Illinois-Indiana Sea Grant (IISG) Research Project Annual Report for Year____2019_____

Title of Project: Failing Septic Systems as a Potential Source of Human Pathogens to Beach Sand

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1. **Table 1. Key results for IISG specialists and communicators.**

List all presentations given by project investigators and partners (include PDF copies of poster or slides)	**Villa CM and Fisher JC. Failing Septic Systems as a Potential Source of Human Pathogens to Beach Sand. Presentation to GLISTEN, Aug 13, 2019.** Presentation by research student funded by the project. [attached]
List all publications in review or in press authored by project investigators and partners (include extension publications and popular articles as well as peer-reviewed articles)	**NA**
List all students and post-docs who have helped with this work even if not directly supported with funding (denote those who are directly funded with a *)	**Catherine Villa* -** IU Northwest, Biology Department, BS Biology 2019, IISG summer 2019. **Ayesha Khan* -** IU Northwest, Biology Department, BS Biology 2019, IISG fall 2019. **Mark Mason** - IU Northwest, Biology Department, BS Biology 2020. **Ben Macuga** - IU Northwest, Geology Department, BS Environmental Studies 2021.
List social media handles/pages of project partners	**Instagram: @fishermicrolab** **Twitter: @dr_jen**

2. **Progress Toward Objectives:**

A. **Field sampling: complete.** Samples were collected from April 2019-September 2019 from water and sand at Long Beach. This sampling framework allowed "pre-bathing season" samples to be collected as a baseline, with additional samples obtained in wet and dry weather throughout the bathing season (Memorial Day to Labor Day). The only deviations from the sampling plan were that no fall 2018 samples were collected, as the grant had not officially started in Oct 2018 and an additional sample "site" of an outfall that discharged directly to the beach was added. An

unintended objective of this study was the observation of extreme beach erosion over the course of the summer. While not directly part of this work, erosion revealed seawalls in areas where they were previously covered by sand and raised the question of how seawalls impact the movement of septage outflows.

Figure 1 Stormwater outfall at Stop 24; discharges consistently in both wet and dry weather directly onto beach.

Results: A total of 34 water samples (including lake, stormwater outfall, and other observed liquid drainage to beach) and 41 sand samples were collected from Long Beach, IN, spanning over 1.5 km of beach along Lake Michigan. Site names were based on the “Stops” (trolley/bus stops along Lakeshore Drive), but specific GPS coordinates were recorded for all samples. Stop 20 was the westernmost site and Stop 28 was the easternmost site. Stop 24 is the public beach access that has been officially “closed” due to erosion, but is still in use.

Figure 2 Evidence of beach erosion at Stop 25. Photo on left taken September 2, 2019; photo on right taken July 25, 2019. Prior to July 25th sampling date, seawall was completely covered by sand. Sand height reduced by >30 cm.

B. ***E. coli* analysis: complete.** *E. coli* concentrations were measure for all sand and water samples collected using the IDEXX Colilert® system, which also provides data on total coliforms. Field and laboratory replicates, blanks, and positive controls were used for QA/QC; all had appropriate results to support the validity and quality of data collected. Water samples were assayed. Sand samples were shaken with sterile phosphate buffered saline (100 g sand/900 mL PBS) to remove bacteria; rinse water was then processed as a water sample.

Results: *E. coli* concentrations in lake water never exceeded the state recreational water quality criterion of 235 MPN E. coli/100 mL. Ten sand samples exceeded this value (although no regulatory value currently exists for sand, with values ranging from 365-3873 MPN/100 mL and observed at multiple sites along the beach on different sampling days. This suggests that *E. coli* contamination of sand is present and widespread, but dynamic or transient and not consistently observed in any one location. The stormwater outfall that discharged directly onto the beach and was flowing in both wet and dry weather had high E. coli concentrations ONLY following rain events. Flow in dry weather was ~20 MPN/100 mL, while flow in wet weather was >1000 MPN/100 mL on average. As there are not sewer lines in this area, the contamination present in the outfall may be of animal origin from runoff or from septage intrusion.

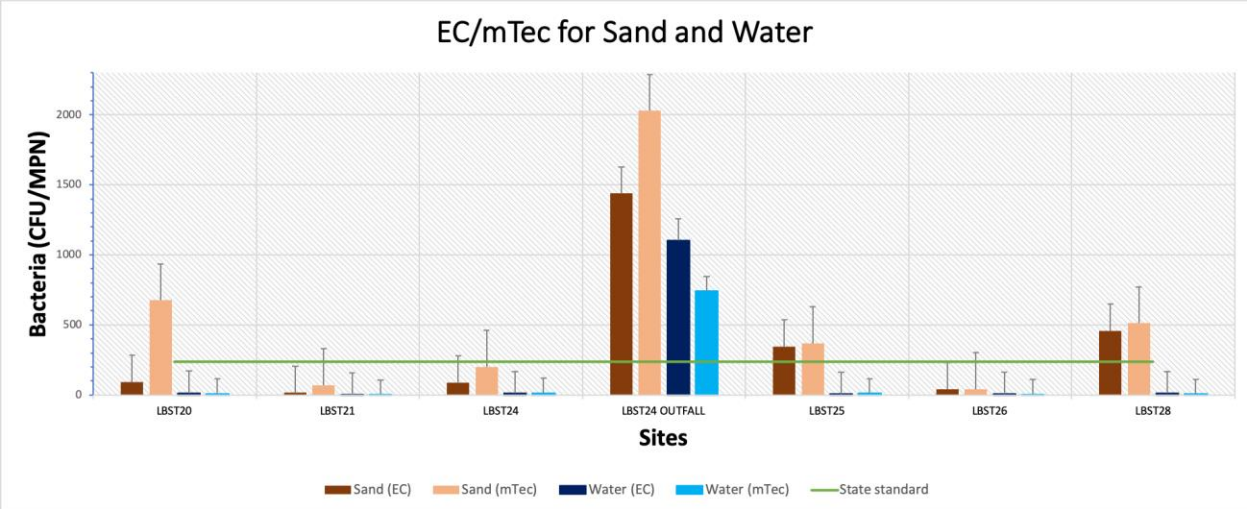

Figure 3 Average E. coli concentrations (Colilert and mTEC) by site for Long Beach sand and water.

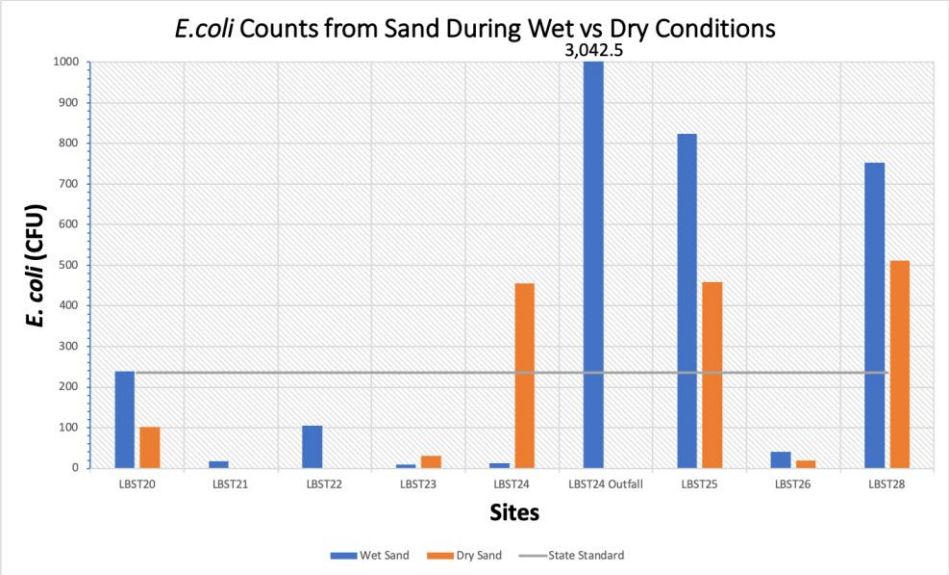

Figure 4 E.coli concentrations in beach sand and stormwater under wet and dry sampling conditions.

C. **DNA collection and extraction: collection complete; most DNA extracted.** DNA samples for water and sand rinse water were preserved by membrane filtration onto 0.22 μm Millipore filters and storage at -80 °C until DNA extraction. Sand samples were portioned into 50 mL tubes and frozen directly. DNA samples from filters were extracted with a Qiagen DNA PowerSoil kit for the first round of extractions. Duplicate samples are in the process of extraction using two additional types of kits – the PowerWater Kit for DNA filters and the DNA PowerMax Soil kit for up to 10 g of sand. To address the comments of one of the proposal reviewers, an experiment

was conducted to differentiate live and dead cells propidium monoazide (PMA) on DNA filters for subset of samples. PMA binds to "free" DNA in compromised/lysed cells and will not amplify in subsequent analyses. This method will help to distinguish live or viable cells present in sand when qPCR analysis and sequencing are done.

D. **Quantitative PCR analysis for Human Bacteroides marker: priority samples complete, reruns and a few additional samples to be analyzed.** The Human Bacteroides assay was developed by the USEPA to serve as an indicator of human fecal pollution. This method was used to analyze DNA from sand and outfall samples that exceeded 235 E. coli/100 g sand or 100 mL water. A few "negative control" sand samples that had low *E. coli* concentrations were also analyzed to validate the correlation between *E. coli* and HB as well as any artefacts of the method (false positives). Reruns with diluted samples are in progress to confirm that negative results are not a result of inhibition.

   Results: Very few samples analyzed for the HB marker were positive, and most of the positive samples were from the outfall or sand near the outfall. However, Site 25 and Site 20 both had 2 positive samples. The outfall samples were >10 times higher than the sand samples. This suggests that little direct septage is reaching the beach sites, but that septage may be collected along with stormwater and deposited on the beach/lake.

E. **DNA Sequencing and Bioinformatic Analysis: DNA sequencing in progress.**

F. **QMRA model: not started; awaiting input from DNA sequencing analysis.**

The overall results of the project thus far indicate 1) sporadic fecal pollution for beach sands in Long Beach, IN and 2) low level, if any, contributions from septic systems to adjacent beach sands. Erosion of beach sands over the course of the summer revealed seawalls previously buried at multiple sampling sites. These seawalls likely play a role in the retention of septage. The major source of human fecal pollution to Long Beach beaches comes from the stormwater outfall at Stop 24. Sequencing of samples with *E. coli* concentrations >235 MPN/100 mL will help determine if the human fecal contamination also carries potential bacterial pathogens.

3. Outputs and Outcomes Not Listed in Table 1:

NA

Reports are due January 31 of each calendar year, regardless of when the project started. Graphs, figures and/or photos should be embedded in your text. We may wish to include these items in IISG publications with the appropriate credits.