The deep-sea dragonfish have higher gut microbiome diversity but lower abundance compared to shallow-water medaka fish

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Abstract

The deep-sea environment is characterized by lower amount of nutrients, compared to relatively shallow water environment. Less nutrient should restrict bacterial growth, limiting both the diversity and abundance of the microbiome. We hypothesized that medaka (shallow water fish) maintained under optimal conditions in the lab should have more abundant and diverse intestinal microflora than dragonfish living in deep-sea. We dissected the fish and isolated their intestines, extracted bacterial DNA from the samples, and then performed Nanopore 16S sequencing to profile the gut microbiota. Medaka fish showed a significantly higher overall abundance of intestinal microbiota, with over 18,000 reads per sample, compared to dragonfish, which has lower than 200 reads per sample. However, medaka showed lower bacterial diversity. Our data indicates that nutrient-dense environments support higher microbiota abundance but may reduce bacterial diversity, while nutrient-poor environments promote greater microbial diversity, but with lower abundance. As many other environmental factors also differ between the two distinct habitats, further studies are needed to verify this pattern.

Introduction

Hypothesis

Shallow water fish (medaka/zebrafish) that live in nutrient-dense environments have more abundant and diverse intestinal microflora than deep sea dragonfish which live in nutrient-deficient areas.

Gut microbiome is affected by the nutrients, diversity of plants, and pollutants in the environment.

The importance of gut microbiome

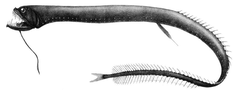
- Affects digestive development, lifespan, and immunity of fish
- Diversity of microbial ecosystem allows for an improved immune systems (Xiong, Nie, Chen, 2019) (Cleveland Clinic, 2023)
- Lack or loss of biodiversity can contribute in immune dysfunction and inflammation (Tasnim, et. al, 2017)

Medaka

- Naturally live in fresh and brackish water (mix of freshwater and seawater)
- Natural habitat contains
  - Minerals: calcium, magnesium, zinc, phosphate
  - Proximate nutrients: proteins, carbohydrates, lipids, ash, fiber, and cartoneids (Hilgers, et. al, 2019)

Dragonfish

- Live in temperatures around 4˚C
- Diet
  - Generally preys on other fish but also consumes detritus floating down from above (Britannica, 2008)



Zebrafish

- Live in freshwater streams and ponds with lush vegetation in South Asia
- Diet
  - Multiple types of algae, plankton, and sediment (Watts, et. al, 2014)

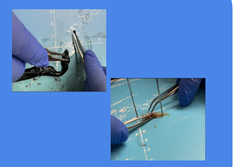
Methodology

Sample Collection

- A dragonfish was collected through net trawling off the coast of Oregon (44.652, -126.05) at the depth of 650m,
- Medaka fish were maintained at the Boz Institute, fed twice daily with processed algae (spirulina 20) ad libitum

Dissection

- One dragonfish and six medaka fish were dissected
- Organs and tissues, including intestine, stomach, eyes, brain and skin, were isolated and frozen at -20°C



DNA Isolation

- Used MO BIO Powersoil kit following bacterial enrichment DNA isolation protocol (Bruggeling, Garza, Achouiti, Mes, Dutilh, Boleij 2021)

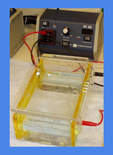
Polymerase Chain Reaction and Agarose Gel Electrophoresis

- Performed PCR on isolated DNA using Promega GoTaq® Green Master Mix
- Amplified 16S V4 region with the following primers:

Forward 5'-GACGCTCTTCCGATCTTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'

Reverse 5'-TGTGCTCTTCCGATCTAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3'

- Checked PCR success with 1.5% agarose gel



Nanopore 16S Sequencing and Data Analysis

- Used 16S Barcoding 24 V14 library preparation kit following the manufacturer protocol
- Used MinION for sequencing and EPI2ME 16S workflow for bacteria species identification

Results

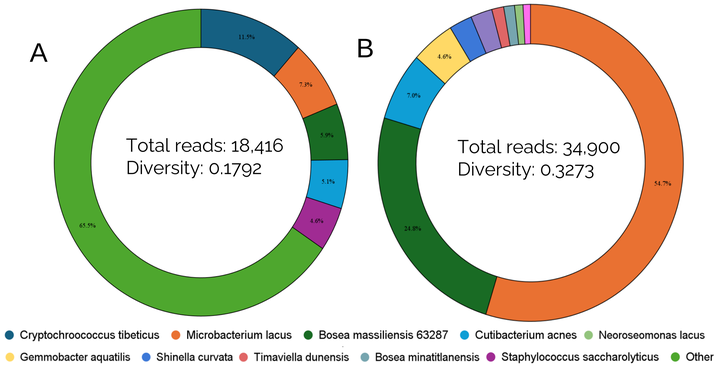


Figure 1: Relative abundance (%) of the most abundant bacteria extracted from the intestines of medaka. (A-B) Total reads and Simpson's Diversity Index (SDI) are indicated at the center of each pie chart. SDI were calculated as $\sum_{i=1}^{S}(\frac{n_i}{N})^2$, where S is the total number of species, n is the total number of individuals in the ith species, and N is the total number of individuals (reads). The most prevalent bacteria are from the *Oculatella* genus.

Figure 2

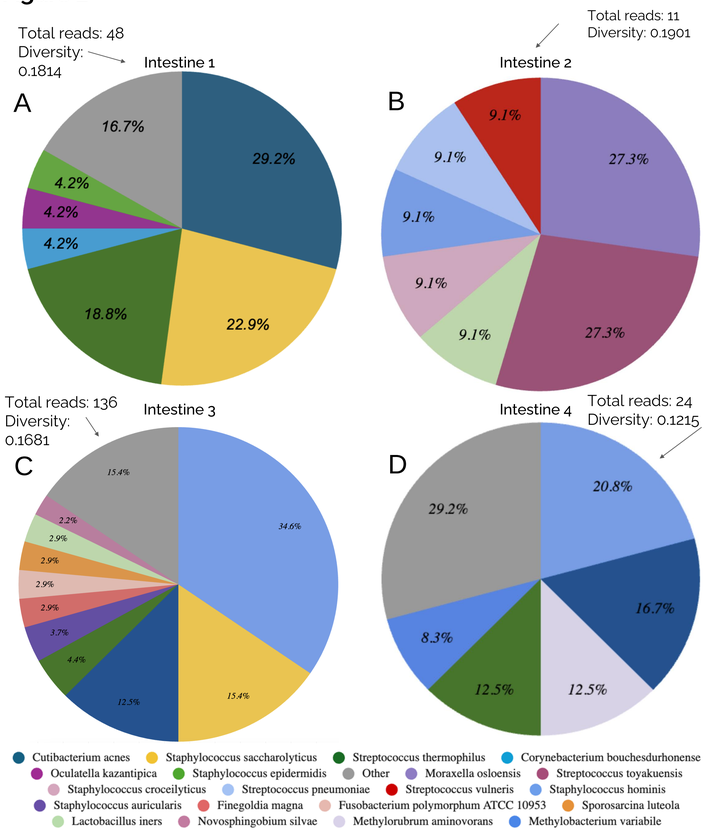


Figure 2: Relative abundance of the most abundant bacteria extracted from the intestines of the Dragonfish. (A-D) Pie charts depict the percentage abundance of 6-10 bacteria species found four technical replicates of the dissected intestine of the dragonfish. Each slice of the pie represents the proportion of a specific bacterial species relative to the total bacterial community. The total reads and Simpson's Diversity Index per sample are indicated at each corner of the chart.

Discussion & Conclusion

Our hypothesis was partially supported by our data.

- One of the medaka intestine samples (Figure 1A) have similar microbiome SDI to the dragonfish ones (Figure 2), while the other (Figure 1B) is much higher, indicating lower diversity.
- The medaka fish have greater abundance of microbiota than dragonfish.
  - Nevertheless, the large total read difference between medaka (> 18,000) and dragonfish (11-136) organisms could be due to technical error during sample processing.

Prevalent bacteria in dragonfish Intestine

- *Oculatella kazantipica* was also present, though in lower abundance in the dragonfish intestine (4.2%).
  - Likely due to the decreased limited light in the deep sea.
- *Halomonas denitrificans* (1.5% abundance) have been found in deep-sea sediment and hyper-saline marine areas. Other bacteria in the gut microbiome of the dragonfish may also have high-tolerance to salt (Mounier et al., 2017).
- Many detected bacteria are commonly found on human skins, and are likely contaminants introduced during the experiments.

Prevalent bacteria in medaka intestine

- The two most abundant bacteria, *O. lusitanica* and *O. kazantipica* are filamentous cyanobacteria.
  - Unicellular organisms both terrestrially and in marine environments.
  - Fix N_2 on aquatic plants, and can be ingested by fish feeding on the plants, playing a crucial role in maintaining the aquatic ecosystem (Gallet et. al, 2023).
  - The environmental conditions of the medaka fish (abundant light and nutrient from fish food) in the lab tanks should favor their growth. (Brito, 2022).
  - It is likely that the fish tanks contained these cyanobacteria, which were ingested by the medaka fish.
- The third-most abundant bacteria, *Neotabrizicola shimadae* (genus is Rhodobacter), is found in mostly brackish-water, the natural environment of medaka fish.

Prevalent bacteria in zebrafish intestine

- We also used the bacteria commonly found in zebrafish intestine as a reference
- *Cetobacterium*: 6.92% of the reads in the zebrafish intestine
  - Produces vitamin B-12 – protects the gut against pathogen infection, maintains gut microbiota, and stabilizes gut redox (Qi et al. 2023, Stephens, et al., 2015).
- *Shewanella*: → 2.65% of the reads in the zebrafish intestine
  - A probiotic, enhances intestine health and improves immunological response (Vidal, et al., 2017, Wei et al., 2021)

Bibliography

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