Gut Microbiome and Liver Pollutant Compositions in Lanternfish and Owlfish

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Abstract

At depths below 200 meters, the deep sea is characterized by low oxygen levels, low temperatures ranging from 4ºC to 20ºC, minimal light, and high pressure (NOAA, 2024). Due to these extreme conditions, the microbiomes of deep-sea fish are uniquely adapted to their environment and may play a role in aiding host survival in such harsh environments. Through targeted chemical analysis, we quantified the persistent organic pollutants present in fish livers. By integrating these findings with prokaryotic metagenomic analysis, my research seeks to explore a relationship between the pollutants found in deep sea fish livers and the bacterial species present in their stomachs. This combined approach could reveal how environmental toxins influence the composition of deep-sea microbes.

Introduction

Hypotheses

- Chemical pollutants that have been banned for longer periods of time will occur in lower concentrations in deep sea fish livers compared to those banned more recently.
- Fish containing similar types and concentrations of pollutants should also have similar stomach microbiome composition.

Background

- Lanternfish and owlfish were selected for chemical and microbiome analysis for their ability to thrive in conditions with no light, high pressure, and low temperatures.
- The unique extreme conditions of the deep sea and the gut microbiome's established role in host immunity and metabolism suggests that the deep sea fish microbiome composition may offer insight to their survival in such an extreme environment.
- PCBs and Pesticides
  - Pesticides are used in agricultural settings for pest control. Common chemical classes include organophosphates and organochlorines.
  - PCBs, or polychlorinated biphenyls, are chemical compounds used in paint, plastic, and rubber manufacture, as well as equipment maintenance and in dyes (EPA, 2024).
  - Ocean dumping and burning of wastes led to deposition of PCBs and pesticides in atmosphere and oceans; regulations began in the 1960s and have been expanded since, but PCBs persist in the environment decades after being banned (NOAA).

Figure 1. The United States National Malaria Eradication Program (1947-1952) used DDT for spraying waterways where mosquitoes bred.*Centers for Disease Control and Prevention (Public Health Image Library(Phil))* (NPS, *A legacy of contamination (U.S. National Park Service)* 2024)

Significance

- Pollution is a global issue, with detrimental environmental and public health implications. Acquisition of information regarding the extent of pollution in deep sea fish in conjunction with information about the bacterial constitution of their microbiomes could yield important data for the future of environmental and human health.

Methodology

Fish Collection and Dissection

- Fish were collected near Oregon coast with a trawling net at 600-800 m below the sea surface
- Liver, stomach, and intestine were dissected from the fish and stored at -20℃ until further processing

DNA Isolation

- Stomach and intestine samples were cut into pieces with ~2 mm diameter
- Followed host-depletion protocol(Bruggeling et al., 2021) to minimize the presence of fish DNA
  - MO BIO Powersoil DNA Isolation kit and QIAGEN DNeasy Blood & Tissue kit were used for bacteria DNA purification to compare extraction efficacy

Chemical Analysis

- Liver samples were sent to Statera Environmental for analysis
  - Pressurized liquid extraction followed by high-performance liquid chromatography was performed to isolate chemicals
  - Gas chromatography-mass spectrometry was performed to quantify extracted pollutants

16S Library Preparation and Sequencing

- Isolated DNA were processed with Oxford Nanopore 16S Barcoding Kit 24 V14 to prepare the sequencing library following manufacturer protocol
- Prepared library was loaded to a Nanopore R10.4.1 flow cell and sequenced with the MinION sequencer

Data Analysis

- Sequencing data were processed with the EPI2ME 16S workflow
- Chemical data was formatted in R version 4.4.2 (R Core Team, 2022) using the dplyr package. Heatmap was generated using the package pheatmap version 1.0.12 (Kolde, 2019), and lollipop plot was generated using the package ggplot2 version 3.4.0 (Wickham, 2016).

Results

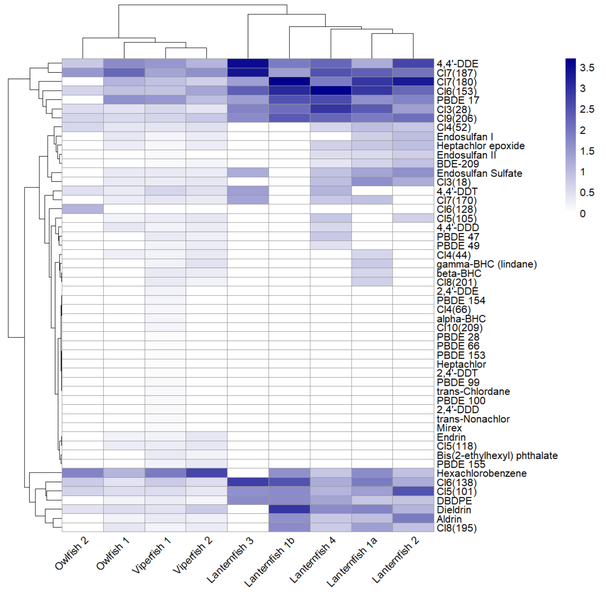

Figure 2. Pollutants are most concentrated in lanternfish livers. The concentration of detected chemicals (ng) per gram of liver tissue. Darker shades of blue represent higher concentrations. Lanternfish1a and 1b represent two parts of the liver isolated from the same lanternfish.

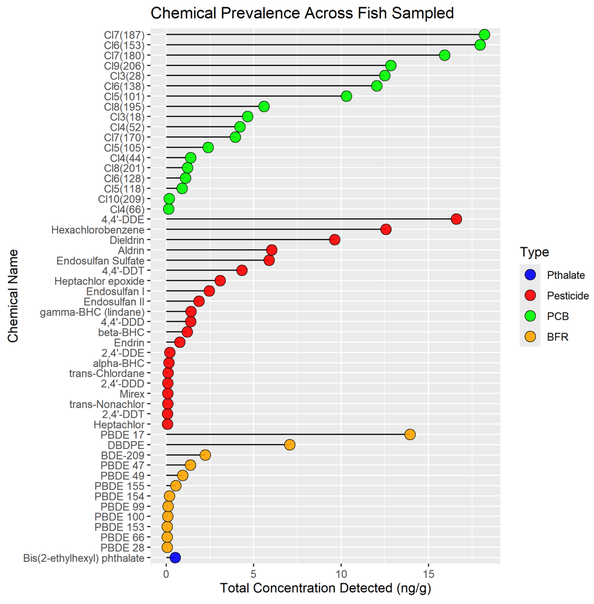

Figure 3. PCBs and DDT make up the majority of chemicals detected. Total chemical concentrations (ng/g) detected across all sampled livers combined is shown. Data is ordered by chemical type in descending order of concentration. Each color corresponds to a separate chemical category. Polychlorinated Biphenyls (PCBs) made up the majority of pollutants detected. 4'4 DDT and its derivatives (4'4 DDE and DDD, 2'4 DDE, DDD, and DDT) make up the majority of pesticides detected.

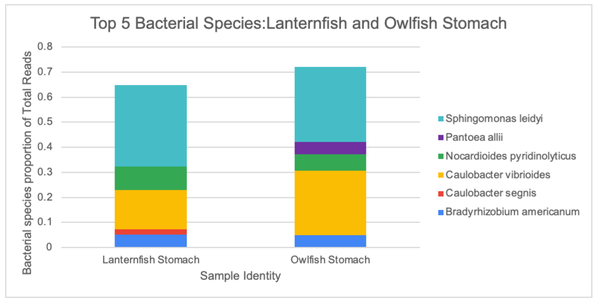

Figure 4. Lanternfish and Owlfish stomach contain similar gut microbiome distribution. Bar graph shows top 5 most prevalent bacteria species in each stomach sample. Sample identity is shown on the x-axis, and proportion of each bacteria in each sample is shown on the y-axis. The top four bacteria are identical between the samples. The fifth bacteria (orange in lanternfish and purple in owlfish) is unique between them.

Conclusion / Discussion

- Most of the tested PCBs were detected in all liver samples (Figure 2).
- 4,4'-DDE, a degradation product of DDT, a pesticide banned in 1972, was the most abundant pesticides tested (Figure 3).
- PCBs were banned by 1979 (EPA, 2024)
  - Despite having been outlawed over 40 years ago, banned pollutants shockingly persist in deep-sea environments
  - Some lanternfish species are known to travel between the deep sea and shallower waters during the day (Eduardo et al., 2021); migratory patterns may explain higher pollutant concentrations
- Metagenomic comparison of Lanternfish and Owlfish stomach samples showed high similarity in the top 5 species (excluding human contaminant bacterial DNA) which contrasts chemical analysis results
  - *Nocardioides pyridinolyticus*, the third most prevalent species in bacterial metagenomic analysis of lanternfish and owlfish, has been shown to degrade organic pollutants. This ability is promising insight into a bacterial species that might mitigate exposure to high levels of persistent organic pollutants (Ma et.al, 2023).

Limitations

- Low sample size limits statistical analysis
- Low yield of bacterial DNA for some samples collected may have compromised the coverage of the total microbiome
- Human bacterial contamination complicated microbiome characterization

Future Directions

- Differences pollutant concentrations across species may match differences in prey and mating activities. Future studies could focus on behavioral patterns and potentially identify pockets of high toxicant levels in the deep sea.
- *N.pyridinolyticus* activities in the fish could be explored in deep-sea fish to determine if they can lower the impact of toxicants internalized by the fish.

Bibliography