Microbial source tracking: using old and new technologies to find out what is in our water

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Introduction

- Background
- Goals
- Sites
- Methods
- Results/Summary
- Future work and next steps

Snow Geese flyway

North Inlet Beach

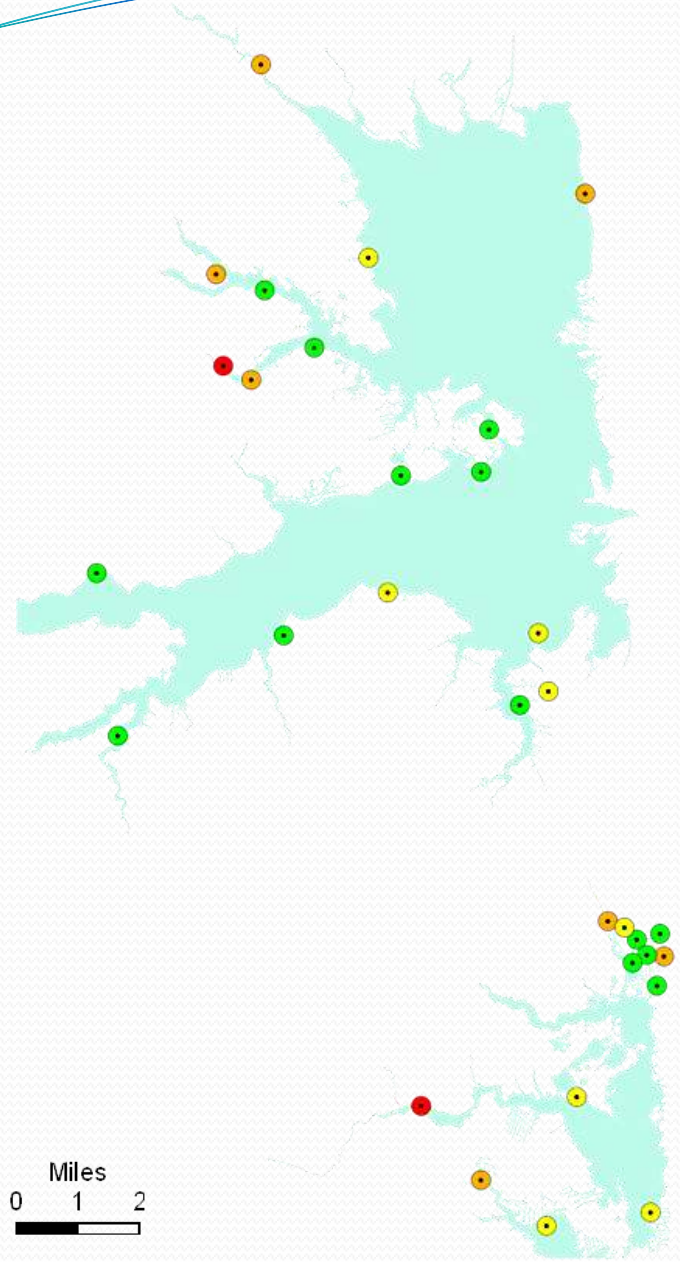
Fecal indicators of contamination

- **FIB –**
  - Non-pathogenic bacteria found in fecal waste
  - Proxies for potential enteric pathogens
  - Threshold determined by quantifiable relationship between:
    - Density of indicator bacteria
    - Health risk to those using the water

Fecal Indicator Bacteria	Recommended by US EPA in:	Threshold for contamination (colonies/100 mL)	Notes
Fecal coliforms	1976	800	
Total coliforms	1976	2400	
Total *Enterococcus*	1986	104	Used by State of Delaware
E. Coli	1986	235 (freshwater only)	Not recommended for marine waters

Fecal indicators, continued

- **Total *Enterococcus* –**
  - Used by Delaware DNREC to determine water quality
  - Guarded beaches
    - Sampled weekly during summer
    - Closed when exceed threshold
  - Non-recreational waters
    - Sampled monthly
    - Historical purposes



Map of monitoring sites as percentage of samples that exceeded the single sample primary recreational water contact standard of 104 Enterococcus cfu/100 mL in the Delaware Inland Bays from 2004 to 2008. Legend: Green = 0 – 10%, Yellow = 10-25%, Orange = 25-75%, red = 75-100%.

From:
Delaware Center for the Inland Bays Environmental Indicators Series 2009-2010, Development of the Recreational Water Quality Indicator

Chris Bason

Human Pathogens in the environment

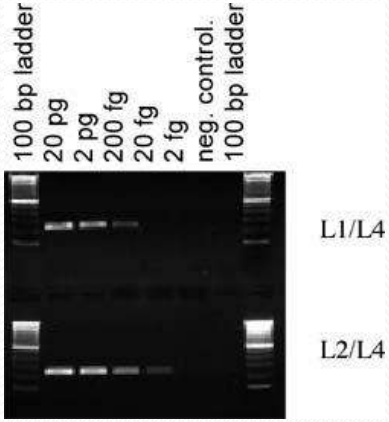
- Detection
  - Culture-based approaches
  - PCR*
  - Microarrays
- Activity
  - Not yet assayed
- Quantification
  - qPCR-based approaches
- Relationship to epidemiology

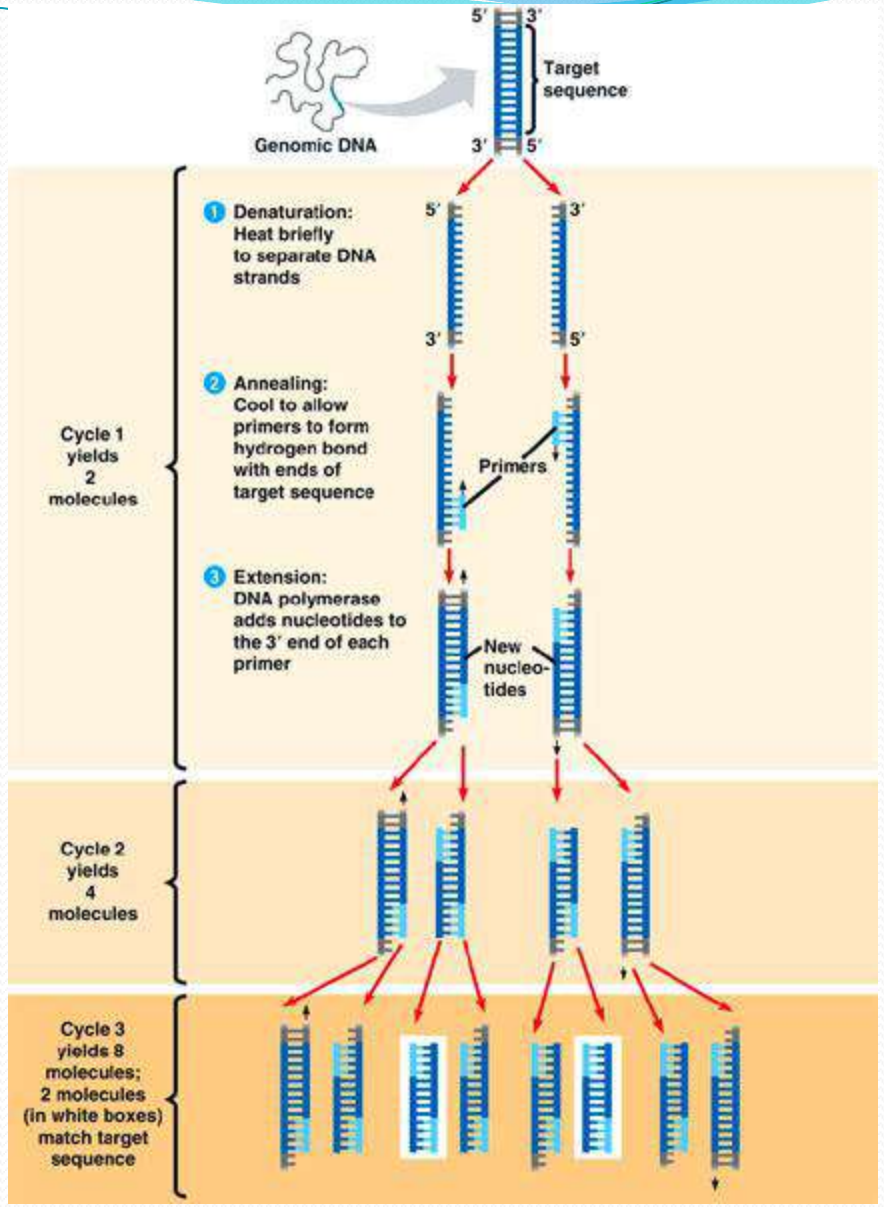
Rehoboth beach

* See next slide for explanation

What is PCR?

-Isolates and amplifies a section of DNA

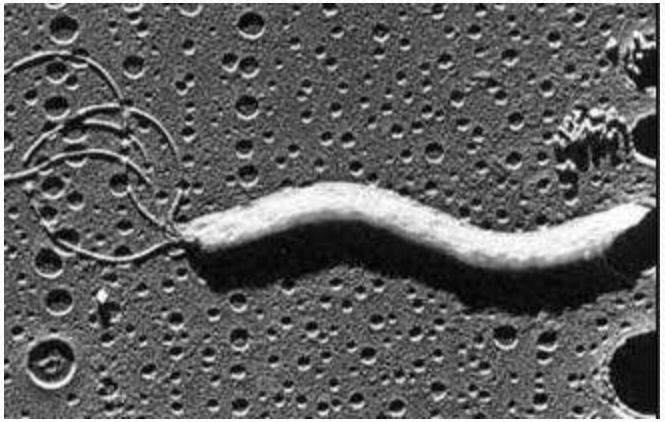




http://schoolworkhelper.net/2011/06/pcr-uses-steps-purpose/

Pathogen-like *Epsilonproteobacteria*

- ***Helicobacter* spp. –**
  - *Helicobacter pylori*
    - Asymptomatically colonizes guts of 20-80% human population in developed countries
    - Causative agent of:
      - Gastritis
      - Peptic ulcers
      - Gastric cancer
    - Presence/absence of virulence factors
  - Transmission route unknown – believed to be fecal-oral
  - Found in VBNC state and associated with zooplankton and particles



Electron micrograph of *H. pylori*
www.health.qld.gov.au

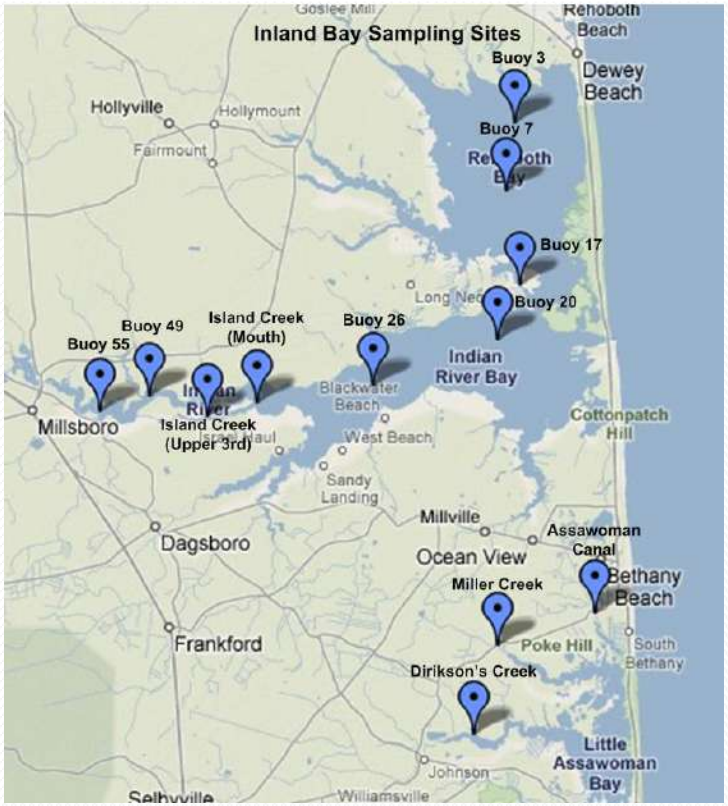
Goals of Project

- Detection of FIB and *Epsilonproteobacteria* in environment, June 2007-August 2008
  - Human-specific *Bacteroidetes* spp.
  - *Helicobacter pylori*, pathogenic *H. pylori*

- Correlation to traditional indicators of fecal pollution
- Correlation to environmental conditions

- Master's student, Katrina Twing

Sites studied

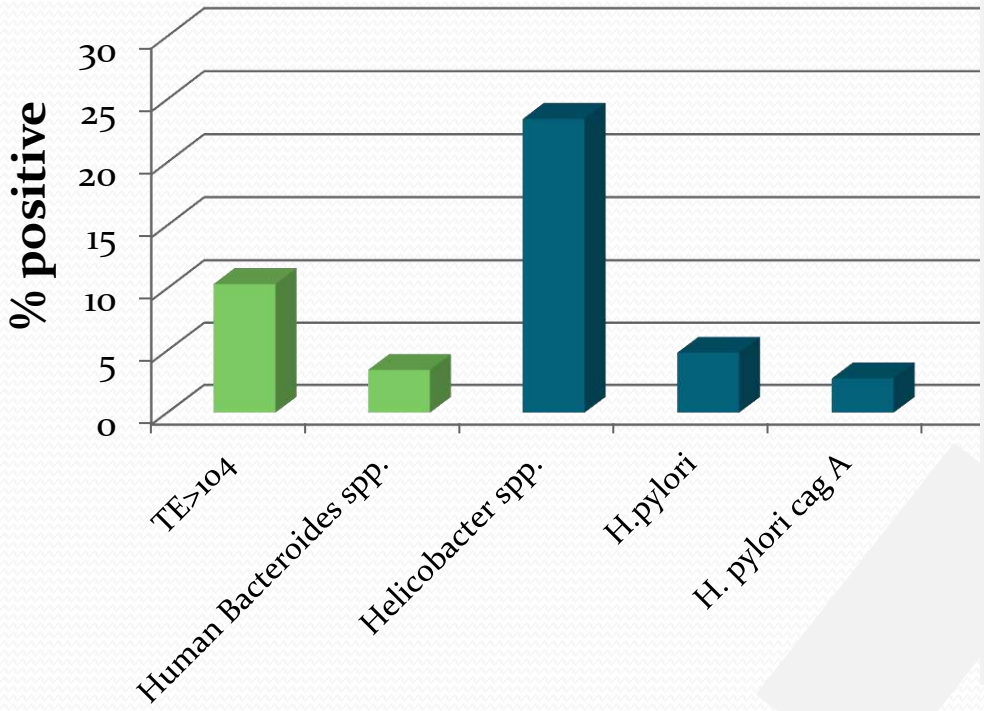


Methods

- Environmental parameters
  - Temp., salinity, DO, chlorophyll a, NO_3, NH_4, PO_4, DO
- Measure FIB via traditional methods (TE counts)
- Measure FIB using molecular methods (PCR with human-specific *Bacteroides* primers)
- Detect *Epsilonproteobacteria* via PCR (whole water)
  - *Helicobacter* spp. primers
  - *Helicobacter pylori* primers
    - 16S
    - cagA (pathogen-specific)

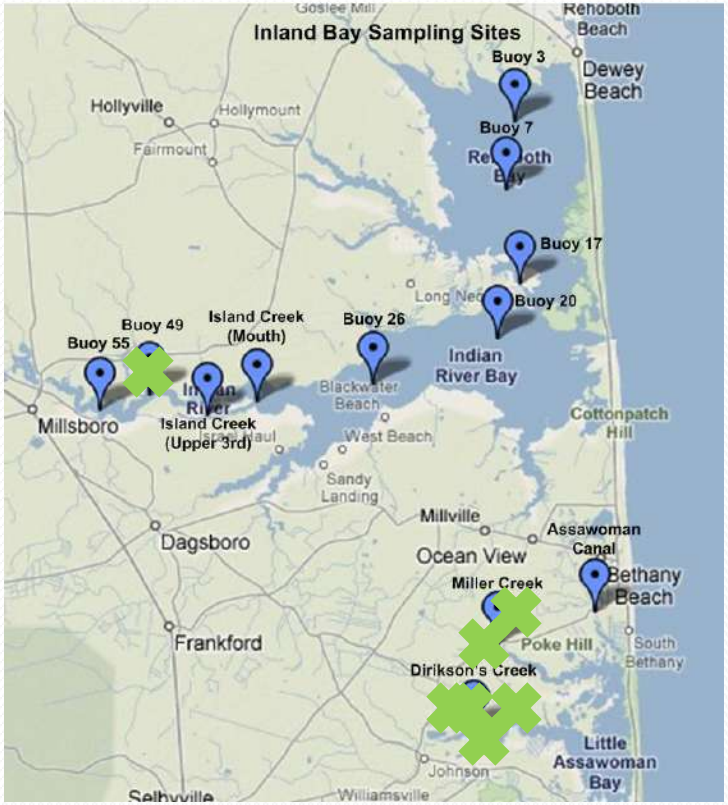
Results

- Approx. 10% samples exceed recommended FIB limit
- Less than 4% are positive for human *Bacteroides*
- About 5% are positive for *Helicobacter pylori*



N=145

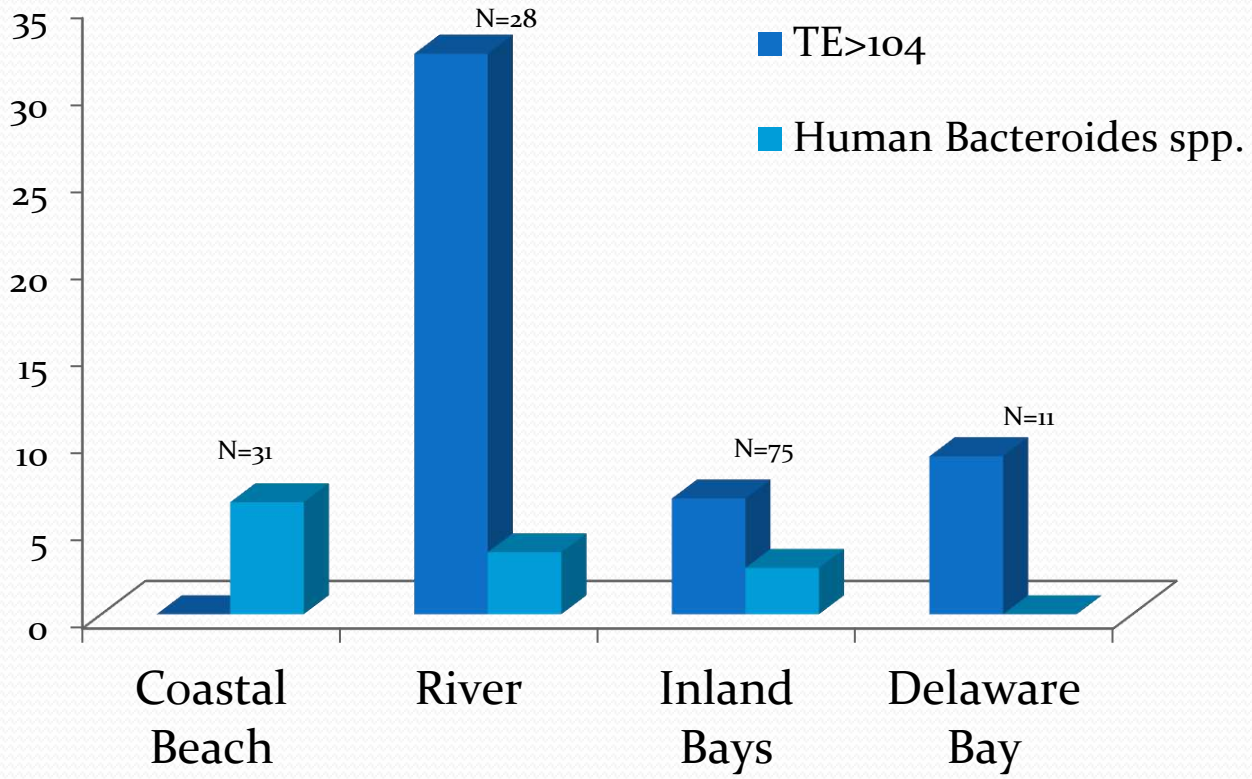
Sites studied- results



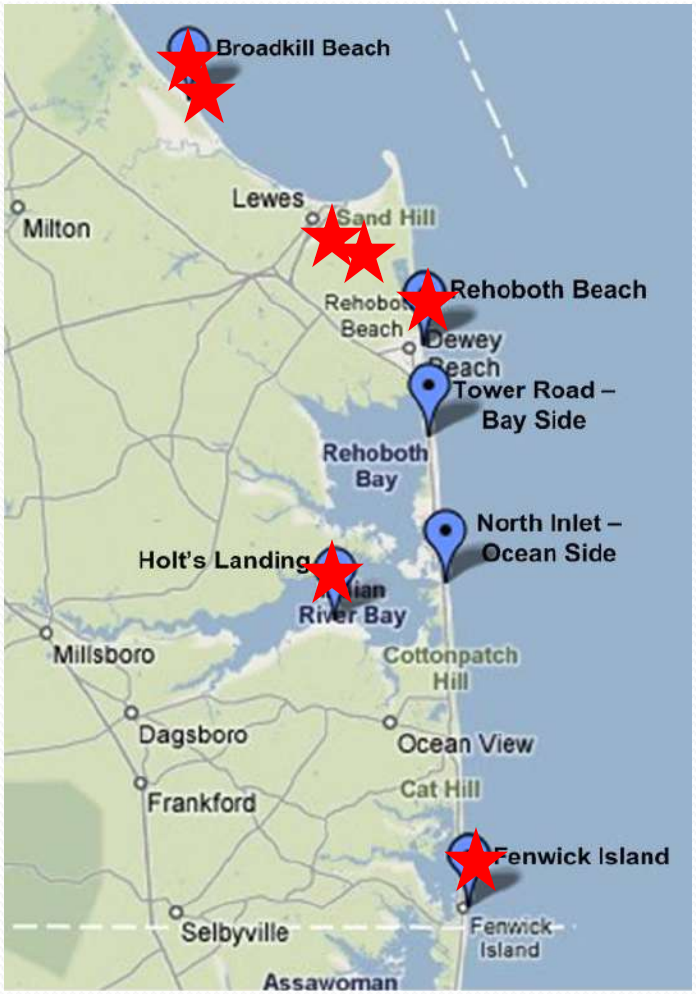
= TE >104

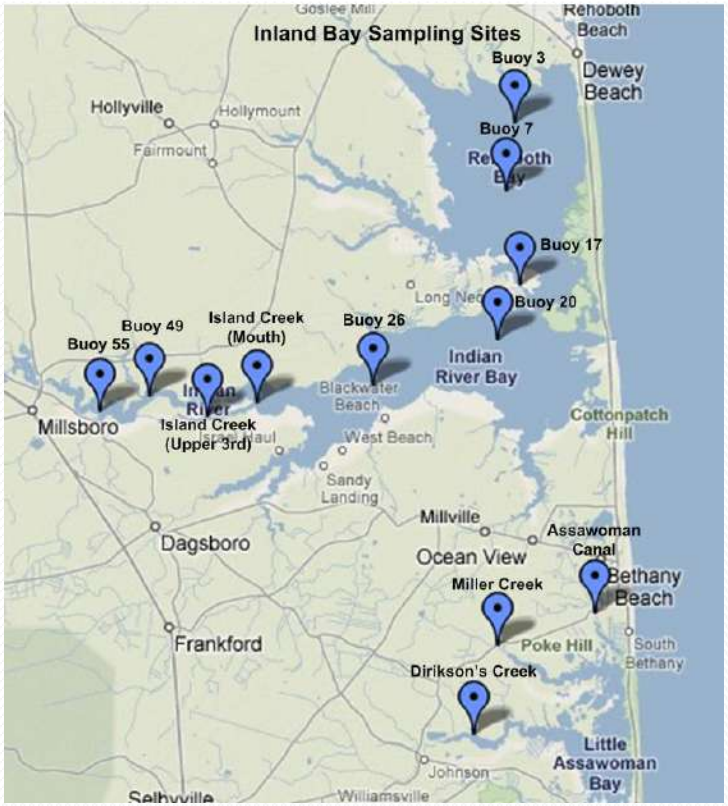
= Human *Bacteroides* spp.

Results by site type



Sites- *Helicobacter pylori*





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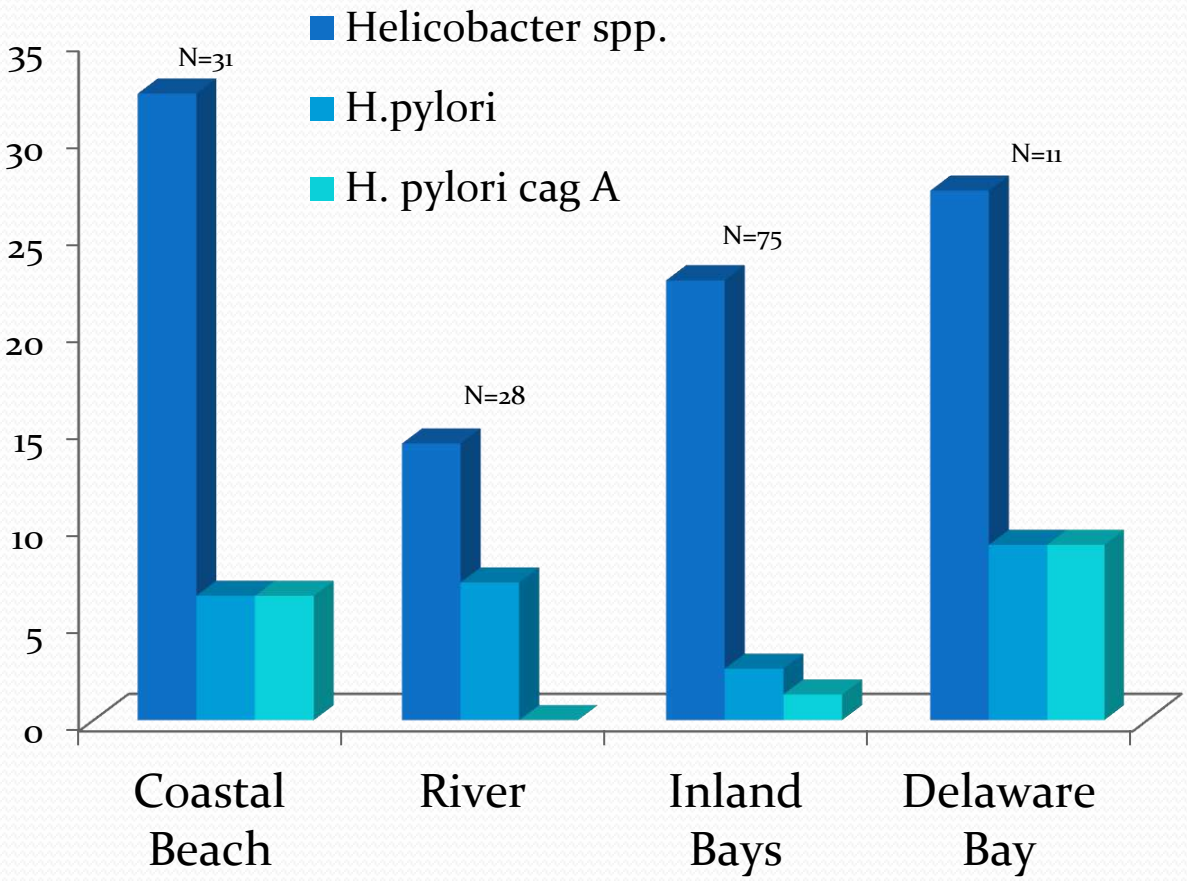
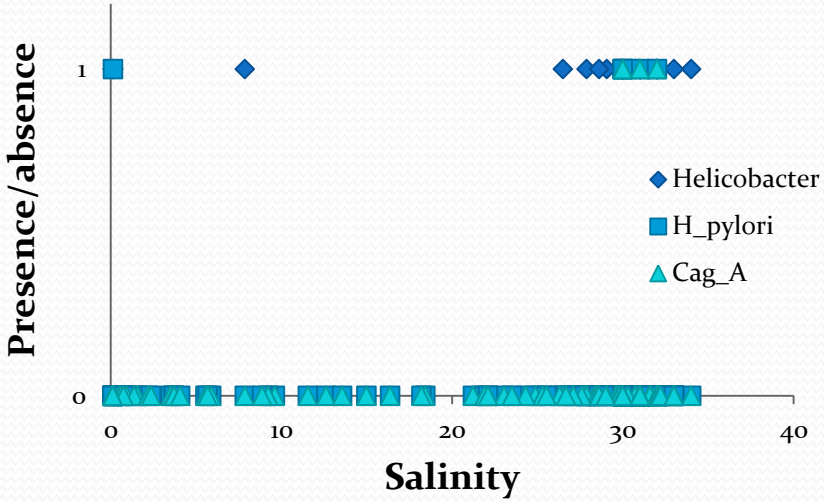


Relationship to environmental parameters

- TE counts >104
  - Low salinity
- Human *Bacteroides* spp.
  - Low nitrate/nitrite
- *H. pylori*
  - High salinity
  - Low chlorophyll a



Summary – past work

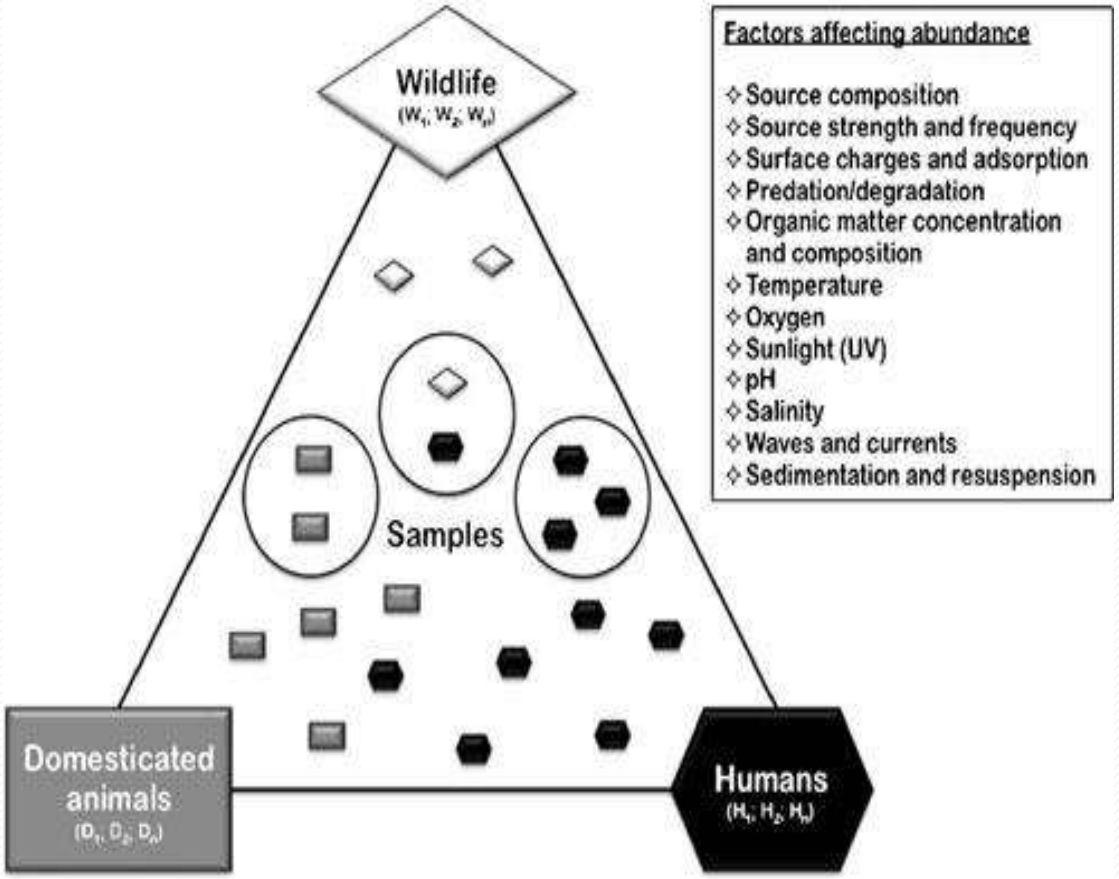
- No correlation between TE counts and the PCR detection of human *Bacteroides* or pathogenic *Epsilonproteobacteria*
- *H. pylori* and pathogen-specific *H. pylori* less prevalent, ranging from 3-9%; mostly in coastal and DE bay sites
- % positives were much greater when separated by year

What's next?

PCR based approaches are sensitive and specific, but…

- We really want to know what the source of the contamination is – is it human or animal?
  - Microbial Source Tracking
  - Use of high throughput sequencing approaches for detection and design of new quantitative PCR primers
- We really want to know how much is there and if it is viable.
  - Quantitation of contaminant by PCR from RNA

Microbial Source tracking



Sources of fecal pollution are typically mixed, but may arise mainly from a single source. Figure adapted from Figure 1 in (Roslev and Bukh 2011).

Microbial Source tracking

- **Purpose**
  - Determine the source of fecal contamination
  - Molecular methods (do not rely on culturing)
  - Indicative of potential pathogens
  - Host specificity

- ***Bacteroides* spp.**
  - Distinct genetic variation among bacteria found in different hosts
  - Strong correlation with enteric pathogens
    - Specifically *Campylobacter* spp.
  - Can not survive for long periods of time outside of host

High throughput sequencing

- **Purpose**
  - Amplify a region of the genomes of all bacteria (ribosomal RNA or ribosomal RNA gene)
  - Produce thousands to millions of sequences from a sample*
  - Analyze the data to characterize the types of bacteria in the sample
  - Can discriminate between sources of contamination, if enough known about the bacteria [sequences] found in different sources
  - Work with Jorge Santo Domingo at EPA who has collections of fecal material from animals

- **Cost effective**
  - Now generally less than $100 a sample

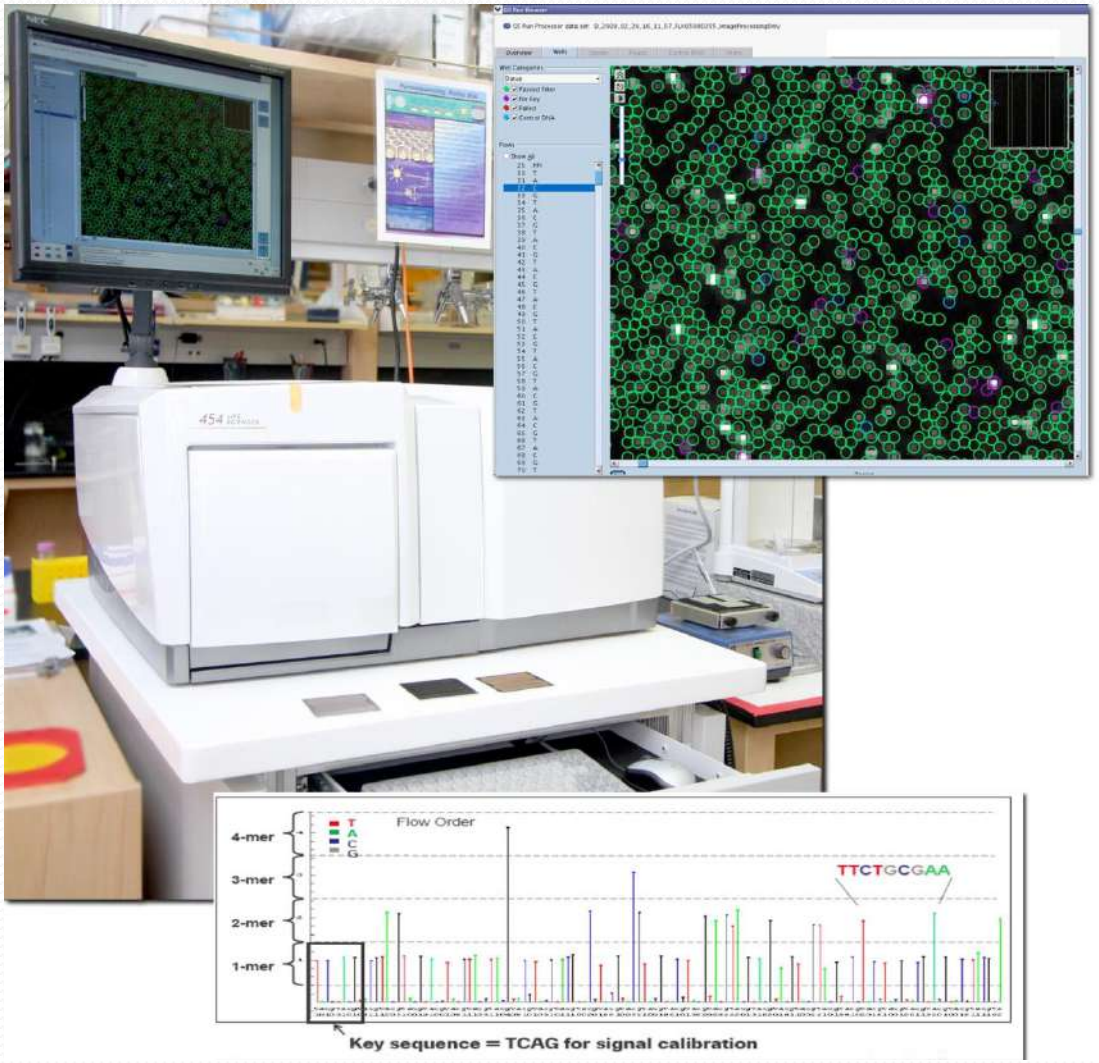
* See next slide for images and information on HTS machines

Roche 454 machine

400+ bp read length

Can multiplex easily

Output ~1 million reads/$10,000



Illumina HiSeq machine

100+ bp read lengths

Can multiplex easily

Output ~100 million reads/$1,000

High throughput sequencing

- **What to do with the data?**
  - Design PCR primers to detect and quantitate specific bacteria from different sources
  - Test on known sources, original samples and new environmental samples

Are these bacteria viable?

- Viable bacteria contain both RNA and DNA in cells
- Dead bacteria generally contain only DNA
- Sometimes have 'naked' DNA in the sample

- Two molecular ways to test for viability:
  - Use RNA instead of DNA in tests
  - Treat samples to get rid of 'naked' DNA prior to cell lysis and extraction

Acknowledgements

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  - David Kirchman
  - **Katrina Twing**
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What I would like from you

- Advise on
  - Where to collect samples
  - How often to collect samples
  - Types of potential sources
  - Archived fecal material from other animals?