# Investigating Vertical Transmission of Microbial Symbionts in Marine Sponges

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## Introduction:

- Many sponges reproduce through a process called brooding and release larvae into the water column during particular times of the year
- Previous research has been done on examining the microbiomes of marine sponges, many of which host symbiotic microbes (1).
- It is unclear which members of these microbiomes are passed from parent to offspring and which are acquired from the environment
- We hypothesized that some but not all of the microbiome members were passed on vertically (from parent sponge) while others were acquired horizontally (from surrounding environment).
- To shed light on this question, we examined the microbiomes of adult sponges and their larvae using next-generation sequencing.

## Methods:

- Three species of sponges were used in this study: *Iotrochota birotulata*, *Niphates erecta*, *Svenzea zeai.*
- Both life stages of each sponge species were used (n=3). All samples were collected at the Smithsonian Tropical Research Institute in Bocas del Toro, Panama in July 2019.
- Larvae were collected by collecting adult individuals from the field and holding them in tanks filled with seawater until larvae were release.
- Larvae were then rinsed in sterile seawater and fixed in RNA later for transport.
- Pieces of adult sponges of each species were also preserved in RNA later for transport.
- DNA was extracted from adult and larvae samples using protocols from the Earth Microbiome Project (EMP).
- Protocols from the EMP were also used to amplify the V4 region of the 16s ribosomal RNA subunit gene using PCR.
- Amplicons were then sequenced using next-gen sequencing on the Illumina MiSeq platform.
- Sequences were then demultiplexed with the bioinformatics platform QIIME.
- Amplicon Sequence Variants (ASV's) were generated using the DADA2 bioinformatics pipeline.
- R package vegan (2) was used to analyze relative abundances of bacteria in adult and larvae life stages between the three species.
- Figures were made with R package ggplot2 (3) and Microsoft Excel.

## Results:

**Figure 1**: From left to right are photos of *I. birotulata (A), N. erecta (B), and S. zeai (C)*

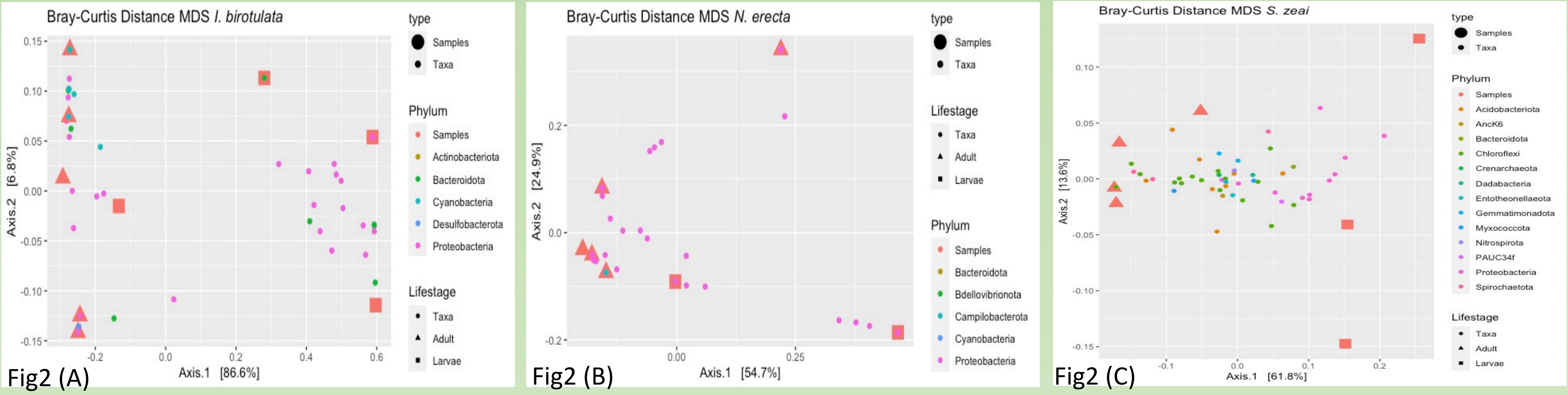

**Figure 2**: Multi-dimensional scaling (MDS) to visualize how similar the communities in adults and larvae are. From left to right the figures represent the communities from: *I. birotulata (A)*, *N. erecta(B), and S. zeai (C).* We noted a significant difference in adult and larvae communities in *I. birotulata* (adonis, df=1, *F*=10.11, $R^2$=0.59, *P*= 0.009), and in *S. zeai (*adonis, df=1, *F*=5.15, $R^2$=0.51, *P*= 0.03). In contrast, the adult and larvae communities found in *N. erecta* were not significantly different *(*adonis, df=1, *F*=2.45, $R^2$=0.33, *P*= 0.11).

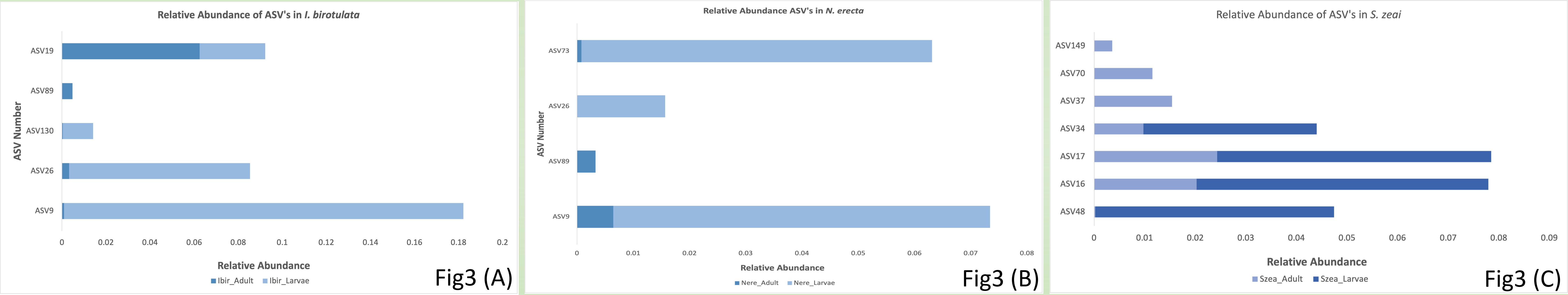

**Figure 3**: Bar plots visualizing abundance of ASV's who showed a significant shift between life stages in *I. birotulata(A)* and *S. zeai*. (C) . (B) A bar plot for *N. erecta* (these ASV's did not show a significant shift in *N. erecta).* Some noteworthy observations from *S. zeai* were: ASV 16, which was identified as being from the class Anaerolinea, a group of thermophilic bacteria. Thermophiles are often archaea, though they can also be bacteria. ASV 17, PAUC34f, is a candidate phylum recently discovered in marine sponges, and is suspected of being mostly composed of sponge symbionts (4). In *I. birotulata*, ASV's 9, 26, and 130, all of which were Proteobacteria, were found in much greater abundance in larvae than in adults. This suggests that at some point perhaps the Proteobacteria are not as significant to the microbiome, and their population numbers decrease.

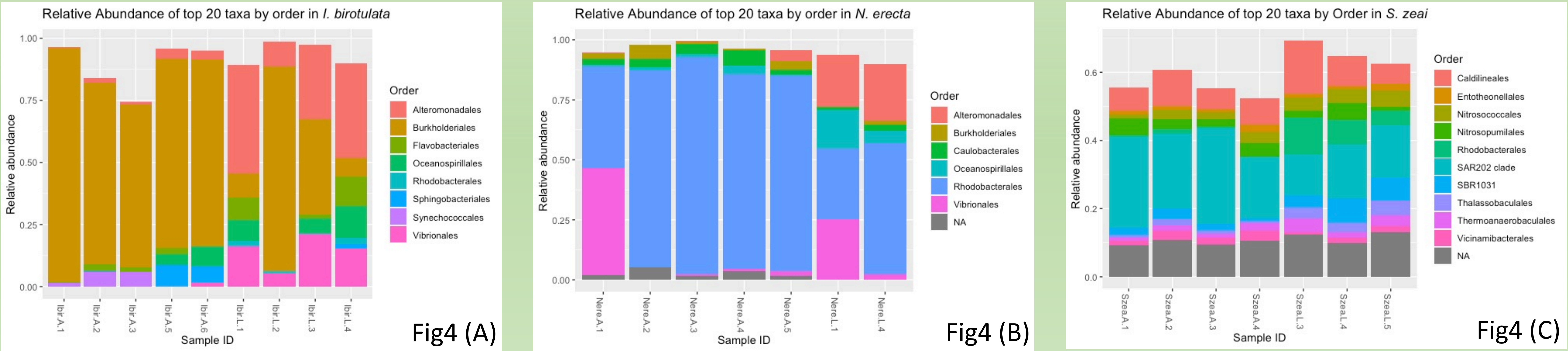

**Figure 4**: Relative abundance bar plots capturing the diversity of the top 20 taxa in each species by taxonomic order. From left to right: *(A) I. birotulata*, *N. erecta (B), and S. zeai (C)*

## Conclusions:

- There was a significant shift in community abundance in two species, *I. birotulata* and *S. zeai,* where several taxa were found in one life stage, and perhaps were absent (or in very low abundance) in the other. This could mean the community shifts are examples of horizontal transmission.
- *N. erecta* did not show any significant community change between life stages, implying that the microbiome members obtained from the parent sponge are mostly preserved (vertical transmission).
- The three species did not share any taxa who significantly contributed to the dissimilarity between the three groups of adult and larvae, suggesting that the community shifts are largely species-specific. This is in line with our current understanding of the sponge microbiome.
- Noting several significant community shifts between larvae and adults raises questions about why certain taxa only inhabit one life stage of a species. Factors such as the environment and food availability could contribute, but future studies will need to be conducted to explore this further.
- While several taxa who were likely symbionts were noted, the mechanism by which they are acquired from the parent or environment is still unknown.
- While this study did not include samples from the surrounding water, it would be beneficial to also compare communities found in water samples to the ones found in the sponge samples, this could provide further evidence as to which taxa were most likely transferred horizontally.

## Acknowledgements:

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## References:

- Photos of the three sponge species in Figure 1 were obtained from:

https://spongeguide.uncw.edu/ and
https://stricollections.org/portal/

- https://earthmicrobiome.org/protocols-and-standards/dna-extraction-protocol/
- https://earthmicrobiome.org/protocols-and-standards/16s/

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(4) Chen Michael L., Becraft Eric D., Pachiadaki Maria, Brown Julia M., Jarett Jessica K., Gasol Josep M., Ravin Nikolai V., Moser Duane P., Nunoura Takuro, Herndl Gerhard J., Woyke Tanja, Stepanauskas Ramunas. (2020). Hiding in Plain Sight: The Globally Distributed Bacterial Candidate Phylum PAUC34f. *Frontiers in Microbiology.* Vol. **11**. pg 357.