TWRI Application

1. **Title:** Source delineation of bacterial contamination in Galveston, TX recreational waters
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    d. Masters Student – expected graduation May 2023, started fall 2021
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4. **Program:** TWRI Mills Scholarship
5. **Previously received scholarship**: No
6. **New or ongoing research:** Ongoing research is funded by TGLO and the Mills Scholarship would cover tuition and fees. The current funds provide samples from February- August of 2022 and the salary of one graduate student. The set end date of the project is May 2023. The project is currently in the early stages of sampling where samples with counts above the acceptable limit. Once samples are selected, they will be sent to Texas A&M College Station for further analysis.
7. **Focus Categories**: Water Quality (WQL), Wastewater (WW)
8. **Research Category**: Water Quality
9. **Keywords:** Microbial source tracking, *Enterococci*, Fecal Pollution
10. **Congressional District:** 17th Congressional District of Texas

11. Abstract:

Fecal contamination impacts water quality along the coast of Texas by threatening ecosystem services and human health. The area of Galveston, Texas is particularly vulnerable to fecal contamination and has been identified by the Texas Beach Watch as a hotspot for bacterial pollution. Quantitative polymerase chain reaction (qPCR) for fecal indicator bacterium will be performed on samples from TGLO specified sites with high pathogenic counts to isolate for human, dog, or seagull sources. This information can then be compared with sewer systems and on-site sewer facilities to determine potential sources and influence management decisions with the goal of improving water quality.

12. Description:

Statement of critical regional or state water problem

Coastal water quality standards were set by the EPA as 104 MPN enterococci per 100mL (USEPA 2012) as part of the Beaches Environmental Assessment and Coastal Health (BEACH) act (USEPA 2000) with the goal of protecting human health. *Enterococcus* is a genus of bacterium that is typically not harmful to humans, but its presence is correlated with other fecal

indicator organisms (Boehm & Sassoubre 2014). A long-term study of 66 Texas beaches revealed that 65 of these beaches within the study exceeded the EPA limit at least once, and 19 beaches exceeded the upper limit of detection for the Enterolert test (>24,196 MPN/100mL) at least once (Powers et al., 2021). The study also found that enterococci counts increased over time and were correlated with population increases and sea level rise.

Galveston is a community along the Texas coast that has been identified by this study (Powers et al., 2021) to be a hotspot for microbial contamination. Exposure to microbial contamination can result in sickness and cause economic loss from costs of treatment and lost productivity (DeFlorio-Barker et al., 2018). Research is needed to examine potential sources of fecal pollution to advise management decisions in the goal to reduce the risks of illness from pathogenic exposure in Galveston.

Statement of expected results or benefits

Based on usage, the most likely sources of enterococci are humans (including onsite wastewater systems), gulls, and dogs. We expect that samples with large enterococci counts to originate from primarily human sources and that there will be higher counts during peak beach seasons. The entire sampling period is from February 2022 through August 2022. The analysis will help to create more informed management decisions through distinguishing sources and identifying problems in infrastructure. The successful completion of this study models the relationship between universities and organizations to promote similar studies in other regions highly affected by microbial contamination in Texas and along the gulf coast.

Nature, scope, and objectives of the research, including a timeline of activities

Potential drivers of pollution will be determined by assessing the density of on-site sewer facilities (OSSF), sewer overflows, potential connectivity between infrastructure and surface water pollution, stormwater and wastewater treatment plant effluent and changes in recreational beach attendance. Geostatistical analysis will be performed to identity potential clustering or spatial patterns. Water samples chosen by Texas Beach Watch will be sent to Texas A&M College Station for MST analysis.

Date	**Task**
February – August 2022	Sample collection and selection of samples with enterococci >104MPN/100mL
September 30th 2022	Detailed LIDAR micro-watershed map complete to identify OSSF and wastewater and treatment plant outfalls
March 31st 2023	qPCR analysis of 100 samples
April 30th 2023	Statistical outputs of significant relationships + geostatistical analysis

Methods, procedures and facilities.

Water samples chosen by Texas Beach Watch, with enterococci counts >104 MPN/100 mL, will be filtered and frozen by a third-party lab and later sent to Texas A&M College Station for MST analysis. Samples will be screened using qPCR for the human HF183 marker using the EPA protocol 1696 (USEPA 2019 & Layton et al., 2013). In total 100 samples will be sent from Galveston for qPCR analysis. A selected 20 samples will additionally be screened for the dog and seagull markers DogBact and LeeSeaGull assay (Dick et al., 2005 & Lee et al., 2013).

OSSF data has been collected through the Texas A&M Bioagricultural Engineering department in an OSSF inventory completed in 2020. This inventory is based on permitted OSSFs within the Galveston County boundary. Not included in the inventory are parcels larger than 10 acres that do not require an OSSF permit and facilities generating more than 5000 gallons per day. LIDAR micro-watershed maps will be developed using ArcGIS that include wastewater treatment plant outfalls and OSSFs compared with enterococci abundance with the goal of determining any statistical relationships. Additionally, enterococci data will be compared with beach attendance to identify any space-time patterns and clustering.

13. Related Research:

Microbial source tracking (MST) is a molecular technique used to detect, distinguish, and quantify different sources of fecal contamination with the use of fecal indicator bacterium. *Escherichia coli (E. coli)* is a common indicator bacterium used for most sources, however the bacterium *Catellicoccus marimammalium* is targeted in seagulls due to its abundance in gull feces. Previous research has identified the markers HF183, DogBact and the LeeSeaGull assay as both specific and sensitive in environmental samples for human, dog, and gull respectively (Layton et al., 2013, Sinigalliano et al., 2010, Lee et al., 2013). Similar studies have been done to identify sources within recreational waters using qPCR, (McKee et al., 2020 & Gyawali et al., 2020) however, little research has incorporated a geographic analysis within the study. This additional geographic analysis compares sewer systems and OSSF with MST data to identify significant spatial patterns and relationships. This unique study bridges the gap between science and management with the goal of using the obtained data to influence management decisions and improve water quality for Galveston.

14. **Training potential:** 1 graduate student for qPCR and geostatistical analysis, twelve undergraduate students will also have the opportunity to learn about this project and methods, including hands-on water sampling and lab analysis as part of the 2022 TAMU Water Quality and Reuse REEU program.
15. **Intended career path:** I aspire to continue my career in environmental microbiology where I can combine my love for the environment with my passion to study microbial organisms. I hope to continue to bridge the gap between science and management through applying my degree and experiences to protect natural resources while serving the community.