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NRRI TECHNICAL REPORT

Bacterial Source Identification Study of Agate Bay, Two Harbors in 2020

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Date: February 6, 2021

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Acknowledgement: This report was prepared by NRRI using Federal funds under award NA19NOS4190063 from the Coastal Zone Management Act of 1972, as amended, administered by the Office for Coastal Management, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce provided to the Minnesota Department of Natural Resources (DNR) for Minnesota's Lake Superior Coastal Program. The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of NOAA's Office of Coastal Management, the U.S. Department of Commerce, or the Minnesota DNR.

Scope of the Study

Fecal contamination is one of the leading causes of impairments for streams, rivers and estuaries across the United States (USEPA 2009). Each year, fecal contamination of recreational waterways results in approximately 90 million illnesses and economic costs of \$2.2- \$3.7 billion nationwide (DeFlorio-Barker et al. 2018). Those values do not account for tourism lost due to recreational beach closures and advisories. Common sources of fecal inputs include agricultural runoff, wildlife deposition, inadequate wastewater treatment, aging sewer infrastructure and faulty septic systems (Johnson et al. 2004, Okabe et al. 2007).

Water quality monitoring data indicate that water quality standards for recreational uses are not being attained in two recreational beaches in Two Harbors, Burlington Bay and Agate Bay Beach and Skunk Creek in Burlington Bay, based on exceedances of numeric criteria for *E. coli*, which is a common fecal indicator bacterium. The applicable water quality standards for *E. coli* are described in amendments to Minnesota's Rule 7050. There are two standards established by the rule for *E. coli*: the single sample water quality standard of 235 most probable number (MPN)/100 milliliters (mL) and the geometric mean water quality standard of 126 MPN/100 mL. Traditional fecal indicator bacteria (FIB), like *Escherichia coli* (*E. coli*) and *Enterococci*, can denote the presence of fecal contamination, but cannot distinguish between sources (Feng et al. 2018).

In 2018-2019, a study was conducted to understand spatial and temporal patterns of *E. coli* levels in Skunk Creek watershed and Agate Bay Beach between sources of fecal contamination within the using culture-independent microbial source tracking methods. The study includes sites along the stream's longitudinal profile, different outfalls (e.g. wastewater effluent and stormwater drain) and beach. The study found that levels of *E. coli* were correlated with stormwater events, while human fecal sources appear to be site-specific and independent from storm events. Agate Bay storm drain was identified as a potential hot spot of human fecal contamination based on great concentration of the human molecular markers. In communication with the City of Two Harbors, the contamination may result from a sewer leak from damaged sewer line to stormwater drain in Agate Bay. The City performed corrective actions on the damage sewer line this area in 2019.

In 2020, we have conducted a follow-up study to monitor bacterial water quality and assess the effectiveness of the corrective action on *E. coli* levels and human fecal contamination in Agate Bay. Water samples were collected from three sites: stormwater drain, wastewater outfall, and beach during June-August. The report includes *E. coli* levels with ancillary water quality parameters and quantification of biomarkers designed for human and waterfowl fecal contamination.

Methods

Sample collection

Water samples were collected by LCSWCD staff from three Agate Bay sites in the summer of 2020 (Figure 1).



Figure 1. Map of the sampling locations in Two Harbors, MN (left). Blue dots represent points in the Skunk Creek watershed while orange dots represent those in Agate Bay. All Sites were sampled during 2018-2019, however only the Agate Bay sites were sampled in 2020. Satellite image of Agate Bay sampling locations (right).

Sample processing

Water samples were immediately transported to the Natural Resources Research Institute (NRRRI) where they were processed and prepared for molecular analysis. 50ml aliquots of raw sample were used for turbidity analysis performed with a Hach 2100 Series Laboratory Turbidimeter. Two-liter water samples were pre-filtered through a 70 micron mesh to remove large particles and debris. The remaining water (~2 L) was then filtered through 5 μ m and 0.22 μ m nitrocellulose membranes (47 mm diameter) using a vacuum filtration system to collect bacterial cells for molecular analysis. Filtered sample volumes were recorded and filters were placed in sterile Whirlpak bags, and frozen at -20°C until DNA extraction. Filter heads and funnels were cleaned between runs using a 10% bleach bath. DNA extraction was performed using the PowerSoil DNA isolation kit and the concentration of the extracted DNA was quantified using a Qubit fluorometer.

Biomarker analysis for fecal sources using qPCR

Quantitative polymerase chain reaction (qPCR) analysis was used to determine the contribution of human and waterfowl fecal sources of *E. Coli* to Skunk Creek and Lake Superior. Table 1 summarizes host-specific biomarkers (primers and probes) of human and avian sources for the qPCR assay. Nine standards (10x serial dilutions) were used to construct each qPCR standard curve and run alongside the samples.

Table 1. Primers, probes, and run specifications for the qPCR biomarkers.

Marker	Forward Primer	Reverse Primer	Probe	Size	Annealing	Ref.
Lachno_3	5-CAACGCGAAGA ACCTTACCAAA- 3	5-CCCAGAGTGCCAC CTTAAAT-3	N/A	186bps	64°C	Feng et al. 2018
HB	5-ATCATGAGTTCA CATGTCCG-3	5-CGTTACCCCGCCTA CTATCTAATG-3	6-FAM- TCCGGTAGACGATGGGA TGC GTT-TAMRA	86 bp	60°C	Feng et al. 2018
GFD	TCGGCTGAGCAC TCTAGGG	GCGTCTCTTTGTAC ATCCCA	N/A	123 bp	56°C	Green et al. 2012
16S	5'- TCCTACGGGAGG CAGCAGT-3'	5' GGA CTA CCA GGG TAT CTA ATC CTG TT 3'	5' CGT ATT ACC GCG GCT GCT GGC AC 3'	466 bp	60°C	Nadkarni et al. 2020

Results

Abundance of culturable E. coli

The overall average abundance of culturable *E. coli* for Agate Bay was comparable between 2019 and 2020 sampling years (Figure 2, $t = 0.80302$, $df = 22.944$, $p\text{-value} = 0.43$) despite decreasing trends. None of the individual sites in Agate Bay shows any significant changes in culturable *E. coli* between 2019 and 2020 (Figure 3). As with 2019, the abundance of culturable *E. coli* was slightly higher after storm events; however unlike 2019, the difference was not significant (Figure 4, $t = -0.84123$, $df = 4.0235$, $p\text{-value} = 0.4473$).

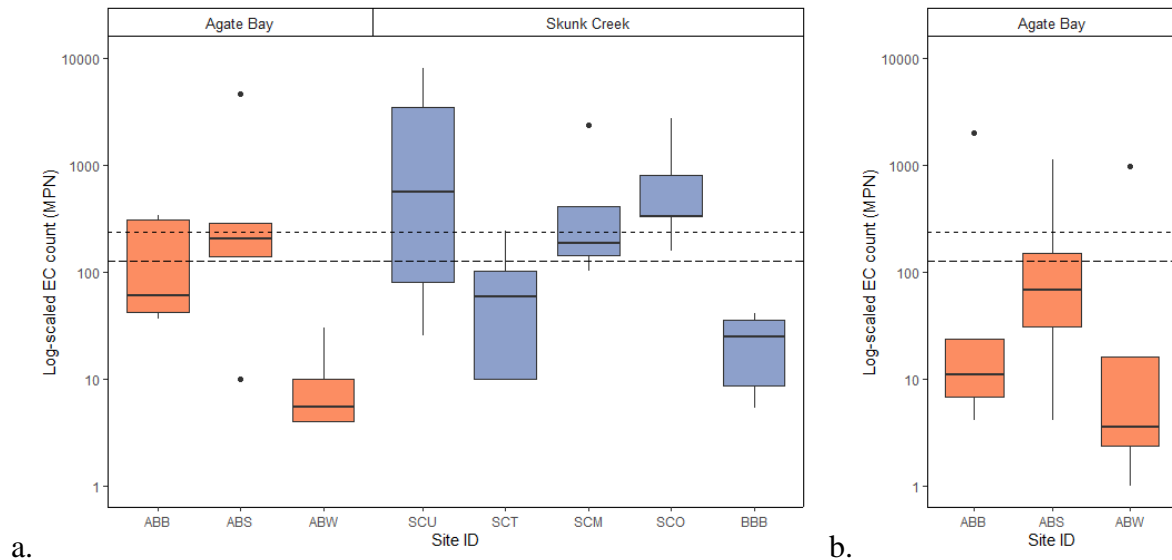


Figure 2. Abundance of culturable *E. coli* for sampling locations in (a.) 2019 and in (b.) summer 2020. The top dashed line is the single-sample threshold (235 MPN/100 ml) and the bottom dashed line is the geometric mean threshold (126 MPN/100 ml from at least five samples within

a 30-day period). There were four values for each site, with the exception of ABB which had five collection dates.

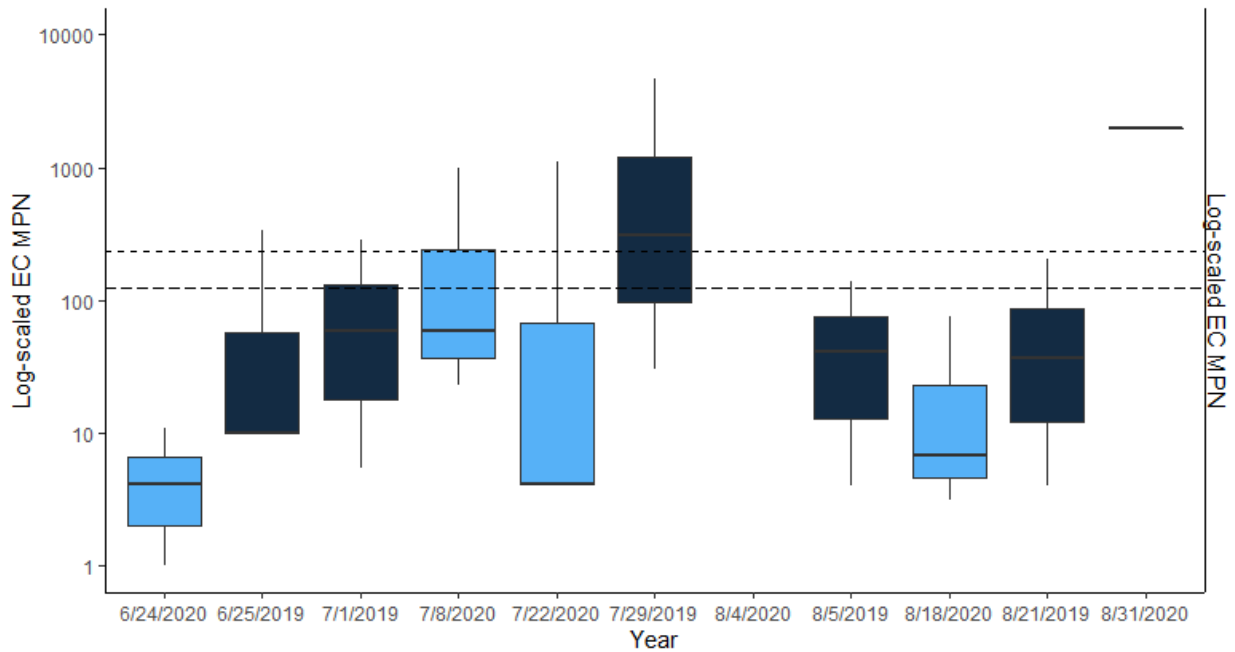


Figure 3. Abundance of culturable *E. coli* for Agate Bay Sites (ABB, ABS, ABW) organized by sampling date within the season. Dark blue boxes are from 2019 and light blue boxes are from 2020.

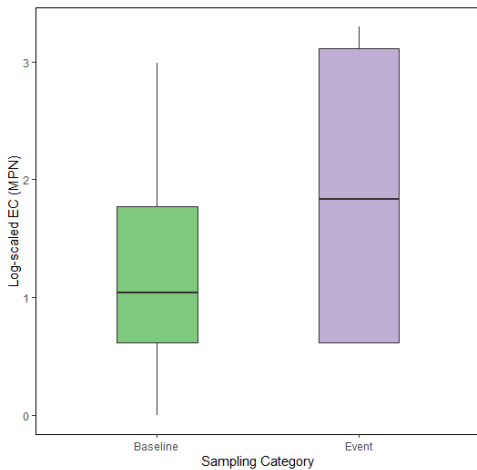


Figure 4. Log-scaled culturable *E. coli* (MPN/100 mL) by sampling category.

Concentration of Human Biomarkers

The concentration of the human biomarker HB in samples collected in 2020 was similar to those in 2019 (Figure 5, $t = -0.29028$, $df = 27.902$, $p\text{-value} = 0.77$). At ABS site where sewer line disconnection was corrected, HB concentration decreased in comparison that in 2019 but the change was not statistically significant ($t = 0.85195$, $df = 7.9756$, $p\text{-value} = 0.4191$). The same trend was seen for the second human biomarker, *Lachno3*, with an insignificant increase Agate Bay's overall concentration (Figure 6, $t = -0.57228$, $df = 26.234$, $p\text{-value} = 0.572$), but insignificant decrease for ABS ($t = 0.82579$, $df = 7.8278$, $p\text{-value} = 0.4334$).

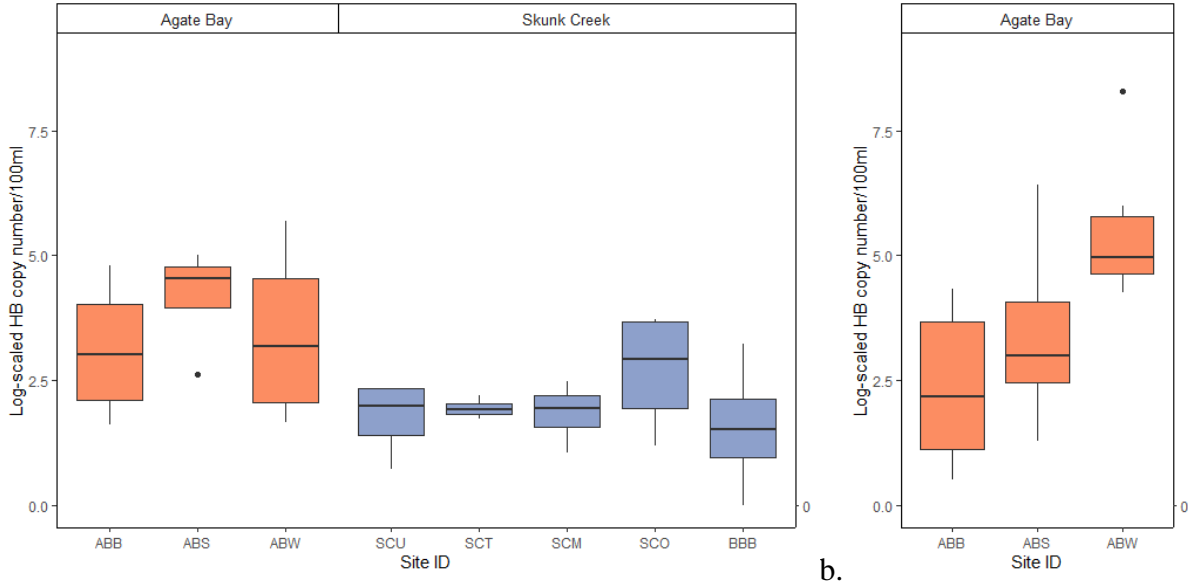


Figure 5. Log-scaled abundance of the human biomarker HB in (a.) 2019 and (b.) 2020 sampling seasons.

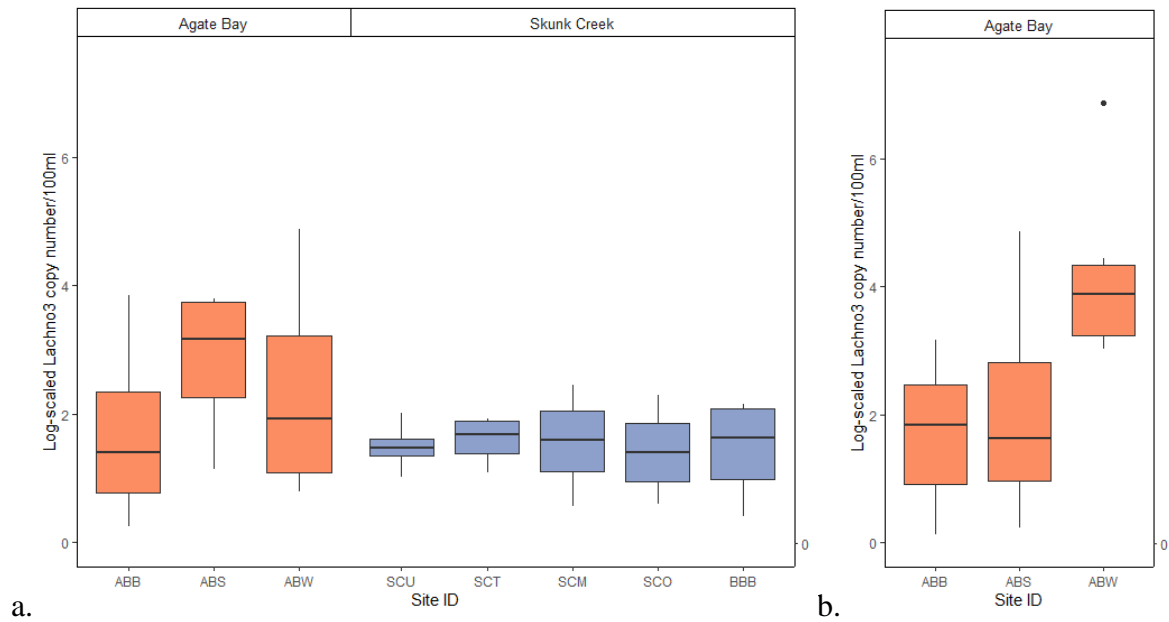


Figure 6. Log-scaled abundance of the human biomarker Lachno3 (gene copies/100ml) during the (a.) 2019 and (b.) 2020 sampling seasons.

Concentration of Avian Biomarkers

There was no change observed for the abundance of avian biomarker, GFD in water samples collected from Agate Bay between 2019 and 2020 ($t = -1.1855$, $df = 27.505$, $p\text{-value} = 0.246$). This is expected as the corrective action was not related with waterfowls.

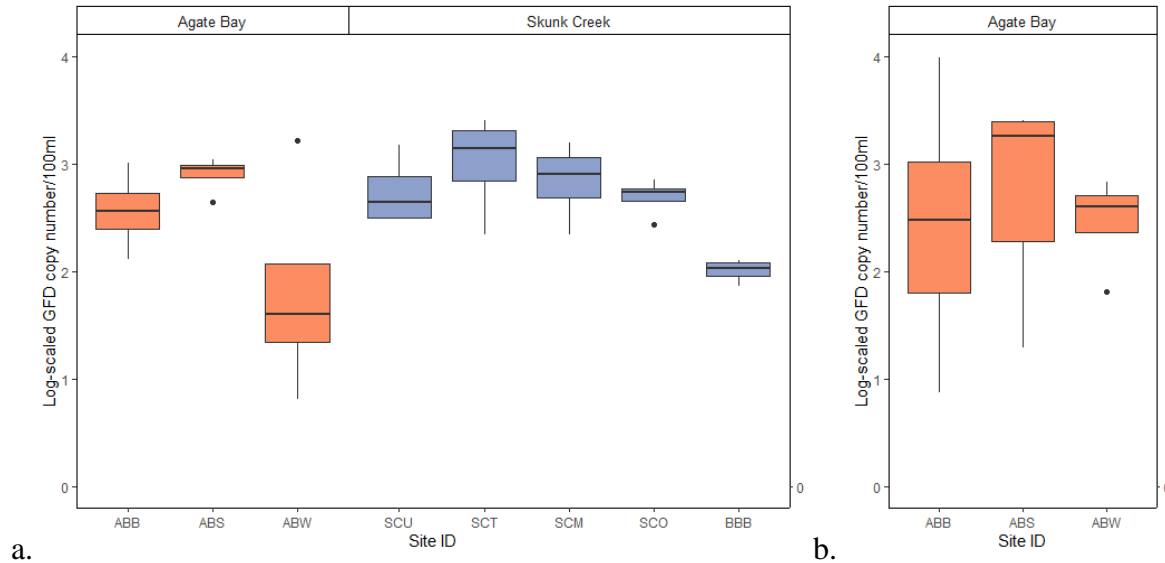


Figure 7. Log-scaled abundance of the avian biomarker GFD (gene copies/100ml) during the (a.) 2019 and (b.) 2020 sampling seasons.

Figure 8 summarizes all results of 2019 and 2020 for ABS where corrective actions on sewer line disconnection were conducted. Mean values of culturable *E. coli* and human biomarkers, HB and Lachno3 in 2020 (post-corrective actions) except for avian biomarker, GFD appear to be lower than in 2019 (pre-corrective actions) but the differences were not statistically significant ($p\text{ value} > 0.5$).

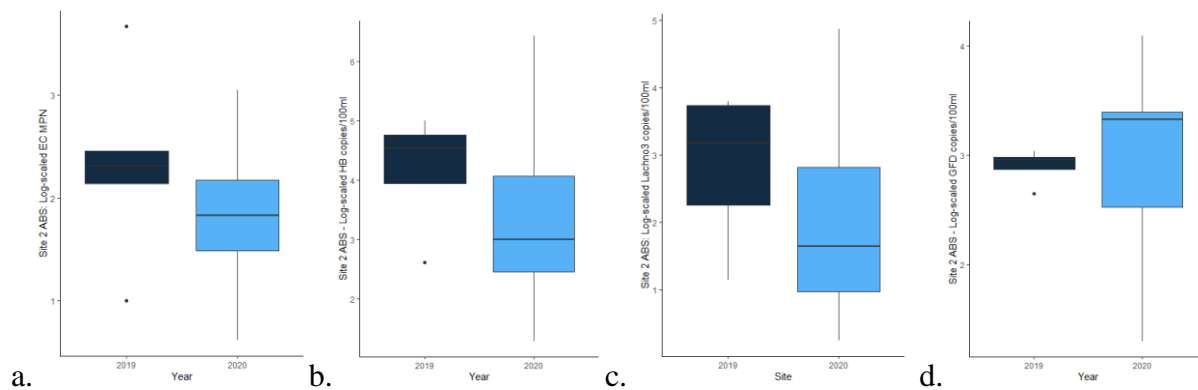


Figure 8. Log-scaled average overall abundance of (a) culturable *E. coli*, (b) human biomarker HB, (c) human biomarker Lachno3, and (d) avian biomarker GFD between the 2019 and 2020 for Agate Bay Stormdrain Outfall (ABS) where corrective actions were conducted.

Summary

Overall, the concentration of *E. coli* and human fecal biomarkers in storm drain outfall, wastewater, and beach in Agate Bay in 2020 were comparable to those in 2019. The corrective actions on sewer line disconnection appear to improve *E. coli* and human fecal biomarkers in the storm drain outfall, but the improvement was not significant. There may be other sewer line influences in the area as sewer infrastructures are aged, suggesting additional inspection. Additionally, it is recommendable to install a biofiltration system to treat stormwater and prevent the introduction of waterfowl waste and suspended solids to Lake Superior.

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