Improving Community Health through Microbial Source Tracking

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Sinéad Ní Chadhain,
with Ania Brown, Penny Demetriades, Bree Janssen

Stakeholder Meeting 6

November 1, 2024

Stakeholders & Community Partners

- Alabama Association of Conservation Districts
- Alabama Department of Conservation & Natural Resourc
- Alabama Department of Environmental Management
- Alabama Marine Mammal Stranding Networl
- Auburn University Shellfish Laboratory
- City of Mobile
- Dog River Clearwater Revival
- FDA Gulf Coast Seafood Laboratory
- Grand Bay National Estuarine Research Reserve
- Mississippi-Alabama Sea Grant Consortium
- Mobile Bay National Estuary Program
- Mobile Baykeeper
- Navy Cove Oyster Company
- The Nature Conservancy
- The Water Institute of the Gulf
- Weeks Bay National Estuarine Research Reserve

Objectives

1. To **define microbial sources** to Alabama waters, with the goal to distinguish at least wastewater treatment plant, septic, wildlife (boar/hog), livestock (chicken, cow), and other non-human (dog) inputs.

2. To **define the conditions, location and timing of indicator microbe influences**, specifically including factors used in determining shellfish area closures.

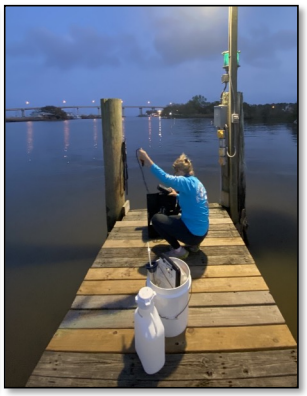
3. To **facilitate data sharing** using an existing online platform, "Our Wastewater Footprint" https://www.disl.org/wastewaterfootprint

4. To **enhance local capacity** for enduring advanced microbial source tracking.

Project updates: Objectives 1 & 2

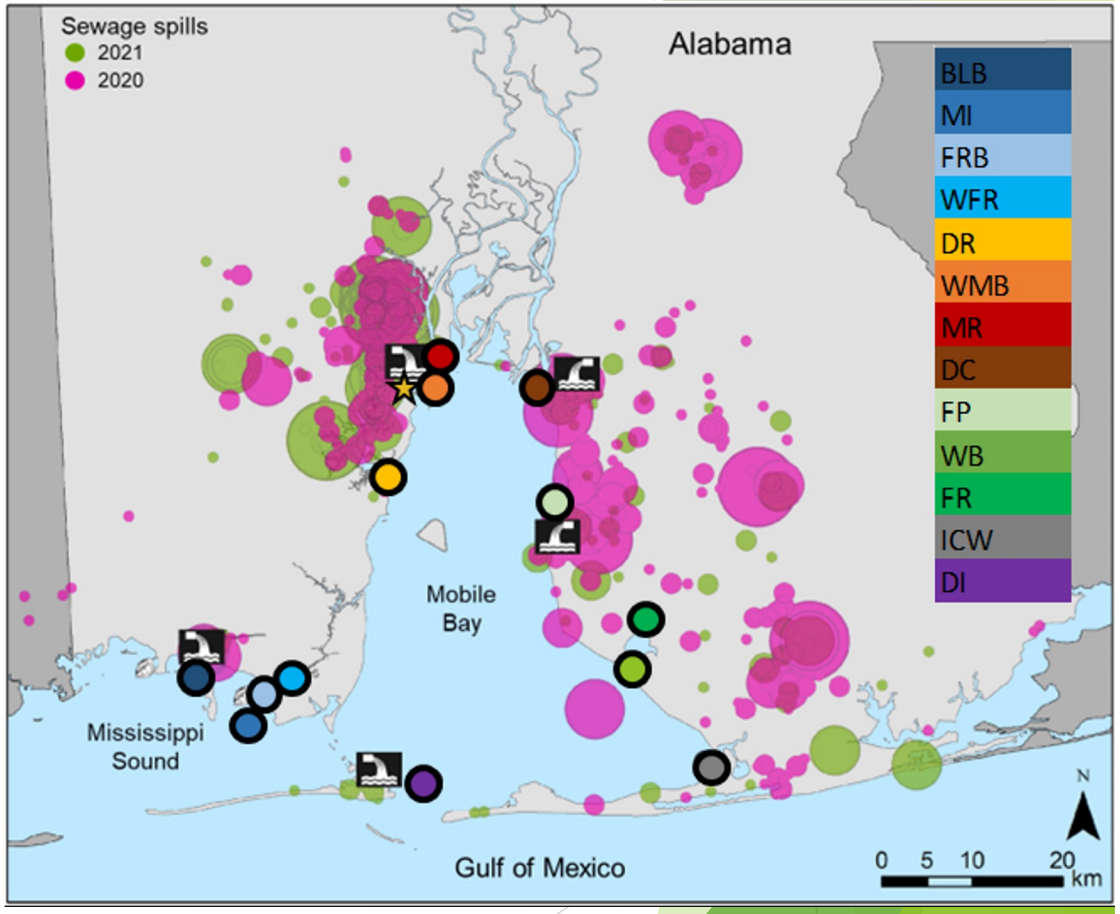
Ania Brown
Penny Demetriades

Project Overview

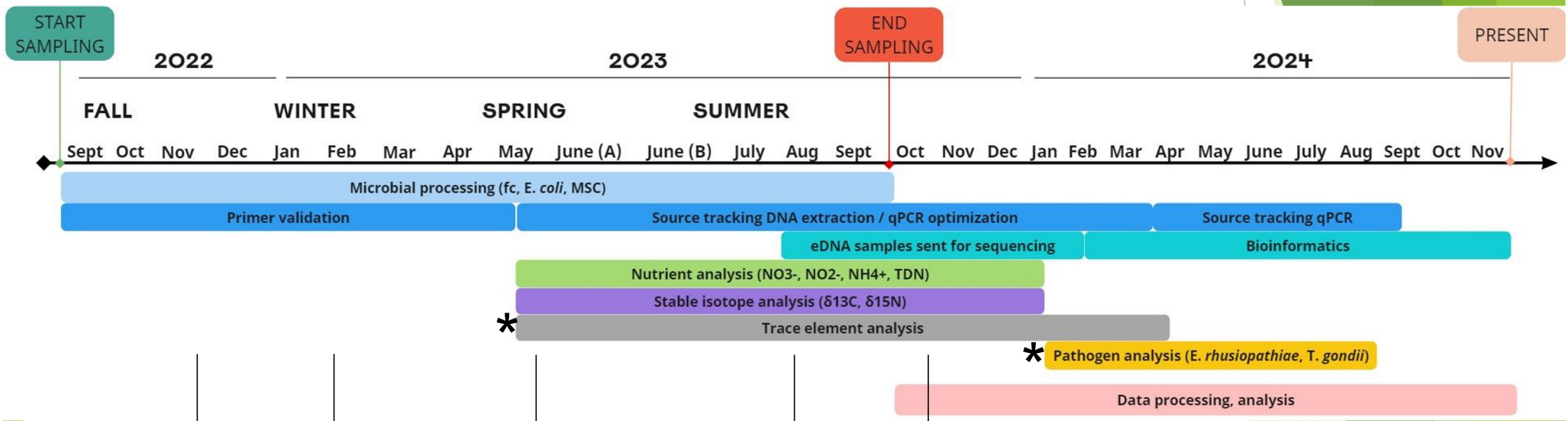


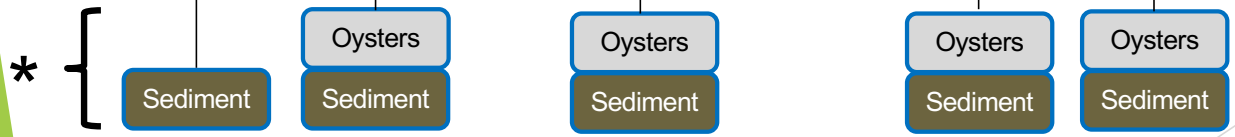
Water Sampling

- 1 year (2022-2023), 4 seasons
- Monthly (4-week intervals); 14 sampling events
- 13 sites (Mobile Bay, EMSS)



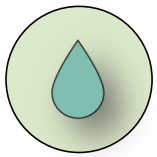
Project Timeline





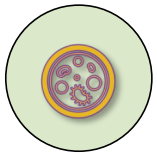
***Resources of current project leveraged to gather additional data**

Project Overview



DISCHARGE/ SALINITY

NUTRIENTS/ STABLE ISOTOPES



INDICATOR MICROORGANISMS

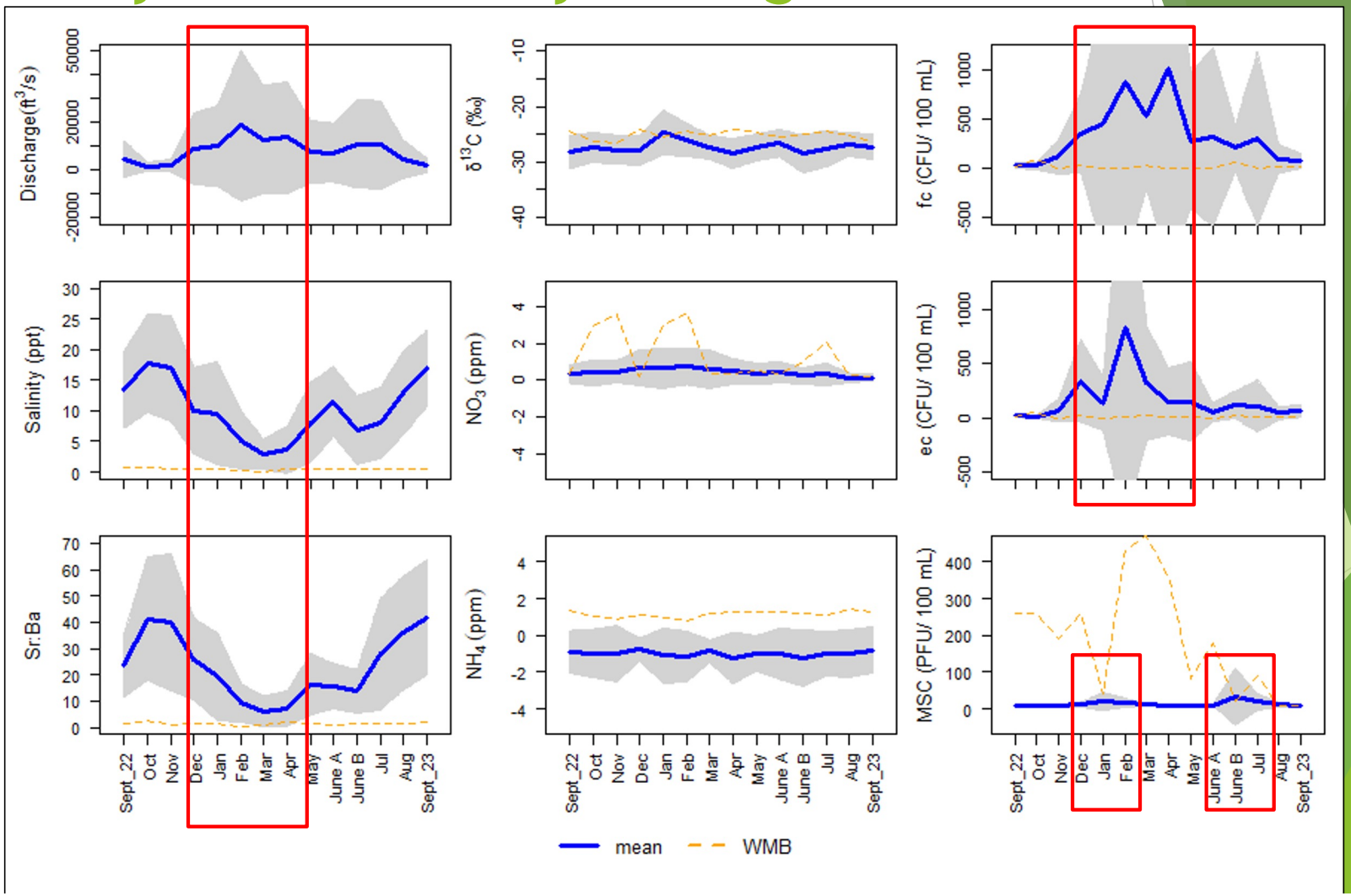
SEASONAL VARIATION

SPATIAL VARIATION

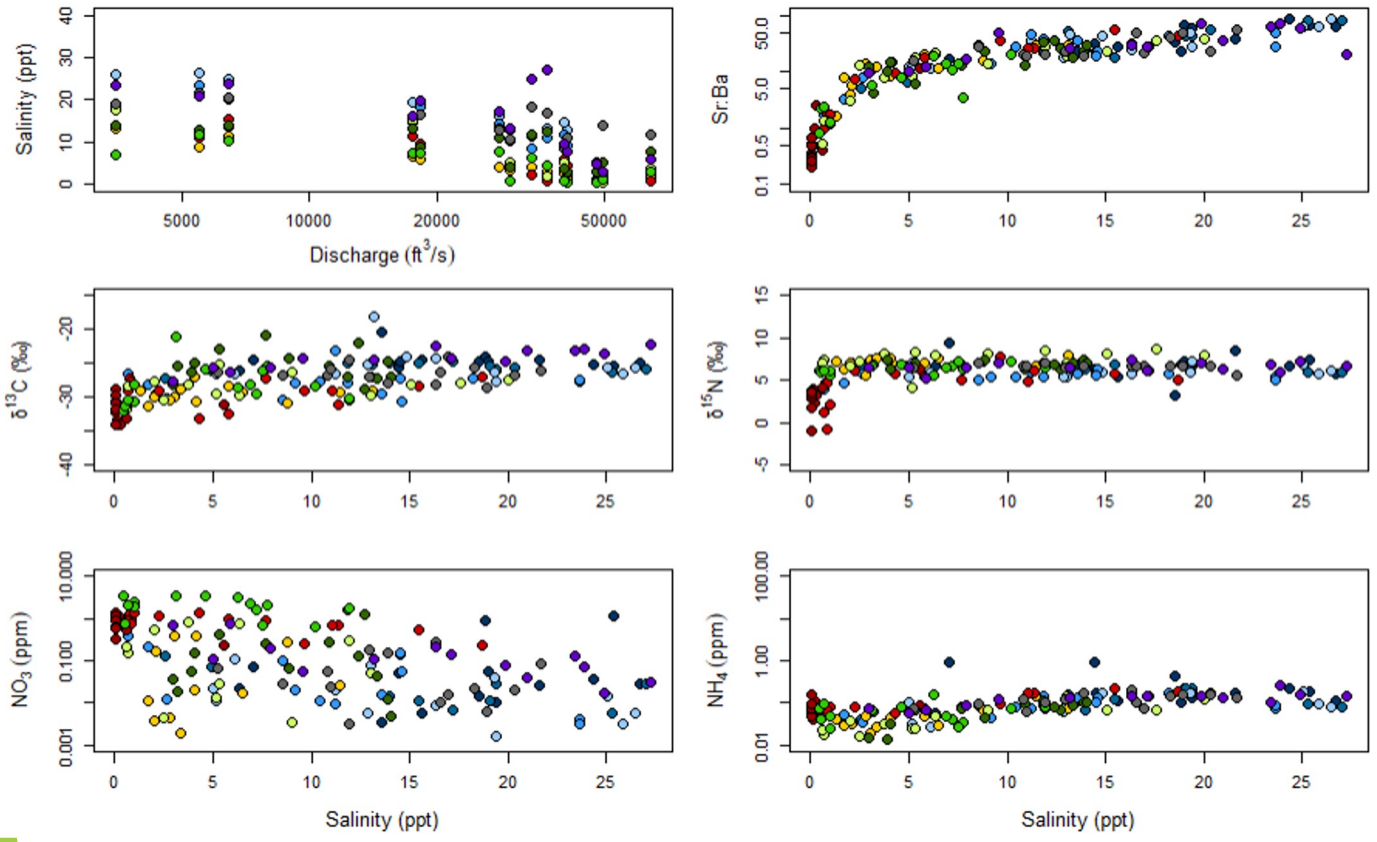
Water Sampling

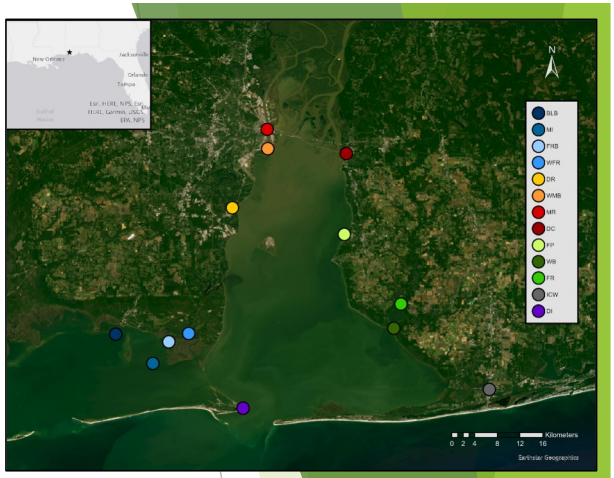
- 180 distinct sampling events
- 7 sites with local discharge data
- 180 samples fc, ec, MSC
- 360 samples nutrients (pseudo-replicates, averaged)
- 360 samples stable isotopes (only 180 analyzed)

Preliminary data: Monthly averages



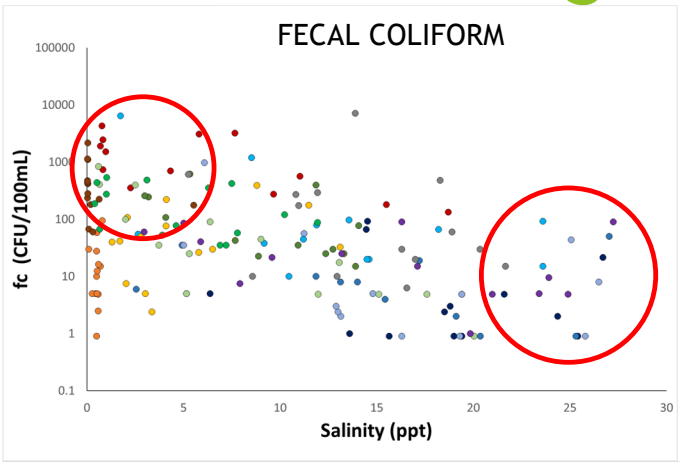
By site: Salinity, SI, and nutrients





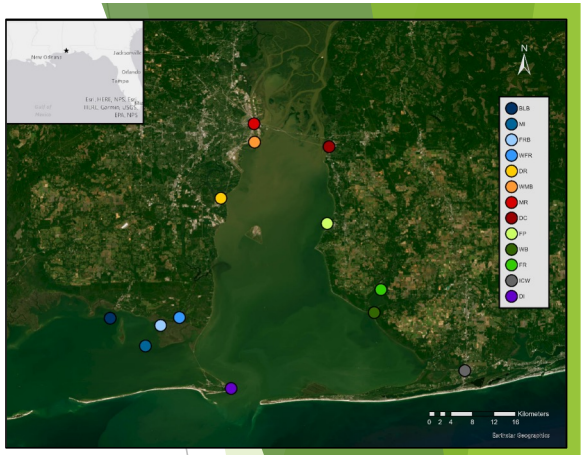
BLB MI FRB WFR DR WMB MR DC FP WB FR ICW DI

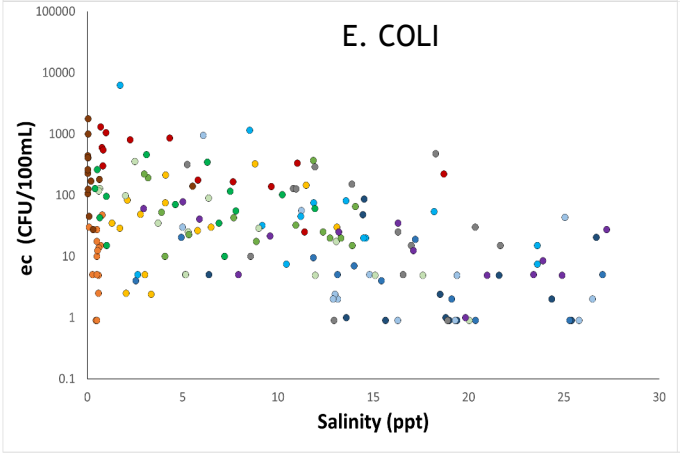
Indicator microorganisms

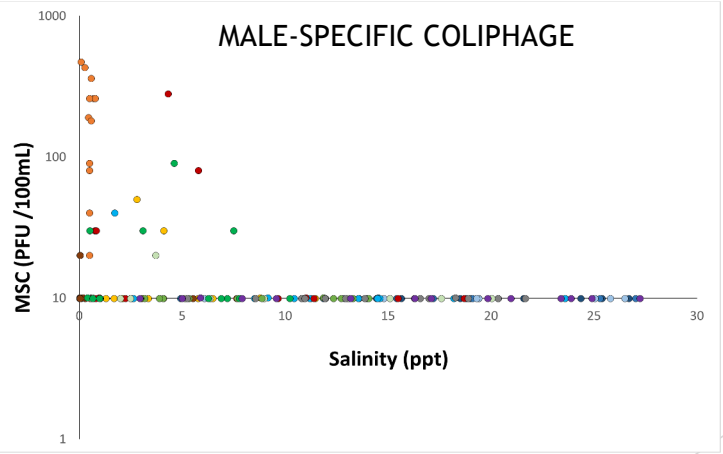


salinity Freshwater

Indicator microorganisms







BLB MI FRB WFR DR WMB MR DC FP WB FR ICW DI

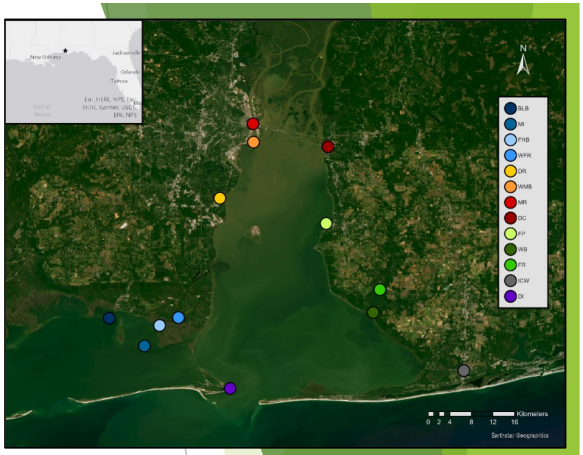
Multiple Indicators: SI, Nutrients

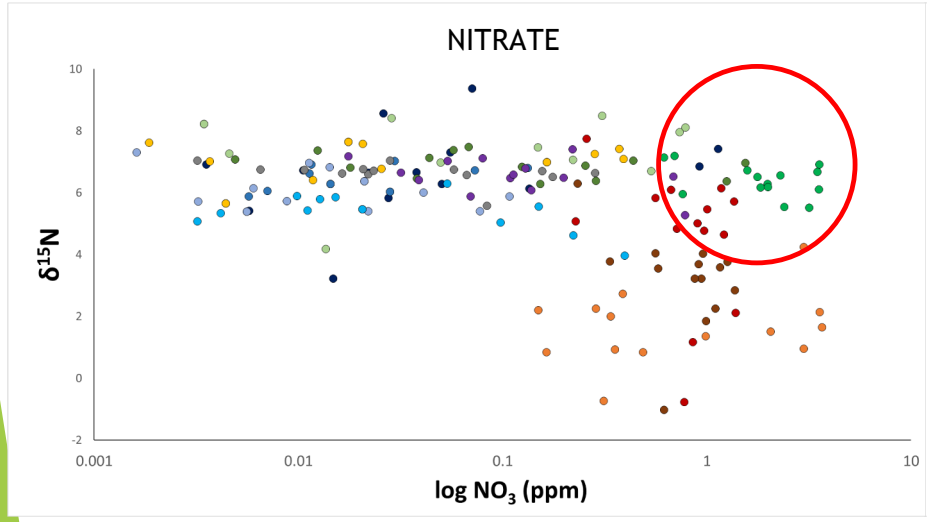
↑ NO_3 ↑ $\delta^{15}N$

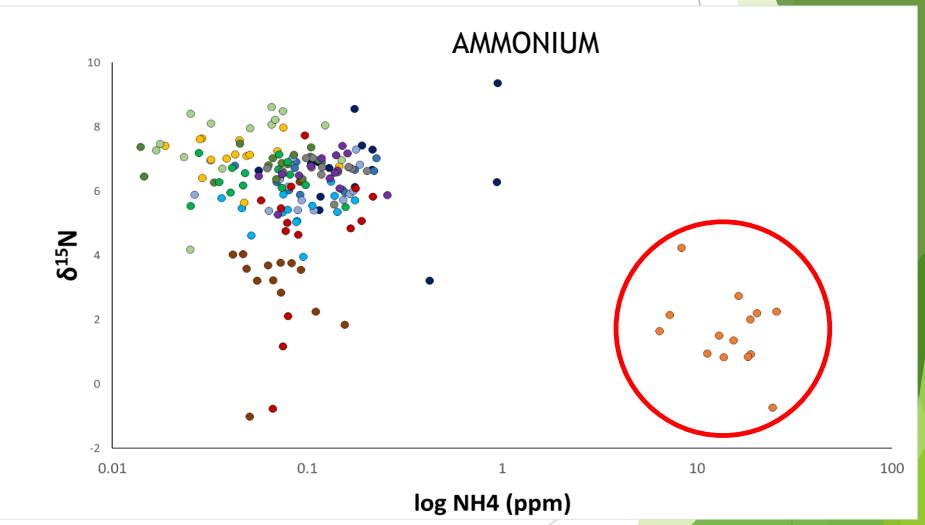
processed waste

↑ NH_4 ↓ $\delta^{15}N$

raw waste



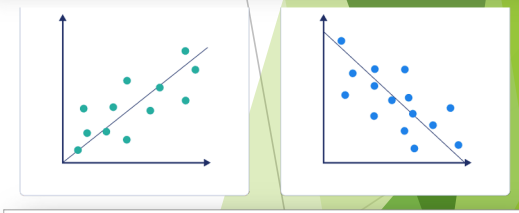


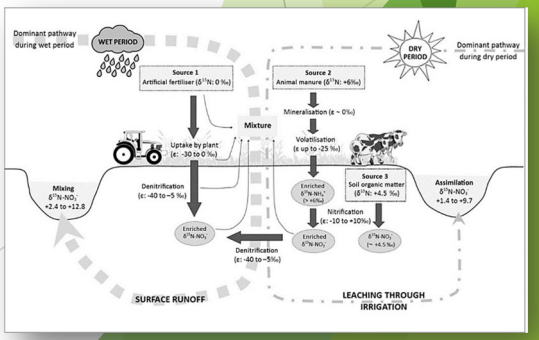


BLB MI FRB WFR DR WMB MR DC FP WB FR ICW DI

Preliminary conclusions

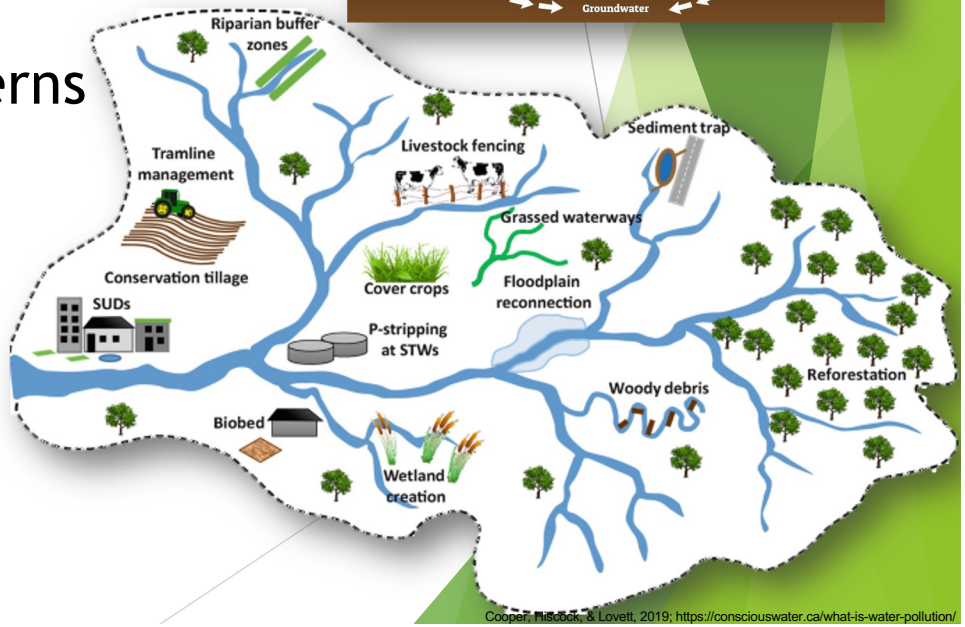
- Salinity correlated with discharge
  - **Sr:Ba** indicator of discharge-related salinity
  - **SI, nutrients, classical microbial indicators** correlated with discharge, salinity
- Distinct signatures of relationships **between indicators** (e.g., NO_3^-, NH_4^+ vs $\delta^{15}N$)
- **Precipitation** not significant driver of overall discharge or salinity
- Site and month affected response variables, with **spatial** variation more important





Implications

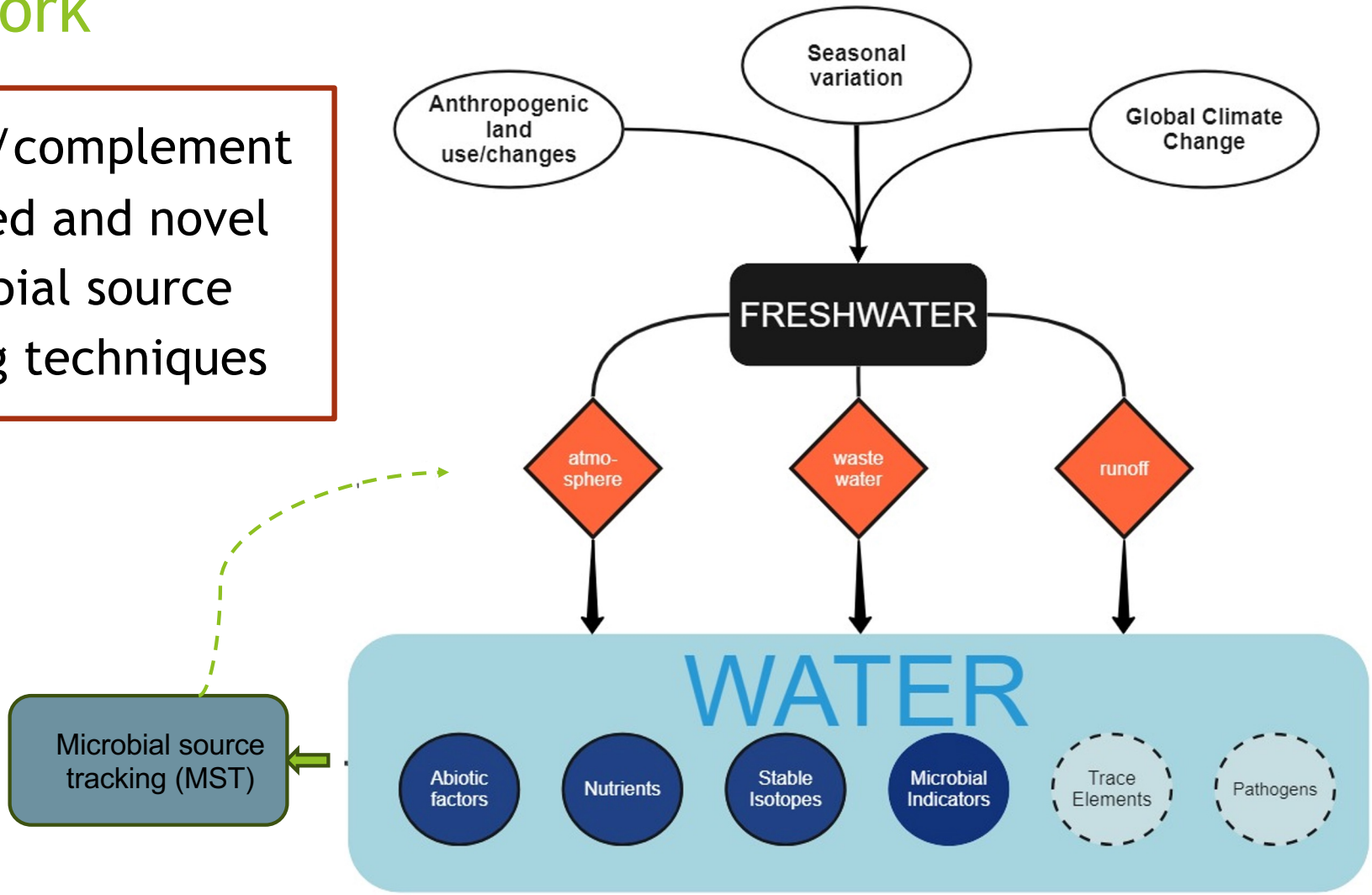
- Use **multiple indicators** to understand:
  - potential contaminant sources
  - pathways
  - Influence of local climatic and land-use conditions
- Evaluate **spatial and temporal** patterns
- Applications for tailored monitoring/management strategies



Cooper, Fiscock, & Lovett, 2019; https://consciouswater.ca/what-is-water-pollution/

Future work

Validate/complement advanced and novel microbial source tracking techniques



Molecular Source Tracking

- Species-specific contributions to microbial indicators in Alabama waters remain largely unknown
- 2 molecular source tracking techniques

Molecular Source Tracking

Quantitative PCR (qPCR) will identify and quantify fecal-associated bacteria by targeting genetically distinct strains of *Bacteroides*

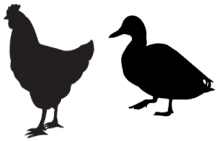

General (All) *Bacteroides* (total)


Human


Pig (including feral hog)

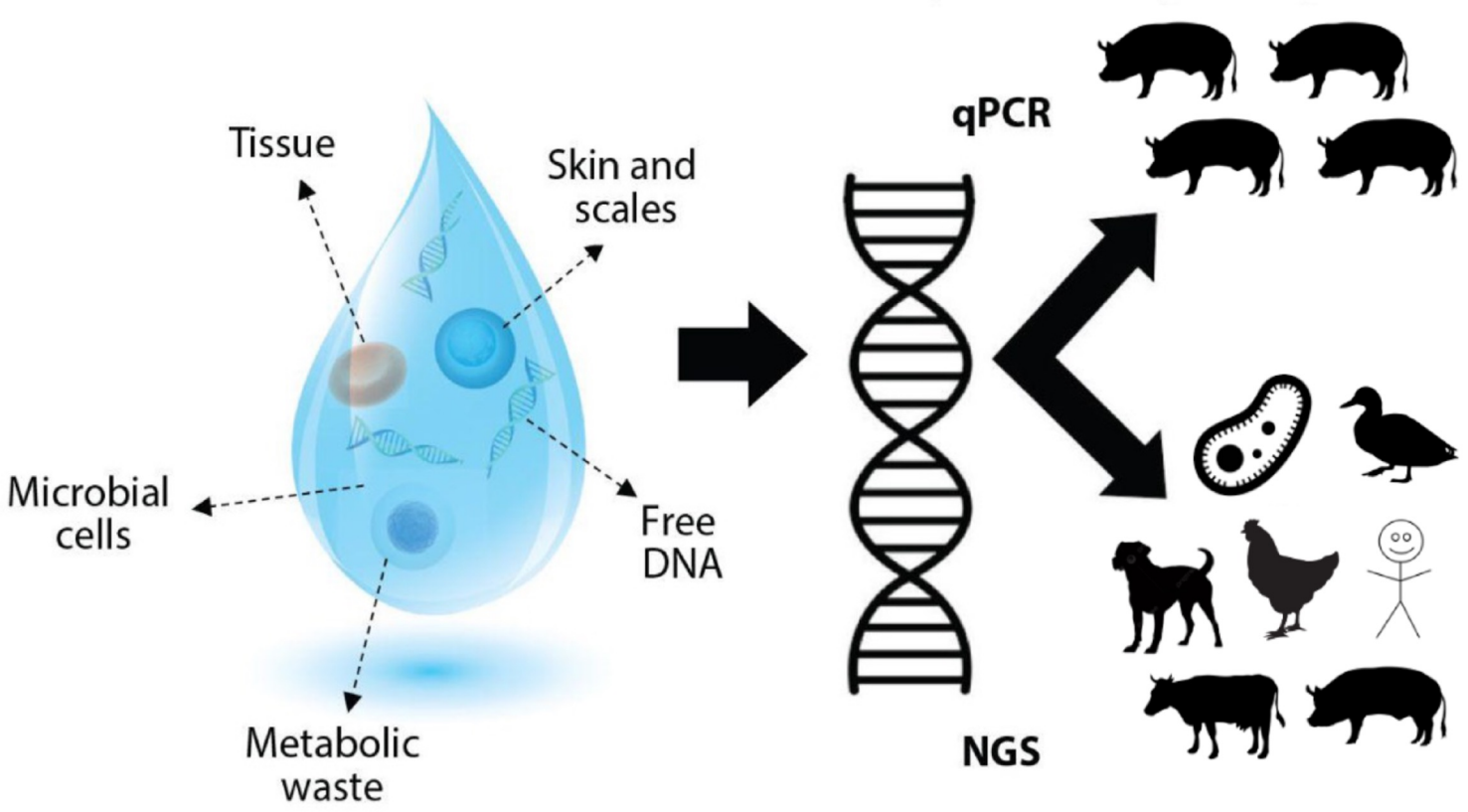

Cow


Dog


Fowl (Chicken/duck)

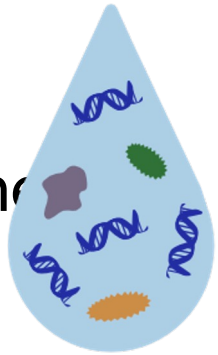
Molecular Source Tracking

eDNA - all DNA present in the environment



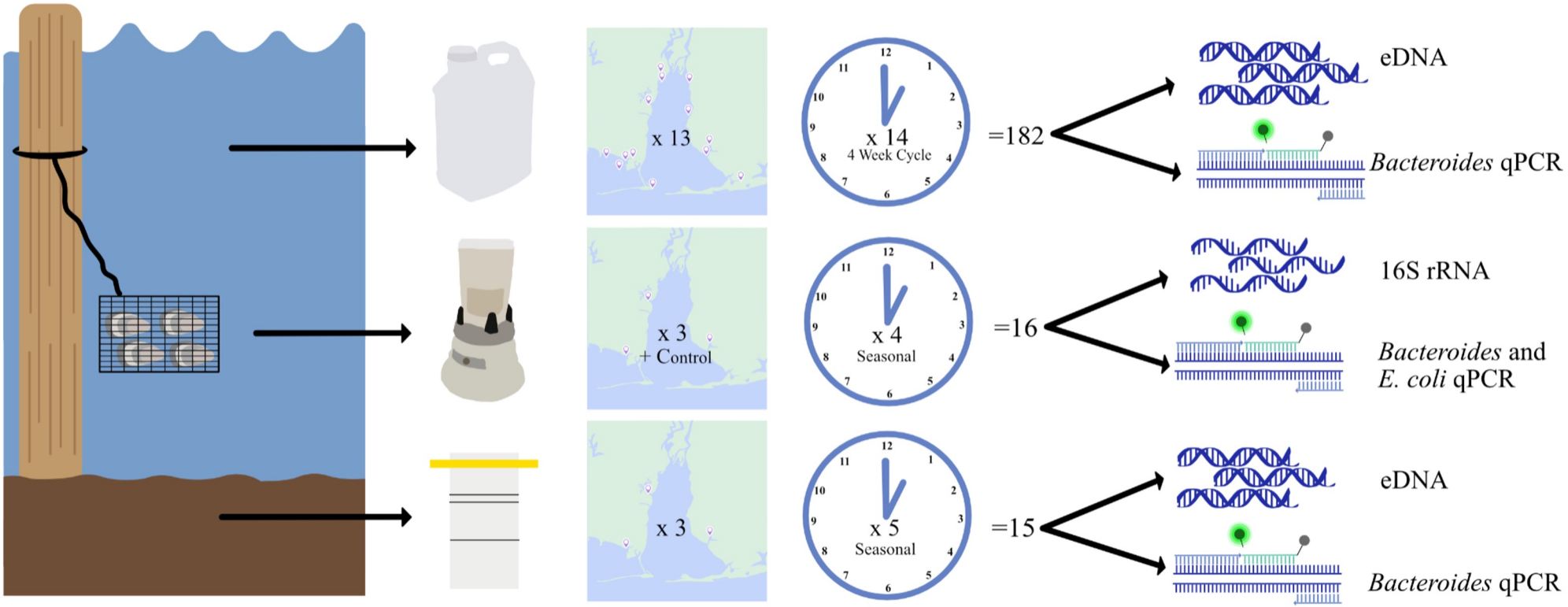
Molecular Source Tracking

Environmental DNA (eDNA) metagenomics will identify all bacterial and DNA-based viral markers within a sample



- Does not depend on prior knowledge of the system
- Has not been implemented as an MST method

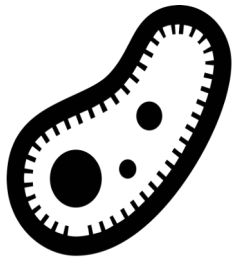
Molecular Matrix

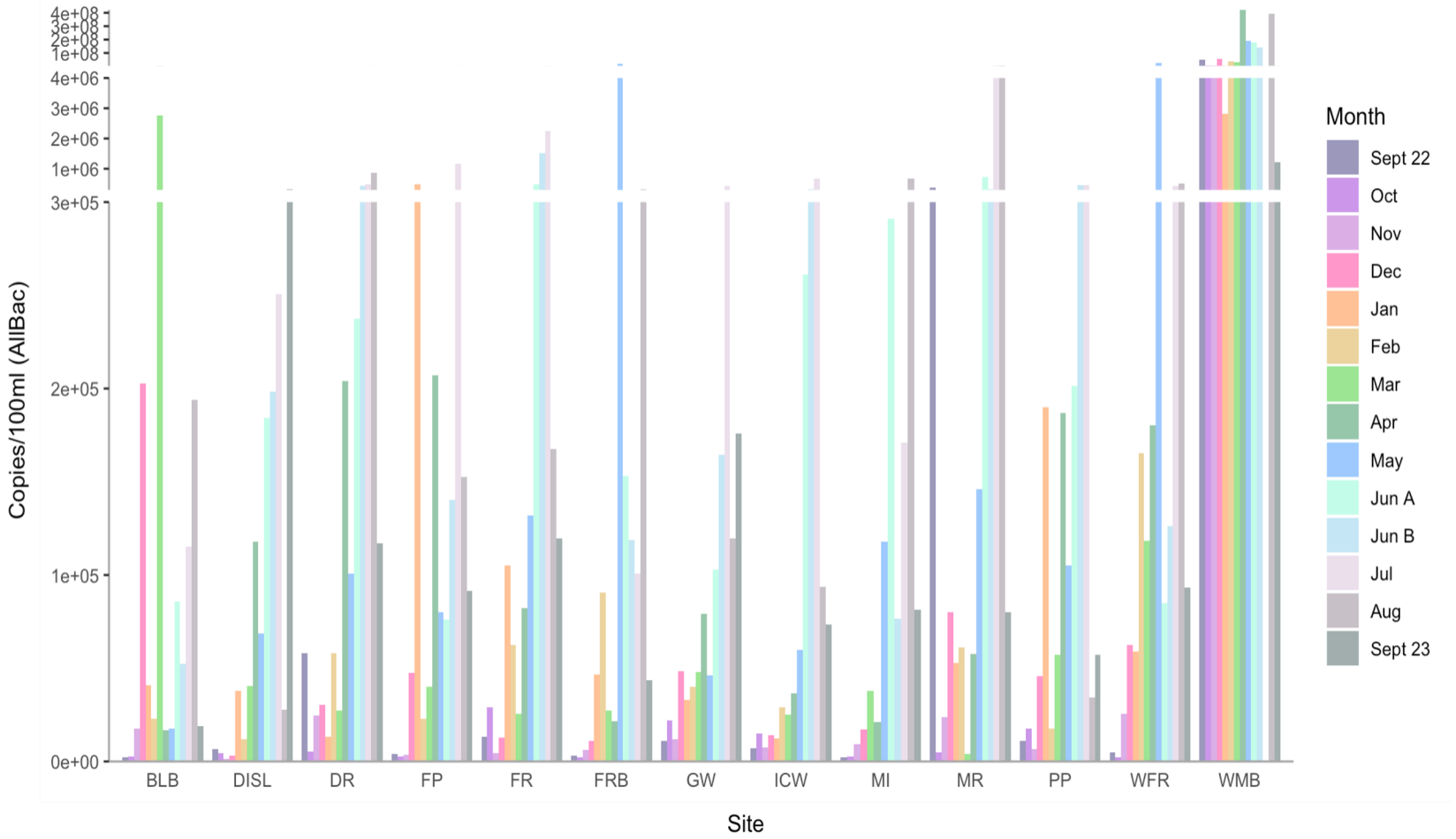


Progress

- Bioinformatic analysis underway
- All source tracking qPCR completed
- Next steps: multivariate statistics to analyze trends and drivers

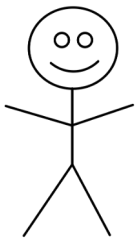
qPCR data - All *Bacteroides*

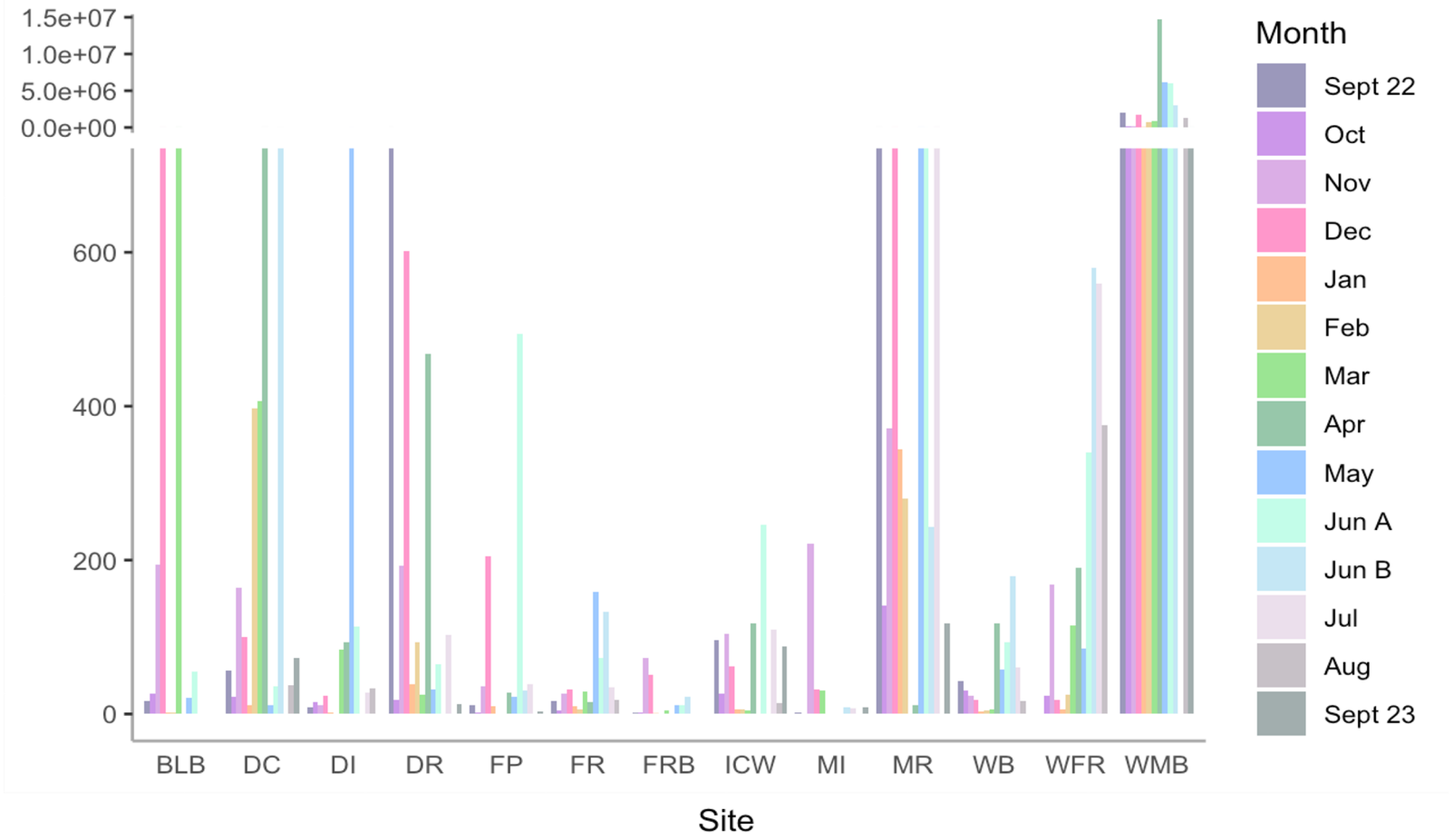




All *Bacteroides* marker was prevalent at all sites over all time points

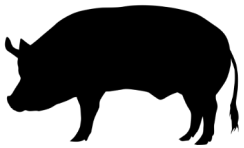
qPCR data - Human

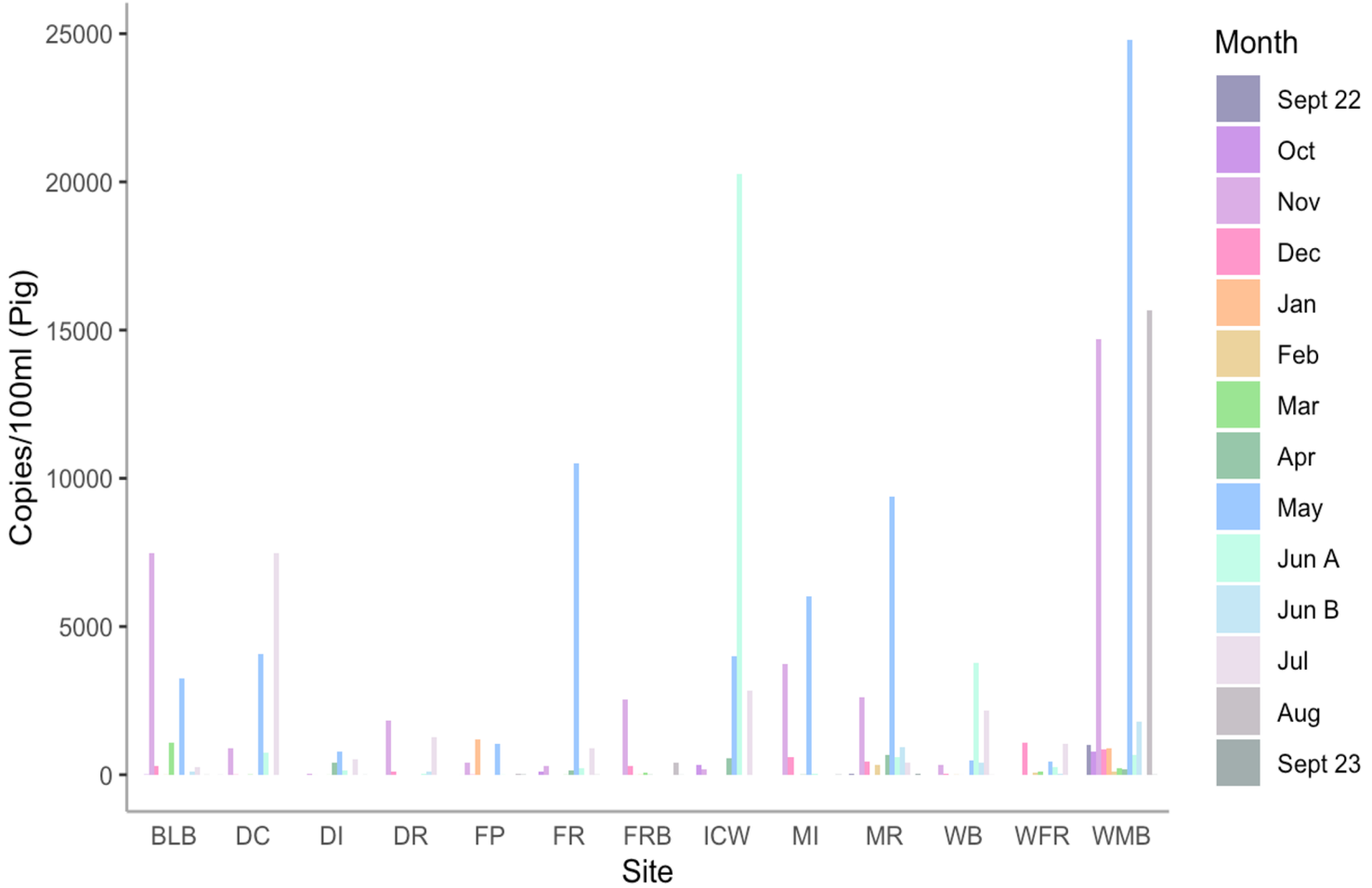




Human marker was found at all sites, and was the major contributor to AllBac

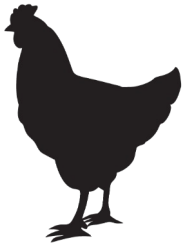
qPCR data - Pig

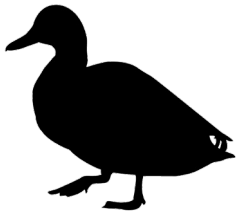


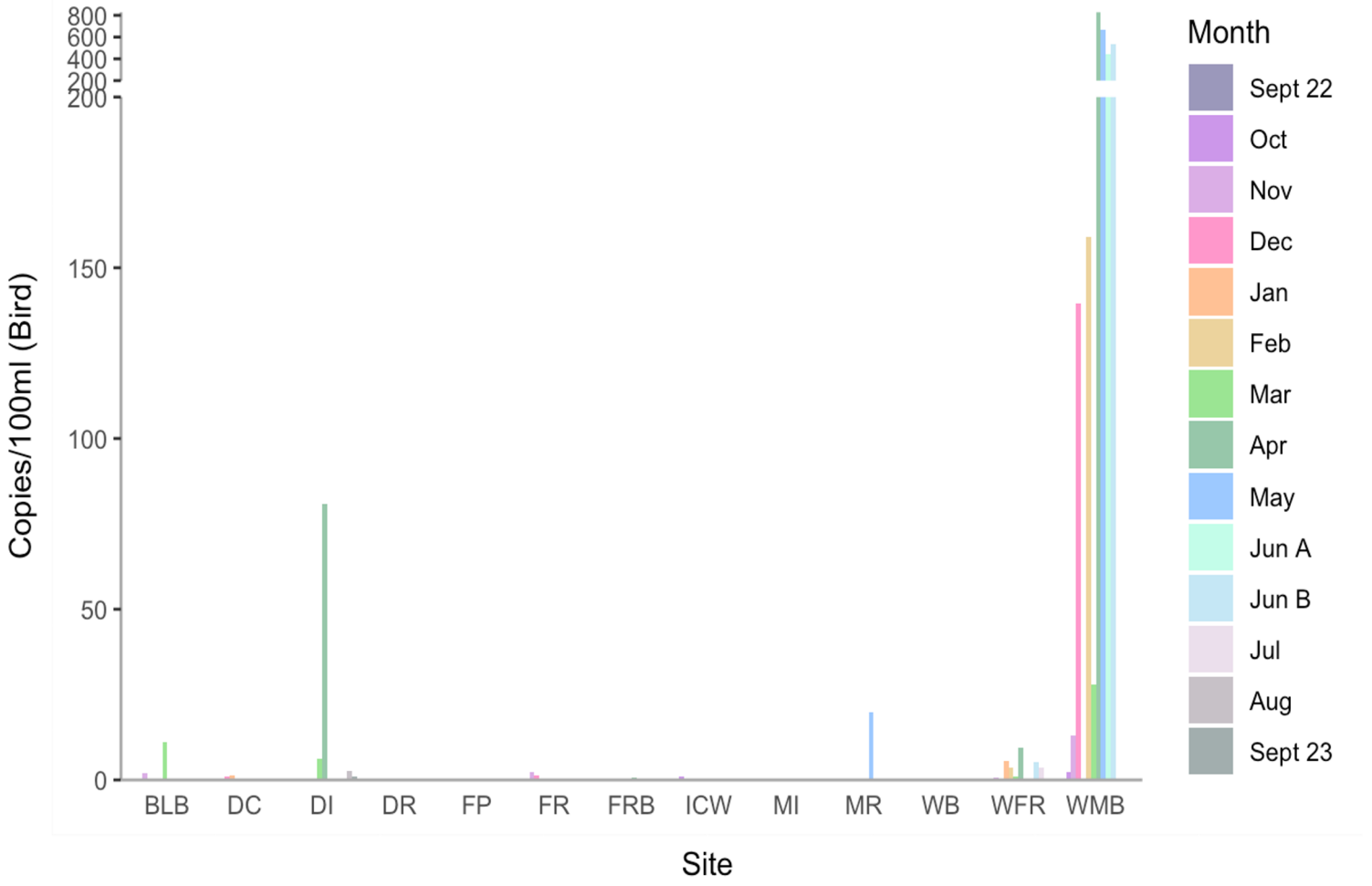


Pig marker was found at all sites episodically

qPCR data - Fowl

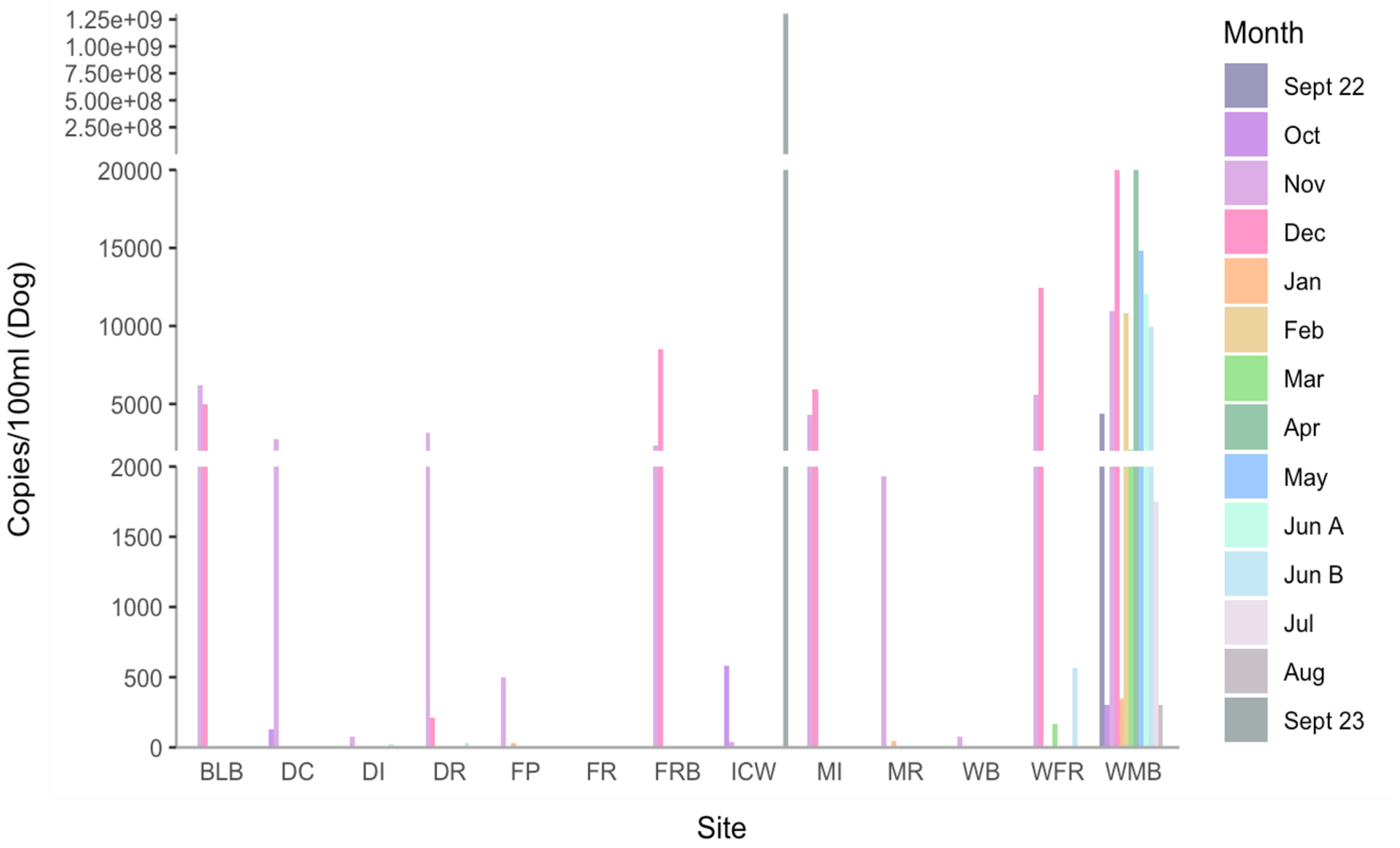






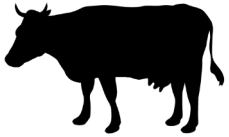
Fowl marker was found at a few sites

qPCR data - Dog



Dog marker was found at most sites, but seems seasonally dependent

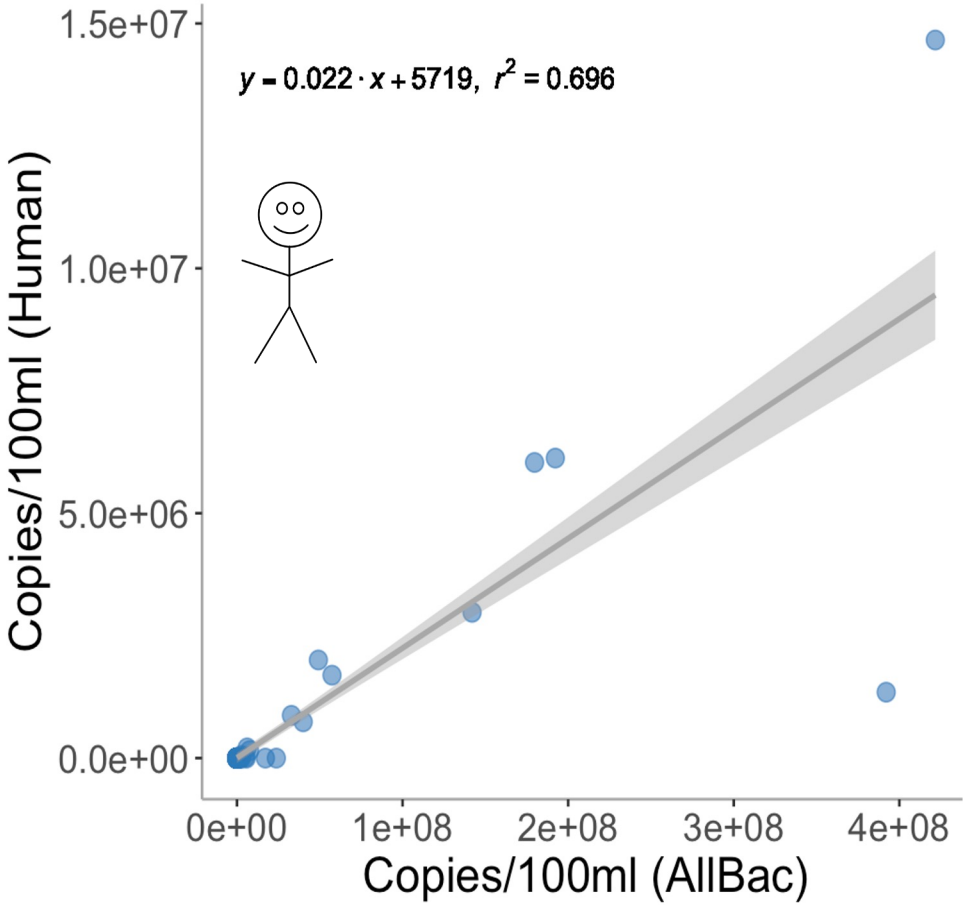
qPCR data - Cow

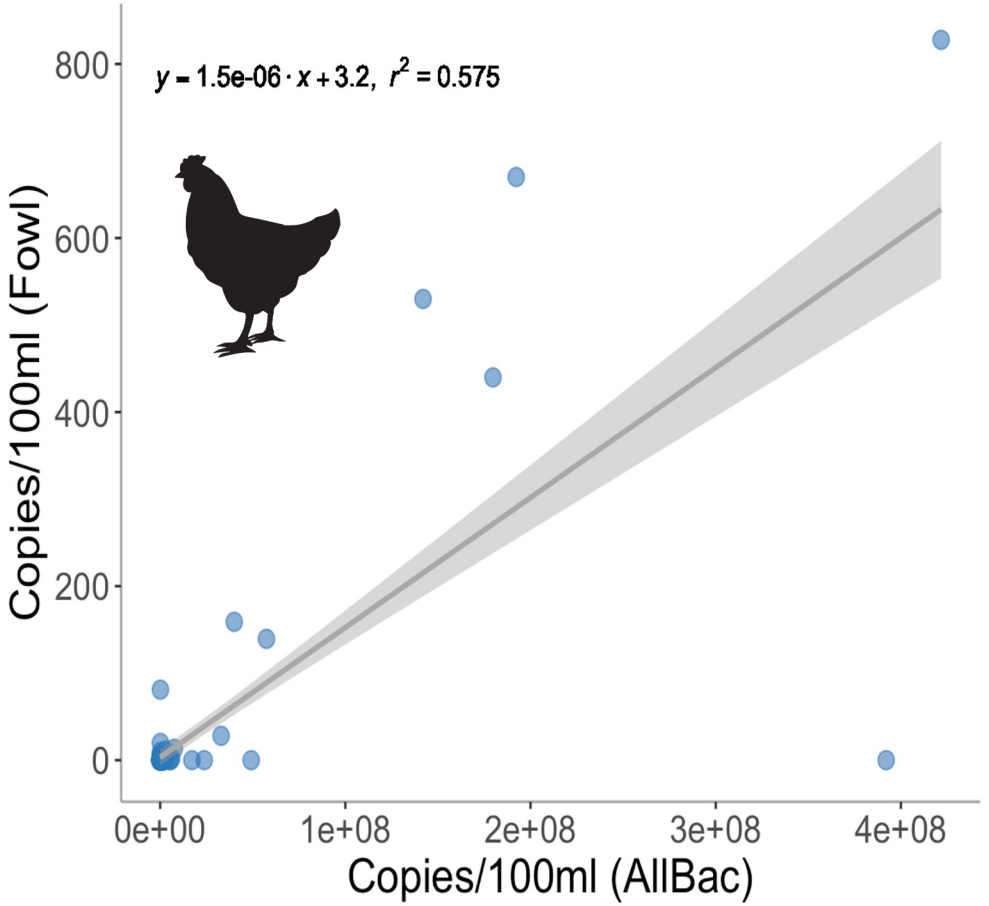


The cow marker was found at 2 sampling events (site 10 in December and site 4 in March)

qPCR data

Human and fowl were significantly correlated with the total (AllBac)





Project Updates: Objective 3

Bree Janssen

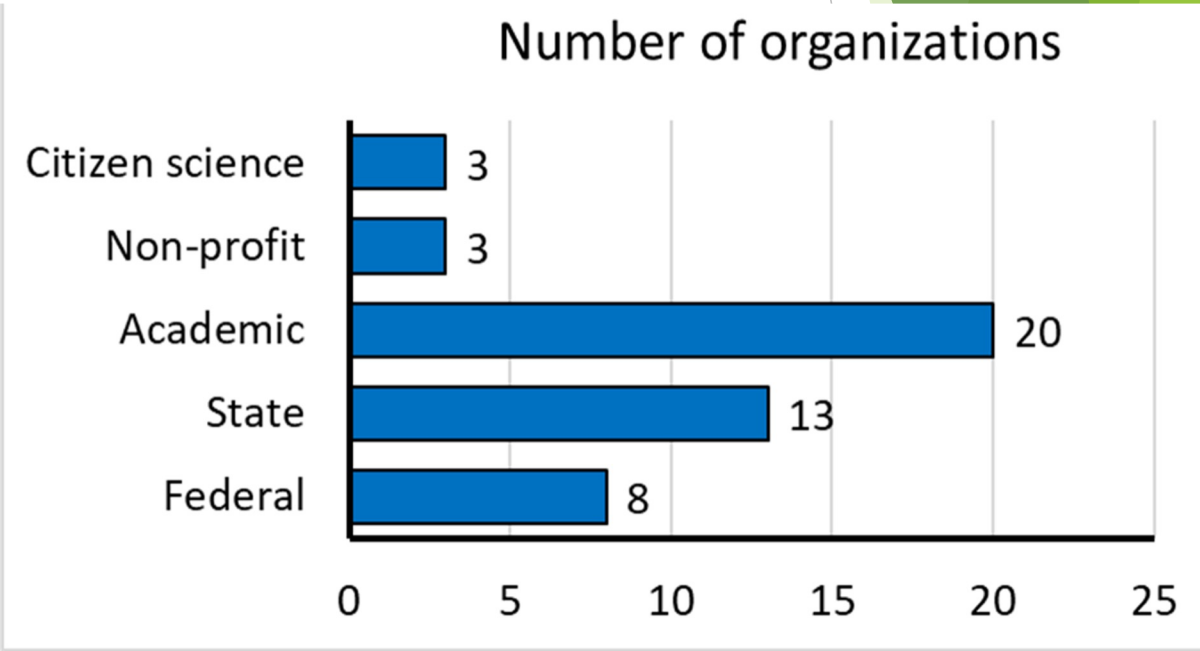
Metadata Clearinghouse

"Our Wastewater Footprint"
https://www.disl.org/wastewaterfootprint

- A living resource to provide up-to-date data on how human wastewater affects water quality on the northern Gulf of Mexico coast data reported for Baldwin County
- Continued support from Pat David (Data Architect-DISL)

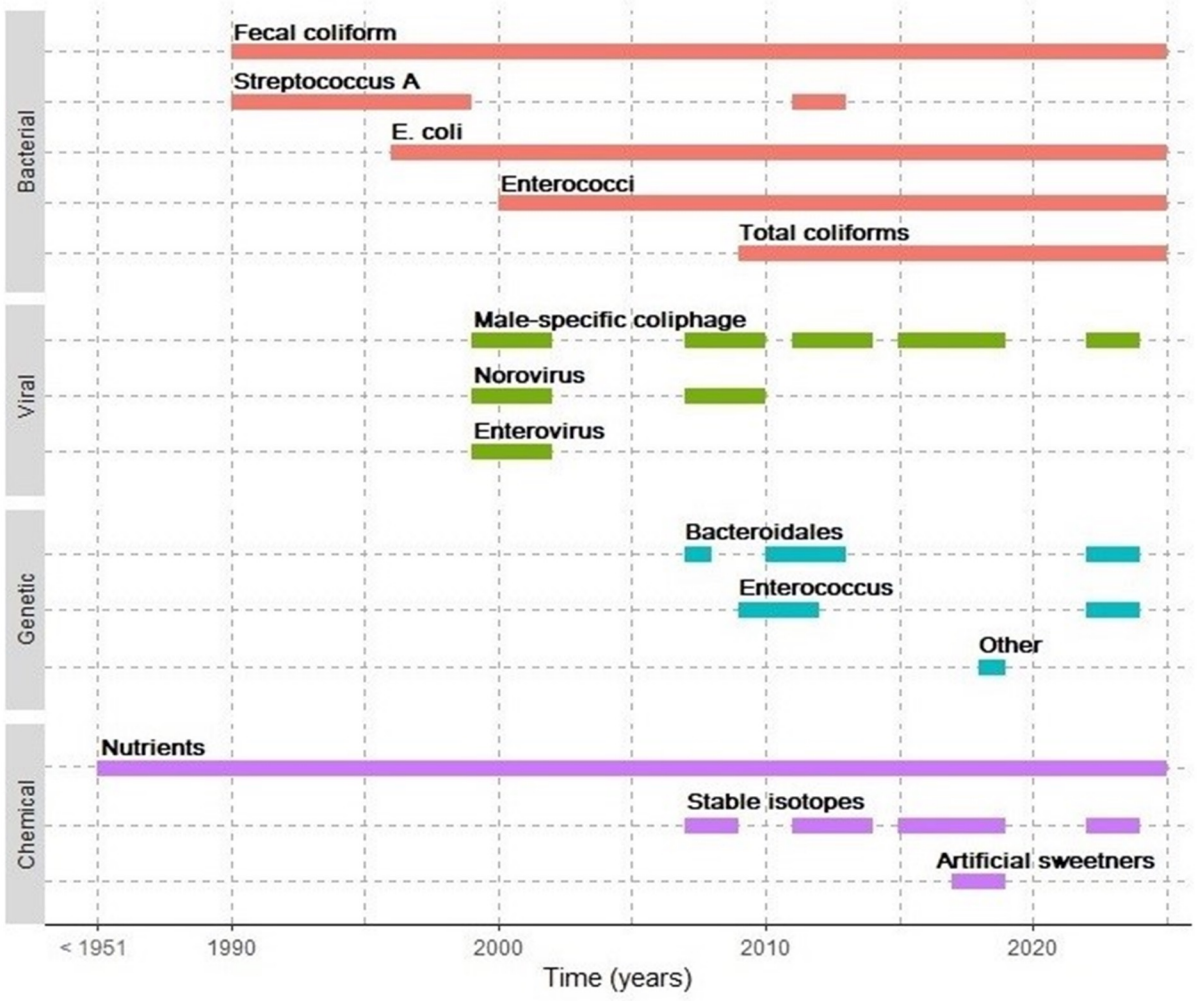
Metadata Clearinghouse

- Compile existing microbial indicator and source tracking data and identify other ongoing projects.
- 30 metadata entries
- Encourage data sharing and use
- Invite contributions to Clearinghouse
  - Website & email
  - Questionnaire



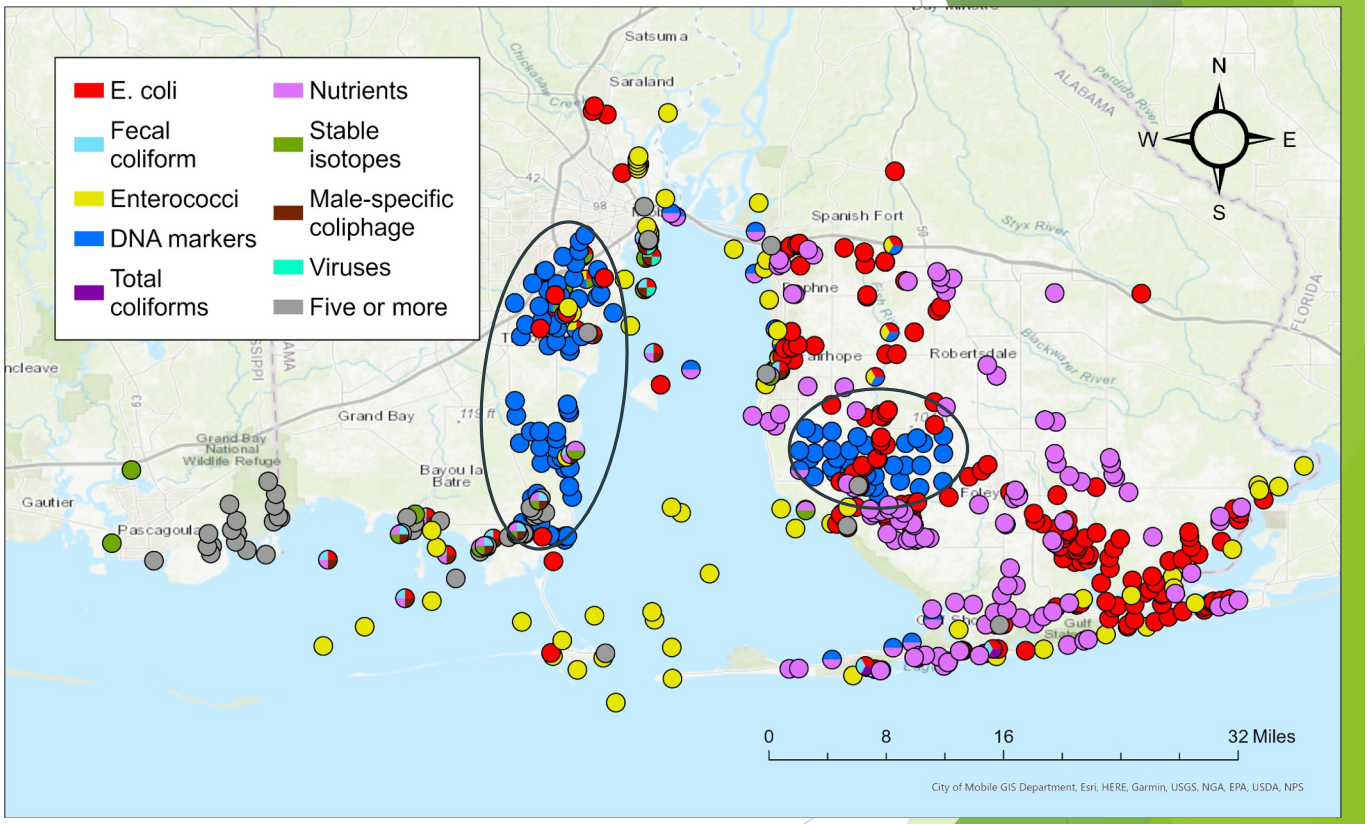
Temporal Analysis

- Four major indicator categories: **bacterial**, **viral**, **genetic**, **chemical**
- Data date back to 1951
  - **Nutrients** are most consistently available
  - **Classical indicators** (fc, E. coli) prominent after 1990
  - **Newer genetic indicators** prominent in the mid-2000s



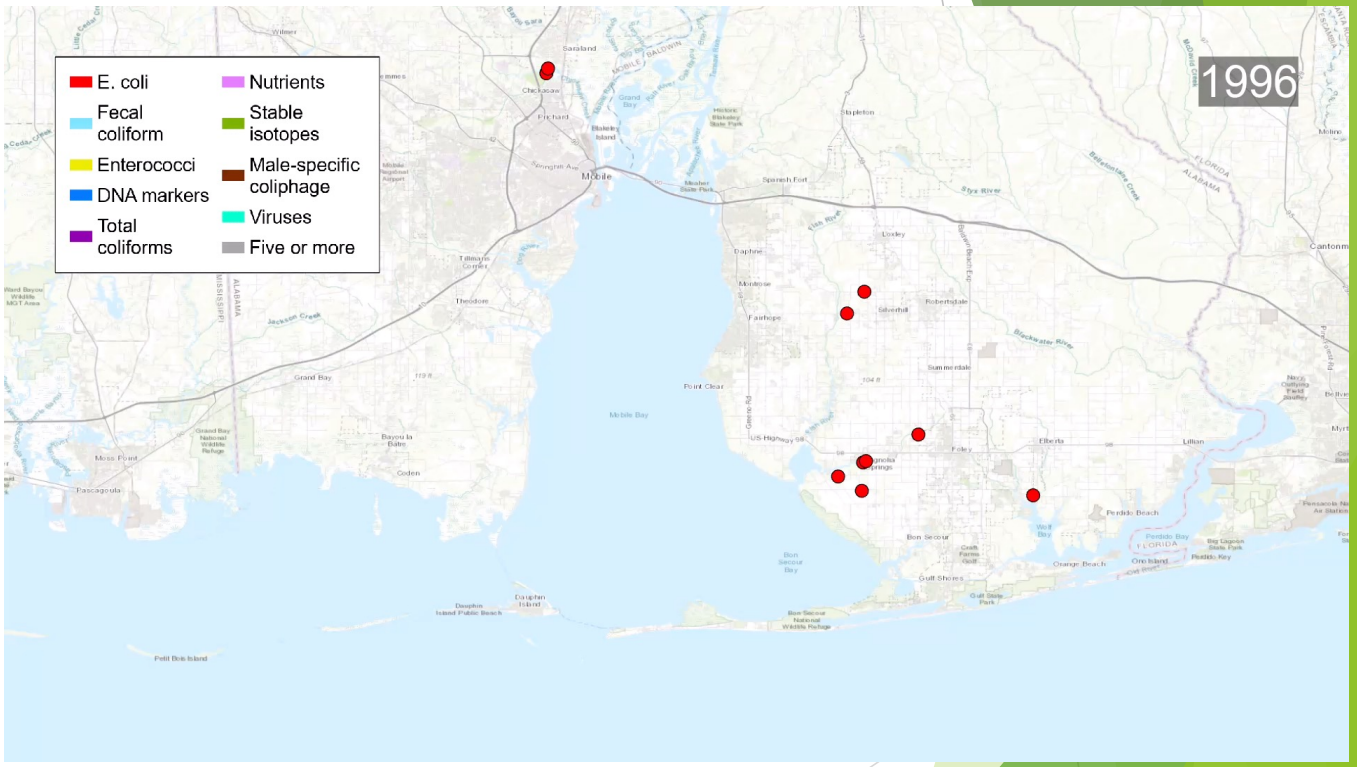
Spatial Analysis

- More data reported for Baldwin County
- Nutrient, E. coli, & enterococci data are widely collected
- DNA markers from one comprehensive study in 2018



Spatiotemporal Analysis

- Shift from “traditional” to “newer” indicators with inclusion of western Mobile Bay
- Lack of long-term monitoring for advanced indicators, but covers a wide range of locations
- Likely due to large, discrete studies

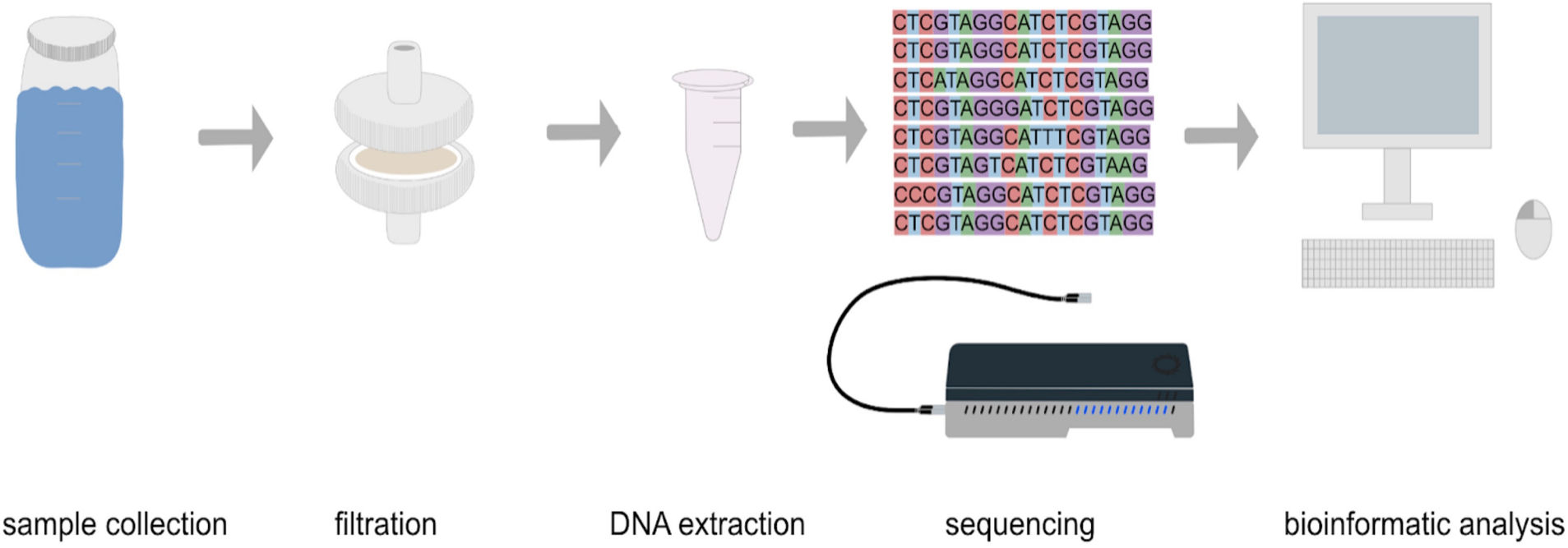


Project Updates: Objective 4
eDNA Workshop

Penny Demetriades

eDNA Source Tracking Toolkit

Implement eDNA as an accessible next generation source tracking tool with the creation of an eDNA toolkit and workshop



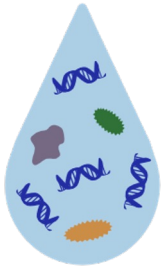
eDNA Source Tracking Toolkit

The workshop will be hosted at the U South Alabama campus

April 25, 2025

https://sites.google.com/view/ednatoolkit/

Register by April 4



eDNA
Source Tracking
Toolkit

eDNA Source Tracking Toolkit

Home Toolkit Workshop Information Resources

The eDNA Source Tracking Toolkit is a resource for implementing eDNA metagenomics as a fecal source tracking tool.

Environmental DNA (eDNA) metagenomics techniques have the capacity to provide important biological context to source tracking identification and fill in data gaps left by source-specific microbial source tracking methods, such as qPCR.

Metagenomics data can show community composition and function to provide a comprehensive assessment of water quality in a system.

This toolkit is hosted through the Kiel Reese lab at the University of South Alabama and the Dauphin Island Sea Lab with funding provided by Environmental Protection Agency Gulf of Mexico Division grant MX-02D17922. Please direct any questions to pdemetriades@disl.org.

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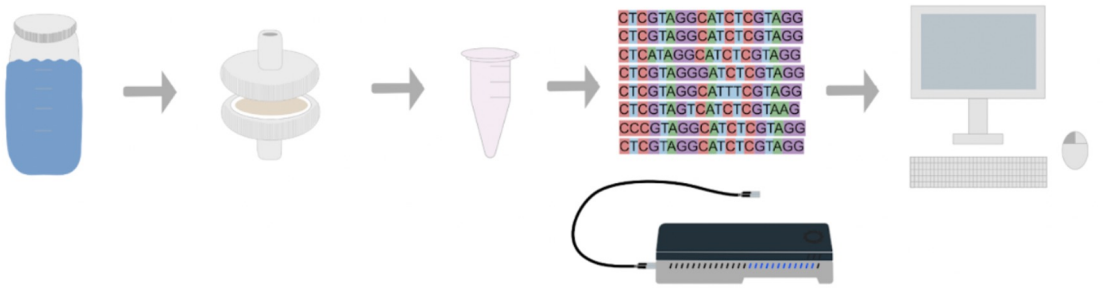
eDNA
Source Tracking
Toolkit

Workshop Information



Learn how to implement eDNA Source Tracking methods during our Toolkit Workshop on **April 25, 2025**!

Participants will learn best practices for eDNA sample collection, filtration, and DNA extraction. Participants will be able to engage in a hands-on sequencing run with a MinION sequencer and will be guided through subsequent bioinformatic analysis and key data visualization.

eDNA Source Tracking Toolkit Workshop Registration

Learn how to implement eDNA Source Tracking methods to enhance fecal contamination monitoring efforts during our Toolkit Workshop. Participants will learn best practices for eDNA sample collection, filtration, and DNA extraction. Participants will be able to engage in a hands-on sequencing run with a MinION sequencer and will be guided through subsequent bioinformatic analysis and key data visualization.

Please use this form to register for the workshop on April 25, 2025 (9am-12pm) at the University of South Alabama.

Registration will close on April 4.

eDNA Source Tracking Toolkit Workshop Registration

Learn how to implement eDNA Source Tracking methods to enhance fecal contamination monitoring efforts during our Toolkit Workshop. Participants will learn best practices for eDNA sample collection, filtration, and DNA extraction. Participants will be able to engage in a hands-on sequencing run with a MinION sequencer and will be guided through subsequent bioinformatic analysis and key data visualization.

Please use this form to register for the workshop on **April 25, 2025** from 9am-12pm. This workshop will be hosted in person on the University of South Alabama campus (EOB Room 101, Stokes School of Marine and Environmental Sciences). Participation will be capped at 12 individuals. **Registration will close on April 4**.

demetriadespenny@gmail.com Switch account

Not shared

Draft saved

* Indicates required question

Email *

Your answer

First and Last Name *

Your answer

Please register by April 4!

Tracking wastewater for health and resilience in Alabama (aka EPAII)

PIs and Partners

- Brandi Kiel Reese, USA/DISL
- Ruth H. Carmichael, DISL/USA
- Sinéad Ní Chadhain, USA
- Brian Dzwonkowski, USA/DISL
- Zhilong Liu, USA/DISL
- Elica Moss, Alabama A&M University
- Kathleen Roberts, Bishop State
- Mobile BayKeeper
- Gulf States Health Policy Center

Tracking wastewater for health and resilience in Alabama (aka EPAII)

1. Conduct community-driven, spatially explicit shoreline **water-quality surveys** in the 4 most disadvantaged and vulnerable coastal communities in Alabama

intersectionality of multiple environmental & demographic stressors

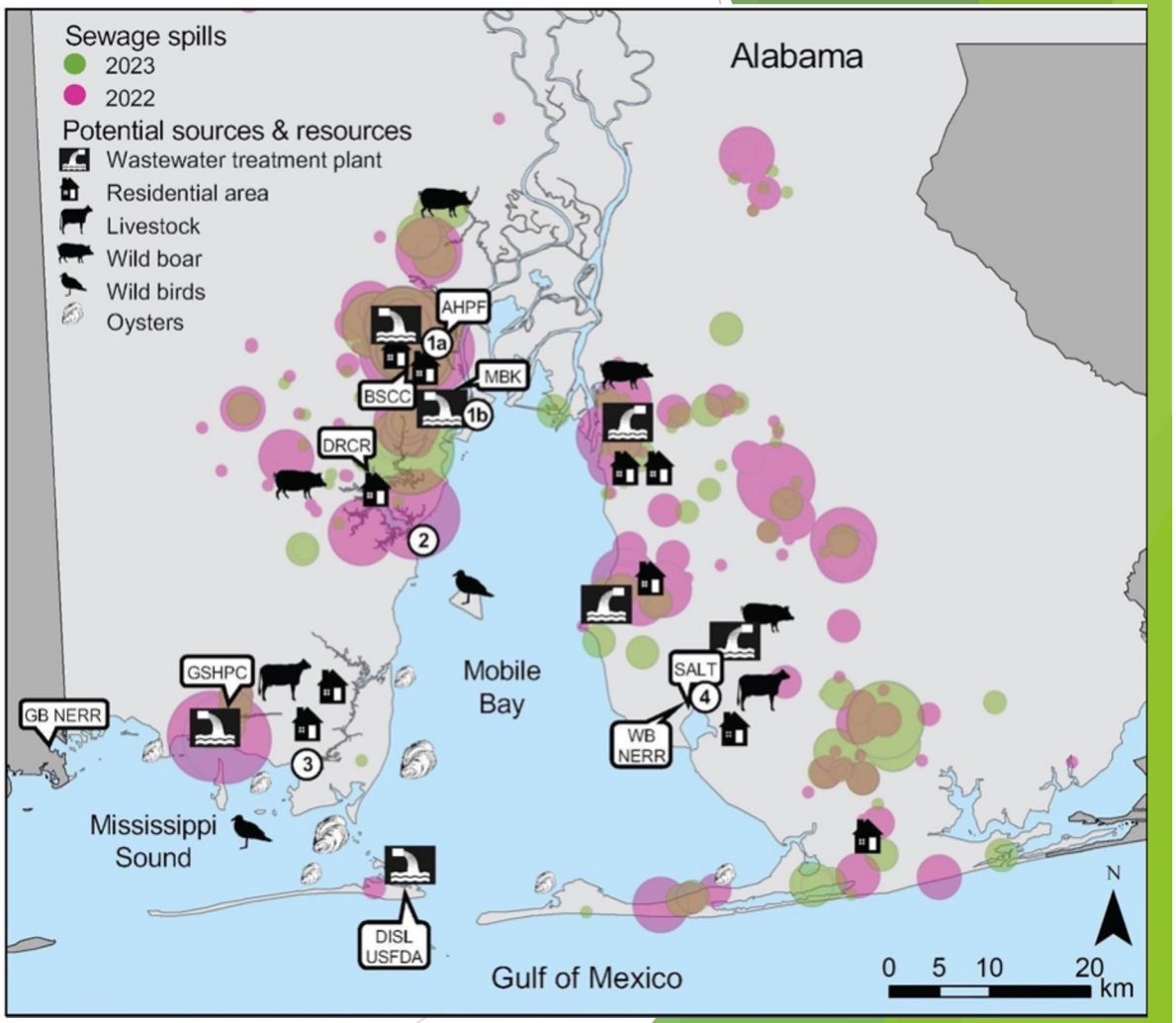
- **Africatown/ Three-mile Creek**
- **Dog River**
- **Fish River**
- **Fowl River**

Tracking wastewater for health and resilience in Alabama (aka EPAII)

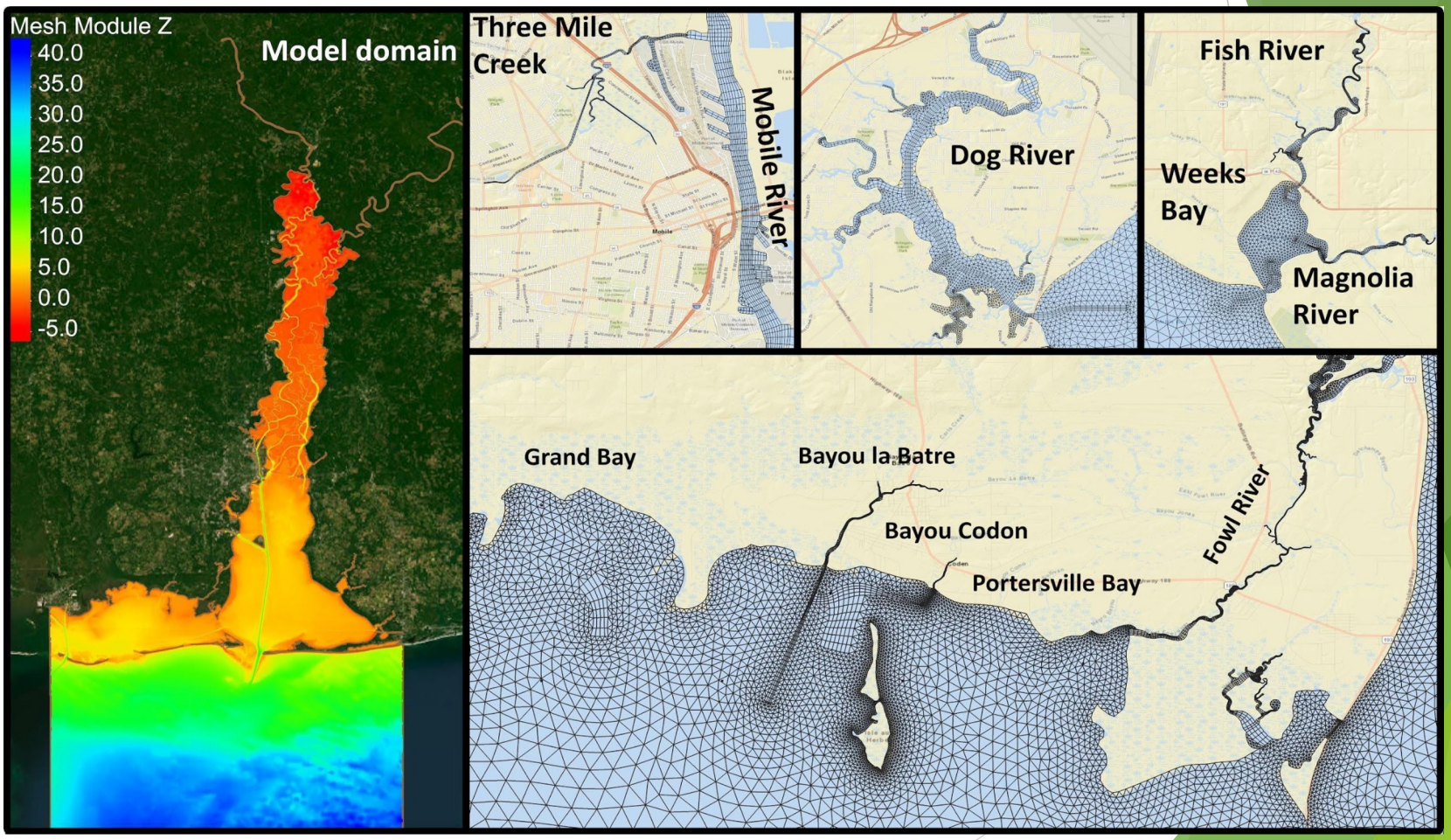
2. **Assess potential health risks** from water use (e.g., fishing, swimming) by defining wastewater-derived microbial sources to the waterways using traditional, biogeochemical, and advanced (genomics) microbial source-tracking approaches
3. Determine if water quality in these areas **affects adjacent communities** by modeling flow to downstream sentinel sites
4. Meaningfully **involve community members** in the project from planning to outreach through partnerships with a Community Engagement Facilitator and stakeholder groups

Sampling Approach

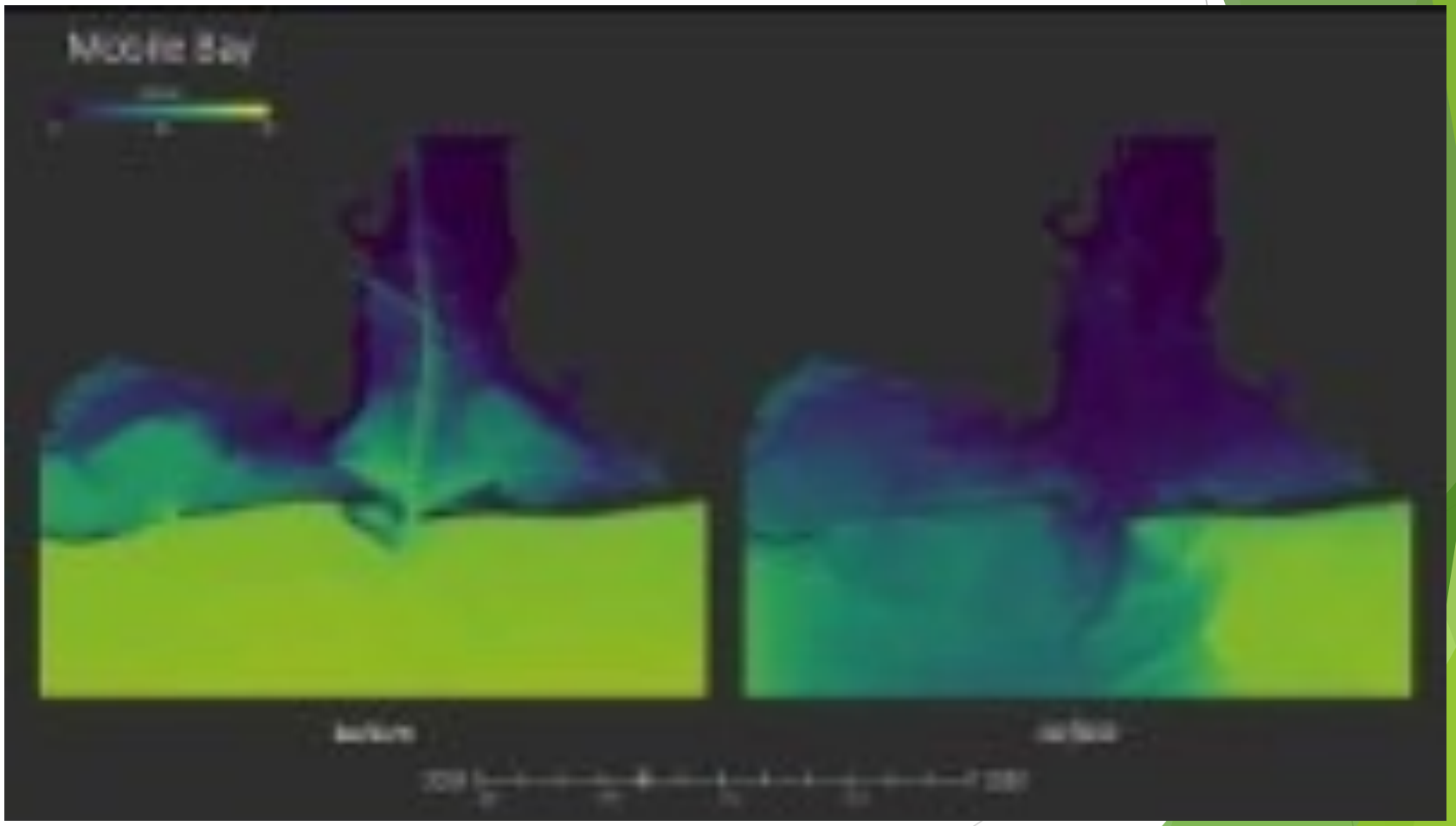
- Shoreline surveys in four local communities (tributaries 1-4)
  - 10 sites along each tributary
  - 2 sentinel sites downstream
- Sampling quarterly
- Tributaries 1 & 2 in Y1-Y2
  Tributaries 3 & 4 in Y3-Y4
- Event sampling



Modeling Approach: Transport & dispersion



Mobile Bay Salt Dynamics -- ROMS



https://www.youtube.com/watch?v=_JKu-bH9K_Y

Community engagement

Align project goals with community interests and values (JFF) through a **Community Engagement Facilitator** (GSHPC)

- Define baseline concerns & conditions
- Refine sampling locations in each community
- Incentivized community meetings
- Community-based sampling (test kits & validation)
- Curriculum workshops & internships for partner HBCU/ MSIs
- Seek new perspectives & stakeholders
- Co-produce outputs of interest and use to improve water quality, community health and resilience

Gulf States Health Policy Center

Timeline

Aim	Task	2025				2026				2027				2028				2029			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
All	Project planning																				
Aim 1	Shoreline surveys																				
	Sentinel site & oyster sampling																				
	Nutrients & Stable isotopes (Carmichael)																				
	Microbial indicators (Carmichael)																				
	Environmental DNA (Kiel Reese)																				
	Sequencing																				
	Bioinformatics																				
	qPCR source specific (Ní Chadhain)																				
	qPCR pathogens (Carmichael)																				
Aim 2	Collect hydrologic data (Dzwonkowski, Liu)																				
	Modeling & Data Integration																				
Aim 3	Citizen Science training & validation																				
	Outreach materials development																				
	Data sharing and Website updates																				
All	Annual reporting & manuscripts																				
	Team meetings																				
	Community meetings																				

How to get involved…

- Attend stakeholder meetings
- Provide feedback
- Provide data: CLEARINGHOUSE
- Tell colleagues
- How will you use these data?
- What would you or your colleagues like to gain from the eDNA toolkit workshop?

Questions or Comments?