



A functional ecological comparison of three sponge species from the Lower Florida Keys

Tyler W. Griffin¹, Clara Prentiss¹, Malcolm Hill², Jeremy B. Weisz¹

¹Department of Biology - Linfield College - McMinnville, OR

²Department of Biology - University of Richmond - Richmond, VA



Introduction

- Sponges are simple, primitive animals that lack true tissues. They are filter feeders that pump water through their body channels and effectively clean the surrounding water.
- Due to climate change and other environmental factors, sponges have replaced corals as the dominant reef inhabitant in the Florida Keys.
- Sponges play host to a variety of symbionts including bacteria, dinoflagellates, and others.
- A sponge's microbiome refers to the bacterial community that lives in or on the sponge. Sponge microbiomes perform ecological roles, most notably nutrient cycling (Fig. 1).

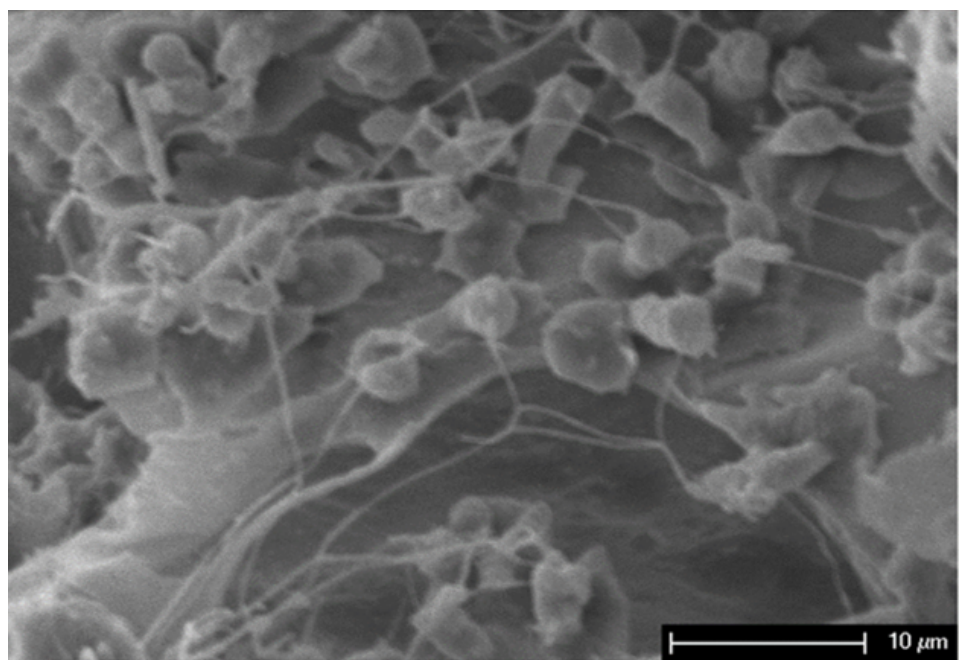


Figure 1: SEM image of microbes inhabiting sponge tissue.

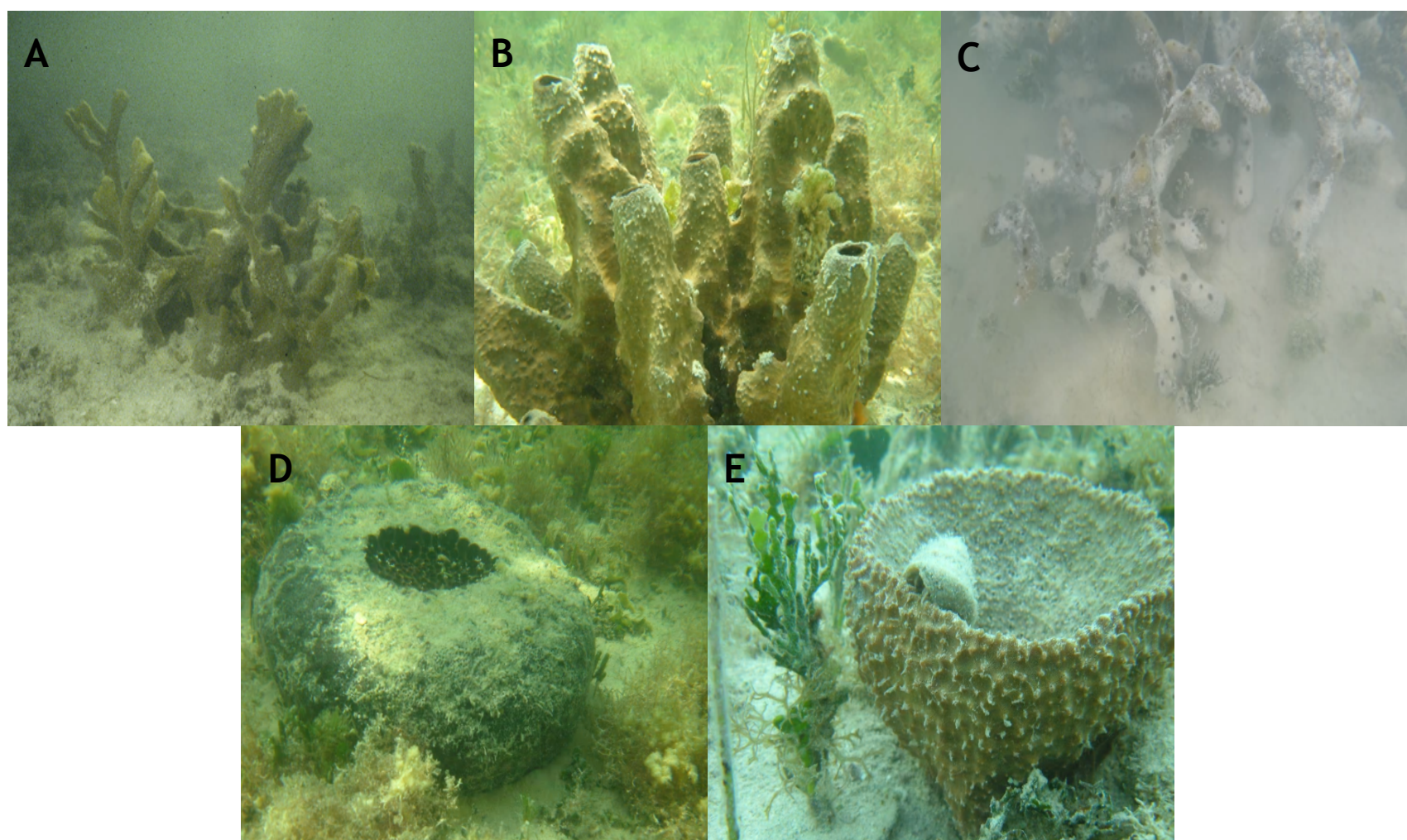


Figure 2: A) *Cliona varians forma varians*. B) *Ircinia variabilis*. C) *Neopetrosia subtriangularis*. D) *Spheciospongia vesparium*. E) *Ircinia campana*.

- Previous work by our research team has shown that *I. variabilis* has a significantly different stable carbon isotope ratio than CVFV and *N. subtriangularis* (Fig. 3).
- This work examined other ecological factors between these three species of interest and other neighboring sponges.
- Specifically, we looked at pumping rates, microbiome compositions, and microbiome functional gene comparisons.

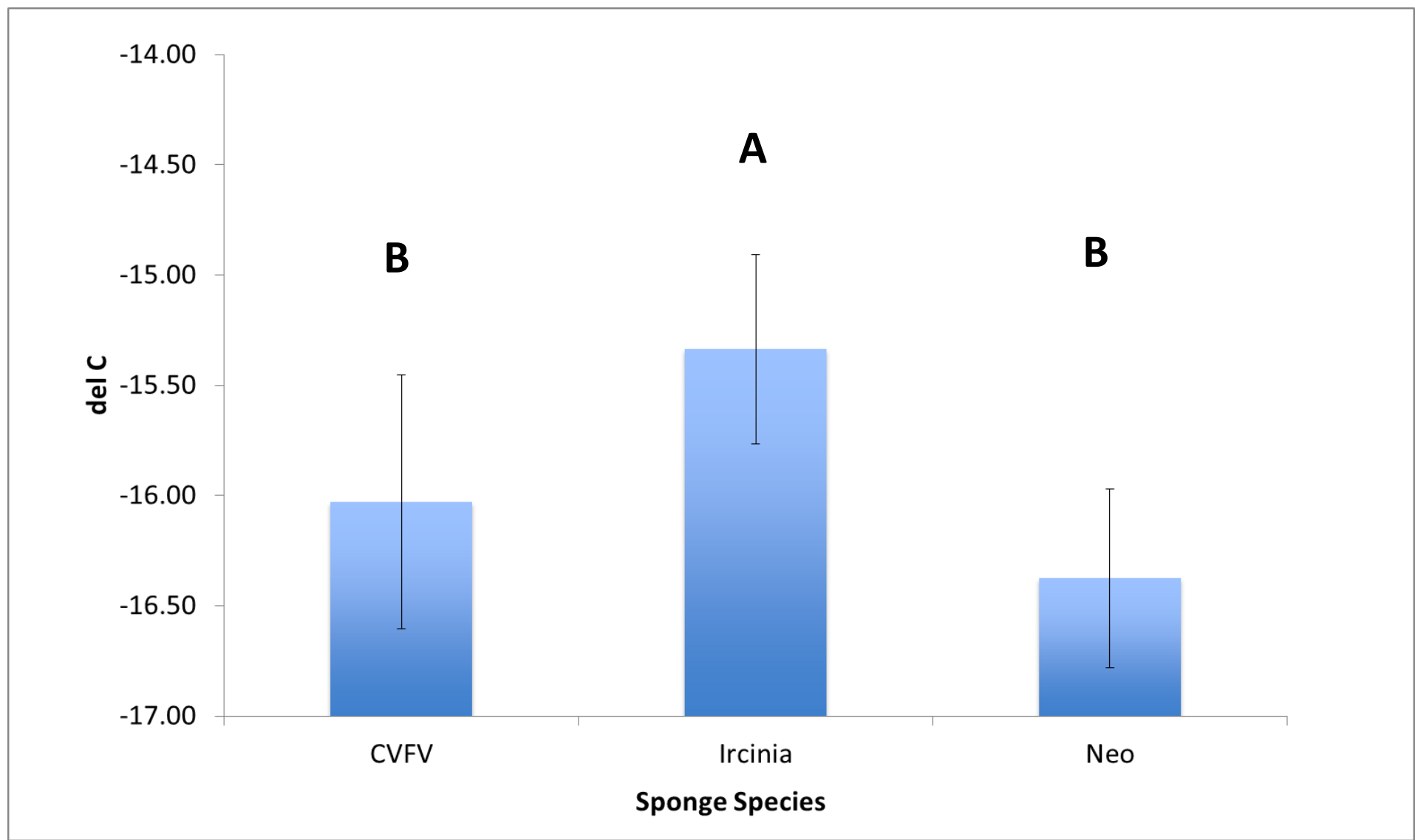


Figure 3: Mean \pm SD $\delta^{13}\text{C}$ for the three species of interest. There was a significant difference among group means $\text{df}=2$, $F=11.51$, $p=0.0003$. Bars sharing the same letter were not significantly different according to the Tukey test ($\alpha=0.05$).

Methods

Fieldwork

- We spent ten days in Summerland Key in June 2016 (Fig. 4) working in sand flats in water up to 1.5 m deep.
- We took tissue samples and videos of dye pumping rates.

Dye Video Analysis

- Non-harmful dye was injected into the water surrounding the sponges. Video was taken as the sponges took up dye, and expelled it through oscula (Fig. 5).
- Using ImageJ, we measured an osculum of each sample sponge and the speed of dye fronts escaping that osculum, which we then used to estimate the pumping rates of individual sponges.

Microbiome Analysis

- Extracted DNA from the three rope sponge species was sequenced on an Illumina MiSeq using primers spanning the V3-V4 region of the 16S rRNA gene.
- Sequence data was processed using standard protocols in Mothur and then analyzed using the vegan package in R.

Functional Gene Search

- We extracted total microbial genomic DNA from tissue samples of all five sponge species.
- Using PCR amplification with specific primer sets, we searched for ecologically relevant functional genes in metagenome of each sponge.
- We searched for the following functional genes: *amoA*, *pmoA*, *nifH*, *nosZ*, *norB*, and *hzo*, which are all genes encoding for enzymes involved in nitrogen cycling or methane metabolism.
- We used gel electrophoresis to visualize the results.

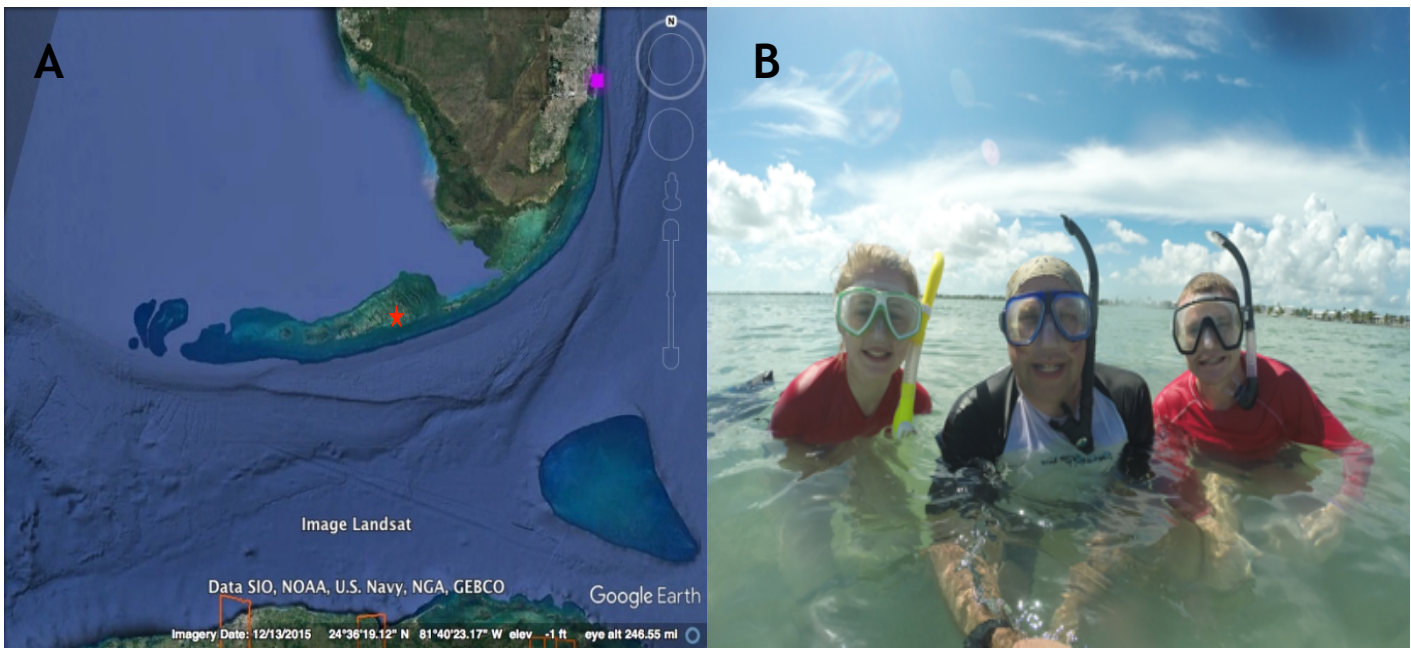


Figure 4: A) Map of South Florida and the Florida Keys, Summerland Key denoted with red star and Miami denoted with pink box. B) Weisz Lab in the field.

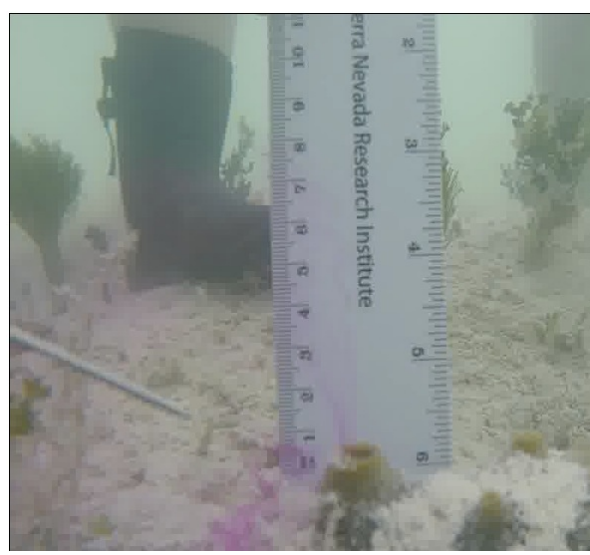


Figure 5: Still-frame image of dye video analysis for CVFV.

Results

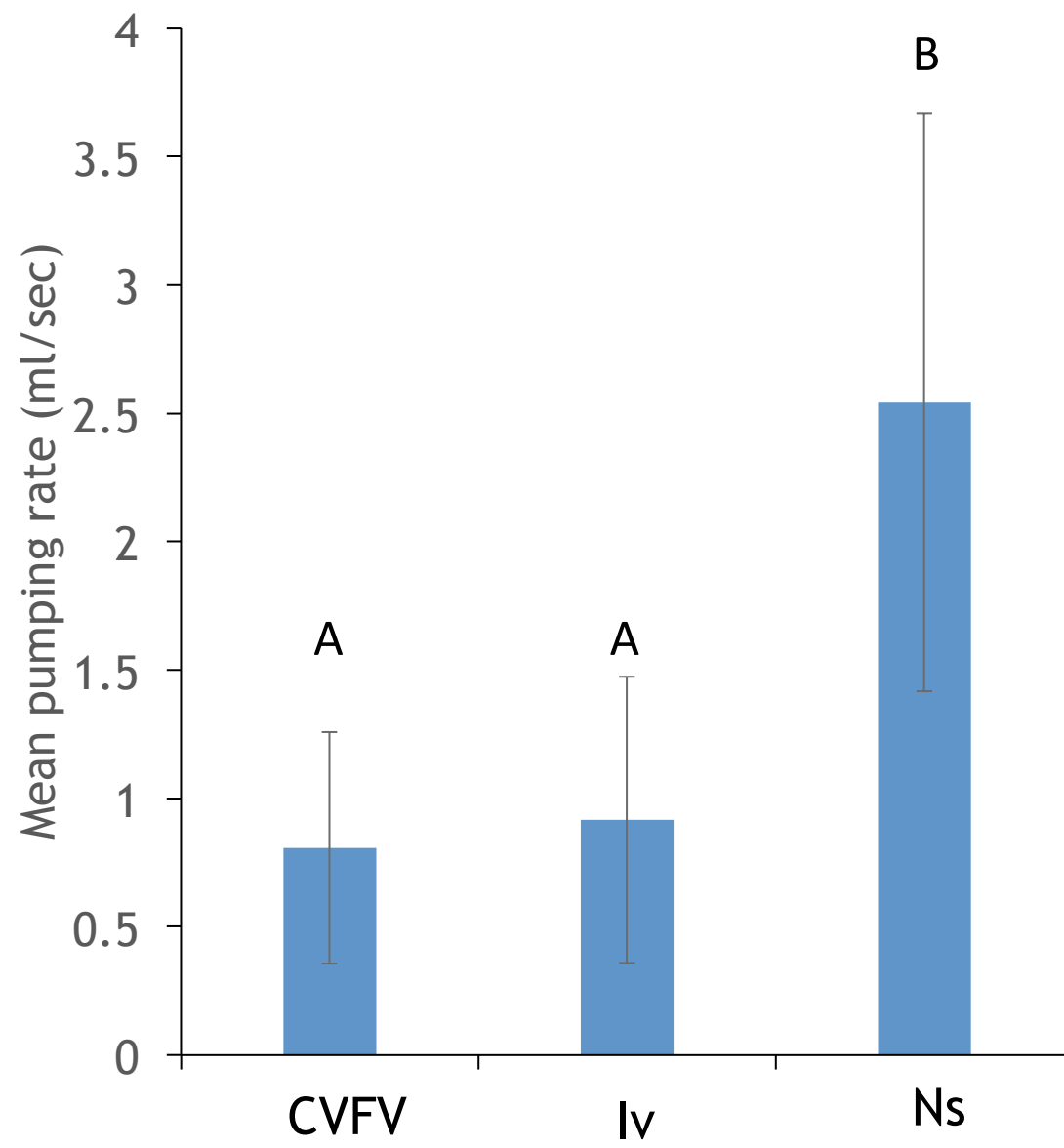
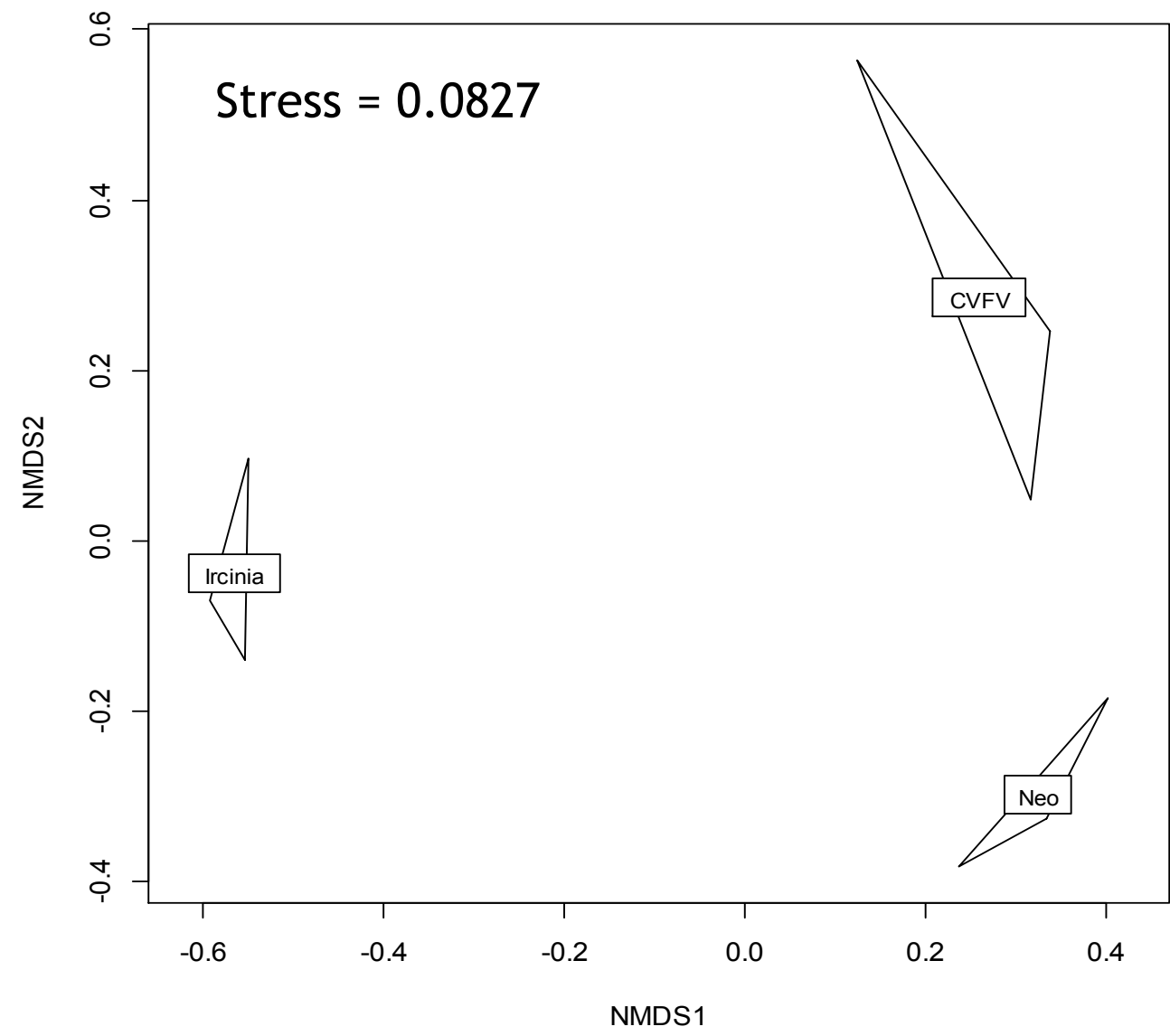


Figure 6: Rates at which seawater is pumped through *Cliona varians forma varians* (CVFV), *Ircinia variabilis* (Iv), and *Neopetrosia subtriangularis* (Ns). There was a significant difference among pumping rates ($\text{df}=2$, $F=5.95$, $p=0.0226$). Bars sharing the same letter were not significantly different according to the Tukey test ($\alpha=0.05$).

Figure 7: Non-metric multidimensional scaling plot for the microbiome compositions of tissue samples from *Cliona varians forma varians* (CVFV), *Ircinia variabilis* (Ircinia), and *Neopetrosia subtriangularis* (Neo). Stress = 0.0827 and polygons do not overlap.



Gene	CVFV	<i>I. variabilis</i>	<i>N. subtriangularis</i>	<i>S. vesparium</i>	<i>I. campana</i>
amoA					
pmoA		X			X
nifH	X			X	X
nosZ					
norB			X		
hzo					

Table 1: Functional gene search via PCR and gel electrophoresis for the sponge tissues of interest.

Discussion and Future Work

- Results revealed a significant difference in pumping rates between *N. subtriangularis* and both CVFV and *I. variabilis* (Fig. 6). This is interesting because it does not follow either of the similarity patterns in stable carbon isotope ratio or photosymbiont regime.
- The microbiome analysis nMDS showed that all three species clustered separately indicating that the bacterial communities differ between *C. varians forma varians*, *I. variabilis*, and *N. subtriangularis* (Fig. 7).
- Furthermore, the non-metric scaling revealed that *N. subtriangularis* and *C. varians forma varians* were more closely related to each other in microbiome composition than either was to *I. variabilis* (Fig. 7). This is the same pattern in similarities as the one observed in the stable carbon isotope ratio analysis (Fig. 3).
- No clear pattern was observed in the functional gene search for the microbiomes of the five species of interest (Table 1). More work is needed to understand these results.

Acknowledgements

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