Supplementary Material

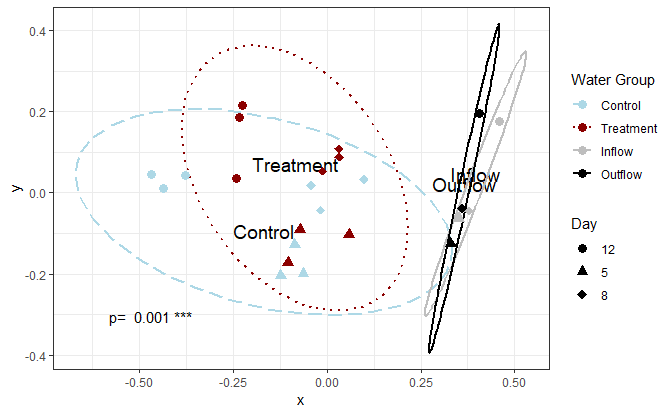
# Supplementary Figures



**Supplementary Figure 1.** Rarefaction curve from all water samples from all three Trials based on taxonomic classification at the order level.



**Supplementary Figure 2.** The relative abundances of the 20 most abundant orders in oyster, swab, and water samples from Trial 1.



**Supplementary Figure 3.** NMDS plot visualization of Bray-Curtis beta-diversity (k=2) at the Order level by Treatment or Water Source of water samples from Trial 3. The ellipse lines show the 95% confidence interval. The water group is indicated by colors (control=light blue dashed, probiotic treatment=dark red dotted, inflow=grey solid, outflow=black solid) and sampling timepoints are indicated by symbols. The inflow water (water piped directly from the environment into the hatchery) and outflow water (inflow water UV-treated and sterilized) are significantly distinct groups, separate from the experimental samples. p-value indicates significance of groupings with adonis2 Permutational Multivariate Analysis of Variance Using Distance Matrices test.



**Supplementary Figure 4.** Percent abundances of *Vibrio* species in all sample types in Trial 1. The total abundance of sequencing reads is shown in the bar graph. The structure of total *Vibrios* is different based on the sample type and time point.



**Supplementary Figure 5.** Percent abundances of *Vibrio* species in rearing water samples from all 3 Trials. The total abundance of sequencing reads is shown in the bar graph. The structure of total *Vibrio* counts in the rearing water is different between Trials and changes over time.

# Supplementary Tables

**Supplementary Table 1.** Kruskal-Wallis Rank Sum Test for percent abundances of *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes* by Sample Type from all trials.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **<All Trials – *Proteobacteria >*** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Sample Type | 2 | 52.745 | 3.521e-12 | \*\*\* |
| Treatment | 1 | 0.75065 | 0.3863 |  |
| **< All Trials – *Cyanobacteria* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Sample Type | 2 | 33.113 | 6.451e-08 | \*\*\* |
| Treatment | 1 | 0.093506 | 0.7598 |  |
| **< All Trials – *Bacteroidetes* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Sample Type | 2 | 63.422 | 1.691e-14 | \*\*\* |
| Treatment | 1 | 0.23442 | 0.6283 |  |

**Supplementary Table 2.** Kruskal-Wallis Rank Sum Test for the Simpson’s Index of Diversity values by Trial, Sample Type, Day, and/or Treatment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< All Trials – Simpson’s Index of Diversity >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Trial | 2 | 38.553 | 4.25e-09 | \*\*\* |
| Trial – water only | 2 | 24.809 | 4.099e-06 | \*\*\* |
| Type | 2 | 51.932 | 5.285e-12 | \*\*\* |
| Day | 2 | 9.1136 | 0.0105 | \* |
| Treatment | 1 | 0.32388 | 0.5693 |  |

**Supplementary Table 3.** Kruskal-Wallis Rank Sum Test for the Simpson’s Index of Diversity values by Day and Treatment in Trial 1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 - Water >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 0.41026 | 0.5218 |  |
| Treatment | 1 | 0.92308 | 0.3367 |  |
| **< Trial 1 – Biofilm Swab >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0.10256 | 0.7488 |  |
| **< Trial 1 – Oyster Larvae >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 6.5641 | 0.01041 | \* |
| Treatment | 1 | 0.41026 | 0.5218 |  |
|  |  |  |  |  |

**Supplementary Table 4.** Kruskal-Wallis Rank Sum Test for the Simpson’s Index of Diversity values by Day and Treatment in Trial 2.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 2 - Water >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 7.4103 | 0.006485 | \*\* |
| Treatment | 1 | 0.10256 | 0.7488 |  |
| **< Trial 2 – Biofilm Swab >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 3.6923 | 0.05466 |  |
| Treatment | 1 | 0.10256 | 0.7488 |  |
| **< Trial 2 – Oyster Larvae >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 0 | 1 |  |
| Treatment | 1 | 0.6 | 0.4386 |  |
|  |  |  |  |  |

**Supplementary Table 5.** Kruskal-Wallis Rank Sum Test for the Simpson’s Index of Diversity values by Day and Treatment in Trial 3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 3 - Water >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 2 | 11.942 | 0.002552 | \*\* |
| Treatment | 1 | 0.32943 | 0.566 |  |

**Supplementary Table 6.** Permutational Multivariate Analysis of Variance Using Distance Matrices (adonis2) for Bray-Curtis beta-diversity (k=2) in each Trial by Sample Type, Day, and Treatment.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **< Trial 1 – Bray-Curtis beta-diversity >** | | | | | | |
|  | **DF** | **Sum of Squares** | **R2** | **F** | **Pr(>F)** |  | |
| Type | 2 | 2.0175 | 0.3053 | 7.2513 | 0.001 | \*\*\* | |
| Residual | 33 | 4.5907 | 0.6947 |  |  |  | |
| Day (Water only) | 1 | 0.71911 | 0.7375 | 28.095 | 0.006 | \*\* | |
| Residual | 10 | 0.25596 | 0.2625 |  |  |  | |
| Treatment (Water only) | 1 | 0.02426 | 0.02488 | 0.2551 | 0.719 |  | |
| Residual | 10 | 0.9508 | 0.97512 |  |  |  | |
| **< Trial 2 – Bray-Curtis beta-diversity >** | | | | | | |
|  | **DF** | **Sum of Squares** | **R2** | **F** | **Pr(>F)** |  | |
| Type | 2 | 2.7762 | 0.57915 | 20.642 | 0.001 | \*\*\* | |
| Residual | 30 | 2.0173 | 0.42085 |  |  |  | |
| Day (Water only) | 1 | 0.18331 | 0.24214 | 3.195 | 0.013 | \* | |
| Residual | 10 | 0.57375 | 0.75786 |  |  |  | |
| Treatment (Water only) | 1 | 0.07318 | 0.09666 | 1.07 | 0.316 |  | |
| Residual | 10 | 0.68389 | 0.90334 |  |  |  | |
| **< Trial 3 – Bray-Curtis beta-diversity >** | | | | | | |
|  | **DF** | **Sum of Squares** | **R2** | **F** | **Pr(>F)** |  | |
| Day | 1 | 0.14732 | 0.32893 | 7.8424 | 0.002 | \*\* | |
| Residual | 16 | 0.30056 | 0.67107 |  |  |  | |
| Treatment | 1 | 0.02889 | 0.06451 | 1.1033 | 0.337 |  | |
| Residual | 16 | 0.41898 | 0.93549 |  |  |  | |
| Water Source Group (Figure S3) | 3 | 0.37087 | 0.57173 | 8.9 | 0.001 | \*\*\* | |
| Residual | 20 | 0.27781 | 0.42827 |  |  |  | |

**Supplementary Table 7.** Kruskal-Wallis Rank Sum Test for relative percent abundance of *Bacillales* reads in water samples per Trial by Day and Treatment Group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 - *Bacillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 0.64103 | 0.4233 |  |
| Treatment: Day 1 | 1 | 1.1905 | 0.2752 |  |
| Treatment: Day 12 | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 2 - *Bacillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 0.23077 | 0.631 |  |
| Treatment: Day 1 | 1 | 0.42857 | 0.5127 |  |
| Treatment: Day 9 | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 3 - *Bacillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 2 | 0.94737 | 0.6227 |  |
| Treatment: All | 1 | 12.789 | 0.0003486 | \*\*\* |
| Treatment: Day 5 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 8 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 12 | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 3 - *Bacillales* Days 5 and 8 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 3 - *Bacillales* Days 5 and 12 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 3 - *Bacillales* Days 8 and 12 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 3.8571 | 0.04953 | \* |

**Supplementary Table 8.** Kruskal-Wallis Rank Sum Test for relative percent abundance of *Oceanospirillales* reads in water samples per Trial by Day and Treatment Group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 - *Oceanospirillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment: Day 1 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 12 | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 2 - *Oceanospirillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 5.7692 | 0.01631 | \* |
| Treatment: Day 1 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 9 | 1 | 1.1905 | 0.2752 |  |
| **< Trial 3 - *Oceanospirillales* >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 2 | 8.2222 | 0.01639 | \* |
| Treatment: Day 5 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 8 | 1 | 3.8571 | 0.04953 | \* |
| Treatment: Day 12 | 1 | 3.8571 | 0.04953 | \* |
| **< Trial 3 - *Oceanospirillales* Days 5 and 8 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 4.3333 | 0.03737 | \* |
| **< Trial 3 - *Oceanospirillales* Days 5 and 12 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 1.2564 | 0.2623 |  |
| **< Trial 3 - *Oceanospirillales* Days 8 and 12 >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 6.5641 | 0.01041 | \* |

**Supplementary Table 9.** Kruskal-Wallis Rank Sum Test for Simpson’s Index of Diversity of *Vibrionales* relative percent reads in Trial 1 per Sample Type by Day and Treatment Group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 All Samples – *Vibrio* diversity >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Type | 2 | 8.4324 | 0.01475 | \* |
| Day | 2 | 10.89 | 0.004318 | \*\* |
| Treatment | 1 | 0.25626 | 0.6127 |  |
| **< Trial 1 Oyster Larvae – *Vibrio* diversity >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 0 | 1 |  |
| Treatment | 1 | 0.41026 | 0.5218 |  |
| **< Trial 1 Biofilm swab – *Vibrio* diversity >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0.025641 | 0.8728 |  |
| **< Trial 1 Water – *Vibrio* diversity >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0.64193 | 0.4233 |  |
| Treatment –  Day 12 | 1 | 3.8571 | 0.04953 | \* |

**Supplementary Table 10.** Kruskal-Wallis Rank Sum Test for relative percent abundance of *Vibrionales* reads in Trial 1 per Sample Type by Day and Treatment Group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 All Samples – *Vibrio* percent abundance >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Type | 2 | 16.722 | 0.0002338 | \*\*\* |
| Day | 2 | 22.651 | 1.206e-05 | \*\*\* |
| Treatment | 1 | 0.0009009 | 0.9244 |  |
| **< Trial 1 Oyster Larvae – *Vibrio* percent abundance >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0.10256 | 0.7488 |  |
| **< Trial 1 Biofilm swab – *Vibrio* percent abundance >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0 | 1 |  |
| **< Trial 1 Water – *Vibrio* percent abundance >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 8.3077 | 0.003948 | \*\* |
| Treatment | 1 | 0.025641 | 0.8728 |  |

**Supplementary Table 11.** Kruskal-Wallis Rank Sum Test for culturable *Vibrio* colony counts in Trial 1 per Sample Type by Day and Treatment Group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **< Trial 1 All Samples – *Vibrio* colony counts >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Type | 2 | 2.4254 | 0.2974 |  |
| Day | 2 | 2.4406 | 0.2951 |  |
| Treatment | 1 | 10.234 | 0.001379 | \*\* |
| **< Trial 1 Oyster Larvae – *Vibrio* colony counts >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 3.7053 | 0.05424 |  |
| Treatment | 1 | 1.8591 | 0.1727 |  |
| **< Trial 1 Biofilm swab – *Vibrio* colony counts >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 2.0769 | 0.1495 |  |
| Treatment | 1 | 3.1026 | 0.07817 |  |
| **< Trial 1 Water – *Vibrio* colony counts >** | | | | |
|  | **DF** | **Chi-Squared** | **P value** |  |
| Day | 1 | 2.0989 | 0.1474 |  |
| Control by Day | 1 | 3.9706 | 0.0463 | \* |
| Treated by Day | 1 | 4.0909 | 0.04311 | \* |
| Treatment | 1 | 8.3958 | 0.003761 | \*\* |