

Bacterial community dynamics during embryonic and larval development of three confamilial echinoids

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SUPPLEMENT 1

Supplemental Note 1

QIIME analysis

Starting with raw read files from MiSeq:

- 1) Pair forward and reverse files using PEAR (pear-0.9.10-bin-64)
- 2) Trim paired files using Trimmomatic (trimmomatic-0.36.jar)
- 3) Convert paired and trimmed .fastq to .fasta using the custom code: `cat [input .fastq] | paste - - - | cut -f 1,2 | sed 's/^/>/' | tr "\t" "\n" >[output .fasta]`
- 4) Validate tab-delimited mapping file using "validate_mapping_file.py"
- 5) Generate meta-.fasta files using "add_qiime_labels.py"
- 6) Detect chimeras from meta-.fasta (called, "combined_seqs.fna")
- 7) Filter chimeras using "filter_fasta.py"
- 8) Pick OTUs using "pick_open_reference_otus.py"
- 9) Filter OTUs with <10 reads and 'o__Cryptophyta' using "filter_otus_from_otu_table.py"
- 10) Determine rarefaction depth using "biom summarize-table"
- 11) Split filtered .biom table to test specific hypotheses using "split_otu_table.py"

Starting with filtered fasta files for comparisons:

- Alpha diversity estimates and observed OTUs were calculated with "alpha_diversity.py"
- Beta diversity via PCoA were calculated using "jackknifed_beta_diversity.py" and compared statically using "compare_categories.py"
- Taxonomic summaries were generated using "summarize_taxa_through_plots.py"
- Shared OTUs and their relative proportions were calculated using the "shared_phylotypes.py"
- Bray-Curtis index was calculated using the "beta_diversity.py" script



Figure S1. Locations of adult urchin collections throughout the Salish Sea with geographic reference points. Specifically, *Strongylocentrotus purpuratus* were hand-collected at Slip Point, Clallam Bay, WA; *S. droebachiensis* were hand-collected at low tide at Cattle Point, San Juan Island, WA; and, *Mesocentrotus franciscanus* were collected by SCUBA off Bell Island, WA.

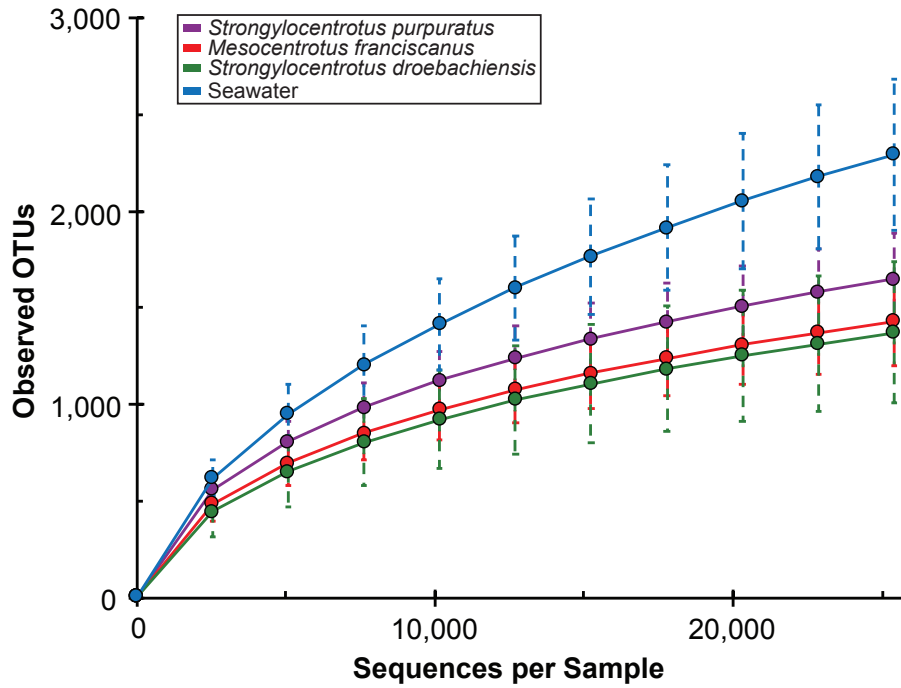


Figure S2. Alpha rarefaction curves for three species of echinoids and of the seawater. Alpha rarefaction curves (mean \pm standard deviation) for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) based on rarefaction depth of 25,396.

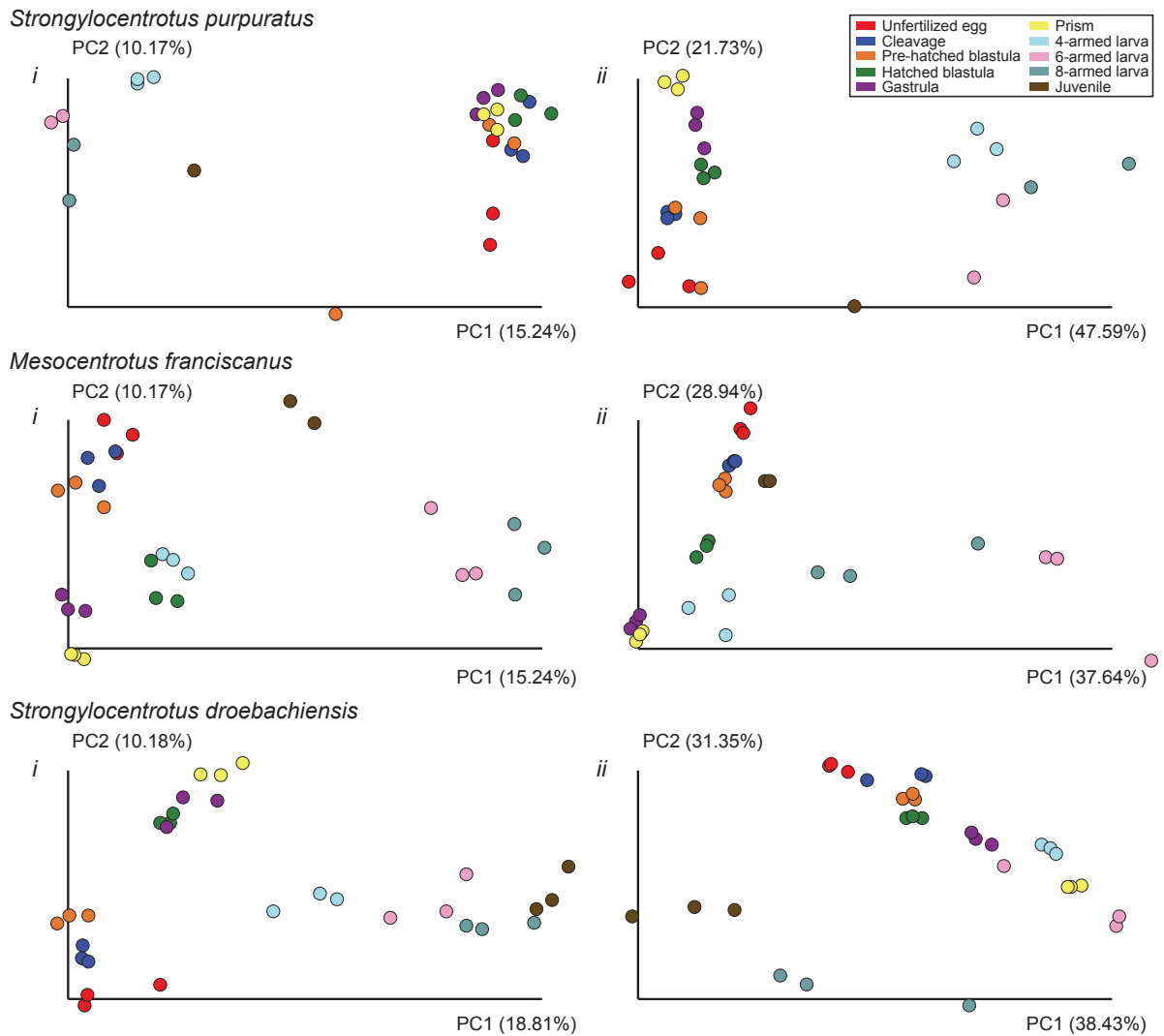
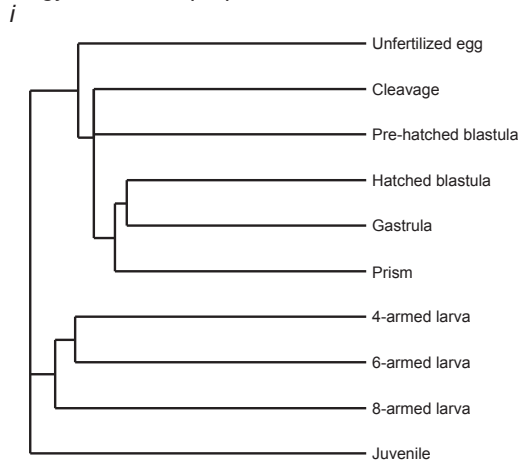
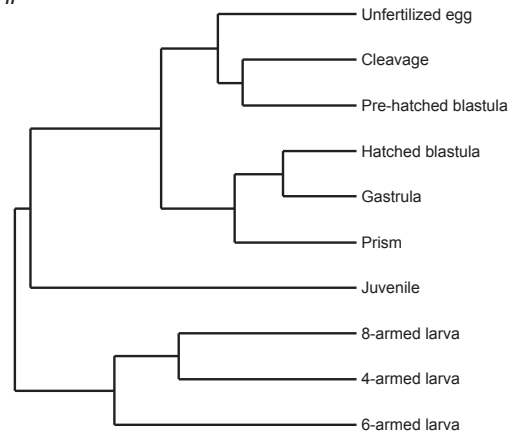


Figure S3. Similarity between the associated bacterial community across development for three species of echinoids. Community similarity for *Strongylocentrotus purpuratus* (top), *Mesocentrotus franciscanus* (middle), and *S. droebachiensis* (bottom) for ten developmental stages when considering the presence/absence of taxa (*i*) and their relative abundance (*ii*).

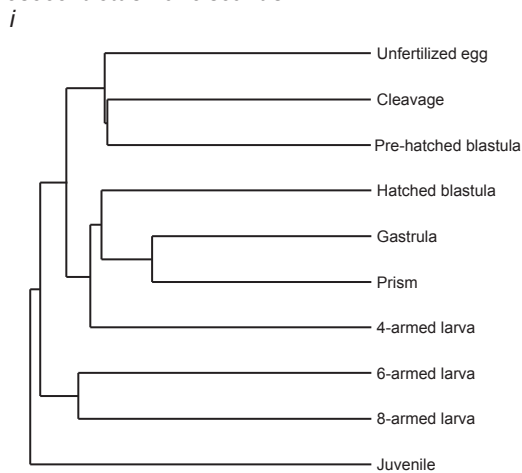
Strongylocentrotus purpuratus



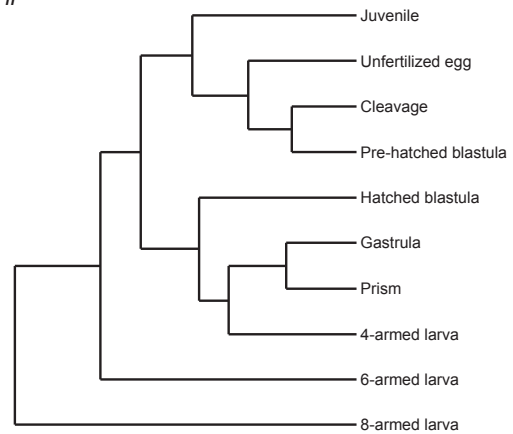
ii



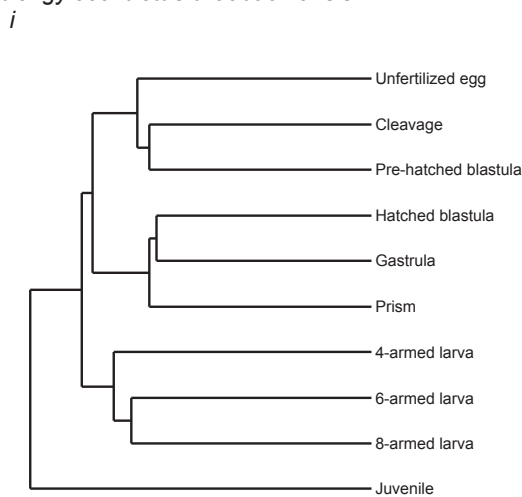
Mesocentrotus franciscanus



ii



Strongylocentrotus droebachiensis



ii

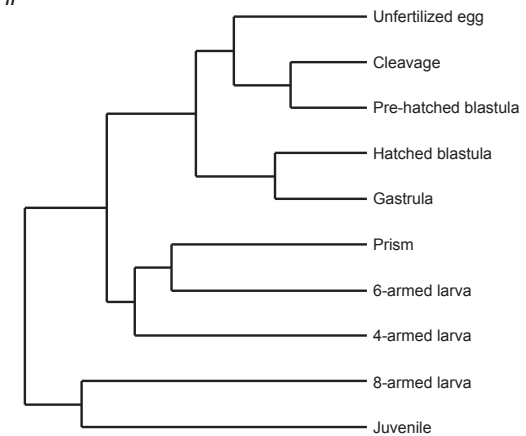


Figure S4. Bacterial community dendrograms across development for three echinoid species. Unweighted (*i*) and weighted (*ii*) bacterial community dendrograms for *Strongylocentrotus purpuratus* (top), *Mesocentrotus franciscanus* (middle), and *S. droebachiensis* (bottom).

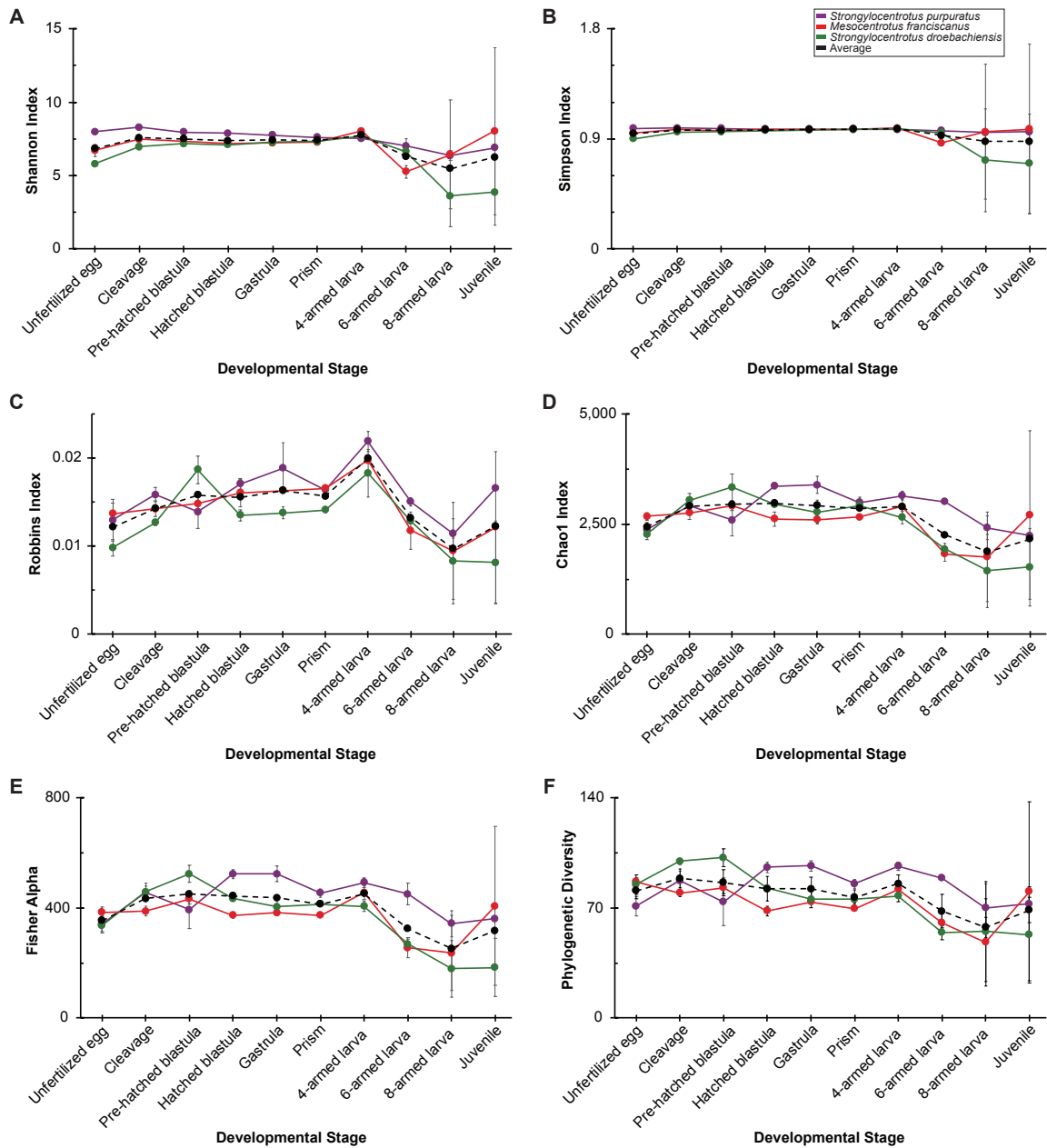


Figure S5. Alpha diversity indices for three echinoid species across development. Shannon (A), Simpson (B), Robbins (C), Chao1 (D), Fisher (E), and Faith's (F) indices (mean \pm standard error) for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), *S. droebachiensis* (green), and the average (black) at ten developmental stages.

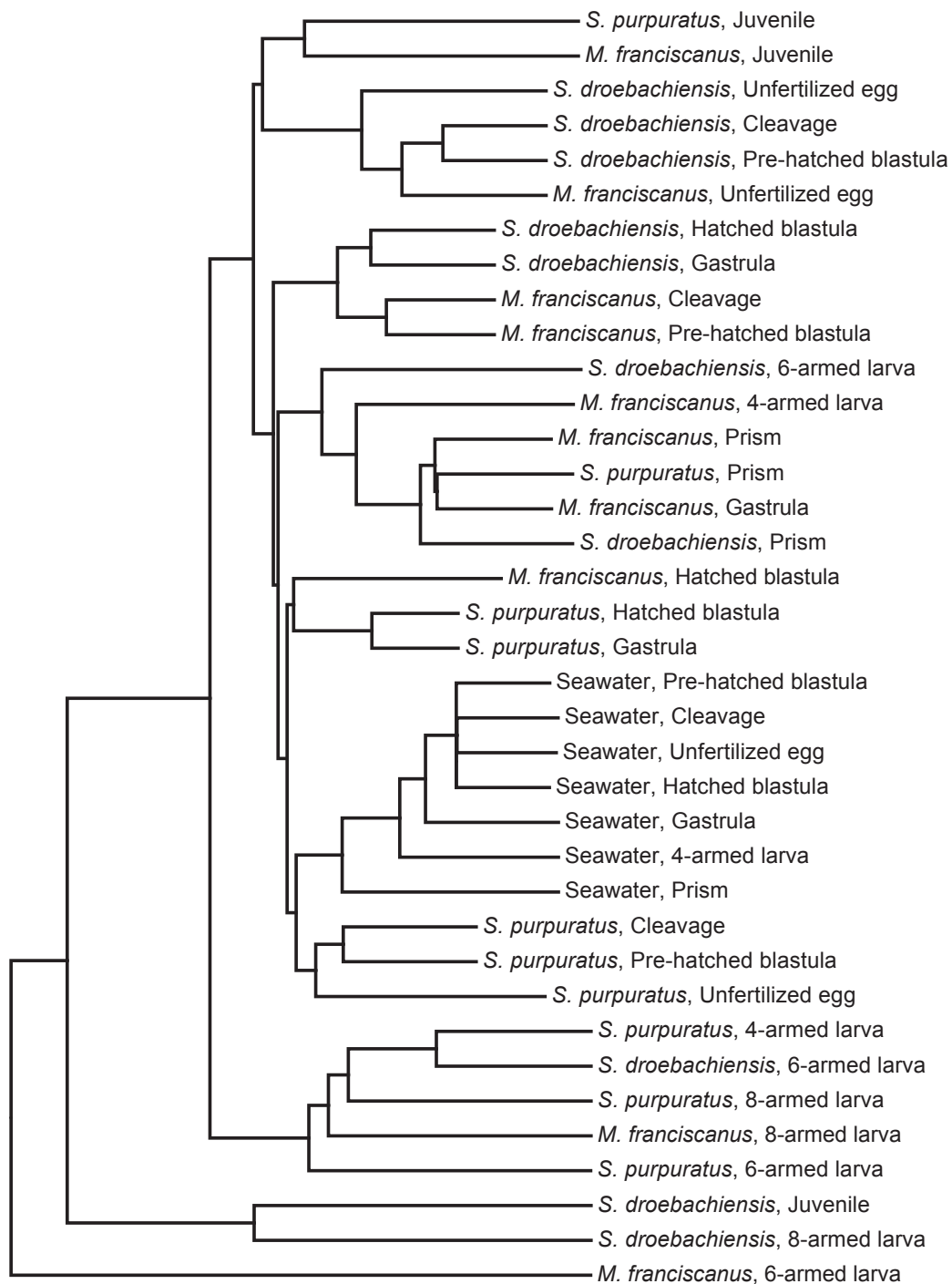


Figure S6. Microbial dendrogram of developmental stages and seawater. Dendrogram of the bacterial communities based on weighted UniFrac for the developmental stages of *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis* as well as the respective environmental bacterial communities.

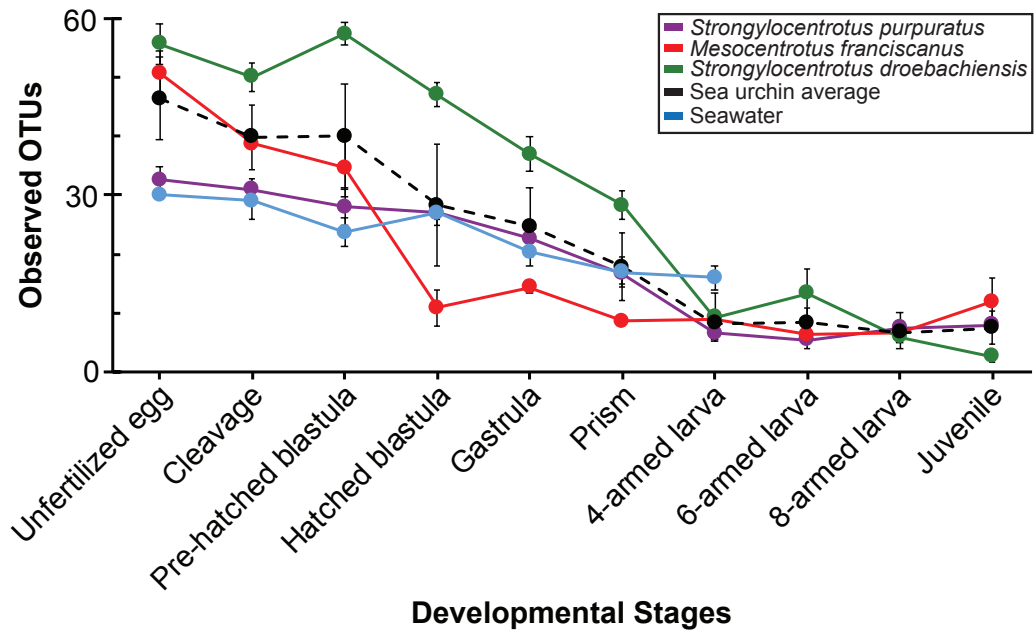


Figure S7. Diversity of *Psychromonas* during development. Total operational taxonomic units (OTUs) (mean \pm standard error) within the bacterial genus *Psychromonas* across embryonic and larval development for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), *S. droebachiensis* (green) as well as the average for the three sea urchin species and the in the seawater.