Dolphin pathogens detected at low and similar abundance levels across dolphin pens in the San Diego Bay

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SUMMARY

- San Diego Bay is surrounded by densely populated urban areas
- We isolated bacterial DNA from mussels collected from dolphin pens in the Bay
- Identified two dolphin pathogens, *S. pasteuri* and *S. hominis*, via Nanopore 16S sequencing
- No significant difference was found among the pathogen abundance levels across the five sampling sites

ABSTRACT

The San Diego Bay has been historically polluted by industrial waste and urban runoff. This study aimed to detect and determine the abundance of bacteria that can be harmful to dolphin populations in the San Diego Bay. Mussels were sampled from 5 sites within the dolphin pens of the Marine Mammal Program's facility. Bacterial DNA isolated from the mussels' digestive system was analyzed, revealing a diverse microbiome, with the presence of dolphin pathogens including *Clostridium perfringens* and *Staphylococcus epidermidis*. There were no statistically significant differences among abundance levels across sampling sites. Future experiments could cover a wider sampling range to determine correlations between variations in bacterial composition and pollution.

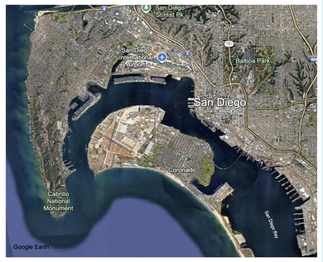
INTRODUCTION

Hypothesis:

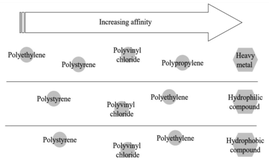
Mussels in San Diego Bay contain high abundance of bacterial pathogens due to pollution.

Background:

- Oysters collected from the San Diego Bay contained levels of Polychlorinated Biphenyls (PCBs) exceeding consumption thresholds, as well as other pollutants including plasticizers[1]



Urban areas with large human populations like San Diego often dispose of waste and harmful materials in nearby waters



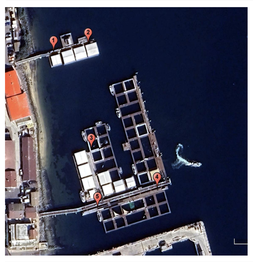
- In the ocean, high levels of contaminants, including heavy metals and organic pollutants, are correlated with high microplastic levels[2]
- Microplastics also carry bacteria, increasing their abundance and geographic range in marine waters[3]
- To identify potential pathogens in San Diego Bay, the 16S gene, which is conserved across bacterial species, can be sequenced

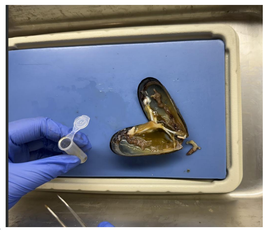
MATERIALS AND METHODS

Sample Collection

Mussels were collected from 5 sites (n = 3 for sites 1-4 and n = 2 for site 5) across the Marine Mammal Program Facility

Site 1: 32°42'18.9"N 117°14'10.7"W; Site 2: 32°42'19.1"N 117°14'09.6"W
Site 3: 32°42'16.4"N 117°14'09.5"W; Site 4: 32°42'15.4"N 117°14'07.4"W
Site 5: 32°42'14.9"N 117°14'09.3"W





Sample Processing

- Dissected digestive tracts of mussels
- Used saponin and DNase to deplete mussel DNA
- Extracted bacterial DNA with Powerfood Microbial DNA Isolation kit

Quality Check

- Used Nanodrop to examine purity and quantity of extracted DNA
- Conducted PCR and agarose gel electrophoresis to confirm 16S rRNA gene presence

Nanopore 16S Library Preparation and Sequencing

- Amplified 16S gene with Nanopore Barcoding Kit 24 V14
- Sequenced prepared library on MinION Sequencer

EPI2ME

R Studio®

Sequencing Data Analysis

- Sequencing reads were aligned to 16S gene sequences with the 16S workflow (wf-16s) on the EPI2ME platform
- Data was processed and visualized in R
- Ran ANOVA calculations to compare abundance levels across sampling sites

RESULTS

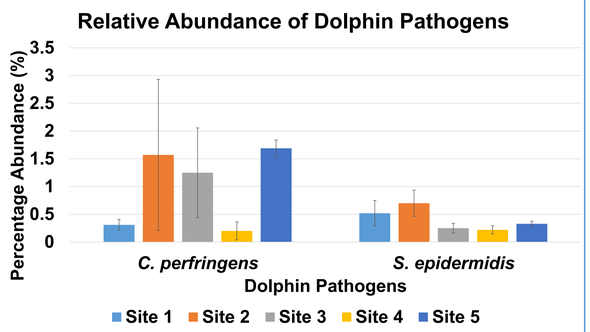


Figure 1: The percent abundance of two dolphin pathogens are not significantly different ($p > 0.05$, ANOVA) across five sites.

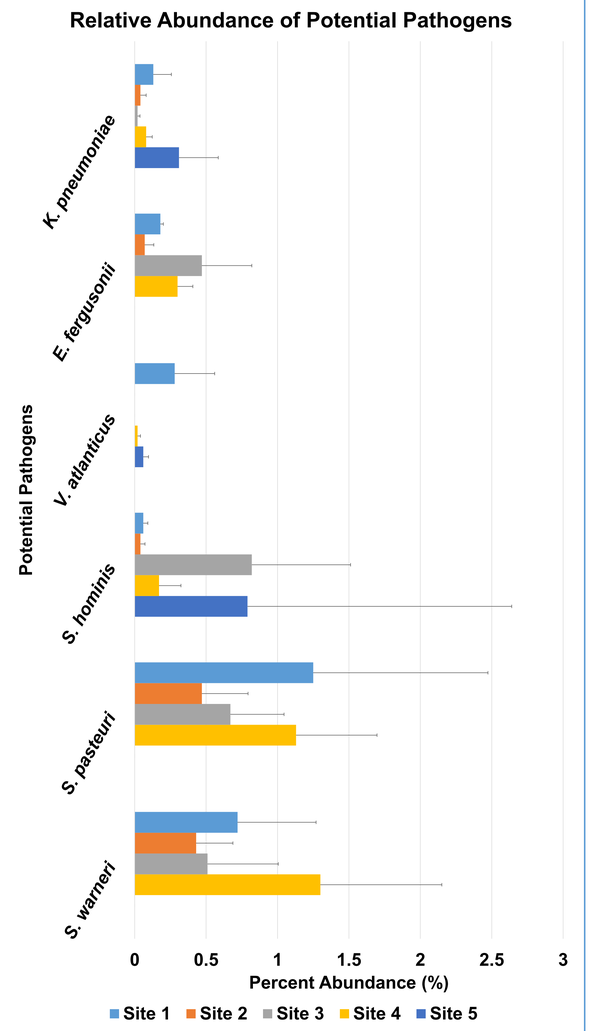


Figure 2. The relative abundance of six bacterial species that are potential dolphin pathogens are not significantly different ($p > 0.05$, ANOVA) across the five sites.

DISCUSSION

- We detected two species of dolphin pathogens, *C. perfringens* and *S. epidermidis*, from the digestive system of mussels in dolphin pens (Figure 1)
  - *C. perfringens* can cause muscle infections, toxemia, necrosis, and gas accumulation[4]
  - *S. epidermidis* infections can lead to pneumonia, respiratory infections, and skin lesions[4]
- Six potential dolphin pathogens were also detected (Figure 2).
  - Three are human pathogens: *S. pasteuri* can cause leukemia and endocarditis[7], *S. hominis* can cause meningitis[8], and *K. pneumoniae* is known to cause inflammation and pneumonia[10]
  - *E. fergusonii* is known to cause intestinal infections in farm mammal animals (cattle, sheep, and pigs)[9]
  - Two are fish pathogens: *S. warneri* infections can result in organ damage[5], and *V. atlanticus* can cause vibriosis[6]
- The abundance levels of pathogens were relatively low (< 3% of all bacteria)
  - Considering dolphin health and enclosures are regularly monitored and well-maintained by the National Marine Mammal Foundation, these pathogens are likely not a health concern for the dolphins
- The abundance levels of each pathogen are not statistically significantly different across the study sites
  - This is likely because the sampling locations are close to each other
  - Future studies can sample across the San Diego Bay and the open ocean habitats of wild dolphins to assess water quality across a wider range

REFERENCES

ACKNOWLEDGEMENTS

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