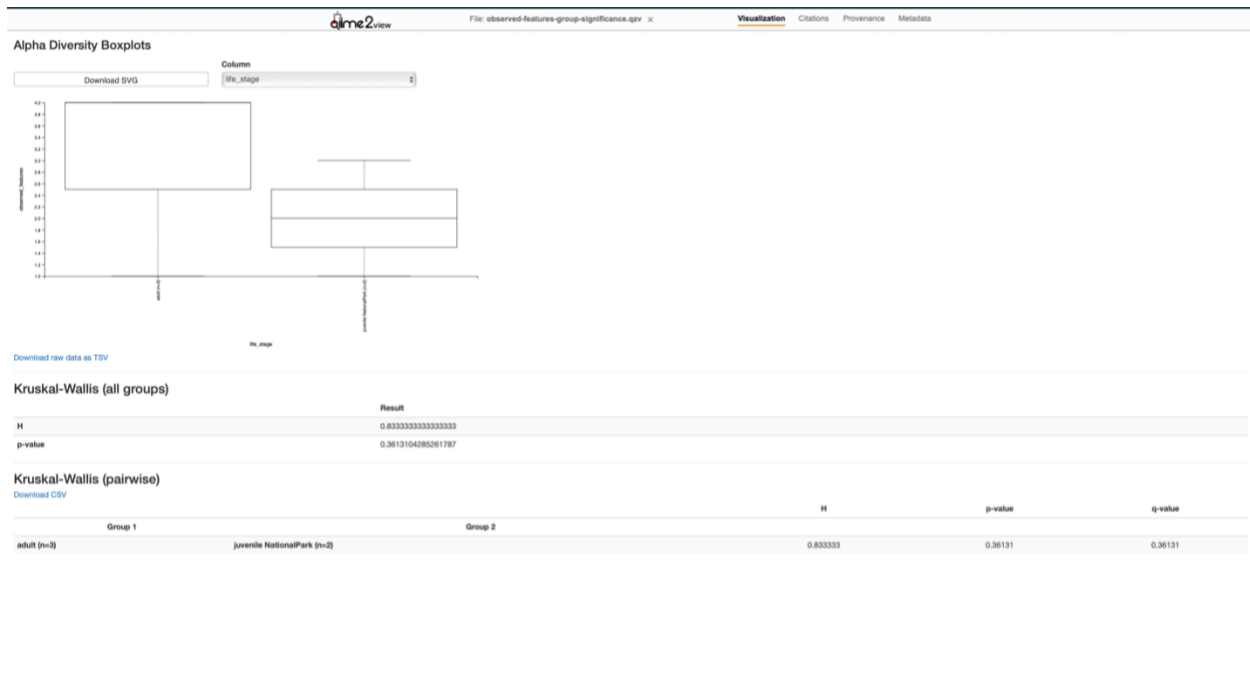
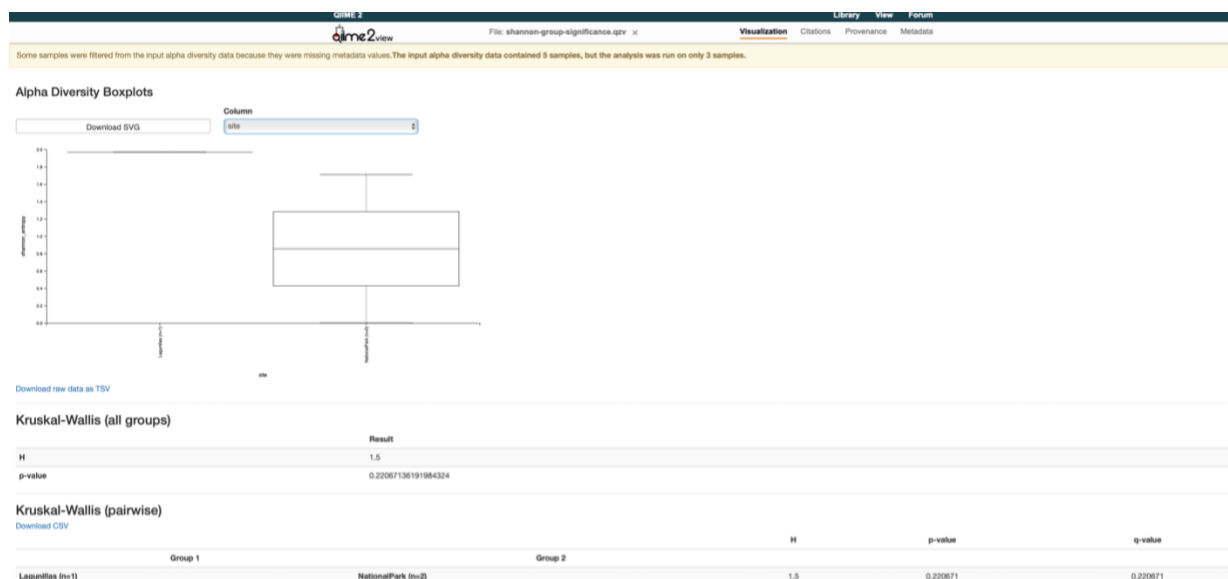


- 1.) All samples had less than 10,000 reads. In total there were 18 samples.
- 2.) Filing by taxon, majority are from the kingdom bacteria. This file shows us the taxonomic classification results from the sequencing data. The output shows the taxon and confidence values.
- 3.) The only thing other than bacteria I see is unassigned. Other things that might show up could be mitochondria, chloroplasts, archaea, and unassigned.
- 4.) When visualizing the taxa bar plot, I notice only the kingdom bacteria was present. Changing level will also include specific classification such as class, order, family...etc. No, there weren't any noticeable changes when sorting to life stage or site of collection.
- 5.) Shannon diversity measures both richness and evenness of the taxa and their abundance while, observed features only measures richness. They both measure the diversity within a sample.
- 6.) No, there weren't any significance as all the p-value > 0.05 . Indicating that there is no correlation between the samples.





- 7.) Alpha and beta diversity differ as alpha forces on diversity within a single sample, while beta compares the difference between the samples. Bray-Curtis distance measures how dissimilar two samples are based on the abundance and presence of the species. Weighted UniFrac distance use phylogenetic relationship between the species and the abundance to measure the difference between their evolutionary history.

- 8.) qiime diversity beta-group-significance \
- ```
--i-distance-matrix diversity-metrics-results/weighted_unifrac_distance_matrix.qza \
--m-metadata-file metadata.txt \
--m-metadata-column life_stage \
--o-visualization diversity-metrics-results/weighted-unifrac-life-stage-significance.qzv \
--p-pairwise
```

```
qiime diversity beta-group-significance \
--i-distance-matrix diversity-metrics-results/weighted_unifrac_distance_matrix.qza \
--m-metadata-file metadata.txt \
--m-metadata-column site \
--o-visualization diversity-metrics-results/weighted-unifrac-site-significance.qzv \
--p-pairwise
```

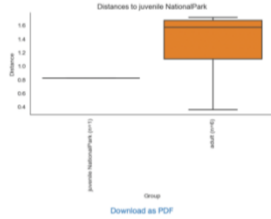
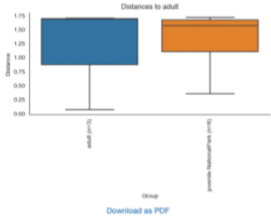
- 9.) No, there weren't any statistically significant different as the p-value is higher than 0.05 for both metrics. For the site comparison, there weren't any significant differences as the p-value was higher than 0.05 as well.

Overview

|                        |           |
|------------------------|-----------|
| method name            | PERMANOVA |
| test statistic name    | pseudo-F  |
| sample size            | 5         |
| number of groups       | 2         |
| test statistic         | 1.847742  |
| p-value                | 0.403     |
| number of permutations | 999       |

Group significance plots

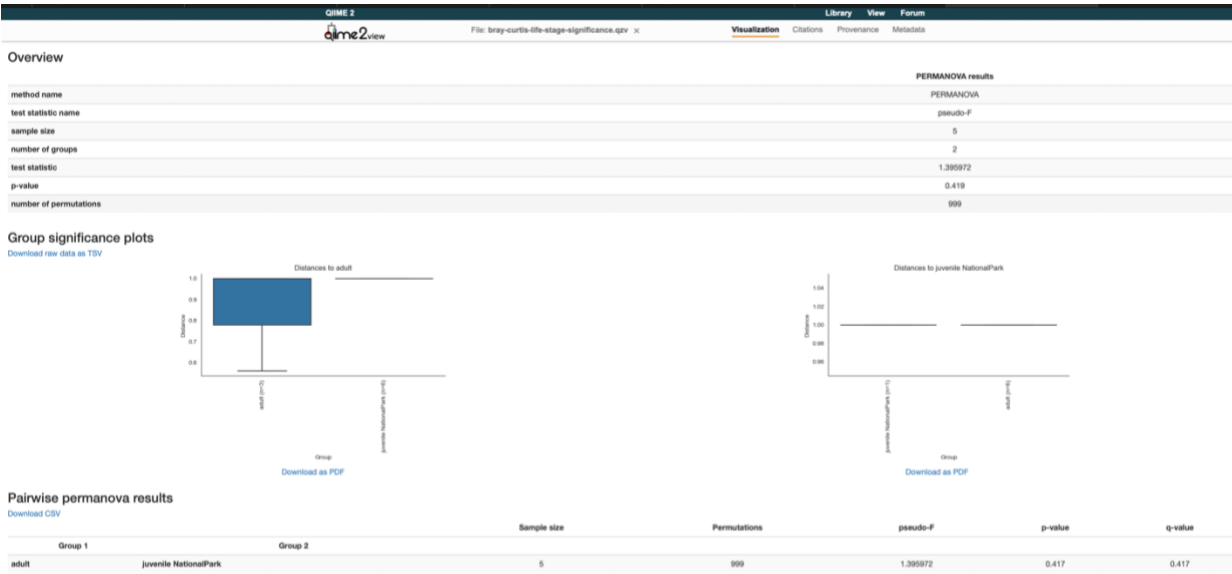
[Download raw data as TSV](#)



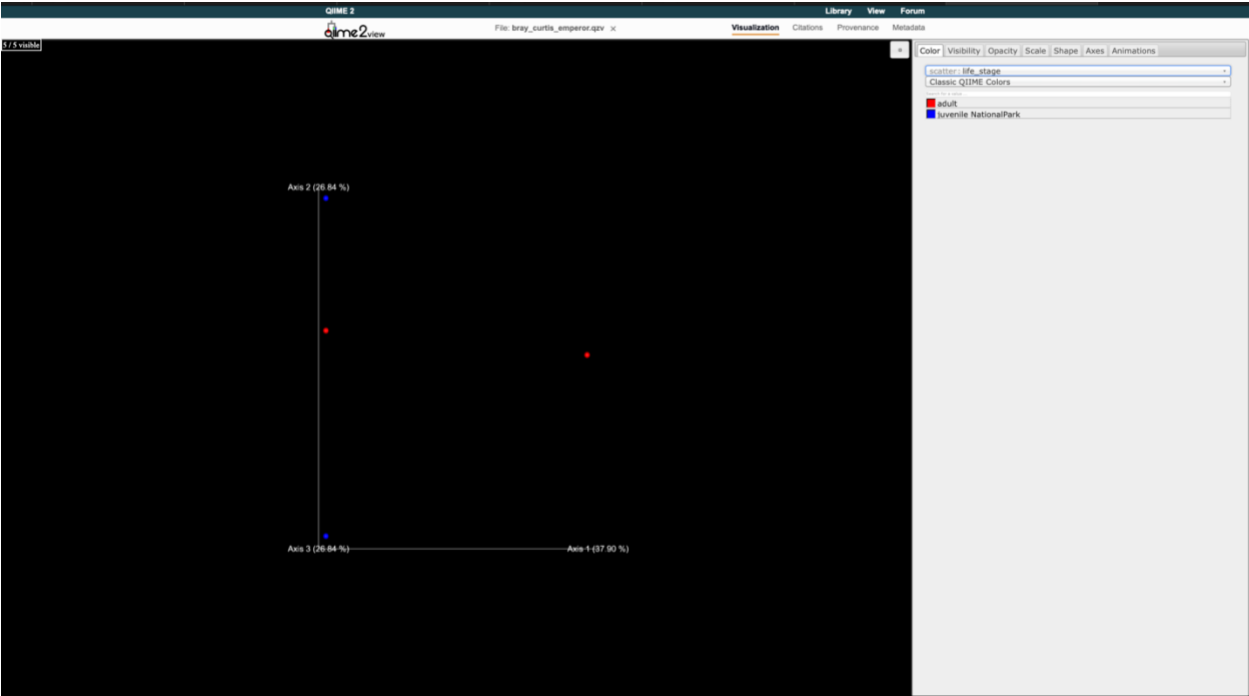
Pairwise permanova results

[Download CSV](#)

| Group 1 |                       | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|---------|-----------------------|---------|-------------|--------------|----------|---------|---------|
| adult   | juvenile NationalPark |         | 5           | 999          | 1.847742 | 0.398   | 0.398   |



10.) As there weren't any differences, the plot showcases that each life stage are randomly placed and show no correlation to one another.





11.) No, bar plot could be generate for the life stage. For the site, the only one that was differentially expressed was featureid 133101da24445e40ef32d134c9296a9f under the kingdom bacteria (no species was shown).