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Research article

Public health use and lessons learned from a statewide SARS-CoV-2 wastewater monitoring program (MiNET)

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ABSTRACT

The global SARS-CoV-2 monitoring effort has been extensive, resulting in many states and countries establishing wastewater-based epidemiology programs to address the spread of the virus during the pandemic. Challenges for programs include concurrently optimizing methods, training new laboratories, and implementing successful surveillance programs that can rapidly translate results for public health, and policy making. Surveillance in Michigan early in the pandemic in 2020 highlights the importance of quality-controlled data and explores correlations with wastewater and clinical case data aggregated at the state level. The lessons learned and potential measures to improve public utilization of results are discussed.

The Michigan Network for Environmental Health and Technology (MiNET) established a network of laboratories that partnered with local health departments, universities, wastewater treatment plants (WWTPs) and other stakeholders to monitor SARS-CoV-2 in wastewater at 214 sites in Michigan. MiNET consisted of nineteen laboratories, twenty-nine local health departments, 6 Native American tribes, and 60 WWTPs monitoring sites representing 45 % of Michigan's population from April 6 and December 29, 2020. Three result datasets were created based on quality control criteria. Wastewater results that met all quality assurance criteria (Dataset Mp) produced strongest correlations with reported clinical cases at 16 days lag (rho = 0.866, $p < 0.05$).

The project demonstrated the ability to successfully track SARS-CoV-2 on a large, state-wide scale, particularly data that met the outlined quality criteria and provided an early warning of

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increasing COVID-19 cases. MiNET is currently poised to leverage its competency to complement public health surveillance networks through environmental monitoring for new and emerging pathogens of concern and provides a valuable resource to state and federal agencies to support future responses.

1. Introduction

The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020 based on the rapid spread and prevalence of SARS-CoV-2 [1]. Globally, as of 11.40am CET, September 21, 2023, there have been 770,778,396 confirmed cases of COVID-19, including 6,958,499 deaths, reported to WHO [2]. As the virus spread around the world, surveillance, prevention, and treatment methods arose as key needs to protect public health. Wastewater surveillance (WS) emerged as one tool for monitoring and assessing community spread of SARS-CoV-2 [3–12]. In early 2020, the Netherlands implemented the first application of WS, targeting nucleocapsid genes N1, N2, N3, and an envelope gene E of the SARS-CoV-2 virus [13]. Wastewater results were quickly shown to be an early indicator of virus circulation, with positive results occurring six days to two weeks before an active outbreak could be identified by clinical surveillance [13,14]. Wastewater testing also detected cases prior to clinical diagnosis in Spain, even when viral prevalence was low during the onset of the pandemic [15]. Public health authorities have used WS to coordinate testing strategies and justify preventative tactics to limit disease spread in high-disease burdened areas [14,15]. Early in the pandemic, researchers in Connecticut, USA were able to identify hot spots where hospital admissions might increase and use proactive reporting to allow medical personnel advance notice of a potential rise in cases [6,16]. WS is a valuable tool in managing pandemic responses in areas where resources are limited, clinical data are unavailable or limited, and where there is underrepresentation of asymptomatic individuals being tested [7].

With the onset of the pandemic, wastewater monitoring expanded very quickly, and many large-scale research and surveillance system networks were established. Within the United States, Michigan was one of the first states to initiate adoption of a larger scale statewide wastewater monitoring program [17] that included both community and congregate facility-level site sampling. Other noteworthy programs referenced by researchers included Utah [18], Ohio [19], Wisconsin [20], and Oregon [21,22]. Additional national networks included the United Kingdom's COVID-19 national surveillance program [23]; the Netherlands [24], Spain [25], Sweden [26], Israel [27], India [28], Pakistan [29], and Australia [30,31]. In addition, the U.S. Centers for Disease Control and Prevention (CDC) and WHO offered guidance on developing a strategy for wastewater sampling, particularly for SARS-CoV-2 RNA detection [3,32]. Finally, multiple global repositories (e.g., CovidPoops19, W-SPHERE) to track wastewater research and sampling efforts have been developed [33,34]. The W-SPHERE database includes 1456 wastewater sampling sites monitored by organizations and individuals across the world, with over 160,000 individual sampling results [33,34].

Despite this intensive global monitoring effort, there was limited documentation in the preliminary stages of the pandemic regarding how a state or country could implement a program to maximize the value of WS for public health uses. Although governments and organizations made funding available, partnerships among public health officials, academic institutions, and independent laboratories were often inadequate. Formation of WS networks is ongoing, but spatial resolution, communication of results and linkage to public health action are limiting factors for implementing and realizing the full benefit of WS [35,36].

Existing laboratory infrastructure and established programs often allowed for expansion of WS for SARS-CoV-2. For example, the WHO poliovirus surveillance system incorporated SARS-CoV-2 monitoring into its pre-existing infrastructure [37]. However, in the preliminary stages of adopting WS, most states or countries found themselves needing to build the necessary laboratory capacity. There are multiple hurdles in establishing a successful WS network at a state or national scale, including ensuring consistency across laboratories with sample collection, sample preparation, analysis, and timely, meaningful reporting. The goals for this paper are to describe Michigan's statewide laboratory network for monitoring SARS-CoV-2 in wastewater that built a growing knowledgebase and to summarize results used for public health action. Specifically, in this paper the following questions are addressed: 1) how was surveillance implemented at the state level? 2) how was quality assurance and quality control (QAQC) addressed within the state databases? and 3) did the wastewater results have meaning in regard to clinical case and laboratory data at the state level? The lessons learned and suggestions to improve public health WS efforts will also be discussed.

2. Methods

2.1. Study Setting: The Michigan Network and sampling sites

The Michigan Network for Environmental Health and Technology (MiNET) began as a group of twelve laboratories using advanced molecular methods for rapid testing of water quality at recreational beaches [38,39]. With the onset of the COVID-19 pandemic, the network expanded and rapidly developed a pilot study to monitor SARS-CoV-2 in wastewater. The Michigan Department of Environment, Great Lakes, and Energy (EGLE) coordinated the pilot project with input and funding from the Michigan Department of Health and Human Services (MDHHS). EGLE and MDHHS structured the pilot project to consist of locally coordinated subprojects, with a focus on required partnerships and communication between laboratories, wastewater utilities, and local health departments. Another focus of the pilot project was to ensure monitoring was representative of the state of Michigan, both in terms of geography and population density. Nineteen laboratories (Fig. 1) coordinated with their corresponding local health department(s) or Native American tribal partner(s) to obtain approval to undertake the testing, along with at least one local utility partner, typically a wastewater

treatment plant (WWTP). These laboratories included 15 universities, two local health departments, one municipal government, and one private laboratory. Each laboratory worked with local community partners as mentioned above, including 29 local health departments, six Native American tribal partners, and 60 WWTPs. The laboratories coordinated with the wastewater collection partners (at WWTPs, sewersheds) to collect and receive samples and with the local health departments to disseminate the data. The laboratories were also required to share results with local partners within 24 h and with state agencies weekly. These results included wastewater concentration metrics and trend data over time. Coordination of the laboratories with an established lead laboratory and state agencies were through email and weekly network calls where progress and barriers were discussed for rapid resolution and guidance. EGLE provided funding for new digital PCR machines for participating laboratories, as well as other necessary equipment and supplies. Laboratories agreed to use a standardized method for surveillance (exceptions for alternative methods were made for two laboratories who had established methods and for two laboratories who made modifications to the standardized method).

The Water Quality and Environmental Microbiology Laboratory at Michigan State University (MSU) served as the lead laboratory for the network. The lead laboratory developed common procedures, including a standard operation procedure (SOP) for the detection of SARS-CoV-2 in wastewater using droplet digital PCR (ddPCR) and a Quality Assurance Project Plan (QAPP) outlining QAQC procedures and responsibilities, while also providing training and technical assistance and provisioning the laboratories with key supplies (e.g., standard reference materials, internal controls, and primer and probe mixes). Additionally, the lead laboratory trained network laboratory staff to implement the ddPCR assay, provided technical support, and created an automated Microsoft excel data analysis file for the developed SOP so all laboratories could conduct their own assays and report results independently. Participating laboratories then followed outlined protocols and generated data using the predetermined QAQC criteria. The lead laboratory hosted weekly calls for network members to share experiences, ask questions, and troubleshoot issues with sampling and analysis.

Network laboratories were initially trained in ddPCR by the lead laboratory in partnership with a field application scientist team from Bio-Rad. A post-training validation exercise was conducted, where each trained laboratory was sent a sample of extracted RNA from a previously confirmed SARS-CoV-2 positive wastewater sample to evaluate the efficacy of the training and the reproducibility of results across laboratories. Each participating trainee laboratory ran the standard ddPCR method and reported gene copies per reaction for the N1, N2, and E genes, along with results of their positive and negative controls. The lead laboratory offered additional training sessions based on the validation exercise results. The lead laboratory also offered additional training and technical assistance for use of the Excel workbook and as requested by participating laboratories throughout the duration of the pilot study. Training sessions were held virtually given pandemic restrictions.

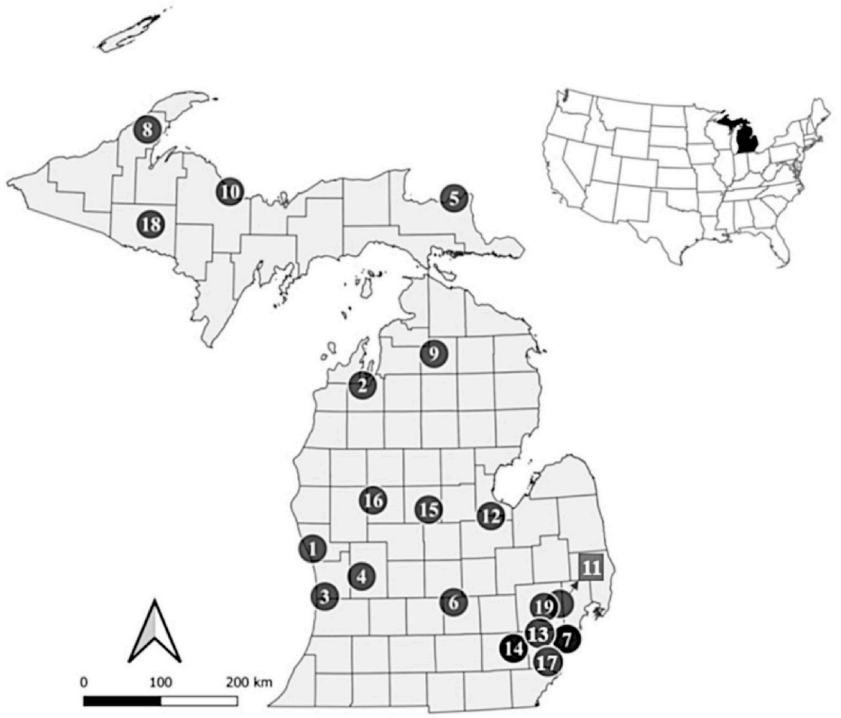

Fig. 1. Michigan Network for Environmental Health and Technology (MiNET) laboratories in the United States of America (n = 19). 1- Annis Water Resources Institute, Grand Valley State University, 2- City of Traverse City Lab, 3- Hope College, 4- Grand Valley State University, 5- Lake Superior State University, 6- Michigan State University, Rose Lab, 7- Michigan State University, Xagoraraki Lab, 8- Michigan Technological University, 9- Northern Michigan Regional Lab, 10- Northern Michigan University, 11- Oakland University, 12- Saginaw Valley State University, 13- University of Michigan, Bakker-Wigginton Lab, 14- University of Michigan, Xi Lab, 15- Central Michigan University, 16- Ferris State University, 17- Wayne State University, 18- White Water Associates, Inc, 19- Oakland County Health Division.

2.2. Sample collection and analysis

MiNET laboratories collected either grab or 24-h composite samples (500–1000 mL) using automatic samplers at WWTPs or installed in sanitary sewers weekly from 214 sites across Michigan between April 6 and December 29, 2020. Samples from sanitary sewer sites (SSS) included those collected at the building level (BL) and from sewer sheds (SS). Wastewater samples which were not immediately processed upon arrival were stored at 4 °C or frozen at −80 °C until analysis. MiNET laboratories stopped freezing samples after September 2020 based on observations that freezing caused reduction in viral counts, which was also reported by others; this was one of the lessons learned early on [40–42]. Samples which were frozen and then analyzed accounted for a small proportion (8.57 %) of the data set and were not used for correlation analyses.

The majority of laboratories (n = 15) utilized a common protocol using a polyethylene glycol (PEG) (8 % w/v) method based on Borchardt et al. (2017) [43] and Flood et al. (2021) [44]. Virus concentration, extraction, and PCR methods used by the network laboratories are listed in Table 1.

The bacteriophage Phi6 was used as a recovery and inhibition control to quantify samples for matrix inhibition [44]. The lead laboratory propagated Phi6 as previously described by Flood et al. (2021) [44] (Phi6 and its bacterial host, *Pseudomonas syringae* were provided by Dr. Krista Wigginton, University of Michigan) and shipped it to the participating laboratories. The phage was used as a SARS-CoV-2 surrogate to assess process efficiency as per Flood et al., 2021 [44]. Two laboratories used bovine coronavirus and one laboratory used human coronavirus OC43 as recovery and inhibition controls (Table 1).

SARS-CoV-2 N1, N2, and E genes were measured using a one-step reverse-transcription droplet digital PCR (RT-ddPCR) method following the CDC-recommended primer probe sets [45,46]. Phi6 RNA was quantified by the RT-ddPCR method, modified from Gendron et al. (2010) [47]. All the primers and probes used in this study are listed in Table 2. Synthetic SARS-CoV-2 RNA purchased from ATCC (ATCC VR-3276SD), and RNA extracted from the Phi6 bacteriophage (internal control assay) were used as positive controls. Droplet digital PCR was performed using QX200 ddPCR system including the QX200 AutoDG droplet generator (Bio-Rad, CA, USA). After ddPCR analysis, SARS-CoV-2 and Phi6 gene copies (GC) per reaction were converted to GC per 100 mL before further data analysis as per Flood et al. (2021) [44]. Two network laboratories used qPCR to quantify SARS-CoV-2 as mentioned in Table 1.

A semi-automated macro-enabled workbook in Excel (Microsoft 365), which imported raw data output files (.csv) from the ddPCR system, was created by the lead laboratory in collaboration with EGLE to streamline reporting of results and reduce data analysis or entry errors across the network. Following the raw data import, laboratory staff were prompted to enter the starting sample and final concentrate volumes; then the workbook calculated the SARS-CoV-2 and Phi6 detection limits and final concentrations in GC per 100 ml as per Flood et al., 2021 [44].

An online dashboard was developed by EGLE and MDHHS to collect and display the SARS-CoV-2 concentrations measured by the nineteen participating laboratories [17]. The laboratories reported results to the dashboard weekly via File Transfer Protocol (FTP). Data from the dashboard were downloaded at the end of the pilot project to use for analysis.

2.3. Quality assurance and quality control

Quality control criteria included the use of method blanks, extraction blanks, spiked internal controls (Phi6), positive ddPCR controls, and non-template controls (Table 3). Each laboratory was responsible for evaluating their samples' data quality using these common criteria prior to reporting results to local partners or uploading to the state dashboard.

Three datasets from the state dashboard download were created based on quality control criteria (Table 3). Dataset M was the complete Michigan dataset, including those samples without one or more internal quality controls (214 sites, n = 2836); Dataset Mp was a subset of Dataset M that passed all quality controls (159 sites, n = 2113); and Dataset Mp_wwtp was a subset of Dataset Mp that only included data from WWTPs that passed all quality controls (53 sites, n = 740) (Table 4). Dataset M contained data from nineteen laboratories covering forty-one counties; Dataset Mp contained data collected from 15 laboratories covering 35 counties; and Dataset Mp_wwtp contained data collected from 13 laboratories covering 29 counties. WWTP data was selected for further analysis based on interest from the state agencies and to enable comparisons to published literature. The comparison of WWTP data versus the entire dataset was used to determine how data combined between sewersheds and WWTPs could provide information on early warning since many monitoring programs sample on both scales. Data from congregate facilities, assisted living facilities, and other restricted sites

Table 1
Summary of lab methods for concentration, extraction, and quantification of SARS CoV-2 targets in wastewater samples.

Method	Number of labs	Virus concentration	RNA Extraction	PCR type	Recovery and Internal Control
Network Standard method	15	PEG Precipitation and Centrifugation [44]	QIAamp viral mini-RNA kit (Qiagen)	ddPCR	Bacteriophage Phi6
Modified Network Standard method	1				Bovine Coronavirus (BCoV)
	1		TRIzol RNA extraction	qPCR	Human Coronavirus (OC43)
4S method	1	Direct Extraction using Silica Column [58]		qPCR	Bovine Coronavirus (BCoV)
Virus adsorption and elution method (Viradel)	1	Nanoceram filtration followed by beef extract elution [57]		ddPCR	Bacteriophage Phi6

Table 2
List of primer and probes used for SARS CoV-2 and Phi6 detection in this study. Additional information on PCR is included in the SOP in Supplementary information.

Target	Primer/Probe name	Primer/Probe Sequence	Reference
SARS CoV-2	2019-nCoV_N1–F	5′-GACCCCAAAATCAGCGAAAT-3′	Lu et al., 2020
	2019-nCoV_N1-R	5′-TCTGGTTACTGCCAGTTGAATCTG-3′	
	2019-nCoV_N1–P	5′-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3′	
	2019-nCoV_N2–F	5′-TTACAAACATTGGCCGCAAA-3′	Lu et al., 2020
	2019-nCoV_N2-R	5′-GCGCGACATTCCGAAGAA-3′	
	2019-nCoV_N2–P	5′-HEX-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3′	
	E_Sarbeco_Forward	5′-ACAGGTACGTTAATAGTTAATAGCGT-3′	Corman et al., 2020
	E_Sarbeco_Reverse	5′-ATATTGCAGCAGTACGCACACA-3′	
	E_Sarbeco_Probe	5′-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3′	
Phi6	Φ6Tfor	5′-TGGCGGCGGTCAAGAGC-3′	Gendron et al., 2010
	Φ6Trev	5′-GGATGATTCTCCAGAAGCTGCTG-3′	
	Φ6Tprobe	5′- FAM-CGGTCGTCGCAGGTCTGACACTCGC-BHQ1-3′	

Table 3
Quality Assurance and Quality Control (QAQC) criteria for SARS-CoV-2 targets (N1/N2/E) and Phi6 Internal control[a] quantification using droplet digital PCR as per Network standard method (included in supplementary information).

No.	Control Type	Control details
1	Method Blank[b]	Prepared using sterile laboratory grade water
2	Extraction Blank[b]	Prepared using elution buffer in place of samples
3	Extraction Blank[b] + internal control[c]	Prepared using elution buffer in place of samples and spiked with Internal control to determine extraction efficiency
4	Positive ddPCR control[c]	ATCC Standard SARS CoV-2 (ATCC VR-3276SD) RNA Phi6 RNA
5	Non-Template ddPCR control[b]	Molecular grade water
6	Sample + internal control[c]	Prepared by spiking sample with internal control to determine extraction efficiency

[a] to determine Extraction efficiency/PCR inhibition.
[b] accepted for ddPCR >9000 droplets, ≤ than 2 positive droplets.
[c] accepted for ddPCR >9000 droplets, ≥ than 3 positive droplets.

Table 4
Description of analyzed datasets.

Dataset	Description of dataset	Number of sites[c] included (n =)	Number of samples included (n =)
M	Complete dataset including samples that fulfill all Control QAQC[a] criteria including those that did not run a Sample + internal control, Criterion 6 in Table 3	214	2836
Mp	Subset of Dataset M which includes all samples that passed all Control QAQC criteria 1–6 in Table 3.	159	2113
Mp_wwtp	Subset of Dataset Mp which includes all WWTP[b] samples that passed Control QAQC criteria 1–6 in Table 3.	53	740

[a] quality assurance quality control.
[b] WWTP wastewater treatment plant.
[c] Sites were sampled weekly.

were excluded to maintain privacy.

2.4. Statistical analysis

Arithmetic means of SARS-CoV-2 gene copies were calculated for samples collected from sites within the same county on the same day (1–20 sites/county). This generated daily county-level wastewater concentration means. County-level wastewater concentration means were used to calculate a statewide mean. The N1, N2 and E genes showed a high level of Spearman correlation with each other [Dataset M: for N1 & N2 (rho = 0.79, $p < 0.05$); N1 & E (rho = 0.86, $p < 0.05$); N2 & E (rho = 0.68, $p < 0.05$). Dataset Mp: for N1 & N2 (rho = 0.74, $p < 0.05$); N1 & E (rho = 0.96, $p < 0.05$); N2 & E (rho = 0.75, $p < 0.05$)]. The N1 gene copy results were used for additional analysis because the most data were available for the N1 target across all sites.

Spearman's rank correlation was used to examine the relationships between wastewater concentrations and clinical cases for all three datasets (M, Mp, Mp_wwtp) from September 1, 2020, to December 29, 2020. Spearman's correlation was chosen since it is less sensitive to outliers and more appropriate for these data sets. County-level case data were extracted from the Michigan COVID-19 Dashboard [48] using reported, confirmed positive cases based on positive NAAT/RT-PCR test results, number of tests, and the

date of onset of symptoms. County population data were obtained from the Johns Hopkins University Center for Systems Science and Engineering data repository dashboard [49]. These data were used to estimate case incidence rates and clinical test positivity rates at the county-level. County-level case incidence rate was calculated as the number of confirmed cases in a county divided by the county's population. County-level clinical test positivity rate was calculated as the number of positive tests in county residents divided by the number of total tests. These two county-level COVID-19 infection variables, case incidence and clinical test positivity rates, were used for the period of wastewater monitoring for all Michigan counties. The state-level arithmetic means were calculated by date for each county-level variable wastewater i.e. SARS-CoV-2 N1 gene copies/100 mL, case incidence rates, and clinical test positivity rates). A seven-day moving average method was used for state-level wastewater and case data which was calculated by taking an average value of 7 days leading up to and including a certain date. This approach was utilized because wastewater concentrations and clinical case counts were not available on a daily basis i.e. Monday-Sunday counts. Time-lagged Spearman's correlations were used to show the time-course dependence between N1 gene copies and COVID-19 case incidence and clinical test positivity on the h-day prior to sampling ($h = 0,..,14$) for the M and Mp datasets. All analyses and plotted results were calculated using R version 4.0.4 (R foundation for statistical computing).

3. Results

3.1. Implementing surveillance across Michigan

The first two cases of COVID-19 in Michigan were identified on March 10, 2020, in Oakland and Wayne counties in the southeastern part of the state, and COVID-19 rapidly spread statewide thereafter. Initial WS for SARS-CoV-2 began in April 2020 with one laboratory sampling four WWTPs; it then expanded to multiple laboratories sampling 31 locations by September and 52 locations in October. At this time, MDHHS and EGLE officially initiated the pilot project, which quickly expanded WS across the state of Michigan (Fig. 2). By December 2020, 19 MiNET laboratories were monitoring 214 locations in 41 counties. These locations represented approximately 45 % of Michigan's total population.

A total of 2836 samples from 214 sites were collected between April and December 2020 (Table 4). Dataset Mp included 2113 samples from 159 locations monitored across the state, of these sites 66.67 % ($n = 106$ sites; 1373 samples) were from sanitary sewer sites, SSS (SS and BL) and 33.33 % ($n = 53$ sites and 740 samples) were from WWTPs. The SS category included 67 sites and 1030 samples, with BL representing 39 building sites and 343 samples. Dataset Mp_WWTP included 53 sites and 740 samples (Table 4). Populations served by the various sampling locations ranged in size from 2261-330,000 persons for the WWTP sites, 70-10,000 persons for BL sites, and 107-32,000 persons for SS sites.

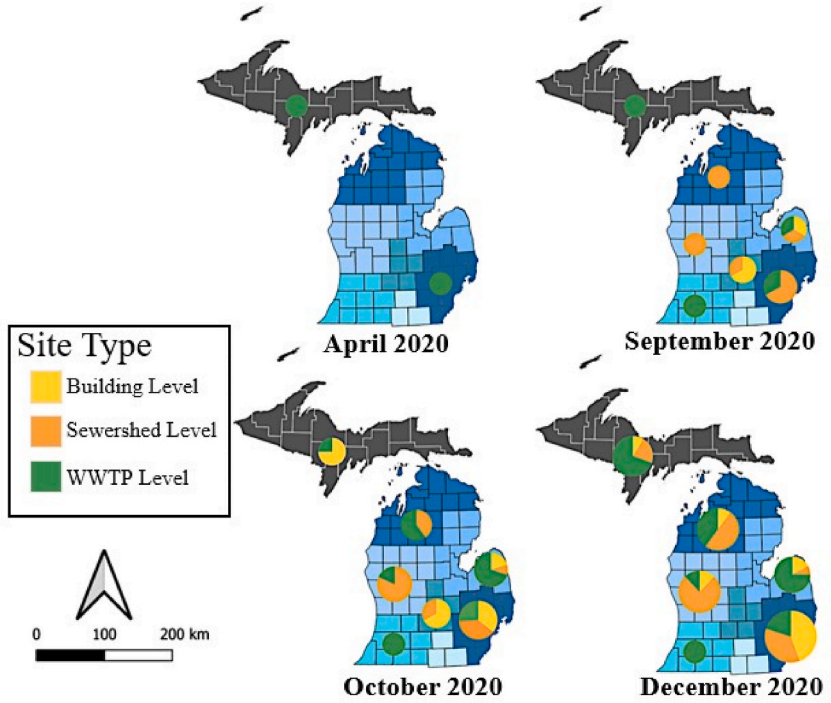

Fig. 2. Pilot Project site types distributed over Michigan Economic Recovery Council (MERC) Regions (shaded) from the months April, September, October, and December 2020. The site distribution includes building level, sewershed level, and wastewater treatment plant (WWTP) level percent of samples per MERC region. The total number of samples per region is displayed by the size ratio of pie charts.

The collected samples were 64 % (n = 1353) composite samples and 36 % (n = 760) grab samples. Approximately half of the WWTP (49 %) and sanitary sewer sites (51 %) used composite sampling. The maximum average concentrations of SARS-CoV-2 N1 GC/100 mL were approximately one log higher for composite samples than grab samples. The percent of positive samples (detection of either N1, N2 or E targets) from composite sites (75 %) was almost twice that of samples from grab sites (44 %). When comparing only composite samples, 63 % of SSS samples and 90 % of WWTP samples were positive.

The mean GC/100 mL for all site types for all three gene targets was 10,000 GC/100 mL (range of 3000–20,000 mean GC/100 mL). The maximum concentration detected was 7,180,000 GC/100 mL at a WWTP in December 2020. The lower detection limit range for samples assessed using ddPCR was 115–6120 GC/100 mL while using qPCR was 37–3993 GC/100 mL. Average recoveries for the samples across all methods ranged from 16.78 to 59.9 %.

3.2. Data quality

Ten laboratories completed the post-training validation exercise by October 2020. Across the participating laboratories, the average GC/reaction calculated were 29.53 ± 5.23 for N1, 25.90 ± 3.78 for N2, and 25 ± 4.26 for E targets (Fig. 3). There was agreement based on the mean and standard deviations for all three gene targets across all laboratories and good reproducibility of results across MiNET laboratories.

The relationships between wastewater SARS-CoV2 concentrations and case data using Dataset M and Dataset Mp were examined (Fig. 4). Dataset M showed a low positive correlation (rho = 0.2–0.4, $p < 0.05$) over the 30-day lag, with the highest correlation at a 5-day lag between cases and wastewater signal (rho = 0.275, $p < 0.05$). Dataset Mp showed a higher positive correlation (rho = 0.6–0.9, $p < 0.05$) over the 30-day lag, with the highest correlation at 16 days lag between the cases and the wastewater signal (rho = 0.866, $p < 0.05$). Since Dataset Mp showed a higher correlation, it was used for further analysis and to explore relationships between wastewater results at WWTPs, COVID-19 cases, and clinical laboratory tests.

3.3. Wastewater surveillance results

Wastewater results from the pilot program corresponded to the Fall 2020 wave of cases in Michigan, during which cases increased beginning in October and peaked at around 8000 cases per day on November 28, 2020 (Fig. 5). Many wastewater sampling sites had their maximum concentrations in November 2020 (38 %, n = 61), followed by December 2020 (30 %, n = 47). During the months of April, and September–December, when clinical cases were high, the percent of positive samples at WWTPs was 88–100 % (Fig. 6). However, the percent of positive samples at WWTPs was much lower between May–August (13–65 %); during this time there was a corresponding decrease in clinical cases, which may have been a result of the State's "stay at home" order in the summer of 2020. During the monitoring period, percent positives at the BL and SS were lower (20–58 %) and more site-specific fluctuations were noted. Overall, the WWTP sample positivity increased and trended well with the case data as cases surged between September–December while increases were noted for SS and BL sites in November December.

Concentrations for datasets Mp compared to Mp_wwtp showed positive correlations with case incidence rate, peaking at a lag of 16 days (rho = 0.866, $p < 0.05$) for Mp as already indicated and 12 days (rho = 0.878, $p < 0.05$) for Mp_wwtp (Fig. 7). Concentrations for Mp and Mp_wwtp datasets also showed positive correlations with clinical laboratory test positivity rates, peaking at a lag of 24 days (rho = 0.887, $p < 0.05$) for Mp and 21 days (rho = 0.870, $p < 0.05$) for Mp_wwtp (Fig. 7).

4. Discussion

This SARS-CoV-2 WS pilot project increased the State of Michigan's capacity for rapid movement into new areas of environmental monitoring and assessment. The project demonstrated the ability to successfully track the virus on a large, statewide scale and that WS

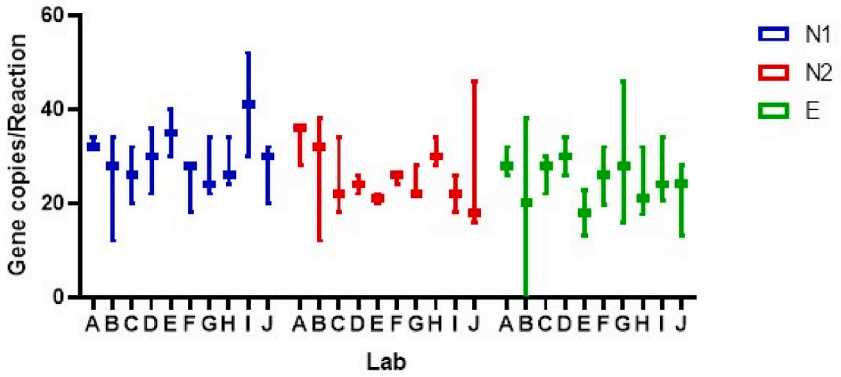

Fig. 3. Summary results of the training validation exercise for 10 trained MiNET labs using a separated box and whiskers plot. SARS CoV-2 gene copy per reaction results for blind sample analysis (n = 1, for triplicate analysis of 3 targets N1, N2 and E) for labs A-J.

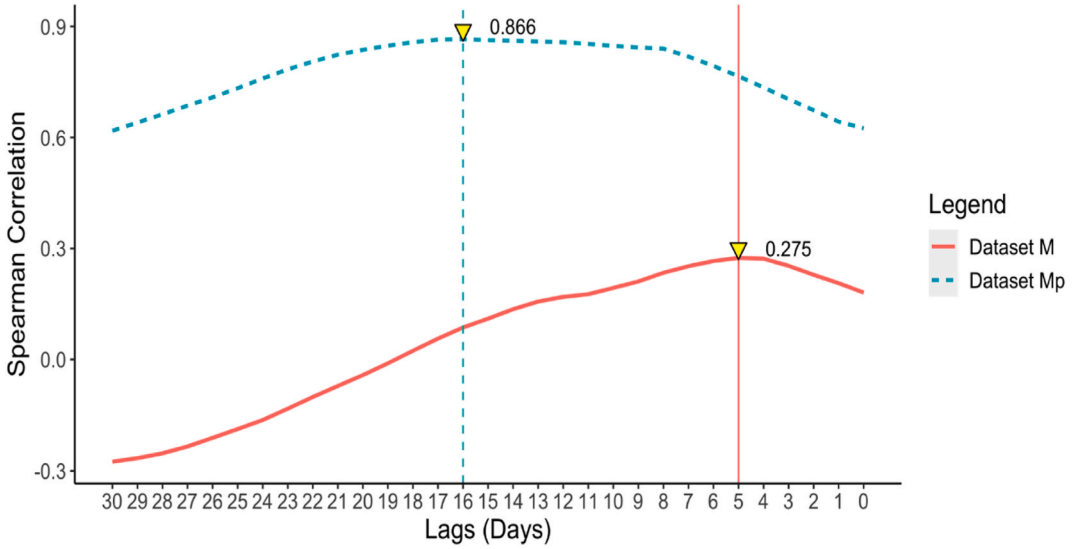

Fig. 4. Wastewater N1 weekly moving average lagged correlations (rho) with incidence rate using confirmed cases (September–December 2020). Dataset M in the solid red line incudes all wastewater data including data which did not pass all internal quality controls. Dataset Mp in the dashed blue line includes a subset of Dataset M which passed all quality control criteria. Correlations for data Mp (rho = 0.866, $p < 0.05$) were better than M (rho = 0.275, $p < 0.05$) and the Dataset Mp indicated highly positive correlations as compared to Dataset M where low positive correlations were seen. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

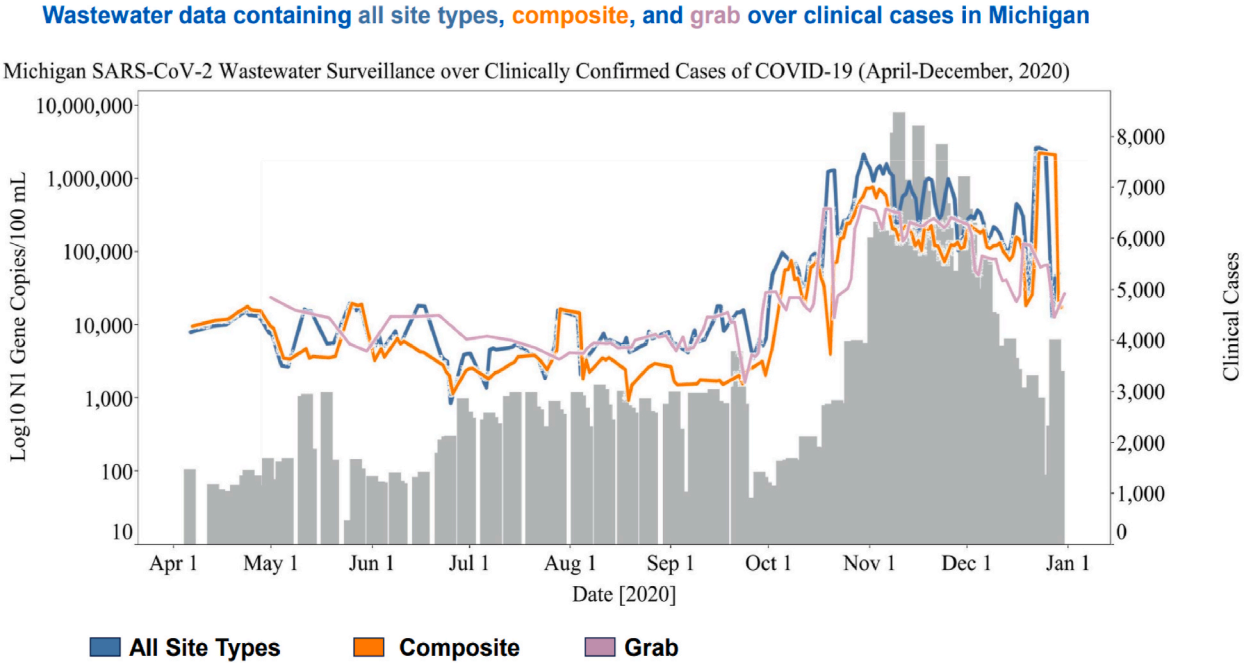

Fig. 5. Weekly wastewater trends (all sites, grabs and composites) against the clinically confirmed cases in Michigan between April and December 2020. Weekly Moving average of wasterwater (log N1 gene copies/100 ml) for Dataset Mp is line plotted with the cases indicated by the bars.

for SARS-CoV-2, particularly for data that met the outlined quality criteria, provided early warning of increasing COVID-19 cases during 2020.

4.1. Impact of Data quality

QAQC information is vital for data interpretation, further advancement of WS methods, and use for public health interventions [50,

Site Type		April	May	June	July	August	September	October	November	December
WWTP		100%	62%	13%	60%	65%	87%	88%	95%	91%
SS		X	50%	20%	38%	31%	34%	27%	58%	51%
BL		X	X	X	X	X	37%	37%	56%	51%
Sample Totals (n)	*WWTP*	4	4	5	6	12	9	22	38	44
	SS	X	4	4	4	13	17	27	47	54
	BL	X	X	X	X	X	5	20	32	30

Percent Positive Samples
0%
1-33%
34-66%
67-100%
Site Type Not Monitored at this Time

Fig. 6. Monthly SARS CoV-2 percent positives for WWTP, SS and BL sites over the 2020 monitoring period. WWTP sites were monitored between April–December 2020, SS sites May–December 2020 and BL sites from September to December 2020. Percent positives at the WWTP sites ranged between 13 and 100 %, SS sites between 20 and 58 % and BL sites between 37 and 56 %.

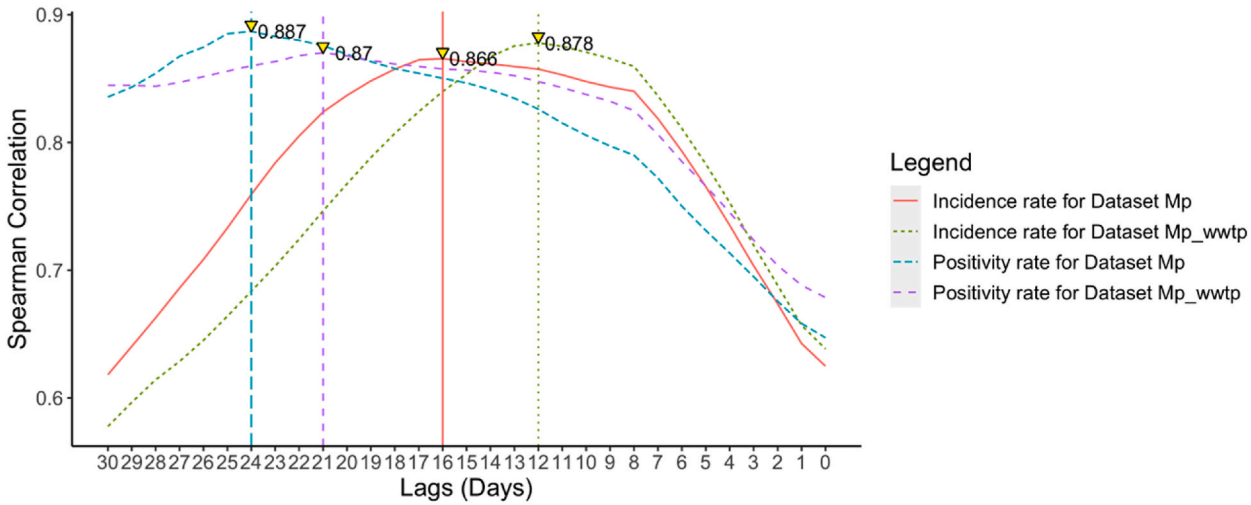

Fig. 7. SARS CoV-2 N1 concentrations correlating a moving average with various lags (rho) with weekly moving average of confirmed cases and positivity rates in Michigan (September–December 2020). County-level incidence rate was computed as the ratio of the number of confirmed cases over county population. County-level positivity rate was obtained by the ratio of the number of positive tests over the number of total tests. The wastewater for datasets Mp and Mp_wwtp showed positive correlations with case incidence rate peaking at a lag of 16 days (rho = 0.866, $p < 0.05$) for Mp and 12 days (rho = 0.878, $p < 0.05$) for Mp_wwtp. The wastewater for datasets Mp and Mp_wwtp showed positive correlations with case positivity rates peaking at a lag of 24 days (rho = 0.887, $p < 0.05$) for Mp and 21 days (rho = 0.870, $p < 0.05$) for Mp_wwtp.

51]. However, information regarding data quality control measures has been lacking in many studies, as the focus of initial work has been on the need to rapidly implement surveillance [52]. Quality assurance parameters were valuable when onboarding and training new laboratories, as it provided a relative standard to validate their operating processes particularly in the initial stages of monitoring. Laboratories experienced multiple challenges in meeting the QAQC criteria, including degradation of standards and reagents during transport or storage, equipment failures, issues with sample handling, accurately assessing recovery efficiencies, and problems with internal controls for inhibition. The major reason for quality assurance failures was determined to be degradation of the recovery/inhibition control solution during transport/storage. Other failures were due to ddPCR droplet count failures or failures of PCR controls. As a result of the failures, 25.50 % of the samples were excluded from dataset Mp. These challenges and others were addressed within MiNET as they arose via weekly network meetings, one-on-one assistance, and additional training sessions. Thus, having a lead support team was critical to the success of the project.

Wastewater results that met all QAQC criteria (Dataset Mp) produced stronger correlations with reported clinical cases than analyses using all results collected (Dataset M). The stringent quality process likely removed outliers that potentially added more variability to the dataset. Based on these results, it is recommended that QAQC parameters be included as a quality review as state or national datasets are compiled. Doing so will improve the use of and confidence in the data to predict disease trends more accurately.

This finding also justified support for following the Minimum Information for Publication of Quantitative PCR Experiments MIQE guidelines [53] to aid in comparability of data from different areas, from different laboratories, and when different protocols may have been employed.

4.2. Statewide wastewater surveillance

This study demonstrated the utility of WS at the state level. Based on these results, wastewater data from WWTPs may serve as a reliable early warning of community level COVID-19 case trends to complement clinical monitoring. The results support the idea that WS of larger populations can serve as a sentinel for disease trends in a state.

Regarding positivity of wastewater samples, between September and December 2020 (a period of high case positivity), the building-level (BL) and sewershed (SS) sites showed similar trends (Fig. 6), indicating that at a statewide scale, building-level trends may mirror community sewershed trends. More local level analysis within nested sampling sites is required to establish relationships for BL and SS wastewater trends. The early warning at the state level was similar in Minnesota, where SARS-CoV-2 RNA levels in wastewater preceded clinical cases by 15–17 days at the statewide level and 4–20 days at the regional level [54].

Given the preliminary analysis in this study, additional work is needed to determine the types of sites (WWTP, SS, BL) that provide the best information for public health actions at a variety of geographic scales (state, county, sewershed or zip code levels). Since this study occurred during a time of high disease incidence and when widespread COVID-19 vaccinations were not available, additional data from times of varying incidence of disease, including times of new variant circulation and changes in vaccination rates, would also be critical to assess. Additionally, early warning periods (lead/lag times) may be contingent on clinical testing in the state and this must be explored in more detail during subsequent monitoring periods. These analyses, using statewide data (2021–2023), are underway and could support future targeted vaccination and educational outreach efforts in communities with sustained or increasing wastewater detections and where clinical testing efforts have declined.[56]

The types of sites to monitor were considered in the context of the goals of the surveillance and the potential public health actions for various populations. For this pilot project, both community level (WWTP and larger SS sites) and congregate facilities (smaller SS and BL sites) were targeted. Large scale community data from WWTPs were informative for overall trends and generalized public communications, while data from smaller sites allowed for specific interventions by public health agencies for these smaller populations, such as targeted clinical testing or building-level quarantines. At present, the state monitoring effort covers over 430 sites which has expanded the monitoring capacity across Michigan after this pilot project in 2020.

In Michigan approximately 30 % of households are on septic tanks instead of municipal sewage systems and thus were not monitored by this project. The occurrence and concentrations of SARS-CoV-2 in septage are currently being explored. However, WWTP, SS and BL sites could be expanded in counties with high septic tank prevalence, and data from such sites could prove useful in supporting public health interventions for rural communities and individuals not on sewer connections.

4.3. Key lessons learned

The pilot project demonstrated that WS could be successfully implemented at a statewide scale within a relatively short timeframe. A variety of collaborative and communication processes were key to the success of the pilot project. For instance, the network provided an outlet for sharing knowledge and resources during a time when it was difficult to meet in person for training or to acquire supplies due to the pandemic. Some of the laboratories involved in the network were either new to this type of testing and surveillance and/or had new, inexperienced analysts. The lead laboratory and vendors were able to organize virtual meetings and training sessions so that the SOP and improvements to the SOP could be implemented properly and consistently across all laboratories. Communication among the network allowed improvements in the protocol to be quickly implemented.

Network laboratories faced multiple challenges to implementing WS during the pilot study. For some laboratories, sample collection was delayed due to the initial coordination that had to be organized among local utilities, municipalities, and environmental contractors. The network also experienced supply chain issues that delayed receipt of equipment and supplies. Staffing issues were a challenge for both laboratories and contractors, resulting in delays in sample collection, analysis, and/or equipment maintenance and repairs. Several laboratories faced difficulties in hiring staff due to pandemic-related hiring freezes. Some academic laboratories had to wait for institutional approvals prior to being allowed to work in their laboratories. Finally, physical access to laboratories was restricted in many locations, as COVID-19 social distancing protocols limited the number of staff who could be in a laboratory at any time. These barriers also hindered cooperative learning and equipment troubleshooting among staff.

Frequent and timely communication with external project stakeholders was critical to establishing support for the project and for ensuring public health utilization of the data. The success of such communications varied by laboratory; some had previous experience in quickly providing results to local health departments, while others had to improve their communication over the course of the project. The data analysis workbook developed for the project enabled consistent reporting of laboratory results in a standardized format. However, many projects found that those results needed additional interpretation for their local stakeholders, with some creating additional report templates and visualization of the data to highlight the implications of the results. Specific actions that were taken by stakeholders because of this project included increasing or relaxing clinical testing in individual residential buildings such as university dormitories and long-term care facilities, subsequent identification, and isolation of both symptomatic and asymptomatic cases through increased clinical testing, and improved communication and interpretation of results to local stakeholders and the public. An initial communication gap was the lack of publicly available data outside of project stakeholders, which led EGLE and MDHHS to develop and publish a Michigan COVID-19 Wastewater Testing Dashboard to make data publicly available [17]. As of

February 2023, the dashboard houses wastewater data (45, 795 data points) generated through MiNET efforts from 2020 to 2023 and contributes to the National Wastewater Surveillance System (NWSS) [55,56].

WS can be readily employed for policy-making and public health decisions; however, a unified, robust network of laboratories that employs quality control procedures during all facets of sample preparation, analysis, and reporting of results with quick turnaround times is recommended. By developing WS networks with a broad set of collaborators, such as academic institutions, government agencies, public health, water utilities, and independent laboratories, WS can leverage resources, address issues rapidly, and effectively support disease surveillance programs. Networks such as MiNET, in partnership with public health agencies and other stakeholders, are poised to quickly address other pathogens or targets that could be present in wastewater, such as monkeypox virus, poliovirus, influenza, respiratory syncytial virus and the next emerging pathogen with pandemic potential.

Data Availability statement

Case data used in this study is available at https://www.michigan.gov/coronavirus/stats.

Wastewater surveillance data is available at https://gisportal.state.mi.us/portal/apps/insights/index.html#/view/52bbb104ed574887918f990af9f3debe.

Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because only publicly available case data was utilized for statistical comparisons. Additionally, wastewater data was collected at congregate level, both data sets were aggregated and sites de identified to ensure anonymity.

CRediT authorship contribution statement

Nishita D'Souza: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alexis M. Porter:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Joan B. Rose:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Erin Dreelin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, MiNET Consortium, Validation, Data curation, Conceptualization. **Susan E. Peters:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. **Penny J. Nowlin:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Samantha Carbonell:** Writing – review & editing, Writing – original draft, Data curation. **Kyle Cissell:** Writing – review & editing, Writing – original draft, Data curation. **Yili Wang:** Writing – original draft, Visualization, Formal analysis, Data curation. **Matthew T. Flood:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis. **Andri T. Rachmadi:** Writing – original draft. **Chuanwu Xi:** Writing – review & editing. **Peter Song:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Shannon Briggs:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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