

Supplementary Material

Impacts of near future ocean warming on microbial community composition of the stomach of the soft bottom sea star *Luidia clathrata* (Say) (Echinodermata: Asteroidea)

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Electronic Supplementary Material 1 Script of all Qiime2 commands used in the analysis of data for this experiment

```
##Import Data into Qiime2 (Fastq Manifest Format)
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path 2020LuidiaManifest.tsv \
  --output-path 2020Luidia-demux.qza \
  --input-format PairedEndFastqManifestPhred33V2
```

```
##Summarize metadata
qiime metadata tabulate \
  --m-input-file 2020LuidiaMetadata.tsv \
  --o-visualization 2020LuidiaMetadata.qzv
```

```
##Summarize demultiplexed sequences
qiime demux summarize \
  --i-data 2020Luidia-demux.qza \
  --o-visualization 2020Luidia-demux.qzv
```

```
##Denoise with DADA2
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs 2020Luidia-demux.qza \
  --p-trim-left-f 13 \
  --p-trim-left-r 13 \
  --p-trunc-len-f 250 \
  --p-trunc-len-r 250 \
  --p-n-threads 0 \
  --o-table table.qza \
  --o-representative-sequences rep-seqs.qza \
  --o-denoising-stats denoising-stats.qza
```

```
##Summarize Feature Table
qiime feature-table summarize \
  --i-table table.qza \
```

```
--o-visualization table.qzv \  
--m-sample-metadata-file 2020LuidiaMetadata.tsv
```

```
##Summarize Sequences  
qiime feature-table tabulate-seqs \  
  --i-data rep-seqs.qza \  
  --o-visualization rep-seqs.qzv
```

```
##Summarize Denoising Stats  
qiime metadata tabulate \  
  --m-input-file denoising-stats.qza \  
  --o-visualization denoising-stats.qzv
```

###TAXONOMIC CLASSIFICATION OF SEQUENCES

##Train Naive Bayes Classifier

#Note: Only needs to be done once for each set of primer pairs/sequence info. Resulting classifier.qza file can be used for future classification of data with same primers/sequence info.

```
##Import SILVA taxonomy files and convert to .qza format  
qiime tools import \  
  --type 'FeatureData[Sequence]' \  
  --input-path silva_132_99_16S.fna \  
  --output-path silva_132_99_16S_otus.qza
```

```
qiime tools import \  
  --type 'FeatureData[Taxonomy]' \  
  --input-format HeaderlessTSVTaxonomyFormat \  
  --input-path taxonomy_7_levels.txt \  
  --output-path ref-taxonomy.qza
```

##Extract Reference Reads Specific to V4 16S Region (515F/806R primers) of Reference Taxonomy

```
qiime feature-classifier extract-reads \  
  --i-sequences silva_132_99_16S_otus.qza \  
  --p-f-primer GTGCCAGCMGCCGCGGTAA \  
  --p-r-primer GGACTACHVGGGTWTCTAAT \  
  --p-trunc-len 0 \  
  --p-min-length 100 \  
  --p-max-length 300 \  
  --o-reads ref-seqs.qza
```

##Train Classifier Using the Reference Reads and Taxonomy Files Just Made

```
qiime feature-classifier fit-classifier-naive-bayes \  
  --i-reference-reads ref-seqs.qza \  
  --i-reference-taxonomy ref-taxonomy.qza \  
  --o-classifier classifier.qza
```

##Test Classifier

```
qiime feature-classifier classify-sklearn \  
  --i-classifier classifier.qza \  
  --i-reads rep-seqs.qza \  
  --o-classification taxonomy.qza
```

```
qiime metadata tabulate \  
  --m-input-file taxonomy.qza \  
  --o-visualization taxonomy.qzv
```

##Visualize taxonomy with barplot before removing non-target sequences

#Not necessary - onoy doing it now ot get a better idea of what my data look like before and after removing non-target sequences

```
qiime taxa barplot \  
  --i-table table.qza \  
  --i-taxonomy taxonomy.qza \  
  --m-metadata-file 2020LuidiaMetadata.tsv \  
  --o-visualization taxa-bar-plots.qzv
```

##Filter out non-target equences

```
qiime taxa filter-table \  
  --i-table table.qza \  
  --i-taxonomy taxonomy.qza \  
  --p-exclude mitochondria,chloroplast,archaea,eukaryota,unknown \  
  --o-filtered-table filtered-table.qza
```

##Visualize taxonomy with barplot after removing non-target sequences

```
qiime taxa barplot \  
  --i-table filtered-table.qza \  
  --i-taxonomy taxonomy.qza \  
  --m-metadata-file 2020LuidiaMetadata.tsv \  
  --o-visualization filtered-taxa-bar-plots.qzv
```

##Summarize feature table

```
qiime feature-table summarize \  
  --i-table filtered-table.qza \  
  --o-visualization filtered-table.qzv \  
  --m-sample-metadata-file 2020LuidiaMetadata.tsv
```

##Alpha-diversity analysis

##Make alpha rarefaction curves

```
qiime diversity alpha-rarefaction \  
  --i-table filtered-table.qza \  
  --m-metadata-file 2020LuidiaMetadata.tsv \  
  --o-visualization alpha-rarefaction.qzv
```

```
--p-max-depth 24573 \  
--o-visualization alpha_rarefaction_curves.qzv
```

```
##Summarize updated metadata file
```

```
##Summarize metadata
```

```
qiime metadata tabulate \  
--m-input-file 2020LuidiaMetadata2.tsv \  
--o-visualization 2020LuidiaMetadata2.qzv
```

```
##Make alpha rarefaction curves with updated metadata file to view curves for each individual  
sample-metadata-file
```

```
qiime diversity alpha-rarefaction \  
--i-table filtered-table.qza \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--p-max-depth 24573 \  
--o-visualization alpha_rarefaction_curves2.qzv
```

```
##Run qiime diversity core metrics to get diversity values
```

```
qiime diversity core-metrics \  
--i-table filtered-table.qza \  
--p-sampling-depth 24573 \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--output-dir .\core-metrics-results
```

```
##Test alpha-diversity significance
```

```
qiime diversity alpha-group-significance \  
--i-alpha-diversity core-metrics-results/observed_otus_vector.qza \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--o-visualization core-metrics-results/observed_otus_group-significance.qzv
```

```
qiime diversity alpha-group-significance \  
--i-alpha-diversity core-metrics-results/evenness_vector.qza \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--o-visualization core-metrics-results/evenness_group-significance.qzv
```

```
qiime diversity alpha-group-significance \  
--i-alpha-diversity core-metrics-results/shannon_vector.qza \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--o-visualization core-metrics-results/shannon_group-significance.qzv
```

```
##Test beta-diversity significance
```

```
qiime diversity beta-group-significance \  
--i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \  
--m-metadata-file 2020LuidiaMetadata2.tsv \  
--m-metadata-column Sample_Type \  
--o-visualization core-metrics-results/bray_curtis_distance_sample-type-significance.qzv
```

```
--p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \  
  --m-metadata-file 2020LuidiaMetadata2.tsv \  
  --m-metadata-column Sample_Group \  
  --o-visualization core-metrics-results/bray_curtis_sample-group-significance.qzv \  
  --p-pairwise
```

```
##Visualize taxonomy with barplot after removing non-target sequences with updated metadata  
file (2020.03.05)
```

```
qiime taxa barplot \  
  --i-table filtered-table.qza \  
  --i-taxonomy taxonomy.qza \  
  --m-metadata-file 2020LuidiaMetadata2.tsv \  
  --o-visualization filtered-taxa-bar-plot2.qzv
```

```
##Group samples to visualize taxonomy with barplot for sample categories instead of 1  
bar/sample
```

```
qiime feature-table group \  
  --i-table filtered-table.qza \  
  --p-axis sample \  
  --m-metadata-file 2020LuidiaMetadata2.tsv \  
  --m-metadata-column "Sample_Group" \  
  --p-mode sum \  
  --o-grouped-table grouped-table.qza \  
  --o-visualization grouped-taxa-bar-plot2.qzv
```

```
##Tabulate file to view as qzv
```

```
qiime feature-table summarize \  
  --i-table grouped-table.qza \  
  --o-visualization grouped-table.qzv
```

```
##Summarize updated metadata file for grouped samples
```

```
qiime metadata tabulate \  
  --m-input-file 2020LuidiaQiimeMetadataGrouped.tsv \  
  --o-visualization 2020LuidiaQiimeMetadataGrouped.qzv
```

```
##Visualize taxonomy with barplot after removing non-target sequences and grouping samples  
by type/treatment
```

```
qiime taxa barplot \  
  --i-table grouped-table.qza \  
  --i-taxonomy taxonomy.qza \  
  --m-metadata-file 2020LuidiaQiimeMetadataGrouped.tsv \  
  --o-visualization grouped-taxa-bar-plots.qzv
```

```
##
```

```
qiime feature-table summarize \  
  --i-table core-metrics-results/bray_curtis_pcoa_results.qza \  
  --o-visualization bray_curtis_pcoa_results.qzv
```

```
qiime tools export \  
  --input-path core-metrics-results/bray_curtis_distance_matrix.qza \  
  --output-path bray_curtis_distance_matrix.fasta
```

```
qiime tools export \  
  --input-path core-metrics-results/bray_curtis_pcoa_results.qza \  
  --output-path bray_curtis_pcoa_results.fasta
```