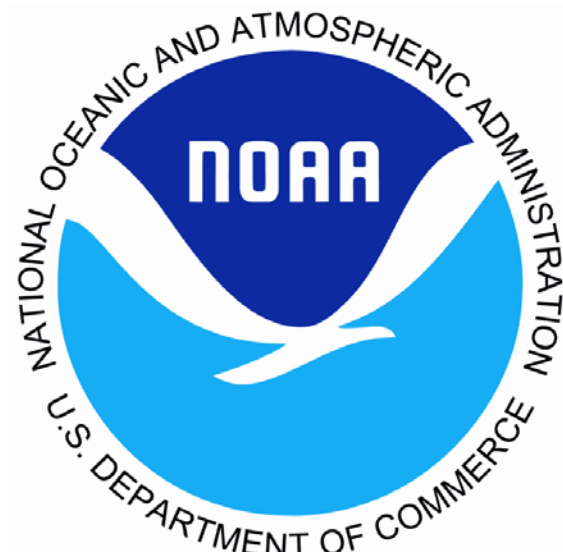
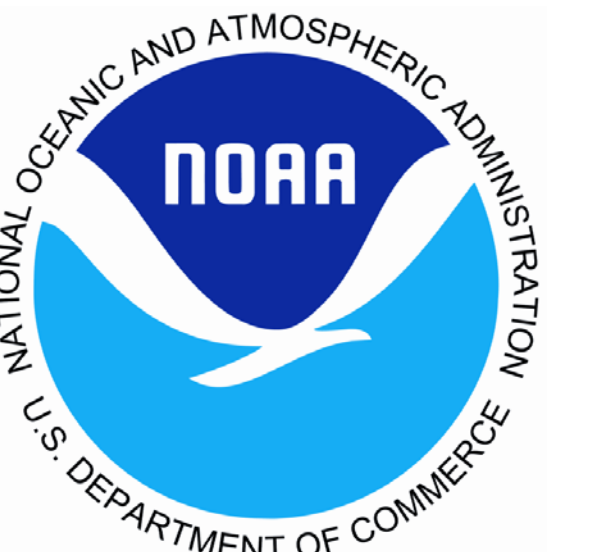




Tracking Microbial Contaminants from Coastal Wastewater Discharges

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Goal

- To determine the level of viable *Enterococci* fecal indicator bacteria and to investigate the abundance of fecal indicators and microbial pathogens in seawater originating from wastewater outfalls based on their genetic signature in the environment.
- Assess the geospatial distribution of fecal indicator bacterial from treated wastewater discharges in along the Southern Florida Coast.

Background



Image of rhodamine dye from the South Central wastewater outfall, Palm Beach County, Florida, July 2008. Vessel shown is R/V F.G. Walton-Smith conducting tracer study for NOAA FACE program.



Image of inter-coastal water exiting the Boynton inlet.

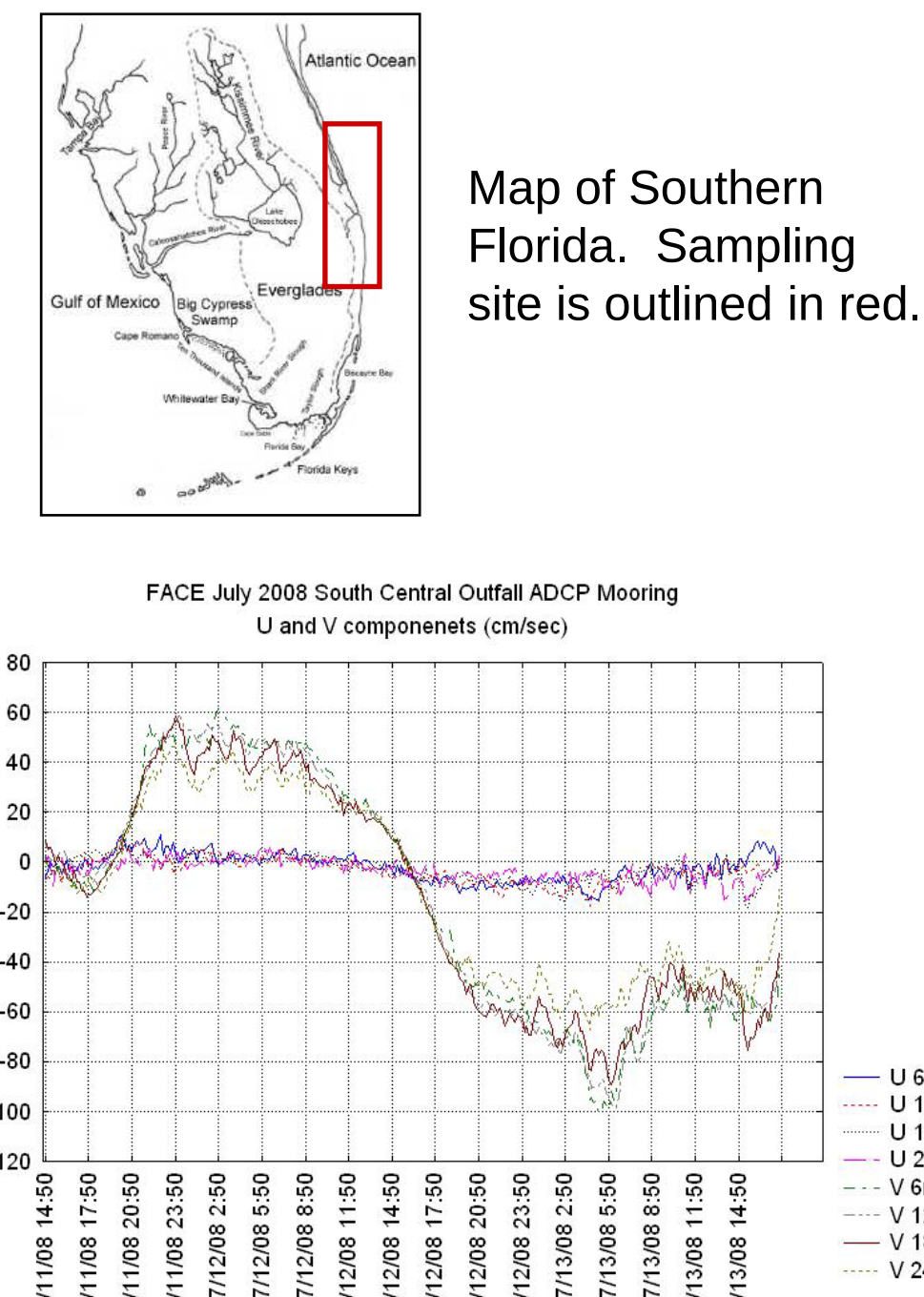
- Public health risks in recreational waters and the decline of coral reefs in southern Florida due to human caused contamination are a significant concern in a region where tourism drives much of the economy.
- The first suspects are typically the wastewater outfalls, which discharge up to 300 million gallons of treated wastewater into the ocean 3km off shore each day.
- However, there are a variety of other human pollution sources in the area, including ground water seepage and a number of coastal inlets, which are contaminated with materials from urban stormwater runoff, septic tanks, landfills, industrial processes, and agriculture.
- It is important to determine the relative contribution of chemical, microbial, and nutrient pollutants from each of these sources in order to ensure that expensive remediation processes accurately and effectively targets the areas that impact the coastal ecosystem and pose the greatest threat to human and reef health.
- The Florida Area Coastal Environment (FACE) program is carrying out a long term assessment study of the South Florida coastal ecosystems to investigate the transport and distribution of nutrients and microbes from the coastal inlets, treated wastewater effluent, and oceanic upwelling.

Wanninkhof, R.; Sullivan, K. F.; Dammann, W. P.; Proni, J. R.; Bloetscher, F.; Soloviev, A. V.; Carsey, T. P. *Environmental Science & Technology* 2005, 39, 8883-8890.

Glassmeyer, S. T.; Furlong, E. T.; Kolpin, D. W.; Cahill, J. D.; Zaugg, S. D.; Werner, S. L.; Meyer, M. T.; Kryak, D. D. *Environ. Sci. Technol.* 2005, 39, 5157-5169.

Coastal Florida Currents

- The July 2008 FACE cruise monitored the South Central waste water outfall, 3 km off the coast of Delray Beach in Southern Florida
- SF₆ gas and rhodamine dye were injected into the treated wastewater effluent the outfall to visualize the effluent plume. The surrounding coastal waters were monitored for the presence of the tracers in order to study the plume composition, distribution, and transport.
- Current Data indicates two distinct flow fields:
 - Northward Current during first half of survey
 - Flow reversal to southward flow during second half of survey
- Samples were collected along transects following the wastewater effluent plume to test for chemical, nutrient, and microbial contaminants

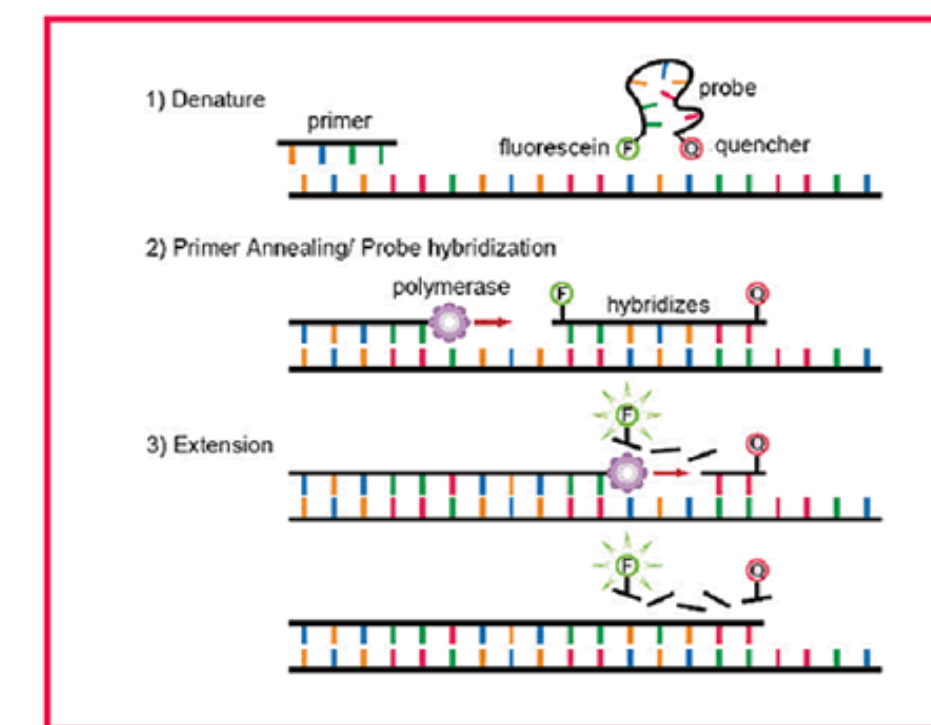


Source Tracking qPCR amplification

- Quantitative Polymerase Chain Reaction (qPCR) is a method of DNA amplification that used to quantitate the presence of specific gene sequences. It is used in microbial source tracking to assess the total amount of specific indicator bacteria present in all seawater samples.

Primers and probes were used against genetic markers for:

- Enterococci* general 23S rRNA gene marker:
 - provides an abundant marker for fecal matter
 - Measures total enterococci; is not host specific
- Human-source *Bacteroides* HF8 gene cluster marker:
 - Bacteroides* marker that is specific for human fecal matter
 - Relatively rare in human population so environmental presence indicates probability of large number of humans contributing to fecal signal, thus it is a good indicator for composite sources such as sewage.



Schematic of TaqMan qPCR mechanism

Culture-Based Viable Bacteria Test

- Viable enterococci levels were determined using the EPA-approved method of Enterolert™ Chromogenic Substrate MPN Assay (IDEXX company).

- Samples are inoculated into Enterolert™ indicator-growth media and are incubated overnight in a 97 well tray.

- A fluorochrome-labeled substrate in the media is cleaved by an enzyme in metabolically active enterococci to produce a fluorescent signal in wells containing the bacteria.

- No viable enterococci were found from ocean water samples near the South Central outfall for this sampling period. Viable enterococci were found at sample stations near the Boynton Inlet.

- With much larger annual discharge volume to the coastal environment of untreated water containing viable fecal indicators, the Boynton Inlet may potentially represent a greater source of viable pathogens to the coastal area than the wastewater outfall.



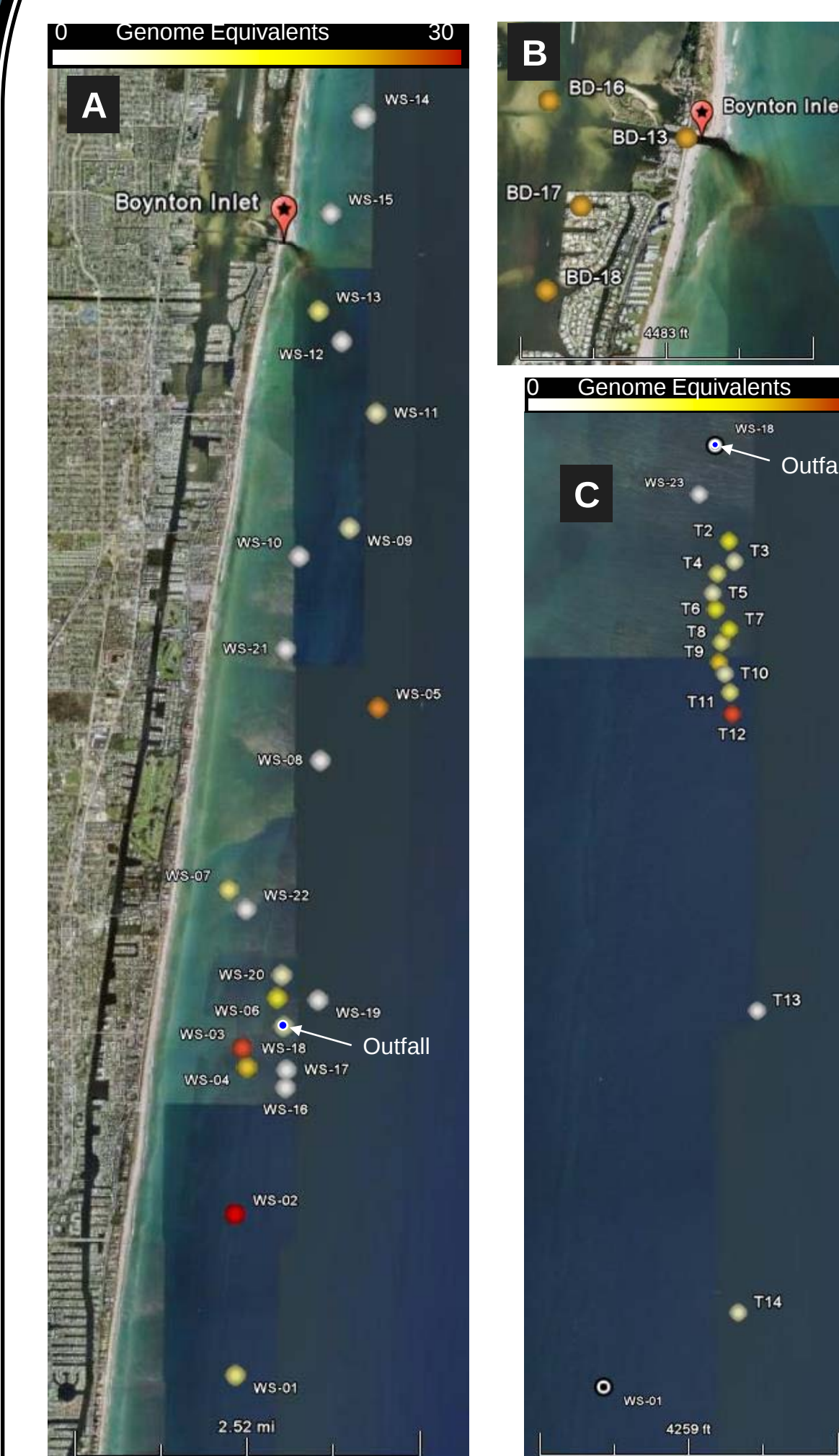
Image of Enterolert Quanti tray under UV lamp. Fluorescent wells indicate specific presence of enterococci.

Enterolert Test Results	
Sample	Enterococci Level (viable bacteria/100mL)
Ocean (85 samples)	0
BD-13	0
BD-16	10
BD-17	20
BD-18	61

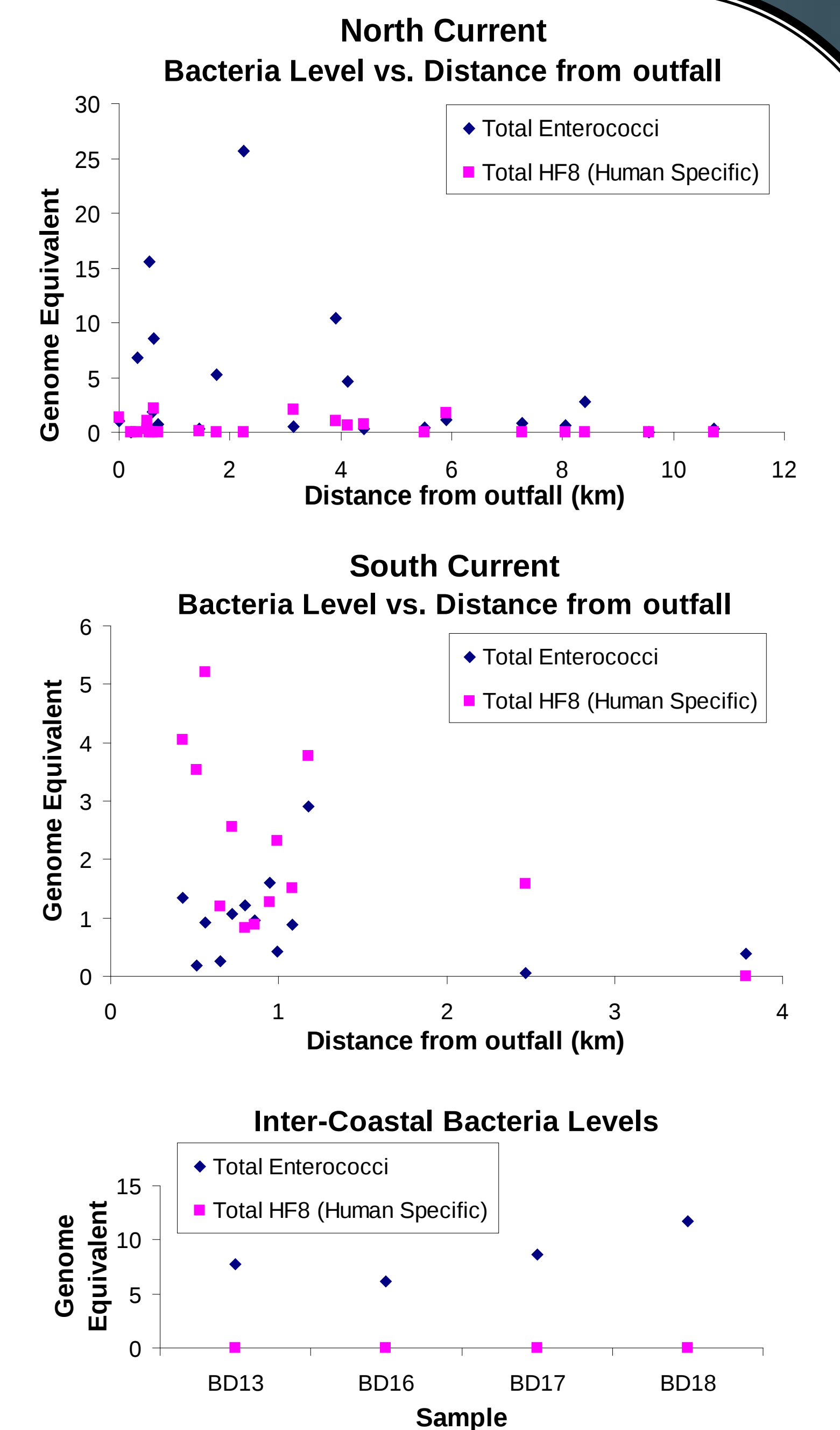
Acknowledgements

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Results



Maps of sample collection sites (A) during a northern current, (B) from the inter-coastal waterway, and (C) during a southern current. Color corresponds to total enterococci level.



- No linear correlation between marker concentration and distance from the outfall indicates that the microbial transport from the boil was not a steady stream.
- The wastewater effluent appears to have exited the outfall as coherent packets of freshwater that may travel along the surface over long distances with little mixing, resulting in the transport of occasional isolated packages of comparatively larger bacterial loads further downstream.
- Additional tracer and current data will be used to more fully characterize the discharge pathways.
- Enterococci and human *Bacteroides* were highest in the region around the boil, indicating that most of the bacterial load from the outfall may be diluting rapidly within the first kilometer downcurrent..
- Observed bacterial loads during this cruise were below levels commonly associated with ecosystem or human health risk.
- Samples collected during the southward current had a greater ratio of HF8 marker (pink) to enterococci (blue) than did the samples collected during the northward moving current. This may indicate that the fecal-associated microbial composition of the wastewater (and hence the associated potential pathogen load) of the effluent changes over time.

Conclusions and Future Directions

- No viable fecal indicator bacteria were found in the wastewater discharge plume
- Indicator bacterial levels determined by IDEXX Enterolert™ and by qPCR analysis were insignificant from a public health perspective. However this does not address potential health risk by other microbial groups such as pathogenic viruses or protozoan cysts. Dilution and distribution characteristics of viruses especially may not be similar to that observed here for bacteria.
- Rhodamine and SF₆ tracer data and current profiles will be used to more fully characterize the discharge pathways, and assess the potential distribution of fecal matter from the effluent plume.
- Extracted DNA samples will be further analyzed for additional source tracking and pathogen markers to more accurately determine which bacterial loads originate from the outfall point source.
- Viral RNA samples will be processed and analyzed to provide information on the distribution of viral pathogens.

Molecular Microbial Source Tracking

- To address the concerns of microbial water quality and ecosystem and public health risk in coastal waters, it is necessary to determine whether the wastewater effluent contains viable pathogens, how far from the point source these pathogens can travel, and how quickly they dilute out.
- However, there are a large number of potential pathogens associated with fecal matter, and often low microbe levels that are difficult and expensive to detect, but which can still be infectious.
- Instead of testing for each potential fecal pathogen, indicator species are used that are present when fecal contamination is present, and are in large enough concentrations to be easily detected in environmental samples. It is assumed that fecal contamination poses the potential risk of pathogen contamination, and human-specific fecal contamination poses the greatest risk of human pathogens.

