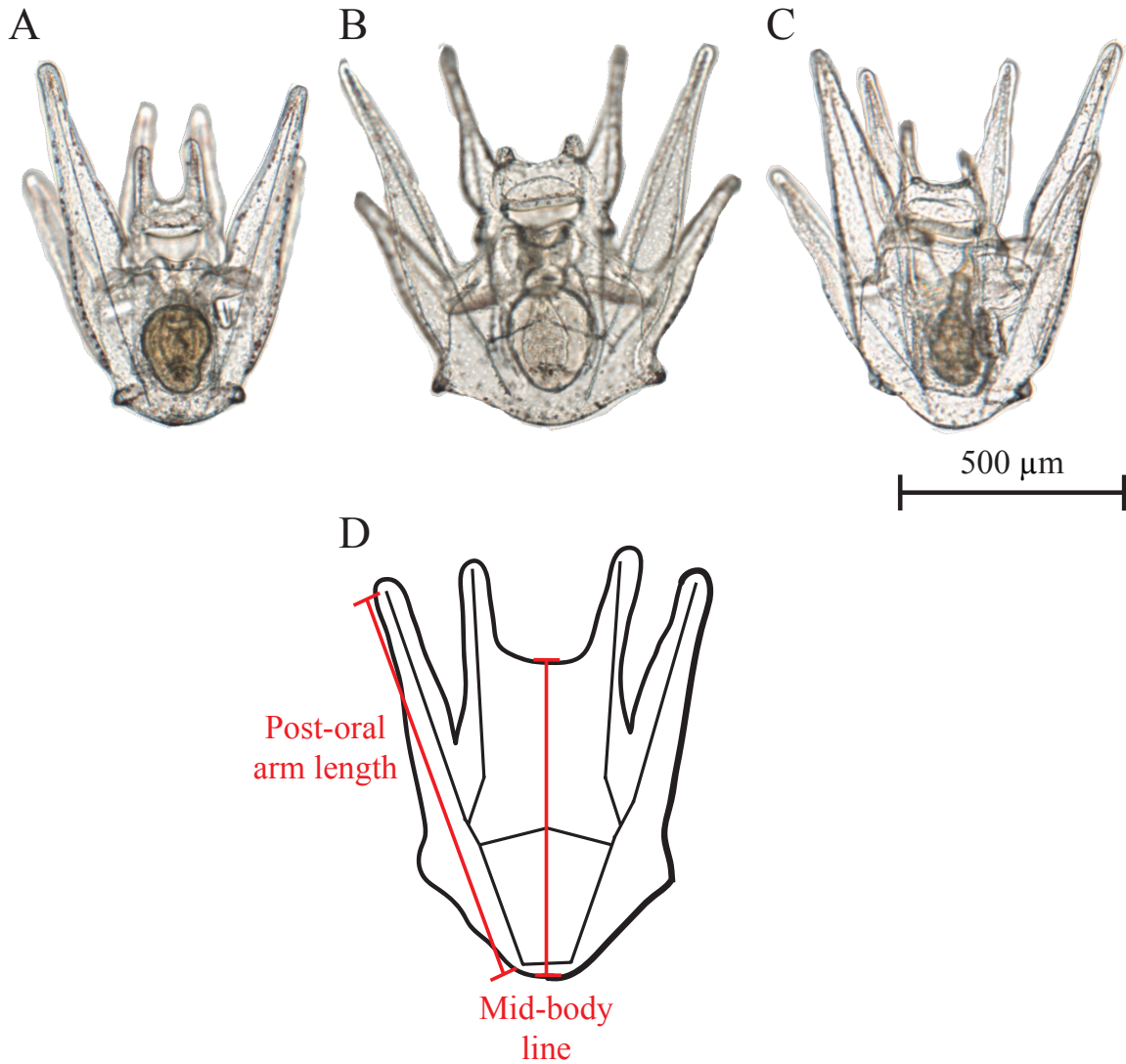


Authors: Tyler J. Carrier and Adam M. Reitzel

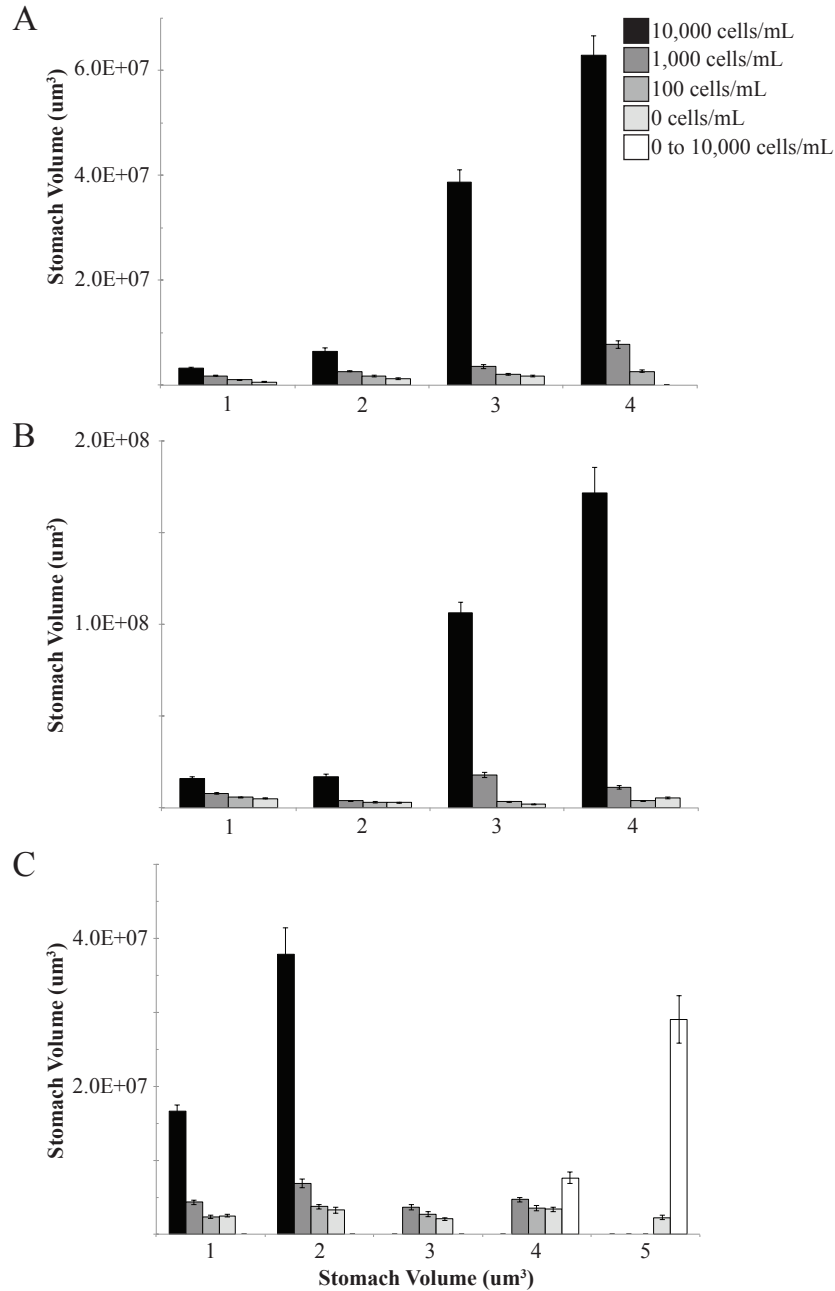
Title: Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes

File name: Supplementary Documentation

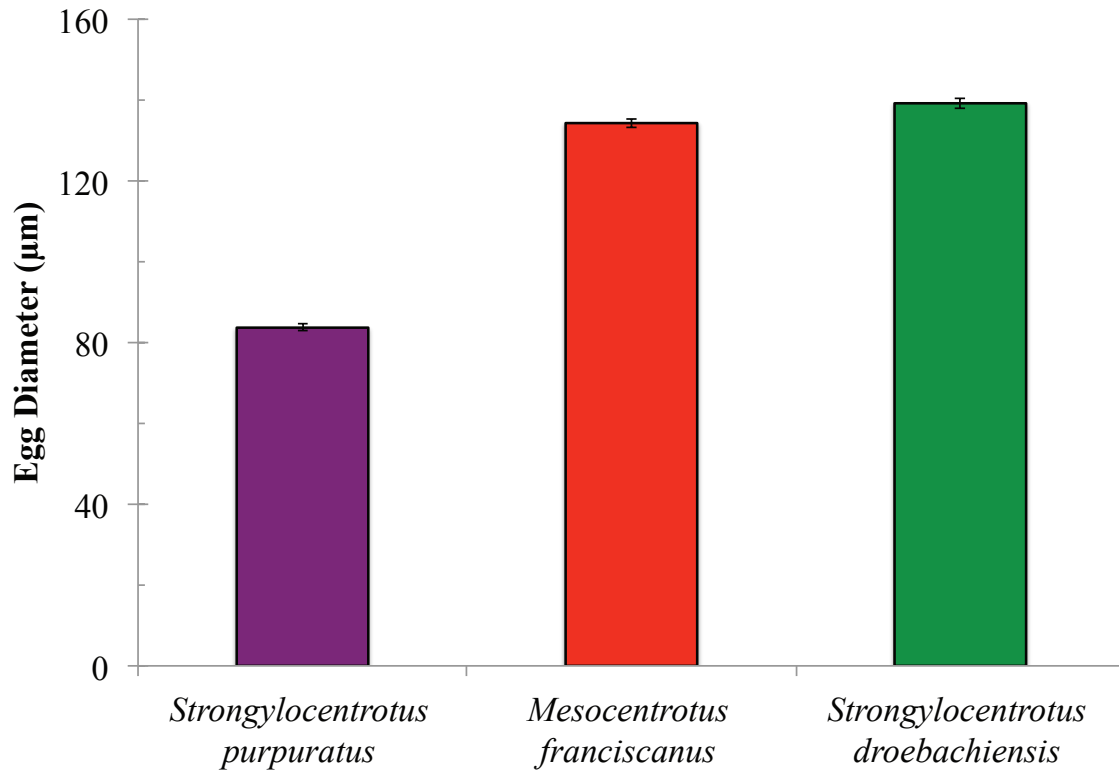
Description: Supplementary Figures and Supplementary Tables



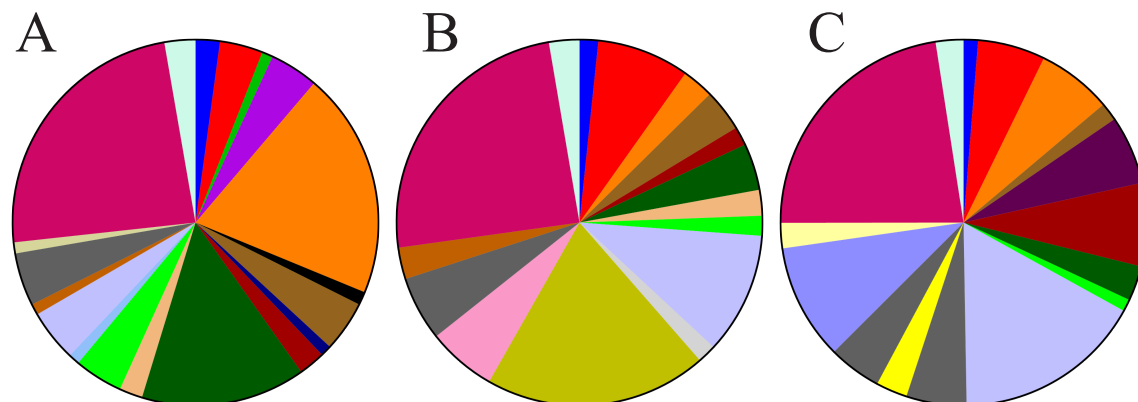
Supplementary Figure 1. Photograph of each species of echinoid larvae and representative cartoon. Microphotograph of 8-arm *Strongylocentrotus purpuratus* (A), 6-arm *Mesocentrotus franciscanus* (B), and 8-arm *S. droebachiensis* (C) larva, and (D) cartoon of 4-arm echinoid larvae with pre-drawn line for post-oral arm and mid-body line measurements.



Supplementary Figure 2. Three species of echinoid larvae alter stomach volume based on feeding environment. Post-oral arm to mid body line ratio (\pm standard error; $n=20$) for *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) larvae having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells \cdot mL $^{-1}$ (light grey). For *S. droebachiensis*, larval stomach volume was also manipulated (white) by being fed 0 cells \cdot mL $^{-1}$ for three weeks then transferred to 10,000 cells \cdot mL $^{-1}$ for three weeks (*i.e.*, until metamorphosis). Data here correspond with Figure 1.

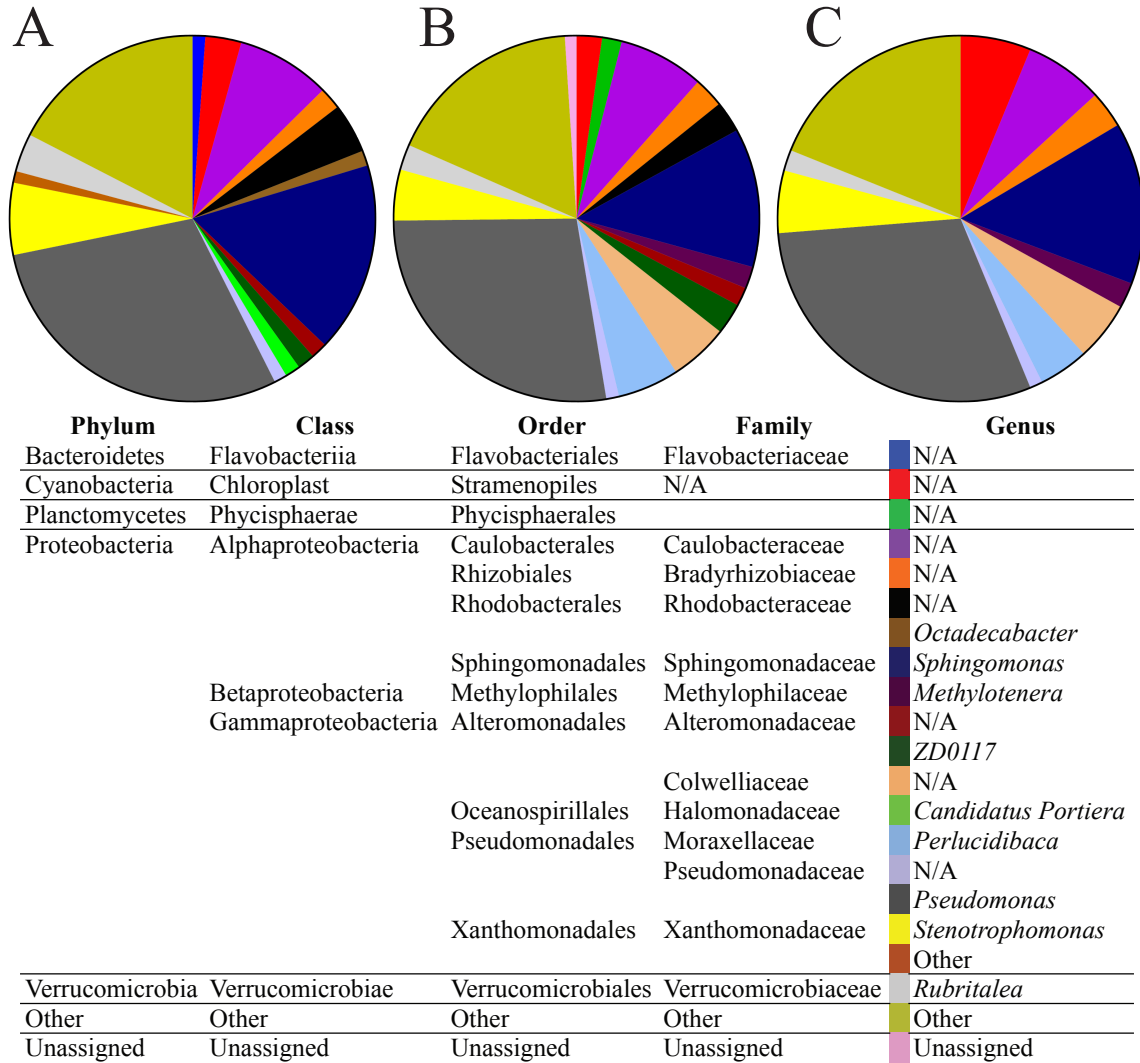


Supplementary Figure 3. Egg diameter for three species of echinoid larvae. Mean egg size (\pm standard error; $n=20$) of unfertilized eggs for *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis*.

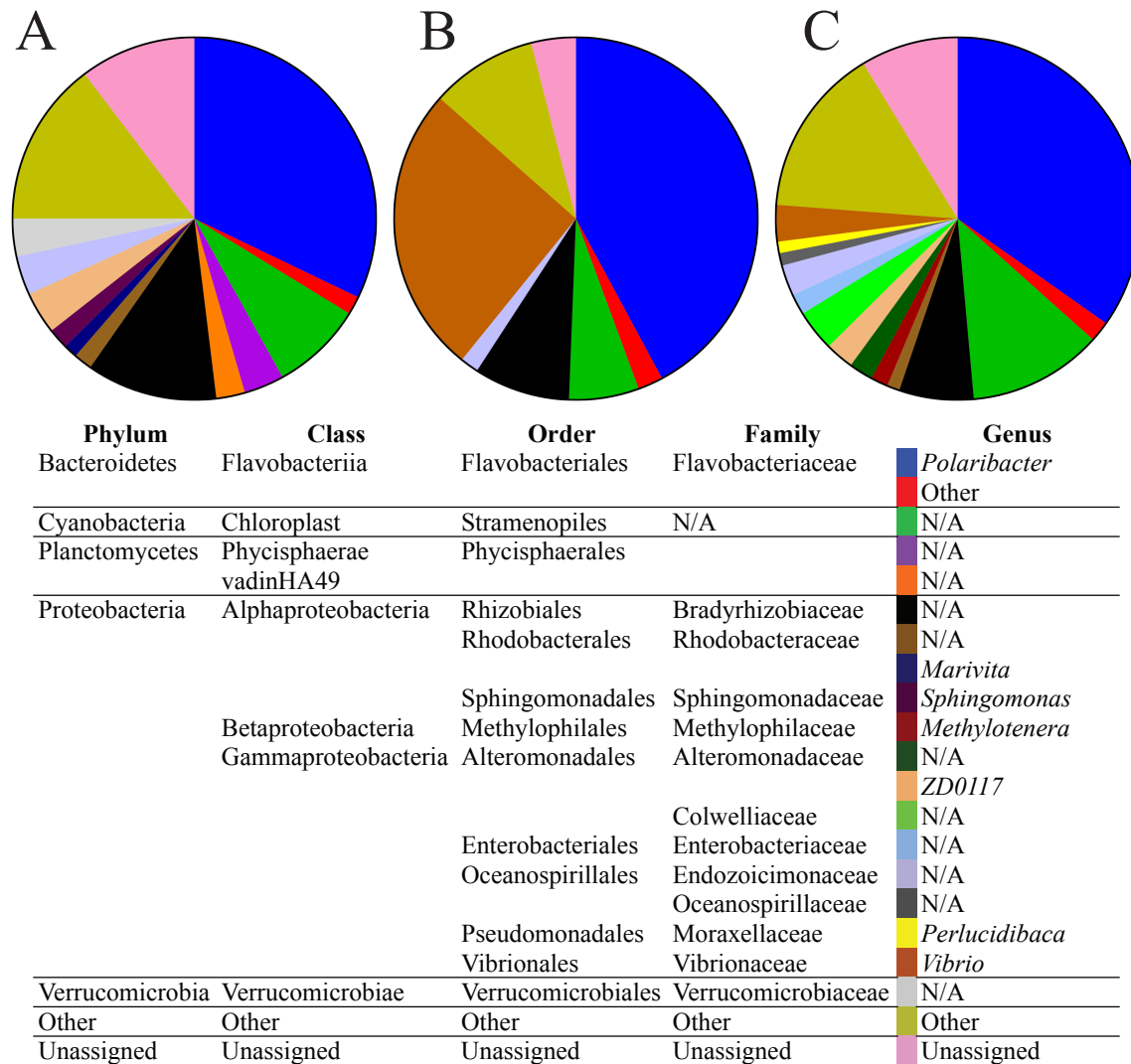


Phylum	Class	Order	Family	Genus		
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	N/A		
Cyanobacteria	Chloroplast	Stramenopiles	N/A	N/A		
Planctomycetes	Phycisphaerae	Phycisphaerales	N/A	N/A		
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	N/A		
		Rhizobiales	Bradyrhizobiaceae	N/A		
		Rhodobacterales	Hyphomonadaceae	N/A		
			Rhodobacteraceae	N/A		
		Sphingomonadales	N/A	N/A		
			Sphingomonadaceae	<i>Sphingomonas</i>		
		Betaproteobacteria	Methylophilales	Methylophilaceae	<i>Methylotenera</i>	
		Gammaproteobacteria	Alteromonadales	Alteromonadaceae	N/A	
					<i>Alteromonas</i>	
						<i>ZD0117</i>
					Other	
				Colwelliaceae	N/A	
					<i>Colwellia</i>	
				J115	N/A	
					Other	
				Oceanospirillales	Oceanospirillaceae	<i>Oleibacter</i>
					<i>Oleispira</i>	
				Other		
			Pseudomonadales	Moraxellaceae	<i>Perlucidibaca</i>	
				Pseudomonadaceae	<i>Pseudomonas</i>	
		Xanthomonadales	Xanthomonadaceae	<i>Stenotrophomonas</i>		
		Other	Other			
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Persicirhabdus</i>		
Other	Other	Other	Other	Other		
Unassigned	Unassigned	Unassigned	Unassigned	Unassigned		

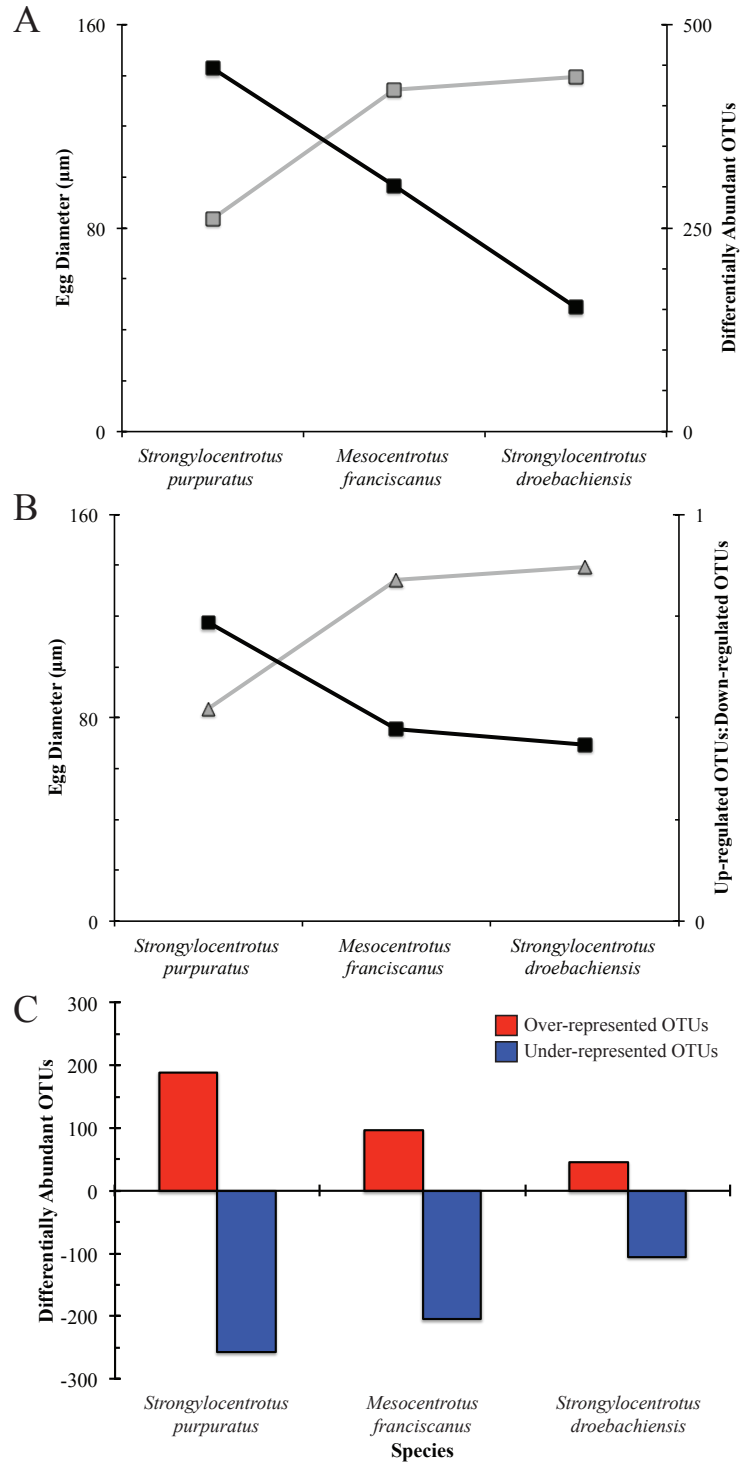
Supplementary Figure 4. Distribution of bacterial taxa associated with *Strongylocentrotus purpuratus* larvae along the phenotypic continuum. Genera associated with *S. purpuratus* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 2A.



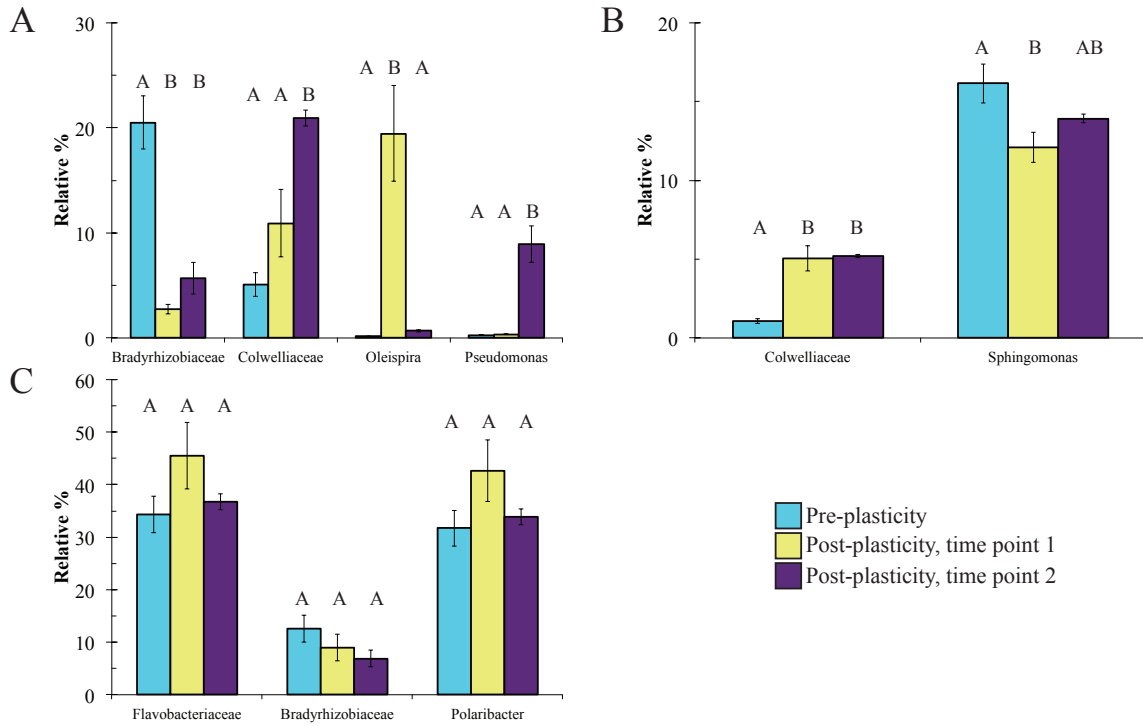
Supplementary Figure 5. Distribution of bacterial taxa associated with *Mesocentrotus franciscanus* larvae along the phenotypic continuum. Genera associated with *M. franciscanus* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 2B.



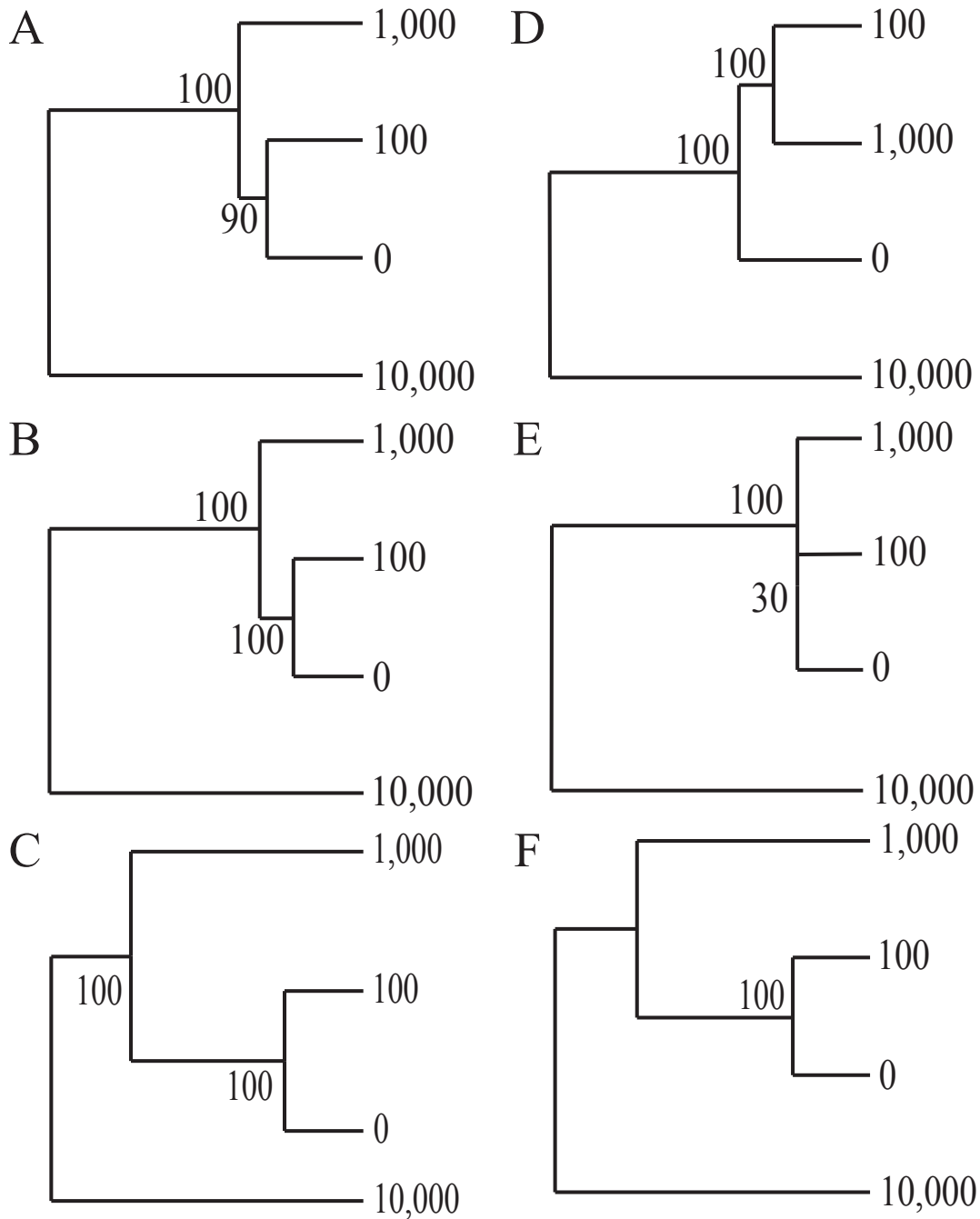
Supplementary Figure 6. Distribution of bacterial taxa associated with *Strongylocentrotus droebachiensis* larvae along the phenotypic continuum. Genera associated with *S. droebachiensis* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Figure 2C.



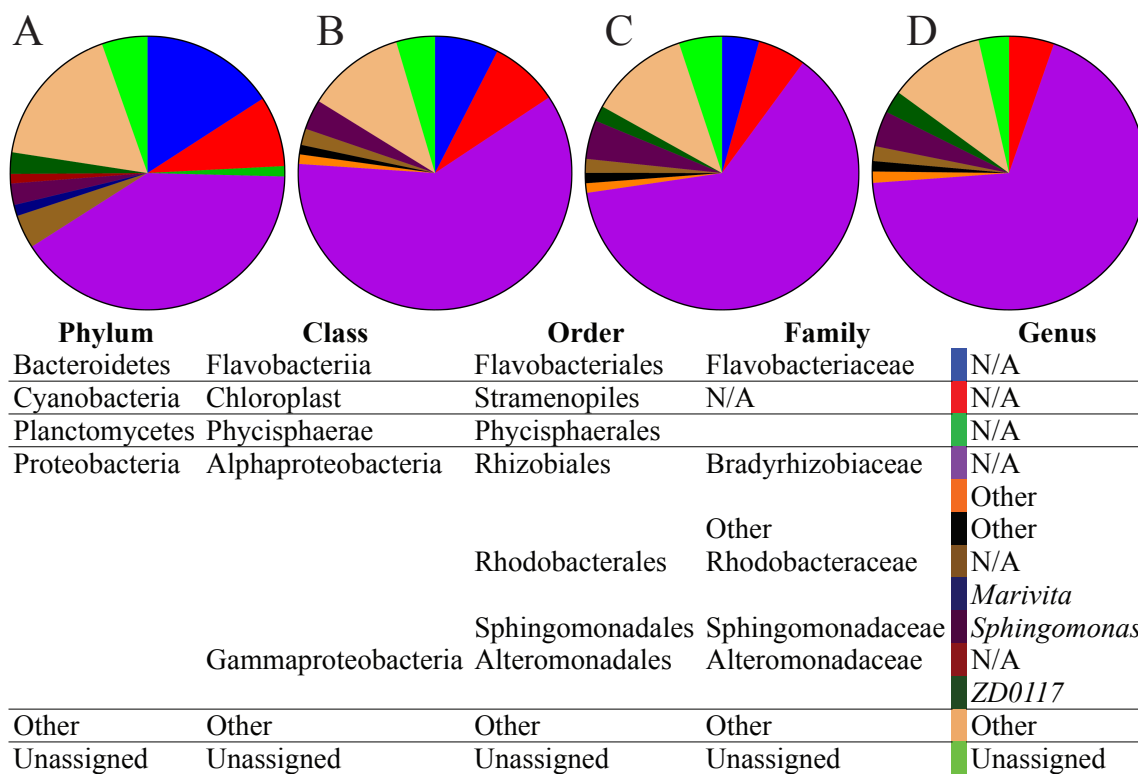
Supplementary Figure 7. Differential abundance of OTUs in relation to egg size for three species of echinoid larvae. Total (A, B) and ratio of (C) over- and under-represented OTUs associated with *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis* larvae following the expression of phenotypic plasticity (black) and in relation to egg size (gray).



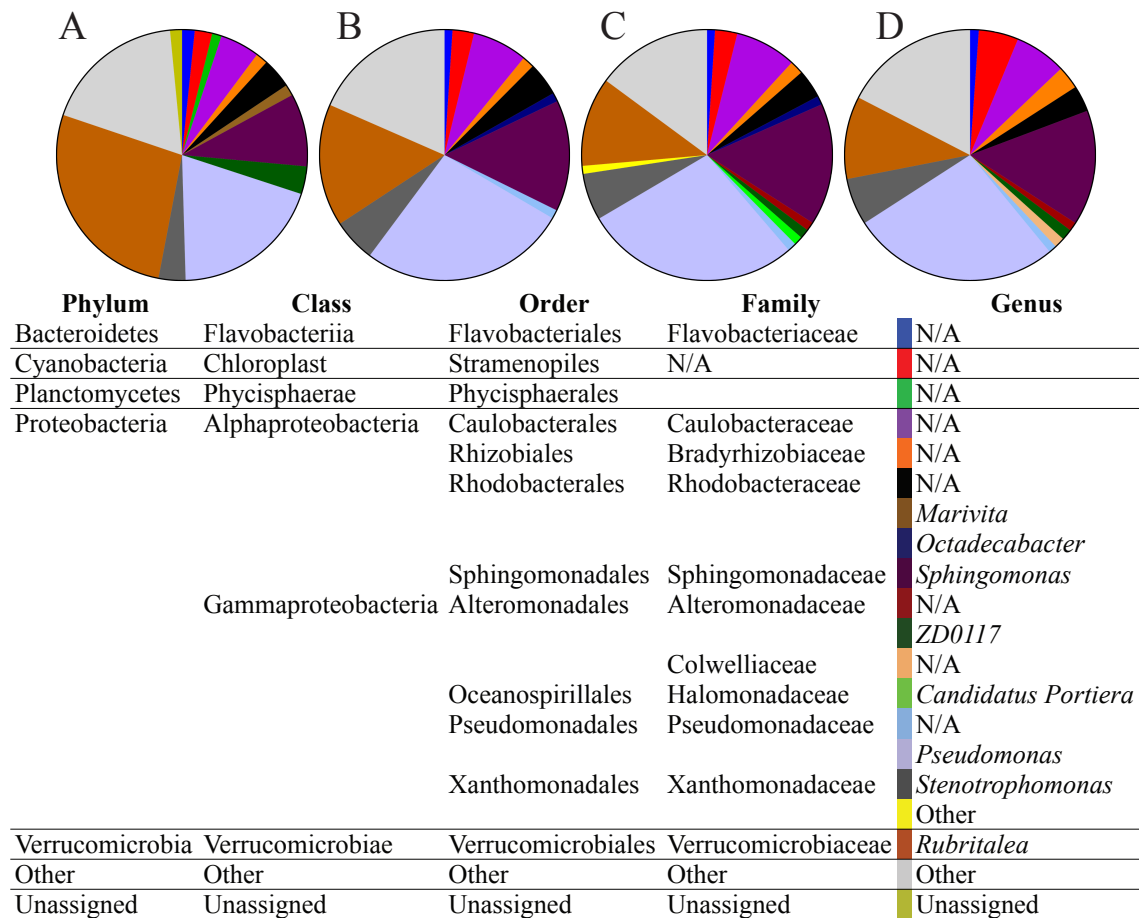
Supplementary Figure 8. Specific bacterial taxa within the proteobacteria differentially associated with larvae from three species of echinoid along the phenotypic continuum. Relative presence of specific bacterial taxa associated with *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) pre- (blue) and post-expression (yellow and purple) of phenotypic plasticity. Data correspond with Figure 2D-F.



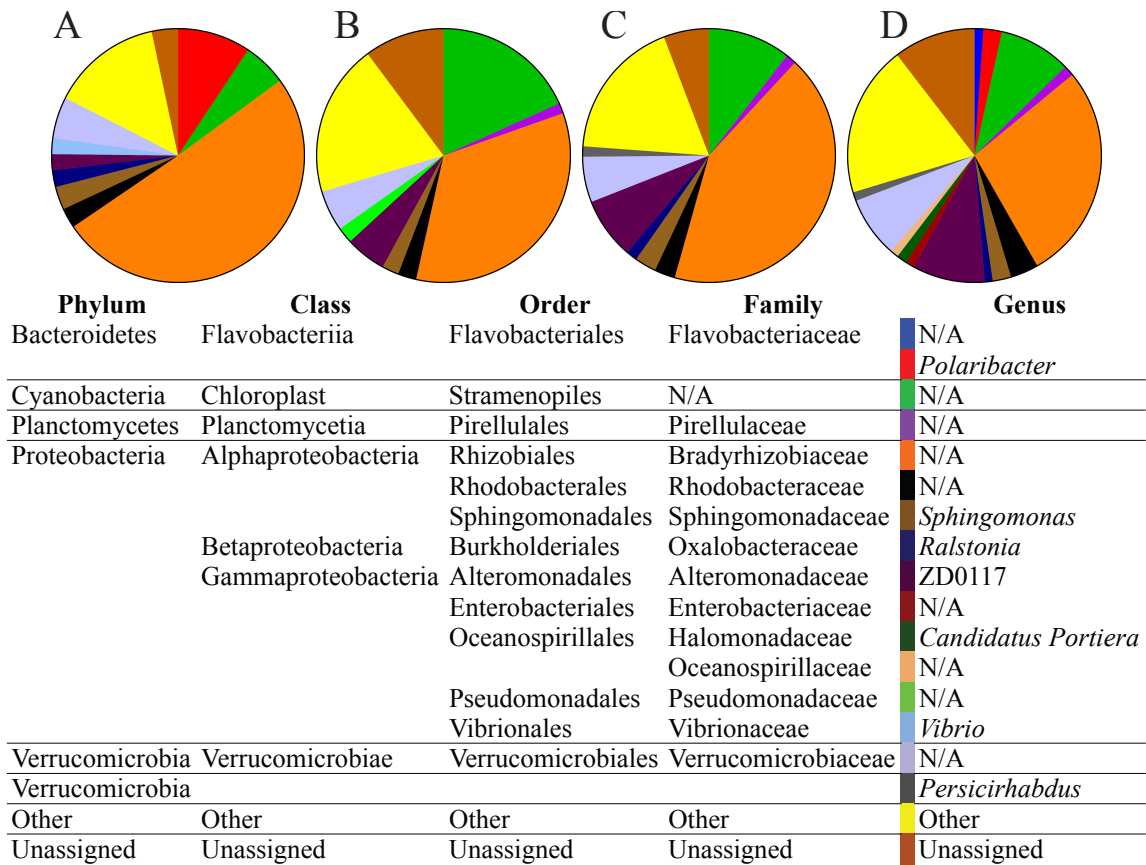
Supplementary Figure 9. Similarity between associated microbial community for three species of differentially fed echinoid larvae. Microbial dendrograms of *Strongylocentrotus purpuratus* (A, D), *Mesocentrotus franciscanus* (B, E), and *S. droebachiensis* (C, F) differentially fed at time points pre- (A-C) and post-expression (D-F) of phenotypic plasticity. Data correspond with Figure 4.



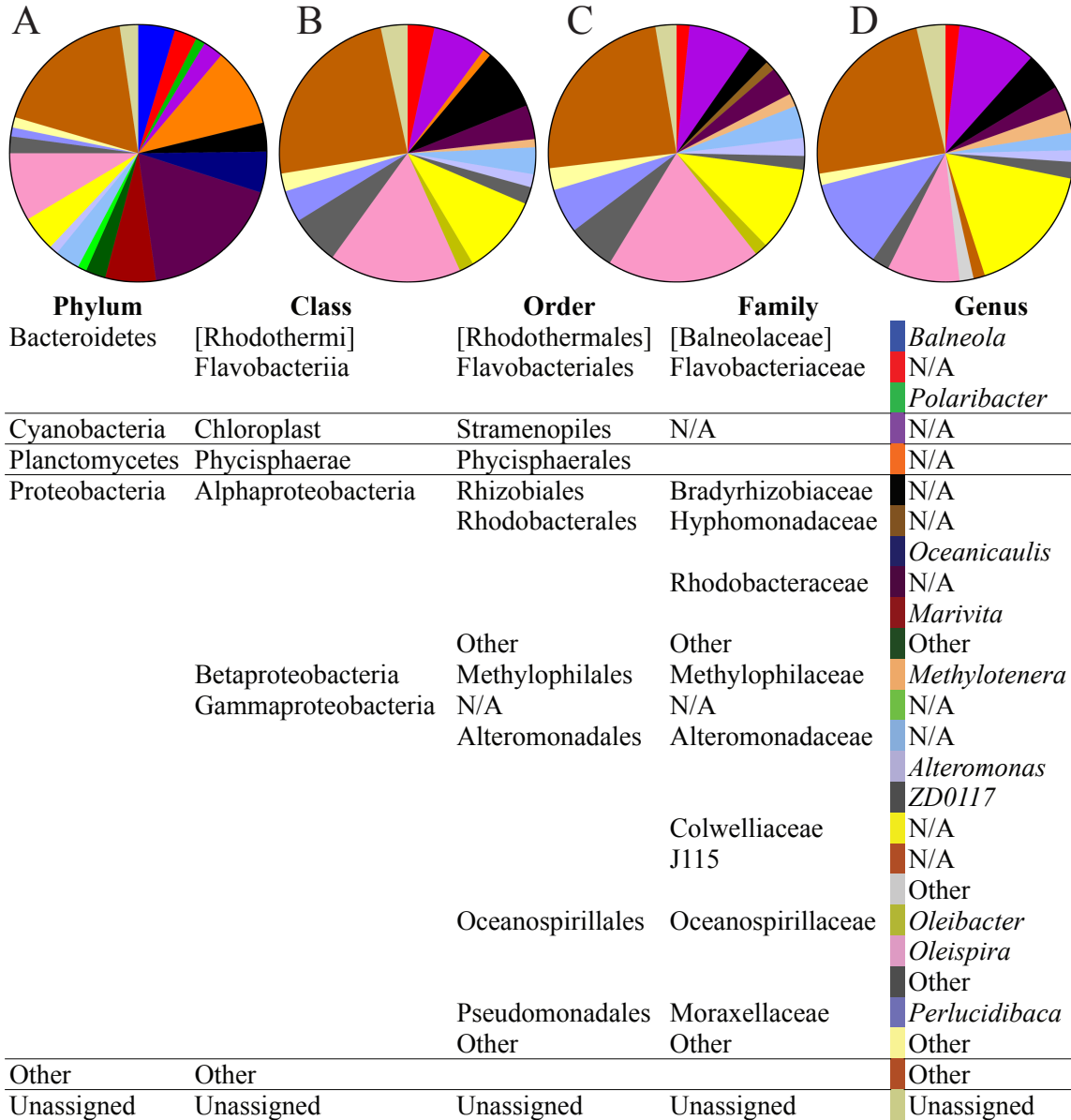
Supplementary Figure 10. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus purpuratus* larvae pre-expression of phenotypic plasticity. Genera associated with *S. purpuratus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4A.



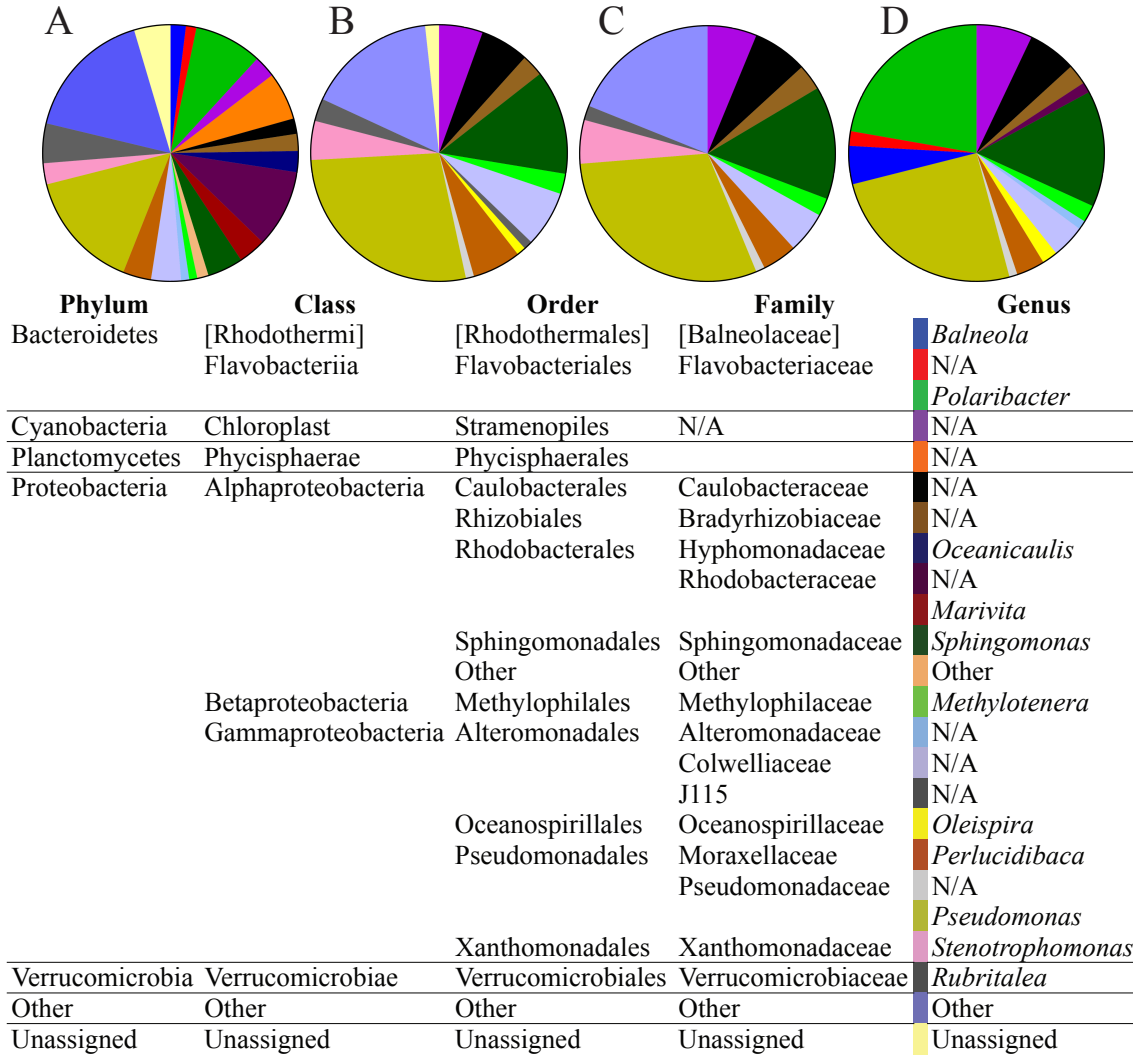
Supplementary Figure 11. Distribution of bacterial taxa associated with differentially fed *Mesocentrotus franciscanus* larvae pre-expression of phenotypic plasticity. Genera associated with *M. franciscanus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4B.



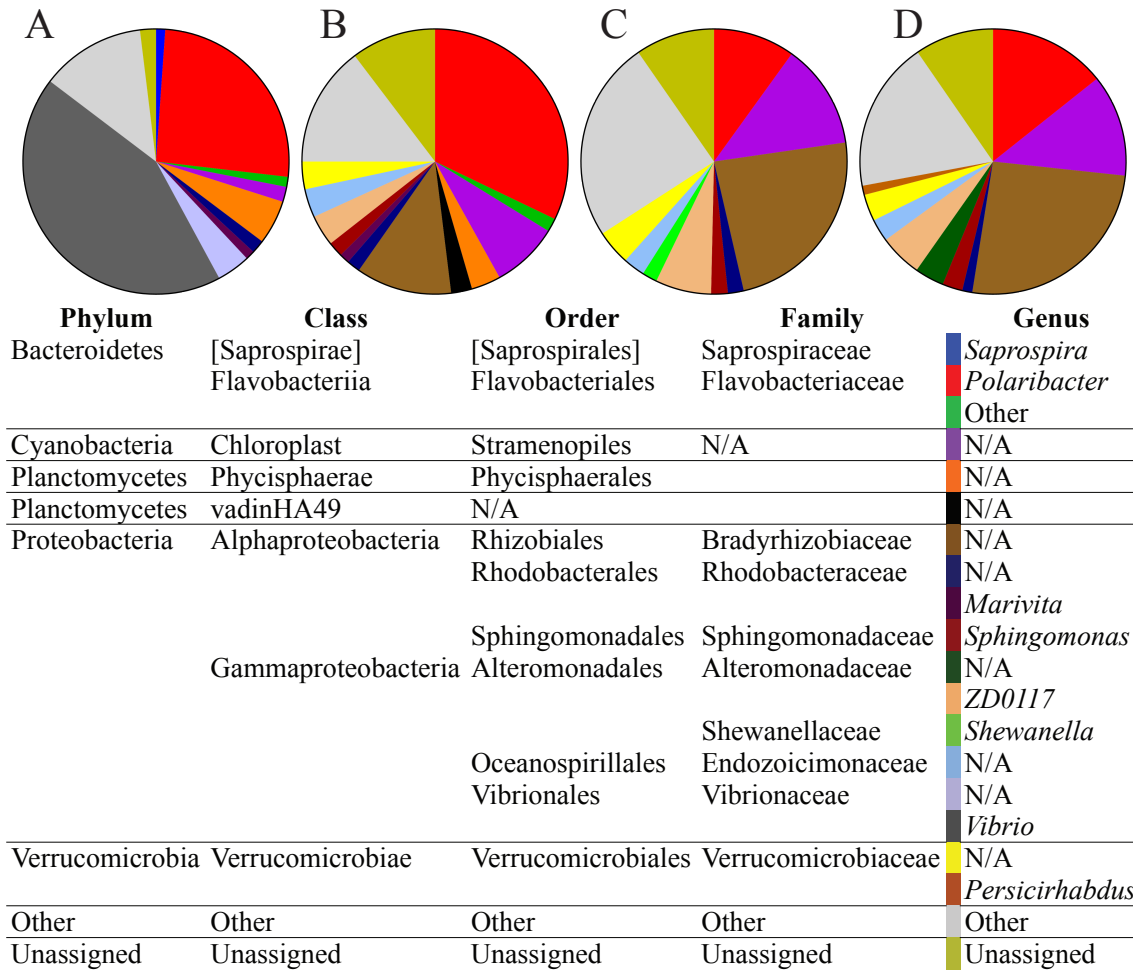
Supplementary Figure 12. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus droebachiensis* larvae pre-expression of phenotypic plasticity. Genera associated with *S. droebachiensis* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4C.



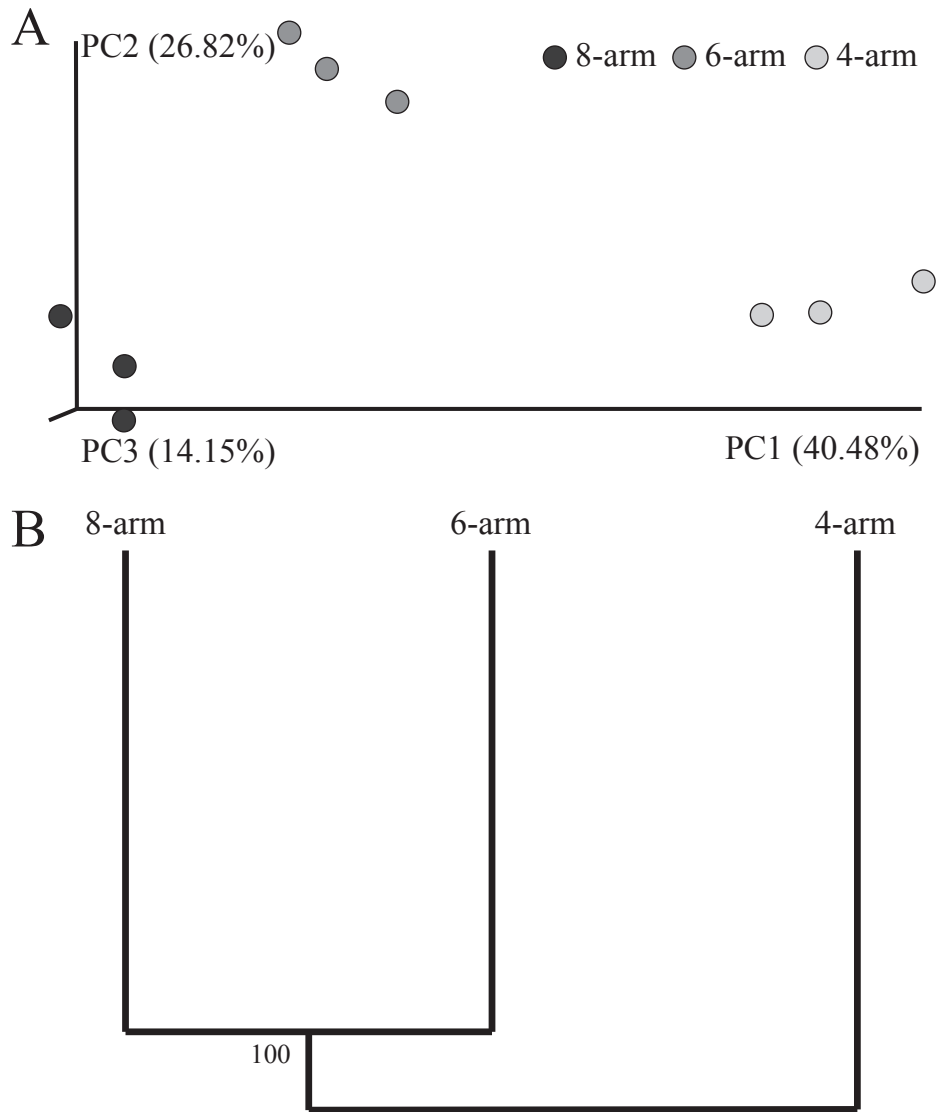
Supplementary Figure 13. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus purpuratus* larvae post-expression of phenotypic plasticity. Genera associated with *S. purpuratus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4D.



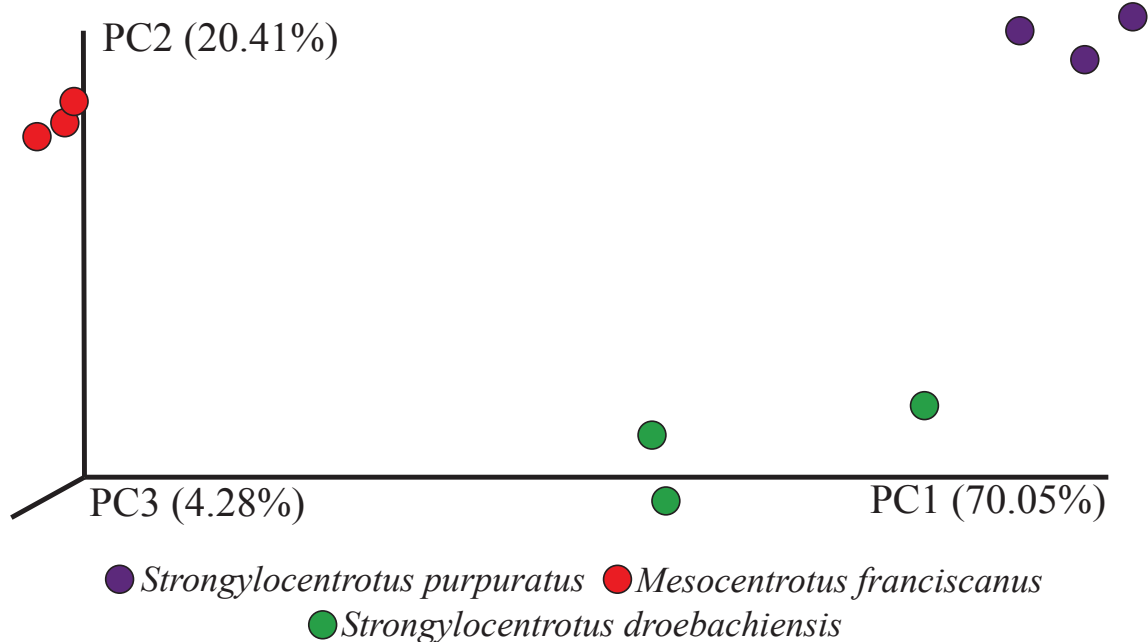
Supplementary Figure 14. Distribution of bacterial taxa associated with differentially fed *Mesocentrotus franciscanus* larvae post-expression of phenotypic plasticity. Genera associated with *M. franciscanus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4E.



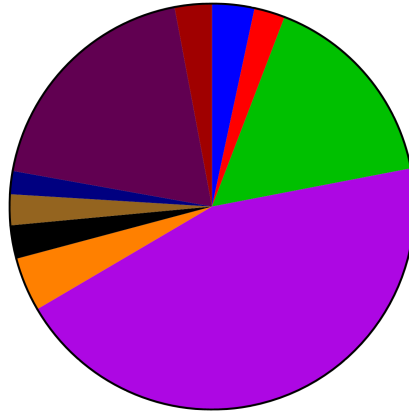
Supplementary Figure 15. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus droebachiensis* larvae post-expression of phenotypic plasticity. Genera associated with *S. droebachiensis* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL⁻¹ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Figure 4F.



Supplementary Figure 16. Developmental stage-specific microbial community for *Strongylocentrotus purpuratus* larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* larvae at the 4- (light grey), 6- (grey), and 8-arm (black) stages (A), with respective microbial dendrogram (B).

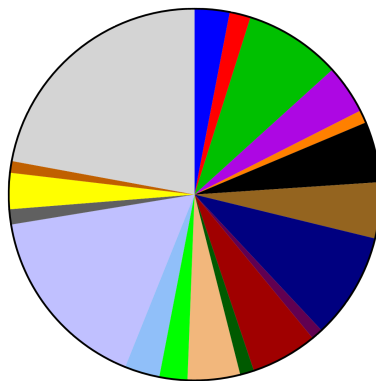


Supplementary Figure 17. Species-specific associated microbial communities for three species of echinoid larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) prior to feeding.



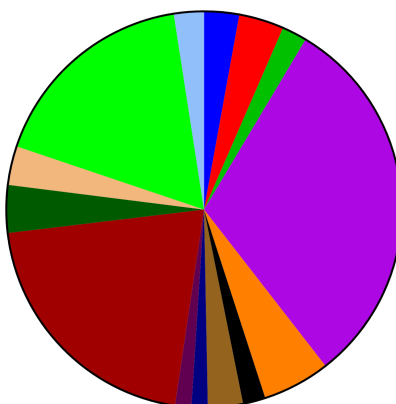
Phylum	Class	Order	Family	Genus
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	N/A
Cyanobacteria	Chloroplast	Stramenopiles	N/A	<i>Polaribacter</i>
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	N/A
		Rhodobacterales	Rhodobacteraceae	N/A
		Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>
	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	N/A
			Colwelliaceae	N/A
Other	Other	Other	Other	Other
Unassigned	Unassigned	Unassigned	Unassigned	Unassigned

Supplementary Figure 18. Bacterial taxa associated with prefeeding *Strongylocentrotus purpuratus* larvae. Genera associated with *S. purpuratus* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Supplementary Figure 18.



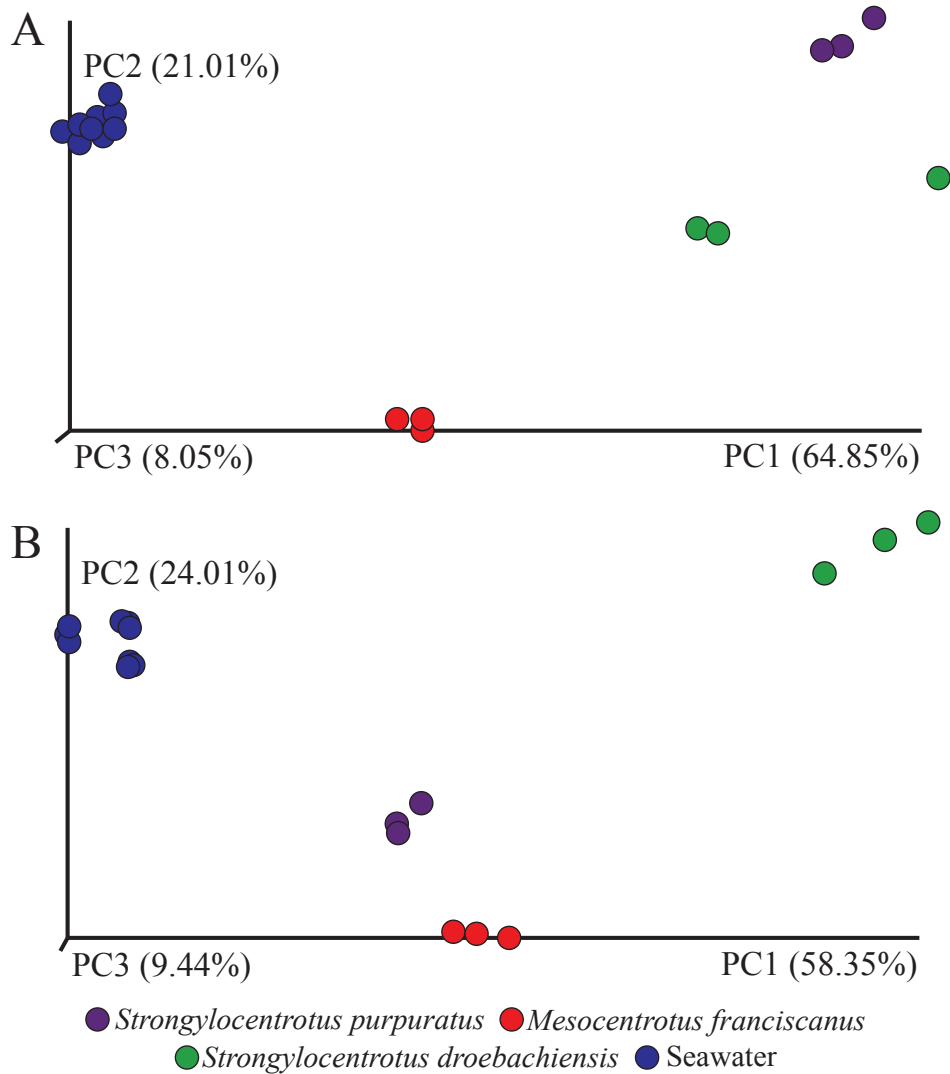
Phylum	Class	Order	Family	Genus	
Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	N/A	
	Flavobacteriia	Flavobacteriales	Cryomorphaceae	<i>Crocinitomix</i>	
			Flavobacteriaceae	N/A	
Cyanobacteria	Chloroplast	Stramenopiles	N/A	N/A	
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	N/A	
		Rhodobacterales	Rhodobacteraceae	N/A	
		Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	
	Gammaproteobacteria	Alteromonadales	Alteromonadales	N/A	N/A
				Alteromonadaceae	N/A
		Oceanospirillales	Colwelliaceae	N/A	
			Oceanospirillaceae	<i>Marinomonas</i>	
			Pseudomonadales	Pseudomonadaceae	<i>Oleispira</i>
		Vibrionales	Pseudoalteromonadaceae	<i>Pseudoalteromonas</i>	
		Xanthomonadales	Xanthomonadaceae	<i>Stenotrophomonas</i>	
	Other	Other	Other		
	Other	Other	Other	Other	

Supplementary Figure 19. Bacterial taxa associated with prefeeding *Mesocentrotus franciscanus* larvae. Genera associated with *M. franciscanus* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Supplementary Figure 18.

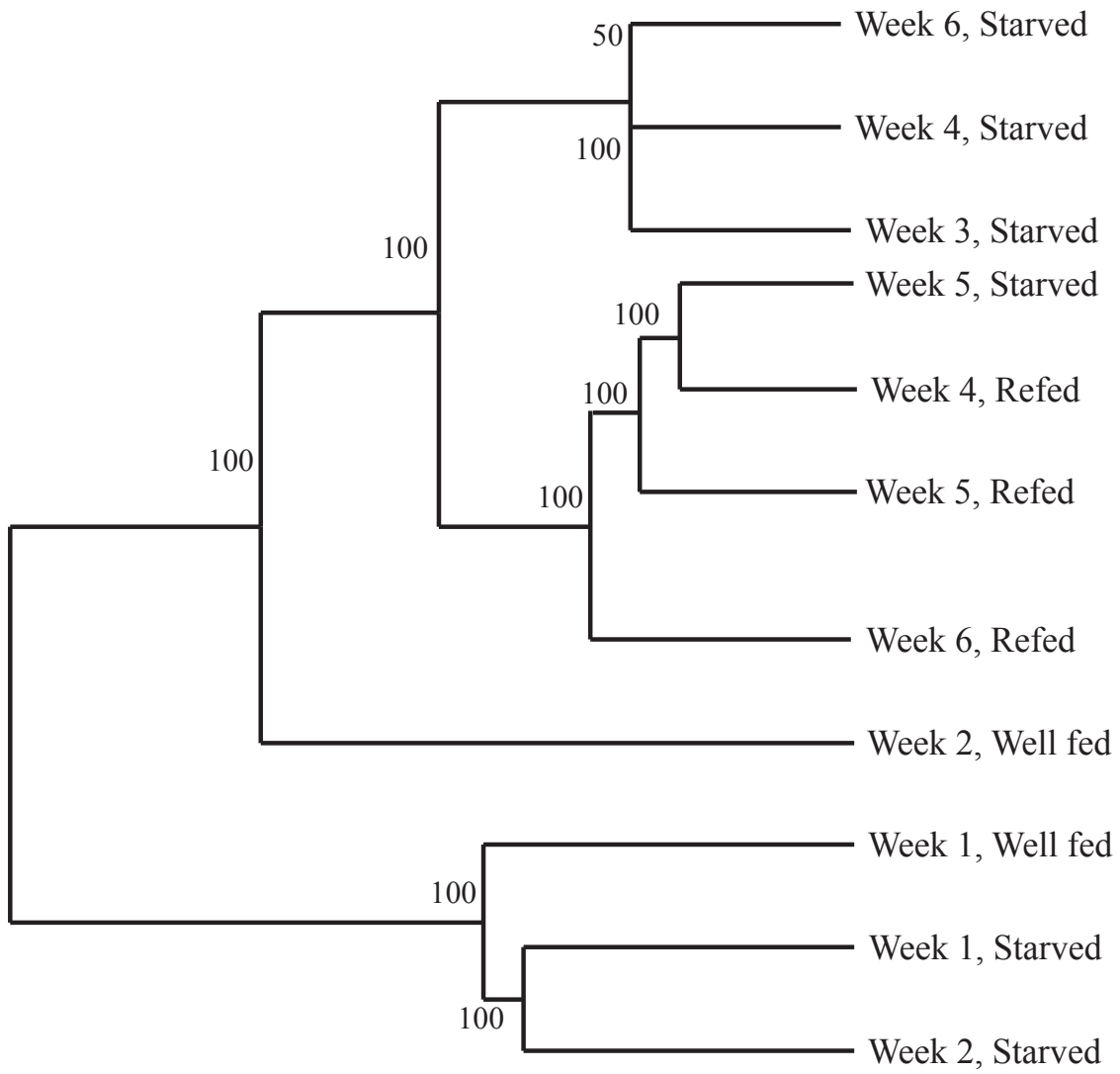


Phylum	Class	Order	Family	Genus
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	N/A
Cyanobacteria	Chloroplast	Stramenopiles	N/A	<i>Polaribacter</i>
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	N/A
		Rhodobacterales	Rhodobacteraceae	N/A
		Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>
	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	N/A
			Colwelliaceae	N/A
		Oceanospirillales	Oceanospirillaceae	N/A
		Vibrionales	Vibrionaceae	<i>Marinomonas</i>
				N/A
				<i>Vibrio</i>
Other	Other	Other	Other	Other
Unassigned	Unassigned	Unassigned	Unassigned	Unassigned

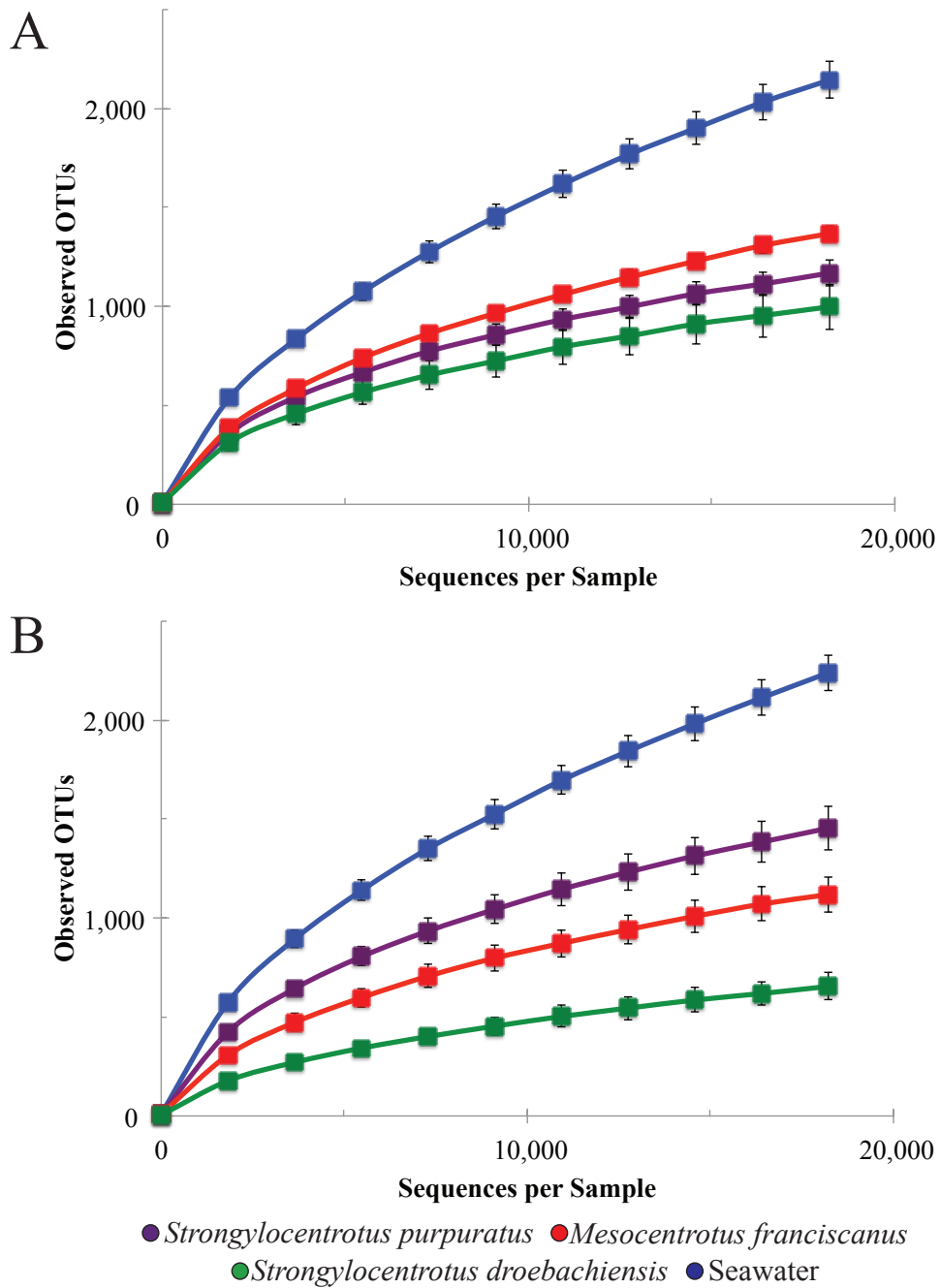
Supplementary Figure 20. Bacterial taxa associated with prefeeding *Strongylocentrotus droebachiensis* larvae. Genera associated with *S. droebachiensis* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as ‘other.’ Data correspond with Supplementary Figure 18.



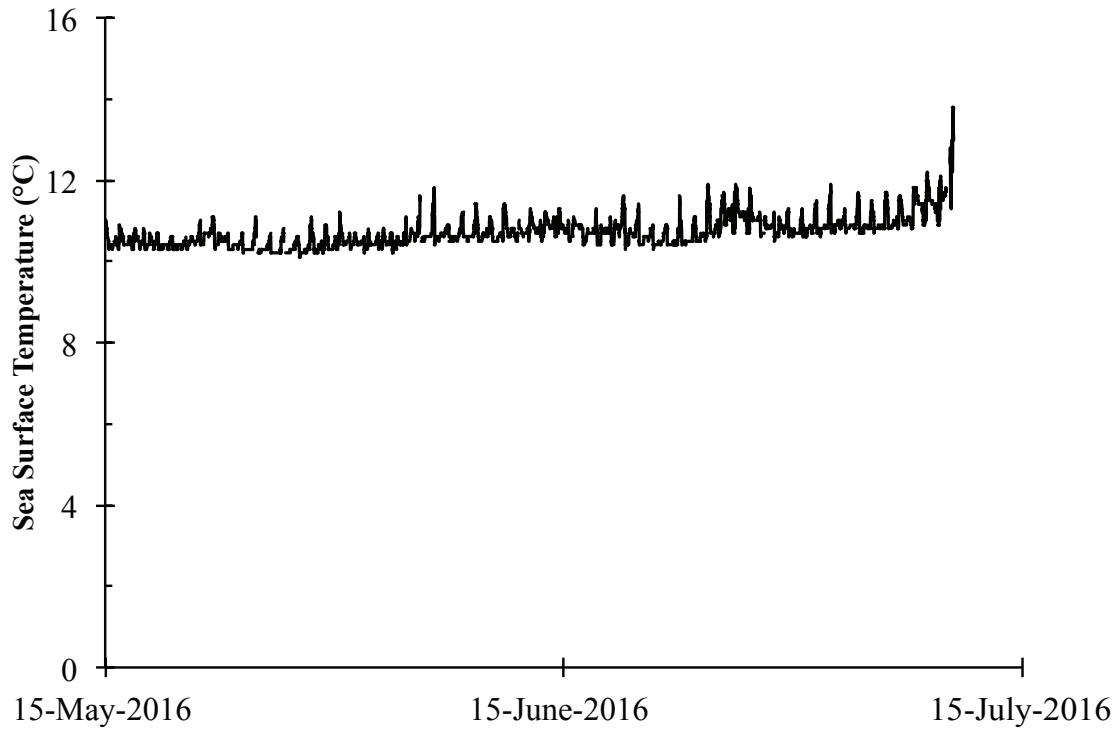
Supplementary Figure 21. Similarity of the associated microbial community for three species of echinoids at two larval stages. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) pre- (A) and post-feeding (B).



Supplementary Figure 22. Similarity between associated microbial communities for dietarily manipulated larvae. Microbial dendrograms of *Strongylocentrotus droebachiensis* larvae fed 10,000 cells•mL⁻¹ until metamorphosis and 0 cells•mL⁻¹ for three weeks, versus larvae fed 0 cells•mL⁻¹ for three weeks then switched to 10,000 cells•mL⁻¹ until metamorphosis. Data correspond with Figure 6.



Supplementary Figure 23. Alpha rarefaction curves for three species of echinoids at two larval stages and of the seawater. Alpha rarefaction curves for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) pre- (A) and post-feeding (B) based on rarefaction depth (*i.e.*, 18,225) used for all PCoA plots.



Supplementary Figure 24. *in situ* measurements of sea surface temperature at Friday Harbor, WA. Sea surface temperature at Friday Harbor, WA (NOAA station, FRDW1; 48°32'43" N 123°0'44" W) for the entirety of larval experimentation (15 May 2016 – 15 July 2016).

Supplementary Table 1. Larval developmental stages across dietary states ('--' designating no sample)

<i>Strongylocentrotus purpuratus</i>	Week 1	Week 2	Week 3	Week 4	
Diet = 10,000	4-arm	4-, 6-arm	8-arm	8-arm	
Diet = 1,000	4-arm	4-arm	6-arm	6-, 8-arm	
Diet = 100	4-arm	4-arm	4-arm	4-arm	
Diet = 0	4-arm	4-arm	4-arm	--	
<i>Mesocentrotus franciscanus</i>	Week 1	Week 2	Week 3	Week 4	
Diet = 10,000	4-arm	8-arm	8-arm	8-arm	
Diet = 1,000	4-arm	6-arm	6-arm	6-arm	
Diet = 100	4-arm	4-arm	4-arm	4-arm	
Diet = 0	4-arm	4-arm	4-arm	4-arm	
<i>Strongylocentrotus droebachiensis</i>	Week 1	Week 2	Week 3	Week 4	Week 5
Diet = 10,000	6-arm	8-arm	--	--	--
Diet = 1,000	6-arm	6-arm	6-arm	6-arm	--
Diet = 100	4-arm	4-arm	4-arm	4-arm	--
Diet = 0	4-arm	4-arm	4-arm	4-arm	4-arm
Diet = 0 to 10,000	--	--	--	6-arm	6-, 8-arm

Supplementary Table 2. Two-way ANOVA results¹ for larval morphology

<i>Strongylocentrotus purpuratus</i>	Effect	df	SS	F-ratio	p
	Week	2	0.522	29.197	< 0.0001
	Diet	2	2.962	165.562	< 0.0001
	Week*Diet	8	1.344	18.780	< 0.0001
	Week 1	3	0.301	11.199	< 0.0001
	Week 2	3	0.675	25.148	< 0.0001
	Week 3	3	1.959	73.004	< 0.0001
	Week 4	3	1.557	58.023	< 0.0001
	Diet = 10,000	3	0.450	16.761	< 0.0001
	Diet = 1,000	3	0.076	2.845	0.038
	Diet = 100	3	0.331	12.326	< 0.0001
	Diet = 0	3	1.092	40.714	< 0.0001
<i>Mesocentrotus franciscanus</i>	Effect	df	SS	F-ratio	p
	Week	2	1.115	28.748	< 0.0001
	Diet	2	9.714	250.410	< 0.0001
	Week*Diet	8	3.627	31.168	< 0.0001
	Week 1	3	0.178	4.586	0.0037
	Week 2	3	2.167	55.853	< 0.0001
	Week 3	3	3.480	89.700	< 0.0001
	Week 4	3	7.577	195.314	< 0.0001
	Diet = 10,000	3	0.864	22.285	< 0.0001
	Diet = 1,000	3	0.317	8.181	< 0.0001
	Diet = 100	3	0.793	20.453	< 0.0001
	Diet = 0	3	2.768	71.359	< 0.0001
<i>Strongylocentrotus droebachiensis</i>	Effect	df	SS	F-ratio	p
	Week	1	0.923	119.776	< 0.0001
	Diet	2	0.137	8.876	0.0002
	Week*Diet	7	0.591	10.956	< 0.0001
	Week 1	3	0.104	4.506	0.0042
	Week 2	3	0.636	27.481	< 0.0001
	Week 3	3	0.109	4.733	0.0031
	Week 4	3	0.507	21.917	< 0.0001
	Diet = 10,000	3	0.807	34.879	< 0.0001
	Diet = 1,000	3	0.295	12.745	< 0.0001
	Diet = 100	3	0.166	7.198	0.0001
	Diet = 0	3	0.738	31.897	< 0.0001

¹ df: degrees of freedom; SS: sum-of-squares; F-ratio: F-statistic

Supplementary Table 3. Two-way ANOVA results² for larval stomach volume

<i>Strongylocentrotus purpuratus</i>	Effect	df	SS	F-ratio	p
	Week	2	4.5E+15	84.754	< 0.0001
	Diet	2	3.3E+16	622.449	< 0.0001
	Week*Diet	8	3.0E+16	140.378	< 0.0001
	Week 1	3	4.0E+14	4.653	0.003
	Week 2	3	3.0E+13	0.335	0.800
	Week 3	3	4.0E+14	5.198	0.002
	Week 4	3	5.0E+16	601.434	< 0.0001
	Diet = 10,000	3	8.0E+13	1.002	0.392
	Diet = 1,000	3	3.0E+14	4.334	0.005
	Diet = 100	3	2.0E+16	247.235	< 0.0001
	Diet = 0	3	5.0E+16	574.317	< 0.0001
<i>Mesocentrotus franciscanus</i>	Effect	df	SS	F-ratio	p
	Week	3	9.4E+16	103.686	< 0.0001
	Diet	3	3.1E+17	343.181	< 0.0001
	Week*Diet	9	2.5E+17	92.261	< 0.0001
	Week 1	3	2.0E+14	0.188	0.9045
	Week 2	3	9.0E+13	0.095	0.9626
	Week 3	3	2.0E+15	2.316	0.0758
	Week 4	3	3.0E+17	377.871	< 0.0001
	Diet = 10,000	3	2.0E+15	1.657	0.1763
	Diet = 1,000	3	3.0E+15	3.187	0.0241
	Diet = 100	3	1.0E+17	164.025	< 0.0001
	Diet = 0	3	4.0E+17	451.096	< 0.0001
<i>Strongylocentrotus droebachiensis</i>	Effect	df	SS	F-ratio	p
	Week	1	1.7E+15	76.456	< 0.0001
	Diet	2	2.0E+14	4.618	0.0107
	Week*Diet	7	2.9E+15	19.198	< 0.0001
	Week 1	3	2.0E+13	0.344	0.7934
	Week 2	3	3.0E+13	0.393	0.7585
	Week 3	3	1.0E+14	1.791	0.1493
	Week 4	3	5.0E+15	69.420	< 0.0001
	Diet = 10,000	3	3.0E+15	42.776	< 0.0001
	Diet = 1,000	3	2.0E+16	254.487	< 0.0001
	Diet = 100	3	1.0E+15	15.247	< 0.0001
	Diet = 0	3	6.0E+14	8.374	< 0.0001

² df: degrees of freedom; SS: sum-of-squares; F-ratio: F-statistic

Supplementary Table 4. One-way ANOVA results³ for trade-off in associating with proteobacteria across phenotypes

<i>Strongylocentrotus purpuratus</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	α -proteobacteria	Phenotype	2	0.059	19.524	0.002
	γ -proteobacteria	Phenotype	2	0.070	17.271	0.003
<i>Mesocentrotus franciscanus</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	α -proteobacteria	Phenotype	2	0.008	12.548	0.011
	γ -proteobacteria	Phenotype	2	0.007	19.194	0.005
<i>Strongylocentrotus droebachiensis</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	α -proteobacteria	Phenotype	2	0.013	2.964	0.142
	γ -proteobacteria	Phenotype	2	0.013	0.532	0.618

³ *df*: degrees of freedom; SS: sum-of-squares; *F*-ratio: F-statistic

Supplementary Table 5. ANOVA results⁴ for differentially-associated taxa across phenotypes

<i>Strongylocentrotus purpuratus</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	Bradyrhizobiaceae	Phenotype	2	0.054	30.575	0.001
	Colwelliaceae	Phenotype	2	0.038	15.692	0.004
	<i>Oleispira</i>	Phenotype	2	0.073	17.583	0.003
	<i>Pseudomonas</i>	Phenotype	2	0.015	24.554	0.001
<i>Mesocentrotus franciscanus</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	Colwelliaceae	Phenotype	2	0.003	15.373	0.007
	<i>Sphingomonas</i>	Phenotype	2	0.002	5.472	0.055
<i>Strongylocentrotus droebachiensis</i>		Effect	<i>df</i>	SS	<i>F</i> -ratio	<i>p</i>
	Flavobacteriaceae	Phenotype	2	0.020	1.596	0.291
	Bradyrhizobiaceae	Phenotype	2	0.004	1.260	0.360
	<i>Polaribacter</i>	Phenotype	2	0.020	1.753	0.265

⁴ *df*: degrees of freedom; SS: sum-of-squares; *F*-ratio: F-statistic

Supplementary Note 1. Informatic pipeline for processing 16S rRNA datasets from MiSeq

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 using "filter_otus_from_otu_table.py"
- 10) Filter 'o__Cryptophyta' using "filter_taxa_from_otu_table.py"
- 11) Determine rarefaction depth using "biom summarize-table"
- 12) Split filtered .biom table to test specific hypotheses using "split_otu_table.py"
- 13) Comparison of microbial communities via PCoA using "jackknifed_beta_diversity.py"
- 14) Statistical comparisons of rarefied matrices using "compare_categories.py"
- 15) Compare taxa from '13' using "summarize_taxa_through_plots.py"