

Evaluating Human Health Risks in Little Bay Final Report

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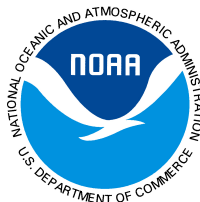
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Executive Summary

Fecal pollution is a leading cause of water quality impairments in coastal Texas. Water quality in marine environments is currently assessed through the measurement of enterococci as a proxy for fecal waste; however, enterococci do not specify the source(s) of fecal pollution or the health risks associated with it. These shortcomings for recreational water quality management can be addressed through an integrated framework utilizing microbial source tracking (MST) in combination with quantitative microbial risk assessment (QMRA), which has been recommended by the United States Environmental Protection Agency (USEPA) and well-supported by the peer-reviewed literature. Microbial source tracking (MST) is a robust scientific method that identifies the specific sources of fecal pollution within the environment through the quantification of host-associated molecular markers. MST data can then be utilized in human health risk modeling, such as QMRA, to estimate the human health risks associated with specific fecal sources and exposure scenarios. These results can then be used to prioritize management strategies that target the fecal sources of greatest health concern. The purpose of this study was to build upon a previous MST study by using the host-associated fecal pollution data to inform a QMRA. The main objectives include: 1) performing QMRA based on host-associated molecular marker concentrations, 2) performing QMRA based on enterococci concentrations, 3) comparing health risks associated with different sites (i.e., Tule Creek, Little Bay, Aransas Bay) and weather conditions (i.e., wet-loading and dry-loading), and 4) assessing potential relationships between the estimated health risks and environmental parameters.

Based on the host-associated marker concentrations, the median estimated health risks in this system were not elevated compared to the USEPA's gastrointestinal illness risk benchmark of 32

illnesses per 1,000 recreation events. However, the human marker (HF183) contributed the most to the overall health risks in this study, followed closely by the canine marker. Wet-loading events were associated with slightly higher risks than dry-loading events, although the risks associated with dry-loading events were more variable, likely due to sporadic but extremely high spikes of HF183 under dry-loading conditions. The health risks based on the MST data were highest in Little Bay, followed closely by Aransas Bay, then Tule Creek.

In contrast to the MST data, the enterococci data showed health risks elevated above the USEPA's risk benchmark, particularly in Tule Creek, where enterococci concentrations were the highest. The enterococci QMRA results also show elevated health risks for swimming in all three locations of the study. However, the QMRA results based on enterococci levels should be interpreted with caution, considering 1) the QMRA included the conservative assumption that enterococci originated from raw sewage rather than the treated effluent that Tule Creek receives and 2) the lack of correlation between HF183 and enterococci indicates that the enterococci likely did not originate from human fecal waste. It is important to note that the MST data, which provides a more accurate representation of fecal sources and potential pathogens, identified that health risks did not exceed the USEPA's risk benchmark. The results of this study provide strong evidence for implementing a combined MST/QMRA framework throughout coastal Texas. While enterococci are the current marine recreational water quality standard in the state, the results presented here highlight a clear discrepancy between enterococci levels and human health risks based on host-associated marker concentrations, which are a more accurate proxy for fecal pathogens present in fecal waste from humans and non-human sources.

Summary of Tables and Figures

Table 1 and Figures 1 and 2 show the relevant information and data collected from (Powers et al., 2021a) that was utilized in this study. Specifically, Table 1 shows the fecal targets and host-associated molecular markers that were detected in the previous MST study. Figure 1 shows a map of the sampling locations, including Tule Creek, Little Bay, and Aransas Bay. Figure 2 shows the host-associated marker concentrations quantified at each site and sampling date.

Tables 2 and 3 show the information that was utilized to inform the QMRA; Table 2 shows the data distributions and parameters for each fecal marker as well as enterococci, and Table 3 shows the dose-response model information. The median estimated health risks based on the QMRA are displayed in Table 4. Figure 3 depicts the health risks based on enterococci concentrations, including all data combined (Figure 3A), comparisons between wet-loading and dry-loading (Figure 3B), and comparisons between the different locations (Figure 3C). Figures 4-6 show the estimated health risks based on the host-associated marker concentrations. The sensitivity results (i.e., which variables and parameters had a stronger influence on the risk results) of the QMRAs are shown in Figures 7-12 for the host-associated markers and Figures 13-18 for enterococci. Figure 19 shows a principal component analysis (PCA) that depicts the relationship between estimated health risks and environmental variables.

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Microbial Source Tracking and Quantitative Microbial Risk Assessment for Evaluation of Health Risks in Little Bay, Texas

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1. Introduction

Fecal pollution is a leading cause of recreational water quality impairments in Texas coastal waters (TCEQ, 2024). Several factors contribute to this pollution and augment its loading into coastal watersheds, including urbanization, extreme weather events, and increasing temperatures. For instance, the population of Texas has increased by nearly 50% statewide in the past two decades (Texas A&M University Natural Resources Institute, 2017) and projections continue to show rapid growth in the upcoming decades through 2050 (Potter & Hoque, 2014). Elevated fecal bacteria levels in the environment have been correlated with this growing population, as well as rising sea levels (Powers et al., 2021b). Additional factors related to climate change, including increased severity and frequency of storm and flooding events, have also been linked with degrading water quality and increased fecal pollution (Carr et al., 2024).

Within coastal Texas, the Little Bay watershed offers a prime example of how water quality directly impacts the livelihoods and well-being of coastal communities. Little Bay is a popular recreational tourism destination in Rockport, Texas, nested within the larger Aransas Bay. It provides residents and tourists with ample opportunity for swimming, fishing, boating, and bird-watching. On an annual basis, over half a million people visit the beaches in Little Bay, particularly during the peak season of June through August (personal communication, Keith Barrett, Harbor Master and Executive Director, Aransas County Navigation District, May 7, 2024). When beach advisories are issued due to elevated enterococci levels, tourism drops drastically, adversely impacting the local economy. An estimated loss of \$2.34-6.42 million (adjusted from \$1.72-4.72 million in 2009) would occur if Rockport Beach (adjacent to Little Bay) were to close down for an entire season (Parsons et al., 2009).

Elevated enterococci levels are frequently reported in Little Bay, particularly after storm events. Such events can introduce stormwater runoff to the environment and lead to the resuspension of sediment, which is a known reservoir of enterococci (Manini et al., 2022). Another pathway in which enterococci can enter this watershed is through Tule Creek, a manmade riparian buffer that transports treated wastewater effluent before it enters Little Bay. Previous work has identified significantly higher levels of enterococci within Tule Creek compared to Little Bay, which in turn was higher than the larger Aransas Bay (Powers et al., 2021a). The same trend was not detected with other fecal markers, leading to the hypothesis that nutrients in Tule Creek were enriching autochthonous enterococci populations (Powers et al., 2021a). This hypothesis suggests that elevated enterococci levels in Little Bay could therefore be leading to beach advisories that are not reflective of recent fecal pollution. Furthermore, enterococci are not host-specific and numerous studies have called into question the utility of enterococci as indicators of fecal waste, particularly in regions that experience nonpoint-source pollution (Colford et al., 2007; Fleisher et al., 2010; Byappanahalli et al., 2012).

Microbial source tracking (MST) provides a methods advancement over monitoring traditional fecal indicator bacteria (i.e., enterococci), as it not only quantifies the pollution but also identifies the specific sources where the pollution originated (Boehm et al., 2013). Identifying the source(s) of pollution is the critical first step for developing mitigation strategies aimed at combating the pollution and reducing beach advisories. Once the sources have been identified, they can be prioritized for mitigation based on which source is contributing the greatest risk to human and environmental health (Zhang et al., 2019). In many cases, the pollutant of greatest concern is

often human fecal waste, as it is known to harbor microorganisms pathogenic to humans. However, other fecal pollution sources, such as domestic animals, natural wildlife, and agricultural livestock, can also be of significant health concern, with studies showing high levels of zoonotic pathogens with varying levels of pathogenicity present in fecal waste from these non-human sources (Dufour & Bartram, 2012).

Human health risk modeling, such as with quantitative microbial risk assessment (QMRA), can utilize MST data (i.e., host-associated fecal pollution data) to estimate the health risks associated with specific fecal sources and exposure scenarios, therefore prioritizing management strategies targeting the fecal sources of greatest health concern (Zhang et al., 2019). QMRA is a mathematical modeling framework that consists of four components - hazard identification, exposure assessment, dose-response, and risk characterization - that can incorporate environmental fecal pollution data in combination with risk-relevant exposure scenarios and pathogen concentrations to estimate specific health risks of concern (Haas, Rose & Gerba, 1999, 2014). Previous integrated MST-QMRA studies have guided the development of risk-based thresholds (RBTs) for host-associated fecal markers (Boehm & Soller, 2020) that correspond to the USEPA's risk benchmark of 32 gastrointestinal illnesses per 1,000 primary contact recreation events (USEPA, 2013). Furthermore, an emerging body of literature shows strong support for this combined MST-QMRA framework (Boehm, Soller & Shanks, 2015; Brown et al., 2017; Boehm, Graham & Jennings, 2018; Boehm & Soller, 2020; Gitter et al., 2023; Burch et al., 2024), and the USEPA has recommended assessing water quality based on site-specific conditions and source-specific contamination (USEPA, 2010). Thus, the aim of this work was to perform an integrated MST-QMRA to assess source-specific human health risks based on

previously quantified fecal markers (i.e., human, canine, gull) as well as enterococci in recreational waters in and around Little Bay (Texas, USA).

2. Methods

2.1 Quantitative Microbial Risk Assessment

2.1.1 Microbial Source Tracking

Data collected during a previous study (Powers et al., 2021a) were utilized in a QMRA to assess human health risks associated with swimming and other contact activities in the Little Bay watershed. A total of 42 water samples were collected from May through November of 2018 along an estuarine ecocline (i.e., Tule Creek, Little Bay, and Aransas Bay), shown in Figure 1. Twelve of the samples were collected after a wet-loading event, and the remaining 30 samples were collected under dry-loading conditions. Enterococci were quantified in each sample via the Enterolert method (IDEXX), and human, canine, and gull-associated fecal markers were quantified with a droplet digital PCR assay (methods described in Powers et al., 2021a). Table 1 shows the host-associated targets, primer sequences, and positive controls utilized in the previous MST study, and Figure 2 shows the concentrations for each sample, according to site and sampling date.

2.1.2 Distribution Fitting for Environmental Data

Best-fit distributions for the human, canine, and gull-associated marker datasets and the enterococci dataset were determined using R (v4.1.2) and RStudio (v2023.06+524). The potential distributions applied to the datasets included normal, lognormal, uniform, gamma, Weibull, beta, exponential, pareto, and triangular. Parameters for each distribution were fit to

each dataset using maximum likelihood estimation (MLE) with the fitdistrplus package (version 1.1-11) (Delignette-Muller & Dutang, 2015) and actuar package (version 3.3-2) (Goulet, 2008). Prior to distribution fitting, all non-detects or zero values within the datasets were replaced with 0.01 (one significant figure higher than 0) for consistent comparisons. Additionally, datasets were transformed to a range of [0,1] for the beta distribution fitting. Every distribution was visually compared and assessed using aic values as well as QQ plots, CDF plots, and PP plots from the fitdistrplus package (version 1.1-11) (Delignette-Muller & Dutang, 2015). Once the most appropriate distribution for each dataset was determined, the parameters were estimated using the EnvStats package (Millard & Kowarik, 2023). Table 2 includes the best-fit distributions and parameters used for each environmental dataset in the QMRA.

2.1.3 Human Health Risk Characterization

The QMRA model utilized environmental data (enterococci and fecal source markers) to estimate the risk of a gastrointestinal (GI) illness when exposed to reference pathogens while engaging in different types of recreation in Little Bay. Three QMRAs were conducted to estimate the risk of a GI illness for swimming, fishing, kayaking, boating, and jet-skiing across varying environmental conditions: 1) all environmental data combined, 2) wet-loading and dry-loading, and 3) across the ecocline (i.e., Tule Creek, Little Bay, and Aransas Bay). All health risks were compared to the USEPA risk benchmark for recreational water quality (0.032 or 32 illnesses per 1,000 recreational events) (USEPA, 2013) and the proposed risk-based thresholds for human marker, HF183 (525 copies/100mL), and gull marker, LeeSeaGull (20,000 copies/100 mL) (Boehm & Soller, 2020).

2.1.3.1 Hazard Identification and Exposure Assessment

Enterococci and host-associated fecal markers are indicators of fecal pollution, therefore warranting the use of reference pathogens to assess human health risks. These reference pathogens, while not directly detected in environmental waters, have been consistently found to co-occur with specific fecal sources and are pathogens of health concern in recreational waters (USEPA, 2010). While enterococci is a fecal indicator that is utilized as a regulatory standard by the USEPA for recreational marine waters, the bacteria is not host-specific and has been found to persist in the environment (Colford et al., 2007; Fleisher et al., 2010; Byappanahalli et al., 2012). Given the lack of correlation among the HF183 marker and enterococci in the samples, it was assumed that 5% of measured enterococci concentrations originated from a human fecal source (e.g., untreated sewage) and the following reference pathogens were utilized to assess human health risks: norovirus, adenovirus, *Cryptosporidium*, *Giardia*, *Campylobacter*, *Salmonella*, and *E. coli* O157:H7 (USEPA, 2010; Soller et al., 2010; Brown et al., 2017; Boehm & Soller, 2020; Gitter et al., 2023). Similarly, the reference pathogens used to estimate health risks associated with the HF183 marker were the same reference pathogens utilized to assess health risks from enterococci (assuming a portion of bacteria were from human fecal waste). For the non-human fecal sources, gull and canine waste, the reference pathogens *Salmonella* and *Campylobacter* have been utilized (Schoen & Ashbolt, 2010; Brown et al., 2017; Brown, Graham & Boehm, 2017). Several QMRA studies have estimated human health risks associated with exposure to gull fecal waste in recreational waters (Schoen & Ashbolt, 2010; Brown et al., 2017; Brown, Graham & Boehm, 2017). While human health risks associated with canine fecal waste have only recently been evaluated (Gitter et al., 2023), it is well known that canine-associated human campylobacteriosis is a health concern for pet owners (Gras et al., 2013; Campagnolo et al.,

2018; Acke, 2018) and the reference pathogen *Campylobacter* can be utilized to estimate potential human health risks.

To estimate a reference pathogen dose associated with individual recreational activities and under varying environmental conditions, the following equation was used (Soller et al., 2010; Brown et al., 2017; Gitter et al., 2023). The health endpoint for all reference pathogens evaluated is a gastrointestinal infection and illness.

$$dose_{RP}^S = \frac{C_{MST}}{F_{MST}^S \times 100} \times R_{RP}^S \times P_S \times V$$

Where S represents the fecal source as identified by the MST fecal markers (human, gull, canine); *RP* reflects the reference pathogen (*Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia*, *E. coli* O157:H7, adenovirus, norovirus); *MST* represents the fecal marker (HF183, LeeSeaGull, and DogBact); C_{MST} is the specific concentration of the MST marker when measured in the environment (gene copies 100mL⁻¹); F_{MST}^S is the concentration of each MST marker in each fecal source (gene copies mL⁻¹ or gene copies g⁻¹); R_{RP}^S is the concentration of the reference pathogen in each fecal source (n g⁻¹ or n L⁻¹); P_S is the pathogenicity of pathogens from a non-human fecal source; and V is the volume of water (mL) ingested per recreational activity (children swimming, adults swimming, fishing, kayaking, boating, and jet-skiing).

The QMRA required several different parameters to estimate a reference pathogen dose based upon host-associated markers (Table 3). Both host-associated marker and reference pathogen concentrations in source-relevant fecal waste were retrieved from the literature (Hurst, McClellan & Benton, 1988; Stampi et al., 1993; Lévesque, 2000; Lemarchand & Lebaron, 2003; Koivunen, Siitonen & Heinonen-Tanski, 2003; Garcia-Aljaro, Bonjoch & Blanch, 2005;

Harwood et al., 2005; Crockett, 2007; Shanks et al., 2010; Chaban, Ngeleka & Hill, 2010; Hewitt et al., 2011; Kitajima et al., 2014; Ervin et al., 2014; Yang et al., 2015; Nasser, 2016; Brown et al., 2017; Eftim et al., 2017; Schoen et al., 2017; Soller et al., 2017). Five different recreational activities involving varying levels of accidental ingestion of water were incorporated into the risk assessment. The following activities were evaluated: swimming (children and adults) and fishing, kayaking, boating, and jet skiing (all for adults only). It was assumed that incidental ingestion would occur upon exposure (such as through direct ingestion, hand-to-mouth transfer and secondary contact to surfaces contaminated with water) in this risk assessment. Swimming is considered a primary contact recreational activity due to the likelihood of immersion, while kayaking, boating, jet skiing, and fishing are considered secondary contact activities (Geosyntec, 2008; USEPA, 2013). Point estimate values were utilized to describe each recreational activity. Ingestion volumes for swimming were retrieved from a study that estimated an average ingestion volume based on the self-reported assessments of 68,000 participants in the United States (DeFlorio-Barker et al., 2018). Fishing and kayaking ingestion volumes were quantified through survey and urine analyses (Dorevitch et al., 2011; Schets, Schijven & De Roda Husman, 2011) while boating and jet skiing ingestion volumes were inferred from a literature review (Geosyntec, 2008).

Lastly, a fraction of pathogenicity was assigned to the gull and canine fecal sources to account for the differing levels of pathogen infectiousness from non-human fecal sources. The gull fecal source was assigned a fraction of pathogenicity that ranged from 0.01 to 0.4 (Fenlon, 1983; Schoen & Ashbolt, 2010) while the canine fecal source had a fraction of pathogenicity that ranged from 0.02 to 0.1 (Gras et al., 2013).

2.1.3.2 Dose-Response

Dose-response equations reflecting each reference pathogen's mathematical relationship between dose and infectivity are retrieved from the literature (Couch et al., 1969; Rose & Gerba, 1991; Medema et al., 1996; Eisenberg et al., 1996; Crabtree et al., 1997; Haas, Rose & Gerba, 1999; Teunis, Nagelkerke & Haas, 1999; USEPA, 2006; Teunis, Ogden & Strachan, 2008) (Table 4). For six of the reference pathogens evaluated, the dose-response equation was either exponential (*Cryptosporidium*, *Giardia*, and adenovirus) or Beta-Poisson (*Salmonella*, *Campylobacter*, and *E. coli* O157:H7). Norovirus was represented by the Fractional Poisson mathematical relationship, assuming full particle disaggregation (Messner, Berger & Nappier, 2014; Vergara, Rose & Gin, 2016; Van Abel et al., 2017). While several dose-response models exist for this virus, there still remains a lack of consensus of which model is most appropriate (Van Abel et al., 2017). However, the Fractional Poisson relationship provides a conservative assessment for the probability of infection. All dose-response relationships estimate the risk of a gastrointestinal infection.

2.1.3.3 Risk Characterization

The USEPA risk benchmark is a threshold assuming the risk of a gastrointestinal illness (U.S. EPA, 2013). To estimate the risk of illness from the risk of infection estimates, the probability of infection (P_{inf}) is multiplied by the morbidity ratio for each pathogen (Table 4) to yield the probability of illness (P_{ill}^S). For fecal sources that include the same reference pathogens (e.g., *Campylobacter* and *Salmonella*), the dose ($dose_{RP}^S$) is estimated independently and then summed together to estimate the total pathogen dose. The cumulative risk of illness associated with exposure to multiple fecal sources were estimated assuming statistically independent exposures (Regli et al., 1991; Soller et al., 2010).

$$P_{ill}^S = 1 - \prod_{RP} (1 - P_{ill,RP}^S)$$

When available, input parameters described by statistical distributions that incorporated variability were included in the QMRA model. Monte Carlo simulations (10,000 iterations) were conducted using the Crystal Ball Pro[®] software (version 11.1.3.0.0) to estimate the probabilities of illness associated with each reference pathogen and fecal source.

2.2 Comparison of Human Health Risks

To assess the impact of location (Tule Creek vs. Little Bay vs. Aransas Bay) and weather conditions (wet-loading vs. dry-loading) on the estimated human health risks, data were subset and re-analyzed based on these categories. The number of water samples included in each subgroup are as follows: Tule Creek (n=7), Little Bay (n=28), Aransas Bay (n=14), wet-loading (n=12), dry-loading (n=30). Best-fit data distributions were determined for each data subset following the methods in section 2.1.2. QMRA models were re-run for each data subset following the methods listed in section 2.1.3.

2.3 Test Utility of Environmental Data

To test the utility of environmental data as an indicator for human health risks, the relationships between point estimates of human health risks and the continuous environmental parameters (e.g., DO, salinity, water temperature, rainfall) were assessed. First, the concentrations of the human, canine, and gull markers were utilized to calculate point estimate health risks for each sample (n=49). The median value of each input parameter utilized in the QMRA model

described above (e.g., marker and reference pathogen concentrations in each fecal source, fraction of pathogenic species) were employed (Table 3).

A principal component analysis (PCA) was performed to visualize relationships between estimated health risks and environmental data. PCA is a statistical method that reduces dimensionality within large datasets that have several independent variables by transforming the dataset into linear representations through principal components. The PCA was performed using R (version 4.1.2) and RStudio (version 2023.06.1+524) with the stats base package, ggplot2 package (version 3.4.2), and ggfortify package (version 0.4.14) (Wickham, 2016; Horikoshi & Tang, 2023). Principal components were generated with the prcomp command, and the PCA was visualized with the autoplot command.

2.4 Dissemination of Project Findings

2.4.1 Community Stakeholder Meetings

Project findings were shared with the local communities of Rockport, TX and Fulton, TX through a series of two public meetings during the project period. During the first meeting on December 12th, 2023, the QMRA approach and preliminary results were introduced to community stakeholders, and community input was sought in order to ensure the most relevant exposure scenarios were included in the analysis. Final project results were shared with community stakeholders during the second public meeting on May 7th, 2024. Both meetings were held at the Bay Education Center in Rockport and marketed through press releases in AgriLife Today, emails to interested community partners, and social media posts.

2.4.2 Additional Dissemination

Project findings were discussed with the Texas General Land Office (GLO) during a series of three online meetings on September 20th, 2023, February 22nd, 2024, and September 20th, 2024. Project data was shared with the GLO and made publicly accessible on the Zenodo online data repository (zenodo.org), which can be accessed with the following DOI: [10.5281/zenodo.13743679](https://doi.org/10.5281/zenodo.13743679) (Gitter et al., 2024). Findings will be further disseminated through this final report, which will be shared with the GLO and published on the Texas Water Resources Institute (TWRI)'s project website (littlebay.twri.tamu.edu). Findings may also be disseminated through future publications and conference presentations after the end of the project period.

2.5 Project Monitoring and Reporting

Quarterly progress reports were submitted to the GLO project manager by the 10th day of each quarter, beginning with April 10th, 2023. Six progress reports were submitted in total. Additional reporting included submission of a project website url, copies of press releases and website updates, notes and presentation slides from community stakeholder meetings, and notes from project meetings with the GLO. Technical milestones including data, tables, and figures were also reported to GLO during the course of the project.

3. Results and Discussion

3.1 Quantitative Microbial Risk Assessment

3.1.1 Human Health Risk Estimates

Distribution parameters for the environmental data utilized in the QMRA are described in Table 2. Estimated human health risks associated with recreation in environmental waters impacted by both enterococci and host-associated fecal markers (HF183, LeeSeaGull, and DogBact) are described in Figures 3-6 and Table 5. The same exposure scenarios were evaluated for both fecal indicators, yet human health risk estimates appeared to vary greatly depending on the indicator evaluated. Given the conservative assumptions utilized in the risk assessment, the median health risks were evaluated for each risk scenario and indicator and were compared to the USEPA risk threshold of 32 illnesses per 1,000 recreators (0.032).

For the QMRA model developed to assess human health risks associated with enterococci, it was assumed that only 5% of the enterococci measured in the environment were originating from a human fecal source (e.g., untreated sewage). Across all risk scenarios evaluated, which included all data combined, wet-loading, dry-loading, and the ecocline (Tule Creek, Little Bay, and Aransas Bay), elevated median human health risks were estimated for all datasets for children swimming (overall median risk for all data: 2.01×10^{-1} ; overall median risk for wet-loading: 2.62×10^{-1} ; overall median risk for dry-loading: 1.81×10^{-1} ; overall median risk for Tule Creek: 4.91×10^{-1} ; overall median risk for Little Bay: 1.55×10^{-1} ; overall median risk of illness for Aransas Bay: 1.13×10^{-1}) which included exceeding the USEPA risk threshold by one order of magnitude (Table 5, Figure 3). For adults, the estimated human health risks associated with all data, wet-loading, dry-loading, and Tule Creek exceeded the USEPA risk threshold (overall median risk

for all data: 1.15×10^{-1} ; overall median risk for wet-loading: 1.61×10^{-1} ; overall median risk for dry-loading: 1.00×10^{-1} ; overall median risk for Tule Creek: 4.12×10^{-1}), whereas the median risk of illness was within the same magnitude for Little Bay (8.42×10^{-2}) and Aransas Bay (5.83×10^{-2}). The median health risks for the secondary contact activities, fishing, jet-skiing, boating, and kayaking, across all risk scenarios and datasets did not exceed the USEPA risk benchmark, yet the upper 95th percentile risk estimates were within the same order of magnitude.

Human health risks associated with the host-associated fecal markers were identified to be at least two orders of magnitude less than the estimated health risks associated with enterococci for all environmental datasets (Table 5, Figures 4-6). The MST markers are host-specific, therefore permitting a more precise health risk estimation of the potential reference pathogens that may be present in the environment. The median human health risks for all risk scenarios never exceeded the USEPA risk threshold. Health risks associated with swimming for both adults and children had a greater risk of infection (overall median risk for all data: children 4.1×10^{-3} and adults 2.0×10^{-3} ; overall median risk for wet-loading: children 6.23×10^{-3} and adults 3.00×10^{-3} ; overall median risk for dry-loading: children 4.42×10^{-3} and adults 2.13×10^{-3} ; overall median risk for Tule Creek: children 3.29×10^{-3} and adults 1.59×10^{-3} ; overall median risk for Little Bay: children 5.29×10^{-3} and adult 2.55×10^{-3} ; overall median risk for Aransas Bay: children 3.98×10^{-3} and adult 1.92×10^{-3}) than for the secondary recreational activities (Table 5).

When comparing wet and dry-loading conditions, the human health risks were slightly greater during wet-loading for human, canine, and overall fecal sources. Although the health risks associated with the human marker during dry-loading conditions were lower than the health risks

associated with wet-loading, the dry-loading risks were more variable (Figure 5), likely due to the occurrence of periodic spikes in HF183 during dry weather. In contrast, the health risks were comparable across conditions for the gull fecal source (Figure 5). Across the ecocline, the greatest human health risks were identified to occur in Little Bay, followed by Aransas Bay and Tule Creek (Figure 6). Similarly, while none of the estimated median health risks (or even the 95th percentile risks) exceeded the USEPA risk threshold, the health risks associated with swimming were greater than the other recreational activities. Most importantly, while the overall health risk, which is an estimated health risk that encompasses all three fecal sources, represented the greatest risk for illness, the risk of illness from the human source and the canine source were comparable across all scenarios and datasets. Identifying that the canine fecal source may contribute a similar risk of illness as the human fecal source and a greater risk than the gull source informs the critical need for dog waste management and mitigating dog fecal pollution from upstream sources and environmental reservoirs.

3.1.2 Sensitivity Analysis

A sensitivity analysis was conducted to identify which of the QMRA model input parameters had the greatest effect on the human health risk estimates and therefore would be most critical for risk mitigation strategies. Using the rank correlation approach, we assessed all parameters that were defined by distributions (Tables 2 and 3). It was found that the QMRA model was most sensitive to the concentration of the human marker, HF183, in the environment, followed by parameters retrieved from the literature (e.g., concentration of the dog marker, DogBact, in feces, and concentration of *Campylobacter* in dog feces). The HF183 marker had a positive correlation indicating that an increase in the value of the marker yielded an increase in the estimated risk

output. In comparison, the assumed value utilized for the dog marker in feces had a negative correlation, indicating that as this parameter increased, risk output values decreased.

Additionally, the concentration of *Campylobacter* in dog feces had a positive correlation. These findings indicate that reducing HF183 contributions in a water body (e.g., mitigating human sources of fecal pollution through effective wastewater treatment and septic system maintenance) is critical for mitigating human health risks. It is also imperative to utilize the best available peer-reviewed data and assumptions given the influence that parameters can have on risk estimates. Results of the sensitivity analysis (for both enterococci and the host-associated markers) showing the factors most influential on the QMRA are shown in Figures 7-18.

3.2 Test Utility of Environmental Data

Figure 19 shows the results of the PCA, displaying relationships between environmental variables and estimated health risks. PC1 explained 25.93% of variability between the samples and was associated with variables related to weather conditions (i.e., days preceding rainfall, higher salinity, wind speed) and the human-associated fecal marker. PC2 explained 18.85% of variability between the samples and was associated with other environmental and water quality variables, including water/air temperature, DO, pH, and transparency. The PCA also highlights the inverse relationship between enterococci and the human marker as well as the lack of relationship between enterococci and the other host-associated markers. This point is further emphasized by the fact that enterococci were strongly associated with samples from Tule Creek (which experiences consistently elevated enterococci concentrations), whereas the human marker was more strongly associated with Little Bay and Aransas Bay.

The estimated human health risks (point estimates) were most strongly associated with HF183 and the number of days preceding rainfall. These results are particularly interesting, considering that HF183, which contributed to the greatest risks in the QMRA, was generally higher under wet-loading conditions (Powers et al., 2021a). However, the occasional yet massive spikes in HF183 detected during dry-loading conditions (shown in Figure 2) were outliers that strongly influenced the PCA. As reported previously, the elevated HF183 under wet-loading conditions could be attributed to stormwater runoff, whereas the large spikes in HF183 under dry-loading conditions could be attributed to occasional leaks in sanitary sewage collection systems or septic systems (Powers et al., 2021a).

3.3 Dissemination of Project Findings

3.3.1 Summary of December 2023 Community Stakeholder Meeting

The first community stakeholder meeting of the project was held on December 12th, 2023. The first half of the meeting introduced MST and QMRA and provided a summary of the project findings to date. The second half of the meeting followed a discussion format where participants asked questions about the QMRA approach and discussed related Little Bay water quality concerns. During the discussion, stakeholders expressed interest in including kayaking and jet skiing in the QMRA. The conversation also included topics such as economic and tourism impacts from bad water quality days, population growth near the bay and impacts to drainage, freshwater dilution and lack of circulation between Little Bay and Aransas Bay, and potential sources of bacteria loading and mitigation for Little Bay.

3.3.2 Summary of May 2024 Community Stakeholder Meeting

The second community stakeholder meeting was held on May 7, 2024. Final QMRA and health risk assessment results were presented to the group, followed by questions and discussion.

Topics of discussion for this meeting included questions about the project results, discussion about sediment in the ski basin being resuspended from boats and jet skis, and the status of installed oyster reefs in the bay. Several stakeholders expressed strong support for a follow-up study, especially one that would apply the MST-QMRA approach to the peak tourism season for Little Bay. Both meetings were attended by Rockport, Fulton, and Aransas County community leaders, small business owners, educators, other interested citizens, and representatives from the Aransas County Navigation District and Texas water agencies.

3.4 Project Monitoring and Reporting

All reports and deliverables for the project were submitted to GLO as outlined in the work plan and Section 2.5.

4. Conclusions

- Project findings provide strong evidence for implementing the MST/QMRA framework in coastal Texas to assess source-specific fecal pollution and associated health risks
- In this system, HF183 and wet-loading weather conditions were associated with higher estimated human health risks
- Human health risks associated with the human marker were greatest, followed by the canine marker

- Enterococci data showed elevated health risks, particularly in Tule Creek, but the lack of correlation between HF183 and enterococci rebut these results
- HF183 data, which provides a more accurate representation of pathogens, showed health risks are not elevated above the USEPA's risk benchmark

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Tables

Table 1. Host-associated molecular markers utilized in the previous MST study (Powers et al., 2021a).

Host target (bacterial target)	Primer name	Primer sequences	Primer reference	Accession number
Human (Bacteroidales)	HF183	Forward: 5'-ATCATGAGTTCACATGTCCG-3' Reverse: 5'-TACCCCGCCTACTATCTAATG-3'	(Bernhard & Field, 2000; Seurinck et al., 2005)	AY618281.1
Canine (Bacteroidales)	DogBact	Forward: 5'-CGCTTGTATGTACCGGTACG-3' Reverse: 5'-CAATCGGAGTTCTTCGTG-3'	(Dick et al., 2005; Sinigalliano et al., 2010)	AY695700.1
Gull (<i>Catellibacoccus</i>)	LeeSeaGull	Forward: 5'-AGGTGCTAATACCGCATAATACAGAG-3' Reverse: 5'-GCCGTTACCTCACCGTCTA-3'	(Lawson et al., 2006; Lu et al., 2008; Lee et al., 2012; Lee, Marion & Lee, 2013)	NR_042357.1

Table 2. Data distribution parameters utilized in QMRA.

Category	Bacterial target	Distribution	Parameter 1	Parameter 2	Parameter 3
All data combined	Enterococci	Weibull	Location: 10	Scale: 121.0580766	Shape: 0.4445485
	Human marker	Weibull	Location: 0	Scale: 28.3856664	Shape: 0.5118443
	Canine marker	Weibull	Location: 0	Scale: 71.6036012	Shape: 1.532525
	Gull marker	Gamma	Location: 7.78	Scale: 41.512596	Shape: 1.324979
Wet-loading	Enterococci	Weibull	Location: 10	Scale: 121.0580766	Shape: 0.4445485
	Human marker	Normal	Mean: 43.74091	SD: 25.36223	NA
	Canine marker	Uniform	Min: 33.34	Max: 120	NA
	Gull marker	Normal	Mean: 64.35500	SD: 35.95878	NA
Dry-loading	Enterococci	Weibull	Location: 10	Scale: 84.6191694	Shape: 0.6173407
	Human marker	Gamma	Location: 0	Scale: 163.5113737	Shape: 0.3567846
	Canine marker	Weibull	Location: 0	Scale: 65.965210	Shape: 1.326526
	Gull marker	Weibull	Location: 7.78	Scale: 1.044568	Shape: 52.265575
Tule Creek	Enterococci	Weibull	Location: 114.5	Scale: 1612.021969	Shape: 0.510815
	Human marker	Gamma	Location: 0	Scale: 97.3405732	Shape: 0.3074331
	Canine marker	Normal	Mean: 59.21286	SD: 25.05508	NA
	Gull marker	Uniform	Min: 7.78	Max: 78.89	NA

Little Bay	Enterococci	Weibull	Location: 10	Scale: 58.2825779	Shape: 0.7184689
	Human marker	Gamma	Location: 0	Scale: 163.7885211	Shape: 0.4076642
	Canine marker	Weibull	Location: 0	Scale: 72.831745	Shape: 1.345417
	Gull marker	Weibull	Location: 7.78	Scale: 68.045950	Shape: 1.208988
Aransas Bay	Enterococci	Weibull	Location: 10	Scale: 28.361959	Shape: 1.279504
	Human marker	Exponential	Rate: 0.03940887	NA	NA
	Canine marker	Uniform	Min: 24.45	Max: 113.34	NA
	Gull marker	Exponential	Rate: 0.03103112	NA	NA

Table 3. Parameters utilized in the QMRA.

Parameter	Units	Concentration	Source
Enterococci in human sewage	CFU L ⁻¹	(4.59, 5.50) ^d	(Montazeri et al., 2015)
HF183 in human sewage	gene copies mL ⁻¹	(5.21, 0.57) ^b	(Shanks et al., 2010)
LeeSeaGull in gull waste	gene copies g ⁻¹	(0.0,8.7,8.3) ^c	(Brown et al., 2017)
DogBact marker in dog waste	gene copies g ⁻¹	(5.0, 9.0) ^d	(Ervin et al., 2014)
Campylobacter in dog feces	organisms g ⁻¹	(3.0, 8.0) ^d	(Chaban, Ngeleka & Hill, 2010)
Campylobacter in gull feces	CFU g ⁻¹	(3.3, 6.0) ^d	(Lévesque, 2000)
Salmonella in gull feces	CFU g ⁻¹	(2.3, 9.0) ^d	(Lévesque, 2000)
Salmonella in sewage	CFU L ⁻¹	(0.5,5.0) ^d	(Lemarchand & Lebaron, 2003; Koivunen, Siitonen & Heinonen-Tanski, 2003)
Campylobacter in sewage	MPN L ⁻¹	(2.9, 4.6) ^d	(Stampi et al., 1993)
E. coli O157:H7 in sewage	CFU L ⁻¹	(-1.0, 3.3) ^{d,e}	(Garcia-Aljaro, Bonjoch & Blanch, 2005)
Cryptosporidium in sewage	oocysts L ⁻¹	(-0.52, 3.7) ^d	(Harwood et al., 2005; Crockett, 2007; Yang et al., 2015; Nasser, 2016; Schoen et al., 2017)
Giardia in sewage	cysts L ⁻¹	(0.51, 4.2) ^d	(Harwood et al., 2005; Kitajima et al., 2014)
Norovirus in sewage	gene copy L ⁻¹	(4.7, 1.5) ^b	(Eftim et al., 2017)
Adenovirus in sewage	IU L ⁻¹	(1.75, 3.84) ^d	(Hurst, McClellan & Benton, 1988; Hewitt et al., 2011; Soller et al., 2017)
Volume water ingested	Swimming adult (mL)	32.3 ^{f,j}	(DeFlorio-Barker et al., 2018)
	Swimming children (mL)	67.7 ^{f,k}	(DeFlorio-Barker et al., 2018)
	Kayaking (mL)	3.8 ^f	(Dorevitch et al., 2011; Schets, Schijven & De Roda Husman, 2011)
	Fishing (mL)	3.6 ^f	(Dorevitch et al., 2011; Schets, Schijven & De Roda Husman, 2011)
	Jet skiing (mL)	4.0 ^f	(Geosyntec, 2008)
	Power Boating (mL)	1.0 ^f	(Geosyntec, 2008)
Fraction of pathogenic species	Gull	0.01-0.4 ^g	(Fenlon, 1983; Schoen & Ashbolt, 2010)
	Sewage	1.0 ^f	Assumed
	Dog	0.02- 0.1 ^g	(Gras et al., 2013)

^aGamma distribution (location, scale, shape)

^bLog₁₀-normal distribution (mean, standard deviation)

^cLog₁₀-weibull distribution (location, scale, shape)

^dLog₁₀-uniform distribution (minimum, maximum)

^eThe lower range was not detected and -1 is used as a lower bound for *E. coli* O157:H7

^fPoint estimate

^gUniform distribution (minimum, maximum)

^jIngestion value for adults age 35 and over recreating in marine water

^kIngestion values for children age 6-12 recreating in marine water

Table 4. Dose-response equations for each reference pathogen.

Pathogen	Probability of Infection (P_{inf})	Morbidity Ratio	Reference
<i>Salmonella</i> spp.	$1-(1+\text{dose}/2884)^{-0.3126}$	0.17-0.4 ^a	(Haas, Rose & Gerba, 1999; Teunis, Nagelkerke & Haas, 1999)
<i>Campylobacter</i>	$1-(1+(\text{dose}/7.59))^{-0.145}$	0.1-0.6 ^a	(Medema et al., 1996)
<i>E. coli</i> O157:H7	$1-(1+(\text{dose}/48.8))^{-0.248}$	0.2-0.6 ^a	(Teunis, Ogden & Strachan, 2008)
<i>Cryptosporidium</i>	$1-\exp(-0.09*\text{dose})$	0.3-0.7 ^a	(USEPA, 2006)
<i>Giardia</i>	$1-\exp(-0.01982*\text{dose})$	0.2-0.7 ^a	(Rose & Gerba, 1991; Eisenberg et al., 1996)
<i>Adenovirus</i>	$1-\exp(-\text{dose} * 0.4172)$	0.5 ^b	(Couch et al., 1969; Crabtree et al., 1997)
<i>Norovirus</i>	$0.72*(1-\exp(-\text{dose}/1))^c$	0.3-0.8 ^a	(Messner, Berger & Nappier, 2014; Van Abel et al., 2017)

^aUniform distribution (minimum, maximum)

^bPoint estimate

^cFull particle disaggregation is assumed with $\mu=1$

Table 5. Median health risks associated with swimming, fishing, kayaking, boating, and jet-skiing in wet- and dry-loading conditions, based on the concentrations of host-associated fecal markers. The USEPA’s risk benchmark is 3.20×10^{-2} . *Overall risks include human, canine, and gull risks combined.

Activity	Fecal source	Median risk of illness (All data)	Median risk of illness (Wet-loading)	Median risk of illness (Dry-loading)	Median risk of illness (Tule Creek)	Median risk of illness (Little Bay)	Median risk of illness (Aransas Bay)
Swimming (children)	Overall*	4.10×10^{-3}	6.23×10^{-3}	4.42×10^{-3}	3.29×10^{-3}	5.29×10^{-3}	3.98×10^{-3}
	Human	1.20×10^{-3}	3.59×10^{-3}	1.54×10^{-3}	6.56×10^{-4}	2.03×10^{-3}	1.47×10^{-3}
	Canine	1.20×10^{-3}	1.63×10^{-3}	1.00×10^{-3}	1.23×10^{-3}	1.10×10^{-3}	1.47×10^{-3}
	Gull	1.80×10^{-4}	2.15×10^{-4}	2.31×10^{-4}	1.43×10^{-4}	2.06×10^{-4}	7.33×10^{-5}
	Enterococci	2.01×10^{-1}	2.62×10^{-1}	1.81×10^{-1}	4.91×10^{-1}	1.55×10^{-1}	1.13×10^{-1}
Swimming (adult)	Overall*	2.00×10^{-3}	3.00×10^{-3}	2.13×10^{-3}	1.59×10^{-3}	2.55×10^{-3}	1.92×10^{-3}
	Human	5.60×10^{-4}	1.72×10^{-3}	7.36×10^{-4}	3.13×10^{-4}	9.70×10^{-4}	7.03×10^{-4}
	Canine	5.60×10^{-4}	7.85×10^{-4}	4.83×10^{-4}	5.89×10^{-4}	5.31×10^{-4}	7.10×10^{-4}
	Gull	8.60×10^{-5}	1.02×10^{-4}	1.10×10^{-4}	6.82×10^{-5}	9.85×10^{-5}	3.50×10^{-5}
	Enterococci	1.15×10^{-1}	1.61×10^{-1}	1.00×10^{-1}	4.12×10^{-1}	8.42×10^{-2}	5.83×10^{-2}
Fishing	Overall*	2.27×10^{-4}	3.40×10^{-4}	2.36×10^{-4}	1.73×10^{-4}	2.95×10^{-4}	2.09×10^{-4}

	Human	6.28×10^{-5}	1.93×10^{-4}	7.83×10^{-5}	3.41×10^{-5}	1.13×10^{-4}	7.49×10^{-5}
	Canine	6.33×10^{-5}	8.89×10^{-5}	5.38×10^{-5}	6.36×10^{-5}	6.14×10^{-5}	7.75×10^{-5}
	Gull	9.73×10^{-6}	1.12×10^{-5}	1.27×10^{-5}	7.73×10^{-6}	1.08×10^{-5}	4.02×10^{-6}
	Enterococci	1.49×10^{-2}	2.27×10^{-2}	1.27×10^{-2}	1.46×10^{-1}	1.04×10^{-2}	6.98×10^{-3}
Jet skiing	Overall*	2.48×10^{-4}	3.79×10^{-4}	2.66×10^{-4}	1.93×10^{-4}	3.20×10^{-4}	2.35×10^{-4}
	Human	6.60×10^{-5}	2.11×10^{-4}	9.08×10^{-5}	3.86×10^{-5}	1.17×10^{-4}	8.81×10^{-5}
	Canine	7.00×10^{-5}	9.94×10^{-5}	5.91×10^{-5}	7.40×10^{-5}	6.89×10^{-5}	8.50×10^{-5}
	Gull	1.04×10^{-5}	1.33×10^{-5}	1.37×10^{-5}	8.58×10^{-6}	1.23×10^{-5}	4.46×10^{-6}
	Enterococci	1.66×10^{-2}	2.51×10^{-2}	1.41×10^{-2}	1.59×10^{-1}	1.16×10^{-2}	7.74×10^{-3}
Boating	Overall*	6.30×10^{-5}	9.51×10^{-5}	6.68×10^{-5}	4.70×10^{-5}	7.87×10^{-5}	5.83×10^{-5}
	Human	1.69×10^{-5}	5.27×10^{-5}	2.31×10^{-5}	9.35×10^{-6}	2.93×10^{-5}	2.20×10^{-5}
	Canine	1.78×10^{-5}	2.53×10^{-5}	1.52×10^{-5}	1.70×10^{-5}	1.65×10^{-5}	2.11×10^{-5}
	Gull	2.67×10^{-6}	3.15×10^{-6}	3.53×10^{-6}	2.10×10^{-6}	3.11×10^{-6}	1.11×10^{-6}

	Enterococci	4.20×10^{-3}	6.45×10^{-3}	3.58×10^{-3}	4.80×10^{-2}	2.92×10^{-3}	1.95×10^{-3}
	Overall*	2.32×10^{-4}	3.52×10^{-4}	2.48×10^{-4}	1.84×10^{-4}	3.12×10^{-4}	2.20×10^{-4}
Kayaking	Human	6.40×10^{-5}	1.98×10^{-4}	8.10×10^{-5}	3.53×10^{-5}	1.17×10^{-4}	8.11×10^{-5}
	Canine	6.56×10^{-5}	9.18×10^{-5}	5.59×10^{-5}	6.78×10^{-5}	6.41×10^{-5}	8.00×10^{-5}
	Gull	1.03×10^{-5}	1.23×10^{-5}	1.34×10^{-5}	8.23×10^{-6}	1.19×10^{-5}	4.17×10^{-6}
	Enterococci	1.57×10^{-2}	2.39×10^{-2}	1.34×10^{-2}	1.52×10^{-1}	1.10×10^{-2}	7.36×10^{-3}

Figures

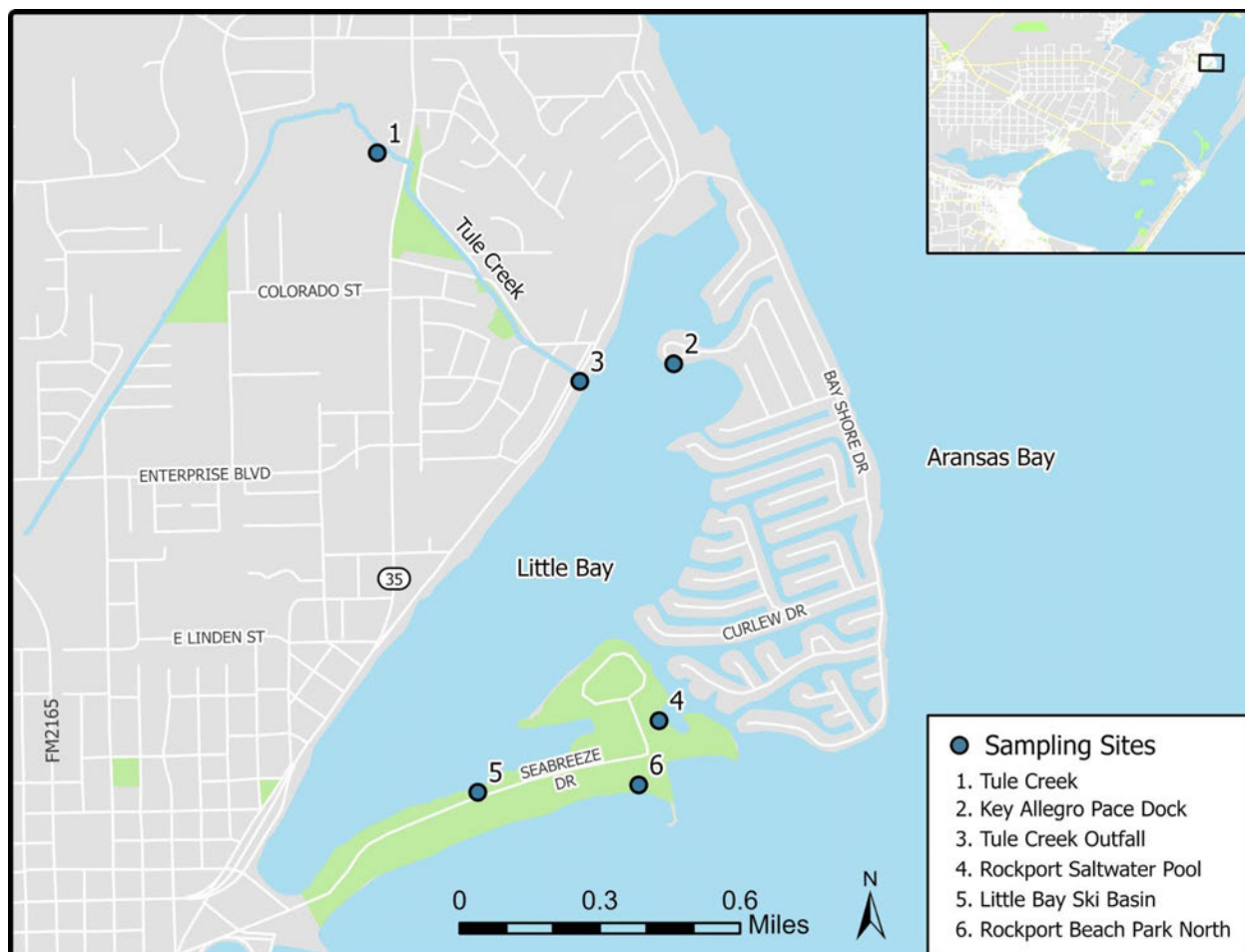


Figure 1. Map of sampling sites.

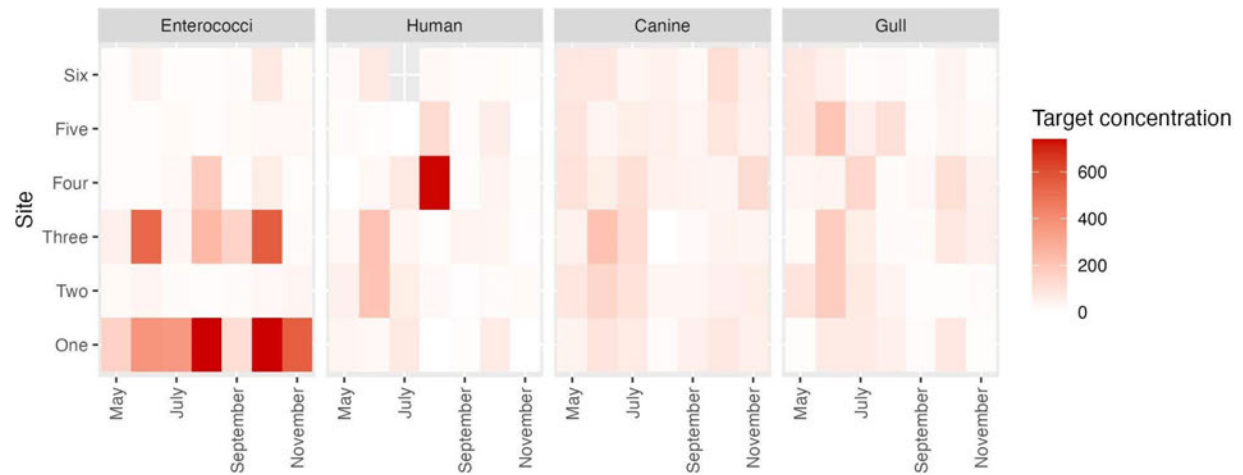


Figure 2. Heatmap showing concentrations of enterococci (MPN/100 mL) and human, canine, and gull-associated markers (gene copies/100 mL) measured in the previous study (Powers et al., 2021a) and were used to inform QMRA in this study.

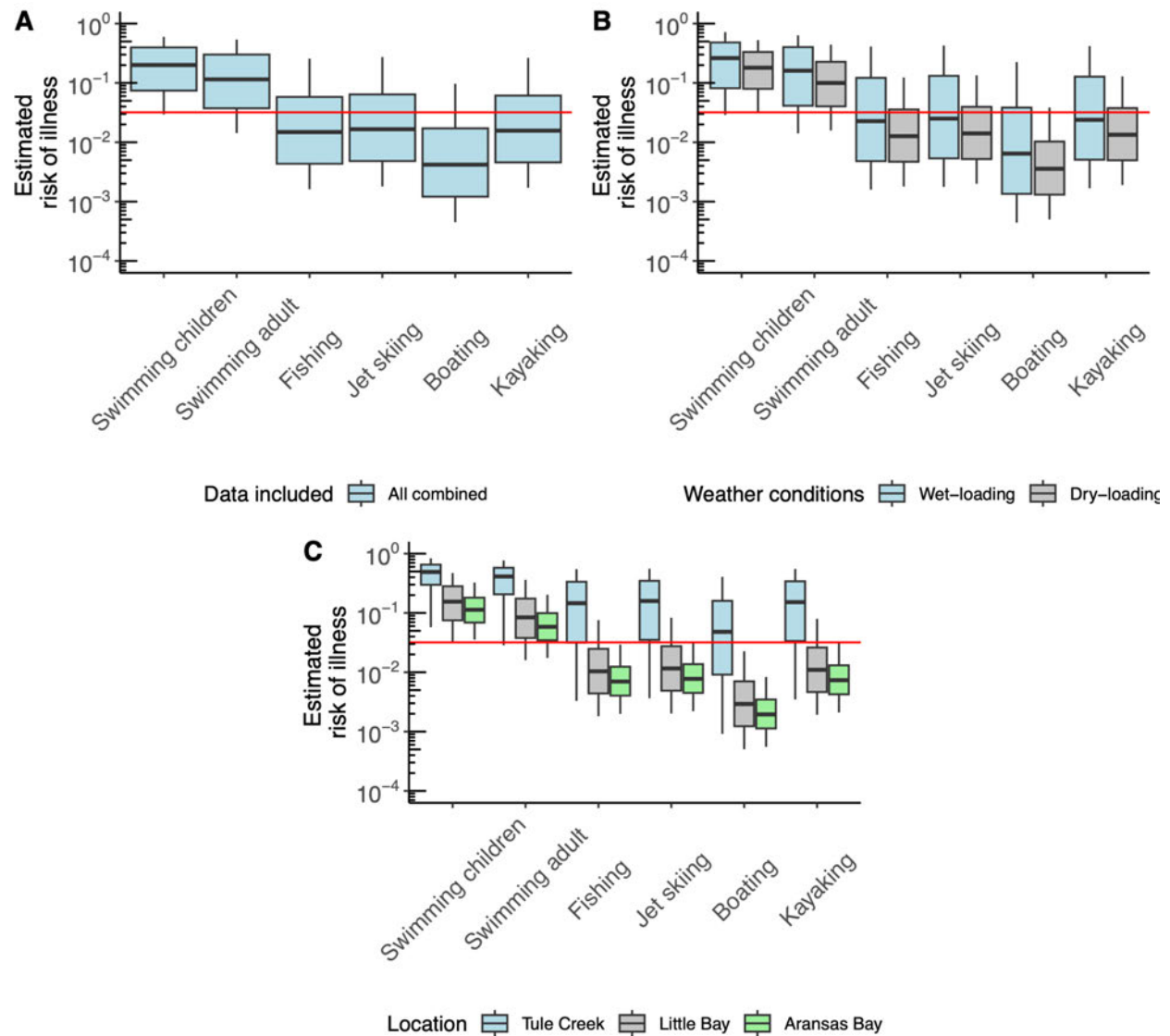


Figure 3. Estimated health risks based on enterococci concentrations (assuming 5% of enterococci originated from raw human sewage) for A) all data combined; B) wet-loading (blue) vs. dry-loading (gray); C) ecocline location (blue = Tule Creek, gray = Little Bay, green = Aransas Bay). The red lines represent the USEPA's illness risk benchmark of 3.2×10^{-2} .

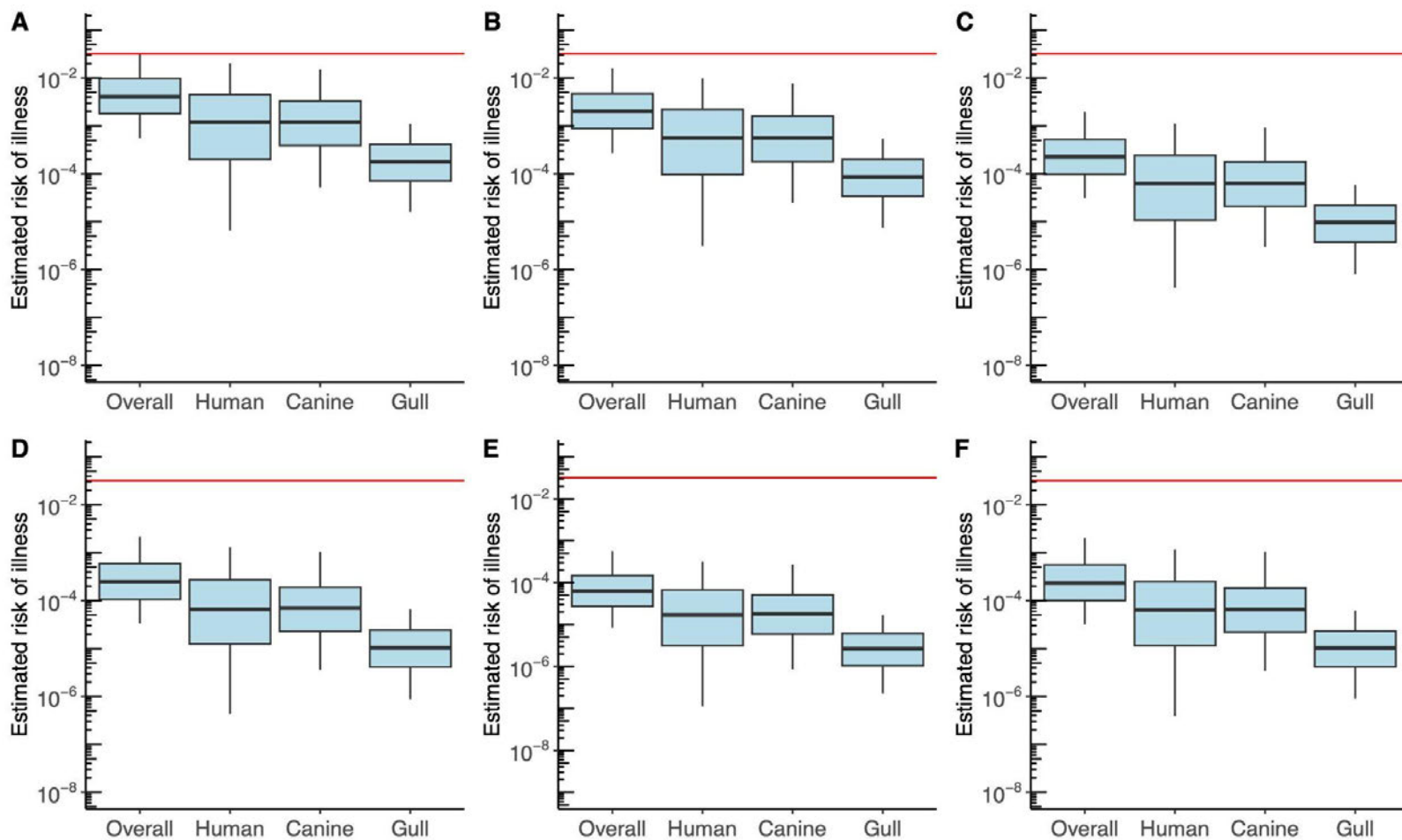


Figure 4. Estimated health risks based on host-associated markers for all data combined for A) children swimming; B) adults swimming; C) adults fishing; D) adults jet-skiing, E) adults boating; F) adults kayaking. The red lines represent the USEPA's illness risk benchmark of 3.2×10^{-2} .

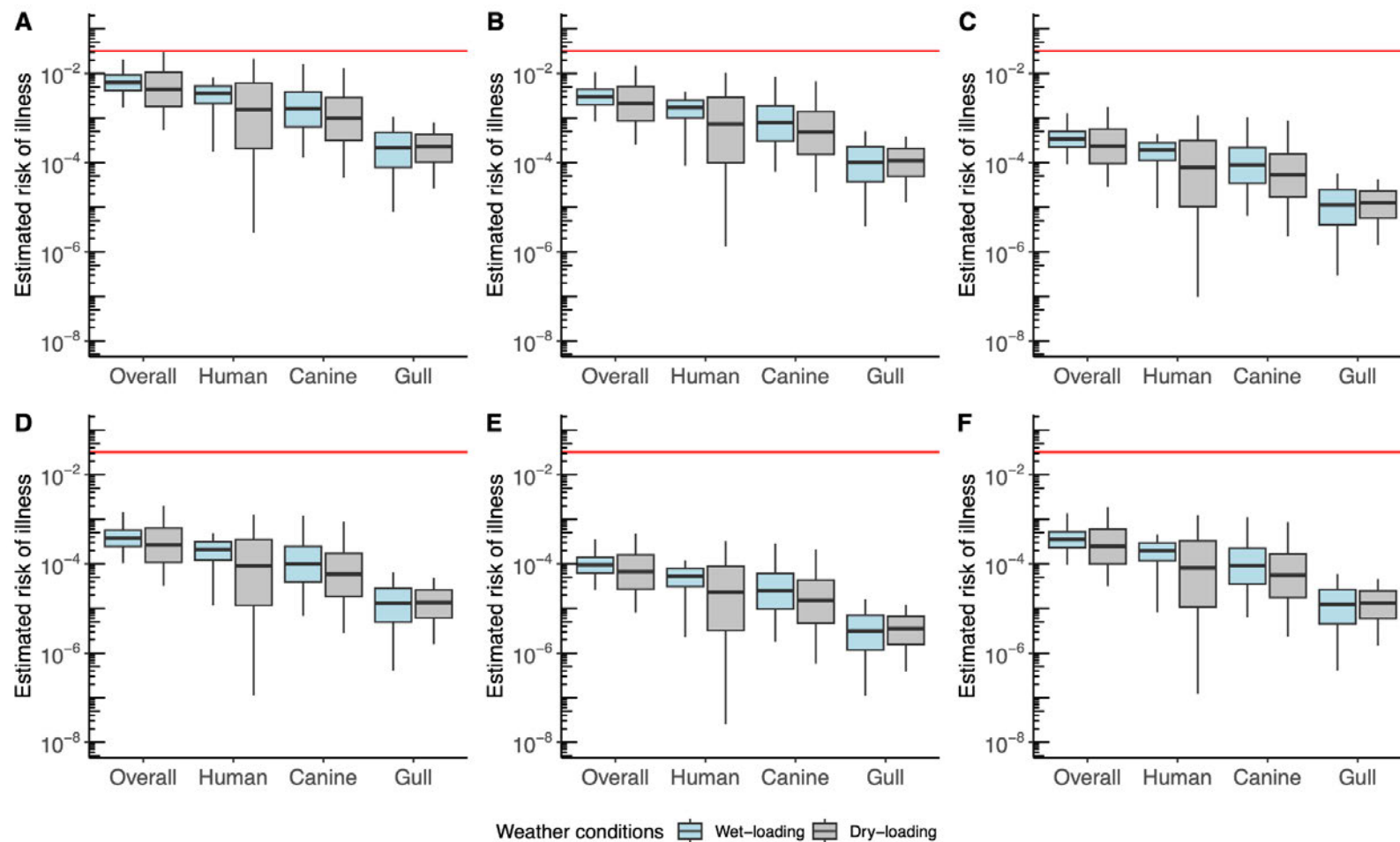


Figure 5. Estimated health risks based on host-associated markers in wet-loading (blue; left) and dry-loading (gray; right) conditions for A) children swimming; B) adults swimming; C) adults fishing; D) adults jet-skiing, E) adults boating; F) adults kayaking. The red lines represent the USEPA's illness risk benchmark of 3.2×10^{-2} .

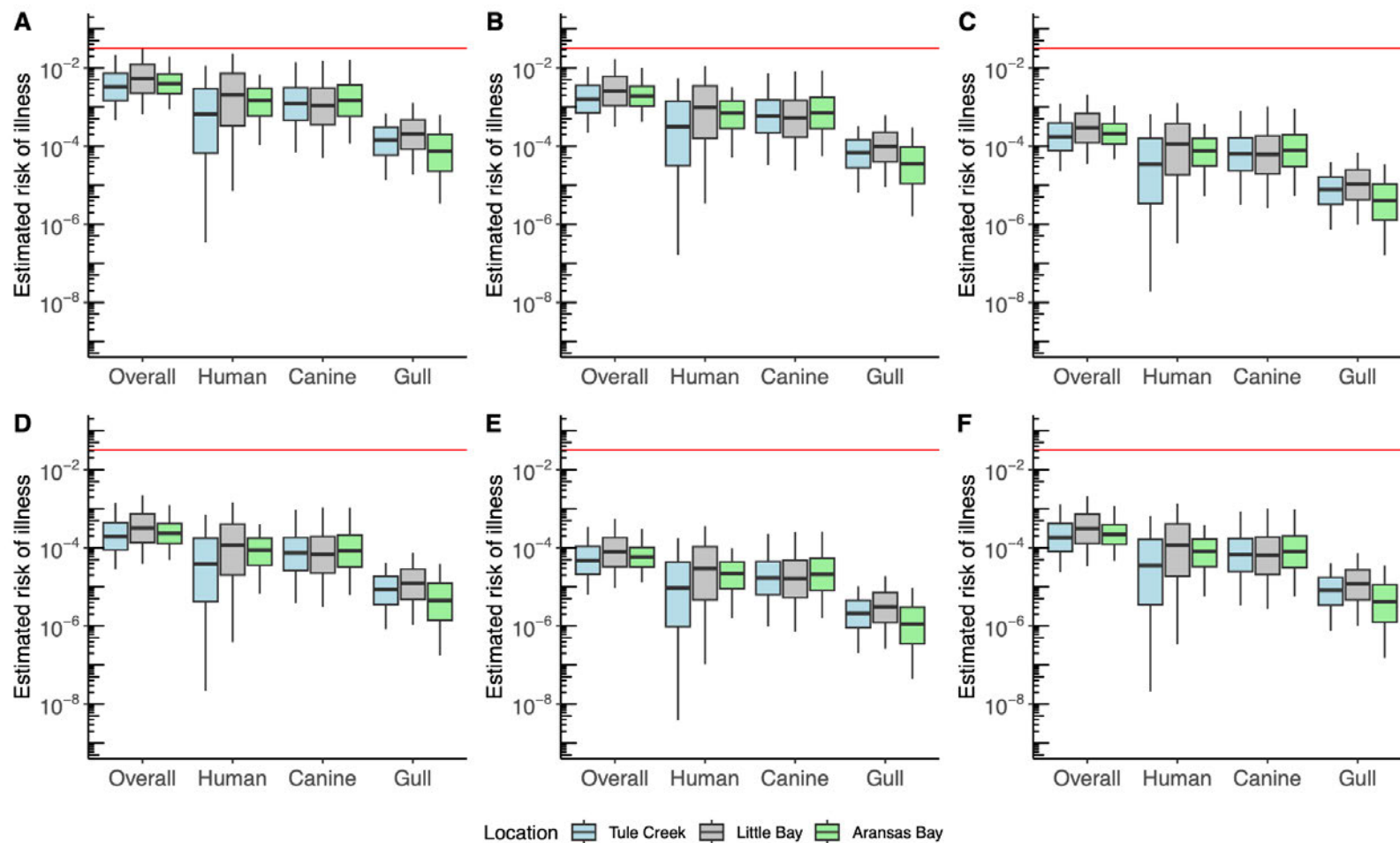


Figure 6. Estimated health risks based on host-associated markers separated by location: Tule Creek (blue; left), Little Bay (gray; middle), and Aransas Bay (green; right) for A) children swimming; B) adults swimming; C) adults fishing; D) adults jet-skiing, E) adults boating; F) adults kayaking. The red lines represent the USEPA's illness risk benchmark of 3.2×10^{-2} .



Figure 7. Sensitivity analysis results for all MST data combined.

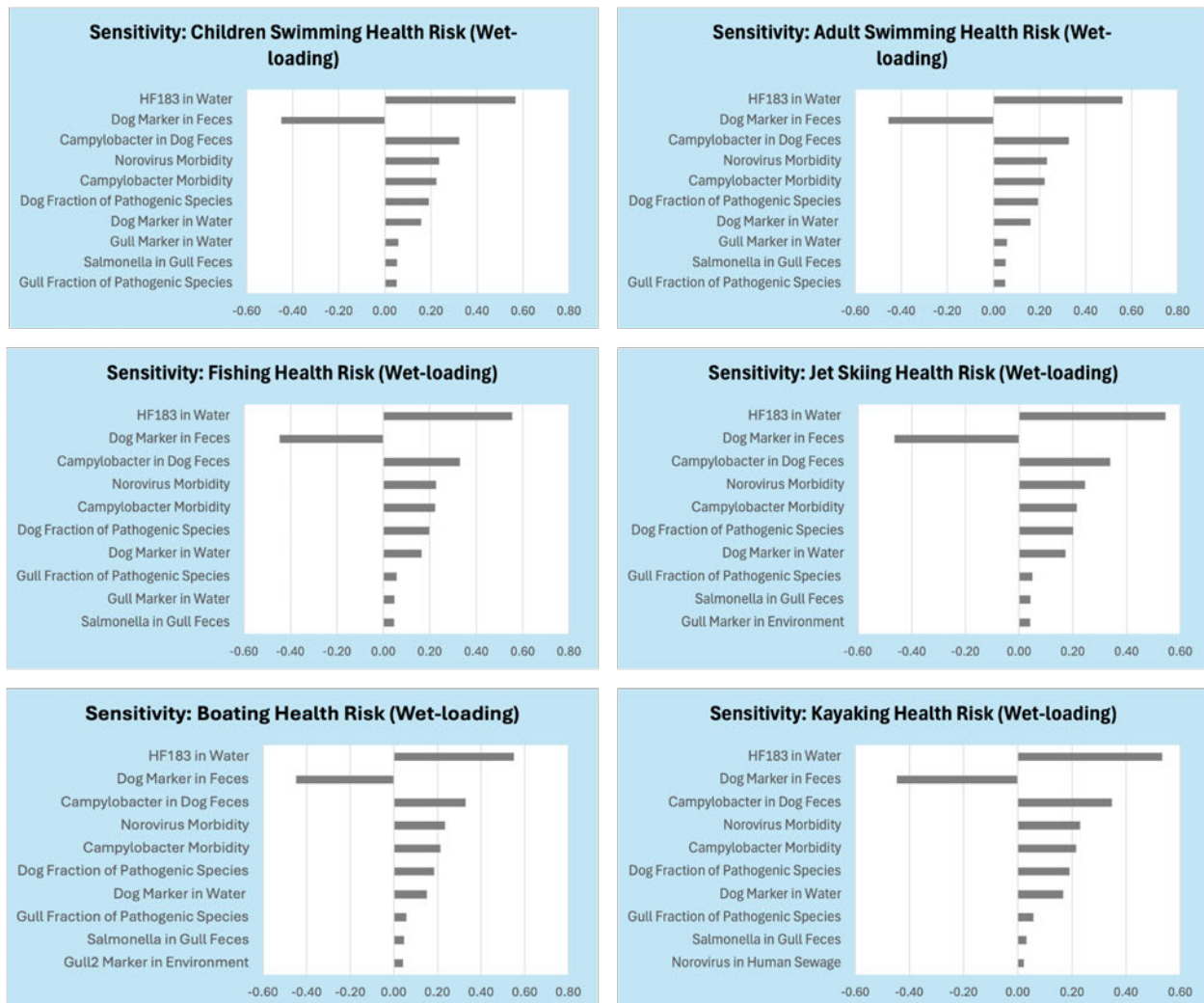


Figure 8. Sensitivity analysis results for MST wet-loading samples.



Figure 9. Sensitivity analysis results for MST dry-loading samples.

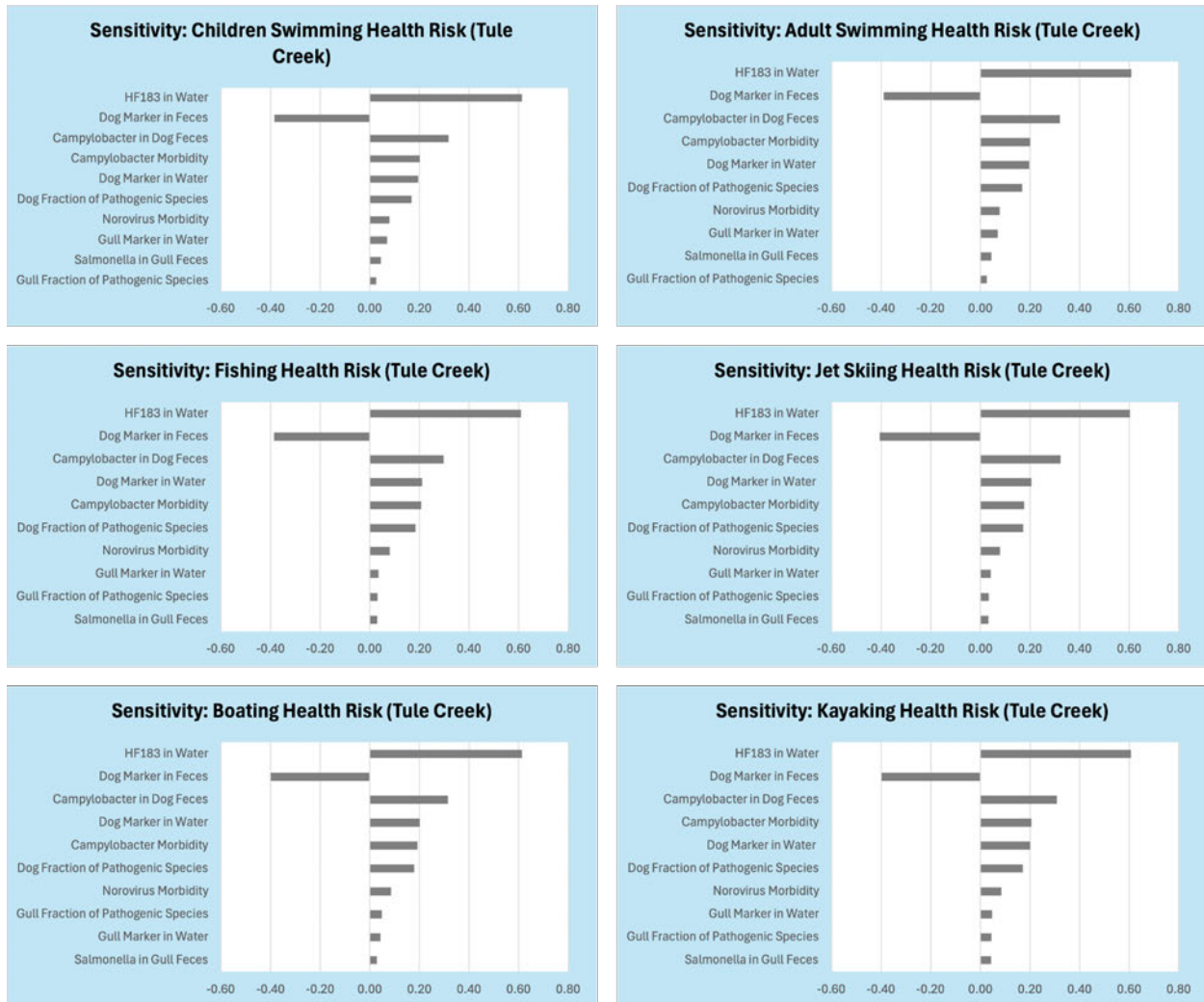


Figure 10. Sensitivity analysis results for MST Tule Creek data.

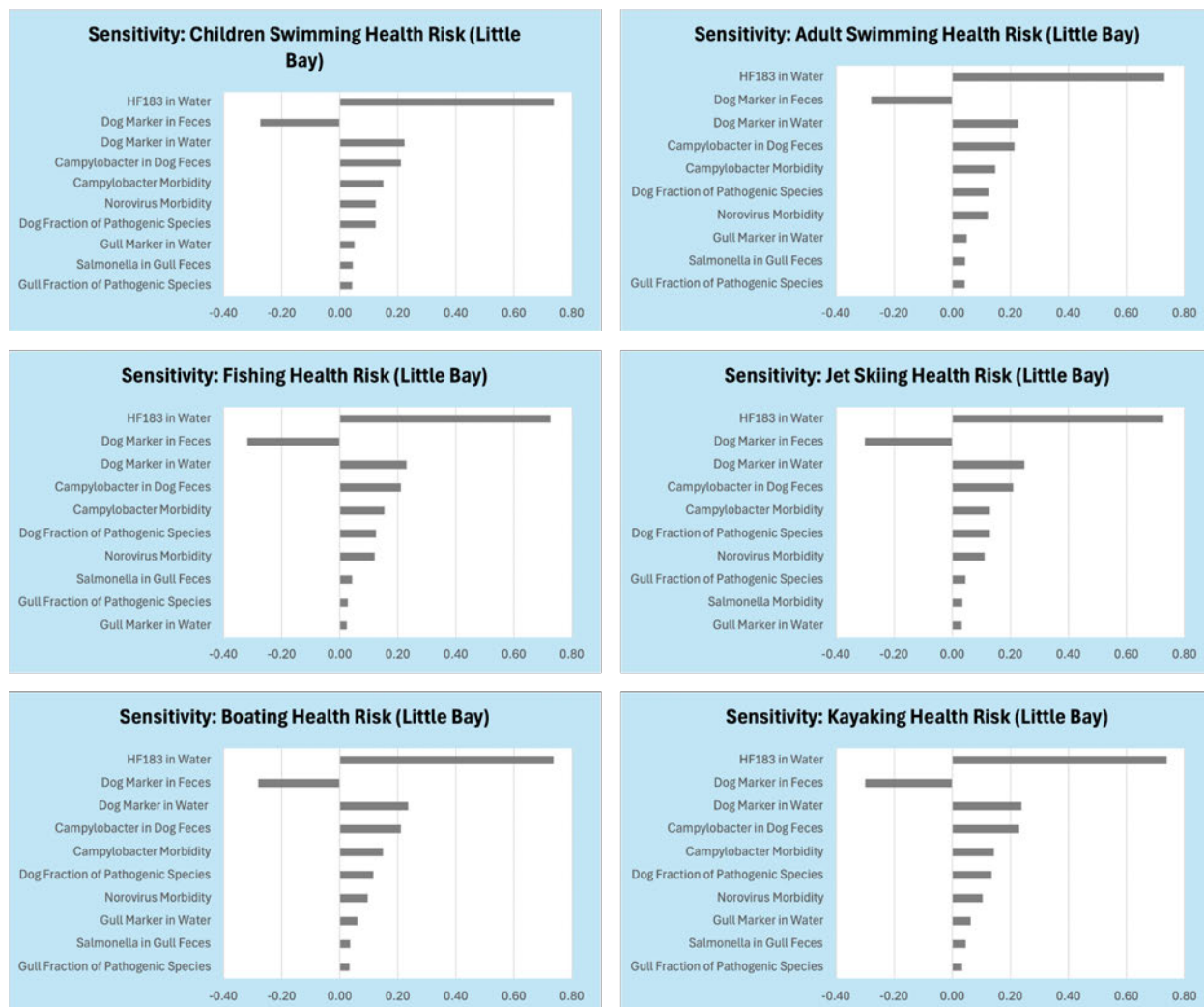


Figure 11. Sensitivity analysis results for MST Little Bay data.

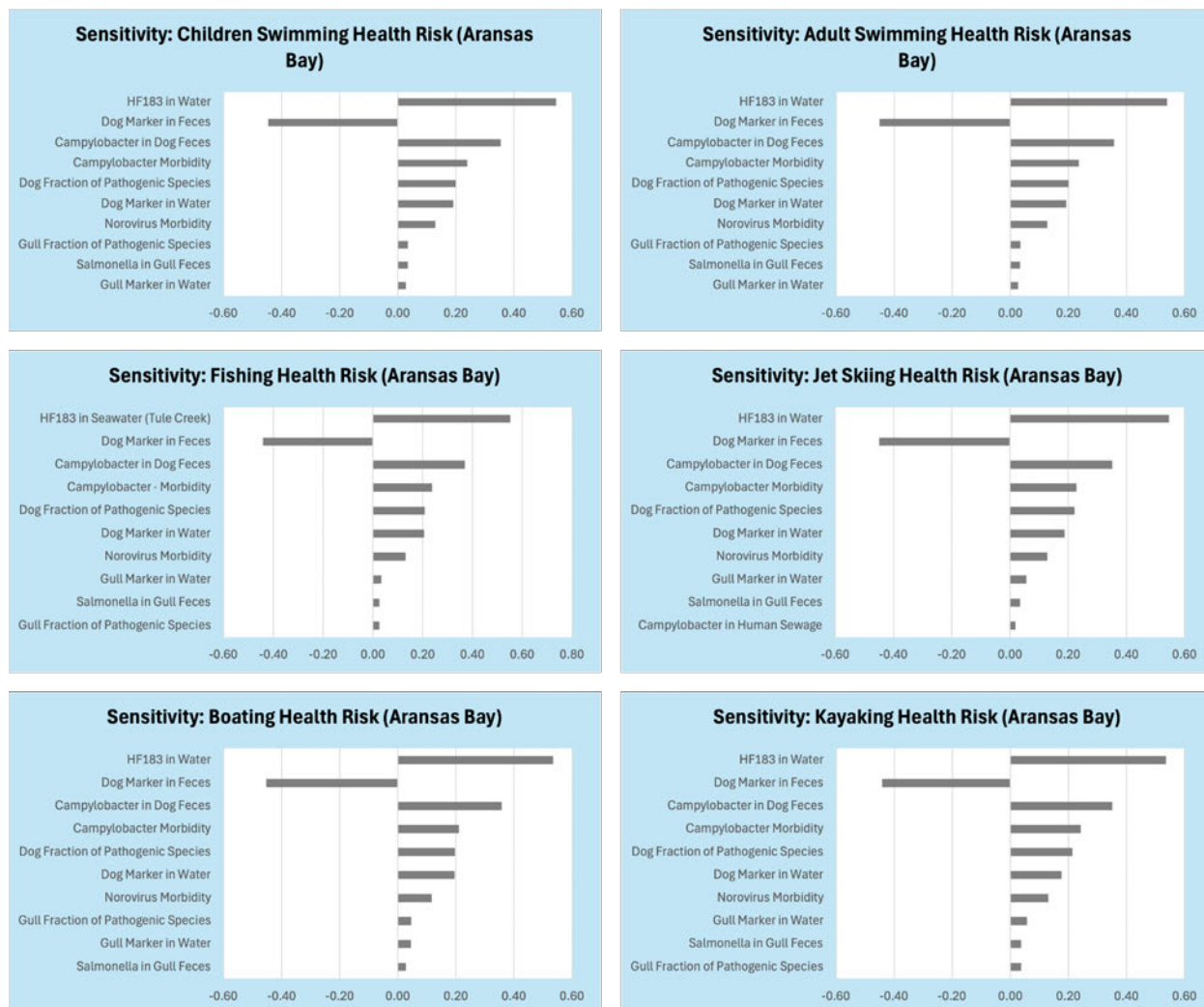


Figure 12. Sensitivity analysis results for MST Aransas Bay data.

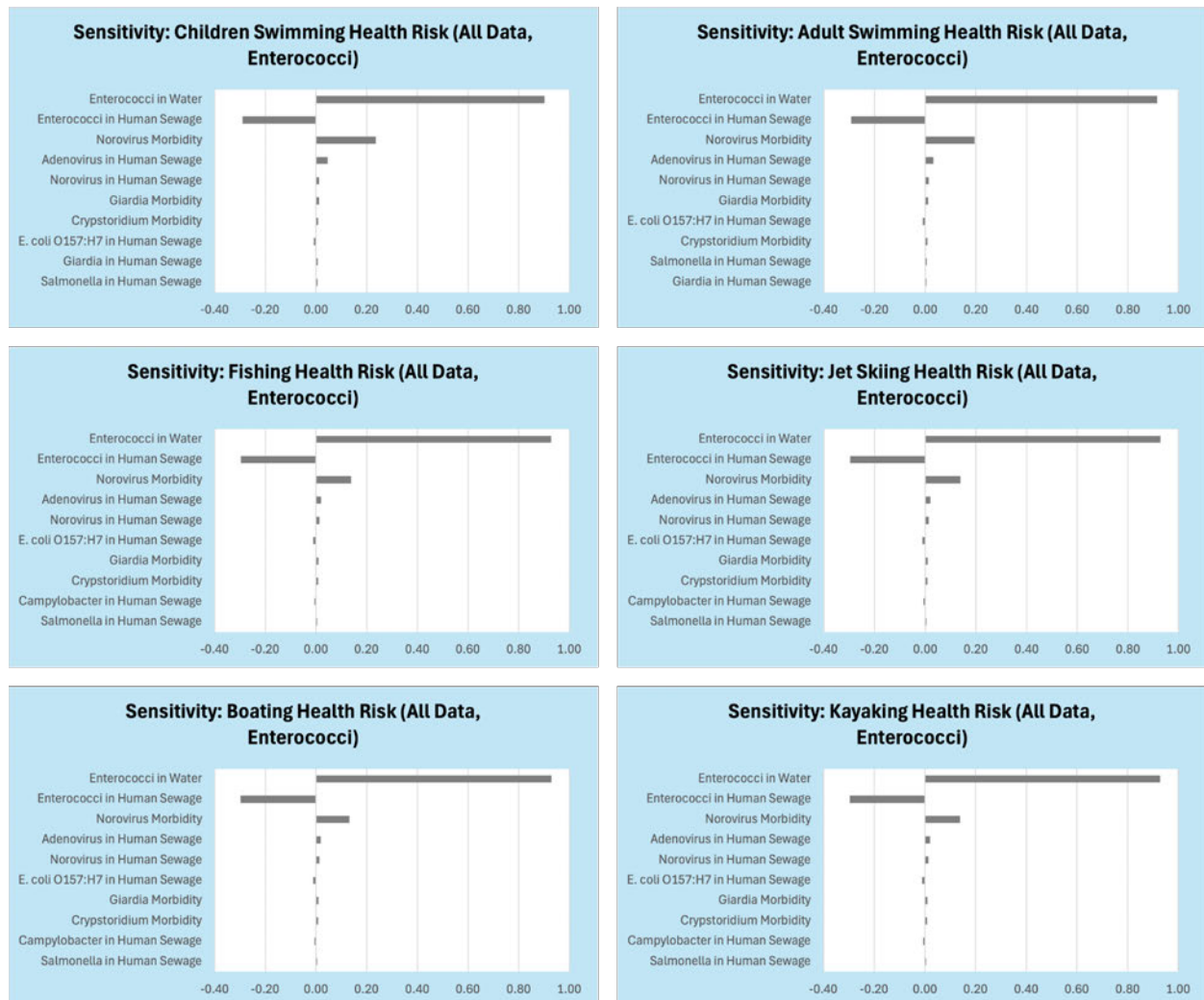


Figure 13. Sensitivity analysis results for all enterococci data combined.

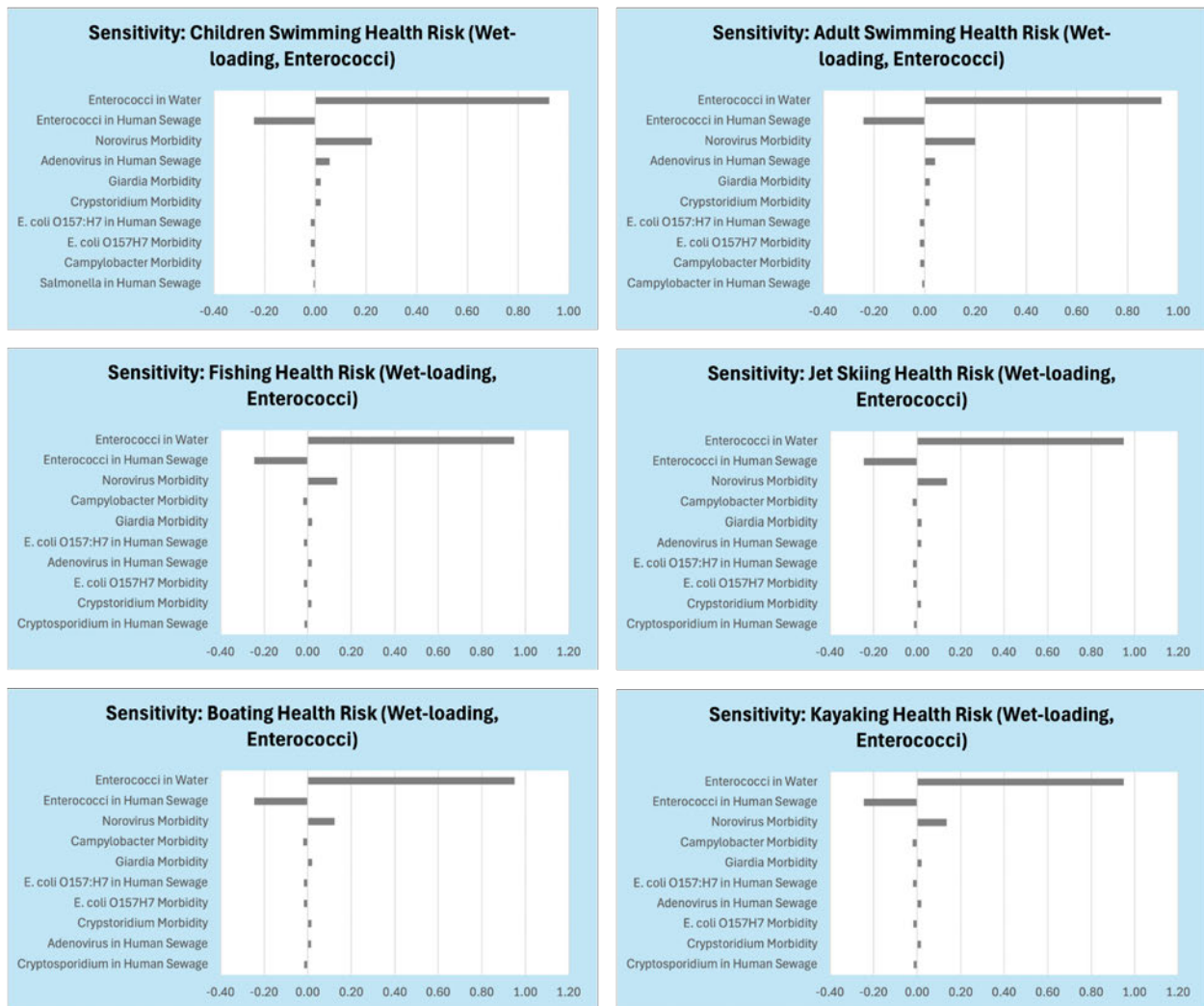


Figure 14. Sensitivity analysis results for enterococci wet-loading data.

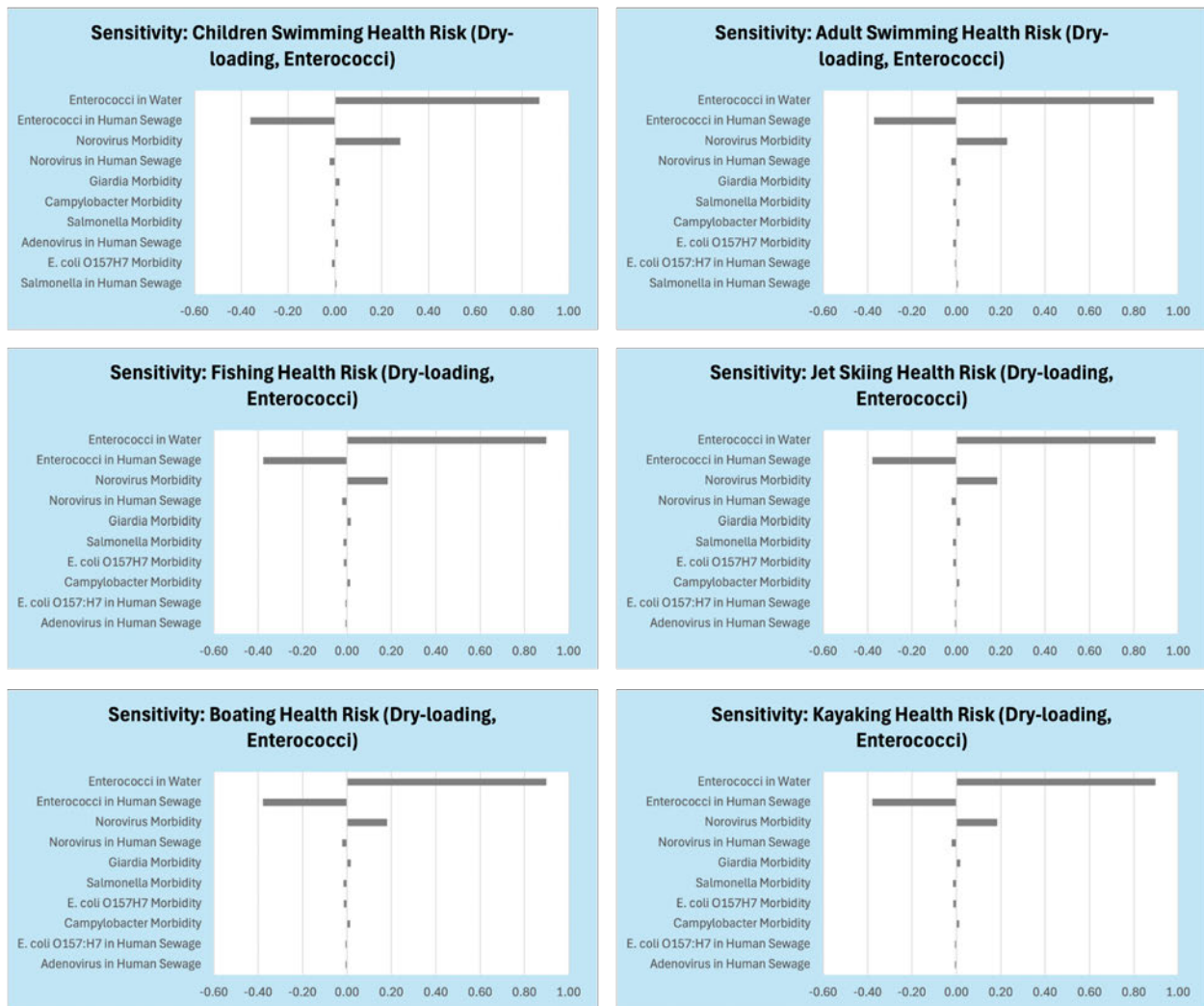


Figure 15. Sensitivity analysis results for enterococci dry-loading data.

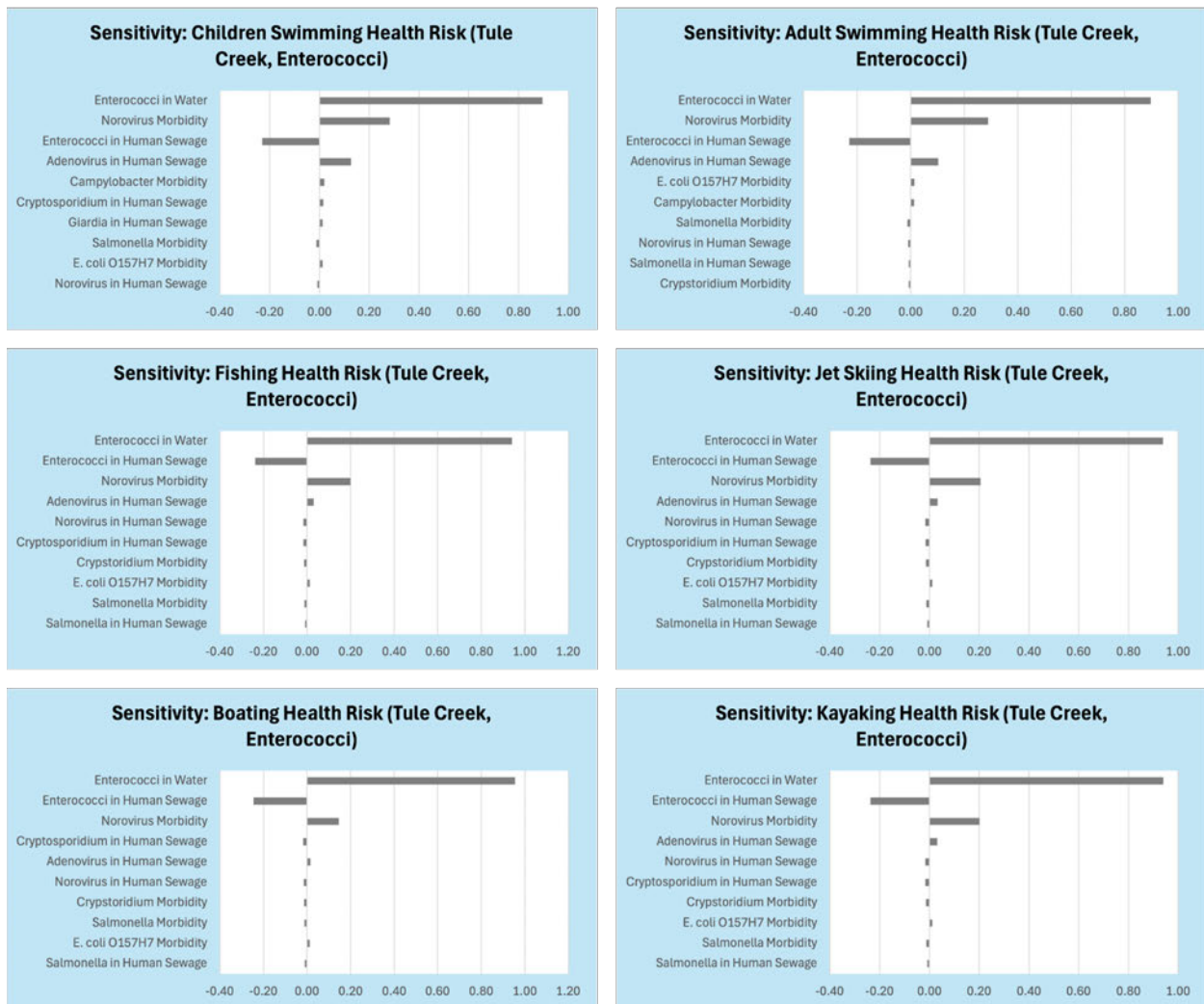


Figure 16. Sensitivity analysis results for enterococci Tule Creek data.

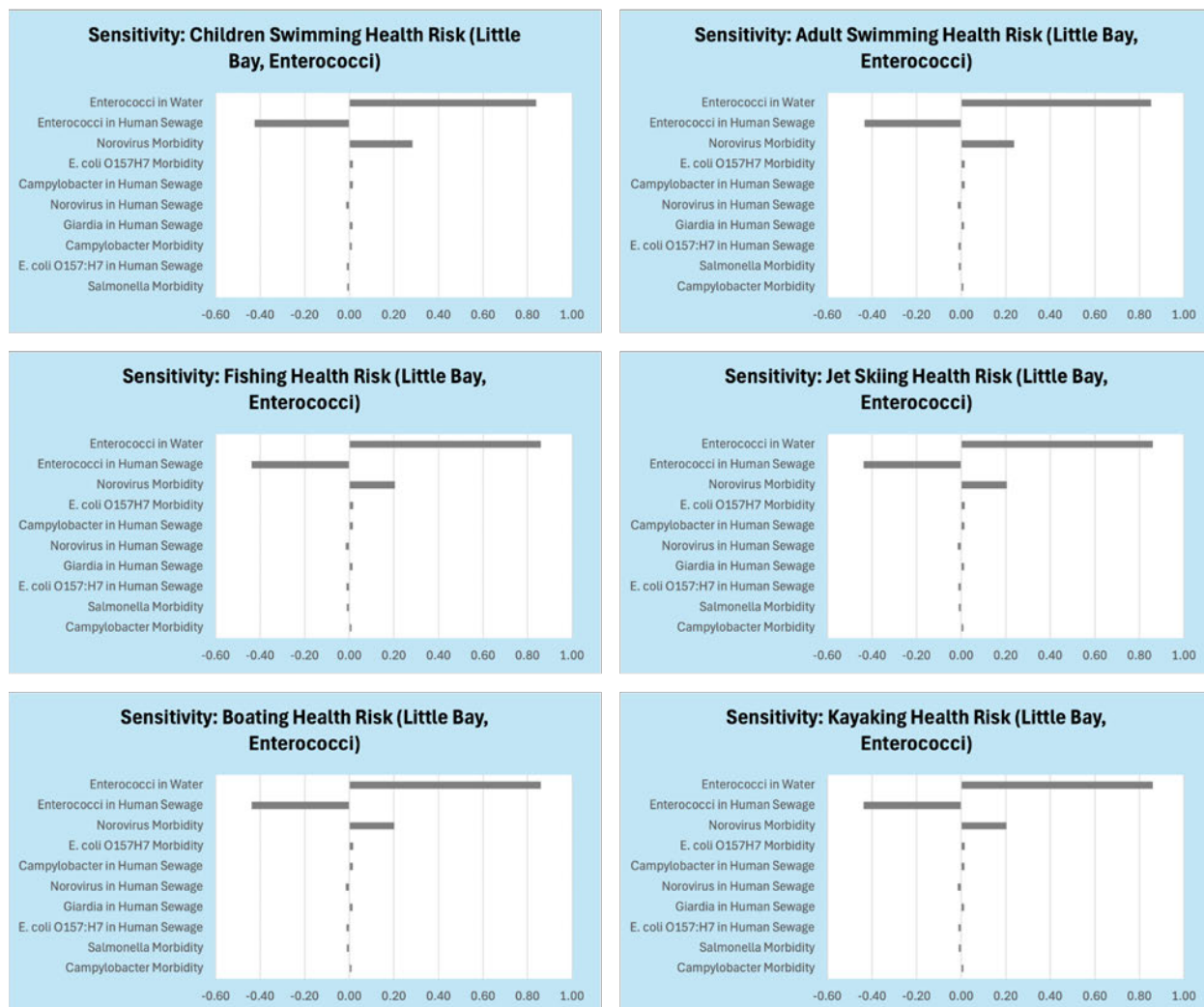


Figure 17. Sensitivity analysis results for enterococci Little Bay data.

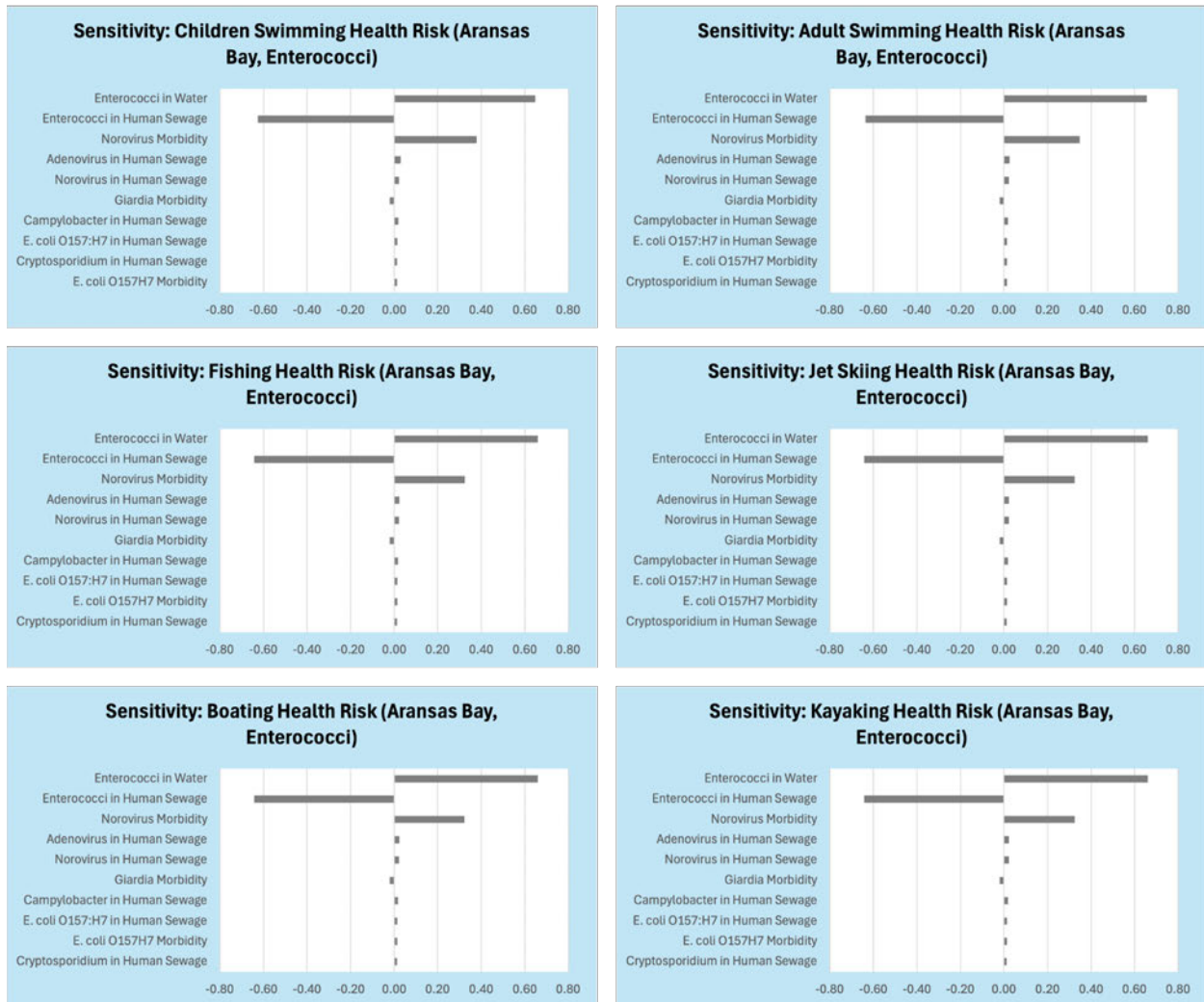


Figure 18. Sensitivity analysis results for enterococci Aransas Bay data.

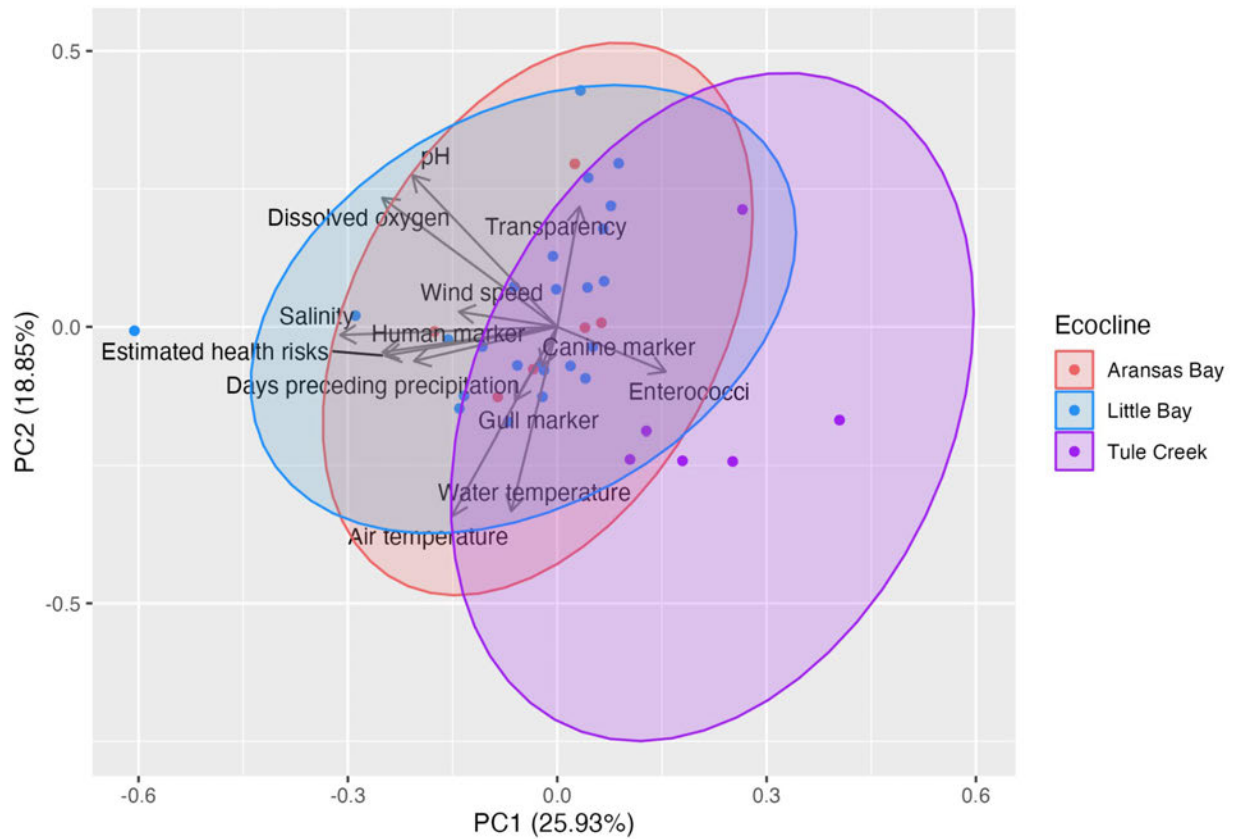


Figure 19. Principal component analysis (PCA) showing relationships between estimated human health risks, fecal indicators, and environmental parameters. The red, blue, and purple circles represent the 95% confidence intervals for Aransas Bay, Little Bay, and Tule Creek samples, respectively.