# Supplement 1

**This supplemental information contains:**

- Text S1: Materials and methods for Becker et al unpublished data used in secondary analysis
- Figure S1. Percent cover of different substrate types in Fl and VI reef systems
- Figure S2. Alpha diversity metrics, particularly Shannon and Simpson's indices, begin to vary at the individual reef level
- Figure S3. ASVs show strong linear relationship between rank abundance and variance. Differentially abundant taxa are also among the most abundant and variable
- Figure S4. Observed ASVs is correlated with sequence depth and does not appear to saturate

**Tables presented separately in Supplement 2 at www.int-res.com/articles/suppl/a088p81_supp2.xlsx**

- Table S1. List of reefs and reef systems sampled in the secondary analysis
- Table S2. Differentially abundant ASVs between reefs in Fl and VI-based study

**Text S1: Materials and methods for Becker et al unpublished data used in secondary analysis**

*Study Area and Sampling*

We sampled coral reef seawater during a research cruise aboard the M/V Alucia between June 5 – 18, 2019 at a total of 27 reefs within the Florida Reef Tract. Reefs were located from the North Key Largo/Biscayne Bay area to the Dry Tortugas National Park (reefs outlined in Table 1). At each reef, accessed via a smaller dive boat, we used a 12V battery-operated groundwater pump (Proactive Mini-Monsoon, EnviroSupply, Irvine, CA, USA) to pump seawater from just above the reef benthos into a 4 L narrow-mouth LDPE Nalgene bottles (Thermo Fisher Scientific, Waltham, MA, USA). We placed all samples on ice inside a cooler until processing within the same day.

Upon returning to the M/V Alucia, 4 L seawater samples for microbial biomass were filtered via peristalsis through silicone tubing (Cole-Parmer, Vernon Hills, IL, USA). To capture technical duplicates, we filtered 2 L at a time through 25 mm 0.2 µm Supor (Pall, Port Washington, NY, USA) filters. After placing the filters in 2 mL cryovials, we froze them at –80°C.

*DNA extraction and sequencing for Seawater Microbiomes*

We extracted DNA from 25 mm filters used for 2 L seawater collections using DNeasy PowerBiofilm (Qiagen, Germantown, MD) kits. To begin, we added the filter directly to the bead tube, then proceeded with the extraction following manufacturer protocols. We also included five DNA extraction controls by extracting DNA from five individual, unused filters.

To amplify the V4 region of the small subunit (SSU) rRNA gene of bacteria and archaea, we used polymerase chain reaction (PCR). For each sample, we included 2 µl of template DNA from the extractions into a 50 µl (total volume) PCR reaction. To include a PCR negative control, we added 2 µl of PCR grade $H_2O$ instead of DNA to one PCR tube. We used Earth Microbiome Project primers, 515F (Parada et al. 2016) and 806R (Apprill et al. 2015), modified to include sample-specific indexes, including an 8 bp barcode, 10 bp pad, and 2 bp link, similar to Kozich et al. (2013). The 50 µl reactions were diluted in UV-sterilized nuclease-free water and contained 2.5 units of GoTaq DNA Polymerase (Promega, Madison, WI, USA), barcoded primers at 0.2 µM, 0.2 mM dNTP mix (Promega), 2.5 mM $MgCl_2$, and 1X colorless GoTaq flexi buffer (Promega). The reactions were run on a Bio-Rad Thermocycler using the following criteria: denaturation at 95°C for 2 min; 28 cycles at 95°C for 20 s, 55°C for 15 s, and 72°C for 5 min; and extension at 72°C for 10 min. We used gel electrophoresis to verify successful amplification using 5 µl of product on a 1% agarose-Tris-borate-EDTA (TBE) gel stained with SYBR Safe gel stain (Invitrogen, Thermo Fisher Scientific). We used the QIAquick 96 PCR Purification Kit (Qiagen) with the QIAvac 96 (Qiagen) and vacuum pressure to purify the remaining 45 µl of PCR products. We applied the HS dsDNA assay on the Qubit 2.0 fluorometer (Thermo Fisher Scientific) to quantify the DNA concentrations then converted to nM assuming an average library size of 450 bp, and average molar mass of DNA nucleotides of 660 g/mol. We diluted individual barcoded PCR products to 10 nM, pooled all samples, and shipped the pooled, ready-to-run library to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing on an Illumina MiSeq using paired-end 250 bp sequencing.

**References**

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137. doi:10.3354/ame01753

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology 79:9. doi:10.1128/AEM.01043-13

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414. doi:10.1111/1462-2920.13023

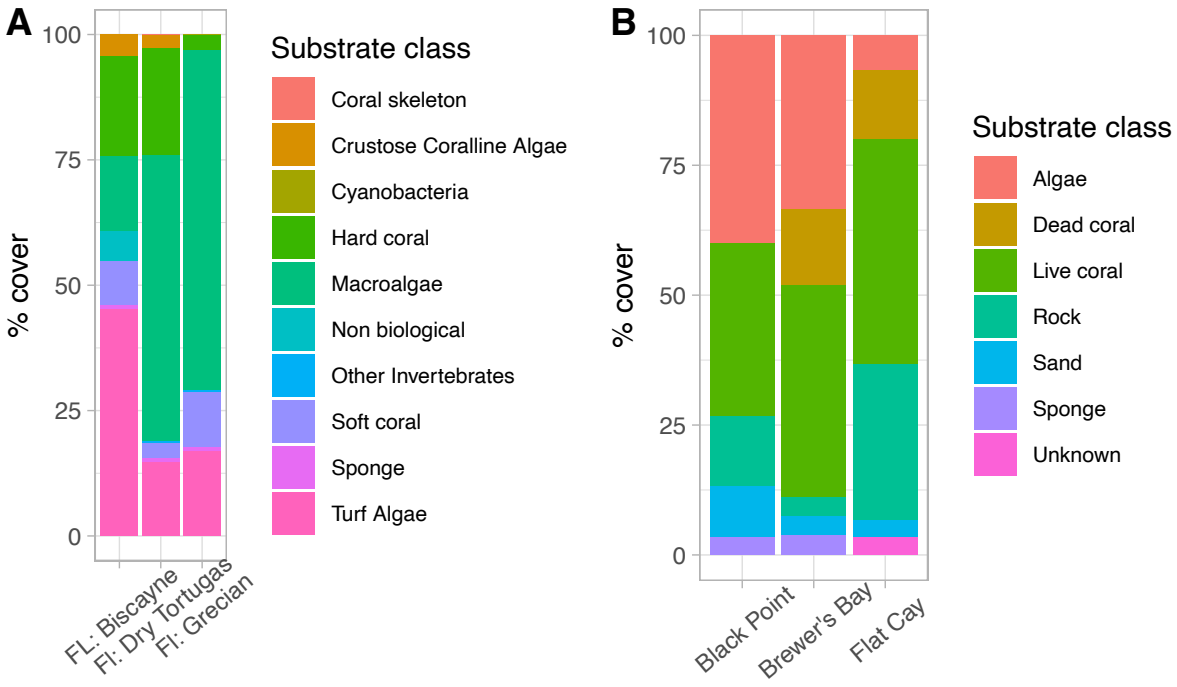


**Figure S1. Percent cover of different substrate types in Fl and VI reef systems** A. Percent cover of benthic substrate types calculated for each Fl reef overall using a photography-based survey. B. Percent cover of benthic substrate types at the VI reefs, determined at each sample location by a diver and video-recording along the transect line.

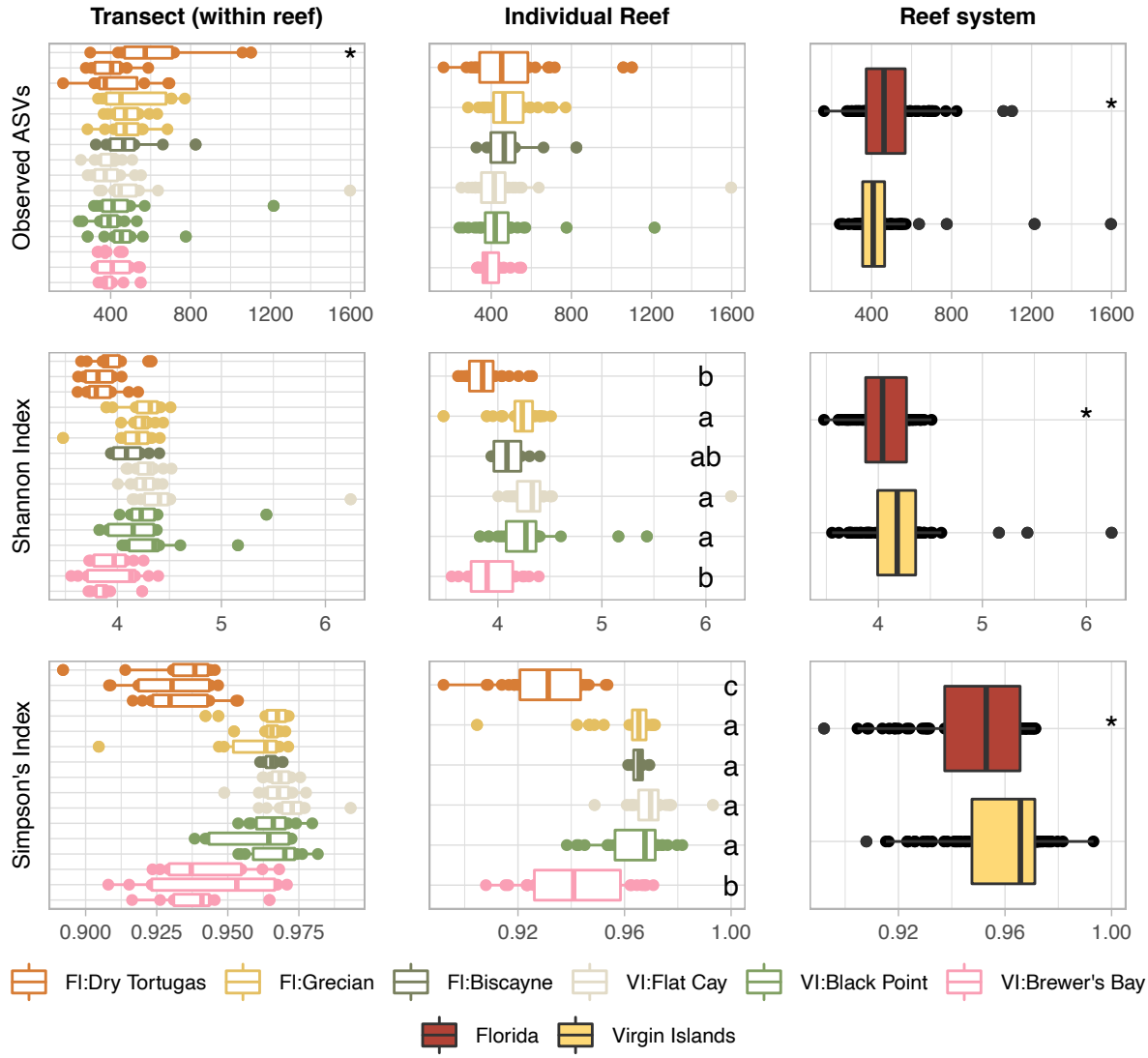


**Figure S2. Alpha diversity metrics, particularly Shannon and Simpson's indices, begin to vary at the individual reef level** Observed ASVs, Shannon index, and Simpsons index are shown here at the transect (within reef), individual reef, and reef system level for the reef seawater microbiomes in the Fl/VI transect-based study. Measures are for unrarefied counts. The vertical line in each boxplot indicates the median, the box hinges represent the first and third quartiles while the whiskers extend to 1.5*IQR (interquartile range). Letters indicate significance groups among reefs and asterisks indicate a significant difference between transects in the same reef or between reef systems (pairwise t test; p-adjusted<0.05).

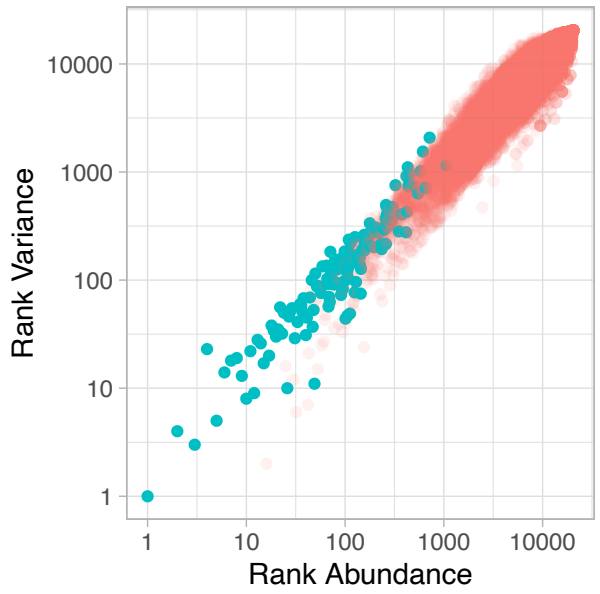


**Figure S3. ASVs show strong linear relationship between rank abundance and variance. Differentially abundant taxa are also among the most abundant and variable** Total read counts for ASVs from the Fl/VI-based study were transformed to ranked abundances, with 1 being the most abundant. Variances were calculated based on relative abundances, to reduce the effect of high read counts, then transformed to ranked variances. Highlighted in teal are the ASVs identified as significantly differentially abundant between reefs.

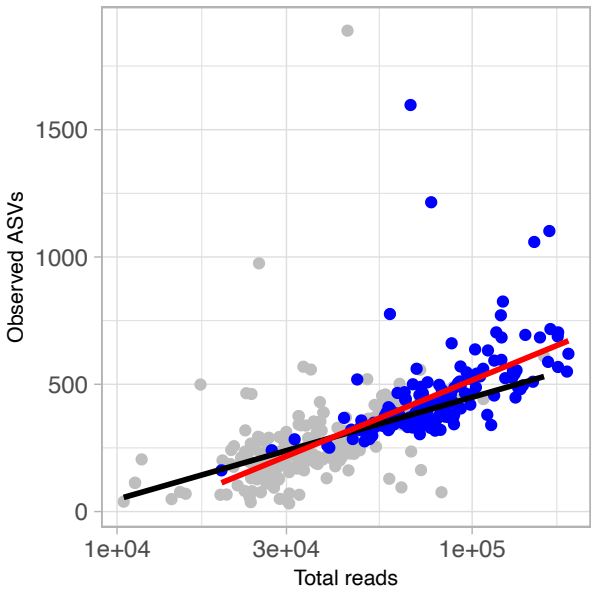


**Figure S4. Observed ASVs is correlated with sequence depth and does not appear to saturate**
The total reads per sample has a positive correlation with the number of observed ASVs. The samples from this present study (in blue) have high read counts but displays the same relationship as the samples in the secondary analysis (in grey). Lines indicate linear regression fit for just the secondary analysis samples (black) and for the FI/VI transect samples (red).