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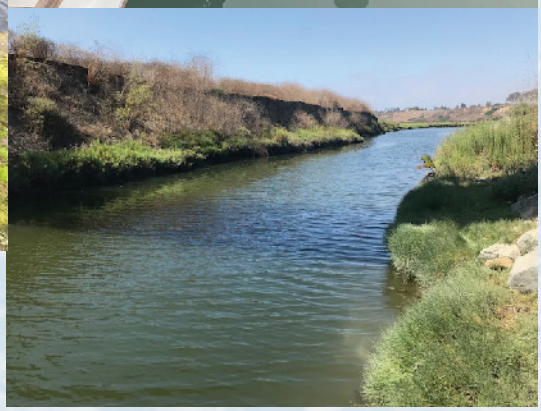

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EXECUTIVE SUMMARY

In California, total and fecal coliform levels in the water column are currently used to evaluate the attainment of the shellfish harvesting (SHEL) beneficial use. The SHEL water quality objectives (WQO) for fecal coliform (FC), which are applicable to the Santa Ana region, and based on U.S. FDA and National Shellfish Sanitation Program (NSSP) standards originally developed for fisheries on the U.S. East Coast and Gulf of Mexico and adopted by California, require that monthly median fecal coliform counts in the water column do not exceed 14 MPN/100 mL and that no more than 10% of samples exceed 43 MPN FC/100 mL. These WQOs are far more difficult to meet than California's REC-1 WQOs, and they apply to many marine and estuarine waters along the California coast. Although microbial water quality in Newport Bay has improved in recent years and often meets WQO's for recreational use, many sites in the bay are out of compliance of the SHEL WQO, especially during the winter season. To date, there have been no locally published studies that evaluate the appropriateness of the current water quality objectives for SHEL. Although, it has been previously hypothesized that current water column-based FC WQO for SHEL may not be relevant for evaluating safety of recreationally harvested shellfish in local waters.

This study aimed to examine the applicability of the current Newport Bay fecal coliform WQO for recreational shellfish harvesting in the dry season, when southern California experiences long periods with no precipitation. To this end, Pacific oysters were deployed at 12 sites within Newport Bay over a six-week period. Oysters were harvested at four different time points, and viral pathogens and fecal bacterial indicators were measured in shellfish tissues. Grab water samples were also collected concomitantly to determine if a relationship existed between water column indicators and viral pathogen detection in oyster tissues.

This study did not find a relationship between fecal coliform levels in the water, upon which the current SHEL WQO is based, and human viral pathogen detection in oyster tissues. Viral pathogen detection in oyster tissues was limited to samples collected during the last week of deployment, coincident with the occurrence of sanitary sewer overflow (SSO) events. This finding was consistent with a recent study conducted by the City of Newport Beach, which did not detect pathogens in shellfish (oyster and mussel) tissues except for samples associated with known sewage spills. The results of this study suggest that viral pathogen presence in the Bay may be low under dry weather conditions, in the absence of sewage spills, and that current WQO for SHEL may not be predictive of viral pathogens in oyster tissue. Alternative indicators that are more predictive of viral pathogen presence than fecal coliforms may need to be explored.

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1. INTRODUCTION

There is currently a fecal coliform TMDL in effect for impairment of the shellfish harvesting (SHEL) beneficial use in Upper and Lower Newport Bay (Resolution No. 99-10). The Water Quality Objective (WQO) for SHEL in the Water Quality Control Plan for the Santa Ana River Basin (Santa Ana Basin Plan) is a monthly median of < 14 MPN FC/100 mL, with no more than 10% of samples exceeding 43 MPN FC/100 mL. This standard applies to many marine and estuarine areas in California regardless of whether shellfish are presently harvested for commercial or recreational purposes.

Bivalve shellfish have the capacity to accumulate pathogens from the surrounding water column in their digestive tracts and tissues (Campos et al. 2013). Although, commercial *E. coli* and fecal coliform water quality standards have historically led to reductions in shellfish consumption related illnesses (Rippey 1994), these bacterial water column standards may not be effective at preventing illnesses associated with viral pathogens in recreationally collected shellfish. The presence of fecal indicator bacteria (FIBs) does not always coincide with pathogen presence (Noble and Fuhrman 2001). Viral pathogens can also persist for extended periods in shellfish tissues, with previous studies detecting viruses in shellfish tissues in the absence of high bacterial counts (Love et al. 2010; Provost et al. 2011)

As a result, the use of male-specific coliphages (MSCs) as a potential proxy for the presence of viral pathogens and sewage-contamination in shellfish has gained attention (Hartard et al. 2018; Hodgson et al. 2017; Kingsley et al. 2019). In fact, the FDA currently uses MSCs to evaluate the impact of sewage spill events on shellfish tissue quality, with shellfish harvesting areas allowed to re-open, following a sewage spill, once levels in shellfish tissues fall below 50 PFU/100 g (NSSP 2019). This is because MSCs are thought to behave more like human enteric viruses, when compared to bacterial indicators, in terms of their morphology, environmental fate and transport, and persistence through the wastewater treatment train (USEPA 2015). Moreover, MSCs occur at consistently high densities in sewage and can be detected relatively simply, making them potentially robust indicators of human viral fecal contamination in both environmental waters (Nappier et al. 2019; Worley-Morse et al. 2019) and shellfish tissues (Hartard et al. 2018).

Another potential approach for evaluating shellfish safety is the direct measurement of human pathogens in shellfish tissues. Measurement methods have advanced in recent years, allowing for improvements in terms of method sensitivity and reliability (Persson et al. 2018; Polo et al. 2016). However, there are challenges associated with direct pathogen detection, including the patchy distribution of many pathogens in the environment and biological systems and the accessibility and cost of performing individual measurements for the entire suite of potential pathogens that may cause illness.

In this study, matched shellfish and water column samples were collected from multiple locations throughout Newport Bay over a 6-week time period. The study was designed to gauge the applicability of the current fecal coliform WQO for protection of the SHEL beneficial use in the dry season and to answer the following questions:

- Do shellfish deployed in Newport Bay bioaccumulate pathogenic viruses or surrogate indicators (e.g., male-specific coliphage) in the dry season, during periods of no precipitation?

- Is there a relationship between water column fecal pollution indicators and shellfish tissue levels of indicators and viral pathogens in Newport Bay?

2. METHODS

2.1 Study Design

The study was completed in Newport Bay, an urbanized estuarine water body located in Southern California. Pacific oysters (*Crassostrea gigas*) were collected from sites in Newport Bay, held in disinfected Newport Bay seawater, and then deployed at 12 sites around the Bay for six-weeks (August to September 2019), during a period of dry weather and no rainfall.

Approximately 1,200 Pacific oysters were initially collected over a three-day period (July 31-August 2, 2019) from Newport Bay, CA. Oysters were transported to holding tanks located at the Kerckhoff Marine Laboratory, Corona Del Mar, CA, within four hours of harvesting.

At the Kerckhoff Marine Laboratory, oysters were arranged on perforated stacked trays in four 282.7 m^3 flow-through seawater tanks for 14 days. An extended depuration period (>5 days) was chosen to enhance reduction of potential viral pathogens, which are expected to have a slower rate of removal when compared to bacterial indicators (FAO, 2008). Seawater was first filtered through a sand filter at 15 – 20 gallons per minute and then further disinfected with a Classic UV 80-Watt Series light (Aqua Ultraviolet, Temecula, California, USA) before entering the holding tanks.

Following the two-week hold time, oysters were deployed across 12 sites in Newport Bay, CA for six weeks. Roughly 100 oysters were deployed in 23-mm plastic mesh oyster bags at each site. At the time of oyster deployment (Week 0), 10-12 oysters were collected and processed from each holding tank to quantify background indicator levels and ensure the absence of pathogens in deployed oysters.

2.2 Site Selection

All 12 oyster deployment locations were located near fixed water quality monitoring stations that are a part of Orange County's historical water quality monitoring program. The 12 sites represented varied water quality conditions, providing spatially representative coverage of the Bay. Sites were selected based on historical monitoring data with the objective of including sites where there would be a range of fecal bacterial levels.

Deployment sites and details are described in Table 1 and illustrated in Figure 1. Oysters were tethered, in mesh bags, to existing structures, except for at sites NSB9, NSB11, and site NBS12 in Upper Newport Bay (described in Table 1). At those three sites, cages were tethered to a wooden stake driven into the substrate to maintain placement. Tethering lengths were variable between sites to ensure that the oysters remained fully submerged throughout the duration of the study.

Potential impacts to water quality during the study include three sanitary sewer overflow (SSO) events that occurred within a 2-mile radius of the sampling locations during the 6-week deployment. However, the transport pathways of these spills were not investigated; thus, we cannot confirm that these events reached Newport Bay or had a definitive impact on the water at the sampling locations. CWIQS database queries indicated that the first SSO event occurred on September 5, 2019 at 1090 Bristol Street, Costa Mesa, CA, approximately 3 days before the week 4 sampling event. An estimated 67 gallons reached surface waters. The second SSO event occurred on September 17, 2019 at 970 Valencia St, Costa Mesa, CA, approximately a week before the week 6 sampling event. An estimated 1,750 gallons reached surface waters. The third SSO event occurred on September 24, 2019 at 1550 Jamboree Road, Newport Beach, CA, the evening of the first week 6 sampling date. An estimated 500 gallons reached surface waters. The SSO event on September 24, 2019 also resulted in a beach closure and public health notification posted by the Orange County public health agency.

2.3 Sample Collection During the 6-week Deployment

Paired oyster and water samples were collected at 4-time points over the six-week deployment, after 1, 2, 4, and 6 weeks. In addition, grab surface water samples were also collected from each of the 12 deployment sites during week 0 at the time of the oyster deployments. Samples were collected at 4 sites on each of three consecutive days during each sampling week. Logistical constraints due to sampling and holding times limited the number of sites that could be processed in one day. The sample collection schedule is described in Appendix A (Table A1). Fecal indicators in the water column were sampled concomitantly with pathogens and indicators in the shellfish tissues on each sampling date.

Approximately 20-24 oysters were collected on each sampling date from each site, transferred to zip-lock bags, immediately placed on ice, and transported to SCCWRP for sample processing. Paired water samples were collected in conjunction with the oysters. A 2-liter surface water grab sample was collected from each site, immediately placed on ice, and transported to the Orange County Public Health Laboratory (OCPHL) for sample processing. All water samples were processed within six hours of collection. Water temperature and salinity were also measured at each site at the time of sample collection with a YSI Model Pro30.

2.4 Sample Processing Methods

2.4.1 Shellfish tissues

On each sampling date, a total of 20-24 oysters per site were aseptically washed and shucked according to recommended guidelines by the FDA (Food and Drug Administration) and the NSSP (National Shellfish Sanitation Program). The tissues of 10 oysters were pooled and blended in a sterile stand blender (Waring, Torrington, CT) for enumeration of culturable indicators (MSC, fecal coliform, *E. coli*), as recommended by the NSSP. In addition, from the remaining 10-12 oysters, the digestive glands were dissected, homogenized, pooled, and frozen in ~1 g portions at -80 deg C for later enumeration of norovirus (NoV) GI and GII, human adenovirus (HAdV), and HF183 human marker by droplet digital PCR (ddPCR) (Figure 2). Processing of digestive glands avoids processing tissues that generally contain limited amounts of virus but significant PCR inhibitors (Lees et al. 2010). Dissection and homogenization

methods followed standard methods, developed in the UK, for the quantification of viral pathogens from shellfish tissues (CEFAS 2019).

Culturable indicators in shellfish tissues

Methods used to analyze culturable indicators, *E. Coli*, fecal coliform, and MSC followed approved methods developed by the FDA and ISSC (Interstate Shellfish Sanitation Conference). There is currently no standard method for the analysis of *Enterococcus* in shellfish tissue. Fecal coliform and *E. coli* concentrations were determined by conventional five-tube multiple dilution most-probable number (MPN) procedure (American Public Health Association 1970). Briefly, a composite sample of 10 oysters per site was utilized as described above. The oyster homogenate was serially diluted in sterile phosphate buffered dilution water; dilutions were equivalent to processing between 1-0.001 g of shellfish tissue, consistent with standard methods. Lauryl tryptose broth (Difco) was utilized for the presumptive growth media, with confirmation performed by inoculating liquid EC-MUG media (Difco) at 44.5 deg C for 24 ± 2 hours.

MSC concentrations in oyster tissues were determined by the Interstate Shellfish Sanitation Conference (ISSC) approved limited use method (Anon 2009). Briefly, a composite sample of 10 oysters per site was utilized as described above. 25 g of the blended oyster meats was aliquoted and diluted 1:2 (wgt:vol) in growth broth. 33 g of the blended oyster homogenate was then centrifuged for 15 minutes at 9000-10000 x g and the supernatant was processed by a modified double-agar overlay method.

Viral pathogens and HF183 human marker in shellfish tissues

Total nucleic acids were extracted from the frozen, homogenized digestive glands. Following a proteinase-k digestion, the NucliSENS magnetic bead extraction kit (bioMerieux) was used to purify total nucleic acids following the CEFAS protocol (CEFAS 2019). NoV GI and GII, HAdV, and HF183 were analyzed in extracted nucleic acids using previously published assays and cycling conditions (Steele et al. 2018) on the QX200 droplet digital PCR system (BioRad). Negative extraction blanks were included in each round of extracted samples and at least three no template control (NTC) reactions were included in each 96-well plate analyzed to ensure that samples were not contaminated.

2.4.2 Water samples

Grab water samples were collected during each processing day. Water samples were processed for MSC and cultivable *Enterococcus, E. coli,* and fecal coliform by OCPHL according to standard methods: EPA Method 1642, EPA Method 1600, EPA Method 1603, and SM 9222-D, respectively. In addition, 100-200 mL of water was filtered to collect bacterial DNA. Filters were stored frozen to preserve nucleic acids for enumeration of human-associated DNA marker by ddPCR (Cao et al. 2015; Griffith et al. 2013). See Figure 3 for the water sample work-flow.

2.5 Statistical Analyses

Pearson correlation tests were utilized to evaluate the relationship between water column and oyster tissue concentrations for each target measured. Correlations were completed using log-transformed data. These were calculated in R using cor.test from the stats package (R Core Team, 2020).

Due to their infrequent detection, biserial point correlations were utilized to further evaluate the relationship between presence/absence of HAdV and MSC in shellfish tissues and concentrations of indicators (fecal coliform, *E. coli*, *Enterococcus*, and MSC) in the water column (Kornbrot 2005). These were calculated in R using cor.test from the stats package (R Core Team 2020).

For comparison of concentrations for the different targets among the 12 sites, Analysis of Variance (ANOVA) tests were conducted in R using the stats package (R Core Team 2020). Individual ANOVA tests were completed separately for each target measured in the water column and oyster tissues. When a significant difference was found, the multcomp package was used to run a post hoc Tukey comparison test for individual pairwise comparisons (Hothorn 2008.

Statistical analyses throughout this report were conducted in R (R Core Team 2020) and figures were generated using the ggplot2 package (Wickham 2016).

3. RESULTS

3.1 Water Quality Results

Fecal coliform was detected in 48 out of the 60 water samples processed, with three of the twelve sites exceeding the fecal coliform SHEL WQO over the course of the six-week study. At stations NBS6 (Arches Drain), NBS12 (San Diego Creek), and NBS13 (Santa Ana-Delhi Channel) median concentrations were greater than 14 MPN/100 mL and more than 10% of samples were above 43 MPN/100 mL (Figure 4). These were the only three stations that exceeded the SHEL WQO for the duration of the study and all three locations were located within or adjacent to major tributary outlets. While the SHEL WQO is based on calculations for monthly median, the values reported here were calculated based on the five water samples taken over the 6-week study for reference purposes.

Enterococcus was detected in 41 out of the 60 water samples processed, with two of the twelve sites exceeding the REC-1 WQO for *Enterococcus* (geometric mean > 30 MPN/100 mL) over the course of the six-week study. At NBS12 (San Diego Creek) and NBS13 (Santa Ana-Delhi Channel), which are both tidally influenced tributary sampling locations, the geometric mean for enterococci was 196 CFU/100 mL and 163 CFU/100 mL, respectively.

Differences in indicator water column concentrations were also apparent among sites (Table 2). Fecal coliform and *E. coli* levels in water samples were significantly higher at NBS12 and NBS13 ($p < 0.05$) than all other sites. *Enterococcus* levels were significantly higher at sites NBS12 and NBS13 than all other sites ($p < 0.05$), with the exception of site NBS6 (Figure 5).

MSC was detected in 22 out of 60 water samples processed. MSC was detected the most frequently and at the highest average concentrations at sites NBS12 and NBS13. However, the highest concentration reported occurred during week 6. At site NBS11 (located within Upper Newport Bay), MSC was detected at a concentration of 2442 PFU/L (Figure 5).

The HF183 human marker was detected infrequently and at low levels, near the limit of detection (LOD), in the water column. The HF183 marker was detected in three samples at concentrations of 56, 88, and 204 copies/100 mL, at the Arches Drain (NBS6), Santa Ana-Delhi Channel

(NBS13) and North Lido Channel (NBS5), respectively. For comparison, the LOD calculated for the HF183 assay was ~45 copies/100 mL (Figure 5).

Temperature and salinity were also measured in parallel during sample collection. Temperature and salinity varied by site, with generally higher temperatures and lower salinities observed at NBS12 (San Diego Creek) and NBS13 (Santa Ana-Delhi Channel), when compared to the other sampling locations (Table 4).

3.1.1 Relationships among microbial targets

Relationships among indicators measured in the water column were evaluated. Fecal coliform, *E. coli*, and *Enterococcus* results tracked each other closely and were highly correlated for the duration of the study (Figure 6B).

3.2 Shellfish tissue results

Differences in fecal coliform and *E. coli* shellfish tissue concentrations were apparent among sites. *E. coli* levels in the oyster tissues were significantly higher at site NBS7 (Dunes Lagoon) than all other sites ($p < 0.05$). Fecal coliform levels were significantly higher at site NBS7 than all other sites, with the exception of site NBS6 ($p < 0.05$). The other fecal indicators measured (HF183 and MSC) were detected infrequently and at low levels throughout the study.

MSC was detected in 12 samples over the course of the study. Concentrations were all detectable but below the ISSC's reported limit of quantification (LOQ), with the exception of one sample. During week 6, at site NBS8, MSC was quantified at 66 PFU per 100 g. This was the highest concentration measured over the course of the study (Figure 7).

HF183 was detected in two shellfish samples over the duration of the study: at site NBS1 during week 1 and at site NBS6 during week 6 (Figure 7). Both detections occurred at levels near the LOD, which was ~60 copies per g.

NoV G1 and G2 were not detected in any shellfish samples for the duration of the study. However, HAdV was detected in oyster tissues at sites NBS7 (Dunes Lagoon) and NBS13 (Santa Ana-Delhi Channel) during week 6 (Figure 8).

In order to evaluate if oysters accumulated indicators over the course of the six-week study, linear regression analyses were completed. The relationship between days since deployment and concentration of MSC, fecal coliform, and *E. coli* was evaluated. No significant relationships were present for any of the assays tested ($p > 0.05$). Average concentrations of each indicator among the weeks sampled were also evaluated with ANOVA. No significant differences were observed ($p > 0.05$). However, concentrations of *E. coli* and fecal coliform did increase from week 0 to week 1. Targets measured were all below the LOD in oysters at the time of deployment (week 0), with concentrations of fecal coliform and *E. coli* increasing significantly by week 1 ($p < 0.05$).

3.2.1 Relationships among microbial targets

Relationships between indicators measured in the shellfish tissues were evaluated. Fecal coliform and *E. coli* results tracked each other closely and were highly correlated for the duration of the

study ($r = 0.95$; $p < 0.01$) (Figure 6A), otherwise there were no significant relationships between the various targets measured in the shellfish tissues.

3.3 Relationships between water column and shellfish tissue measurements

Relationships between indicator and pathogen measurements in the shellfish tissues and indicators in the water column were analyzed two ways. First, correlations were completed between water column and shellfish tissue measurements. In addition, the relationship between detection of HF183, MSC, and HAdV (presence/absence) in the oyster tissues and indicator levels (MSC, *Enterococcus*, Fecal Coliform) in the water column was tested using point biserial correlations. No significant relationships were observed between measurements made in the shellfish tissues and water column.

4. DISCUSSION

This study aimed to examine the applicability of the current fecal coliform WQO for protection of the SHEL beneficial use. Overall, viral pathogens were infrequently detected in shellfish tissues with differences observed between sampling dates.

Shellfish did not consistently accumulate viral pathogens or surrogates (MSC) with regularity over the course of the 6-week deployment. Detection of HAdV or detection of MSC above FDA food safety thresholds in oyster tissues were both limited to the last week of sampling (week 6) at a total of three sampling locations. A potential source of the pathogens detected are three SSO events that occurred within a 2-mile radius of the Bay coincident with the week 6 sampling event. However, this study did not explicitly investigate transport pathways between the location of the SSO events and Newport Bay, so we cannot confirm that fecal material from these events reached and impacted Bay water quality or were the only potential source of pathogens to the sampling locations.

The MSC detection at site NBS8 during week 6 was the only sample where oyster tissues exceeded the FDA threshold of 50 PFU/100 g, resulting in shellfish that are potentially unsafe for consumption based on current guidance by the NSSP and FDA. Otherwise, MSC was detected infrequently in shellfish tissues and at levels that were generally below what has been reported in previous efforts (Biancani et al. 2012; Sheih et al. 2003). However, these studies focused on shellfish located in waters in close proximity to sewer outfalls. In Newport Bay, we expect levels to be lower since waters are not directly impacted by an outfall. In the water column, the highest concentration of MSC observed also occurred during week 6.

In contrast, NoV GI or GII were not detected in any samples, even during week 6, when other viral pathogens were detected. The presence of NoV in wastewater is dependent on active infections and viral shedding in the human population. Thus, NoV occurs at low levels, or not at all, in wastewater with detections generally increasing during the winter months (Shamkhali Chenar & Deng 2017; Wang & Deng 2016). Its inconsistent presence in wastewater makes it a less ubiquitous indicator for the presence of sewage contamination. However, due to its known ability to persist for extended periods of time in shellfish tissues (McLeod et al. 2017), it is

important to continue to evaluate how the presence of NoV relates to other potential indicators, including those considered in this study.

There were no significant changes in fecal coliform or *E. coli* levels in shellfish tissues over the duration of the study or associated with week 6, including in those shellfish samples with detectable HAdV. Presence of HAdV did not coincide with an increase in traditional FIB levels in oyster tissues. This suggests that alternatives to bacterial indicators are needed to demonstrate the presence of viral pathogens in shellfish tissues.

Likewise, detection of HF183 in both the shellfish tissues and water column was infrequent and its presence did not parallel detection of MSC or HAdV, apart from one sample. During week 6, HF183 was detected in the water column coincident to detection of HAdV in oyster tissues at site NBS13. However, overall, pathogens and HF183 were both detected infrequently, which limits our ability to identify if consistent relationships may exist. HF183 has also been shown previously to exhibit differential decay in the environment, when compared to pathogens. Ahmed et al. 2021 reported significantly faster decay of HF183 than other enteric pathogens, with pathogen and indicator rates known to be highly dependent on a variety of environmental conditions including temperature, sunlight radiation, and presence of other microbiota, among other factors.

Overall, the likelihood of viral pathogen occurrence in shellfish appears to be low in Newport Bay under dry weather conditions in the absence of sewage spills. In addition, there were no statistically significant relationships between any of the indicators measured in the water column and measurements made in the shellfish tissues. Limited number (5 total per site) of grab water samples were taken from each site at intervals of 1 or 2 weeks. Given the dynamic nature of Newport Bay, and variability in water quality due to tides, waves, and wind, among other factors, these five samples may not have captured all variations in water quality conditions in the Bay. In contrast to grab water samples, Pacific oysters filter on average 40 gallons of water daily, with filtration capacity dependent on oyster size and environmental conditions. The deployed shellfish may reflect more general microbial water quality conditions of the Bay during this six-week study period. Previous studies have reported that shellfish tissues can serve as useful sentinels for capturing more general seawater quality trends and for the monitoring of microorganisms (Desdouits et al. 2021; Winterbourn et al. 2016).

In this study, our ability to detect significant relationships between pathogen presence in the shellfish tissues and other measurements in the water column was limited due to the infrequent detection of pathogens for the duration of the 6-week deployment. Additional studies evaluating alternative shellfish species and different environmental conditions are needed. Shellfish are known to depurate viral and bacterial particles at different rates, with these rates dependent on a complex mixture of factors including temperature and salinity (Choi & Kingsley 2016; Nappier et al. 2008). Oysters also tend to filter less during colder temperatures, resulting in the potential for increased accumulation and persistence of viruses in shellfish tissues with decreasing temperatures (Choi & Kingsley 2016). This points to the particular importance of evaluating these relationships in more than one season.

5. CONCLUSIONS

- Deployed oysters in Newport Bay, CA did not consistently accumulate pathogens during a six-week period of dry weather. Detection of HAdV was limited to the last week of the study and coincided with the occurrence of SSO events, and norovirus was not detected in any of the shellfish samples tested.

- Additional studies during different seasons, including during winter and wet weather, are recommended.

- Pathogen detection in oyster tissues, although limited, was not related to exceedance of either the fecal coliform SHEL WQO or the *Enterococcus* REC-1 WQO.

- Measurement of MSC at levels above FDA food safety thresholds and detection of HAdV coincided with the occurrence of SSO events; their potential as useful indicators of viral fecal contamination in shellfish tissues warrants further investigation.

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FIGURES AND TABLES

Table 1. Description of the 12 field sites.

Site ID	Description	Lat	Long	Deployment Details	Access	Ownership
NBS1	Bayside Dr Beach Dock	33.603	-117.884	Cages tethered to floating dock (near pump out station)	Land	County
NBS2	Sapphire Ave Dock, Balboa Isl (North Side)	33.604	-117.893	Cages tethered to floating dock	Land	City
NBS3	10th St Beach	33.606	-117.912	Cages tethered to swim area marker	Boat	City
NBS4	Coast Hwy Bridge	33.616	-117.904	Cages tethered to eye bolts attached to seawall	Boat	Private
NBS5	OCC Dock	33.617	-117.918	Cages tethered to Orange Coast College dock	Land	OCC
NBS6	Newport Blvd Bridge dock	33.620	-117.929	Cages tethered to floating dock, near trash skimmer	Land	Private
NBS7	Dunes Lagoon Pedestrian Bridge	33.619	-117.894	Cages tethered to pedestrian bridge	Boat	County
NBS8	Back Bay Science Center Dock	33.621	-117.893	Cages tethered to floating dock	Land	State
NBS9	Big Canyon Buoy	33.631	-117.887	Cages tethered to mudflats	Boat	State
NBS11	Salt Dike Channel Marker	33.647	-117.884	Cages tethered to channel marker	Boat	State
NBS12	SD Creek	33.651	-117.866	Cages tethered to mudflats	Land	County
NBS13	Santa Ana-Delhi at University	33.653	-117.884	Cages tethered to existing bridge infrastructure	Land	County

Table 2. Arithmetic mean, minimum, and maximum concentrations by site for each indicator in the water column samples. Samples that were non-detects were included in mean calculations as LOD/2. ND=not detected for the duration of the study.

Site ID	FC [CFU/100 mL]			EC [CFU/100 mL]			ENT [CFU/100 mL]			MSC [PFU/L]		
	Mean	Min	Max	**Mean**	Min	Max	**Mean**	Min	Max	**Mean**	Min	Max
NBS1	**3**	<2	4	**3**	<2	7	**3**	<2	9	**ND**	<1	<1
NBS2	**6**	<2	9	**3**	<2	5	**2**	<2	4	**1**	<1	2
NBS3	**6**	<2	20	**3**	<2	5	**3**	<2	5	**2**	<1	5
NBS4	**4**	<2	7	**4**	<2	7	**4**	<2	9	**4**	<1	11
NBS5	**6**	2	10	**4**	<2	10	**2**	<2	4	**1**	<1	2
NBS6	**55**	5	180	**36**	4	78	**87**	7	400	**15**	<1	54
NBS7	**4**	<2	7	**3**	<2	4	**2**	<2	2	**1**	<1	2
NBS8	**2**	<2	4	**2**	<2	2	**3**	<2	5	**3**	<1	8
NBS9	**3**	<2	7	**2**	<2	4	**7**	<2	20	**ND**	<1	<1
NBS11	**11**	2	24	**12**	<2	29	**12**	<2	25	**489**	<1	2442
NBS12	**2218**	58	9700	**1467**	46	6000	**965**	9	2500	**91**	<1	374
NBS13	**1616**	214	3860	**1380**	120	3080	**399**	24	1300	**14**	2	61

FC LOD is 2 CFU/100 mL; EC LOD is 2 CFU/100 mL; ENT LOD is 2 CFU/100 mL;
MSC LOD is 1 PFU/L

Table 3. Arithmetic mean, minimum, and maximum concentration by site for each indicator measured in the oyster tissues. Samples that were non-detects were included in mean calculations as LOD/2. ND = not detected for the duration of the study.

Site ID	FC [MPN/100 g]			EC [MPN/100 g]			MSC [PFU/100 g]		
	Mean	Min	Max	**Mean**	Min	Max	**Mean**	Min	Max
NBS1	**207**	78	490	**173**	45	490	**ND**	<7	ND
NBS2	**230**	130	490	**141**	45	330	**9***	<7	23.5
NBS3	**50**	<10	140	**43**	<10	140	**14***	<7	23.5
NBS4	**246**	20	790	**227**	20	790	**9***	<7	23.5
NBS5	**315**	20	790	**129**	20	340	**9***	<7	23.5
NBS6	**1083**	<10	3500	**387**	<10	1300	**14***	<7	23.5
NBS7	**2908**	330	5400	**2908**	330	5400	**ND**	<7	ND
NBS8	**32**	<10	68	**20**	<10	40	**19***	<7	66
NBS9	**35**	<10	45	**29**	<10	45	**ND**	<7	ND
NBS11	**20**	<10	20	**13**	<10	20	**ND**	<7	ND
NBS12	**167**	<10	490	**150**	<10	490	**19***	<7	23.5
NBS13	**285**	68	790	**262**	68	790	**9***	<7	23.5

*Mean concentration was below the method limit of quantification (LOQ).
FC LOD is 20 MPN/100 g; EC LOD is 20 MPN/100 g; MSC LOD is 7 PFU/100 g and LOQ is 47 PFU/100 g

Table 4. Arithmetic mean, minimum, and maximum temperature and salinity observed at each site for the duration of the study.

Site ID	Salinity [ppt]			Temperature [deg C]		
	Mean	Min	Max	**Mean**	Min	Max
NBS1	**33.8**	33.3	34.0	**21.3**	20.4	22.5
NBS2	**33.9**	33.8	34.0	**22.3**	21.7	22.9
NBS3	**33.7**	32.8	34.2	**23.0**	22.4	23.3
NBS4	**33.8**	33.6	34.0	**22.4**	21.1	22.9
NBS5	**33.5**	33.1	33.7	**22.9**	22.5	23.3
NBS6	**33.4**	32.5	33.9	**22.8**	22.0	23.6
NBS7	**33.3**	33.2	33.5	**23.5**	23.2	23.8
NBS8	**33.6**	33.4	33.9	**23.1**	22.6	23.6
NBS9	**32.6**	30.1	33.7	**24.0**	23.0	25.2
NBS11	**31.1**	27.9	33.6	**24.0**	22.9	25.0
NBS12	**14.8**	7.0	23.1	**25.3**	22.5	26.8
NBS13	**23.4**	17.8	29.8	**25.3**	24.7	25.9

Figure 1. Top photo is a map of the 12 sampling locations. Each site is indicated with an orange circle. Bottom photo is a picture of the oysters in mesh bags, pre-deployment.

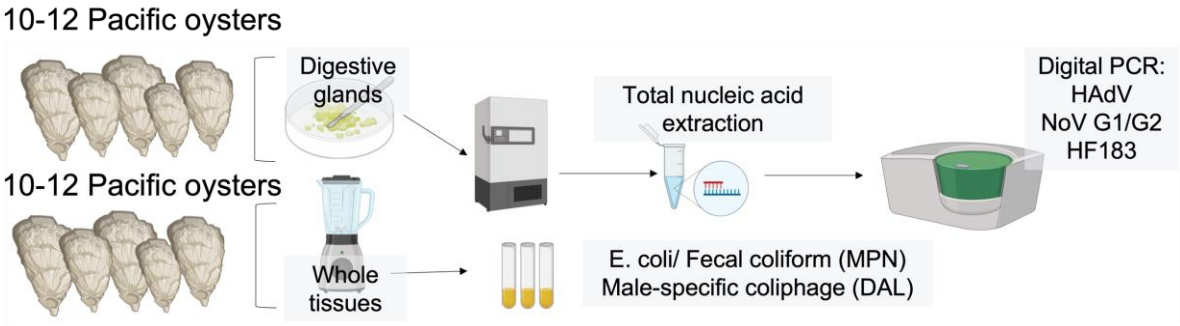

Figure 2. Work-flow for processing shellfish tissues.

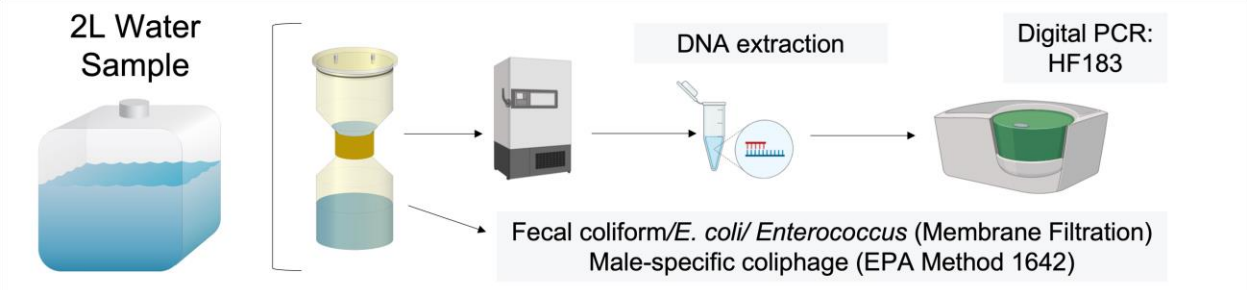

Figure 3. Work-flow for processing grab water samples.

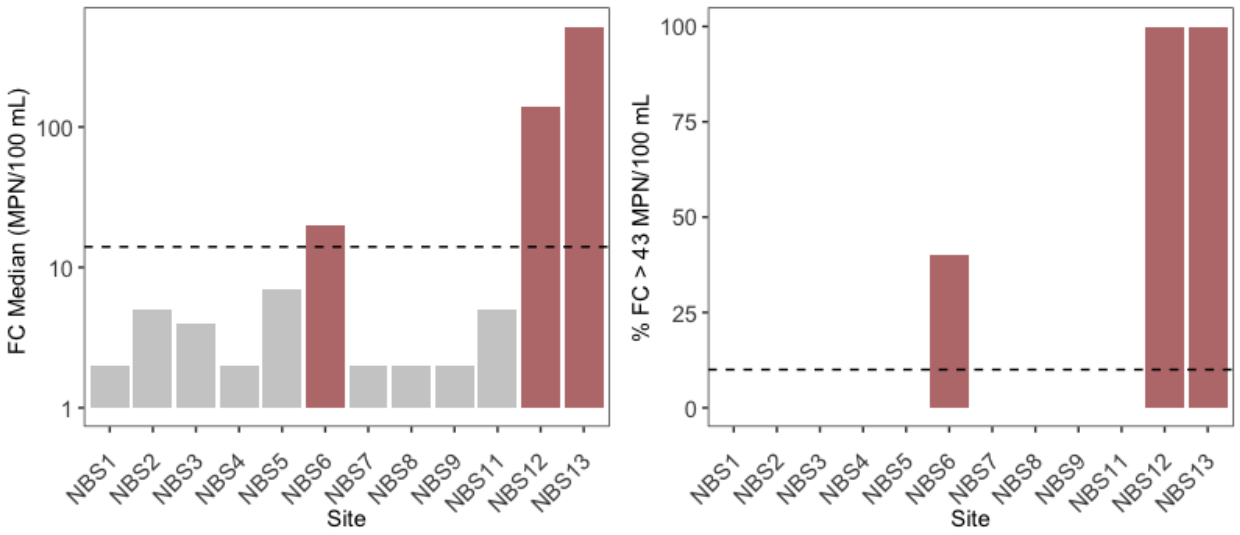

Figure 4. Exceedance of SHEL fecal coliform WQOs over the course of the 6-week study. On the left, median fecal coliform concentrations are shown. Sites where the median was > 14 MPN per 100 mL are highlighted in maroon. On the right, % of samples at each site that were above 43 MPN per 100 mL. Black dashed line indicates 10%.

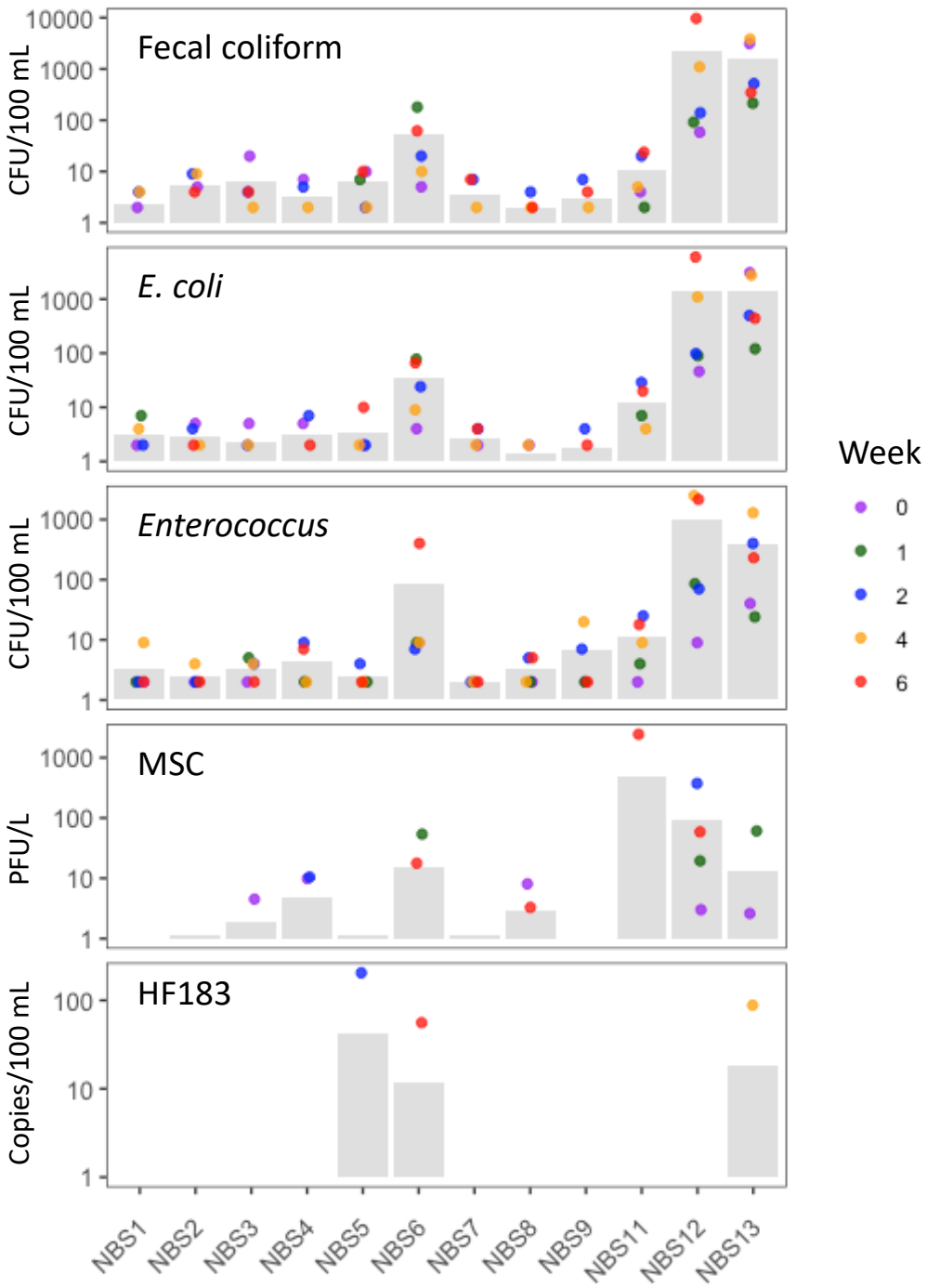

Figure 5. Average concentrations for each target measured in the water column (gray bars). Individual measurements made each week are indicated by the different colored dots, with color representing the week the sample was collected. Samples that were non-detects are not shown but were included in the calculation of site averages.

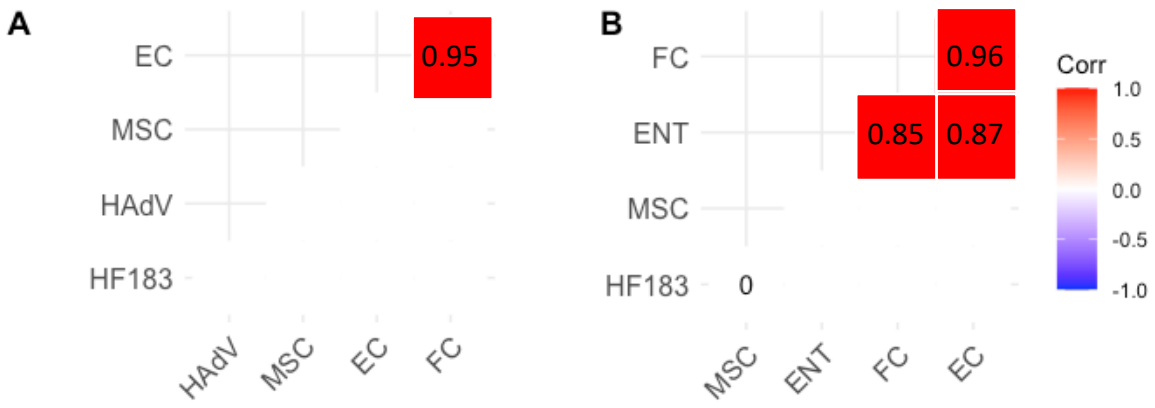

Figure 6. Correlations within A) oyster and B) water matrices. Only significant correlations (at $p < 0.05$) are shown.

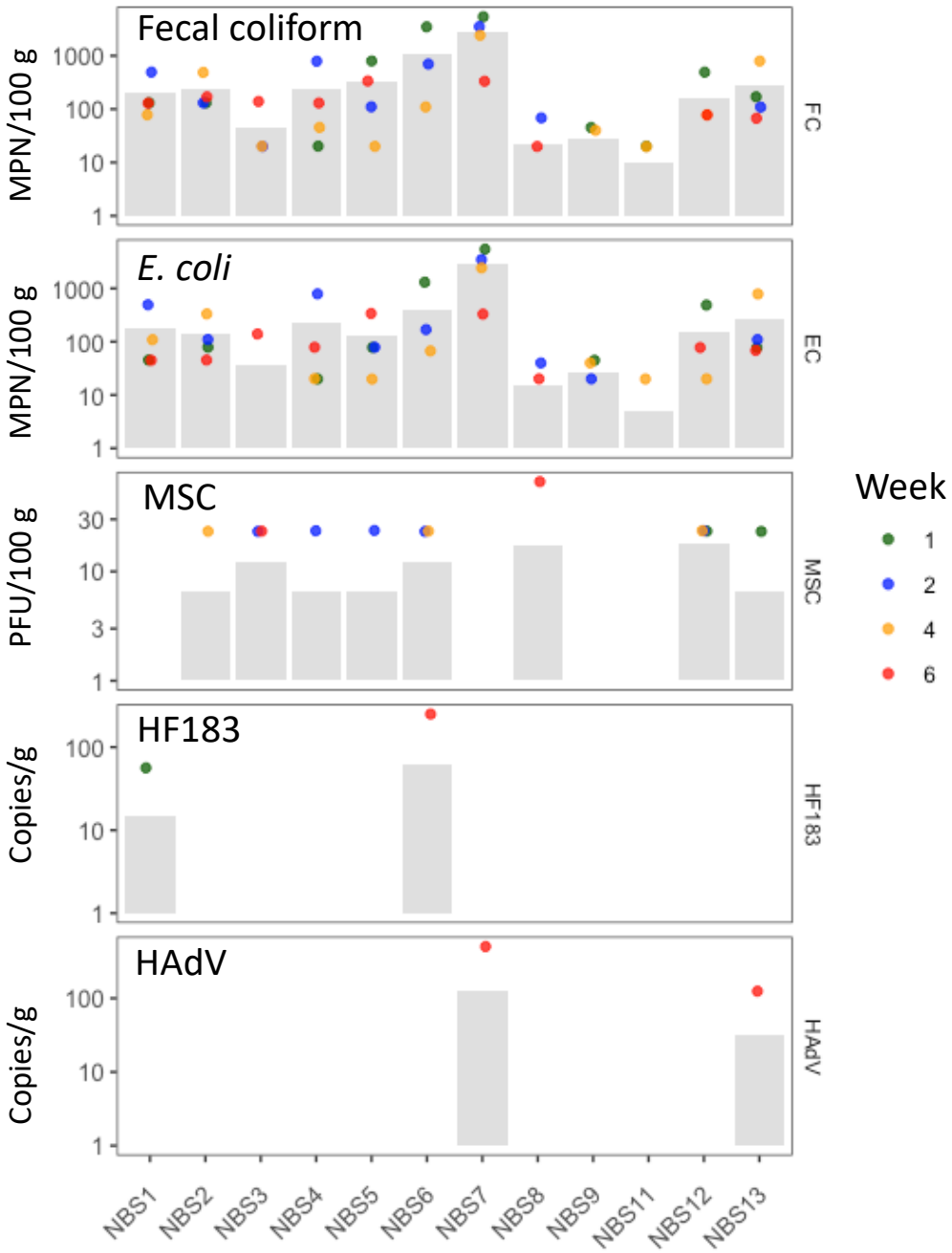

Figure 7. Average concentrations for each target measured in the oyster tissues (gray bars). Individual measurements made each week are indicated by the different colored dots, with color representing the week the sample was collected. For MSC, samples that were detectable but non-quantifiable (DNQ) were plotted at 23.5 (LOQ/2). Samples that were non-detects are not shown but were included in the calculation of site averages.

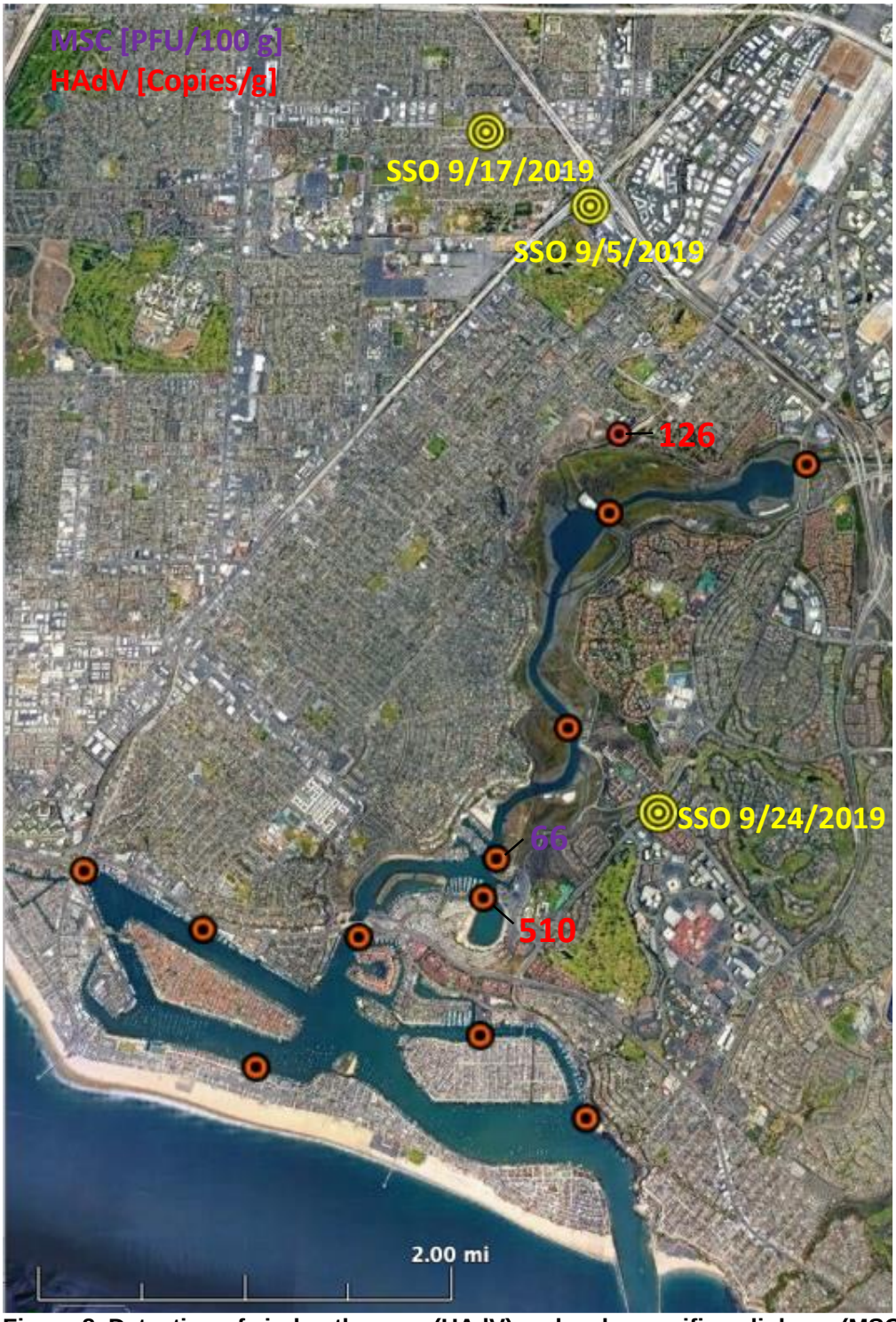

Figure 8. Detection of viral pathogens (HAdV) and male specific coliphage (MSC) above the FDA threshold during week 6 (9/24-9/26/19). Location and dates of the three SSO events are shown with yellow circles. Sampling locations are shown with orange circles.

APPENDIX A. SAMPLING DATES

Table A1. Description of samples collected on each sampling date. n describes the number of oyster samples processed where each sample represents a composite of 10-12 oysters.

Date	Task	Description	Sample Processing	
			Oysters (n)	Water (n)
7/31/19	Background measurements	Background measurements in holding tank intake water	NA	1
7/31/2019-8/2/2019		Background measurements in collected oysters	3	NA
8/13/19	Measurement of deployed test oysters and paired water samples	Background measurements in depurated oysters/ holding take intake water	3	1
8/13/19	Measurement of water samples at deployment sites: Week 0	Sites: NBS1/NBS2/NBS12/NBS13	NA	4
8/14/19		Sites: NBS4/NBS7/NBS9/NBS11	NA	4
8/15/19		Sites: NBS3/NBS5/NBS6/NBS8	NA	4
8/20/19	Measurement of deployed oysters and paired water samples: Week 1	Sites: NBS1/NBS2/NBS12/NBS13	4	4
8/21/19		Sites: NBS4/NBS7/NBS9/NBS11	4	4
8/22/19		Sites: NBS3/NBS5/NBS6/NBS8	4	4
8/27/19	Measurement of deployed oysters and paired water samples: Week 2	Sites: NBS1/NBS2/NBS12/NBS13	4	4
8/28/19		Sites: NBS4/NBS7/NBS9/NBS11	4	4
8/29/19		Sites: NBS3/NBS5/NBS6/NBS8	4	4
9/10/19	Measurement of deployed oysters and paired water samples: Week 4	Sites: NBS1/NBS2/NBS12/NBS13	4	4
9/11/19		Sites: NBS4/NBS7/NBS9/NBS11	4	4
9/12/19		Sites: NBS3/NBS5/NBS6/NBS8	4	4
9/24/19	Measurement of deployed oysters and paired water samples: Week 6	Sites: NBS1/NBS2/NBS12/NBS13	4	4
9/25/19		Sites: NBS4/NBS7/NBS9/NBS11	4	4
9/26/19		Sites: NBS3/NBS5/NBS6/NBS8	4	4

APPENDIX B. WATER COLUMN AND OYSTER TISSUE RESULTS

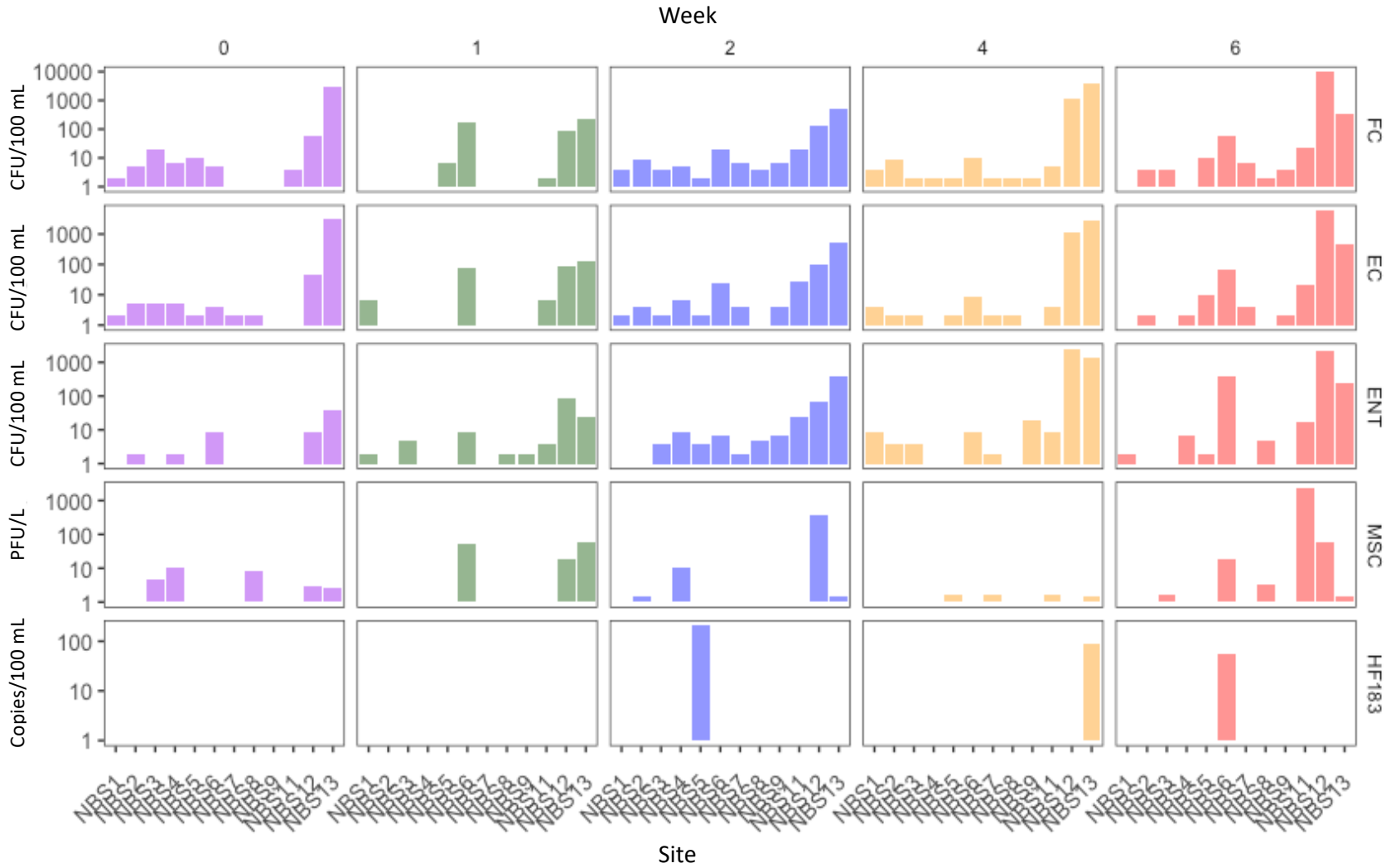

Figure B1. Fecal indicator concentrations measured in water samples for the 12 sites sampled. Each column indicates the week sampling occurred. Each row indicates the different indicators measured in the water column, with target listed on the secondary y-axis. FC = fecal coliform, EC = E. coli, ENT = Enterococcus, MSC = Male-specific coliphage. HF183 = HF183 human-associated marker. Non-detects are not shown.

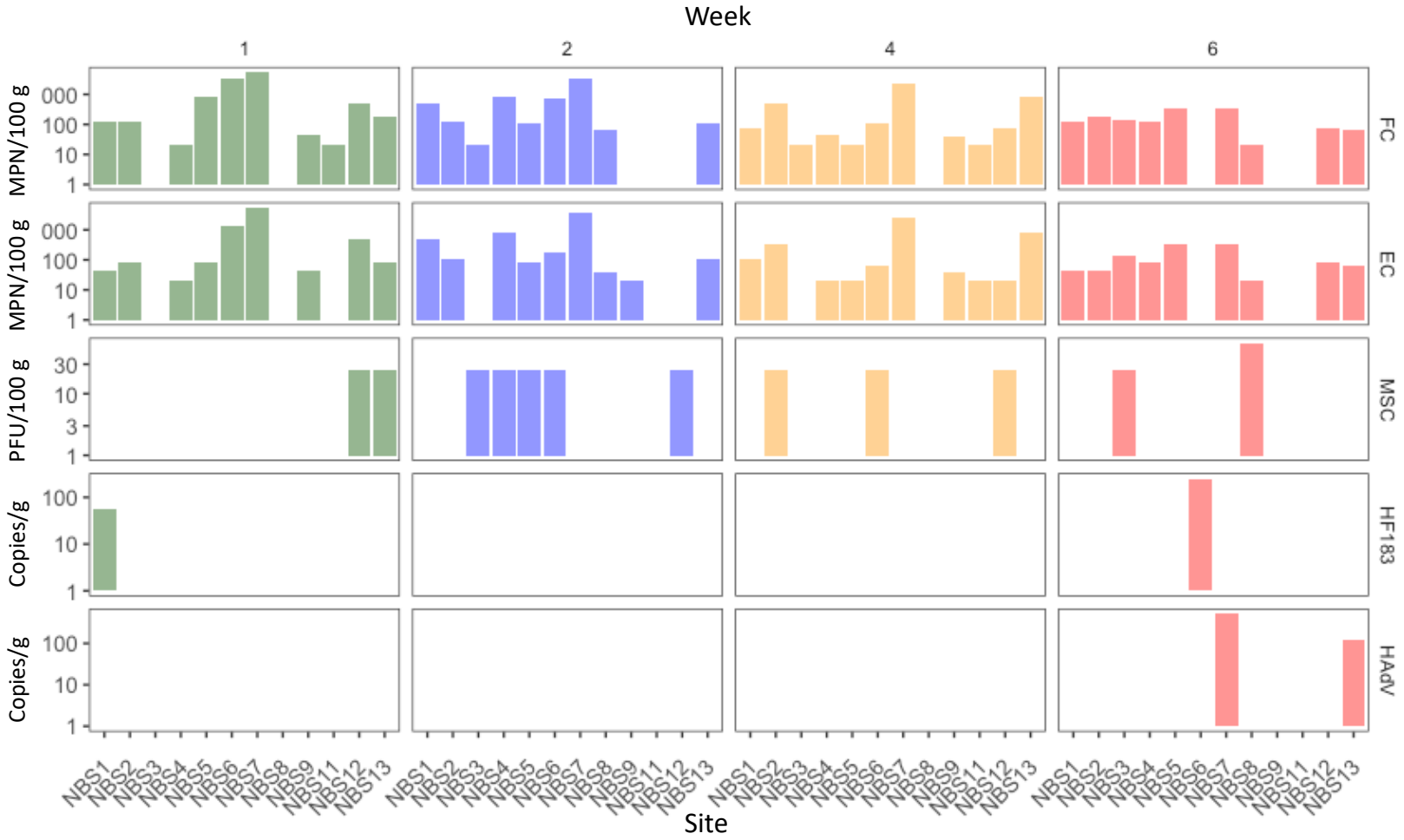

Figure B2. Fecal indicator and pathogen concentrations measured in oyster tissues for the 12 sites sampled. Each column indicates the sampling week. Each row indicates the different targets measured, which are also listed on the secondary y-axis. FC=fecal coliform, EC = E. coli. MSC = Male-specific coliphage, HF183 = HF183 human-associated marker, HAdV = human adenovirus. Non-detects are not shown.

Table B1. Fecal indicator and pathogen concentrations measured in oyster tissues for the 12 sites sampled for the duration of the six-week study.

Station	Week	Date	E. coli	Fecal coliform	MSC	HF183	HAdV	NoV GI	NoV GII
			MPN/100 g	MPN/100 g	PFU/100 g	Copies/ g	Copies/ g	Copies/ g	Copies/ g
NBS1	1	8/20/19	45	130	<7	57	ND	ND	ND
NBS2		8/20/19	78	130	<7	ND	ND	ND	ND
NBS3		8/22/19	<20	<20	<7	ND	ND	ND	ND
NBS4		8/21/19	20	20	<7	ND	ND	ND	ND
NBS5		8/22/19	78	790	<7	ND	ND	ND	ND
NBS6		8/22/19	1300	3500	<7	ND	ND	ND	ND
NBS7		8/21/19	5400	5400	<7	ND	ND	ND	ND
NBS8		8/22/19	<20	<20	<7	ND	ND	ND	ND
NBS9		8/21/19	45	45	<7	ND	ND	ND	ND
NBS11		8/21/19	<20	20	<7	ND	ND	ND	ND
NBS12		8/20/19	490	490	23.5	ND	ND	ND	ND
NBS13		8/20/19	78	170	23.5	ND	ND	ND	ND
NBS1	2	8/27/19	490	490	<7	ND	ND	ND	ND
NBS2		8/27/19	110	130	<7	ND	ND	ND	ND
NBS3		8/29/19	<20	20	23.5	ND	ND	ND	ND
NBS4		8/28/19	790	790	23.5	ND	ND	ND	ND
NBS5		8/29/19	78	110	23.5	ND	ND	ND	ND
NBS6		8/29/19	170	700	23.5	ND	ND	ND	ND
NBS7		8/28/19	3500	3500	<7	ND	ND	ND	ND
NBS8		8/29/19	40	68	<7	ND	ND	ND	ND
NBS9		8/28/19	20	<20	<7	ND	ND	ND	ND
NBS11		8/28/19	<20	<20	<7	ND	ND	ND	ND
NBS12		8/27/19	<20	<20	23.5	ND	ND	ND	ND
NBS13		8/27/19	110	110	<7	ND	ND	ND	ND
NBS1	4	9/10/19	110	78	<7	ND	ND	ND	ND
NBS2		9/10/19	330	490	23.5	ND	ND	ND	ND
NBS3		9/12/19	<20	20	<7	ND	ND	ND	ND
NBS4		9/11/19	20	45	<7	ND	ND	ND	ND
NBS5		9/12/19	20	20	<7	ND	ND	ND	ND
NBS6		9/12/19	68	110	23.5	ND	ND	ND	ND
NBS7		9/11/19	2400	2400	<7	ND	ND	ND	ND
NBS8		9/12/19	<20	<20	<7	ND	ND	ND	ND
NBS9		9/11/19	40	40	<7	ND	ND	ND	ND
NBS11		9/11/19	20	20	<7	ND	ND	ND	ND
NBS12		9/10/19	20	78	23.5	ND	ND	ND	ND
NBS13		9/10/19	790	790	<7	ND	ND	ND	ND
NBS1	6	9/24/19	45	130	<7	ND	ND	ND	ND
NBS2		9/24/19	45	170	<7	ND	ND	ND	ND
NBS3		9/26/19	140	140	23.5	ND	ND	ND	ND
NBS4		9/25/19	78	130	<7	ND	ND	ND	ND
NBS5		9/26/19	340	340	<7	ND	ND	ND	ND
NBS6		9/26/19	<20	<20	<7	251	ND	ND	ND
NBS7		9/25/19	330	330	<7	ND	510	ND	ND
NBS8		9/26/19	20	20	66	ND	ND	ND	ND
NBS9		9/25/19	<20	<20	<7	ND	ND	ND	ND
NBS11		9/25/19	<20	<20	<7	ND	ND	ND	ND
NBS12		9/24/19	78	78	<7	ND	ND	ND	ND
NBS13		9/24/19	68	68	<7	ND	126	ND	ND

*Samples where the MSC concentration measured was detect but not quantifiable (DNQ) were assigned a value of LOQ/2 (23.5).

Table B2. Indicator concentrations measured in the water column for the 12 sites sampled.

Station	Week	Date	Enterococcus	E. coli	Fecal coliform	MSC	HF183
			CFU/100 mL	CFU/100 mL	CFU/100 mL	PFU/L	Copies/100 mL
NBS1	0	8/13/19	<2	<2	2	<1	ND
NBS2		8/13/19	2	5	5	<1	ND
NBS3		8/15/19	<2	5	20	4.5	ND
NBS4		8/14/19	2	5	7	9.9	ND
NBS5		8/15/19	<2	<2	10	<1	ND
NBS6		8/15/19	9	4	5	<1	ND
NBS7		8/14/19	<2	<2	<2	<1	ND
NBS8		8/15/19	<2	<2	<2	8.1	ND
NBS9		8/14/19	<2	2	<2	<1	ND
NBS11		8/14/19	<2	2	4	<1	ND
NBS12		8/13/19	9	46	58	3	ND
NBS13		8/13/19	40	3080	3140	2.6	ND
NBS1	1	8/20/19	2	7	<2	<1	ND
NBS2		8/20/19	<2	<2	<2	<1	ND
NBS3		8/22/19	5	<2	<2	<1	ND
NBS4		8/21/19	<2	<2	<2	<1	ND
NBS5		8/22/19	<2	<2	7	<1	ND
NBS6		8/22/19	9	78	180	53.9	ND
NBS7		8/21/19	<2	<2	<2	<1	ND
NBS8		8/22/19	2	<2	<2	<1	ND
NBS9		8/21/19	2	<2	<2	<1	ND
NBS11		8/21/19	4	7	2	<1	ND
NBS12		8/20/19	86	90	92	19.5	ND
NBS13		8/20/19	24	120	214	61	ND
NBS1	2	8/27/19	<2	2	4	<1	ND
NBS2		8/27/19	<2	4	9	1.5	ND
NBS3		8/29/19	4	2	4	<1	ND
NBS4		8/28/19	9	7	5	10.5	ND
NBS5		8/29/19	4	2	2	<1	204
NBS6		8/29/19	7	24	20	<1	ND
NBS7		8/28/19	2	4	7	<1	ND
NBS8		8/29/19	5	<2	4	<1	ND
NBS9		8/28/19	7	4	7	<1	ND
NBS11		8/28/19	25	29	20	<1	ND
NBS12		8/27/19	70	98	140	373.6	ND
NBS13		8/27/19	400	500	520	1.5	ND
NBS1	4	9/10/19	9	4	4	<1	ND
NBS2		9/10/19	4	2	9	<1	ND
NBS3		9/12/19	4	2	2	<1	ND
NBS4		9/11/19	<2	<2	2	<1	ND
NBS5		9/12/19	<2	2	2	1.6	ND
NBS6		9/12/19	9	9	10	<1	ND
NBS7		9/11/19	2	2	2	1.7	ND
NBS8		9/12/19	<2	2	2	<1	ND
NBS9		9/11/19	20	<2	2	<1	ND
NBS11		9/11/19	9	4	5	1.6	ND
NBS12		9/10/19	2500	1100	1100	<1	ND
NBS13		9/10/19	1300	2760	3860	1.5	88

Station	Week	Date	Enterococcus	E. coli	Fecal coliform	MSC	HF183
			CFU/100 mL	CFU/100 mL	CFU/100 mL	PFU/L	Copies/100 mL
NBS1	6	9/24/19	2	<2	<2	<1	ND
NBS2		9/24/19	2	2	4	<1	ND
NBS3		9/26/19	<2	<2	4	1.6	ND
NBS4		9/25/19	7	2	<2	<1	ND
NBS5		9/26/19	2	10	10	<1	ND
NBS6		9/26/19	400	66	62	17.8	56
NBS7		9/25/19	<2	4	7	<1	ND
NBS8		9/26/19	5	<2	2	3.3	ND
NBS9		9/25/19	<2	2	4	<1	ND
NBS11		9/25/19	18	20	24	2442	ND
NBS12		9/24/19	2160	6000	9700	58.8	ND
NBS13		9/24/19	231	440	347	1.5	ND

APPENDIX C. SITE-AGGREGATED OYSTER AND WATER COMPARISONS

Methods

In order to evaluate more general relationships between oyster tissues and water column measurements over the course of the 6-week study, aggregated data (by site) was compared between the two matrices for fecal coliform and MSC.

Log-normalized average concentrations for fecal coliform in the oyster tissues and water column were calculated at each site over the 6-week study. Pearson correlation was then used to evaluate if there was an association between the average concentrations.

Since generally low levels of MSC were detected in both the water column and oyster tissues, overall detection frequencies (% of samples where MSC was detected) at each site were calculated, as opposed to average concentration. Spearman correlation was used to test for an association between detection frequencies at the different sites.

Results

There was not a significant correlation between the averaged water column and shellfish tissue fecal coliform results ($p > 0.05$).

There was a significant correlation between frequency of MSC detection in the oyster tissues and water column ($r = 0.64$, $p < 0.05$) (Figure C1). However, this relationship was driven in part by the higher detection frequency of MSC at NBS12 in both matrices. When NBS12 was removed, the relationship was no longer significant ($p = 0.07$).

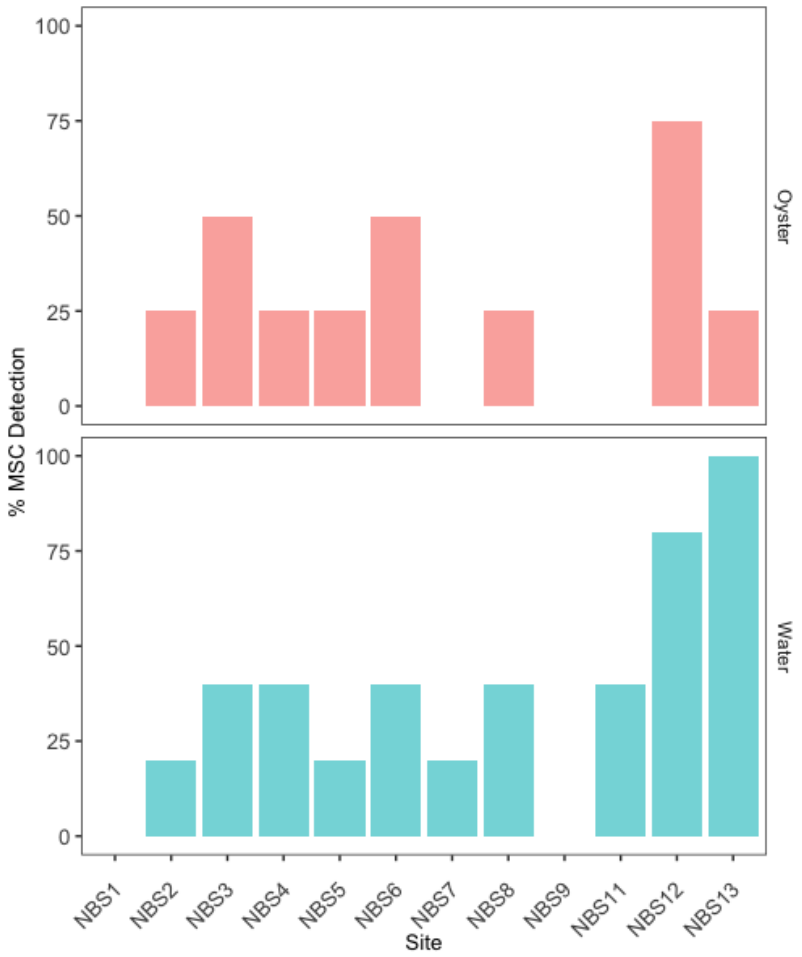

Figure C1. Detection frequency for MSC in the oyster tissues (top panel) and the water column (bottom panel) at each site for the duration of the six-week study.

APPENDIX D. SPLIT SAMPLE ANALYSIS OF WATER COLUMN SAMPLES FOR HF183

Analysis of water samples for HF183 was conducted by two labs, OCPHL and SCCWRP. The two labs tested the samples on the same platform but differed in extraction kit as well as the primer/probe sequences used. OCPHL used the DNA-EZ extraction kit (Gene-Rite) and EPA HF183 primer/probe sequences (USEPA 2019). SCCWRP used the PowerWater DNA Isolation Kit (Qiagen) and HF183 primer/probe sequences described in Cao et al. 2015.

Despite methodological differences, the two labs were in agreement in terms of HF183 detection with the exception of one sample. HF183 was detected at a concentration of 56 copies per 100 mL on 9/26/2019 by SCCWRP; HF183 was not detected in this sample by OCPHL. Otherwise, HF183 was detected in a total of two other samples by both labs (Table D1).

Table D1. Human-associated marker concentrations (HF183) from water column samples run by SCCWRP and OCPHL. ND is not detected.

Station	Week	Date	SCCWRP HF183 [copies/100 mL]	OCPHL HF183 [copies/100 mL]
NBS1	0	8/13/19	ND	ND
NBS2	0	8/13/19	ND	ND
NBS3	0	8/15/19	ND	ND
NBS4	0	8/14/19	ND	ND
NBS5	0	8/15/19	ND	ND
NBS6	0	8/15/19	ND	ND
NBS7	0	8/14/19	ND	ND
NBS8	0	8/15/19	ND	ND
NBS9	0	8/14/19	ND	ND
NBS11	0	8/14/19	ND	ND
NBS12	0	8/13/19	ND	ND
NBS13	0	8/13/19	ND	ND
NBS1	1	8/20/19	ND	ND
NBS2	1	8/20/19	ND	ND
NBS3	1	8/22/19	ND	ND
NBS4	1	8/21/19	ND	ND
NBS5	1	8/22/19	ND	ND
NBS6	1	8/22/19	ND	ND
NBS7	1	8/21/19	ND	ND
NBS8	1	8/22/19	ND	ND
NBS9	1	8/21/19	ND	ND
NBS11	1	8/21/19	ND	ND
NBS12	1	8/20/19	ND	ND
NBS13	1	8/20/19	ND	ND
NBS1	2	8/27/19	ND	ND
NBS2	2	8/27/19	ND	ND
NBS3	2	8/29/19	ND	ND
NBS4	2	8/28/19	ND	ND
NBS5	2	8/29/19	**204**	**216**
NBS6	2	8/29/19	ND	ND
NBS7	2	8/28/19	ND	ND
NBS8	2	8/29/19	ND	ND
NBS9	2	8/28/19	ND	ND
NBS11	2	8/28/19	ND	ND
NBS12	2	8/27/19	ND	ND
NBS13	2	8/27/19	ND	ND

Station	Week	Date	SCCWRP HF183 [copies/100 mL]	OCPHL HF183 [copies/100 mL]
NBS1	4	9/10/19	ND	ND
NBS2	4	9/10/19	ND	ND
NBS3	4	9/12/19	ND	ND
NBS4	4	9/11/19	ND	ND
NBS5	4	9/12/19	ND	ND
NBS6	4	9/12/19	ND	ND
NBS7	4	9/11/19	ND	ND
NBS8	4	9/12/19	ND	ND
NBS9	4	9/11/19	ND	ND
NBS11	4	9/11/19	ND	ND
NBS12	4	9/10/19	ND	ND
NBS13	4	9/10/19	**88**	**118**
NBS1	6	9/24/19	ND	ND
NBS2	6	9/24/19	ND	ND
NBS3	6	9/26/19	ND	ND
NBS4	6	9/25/19	ND	ND
NBS5	6	9/26/19	ND	ND
NBS6	6	9/26/19	**56**	ND
NBS7	6	9/25/19	ND	ND
NBS8	6	9/26/19	ND	ND
NBS9	6	9/25/19	ND	ND
NBS11	6	9/25/19	ND	ND
NBS12	6	9/24/19	ND	ND
NBS13	6	9/24/19	ND	ND