E+03 (DNQ)	5.7E+03
SD	11/17/20	1.1E+05	4.6E+03	9.9E+04	41%	45%	2.6E+05	1.1E+04	2.3E+05
SD	12/9/20	ND	2.6E+03 (DNQ)	ND	68%	47%	ND	4.5E+03 (DNQ)	ND
SD	1/10/21	ND	ND	4.0E+03 (DNQ)	66%	13%	ND	ND	1.0E+04 (DNQ)
SD	1/25/21	5.9E+05	2.3E+04	1.6E+04	41%	45%	1.4E+06	5.3E+04	3.7E+04
SFPUC	12/11/19	2.3E+03 (DNQ)	2.8E+04	2.0E+05	12%	53%	7.1E+03 (DNQ)	8.6E+04	6.2E+05
SFPUC	12/29/19	ND	6.6E+03 (DNQ)	1.5E+05	102%	103%	ND	6.4E+03 (DNQ)	1.5E+05
SFPUC	1/13/20	2.1E+06	2.0E+05	8.6E+05	10%	130%	3.0E+06	2.9E+05	1.2E+06
SFPUC	1/28/20	2.8E+03 (DNQ)	1.4E+03 (DNQ)	2.1E+05	12%	53%	8.6E+03 (DNQ)	4.3E+03 (DNQ)	6.5E+05

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
SFPUC	2/12/20	2.8E+04 (DNQ)	1.8E+04	2.0E+05	64%	32%	5.8E+04 (DNQ)	3.8E+04	4.2E+05
SFPUC	3/9/20	ND	ND	2.6E+03 (DNQ)	15%	81%	ND	ND	5.4E+03 (DNQ)
SFPUC	3/23/20	ND	ND	6.6E+03	42%	74%	ND	ND	1.1E+04
SFPUC	4/13/20	3.3E+04 (DNQ)	9.8E+03 (DNQ)	2.4E+04 (DNQ)	31%	56%	7.6E+04 (DNQ)	2.3E+04 (DNQ)	5.5E+04 (DNQ)
SFPUC	4/29/20	ND	ND	ND	21%	31%	ND	ND	ND
SFPUC	5/18/20	ND	ND	ND	19%	37%	ND	ND	ND
SFPUC	6/1/20	ND	ND	6.4E+04	80%	99%	ND	ND	7.2E+04
SFPUC	6/16/20	ND	5.2E+03	ND	20%	35%	ND	1.9E+04	ND
SFPUC	7/8/20	ND	2.9E+03 (DNQ)	2.8E+05	18%	39%	ND	1.0E+04 (DNQ)	9.8E+05
SFPUC	7/27/20	ND	ND	ND	14%	32%	ND	ND	ND
SFPUC	7/27/20	ND	ND	ND	141%	161%	ND	ND	ND
SFPUC	9/1/20	1.1E+04	2.3E+03 (DNQ)	7.1E+03	10%	24%	6.5E+04	1.4E+04 (DNQ)	4.2E+04
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/16/20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	9/22/20	ND	ND	ND	73%	98%	ND	ND	ND
SFPUC	10/5/20	ND	ND	ND	12%	38%	ND	ND	ND
SFPUC	10/19/20	ND	ND	ND	5%	35%	ND	ND	ND
SFPUC	11/2/20	ND	ND	ND	9%	22%	ND	ND	ND
SFPUC	11/2/20	ND	ND	ND	7%	29%	ND	ND	ND
SFPUC	11/10/20	ND	ND	ND	7%	19%	ND	ND	ND
SFPUC	12/2/20	2.2E+04	5.2E+03	2.8E+04	14%	52%	6.7E+04	1.6E+04	8.5E+04
SFPUC	12/14/20	9.8E+03	ND	1.9E+04	14%	34%	4.1E+04	ND	7.9E+04

WWTP	Date	Molecular Norovirus GIA Uncorrected Conc. (GC/L)	Molecular Norovirus GIB Uncorrected Conc. (GC/L)	Molecular Norovirus GII Uncorrected Conc. (GC/L)	MS2 Recovery	PhiX174 Recovery	Molecular Norovirus GIA Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GIB Recovery-Corrected Conc. (GC/L)	Molecular Norovirus GII Recovery-Corrected Conc. (GC/L)
SFPUC	1/4/21	ND	2.6E+03 (DNQ)	4.4E+03 (DNQ)	9%	3%	ND	4.3E+04 (DNQ)	7.3E+04 (DNQ)
SFPUC	1/19/21	ND	6.1E+03	8.5E+03	15%	16%	ND	3.9E+04	5.5E+04

N/A = Not analyzed for this parameter in this sample

ND = Not detected (concentration below the limit of detection)

DNQ = Detected but not quantifiable (concentration below the limit of quantification); value shown is an approximation.

Recovery of was measured using two surrogates, MS2 and PhiX174, in every other sample from a given WWTP. The recovery was interpolated for samples where recovery of MS2 and PhiX174 was not directly measured. To determine the recovery corrected concentrations, the average of the MS2 and PhiX174 recovery for a given sample was used.

Table A-5. Molecular SARS-CoV-2 (N1, N2, and E Primer/Probe Sets) Uncorrected Concentrations, Recoveries, and Corrected Concentrations Measured Using the Optimized Ultrafiltration Method.

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
LACSD	12/1/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	12/30/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	1/21/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	1/29/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	2/9/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	3/2/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	3/17/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LACSD	4/8/20	BCS	1.6E+02	2.3E+02	ND	2%	8.0E+03	1.1E+04	ND
LACSD	4/26/20	Cel	ND	6.2E+04	1.5E+04	18%	ND	3.4E+05	8.6E+04
LACSD	5/11/20	Cel	1.6E+05	3.5E+05	1.2E+05	18%	8.9E+05	2.0E+06	6.9E+05
LACSD	5/26/20	BCS	1.9E+02	2.5E+03	ND	2%	9.7E+03	1.3E+05	ND
LACSD	6/10/20	Cel	3.2E+04	6.9E+04	ND	18%	1.8E+05	3.8E+05	ND
LACSD	6/28/20	Cel	8.5E+03	ND	ND	12%	7.1E+04	ND	ND
LACSD	7/20/20	BCS	1.5E+04	3.1E+03	1.5E+04	11%	1.4E+05	2.8E+04	1.4E+05
LACSD	8/11/20	BCS	1.0E+04	3.8E+03	1.3E+02	28%	3.6E+04	1.4E+04	4.8E+02
LACSD	8/19/20	BCS	8.9E+03	2.4E+04	N/A	23%	3.9E+04	1.0E+05	N/A
LACSD	8/19/20	BCS	ND	ND	N/A	14%	ND	ND	N/A
LACSD	8/19/20	BCS	1.4E+04	1.9E+04	N/A	18%	8.2E+04	1.1E+05	N/A
LACSD	8/19/20	Cel	ND	ND	N/A	1%	ND	ND	N/A
LACSD	8/19/20	Cel	ND	ND	N/A	2%	ND	ND	N/A
LACSD	9/2/20	Cel	8.8E+03	2.1E+04	ND	22%	4.0E+04	9.5E+04	ND
LACSD	9/13/20	BCS	ND	1.2E+03	ND	31%	ND	3.9E+03	ND
LACSD	10/5/20	BCS	3.2E+04	3.4E+03	ND	24%	1.3E+05	1.4E+04	ND
LACSD	10/27/20	Cel	1.2E+04	2.2E+03	ND	16%	7.5E+04	1.4E+04	ND

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
LACSD	11/11/20	BCS	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC
LACSD	11/30/20	BCS	1.6E+04	1.9E+03	3.3E+04	42%	3.7E+04	4.5E+03	7.9E+04
LACSD	12/14/20	Cel	1.4E+05	5.9E+04	2.5E+05	31%	4.4E+05	1.9E+05	8.2E+05
LACSD	1/5/21	BCS	3.2E+04	8.1E+04	1.9E+04	29%	1.1E+05	2.8E+05	6.4E+04
LACSD	1/20/21	BCS	5.9E+03	1.0E+04	2.9E+03	8%	7.4E+04	1.3E+05	3.6E+04
LASAN	12/9/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	1/7/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	1/22/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	2/2/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	2/17/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	3/4/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	3/18/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
LASAN	3/29/20	BCS	2.5E+04	7.2E+04	N/A	5%	5.0E+05	1.4E+06	N/A
LASAN	4/20/20	Cel	4.4E+04	4.1E+04	2.6E+03	2%	2.2E+06	2.0E+06	1.3E+05
LASAN	5/5/20	BCS	1.5E+03	1.3E+02	ND	5%	3.0E+04	2.5E+03	ND
LASAN	6/3/20	BCS	5.1E+02	3.0E+03	1.5E+03	5%	1.0E+04	6.0E+04	3.0E+04
LASAN	6/14/20	Cel	8.0E+03	ND	ND	2%	4.0E+05	ND	ND
LASAN	6/29/20	BCS	7.2E+04	6.6E+04	4.2E+04	19%	3.8E+05	3.5E+05	2.2E+05
LASAN	7/14/20	Cel	5.2E+03	1.3E+04	2.2E+04	10%	5.2E+04	1.3E+05	2.2E+05
LASAN	8/5/20	Cel	3.3E+03	1.3E+04	6.7E+04	17%	1.9E+04	7.5E+04	3.9E+05
LASAN	8/17/20	BCS	6.5E+03	1.0E+04	N/A	19%	3.4E+04	5.2E+04	N/A
LASAN	8/17/20	BCS	3.8E+03	1.8E+04	N/A	23%	1.6E+04	7.8E+04	N/A
LASAN	8/17/20	BCS	2.1E+04	ND	N/A	16%	1.4E+05	ND	N/A
LASAN	8/17/20	Cel	9.5E+04	2.6E+05	N/A	4%	2.2E+06	6.0E+06	N/A
LASAN	8/17/20	Cel	1.5E+05	1.1E+05	N/A	7%	1.9E+06	1.5E+06	N/A

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
LASAN	8/17/20	Cel	ND	2.3E+04	N/A	4%	ND	5.8E+05	N/A
LASAN	8/23/20	BCS	3.0E+04	7.9E+03	1.6E+02	13%	2.3E+05	6.1E+04	1.2E+03
LASAN	9/14/20	BCS	1.5E+03	1.8E+03	ND	20%	7.5E+03	9.0E+03	ND
LASAN	9/29/20	Cel	ND	ND	ND	19%	ND	ND	ND
LASAN	10/21/20	BCS	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC	Fail QA/QC
LASAN	11/1/20	Cel	ND	ND	ND	4%	ND	ND	ND
LASAN	11/30/20	Cel	4.3E+04	5.1E+04	1.6E+04	7%	6.2E+05	7.3E+05	2.2E+05
LASAN	12/15/20	BCS	2.4E+04	7.6E+04	4.6E+04	26%	9.3E+04	2.9E+05	1.8E+05
LASAN	1/6/21	Cel	4.8E+03	ND	ND	3%	1.6E+05	ND	ND
LASAN	1/24/21	Cel	6.9E+04	ND	3.1E+04	6%	1.1E+06	ND	5.2E+05
OCSD	12/2/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	12/16/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	1/8/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	1/21/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	2/5/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	2/19/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	3/3/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	3/17/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	3/17/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
OCSD	4/20/20	BCS	ND	ND	ND	3%	ND	ND	ND
OCSD	5/4/20	BCS	2.7E+02	5.3E+02	ND	3%	9.1E+03	1.8E+04	ND
OCSD	5/18/20	Cel	ND	3.3E+03	1.4E+06	2%	ND	1.6E+05	6.9E+07
OCSD	6/2/20	BCS	2.7E+02	1.9E+03	ND	3%	9.1E+03	6.5E+04	ND
OCSD	6/24/20	Cel	7.4E+02	2.0E+03	1.2E+02	2%	3.7E+04	1.0E+05	5.9E+03
OCSD	7/13/20	Cel	2.5E+03	3.9E+03	2.3E+05	3%	8.4E+04	1.3E+05	7.8E+06

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
OCSD	8/4/20	BCS	1.0E+04	1.6E+03	3.5E+03	7%	1.4E+05	2.3E+04	5.0E+04
OCSD	8/25/20	Cel	ND	ND	ND	45%	ND	ND	ND
OCSD	9/16/20	Cel	1.1E+04	3.4E+04	3.4E+04	42%	2.6E+04	8.1E+04	8.0E+04
OCSD	10/12/20	BCS	2.2E+03	ND	ND	4%	5.6E+04	ND	ND
OCSD	10/26/20	BCS	ND	1.4E+04	1.1E+04	2%	ND	7.1E+05	5.6E+05
OCSD	11/17/20	Cel	1.4E+05	1.8E+05	6.0E+04	26%	5.2E+05	7.0E+05	2.3E+05
OCSD	12/9/20	Cel	8.0E+04	1.6E+05	3.3E+04	28%	2.9E+05	5.8E+05	1.2E+05
OCSD	12/28/20	BCS	5.5E+04	1.3E+05	7.0E+04	30%	1.8E+05	4.4E+05	2.3E+05
OCSD	1/18/21	Cel	1.7E+05	1.1E+05	8.4E+04	83%	2.1E+05	1.3E+05	1.0E+05
SD	12/3/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	12/18/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	1/6/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	1/20/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	2/4/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	2/26/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	3/9/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	3/30/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD	4/14/20	BCS	5.1E+01	4.3E+02	ND	3%	1.7E+03	1.4E+04	ND
SD	5/13/20	BCS	ND	ND	ND	3%	ND	ND	ND
SD	5/27/20	Cel	6.1E+03	8.9E+03	ND	49%	1.2E+04	1.8E+04	ND
SD	6/8/20	BCS	ND	2.7E+02	ND	3%	ND	8.9E+03	ND
SD	6/30/20	BCS	1.1E+04	3.3E+04	2.1E+03	32%	3.5E+04	1.0E+05	6.5E+03
SD	7/15/20	Cel	7.1E+03	8.7E+03	3.3E+03	49%	1.4E+04	1.8E+04	6.8E+03
SD	7/26/20	BCS	5.0E+04	4.9E+04	6.9E+03	26%	1.9E+05	1.9E+05	2.7E+04
SD	8/10/20	Cel	5.2E+03	4.6E+02	3.9E+02	34%	1.5E+04	1.4E+03	1.2E+03

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
SD	9/8/20	Cel	6.9E+02	2.1E+04	ND	25%	2.8E+03	8.4E+04	ND
SD	9/30/20	BCS	ND	5.2E+03	ND	17%	ND	3.1E+04	ND
SD	10/11/20	Cel	ND	ND	2.3E+04	26%	ND	ND	9.0E+04
SD	11/2/20	Cel	ND	ND	ND	9%	ND	ND	ND
SD	11/17/20	BCS	3.0E+04	8.6E+02	1.4E+04	35%	8.6E+04	2.5E+03	3.9E+04
SD	12/9/20	Cel	7.2E+04	1.2E+05	1.8E+04	42%	1.7E+05	2.7E+05	4.3E+04
SD	1/10/21	Cel	6.0E+04	8.9E+03	1.2E+04	22%	2.7E+05	4.1E+04	5.4E+04
SD	1/25/21	BCS	2.2E+03	1.7E+04	1.5E+04	19%	1.2E+04	8.7E+04	7.9E+04
SFPUC	12/11/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	12/29/19		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	1/13/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	1/28/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	2/12/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	3/9/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	3/23/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	4/13/20	Cel	8.9E+03	2.9E+04	2.7E+04	7%	1.3E+05	4.1E+05	3.8E+05
SFPUC	4/29/20	BCS	ND	4.4E+02	ND	4%	ND	1.1E+04	ND
SFPUC	5/18/20	Cel	ND	2.0E+05	1.4E+06	7%	ND	2.8E+06	2.0E+07
SFPUC	6/1/20	Cel	3.5E+04	9.0E+04	ND	7%	5.0E+05	1.3E+06	ND
SFPUC	6/16/20	BCS	2.3E+01	3.6E+02	ND	4%	5.8E+02	9.0E+03	ND
SFPUC	7/8/20	BCS	1.0E+04	2.0E+03	4.2E+02	13%	7.8E+04	1.5E+04	3.2E+03
SFPUC	7/27/20	BCS	1.6E+03	1.5E+03	8.2E+04	3%	5.2E+04	4.9E+04	2.7E+06
SFPUC	7/27/20	Cel	7.5E+05	2.8E+07	3.9E+06	2%	5.0E+07	1.9E+09	2.6E+08
SFPUC	9/1/20	BCS	1.1E+04	3.3E+03	5.3E+03	8%	1.4E+05	4.1E+04	6.6E+04
SFPUC	9/16/20	Cel	1.3E+04	1.4E+05	1.1E+04	24%	5.5E+04	6.0E+05	4.5E+04

WWTP	Date	Lab	Molecular SARS-CoV-2 N1 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Uncorrected Concentration (GC/L)	Molecular SARS-CoV-2 E Uncorrected Concentration (GC/L)	OC43 Recovery	Molecular SARS-CoV-2 N1 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 N2 Recovery-Corrected Concentration (GC/L)	Molecular SARS-CoV-2 E Recovery-Corrected Concentration (GC/L)
SFPUC	9/16/20	Cel	ND	2.8E+05	1.9E+04	34%	ND	8.3E+05	5.6E+04
SFPUC	9/16/20	Cel	2.8E+04	7.5E+04	5.0E+04	51%	5.6E+04	1.5E+05	9.7E+04
SFPUC	9/16/20	BCS	ND	2.0E+03	ND	10%	ND	2.0E+04	ND
SFPUC	9/16/20	BCS	1.6E+03	3.1E+03	ND	12%	1.3E+04	2.6E+04	ND
SFPUC	9/16/20	BCS	5.0E+02	6.1E+02	ND	11%	4.5E+03	5.6E+03	ND
SFPUC	9/22/20	Cel	1.9E+03	5.9E+04	6.7E+04	42%	4.5E+03	1.4E+05	1.6E+05
SFPUC	10/5/20	BCS	ND	ND	9.3E+02	7%	ND	ND	1.3E+04
SFPUC	10/19/20	Cel	3.5E+04	7.1E+04	3.5E+04	19%	1.9E+05	3.7E+05	1.8E+05
SFPUC	11/2/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	11/2/20		N/A	N/A	N/A	N/A	N/A	N/A	N/A
SFPUC	11/10/20	Cel	3.2E+04	ND	ND	25%	1.3E+05	ND	ND
SFPUC	12/2/20	BCS	3.0E+04	7.2E+03	1.3E+04	129%	2.3E+04	5.5E+03	1.0E+04
SFPUC	12/14/20	BCS	8.4E+03	4.1E+04	3.7E+04	158%	5.3E+03	2.6E+04	2.4E+04
SFPUC	1/4/21	Cel	9.0E+02	1.2E+05	2.2E+04	36%	2.5E+03	3.4E+05	6.1E+04
SFPUC	1/19/21	BCS	1.1E+04	1.1E+04	4.1E+04	51%	2.2E+04	2.2E+04	7.9E+04

N/A = Not analyzed for this parameter in this sample using this method

ND = Not detected. For SARS-CoV-2, the majority of the samples were below the method LOD and/or LOQ. Because there was still value to be gained from these results, a sample was only considered non-detect if the fluorescence was below the instrument threshold at a cycle number of 40.

Fail QA/QC = Samples with an OC43 recovery less than 1% were considered to have failed the QA/QC criteria.

Table A-6. Native Male-Specific Coliphage Concentrations.

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
LACSD	12/1/19	5.7E+07
LACSD	12/30/19	6.8E+05
LACSD	1/21/20	2.3E+06
LACSD	1/29/20	4.4E+05
LACSD	2/9/20	6.0E+05
LACSD	3/2/20	1.5E+06
LACSD	3/17/20	4.0E+06
LACSD	4/8/20	2.0E+05
LACSD	4/26/20	5.0E+05
LACSD	5/11/20	2.0E+05
LACSD	5/26/20	2.2E+05
LACSD	6/10/20	4.0E+06
LACSD	6/28/20	1.0E+05
LACSD	7/20/20	3.3E+05
LACSD	8/11/20	3.1E+05
LACSD	8/19/20	N/A
LACSD	8/19/20	N/A
LACSD	8/19/20	N/A
LACSD	8/19/20	N/A
LACSD	8/19/20	N/A
LACSD	9/2/20	3.0E+05
LACSD	9/13/20	7.7E+04
LACSD	10/5/20	1.6E+05
LACSD	10/27/20	9.0E+05
LACSD	11/11/20	1.3E+05

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
LACSD	11/30/20	1.1E+05
LACSD	12/14/20	3.0E+05
LACSD	1/5/21	4.6E+05
LACSD	1/20/21	1.6E+05
LASAN	12/9/19	2.2E+05
LASAN	1/7/20	6.3E+05
LASAN	1/22/20	6.2E+05
LASAN	2/2/20	2.1E+05
LASAN	2/17/20	5.0E+05
LASAN	3/4/20	2.0E+07
LASAN	3/18/20	1.8E+05
LASAN	3/29/20	2.7E+05
LASAN	4/20/20	3.0E+05
LASAN	5/5/20	3.5E+05
LASAN	6/3/20	2.2E+05
LASAN	6/14/20	3.0E+04
LASAN	6/29/20	7.3E+05
LASAN	7/14/20	1.0E+05
LASAN	8/5/20	4.0E+05
LASAN	8/17/20	N/A
LASAN	8/17/20	N/A
LASAN	8/17/20	N/A
LASAN	8/17/20	N/A
LASAN	8/17/20	N/A
LASAN	8/17/20	N/A

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
LASAN	8/23/20	2.2E+05
LASAN	9/14/20	8.3E+04
LASAN	9/29/20	6.0E+05
LASAN	10/21/20	2.0E+05
LASAN	11/1/20	5.0E+04
LASAN	11/30/20	2.0E+06
LASAN	12/15/20	2.8E+05
LASAN	1/6/21	7.0E+04
LASAN	1/24/21	2.0E+06
OCSD	12/2/19	8.6E+05
OCSD	12/16/19	1.5E+03
OCSD	1/8/20	3.2E+06
OCSD	1/21/20	4.0E+05
OCSD	2/5/20	3.8E+06
OCSD	2/19/20	6.1E+05
OCSD	3/3/20	1.0E+06
OCSD	3/17/20	3.3E+05
OCSD	3/17/20	9.0E+06
OCSD	4/20/20	3.2E+05
OCSD	5/4/20	1.0E+06
OCSD	5/18/20	9.0E+06
OCSD	6/2/20	1.6E+05
OCSD	6/24/20	4.0E+06
OCSD	7/13/20	4.0E+05
OCSD	8/4/20	5.4E+05

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
OCSD	8/25/20	2.0E+06
OCSD	9/16/20	1.0E+05
OCSD	10/12/20	6.7E+05
OCSD	10/26/20	1.1E+06
OCSD	11/17/20	8.0E+06
OCSD	12/9/20	2.0E+06
OCSD	12/28/20	5.0E+05
OCSD	1/18/21	7.0E+06
SD	12/3/19	6.6E+04
SD	12/18/19	1.4E+05
SD	1/6/20	2.6E+04
SD	1/20/20	3.8E+05
SD	2/4/20	2.3E+05
SD	2/26/20	8.0E+03
SD	3/9/20	2.0E+05
SD	3/30/20	6.5E+04
SD	4/14/20	7.6E+04
SD	5/13/20	5.3E+04
SD	5/27/20	9.0E+04
SD	6/8/20	1.8E+04
SD	6/30/20	8.8E+04
SD	7/15/20	6.0E+04
SD	7/26/20	2.3E+04
SD	8/10/20	3.0E+04
SD	9/8/20	6.0E+04

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
SD	9/30/20	5.0E+04
SD	10/11/20	2.0E+05
SD	11/2/20	2.0E+05
SD	11/17/20	5.1E+04
SD	12/9/20	3.0E+06
SD	1/10/21	5.0E+05
SD	1/25/21	1.4E+05
SFPUC	12/11/19	2.2E+05
SFPUC	12/29/19	6.8E+07
SFPUC	1/13/20	4.2E+05
SFPUC	1/28/20	7.7E+05
SFPUC	2/12/20	7.0E+05
SFPUC	3/9/20	2.2E+05
SFPUC	3/23/20	1.5E+07
SFPUC	4/13/20	3.0E+05
SFPUC	4/29/20	1.1E+05
SFPUC	5/18/20	4.0E+06
SFPUC	6/1/20	4.0E+05
SFPUC	6/16/20	7.8E+05
SFPUC	7/8/20	3.5E+05
SFPUC	7/27/20	9.5E+04
SFPUC	7/27/20	4.0E+04
SFPUC	9/1/20	4.2E+05
SFPUC	9/16/20	N/A
SFPUC	9/16/20	N/A

WWTP	Date	Native Male-Specific Coliphage Concentration (PFU/L)
SFPUC	9/16/20	N/A
SFPUC	9/16/20	N/A
SFPUC	9/16/20	N/A
SFPUC	9/16/20	N/A
SFPUC	9/22/20	2.0E+05
SFPUC	10/5/20	9.0E+04
SFPUC	10/19/20	1.0E+05
SFPUC	11/2/20	N/A
SFPUC	11/2/20	N/A
SFPUC	11/10/20	1.0E+06
SFPUC	12/2/20	1.2E+05
SFPUC	12/14/20	1.1E+05
SFPUC	1/4/21	4.0E+06
SFPUC	1/19/21	1.6E+06

N/A = Not analyzed for this parameter in this sample

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