

Human Pathogens Consistently Detected in Bivalves Across San Diego Bay

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Summary

- Bacterial DNA samples were isolated from oysters and mussels across sites in San Diego Bay with varying risks of sewage pollution, and pathogenic bacterial abundance within the gut microbiota was compared
- No site-specific pathogenic bacteria patterns were identified

Abstract

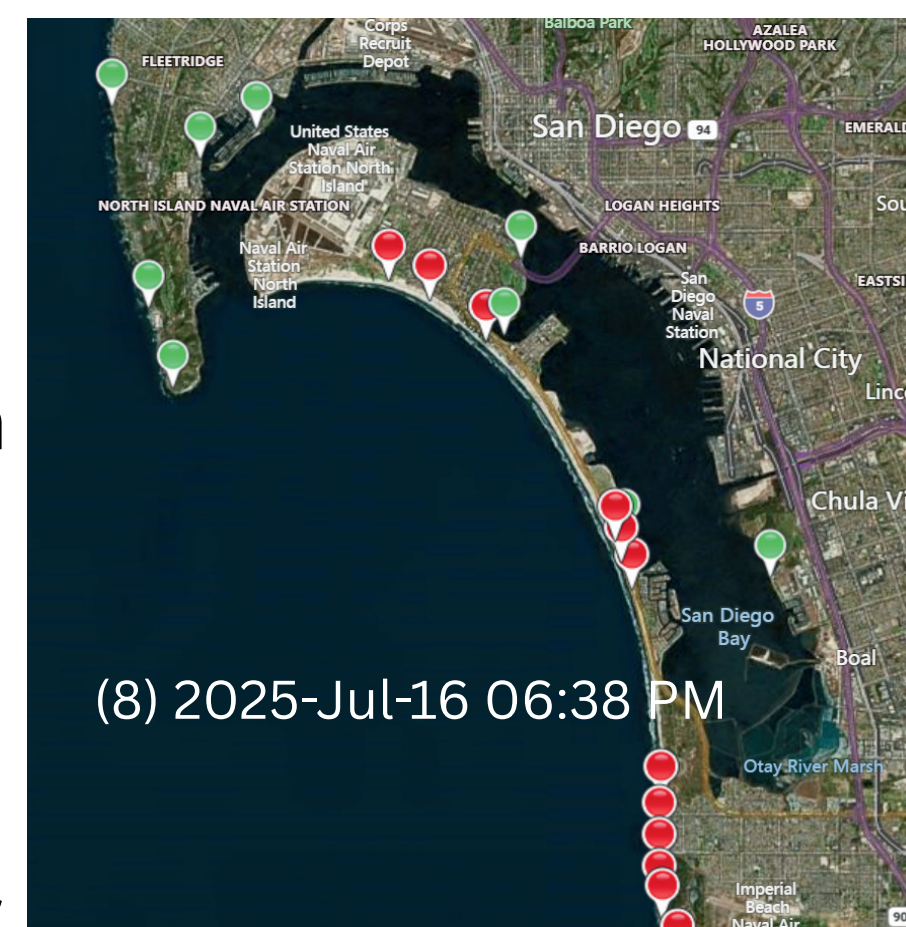
Bivalves are effective bioindicators of pollution as their filter-feeding tendencies can accumulate chemical pollutants and pathogens in their tissues. To determine if sewage pollution impacts pathogenic bacteria levels in the San Diego Bay, mussels and oysters were collected from 9 geographic locations. Bacterial DNA was isolated from bivalve gastrointestinal tissue, amplified, and then sequenced. We identified pathogens associated with sewage in all samples, and then compared pathogenic bacterial profiles across sites. While the abundance of pathogenic bacteria differs between samples, no consistent patterns of differing by location were identified.

Introduction

Hypothesis: Human pathogenic bacteria abundance in the San Diego Bay is influenced by sewage pollution.

Sewage pollution

- Poorly treated sewage increases pathogen levels in ecosystems¹ and enters bodies of water due to overflows, leaks, storm drains, and natural disasters^{2,3}
- Pathogens can spread in water, stick to sediments, and enter the bodies of organisms³.
- This poses serious health risks through direct exposure, food chain contamination, and disruption of microbial ecosystems⁴.
- San Diego Bay receives large discharges of untreated wastewater from Tijuana via the Tijuana River⁵, and poor water quality has closed down multiple beaches due to high bacteria levels⁶.



Red pins represent beach closures due to high bacteria levels

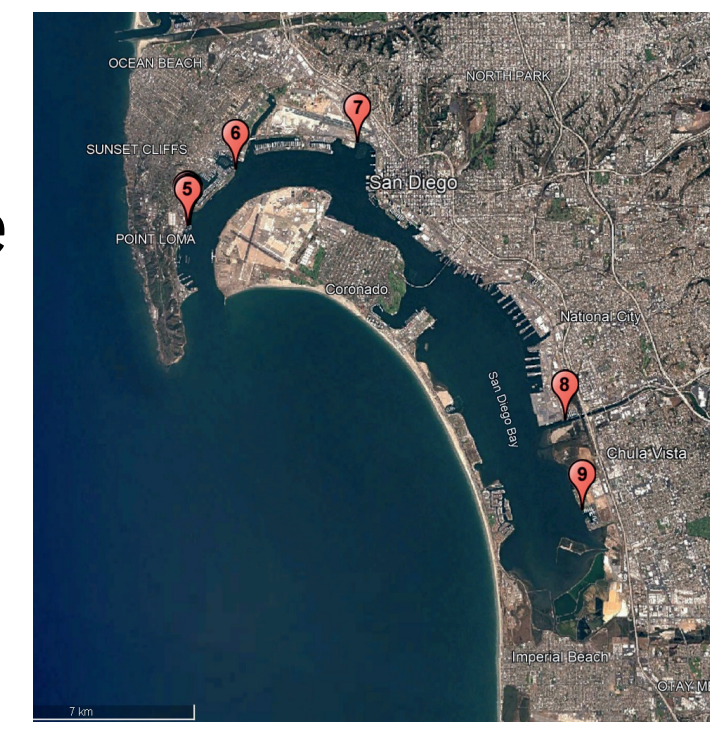
Bivalves as bioindicators of pollution

- Bivalves, including mussels and oysters, are filter feeders and can take in pollutants and pathogens associated with water when they feed⁷.
- The composition of the gut microbiome, microorganisms that live in the digestive tract, may be influenced by an organism's living environment⁸.
- Bivalve microbiota can provide insights into the impacts of sewage runoff in the San Diego Bay.

Materials & Methods

Bivalve Collection

Fifteen mussels from sites 1-5 near the marine mammal facility and eight oysters from sites 6-9 around San Diego Bay were collected



Dissection

Mussels and oysters across 9 sites were dissected, and gastrointestinal (GI) tracts were removed



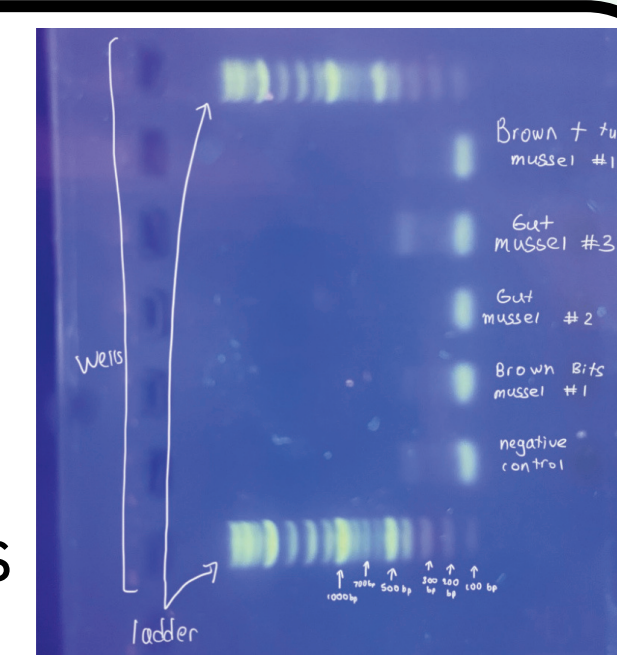
Bacterial DNA Isolation

MoBio PowerFood Microbial DNA Isolation Kit was used to isolate bacterial DNA from each sample. A modified protocol was used for host DNA depletion⁹



Quality Control

The V4 region of the 16S gene was amplified using polymerase chain reaction and quality checked with agarose gel electrophoresis



Nanopore sequencing

The 16S Barcoding Kit 24 V14 was used for preparing the library, which was sequenced with Oxford Nanopore's MinION sequencer



Data Analysis

Sequencing data was processed with EPI2ME for species identification, then analyzed and visualized with R (4.5.0) using tidyverse (2.0.0), vegan (2.7-1), ggplot2 (3.5.2), dplyr (1.1.4), tidyr (1.3.1), and pheatmap (1.0.13)

Results

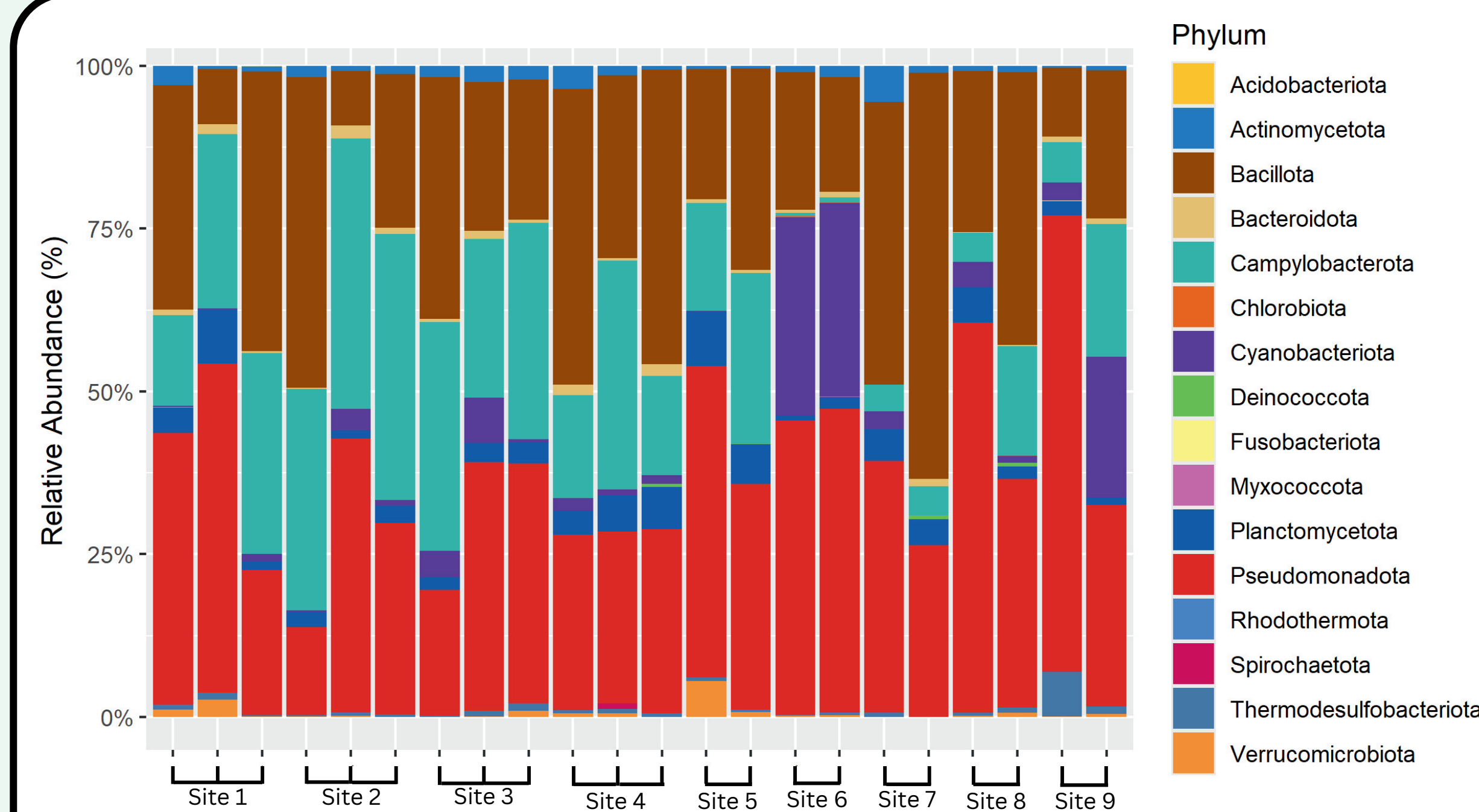


Figure 1: Relative abundance of bacterial phyla per bivalve shows high levels of *Bacillota*, *Campylobacterota*, and *Pseudomonadota*. Stacked bars represent the different phyla found in each of the collected bivalves, grouped by site.

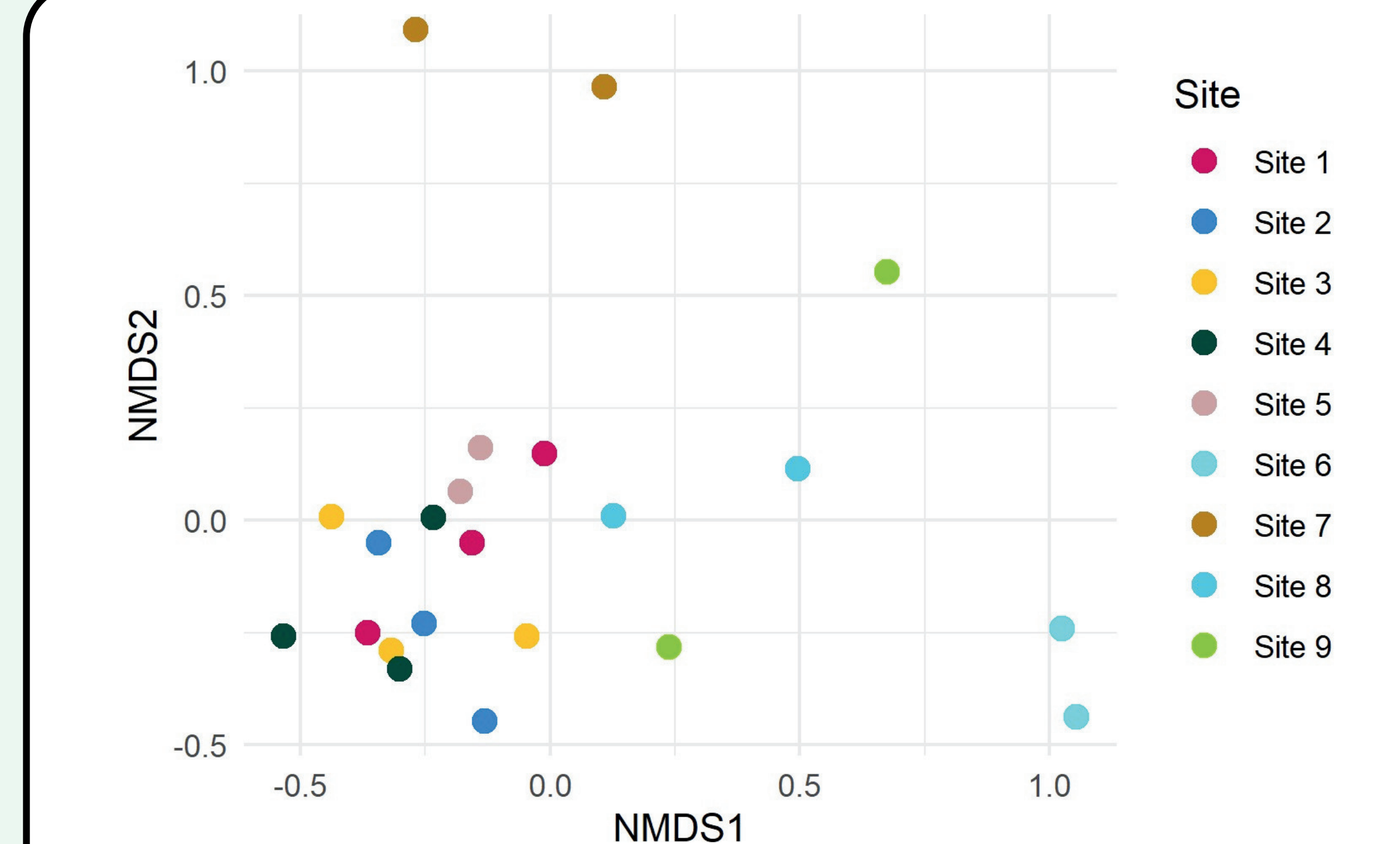


Figure 2: Beta Diversity NMDS of bivalve samples clusters sites 1-5. Non-metric Multidimensional Scaling (NMDS) using Bray-Curtis dissimilarity clusters bivalves by similar bacterial genera composition, and bivalves from the same collection sites are grouped by color. Stress = 0.129.

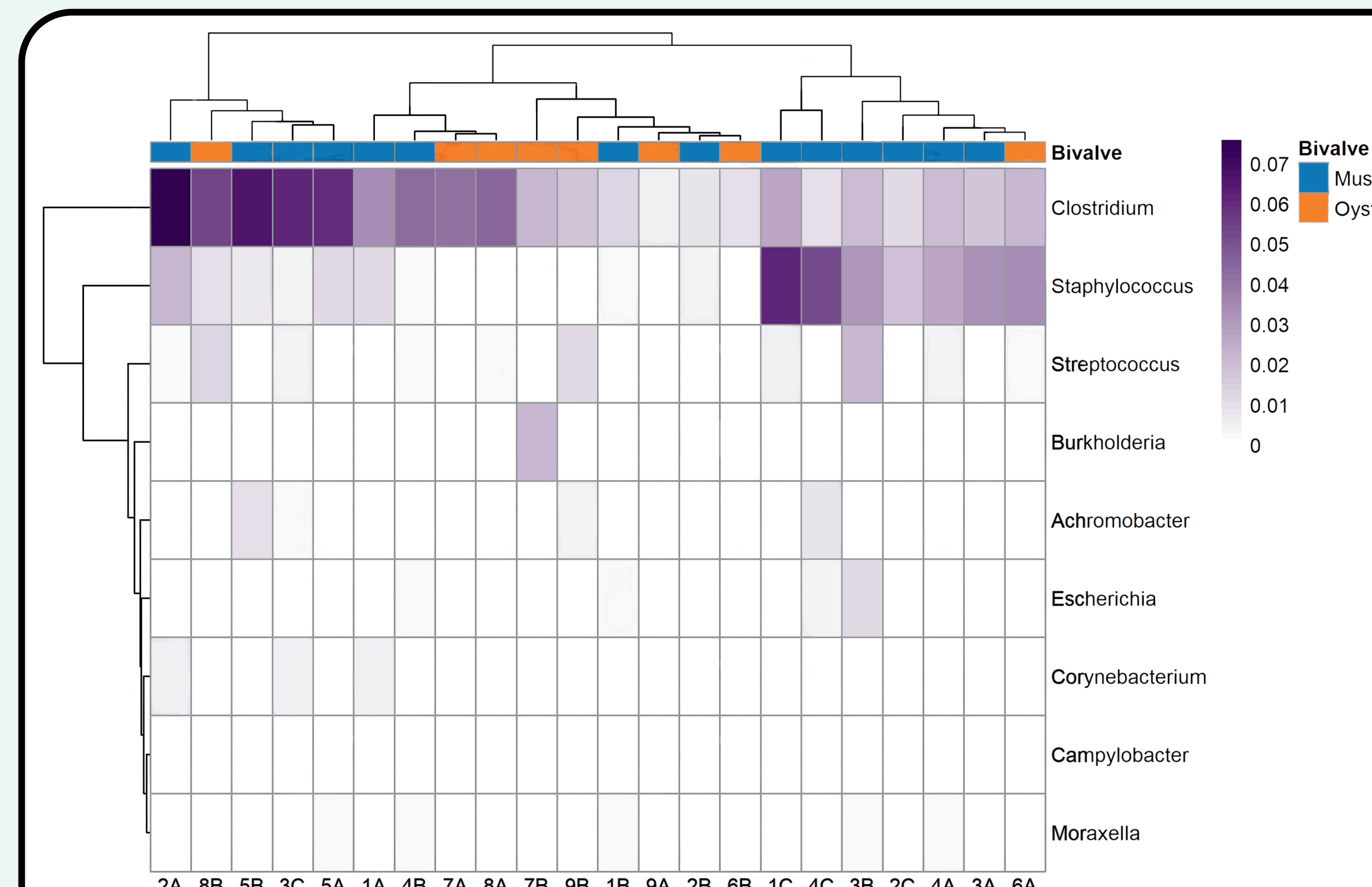


Figure 3: *Clostridium* and *Staphylococcus* are the most abundant genera containing pathogenic bacteria. Rows represent genera and columns represent samples, hierarchically clustered by relative abundance. Darker purple colors indicating higher abundance. Each sample's species is annotated along the top.

Discussion & Future Directions

Bacterial Composition Trends

- Bivalves consistently showed high levels of bacterial phyla Bacillota, Campylobacterota, and Pseudomonadota (Fig 1), all commonly found in marine habitats^{10,11,12}.
- Mussel and oyster gut microbiomes are generally similar⁸, so the clustering in bacterial profiles in mussels versus oysters is likely due to environmental differences (Figure 2).
- Sites 6 and 8 are near marinas¹³ and frequent boating activity may alter microbiota in this area¹⁴.

Pathogenic Bacteria

- Pathogenic bacteria range from one to eleven percent of the bacterial community in each sample (Figure 3).
- Clostridium*, the most abundant genus with pathogenic species detected (Figure 3), is associated with sewage pollution¹³
- The second most abundant genus, *Staphylococcus* (Figure 3), contains the species *S. aureus*. It has been found in sewage and can cause severe infections¹⁰.
- Although pathogenic bacteria were detected in all samples, there was no clear trend in bacterial composition across sites.

Limitations and Future Directions

- Samples were taken from multiple species, which may have contributed to bacterial composition variations between sites
- Modeling ocean currents could uncover sewage pollution movement
- Chemical analysis of ocean water and tissue samples can characterize pollutant distribution

Works Cited

