# Who’s Polluting the Water? Pointing the Finger in the Right Direction

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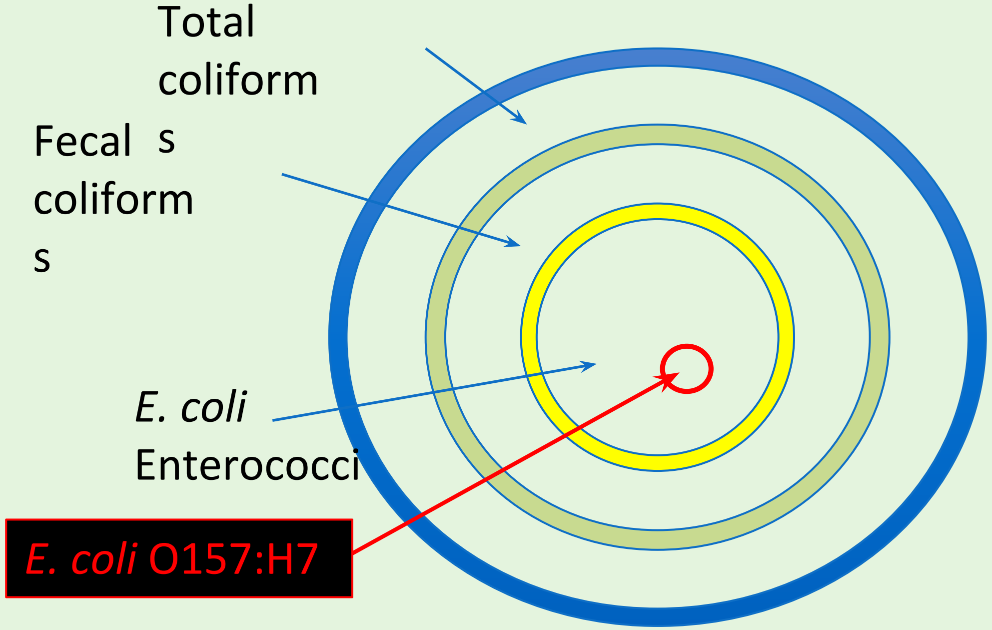
# How is Water Quality Monitored?

- Collect water samples from beaches and potential source areas
- Analyze samples for detection of fecal pollution
- Use Fecal Indicator Bacteria
  - *Escherichia coli*, **<u>enterococci</u>**, fecal coliforms
  - Highly abundant in all feces
  - <u>Not source-specific!</u>
- Compare results to standards related to public health risks
- The States of Maine & New Hampshire have set standard **enterococci** level criteria for posting advisories at marine beaches that are consistent with EPA guidelines



# When Enterococci Concentrations are Elevated…

- Water quality standards are set to guide prevention of illness above a threshold level (US EPA)
    - >30/1000 predicted GI illness
- Based on epidemiological studies
    - Significant human/sewage source
- Current regulations:
    - Advisories
    - Closures
    - TMDLs

Welcome!
This beach is
part of the Maine Healthy
Beaches Program.

ATTENTION
Swimming
and water contact
activities are not
advised at this time

Think Healthy. Act Healthy. Swim Healthy.

Symptoms of water-related illness can include stomachache, vomiting, diarrhea, and infections of ear, eye and skin. If you have any of these symptoms within 7 days of swimming and suspect they were due to water contact at a Maine beach, notify your physician and contact the Maine Department of Health & Human Services (800)821-5821. Risk of illness may be greater for people with compromised immune systems.

Water quality can change unexpectedly. Swim at your own risk.

For more information visit:
www.mainehealthybeaches.org

Or contact:

NOTICE

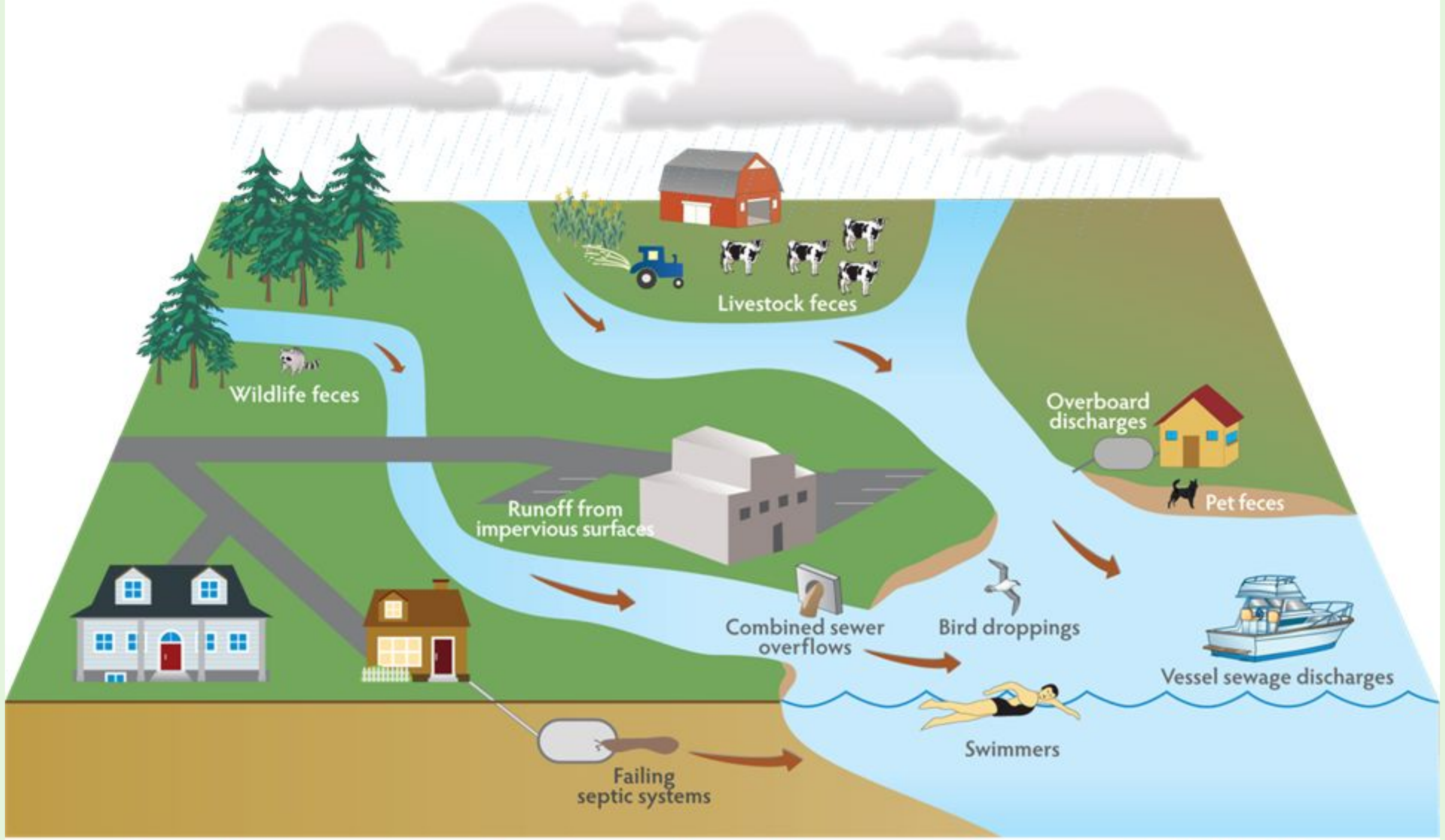
THIS BEACH AREA MAY NOT
BE SUITABLE FOR SWIMMING
DUE TO
HIGH LEVELS OF BACTERIA

Per Commissioner
NH Department of Environmental Services

Please:
- Do not drink the water or let children drink the water!
- Do not wade or swim in the water!

# What is Microbial Source Tracking?

- Usage of molecular-based techniques to determine the source of fecal-associated bacterial contaminants
- Evaluate fecal contamination in water from humans, domestic animals, wildlife sources



Disease-causing pathogens can enter Casco Bay coastal waters from multiple sources, leading to potential public health risks.
Illustration by Waterview Consulting including symbols adapted from the Integration and Application Network, University of Maryland Center for Environmental Science.

# Why use Microbial Source Tracking?

- Minimize human health risks
  - Contact with pathogens from fecal contamination can cause illnesses, often gastroenteritis
- Keep beaches open
  - Avoid economic losses associated with closed waterways
- Gain insight on pollution sources in areas with unacceptable water quality
- Save resources
  - Identify the most important pollution sources

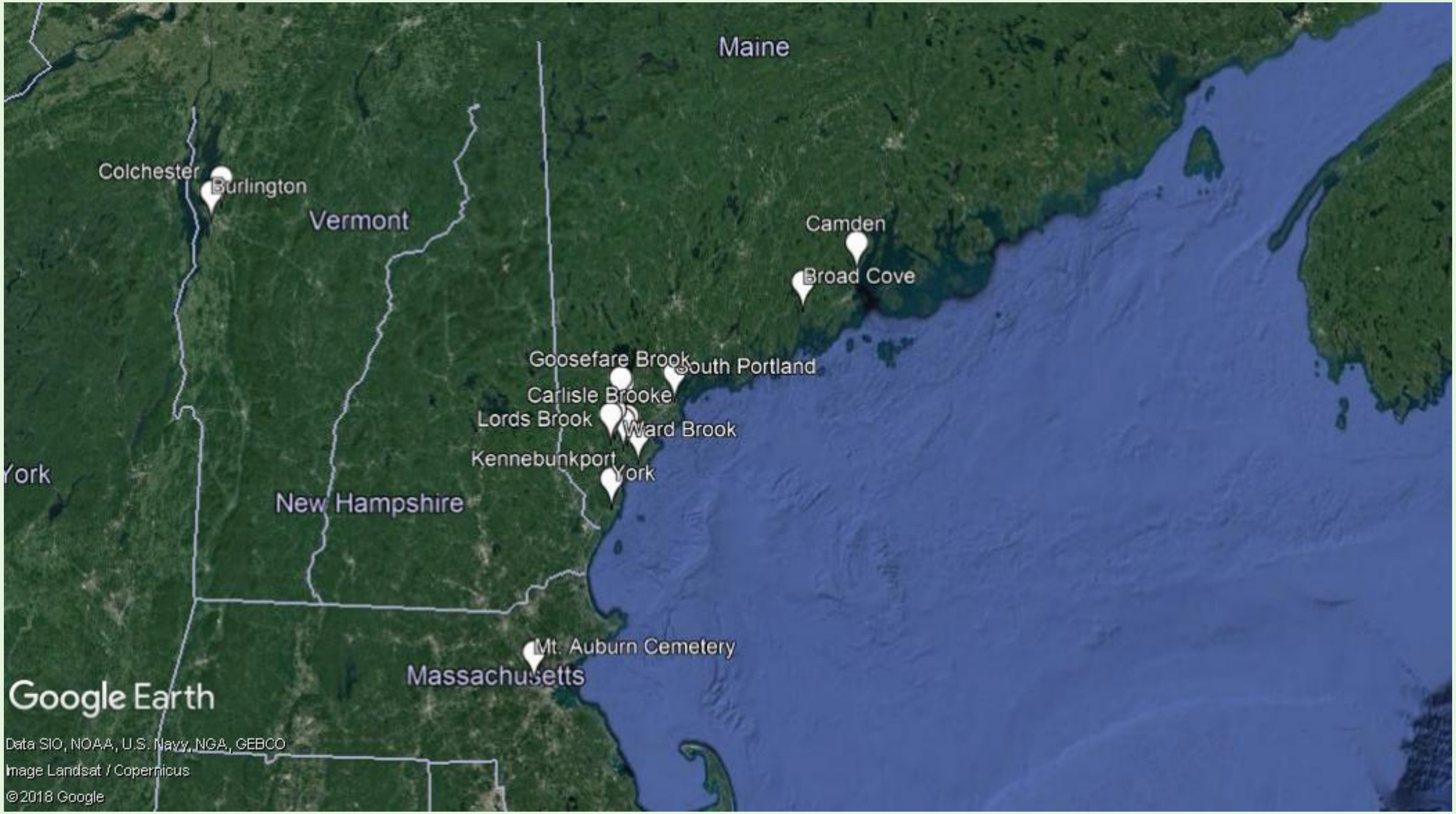
# Microbial Source Tracking Techniques at UNH-Jones Lab

- Polymerase Chain Reaction (PCR)
  - Utilize known DNA sequences of animal-associated fecal bacteria (typically Bacteroidales) to develop primers
  - **Determine presence/absence** of specific sequences
    - TARGETS: Mammal, human, ruminant, cow, horse, dog, bird, gull
- Quantitative Polymerase Chain Reaction (qPCR)
  - Addition of measurable fluorescence to **determine relative concentration** of specific sequences
    - TARGETS: Mammal, human, bird

# Current MST Pipeline

1. Client collects water samples from areas of concern
   - high Enterococci/fecal coliform levels, contaminated storm drains, high urbanization, leaky sewage conveyance system, concentrated animal areas (pets, livestock, wildlife)
2. Water samples are shipped or driven (cold) to UNH
3. Water samples are filtered to catch all bacterial cells
4. DNA is extracted from the bacterial cells caught on filters
5. DNA is tested with PCR and/or qPCR with QA/QC & controls
6. Results are submitted to client

# MST Study Sites: 2017-18



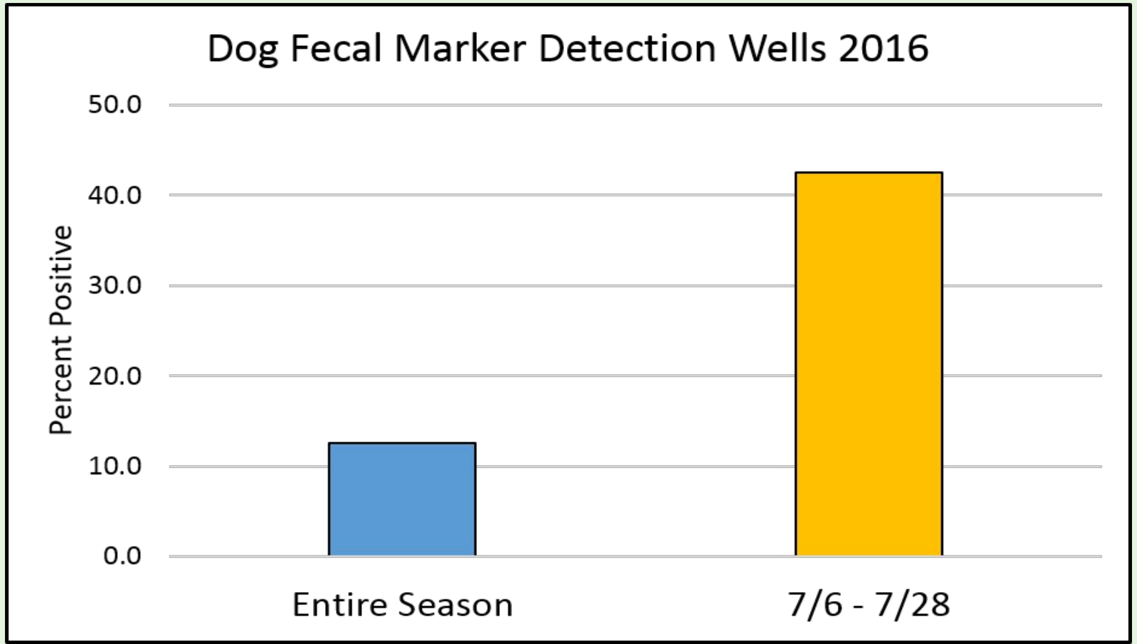
# Targeted sources, sample numbers and % positive PCR results: 2017-18

| Year | Mammal | Human | Gull | Dog | Ruminant | Bird | Horse |
|---|---|---|---|---|---|---|---|
| 2017 | 53/53<br>(100%) | 43/53<br>(81%) | 0/17<br>(0%) | 6/34<br>(18%) | 0/9<br>(0%) | 20/33<br>(61%) | N/A |
| | | | | | | | |
| 2018 | 10/11<br>(91%) | 4/11<br>(36%) | 3/17<br>(18%) | 20/64<br>(31%) | 0/54<br>(0%) | 3/11<br>(27%) | 0/5<br>(0%) |

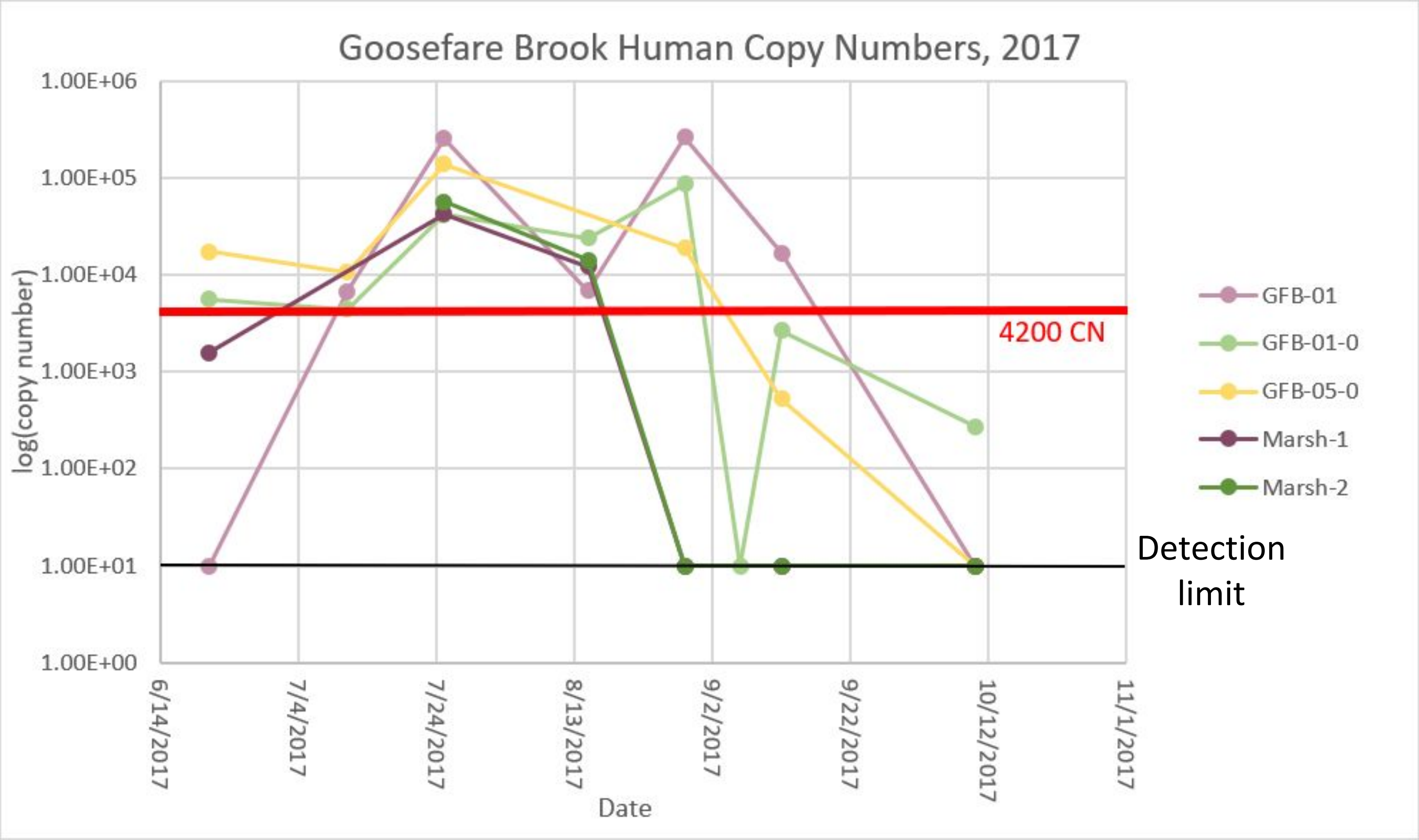
*These results are influenced to some extent by what target sources were requested for analysis, based in part on past knowledge and evidence for suspected sources in the study areas.*

# Positive PCR results by month: 2018

| Month | Mammal | Human | Gull | Dog | Ruminant | Bird |
|---|---|---|---|---|---|---|
| June | 8/8 (100%) | 8/8 (100%) | 0/8 (0%) | 0/8 (0%) | 0/5 (0%) | 3/6 (50%) |
| July | 14/14 (100%) | 13/14 (93%) | 0/3 (0%) | 1/8 (13%) | 0/0 (0%) | 7/9 (78%) |
| August | 12/12 (100%) | 10/12 (83%) | 0/2 (0%) | 1/5 (20%) | 0/0 (0%) | 4/5 (80%) |
| September | 10/10 (100%) | 8/10 (80%) | 0/0 (0%) | 1/6 (17%) | 0/0 (0%) | 3/6 (50%) |
| October | 9/9 (100%) | 4/9 (44%) | 0/4 (0%) | 3/7 (43%) | 0/4 (0%) | 3/7 (43%) |

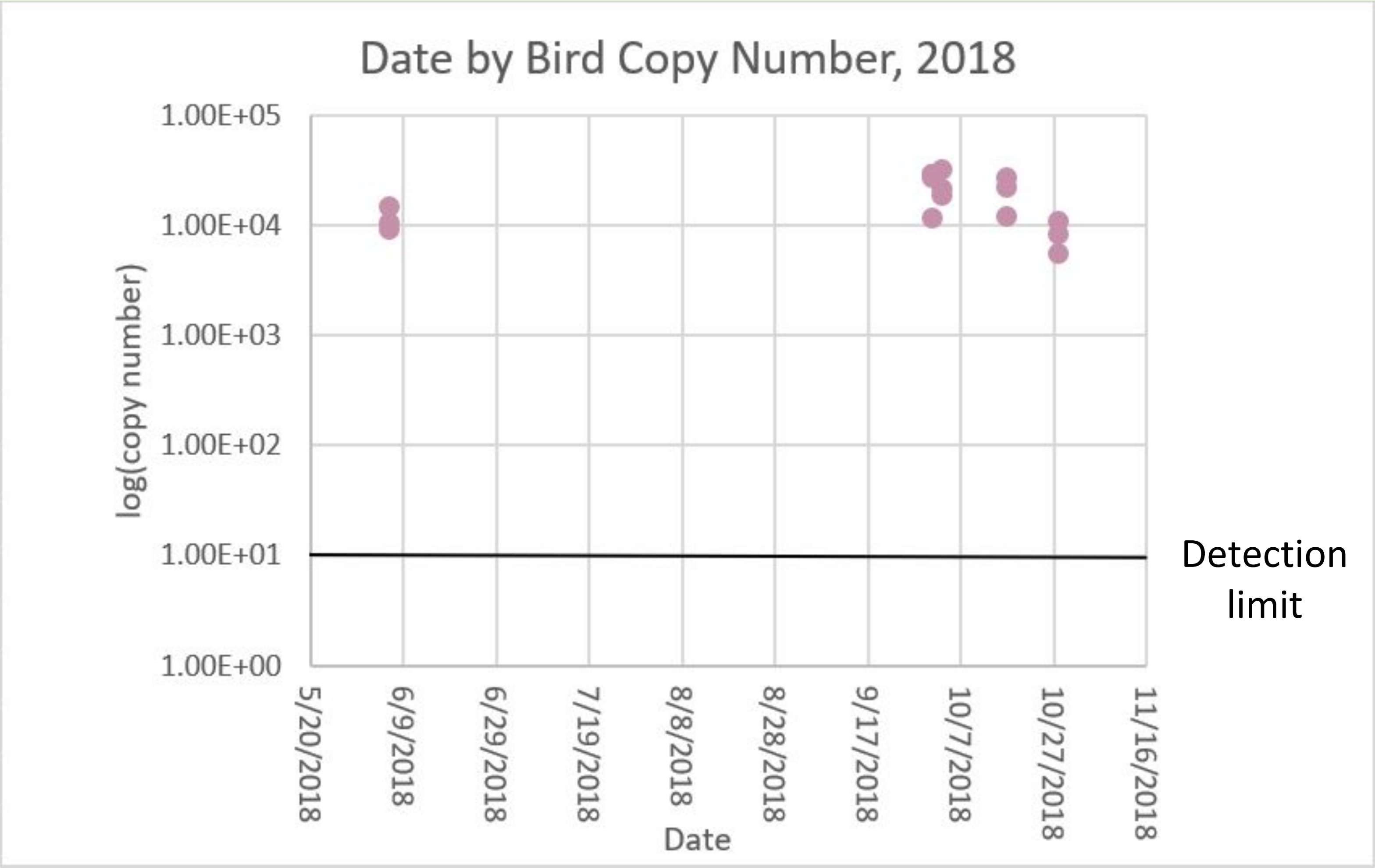


# All Human qPCR copy number data were above EPA illness threshold (4200/100 ml: red line) throughout July and August



Detection limit

# Water samples with Bird detection by qPCR had relatively high copy numbers throughout 2018- *What is the public health significance of these numbers?*



# Conclusions

- Fecal contamination should be monitored over time (multiple collections from the same sites)
- Humans, dogs, and birds have been the most common contributors to fecal contamination of study area waterways
  - Humans during summer
  - Birds likely throughout the year, peak in summer
  - Dogs late summer- fall, though potentially more episodic presence (2016)
  - Dog qPCR assays might be a beneficial analysis to add

# What is the relationship between different fecal sources identified in the environment and the presence of actual bacterial pathogens?

# …do specific fecal sources relate to differential levels of potential pathogens?

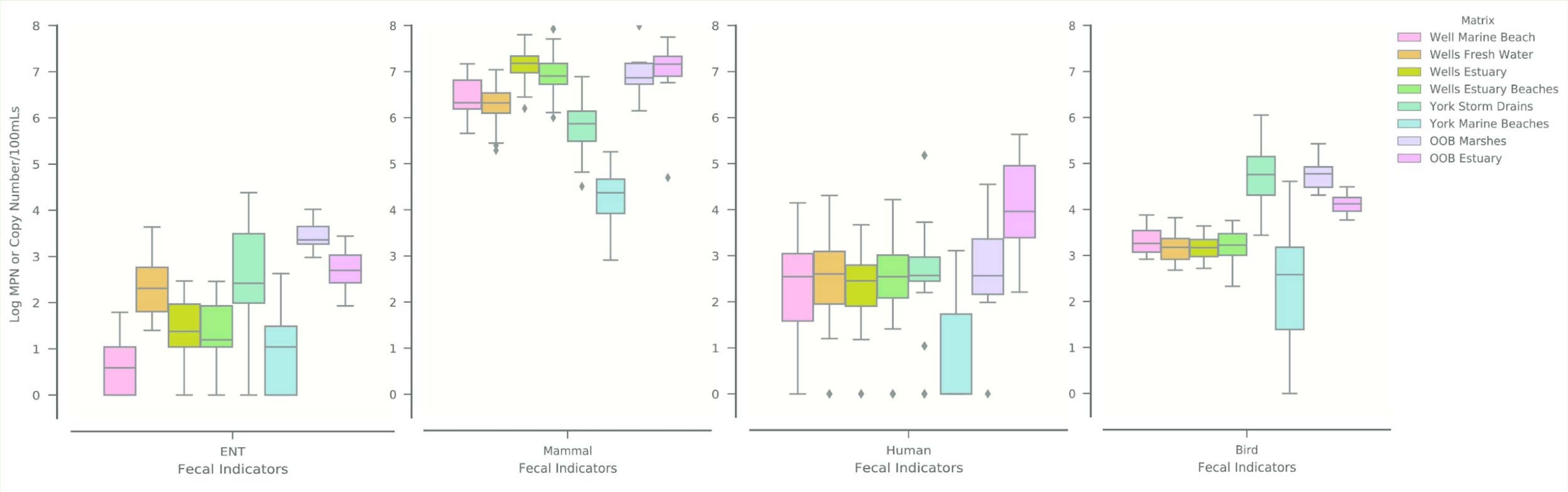
- Limited research has been conducted on the relationships between fecal sources and a full range of potential pathogens in water.
  - There is a wide range of potential bacterial (and viral & protozoan) pathogens
  - Researchers are now applying bacterial community and targeted DNA sequence analysis approaches to identify potential pathogens in water.

# Study Areas (2015-2016)

- York, ME
  - Storm drains & beach water
- Wells, ME
- Old Orchard Beach
  - Marsh and tidal river

# Regional Differences in Enterococci and MST Fecal Markers

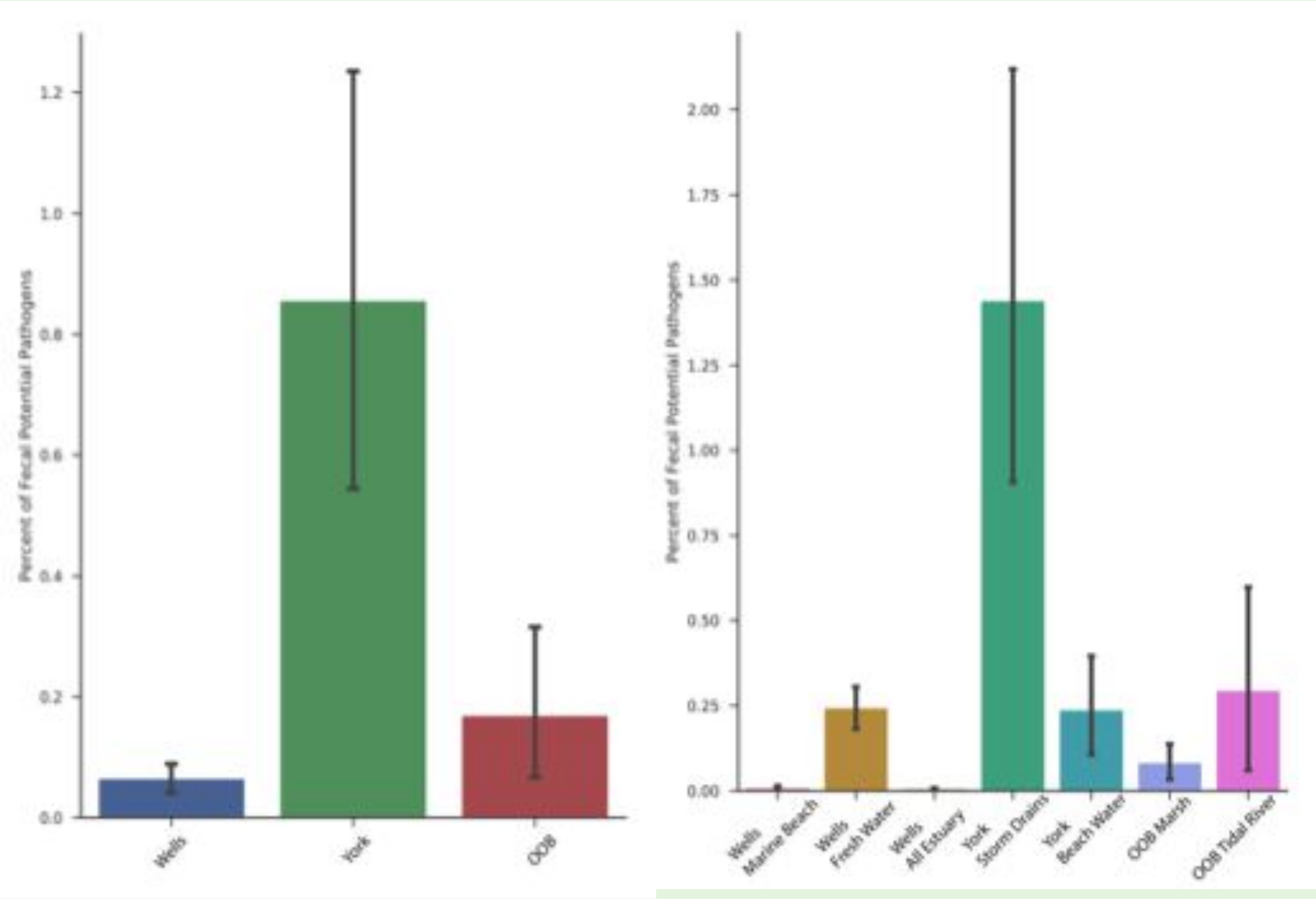
- Regional comparisons aid interpretation of MST study results
- Old Orchard Beach study & York storm drains – elevated concentrations



***Do elevated levels of enterococci and/or MST fecal markers correspond to an increase in levels of potential pathogens?***

# Study and Water-Type Pattern

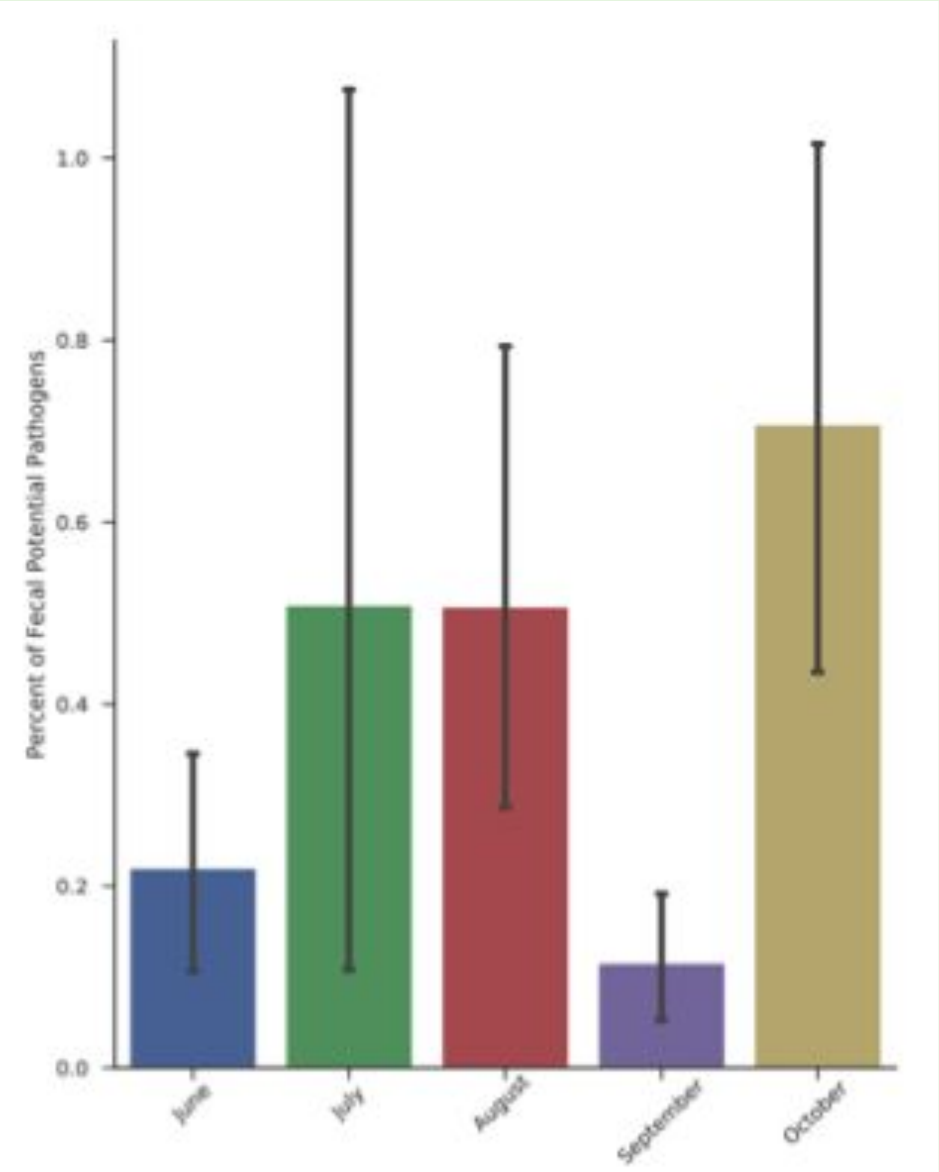
- Study location and water-type are important factors
- **Storm drains** contained the highest percent (1.4%) of fecal potential pathogens



# Monthly detection of fecal specific potential pathogens and relationship to fecal source

- Elevated fecal potential pathogens during July, August, & October;
- Significant relationships between fecal potential pathogens and enterococci;
- Significant relationship with human fecal source (August and October);
- Bird only marker to show significant relationship every month to percent of fecal potential pathogens

| ENT or Fecal Source | $r^2$ | p-value |
|---|---|---|
| **Enterococci** | **0.25** | **< 0.05** |
| Mammal | -0.00012 | 0.99 |
| Human | 0.22 | 0.082 |
| **Bird** | **0.27** | **<0.05** |



# Study Limitations

- Not detecting actual pathogens or genetic traits
- True pathogens are likely lower in concentration
- First time applying method to recreational waters
- Results indicate interesting trends
- Further Shotgun sequencing for identifying actual pathogens

# Study Conclusions

- **Total** potential pathogens (included *Vibrio* spp. as dominant pathogen in saline water) showed no relationship to enterococci or fecal source concentrations
- **Fecal specific** potential pathogens were significantly related to enterococci and bird fecal source concentrations
- Study location and water-type were important considerations- **<u>storm water</u>** was the most significant factor

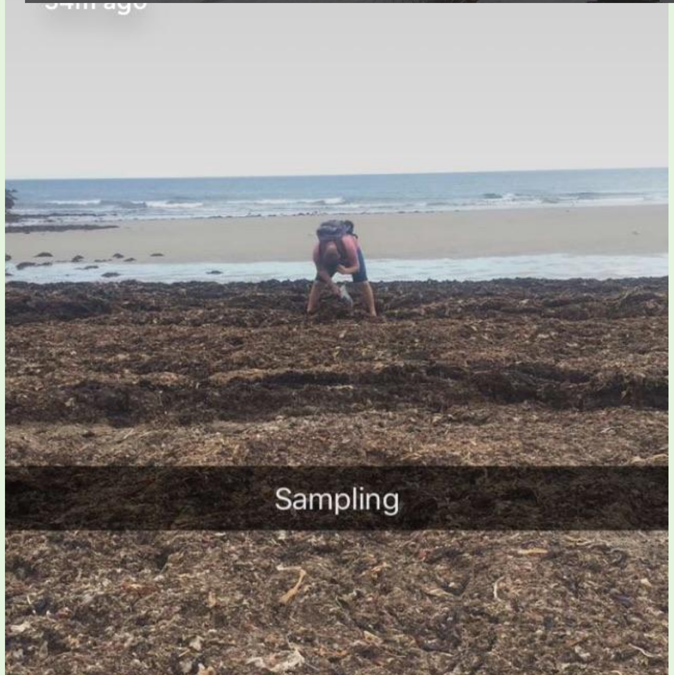
# Acknowledgements

- Derek Rothenheber
- Meagan Sims and the MHB Program
- Towns of York, Wells, Old Orchard Beach, Saco, Kennebunkport and others
- Jones Lab field and lab technicians

MAINE Healthy Beaches

Sea Grant New Hampshire

NEW HAMPSHIRE EPSCoR



Questions?