More pathogens, including *Acinetobacter* and *Streptococcus* species, were detect in oysters near heavily industrialized areas across the San Diego Bay

Tong, T.[1], Macina, D.[2], Ning, Z.[3], Ching. H.[4], Feng, Z.[5], Deviney, B.[5,6], Jedlicki, J.[5,6], Lu, J.[5,6], McClain, A.[5,7], Bozinovic, G.[5]

[1]Westview High School, [2]Point Loma High School, [3]University High School, [4]San Diego Met High School, [5]Boz Life Science Research and Teaching Institute, [6]UC San Diego Division of Extended Studies, [7]National Marine Mammal Foundation

Summary

- Oysters were collected across the San Diego Bay
- Bacterial DNA were extracted and sequenced
- Dolphin pathogens were detected across study sites

Abstract

San Diego Bay has been polluted by human activities. We collected oysters across the bay and dissected them for chemical and microbiome analyses. Bacterial DNA were extracted from the digestive organs, and the 16S rRNA gene was sequenced. More dolphin pathogens were detected in more industrialized sites, including *Escherichia*, *Streptococcus*, and *Acinetobacter* species. This data will be correlated with chemical analysis to evaluate the water quality in the San Diego Bay.

Introduction

Hypothesis: More industrial activity correlates with greater abundance of pathogenic bacteria.

Figure 1. San Diego rainstorm causes pollution in the South Bay.[2]

Figure 2. Large South Bay industrial site for fleet storage and maintenance at 901 Bay Marina Drive in National City. Photo credit: Cushman & Wakefield.

Ocean Pollution:

- More than 80% of ocean pollution is caused by land-based sources[1]
- Increasing urbanization is associated with greater coastal pollution[2]
- There is major boat traffic in San Diego Bay[3], which can generate waste streams[6]
- Common dolphin pathogens include *Clostridium perfringens*, *Campylobacter sp.*, and *Staphylococcus sp.*[4]
- Bacteria in sea water can be concentrated in filter-feeding organisms like mussels and oysters[3]

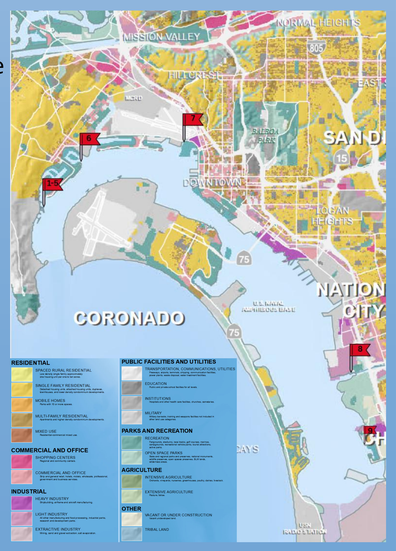
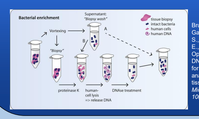


Figure 3. San Diego Region 2019 existing generalized land use map[6] with sampling sites labeled with Canva.

Materials & Methods

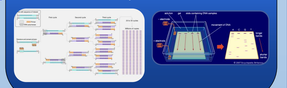
Sample Collection
- As part of a larger water quality assessment study, oyster were from four sampling sites 6 (32°43'11.4"N 117°13'12.5"W), 7 (32°43'37.7"N 117°10'46.7"W), 8 (32°38'58.3"N 117°06'39.7"W), and 9 (32°37'28.7"N 117°06'20.0"W) (Figure 3) and stored at -80°C until dissection

Dissection & DNA Isolation
- Dissected oyster gut contents
- Obtained bacterial cell pellet
- Host Depletion & PowerFood DNA Isolation

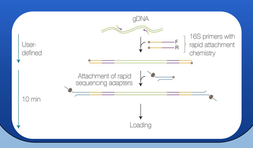


Bruggeling, C. E., Garza, D. R., Achouiti, S., Mes, W., Duith, B. E., & Boleij, A. (2021). Optimized bacterial DNA isolation method for microbiome analysis of human tissues. *Microbiologyopen*, *10*(3), e1191.

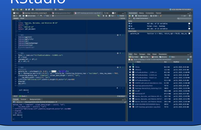
PCR and Agarose Gel Electrophoresis Quality Check
- Amplified 16S rDNA with PCR
- Observed gel results to confirm amplification success



Library Prep and 16S rDNA Nanopore Sequencing
- Prepared DNA library with 16S Barcoding Kit 24 V14
- Sequenced the library using Nanopore MinION Sequencer



Sequencing Data Analysis
- Sequencing reads were processed with EPI2ME to identify bacteria
- Bacterial data were analyzed and visualized in RStudio



EPI2ME

R Studio

Sample collection for chemical analysis

Composite Integrative Passive Samplers (CIPS) were deployed and retrieved at the same sampling sites (Figure 3)
- CIPS bind hydrophobic and hydrophilic pollutants from water
- Oyster tissues and CIPS sent out for chemical analysis

Results

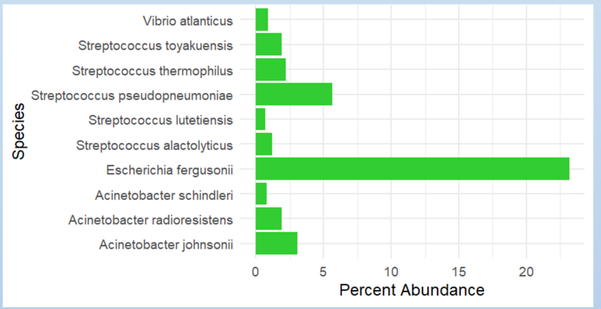


Figure 4. *Escherichia fergusonii* is the most abundant pathogenic species detected. Read counts per species were pooled across all samples and normalized to the total number of reads.

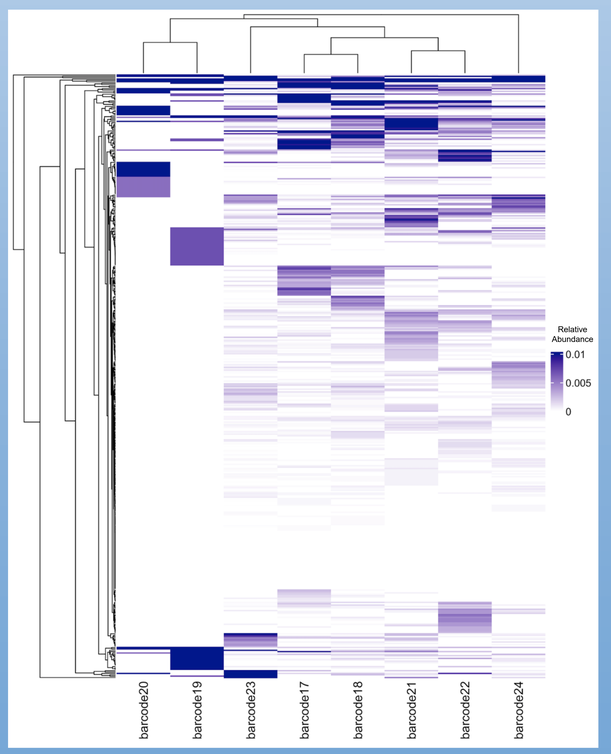


Figure 5. Hierarchical clustering highlights similar relative abundance levels across bacterial species within Sites 6 (Barcodes 17-18), 7 (Barcodes 19-20), and 8 (Barcodes 21-22). The microbiome between the two oysters from Site 9 (Barcodes 23-24) are more different and were not clustered together.

Discussion/Conclusion

- Our hypothesis was supported by the data, as some of the pathogens were more abundant in samples from more industrialized areas
- Among the detected pathogens, *Acinetobacter johnsonii*, *Streptococcus alactolyticus*, and *Streptococcus lutetiensis* are more abundant at sites 8 and 9, while the others were at similar levels.
- **Urban runoff, sewage, and industrial activity** tend to **increase** bacterial pollution and environmental health risks for marine mammals.
- The presence of fecal-associated bacteria like *Escherichia* may reflect **wastewater discharge** or **stormwater contamination** in more developed shoreline areas.
- Hierarchical clustering highlights *Methyloceanibacter caenitepidi* as a highly abundant (> 1%) bacteria across Sites 6-9 (Figure 5)
  - This species can use one-carbon compounds like methane and methanol as sole sources of carbon and energy.[19] There could be a relatively large amount of such compounds released into coastal waters by human activities.

Study Limitations and Future Directions:

- Only bacterial data were available. Chemical analysis will be performed to confirm water pollution by industrial activities
- Sample size was small (n = 2 per site), and more samples should be collected and processed
- Only land industrial activity was considered, not any other potential sources of pollution
- Did not account for oyster health before data collection (i.e., if they had existing illness caused by bacterial infection)

Acknowledgements

We thank the National Marine Mammal Foundation for enabling this research opportunity.

References

SCAN ME