Table 1. Summary of probiotic trial information and sequencing data. W=water, S=swabs, O=oysters

	Trial 1	**Trial 2**	**Trial 3**
Sample Types	Water, Swabs, Oysters	Water, Swabs, Oysters	Water
Sampling Days (0=spawn)	W:1,12 / OS:5,12	W:1,9 / OS:6,9	W: 5,8,12
Water Filtered	410-750ml	7-10ml	1300-1700ml
Trial Dates	July 11-23, 2012	Jan 9-18, 2013	June 3-15, 2016
Bacterial reads from 12 water samples	1.3 million	1.8 million	5.7 million
Methods	MoBio extraction MiSeq, 2x250 PE	MoBio extraction MiSeq, 2x250 PE	Puregene extraction HiSeq, 2x100 PE
16S region	V4	V4	V6

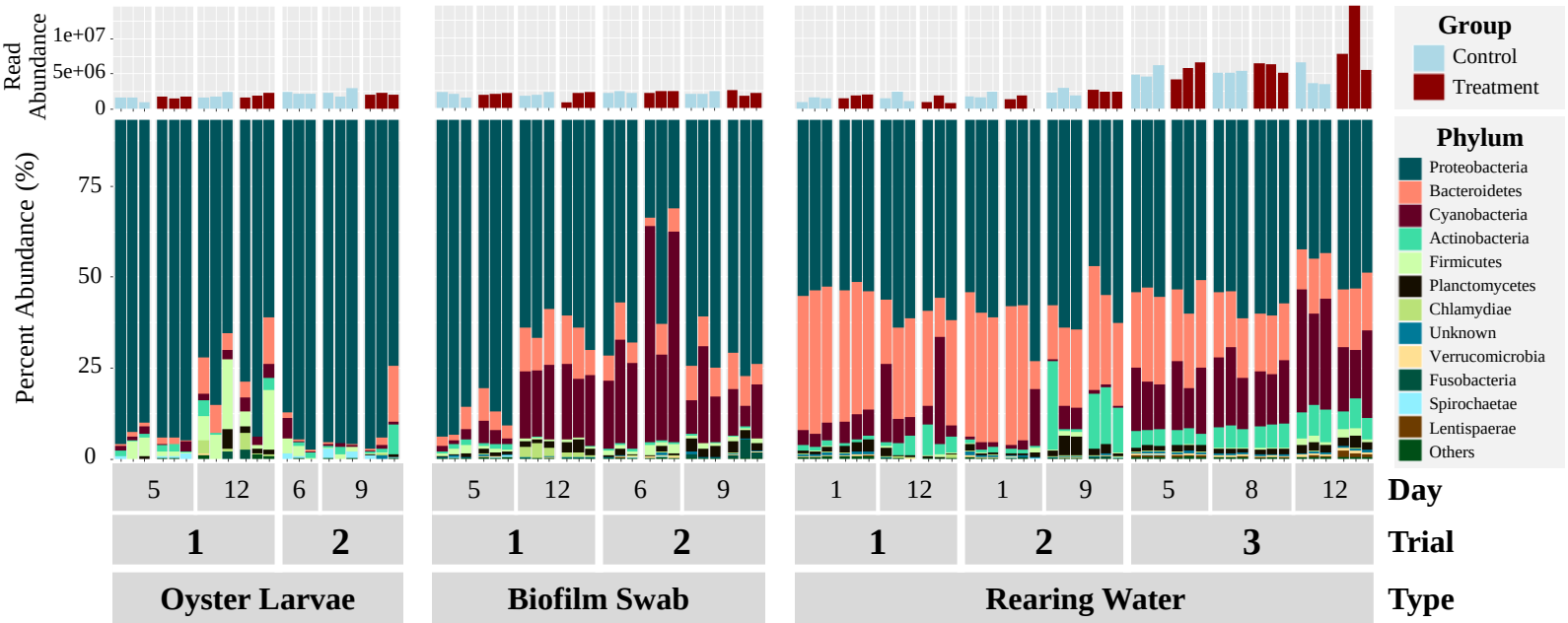

Figure 1. Percent abundances of the 12 most abundant phyla in all oyster larvae, biofilm swab, and rearing water samples from all 3 Trials based on 16S rDNA amplicon sequencing data (bottom). The total abundance of quality filtered sequencing reads is shown in the bar graph (top). The 12 dominant phyla include *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Deferribacteres*, *Firmicutes*, *Fusobacteria*, *Lentisphaerae*, *Planctomycetes*, *Proteobacteria*, *Spirochaetae*, *Verrucomicrobia*, and Unknown. Note: there are no treated oyster larvae samples from Trial 2, Day 6.

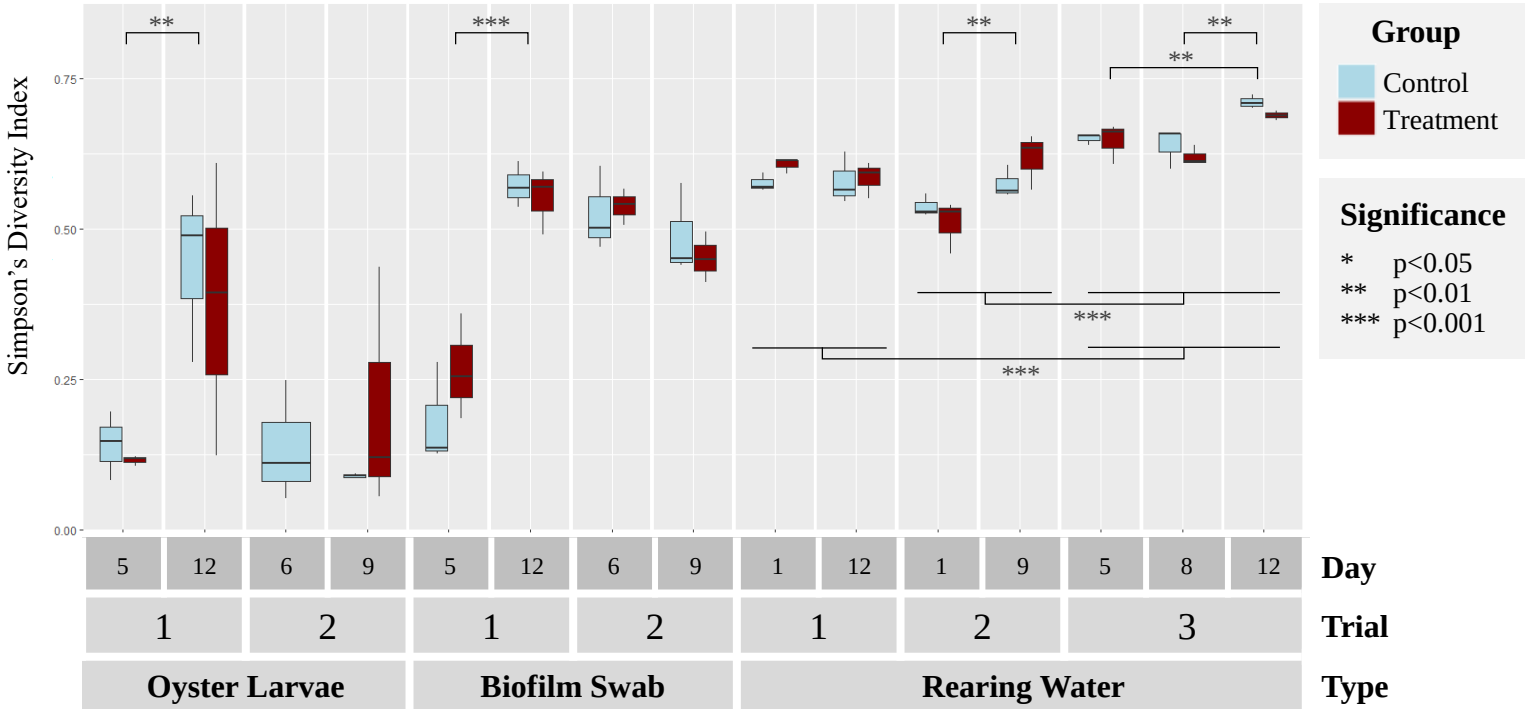

Figure 2. Simpson's index of diversity of bacterial communities by sample (larvae, swab, water) and trial (n=3). No significant differences in diversity were found between control (light blue) and treatment (dark red) within each sample type and trial. Bacterial community diversity significantly increased over time in larvae, swab, and water samples from Trial 1, and water samples from Trial 3. Diversity in water was significantly higher in Trial 3 than Trials 1 and 2. Note: there are no treated oyster larvae samples from Trial 2, Day 6.

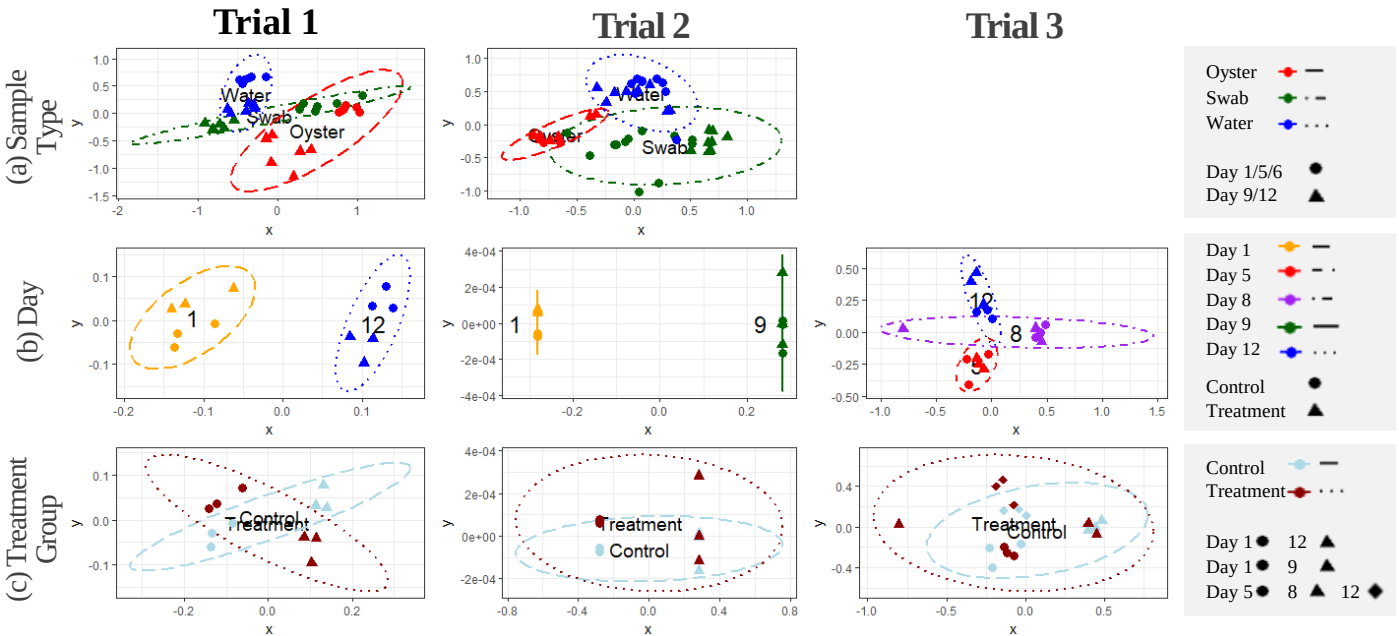

Figure 3. NMDS plot visualization of Bray-Curtis beta-diversity (k=2) at the Order level by (a) sample Type, (b) sampling Day, and (c) treatment. (b) and (c) were calculated using only water samples. The ellipse lines show the 95% confidence interval. (a) The different types of samples are indicated by colors (Oyster=dashed red, Swab=dashdot green, Water=dotted blue) and the days are indicated by symbols (Timepoint 1=circle, Timepoint 2=triangle). The water and swab communities were significantly distinct from each other in both trials. (b) The sampling timepoints are indicated by colors (1=longdash yellow, 5=shortdash red, 8=dashdot purple, 9=solid green, 12=dotted blue) and the treatment group is indicated by symbols (control=circle, probiotic treatment=triangle). The water community was significantly different between timepoints. (c) The treatment group is indicated by colors (control=light blue dashed, probiotic treatment=dark red dotted) and sampling timepoints are indicated by symbols. No significant differences in community structure in water from control and probiotic-treated tanks was detected when samples from all time points were analyzed together.

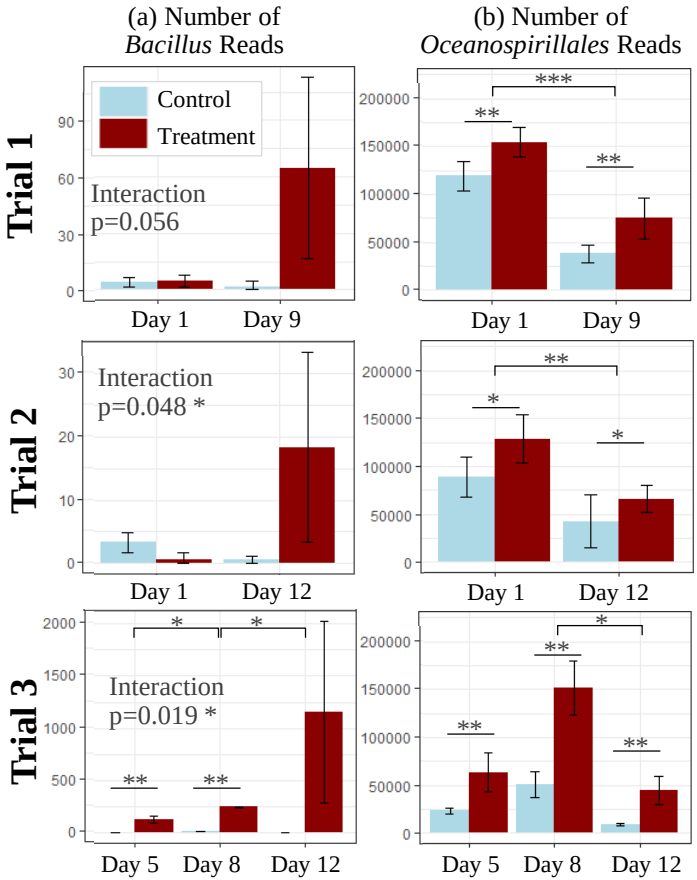

Figure 4. Effect of probiotic treatment on abundance of (a) *Bacillus* and (b) *Oceanospirillales* in water. Number of reads in treated (dark red) and control (light blue) samples (n=3) are represented for each sampling day and trial. (a) *Bacillus* abundance was significantly higher in the treated than the control water after 5 days of treatment, and (b) *Oceanospirillales* were consistently more abundant in probiotic-treated tank rearing water, and decreased with time. **Significance**: *p<0.05, **p<0.01, ***p<0.001

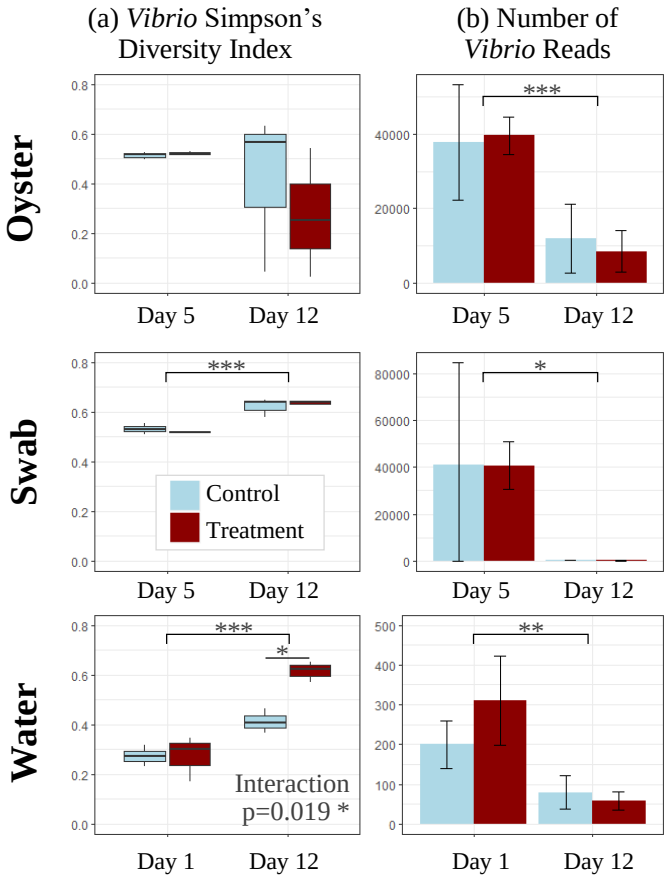

Figure 5. Effect of treatment, time, and samples type on Simpson's Index of Diversity for *Vibrio* (a, boxplots) and Total *Vibrio* read abundance (b, bar graph). Representative data from Trial 1 (n=3 tanks per treatment). Note different scales for (b). **Significance**: *p<0.05, **p<0.01, ***p<0.001

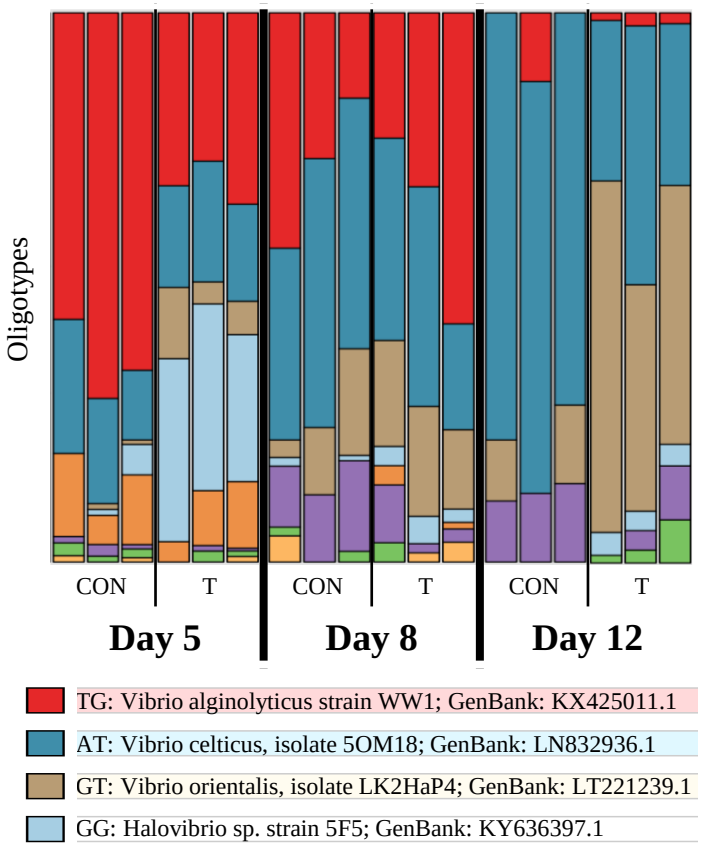

Figure 6. *Vibrio* spp. oligotypes in Control (CON) and Treatment (T) water samples on Days 5, 8, and 12 from Trial 3. These 8 oligotypes were generated from changes in positions 23 and 37 in a total of 1727 sequences. *Vibrio* oligotypes showed differences in succession of species over time between control and treatment rearing water.

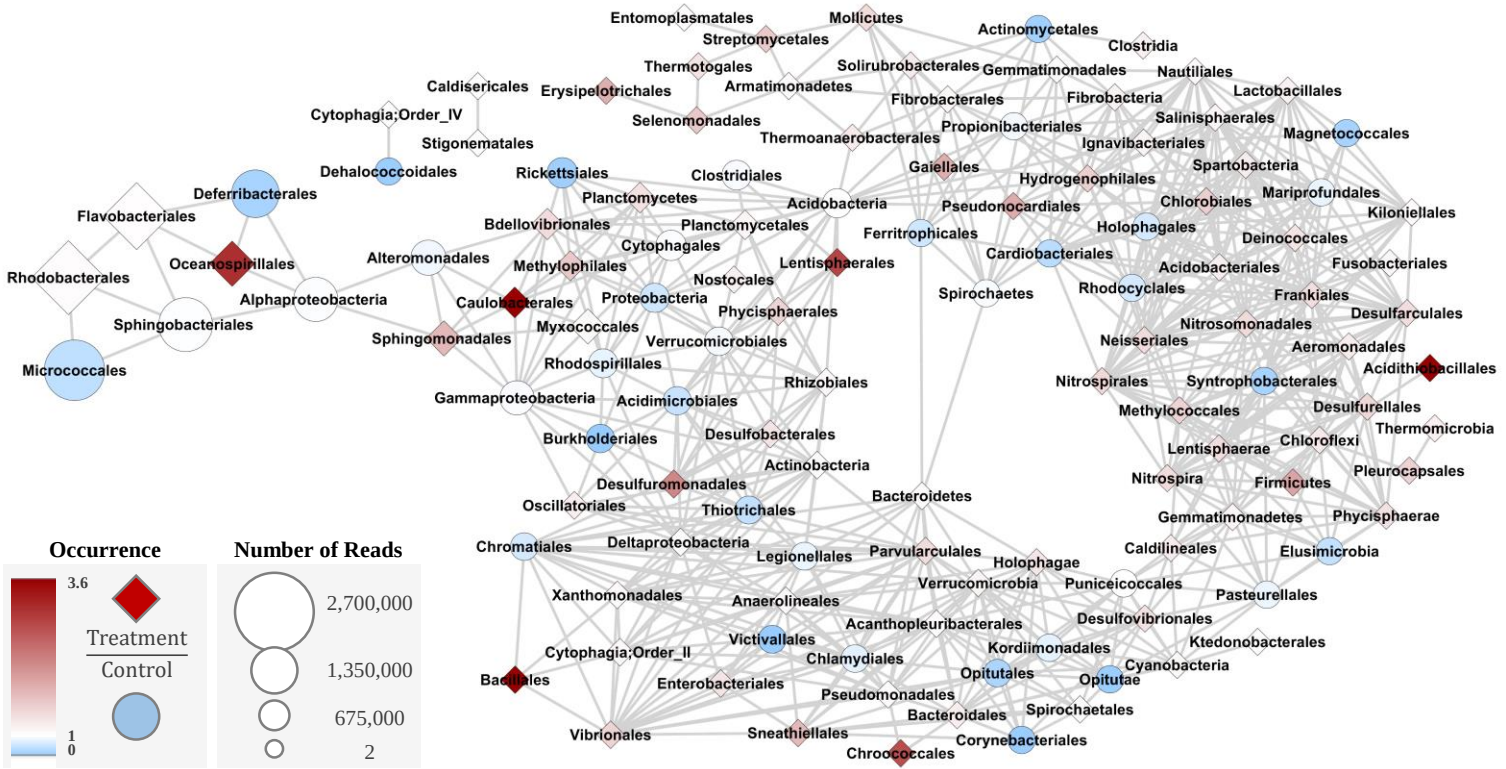

Figure 7. Co-occurrence network analysis based on Bray-Curtis dissimilarity metric (max distance =0.5, Order level) for water samples from Trial 3 (n=18). Taxa that change in the same way share an edge; nodes that have edges occur in the same proportions and in the same samples. Darker blue circle nodes indicate taxa that occurs in the Control significantly more than Treated water samples. White nodes have equal occurrence in treated and control water samples. Darker red diamond nodes indicated taxa that occurs in the Treated significantly more than Control water samples.

Supplementary Figures & Tables

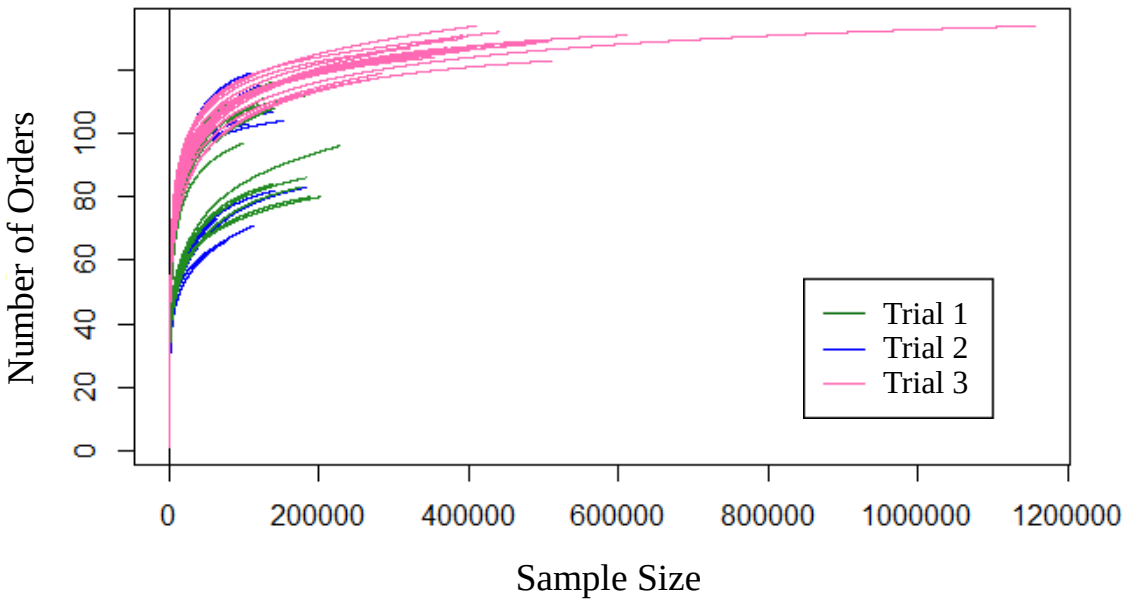

Figure S1. Rarefaction curve from all water samples from all three Trials based on taxonomic classification at the order level.

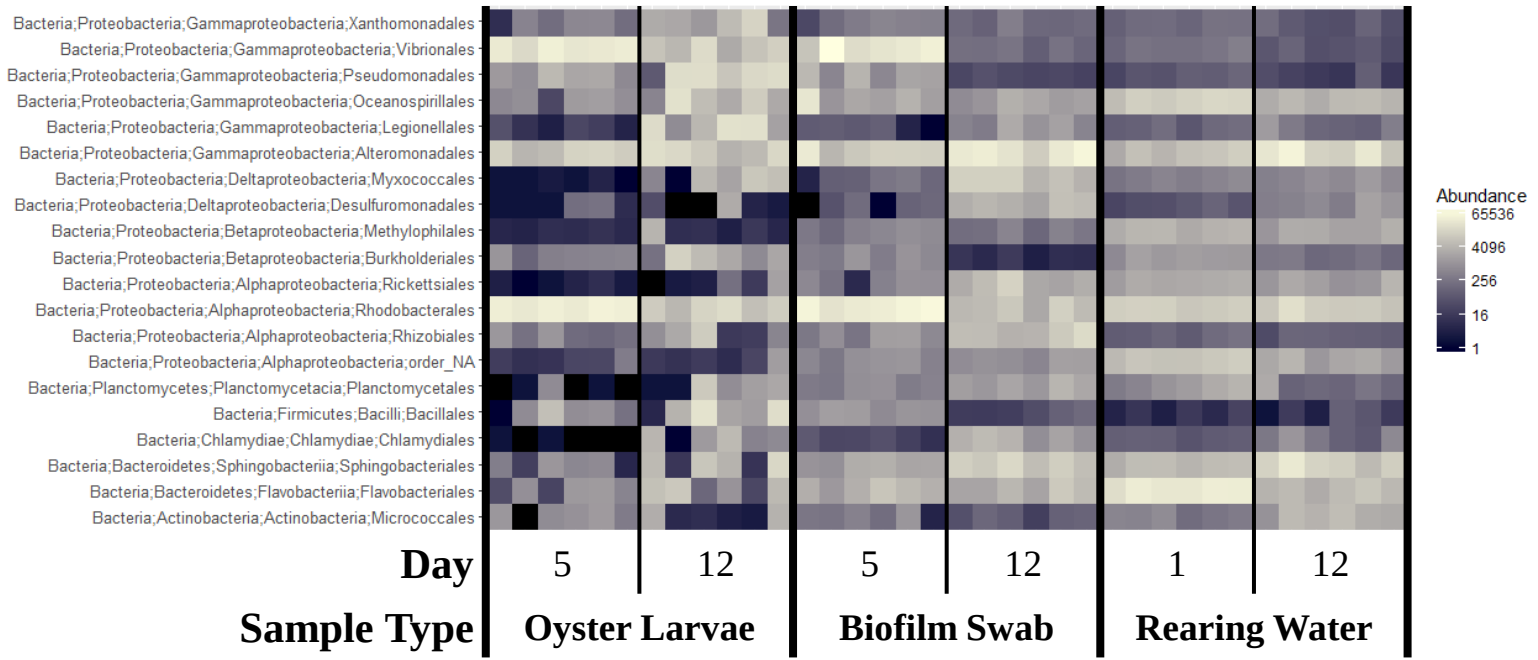

Figure S2. The relative abundances of the 20 most abundant orders in oyster, swab, and water samples from Trial 1.

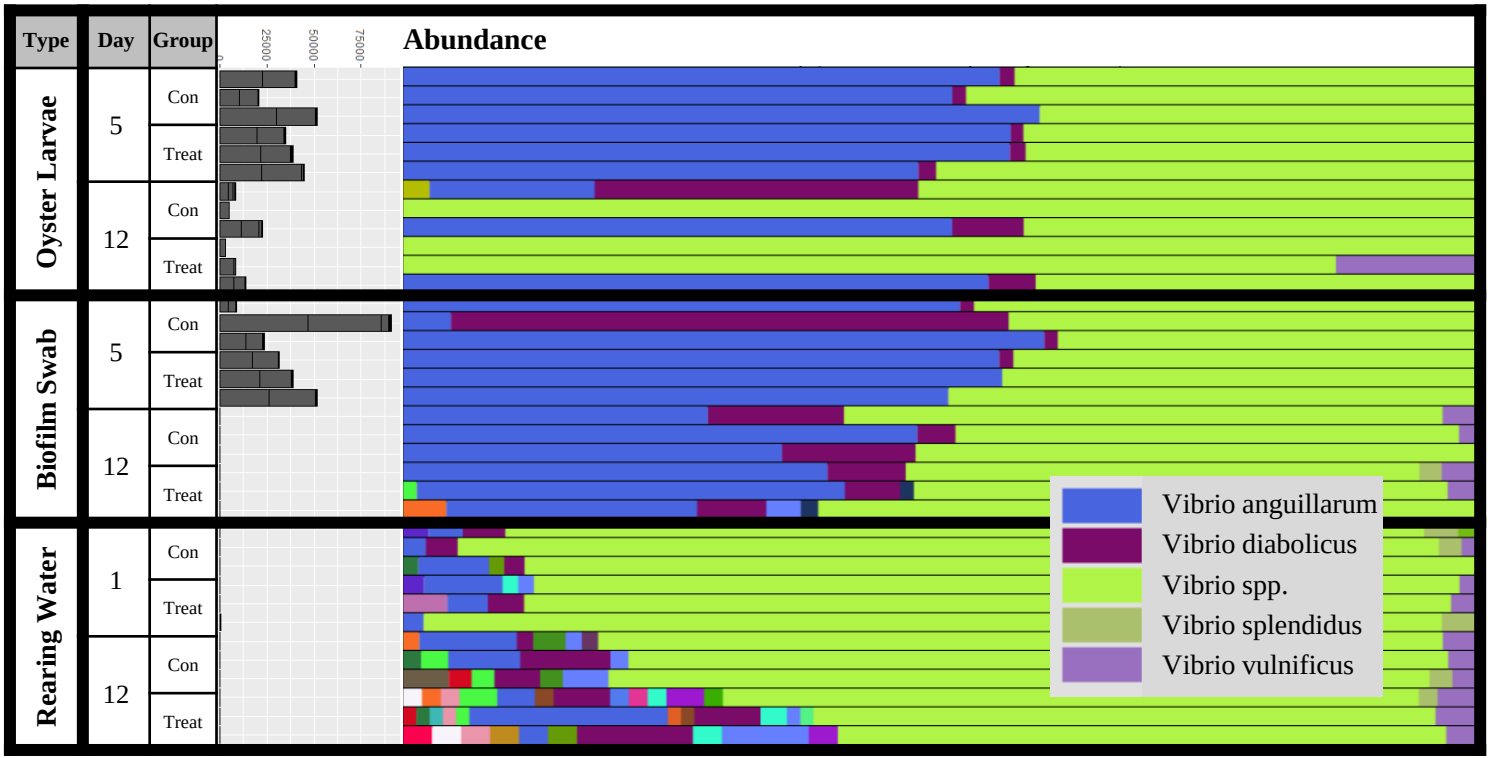

Figure S3. Percent abundances of *Vibrio* species in all sample types in Trial 1. The total abundance of sequencing reads is shown in the bar graph. The structure of total *Vibrios* is different based on the sample type and time point.

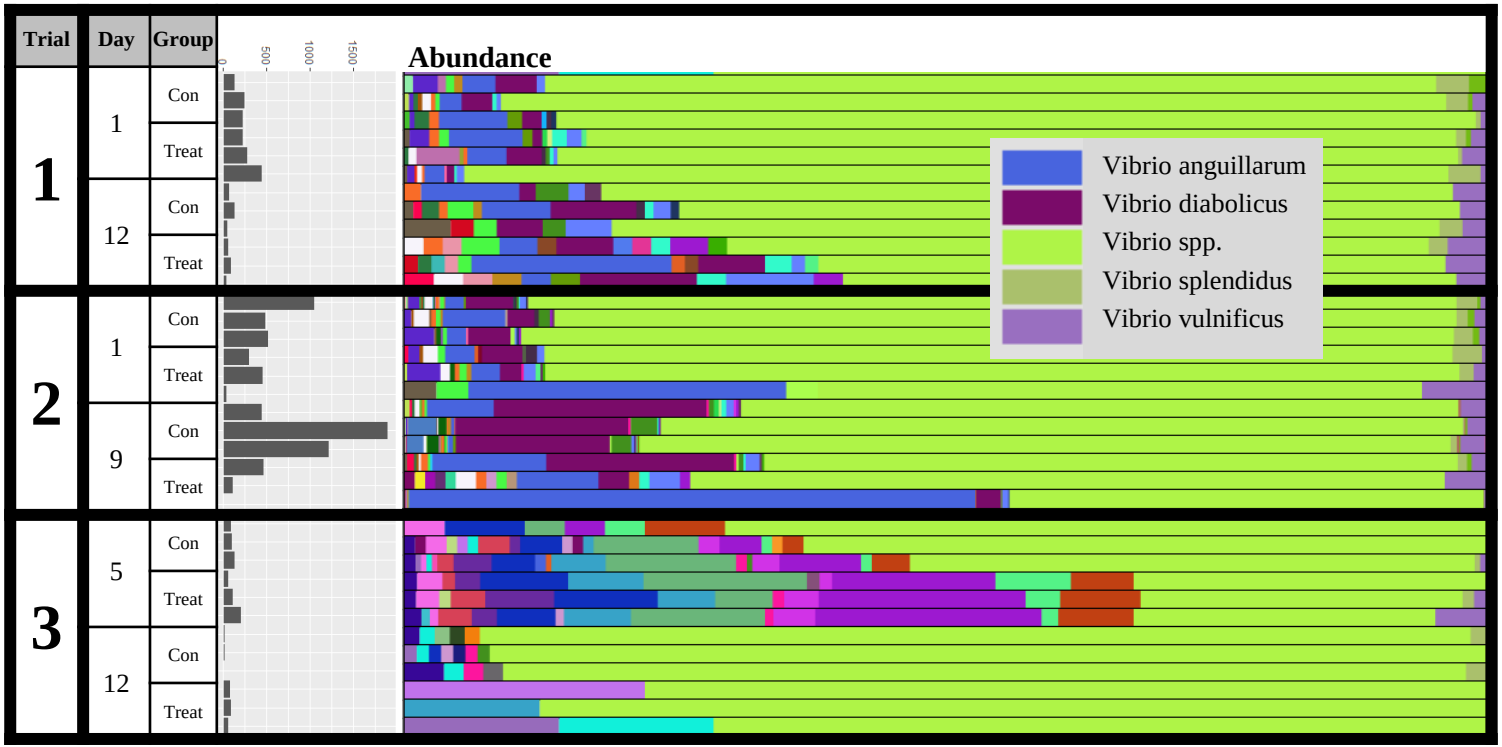

Figure S4. Percent abundances of *Vibrio* species in rearing water samples from all 3 Trials. The total abundance of sequencing reads is shown in the bar graph. The structure of total *Vibrio* counts in the rearing water is different between Trials and changes over time.

Table S1. ANOVA for abundance of *Proteobacteria* and *Cyanobacteria* by Sample Type.

< All Trials - *Proteobacteria* >

ANOVA table	DF	SS	MS	F	P value	
Sample Type	1	6.57E+11	6.57E+11	19.75	2.65E-05	***
Residuals	85	2.83E+12	3.33E+10			

< All Trials - *Cyanobacteria* >

ANOVA table	DF	SS	MS	F	P value	
Sample Type	1	4.35E+11	4.35E+10	2.24	0.138	
Residuals	85	1.65E+12	1.94E+10			
Sample Type – no water	1	9.19E+11	4.19E+11	23.51	1.66E-05	***
Residuals	43	7.67E+11	1.78E+10			

Table S2. Two-way ANOVAs for the Simpson's Index of Diversity values by Trial, Sample Type, Day, and/or Treatment.

< All Trials – Simpson's Index of Diversity >

ANOVA table	DF	SS	MS	F	P value	
Trial	1	0.5689	0.5689	43.4	4.16E-09	***
Type	2	1.6714	0.8357	63.76	< 2e-16	***
Trial:Type	2	0.1628	0.0814	6.21	0.0031	**
Residuals	81	1.0617	0.0131			

< All Trials – Simpson's Index of Diversity >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.07	0.06988	1.72	0.193
Group	1	0.016	0.01577	0.388	0.535
Day:Group	1	0.007	0.00711	0.175	0.677
Residuals	83	3.372	0.04063		

< All Trials – Water Only – Simpson's Index of Diversity >

ANOVA table	DF	SS	MS	F	P value	
Trial	1	0.04629	0.04629	19.46	7.55E-05	***
Residuals	40	0.09514	0.00238			

Table S3. Two-way ANOVAs for the Simpson's Index of Diversity values by Day and Treatment in Trial 1.

< Trial 1 - Water >

ANOVA table	DF	SS	MS	F	P value	
Day	1	0.000277	0.0002775	0.35	0.571	
Treatment	1	9.36E-04	9.37E-04	1.18	0.309	
Day:Treatment	1	4.94E-04	4.94E-04	0.623	0.453	
Residuals	8	0.006347	0.0007933			

< Trial 1 - Biofilm Swab >

ANOVA table	DF	SS	MS	F	P value	
Day	1	0.3443	0.3443	71.378	2.94E-05	***
Treatment	1	0.0032	0.0032	0.663	0.439	
Day:Treatment	1	8.40E-03	8.40E-03	1.75	0.222	
Residuals	8	3.86E-02	4.80E-03			

< Trial 1 - Oyster Larvae >

ANOVA table	DF	SS	MS	F	P value	
Day	1	0.2348	0.2348	11.312	0.00988	**
Treatment	1	0.00625	0.00625	0.301	0.59824	
Day:Treatment	1	0.00121	0.00121	0.058	0.8155	
Residuals	8	0.16606	0.02076			

Table S4. Two-way ANOVAs for the Simpson's Index of Diversity values by Day and Treatment in Trial 2.

< Trial 2 - Water >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.016033	0.016033	12.27	0.00805 **
Treatment	1	0.000141	0.000141	0.108	0.75137
Day:Treatment	1	0.003703	0.003703	2.834	0.13078
Residuals	8	0.010454	0.001307		

< Trial 2 - Biofilm Swab >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.01117	0.01117	3.346	0.105
Treatment	1	0.000421	0.000421	0.126	0.732
Day:Treatment	1	0.001787	0.001787	0.535	0.485
Residuals	8	0.026704	0.003338		

< Trial 2 - Oyster Larvae >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.00021	0.000206	0.012	0.917
Treatment	1	0.01966	0.019658	1.136	0.327
Resduals	6	0.10379	0.017298		

Table S5. Two-way ANOVA for the Simpson's Index of Diversity values by Day and Treatment in Trial 3.

< Trial 3 - Water >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.009329	0.009329	10.68	0.00561 **
Treatment	1	0.001006	0.001006	1.152	0.30125
Day:Treatment	1	0.000254	0.000254	0.291	0.59832
Residuals	14	0.012229	0.000873		

Table S6. Two-way ANOVAs for abundance of *Bacillus* spp. reads in water samples per Trial by Day and Treatment Group.

< Trial 1 – *Bacillus* spp. >

ANOVA table	DF	SS	MS	F	P value
Day	1	2494	2494.1	4.282	7.23E-02 .
Treatment	1	3040	3040.1	5.219	0.0517 .
Day:Treatment	1	2.91E+03	2.91E+03	5.003	0.0557 .
Residuals	8	4.66E+03	5.83E+02		

< Trial 2 – *Bacillus* spp. >

ANOVA table	DF	SS	MS	F	P value
Day	1	168.8	168.75	2.956	0.1239
Treatment	1	168.7	168.75	2.956	0.1239
Day:Treatment	1	310.1	310.08	5.432	0.0481 *
Residuals	8	456.7	57.08		

< Trial 3 – *Bacillus* spp. >

ANOVA table	DF	SS	MS	F	P value
Day	1	834952	834952	6.866	0.02017 *
Treatment	1	1142568	1142568	9.395	0.00839 **
Day:Treatment	1	848829	848829	6.98	0.01933 *
Residuals	14	1702573	121612		

Table S7. Two-way ANOVAs for abundance of *Oceanospirillales* reads in water samples per Trial by Day and Treatment Group.

< Trial 1 – *Oceanospirillales* >

ANOVA table	DF	SS	MS	F	P value
Day	1	1.93E+10	1.93E+10	74.823	2.48E-05 ***
Treatment	1	3.91E+09	3.91E+09	15.202	0.00455 **
Day:Treatment	1	1.96E+06	1.96E+06	0.008	0.93269
Residuals	8	2.06E+09	2.57E+08		

< Trial 2 – *Oceanospirillales* >

ANOVA table	DF	SS	MS	F	P value
Day	1	8.92E+09	8.92E+09	17.412	0.00311 **
Treatment	1	2.96E+09	2.96E+09	5.782	0.04287 *
Day:Treatment	1	2.09E+08	2.09E+08	0.408	0.54084
Residuals	8	4.10E+09	5.12E+08		

< Trial 3 – *Oceanospirillales* >

ANOVA table	DF	SS	MS	F	P value
Day	1	1.56E+09	1.56E+09	0.921	0.35355
Treatment	1	1.56E+10	1.56E+10	9.236	0.00884 **
Day:Treatment	1	7.84E+07	7.84E+07	0.046	0.83267
Residuals	14	2.37E+10	1.69E+09		

Table S8. Two-way ANOVAs for Simpson's Index of Diversity of *Vibrio* reads in Trial 1 per Sample Type by Day and Treatment Group.

< All Samples – *Vibrio* diversity >

ANOVA table	DF	SS	MS	F	P value	
Type	2	0.2249	0.11245	7.261	0.00268	**
Day	1	0.0475	0.04752	3.069	0.09004	.
Type:Day	2	0.2494	0.12471	8.053	0.00159	**
Residuals	30	0.4646	0.01549			

< Oyster Larvae– *Vibrio* diversity >

ANOVA table	DF	SS	MS	F	P value
Day	1	0.0902	0.09025	2.118	.184
Treatment	1	1.27E-02	1.27E-02	0.298	.600
Day:Treatment	1	1.68E-02	1.68E-02	0.395	0.547
Residuals	8	3.41E-01	4.26E-02		

< Biofilm swab– *Vibrio* diversity >

ANOVA table	DF	SS	MS	F	P value	
Day	1	0.03264	0.03264	66.039	3.90E-05	***
Treatment	1	0.00001	0.00001	0.02	0.891	
Day:Treatment	1	0.00064	0.00064	1.287	0.29	
Residuals	8	3.95E-03	4.90E-04			

< Water – *Vibrio* diversity >

ANOVA table	DF	SS	MS	F	P value	
Day	1	0.17404	0.17404	48.177	0.000119	***
Treatment	1	0.02997	0.02997	8.295	0.020507	*
Day:Treatment	1	0.03068	0.03068	8.493	0.019459	*
Residuals	8	2.89E-02	3.61E-03			

Table S9. Two-way ANOVAs for abundance of *Vibrio* reads in Trial 1 per Sample Type by Day and Treatment Group.

< All Samples – *Vibrio* reads >

ANOVA table	DF	SS	MS	F	P value	
Type	2	4.08E+09	2.04E+09	12.62	0.000105	***
Day	1	3.95E+09	3.95E+09	24.47	2.71E-05	***
Type:Day	2	3.44E+09	1.72E+09	10.65	0.000319	***
Residuals	30	4.85E+09	1.62E+08			

< Oyster Larvae– *Vibrio* reads >

ANOVA table	DF	SS	MS	F	P value	
Day	1	2.43E+09	2.43E+09	25.417	1.00E-03	***
Treatment	1	2.48E+06	2.48E+06	0.026	0.876	
Day:Treatment	1	2.09E+07	2.09E+07	0.219	0.653	
Residuals	8	7.65E+08	9.56E+07			

< Biofilm swab– *Vibrio* reads >

ANOVA table	DF	SS	MS	F	P value	
Day	1	4.97E+09	4.97E+09	9.791	0.014	*
Treatment	1	7.55E+04	7.55E+04	0	0.991	
Day:Treatment	1	2.52E+04	2.52E+04	0	0.995	
Residuals	8	4.06E+09	5.07E+08			

< Water – *Vibrio* reads >

ANOVA table	DF	SS	MS	F	P value	
Day	1	1.04E+05	1.04E+05	22.534	0.00145	**
Treatment	1	6.03E+03	6.03E+03	1.307	0.28601	
Day:Treatment	1	1.31E+04	1.31E+04	2.847	0.13005	
Residuals	8	3.69E+04	4.61E+03			