Pollutant and Gut Microbiome Analyses of Deep-sea Fish from Oregon Coastal Waters

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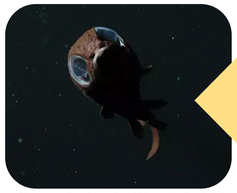
Background



Monterey Bay Aquarium "Meet the Owlfish"

The deep Pacific Ocean is a high pressure, low oxygen, and low light environment. These conditions favor extremophiles, such as the large-eyed owlfish.

NOAA "What are phytoplankton?"

Little is known about deep-sea bacteria and possible hosts. Metagenomic surveys of bacteria in deep-sea fish guts will allow insight into a largely unknown environment.

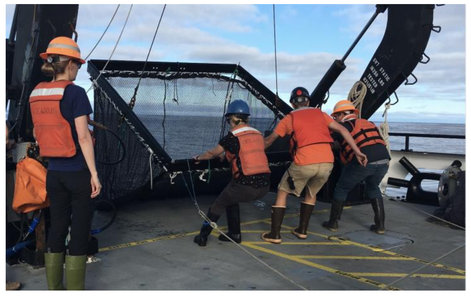
NOAA Office of Response and Restoration

Researchers examine ocean fauna to gauge ecosystem health in the context of historical pollution.

Hypotheses

1. Organic chemical pollutants are present in deep-sea fish.
2. Owlfish and lanternfish have unique gut microbiomes.

Fish Sample Collection



Lab group with net from PSU "CLEE Director" Webpage on Dr. Lindgren

- Fish were collected from off the Oregon Coast
- They were frozen and barcoded by Center for Life in Extreme Environments (CLEE)
  - Special thanks to Dr. Lindgren
- Likely deep sea fish identities were selected based on anatomy
  - 9 fish for pollutant analysis
  - 3 fish for gut microbiome sequencing

Chemical Analysis Methods

Fish Dissection and Liver Collection

5 Lanternfish Specimens | 2 Viperfish Specimens | 2 Owlfish Specimens

Extraction/Clean-up and Quantitative Target Compound Analysis

Procedure performed by Statera Environmental

Pressurized liquid extraction (DCM solvent) → HPLC fractionation → Evaporate extract to 300 µl → Analyze each extract with GC/MS *

Buchi E-916 Speed Extractor

Example Silica Columns for HPLC

Buchi SyncorePlus Analyst

Agilent 7010 Triple Quadrupole GC/MS

** = analysis precision with deuterated internal standards*

Chemical Analysis Results

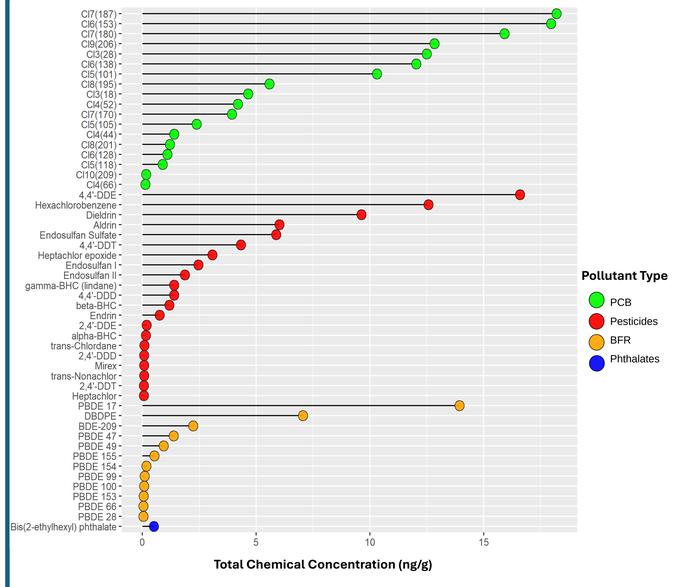


Figure 1. Total pollutants detected across all fish liver samples. Total concentration (chemical abundance by liver wet weight) of each chemical across all liver samples. Chemical groups are arranged in descending order by total concentration, with PCBs being the highest. Figure generated with R and ggplot2.

Microbiome Analysis Methods

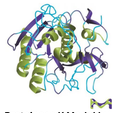
Fish Dissection and Organ Collection

2 Dragonfish intestine replicates | 2 Lanternfish stomach replicates | 5 Owlfish Intestine replicates | 3 Owlfish stomach replicates

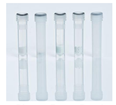
Dissection → Organ separation and tissue lysis → Fish DNA host depletion with Proteinase K, saponin and TurboDNAse → Mutanolysin and bead-beating to extract bacterial DNA

Owlfish-like Specimen with Barcode

Owlfish-like Specimen Stomach



Proteinase K Model by Sigma Aldrich



Qiagen PowerBead Tubes

Modified from Bruggeling et al. (2021)

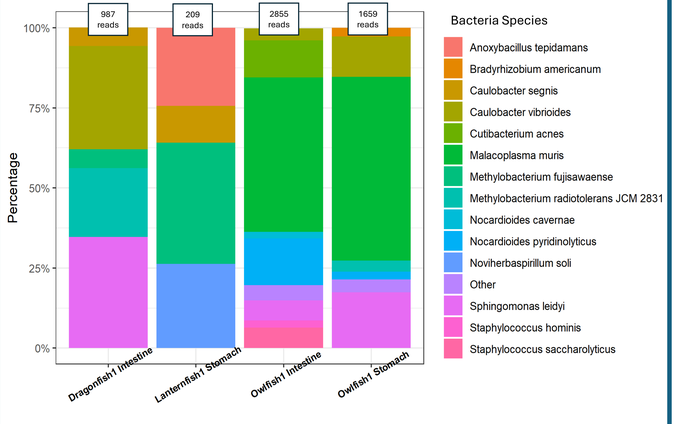
16S rDNA PCR amplification → DNA barcoding and adapter ligation → DNA sequencing

Promega GoTaq Green Mastermix for PCR

Nanopore 16S Barcoding Kit 24 V14

Nanopore MinION

Metagenomic Analysis Results



Fish Sample and Organ

Figure 2. Bacteria species relative abundance by sample organized by deep-sea fish identity and tissue type. Host identities are determined by morphology. Any bacterial species with only one occurrence across all fish technical replicates were excluded. Gastrointestinal samples were compared against skin samples from the same host, and only bacterial species unique to gut tissue were kept. Data was combined based on fish and tissue type. Figure generated with R and ggplot2.

Conclusions

Owlfish contained similar microbiome make up in both stomach and intestine samples, differing from that of the lanternfish and dragonfish. A few notable genera include:

- Caulobacter: oligotrophic alphaproteobacteria that live in conditions with very low nutrient sources.
- Methylobacteria: alphaproteobacteria known for their ability to metabolize single carbon compounds like methanol and methane.
- Nocardiodes: bacteria that quickly degrade many pollutants including polymeric polyesters.

Two staphylococci were present in multiple fish gut samples but not in each host's skin samples and were included. These could have come from human contamination during handling or processing.
Whether mutualistic, parasitic or just fish food, these bacteria make their home in the deep Pacific Ocean.

There were high amounts of polychlorinated biphenyls (PCBs) detected in these fish. PCBs were outlawed in the USA 1979 because they act as a hormone disruptor. It is possible these fish endocrine systems were impacted by PCBs in their environment. Pesticides are substances that destroy unwanted plant or animal or mitigate harm to crops (40 CFR 152.3). It is possible that insecticides DDT and DDE affected these specimens' immune systems, central nervous systems and livers.
Brominated fire retardants have also been found to be toxic.

Future Directions

Future cohorts of the Boz Institute Metagenomics Research Initiative can continue to partner with the Center for Life in Extreme Environments to further illuminate the unique organisms of the Pacific Twilight Zone. Interesting future studies could include:

1. Re-evaluating the top ten bacteria species found in these technical replicates and selecting for bacteria found both on the skin and in the gut
2. Performing chemical analysis of the remaining liver and skin samples of the three fish with gut microbiome data
3. Fully sequencing the genome of each deep-sea fish species
4. Performing heavy metal specific analysis of brain samples from these fish
5. Partnering with industry representatives to determine therapeutic potential of bacteriocins produced in deep sea fish microbiomes

References

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