

Microbiome of Pacific Whiteleg shrimp reveals differential bacterial community composition between wild, aquacultured and AHPND/EMS outbreak conditions

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Supplementary Fig. S1 Reads percentage distribution in each 16S rRNA sequenced hypervariable region for all samples

Supplementary Fig. S2 Reads percentage coverage across all sequenced 16S rRNA hypervariable regions for all groups.

Supplementary Fig. S3 Venn diagrams between assigned OTUs for every hypervariable region for a) intestine, b) hepatopancreas, c) pond sediment.

Supplementary Fig. S4 Percentage of unknown OTU reported in GreenGenes database. a) Percentage of unknown reads for all the sequenced samples. b) Average percentage of unknown reads and standard deviation bars for all the sequenced groups.

Supplementary Fig. S5 Alpha diversity rarefaction curves. a) Shannon index until the library with the most reads. b) Observed OTUs for all the 24 samples. c) Observed OTUs for all the sequenced samples grouped by treatment.

Supplementary Fig. S6 PCoA using weighted UniFrac distances. a) tagged as wild type and cultured and b) samples tagged by shrimp organ or sediment.

Supplementary Fig. S7 UPGMA tree of weighted UniFrac distances. Node value represents the jackknife support using 1,000 replicates.

Supplementary Fig. S8 LDA of the KEGG pathways at the level three between wild type and cultured samples. a) intestine and b) hepatopancreas samples.

Supplementary Fig. S9. Venn diagrams showing the shared OTUs. a) wt vs cultured healthy shrimp intestine. b) wt vs cultured healthy shrimp hepatopancreas. c) healthy intestine vs hepatopancreas vs pond sediment.

Supplementary Fig. S10 PCoA of UniFrac distances. a) unweighted and b) weighted. The samples are tagged as healthy and diseased.

Supplementary Fig. S11 UPGMA tree of a) unweighted and b) weighted UniFrac distances. The blue box highlights the diseased samples. Node value represents the jackknife support using 1,000 replicates.

Supplementary Fig. S12 Venn diagrams showing shared OTUs between healthy and diseased samples. a) healthy vs diseased intestine. b) diseased intestine vs diseased hepatopancreas vs diseased pond sediment. c) healthy vs diseased hepatopancreas. d) healthy vs diseased pond sediment.

Supplementary Fig. S13 LDA of the KEGG pathways at the level three between healthy and diseased cultured samples. a) hepatopancreas, b) intestine and c) pond sediment samples.

Supplementary Fig. S14 PCoA using unweighted UniFrac distances without 7 age discriminatory OTUs. a) tagged as wild type and cultured and b) tagged by shrimp organ or sediment

Supplementary Fig. S15 Photographs of a) healthy cultured shrimp. b) diseased cultured shrimp. The diseased shrimps presented the phenotypic characteristics of the APHND/EMS disease such as: lethargy, empty intestine and pale and white aqueous hepatopancreas. c) wild type shrimp.

Supplementary Table S1. Relative abundance of OTUs with an abundance >0.1% at phylum level.

Supplementary Table S2. Average relative abundance of top 5 OTUs with an abundance >0.1% at family and genus level.

Supplementary Table S3. Spearman correlation test between group triplicates.

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Supplementary Table S5. Good's Coverage of sequenced samples (10,000 iterations at 2,078 sequence depth).

Supplementary Table S6. Linear Discriminant Analysis of enriched taxonomies in wt and cultured intestine samples using LEfSe.

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Supplementary Table S10. Relative frequency of unique genera and species in sequenced wt and cultured samples.

Supplementary Table S11. Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased intestine using LefSe.

Supplementary Table S12. Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased hepatopancreas using LefSe.

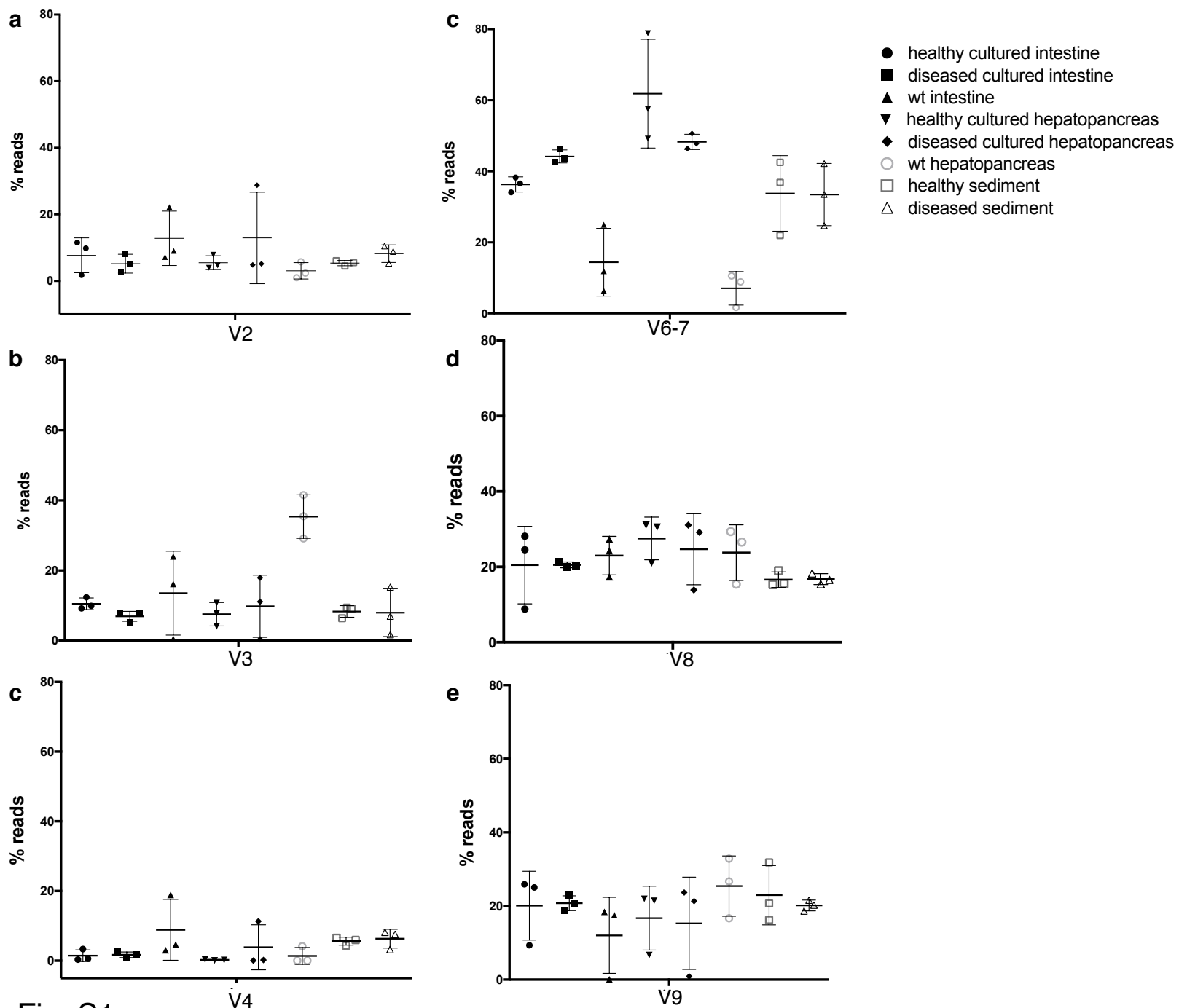
Supplementary Table S13. Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased sediment using LefSe.

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples.

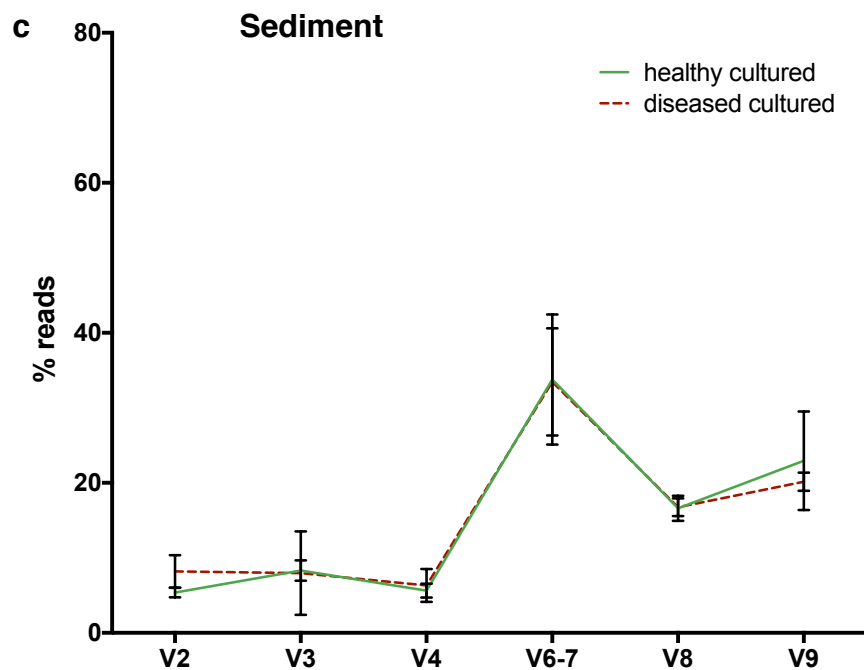
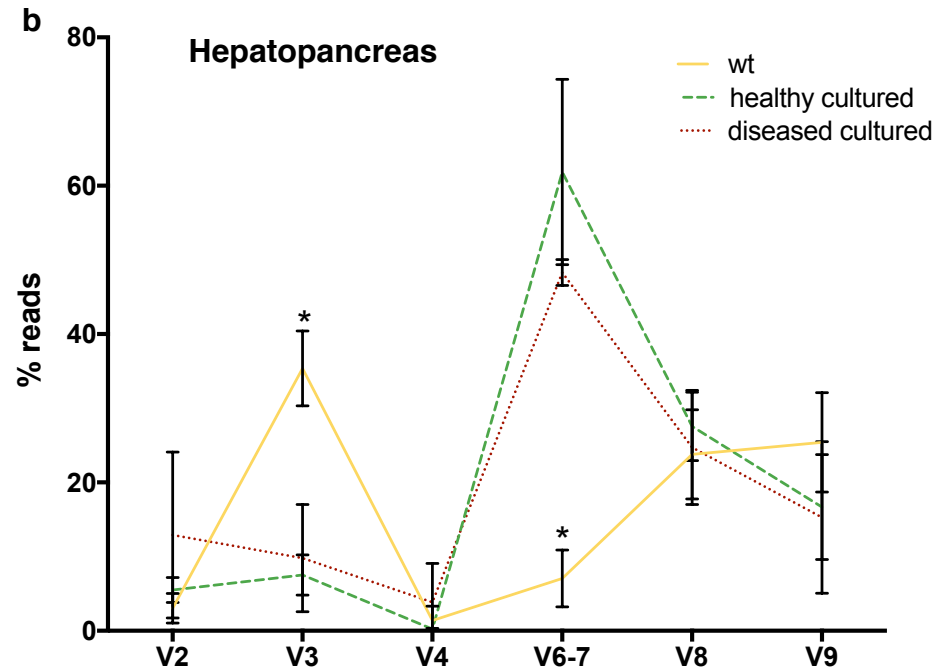
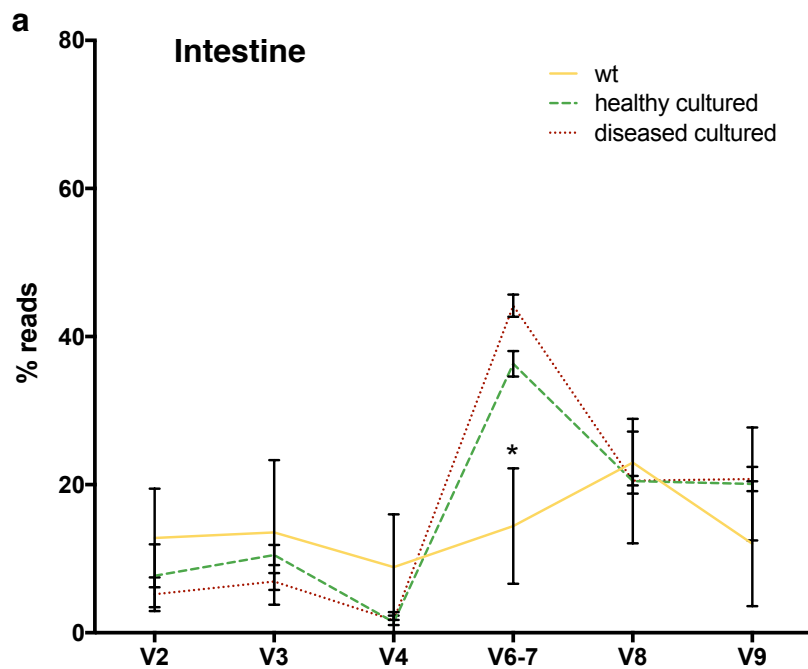
Supplementary Table S15. AP3 PCR diagnostic results.

Supplementary Table S16. Relative abundance of 7 age discriminating OTUs present in the sequenced samples.

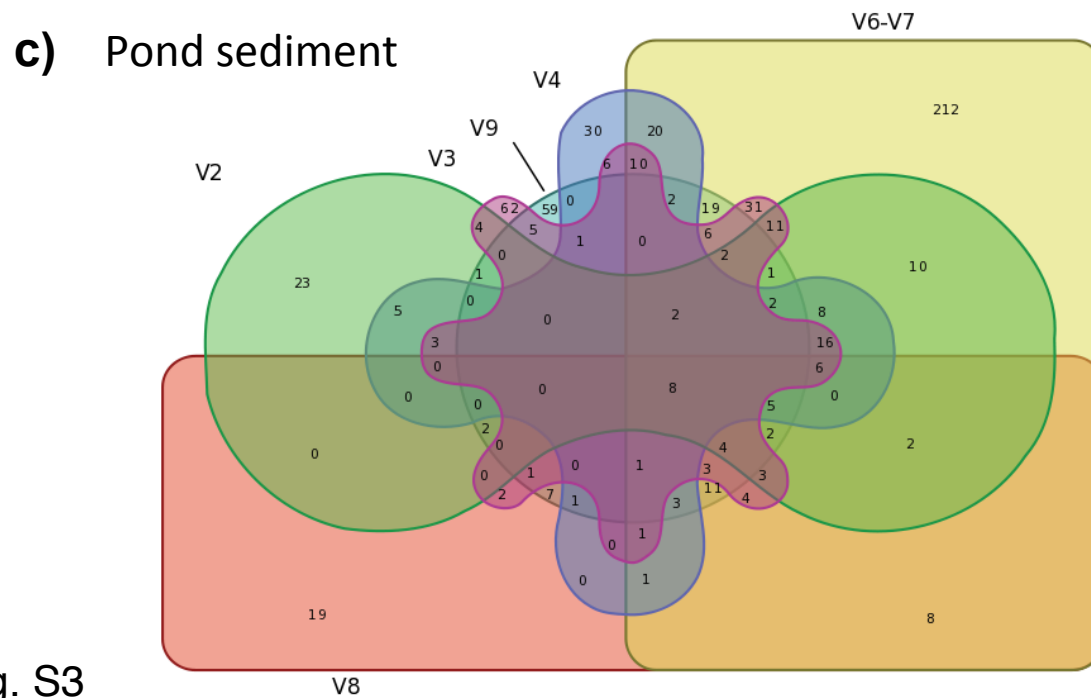
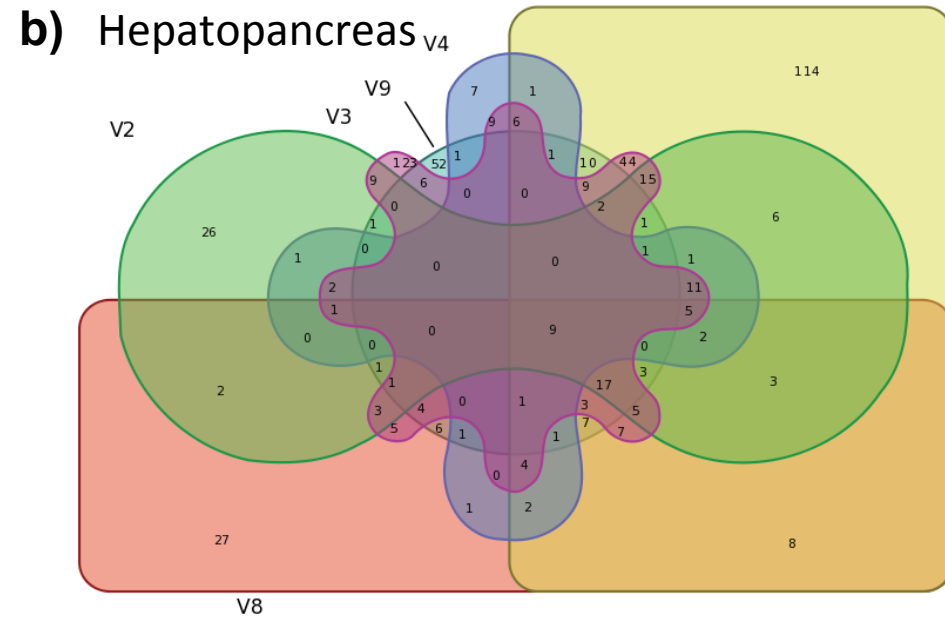
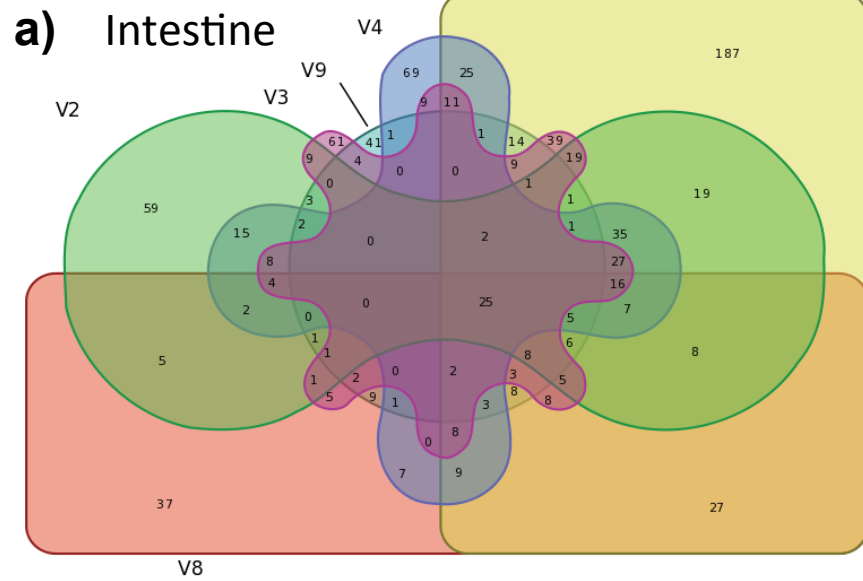
Supplementary Table S17. Weight of the wt and cultured shrimps used for the extraction of sequenced intestines and hepatopancreas.



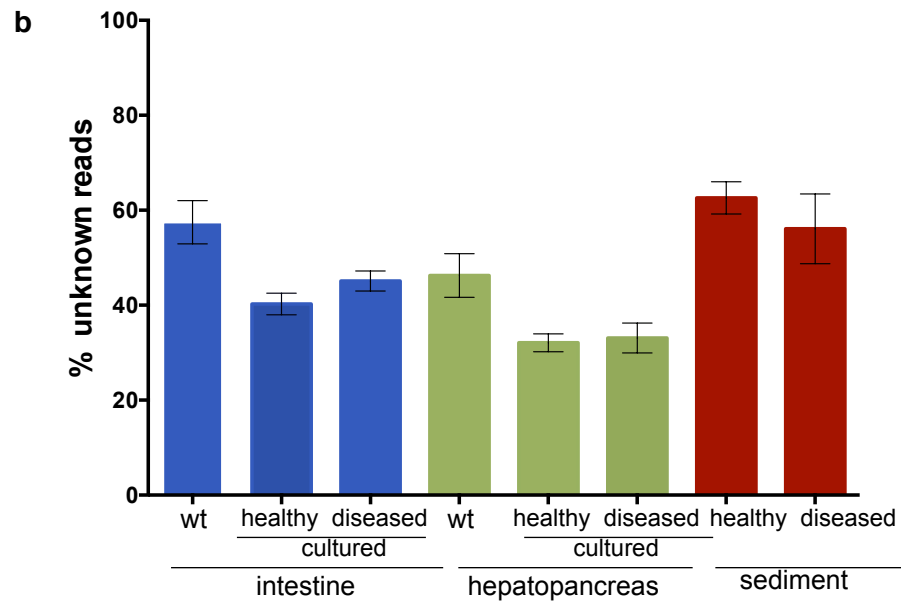
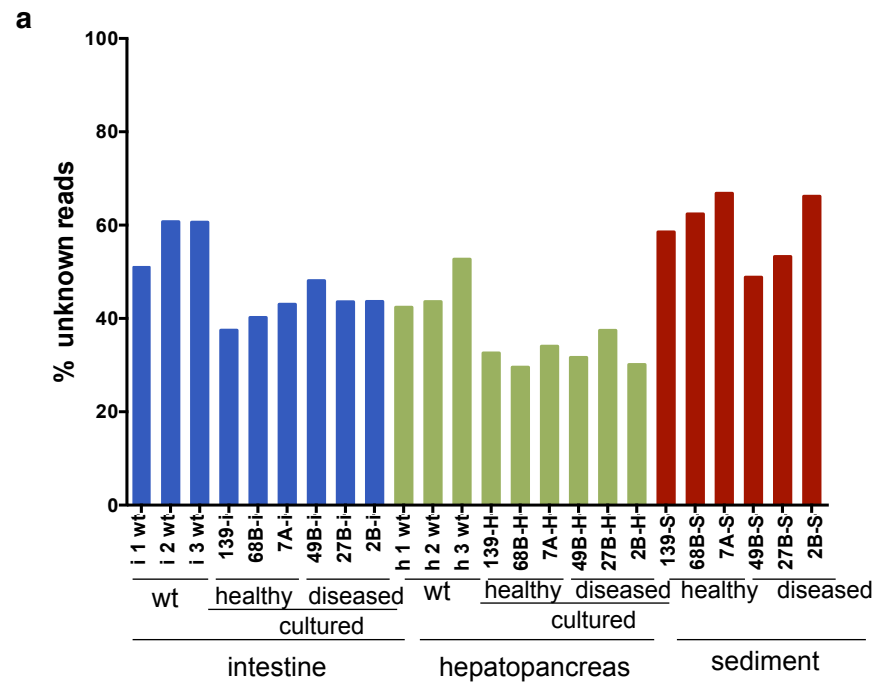
Supplementary Fig. S1



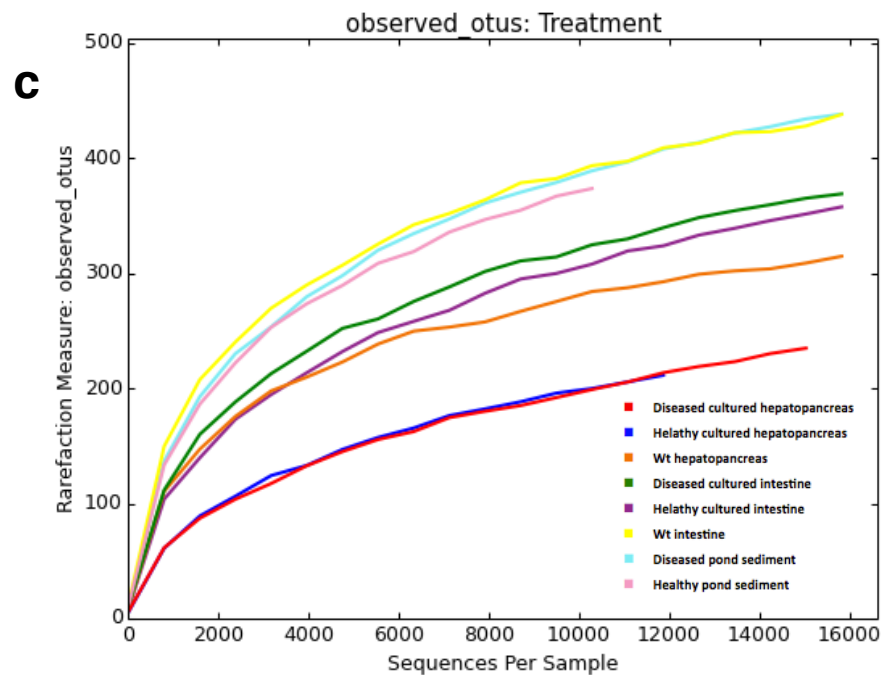
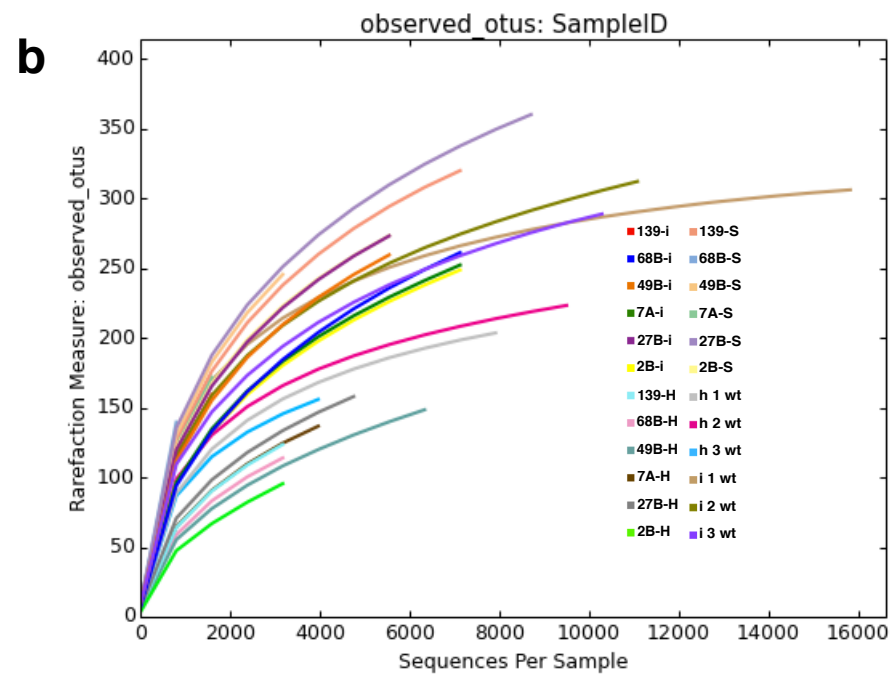
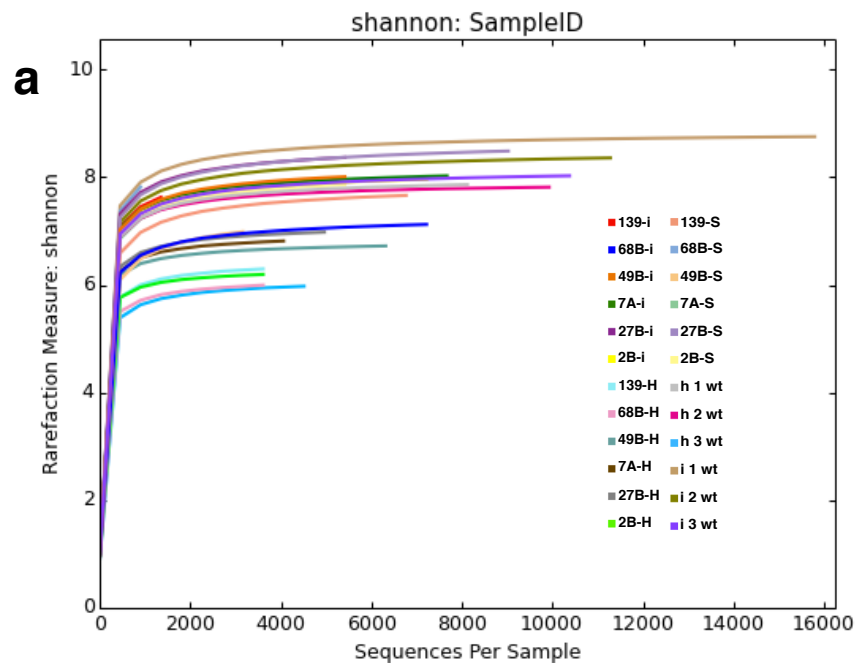
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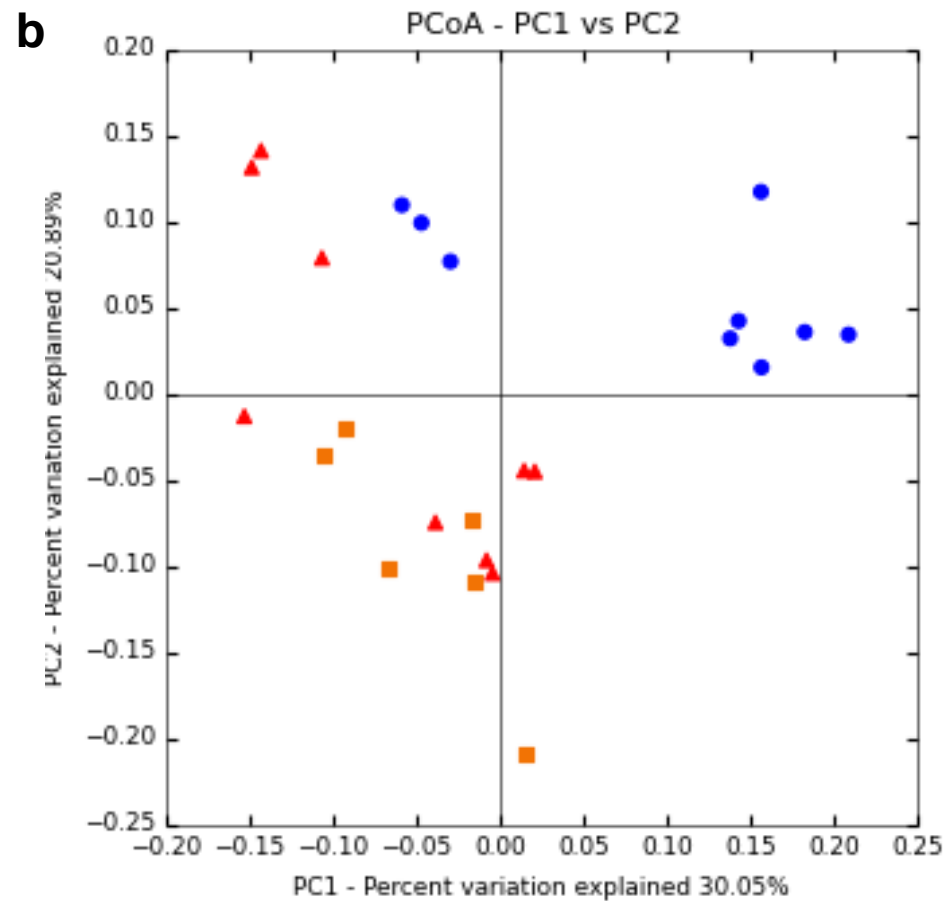
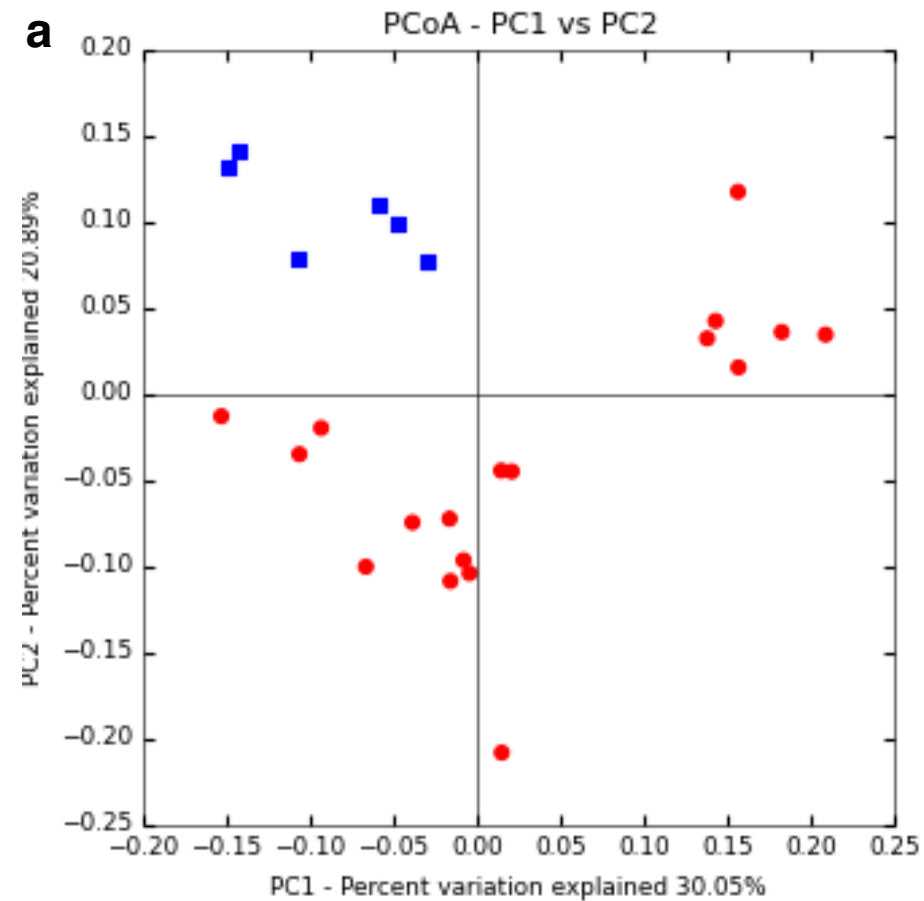
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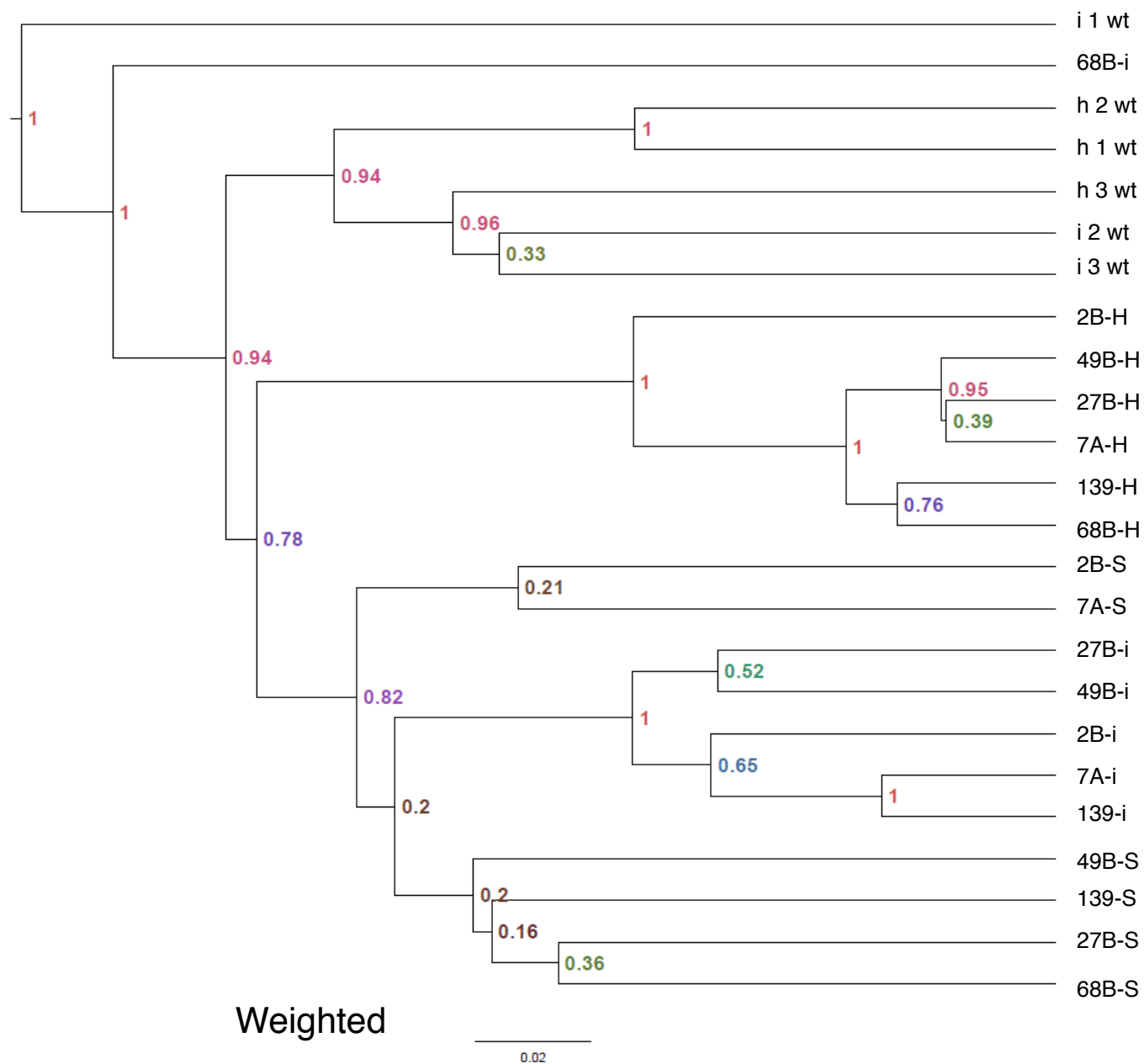
Supplementary Fig. S4



Supplementary Fig. S5

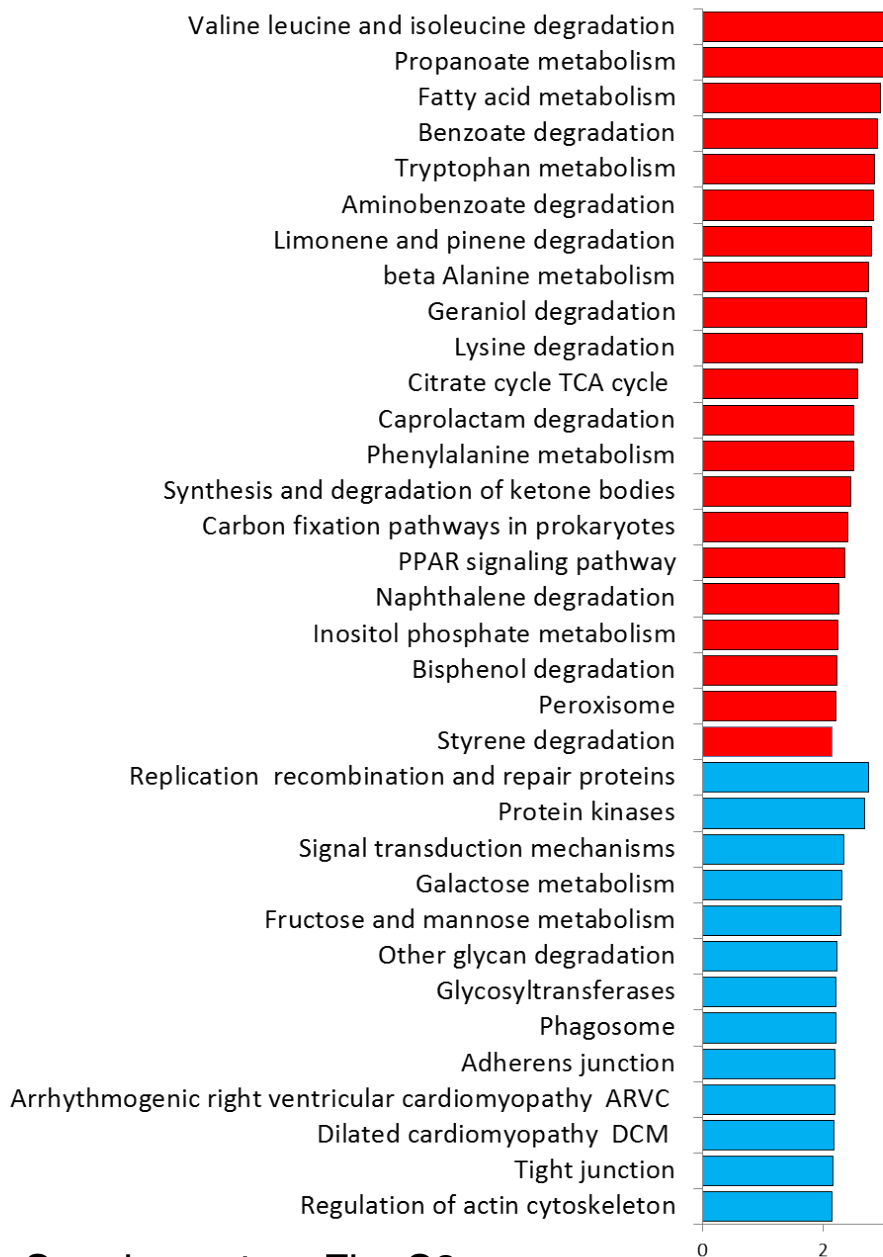


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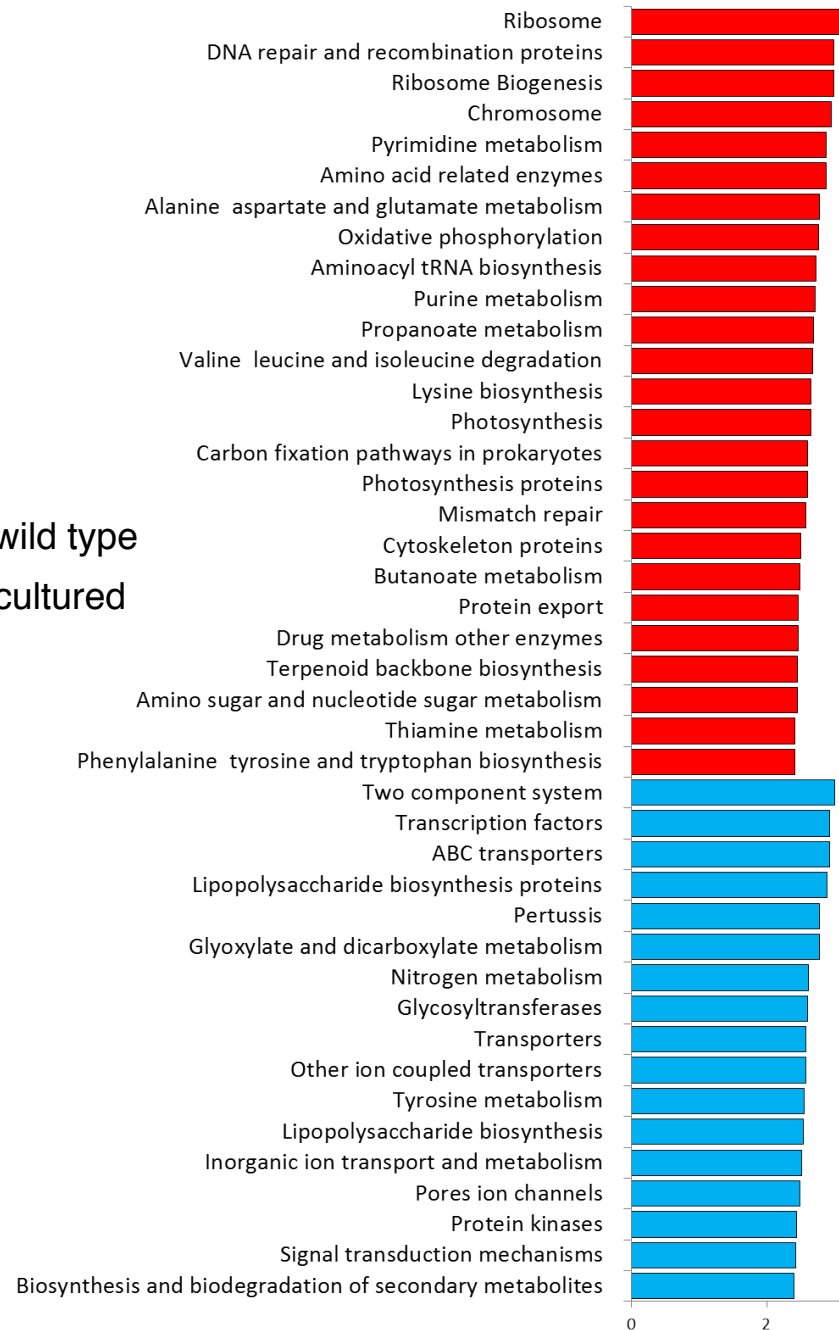


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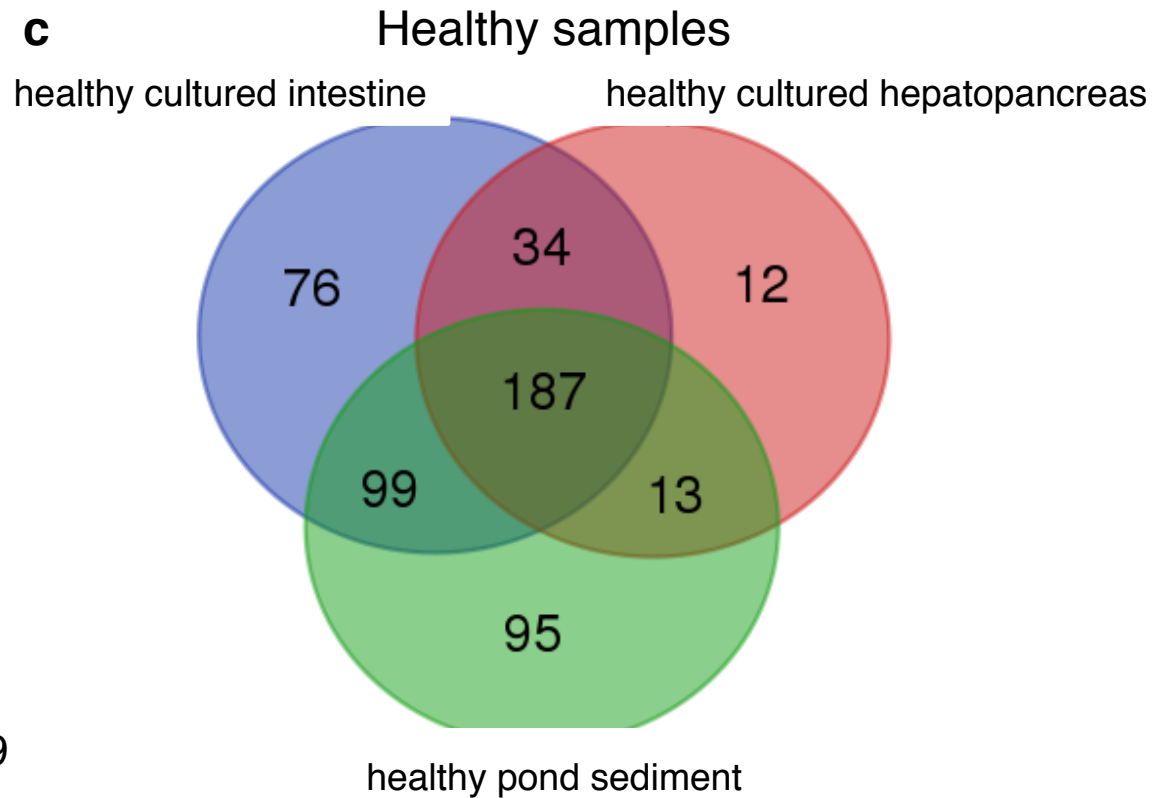
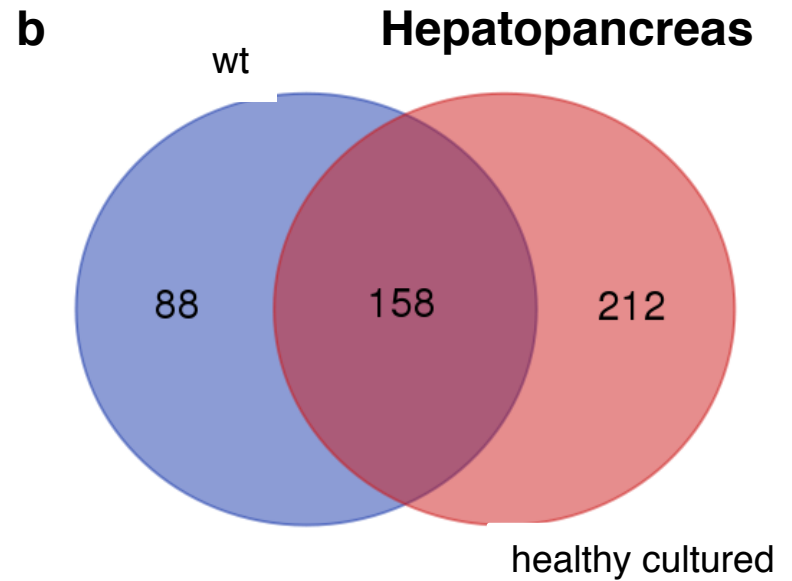
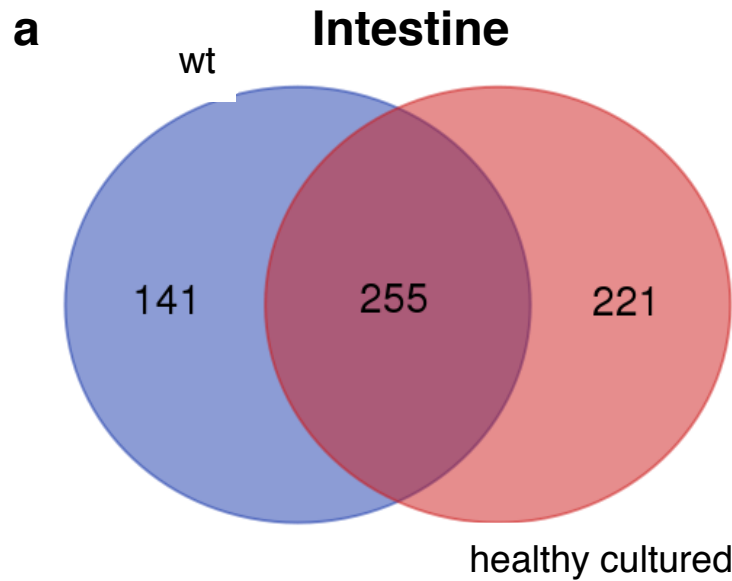
a Intestine



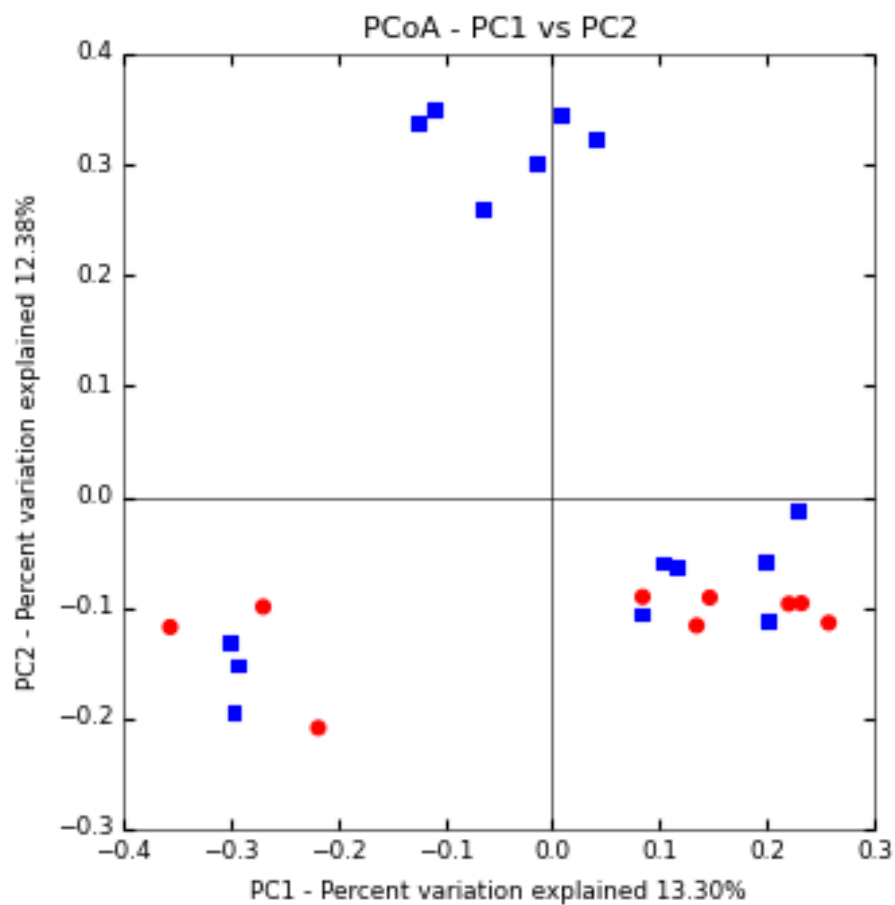
b Hepatopancreas



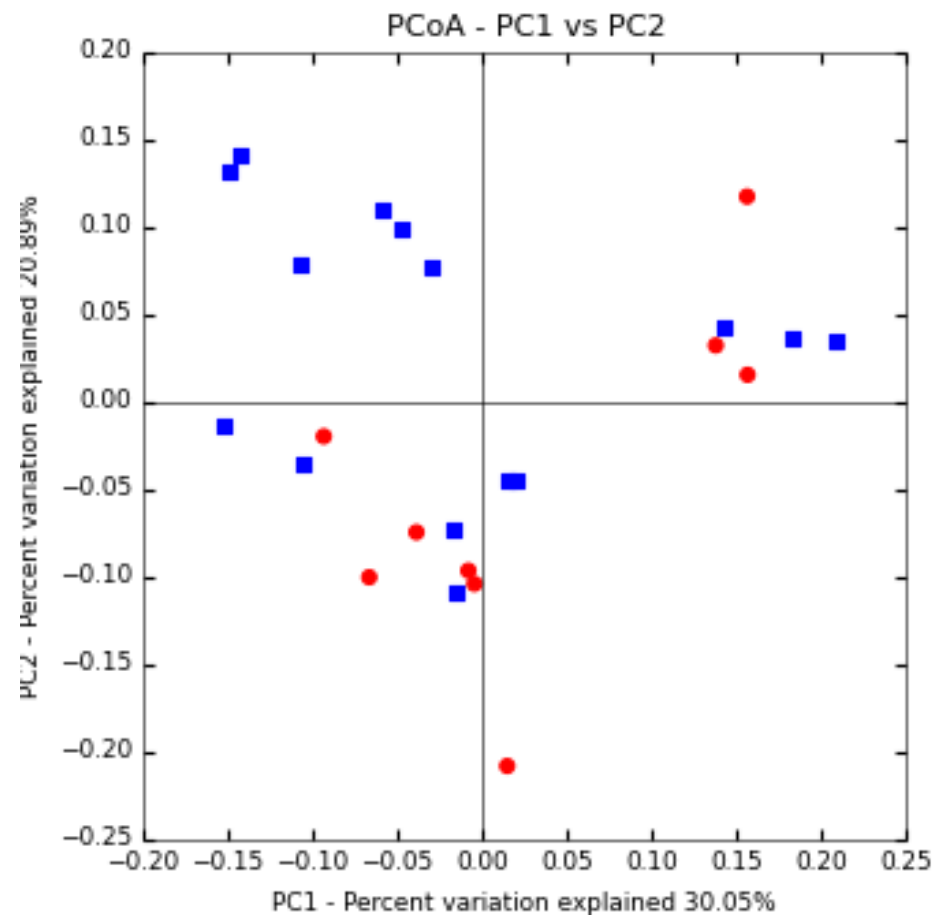
Supplementary Fig. S8



Supplementary Fig. S9

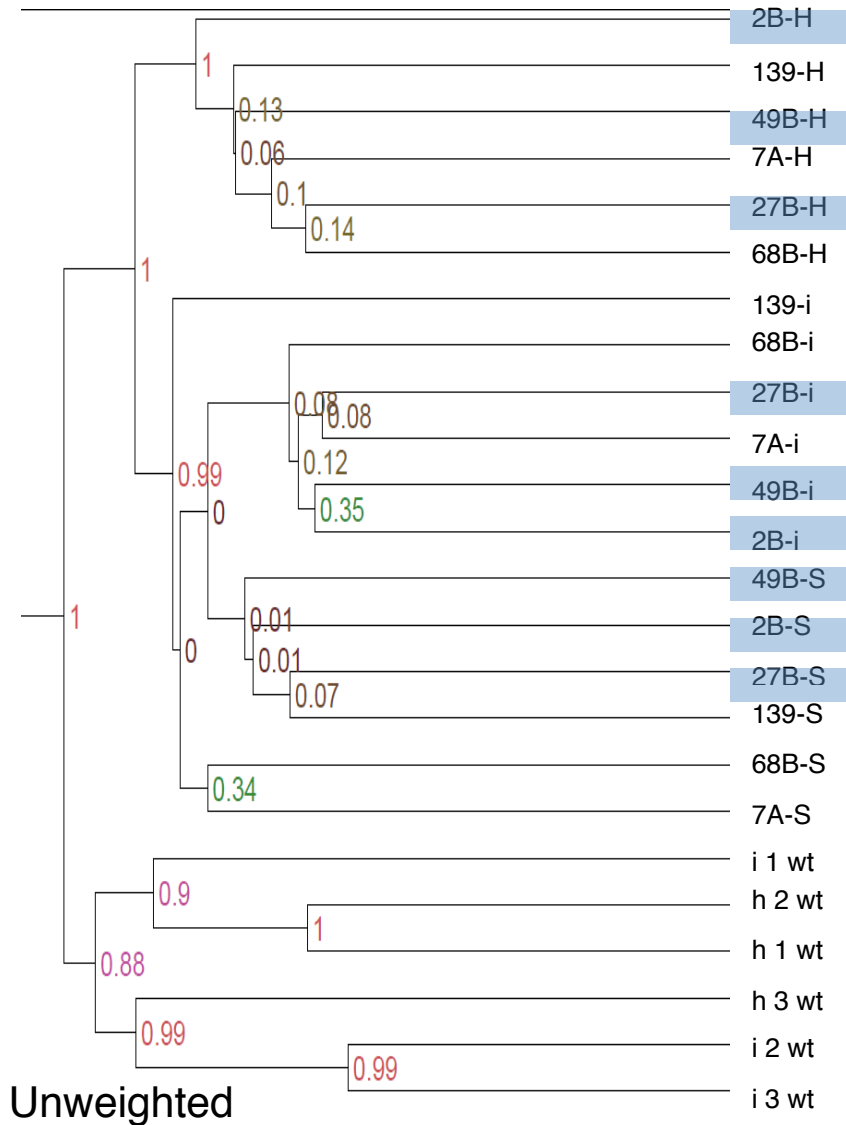
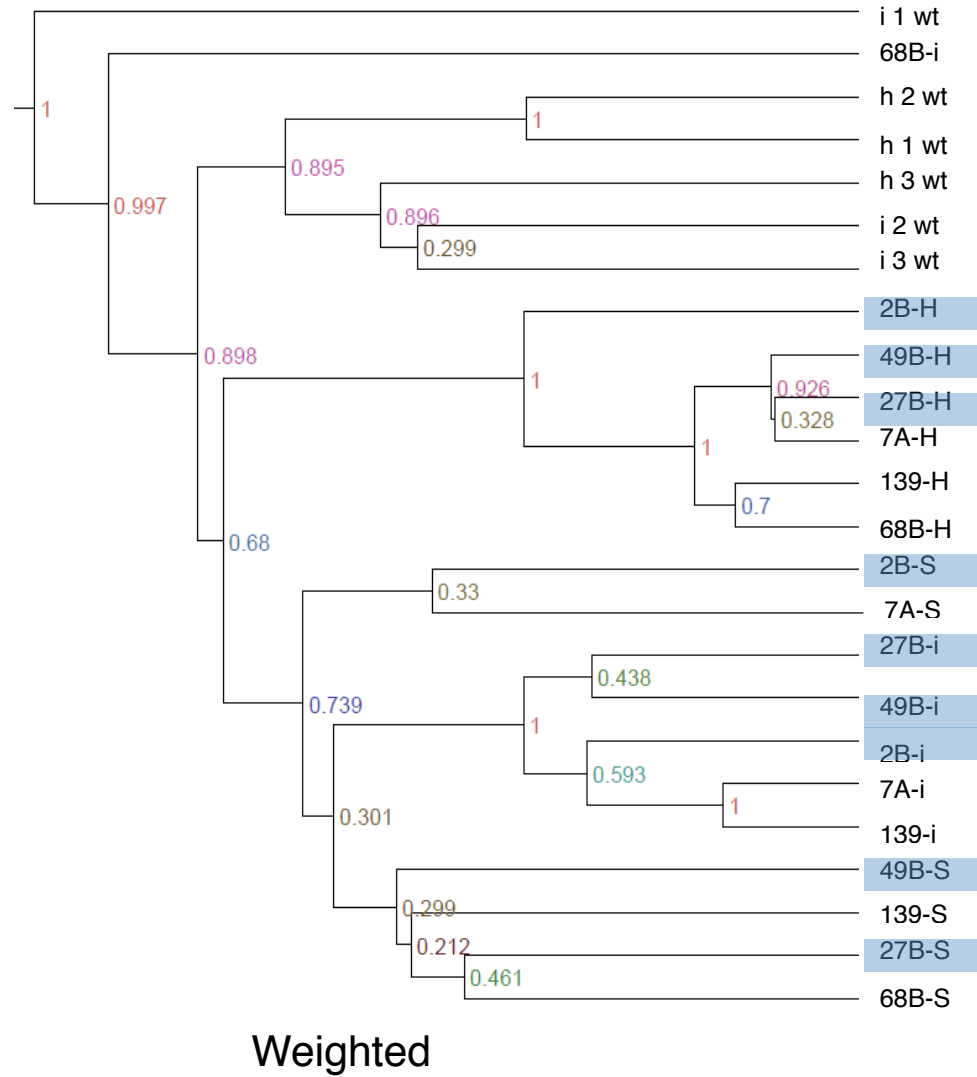
a

Unweighted

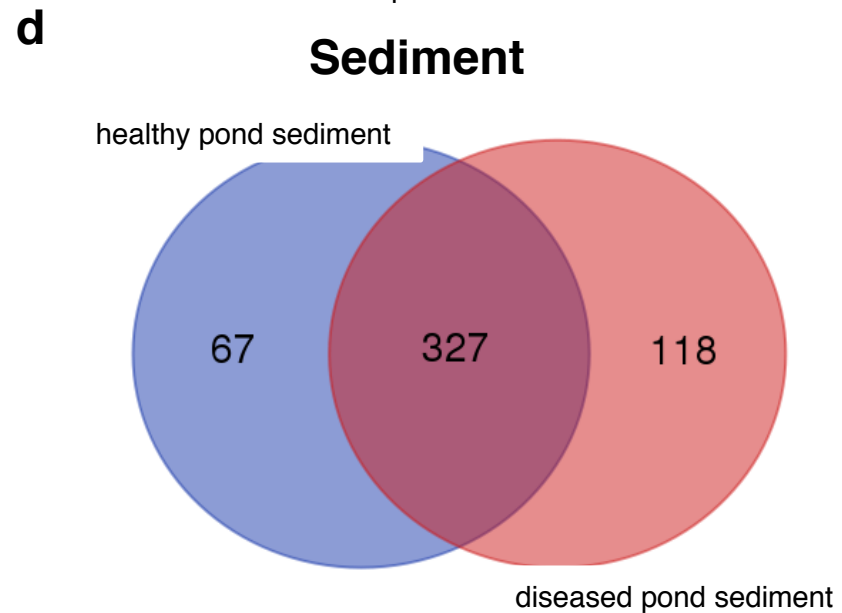
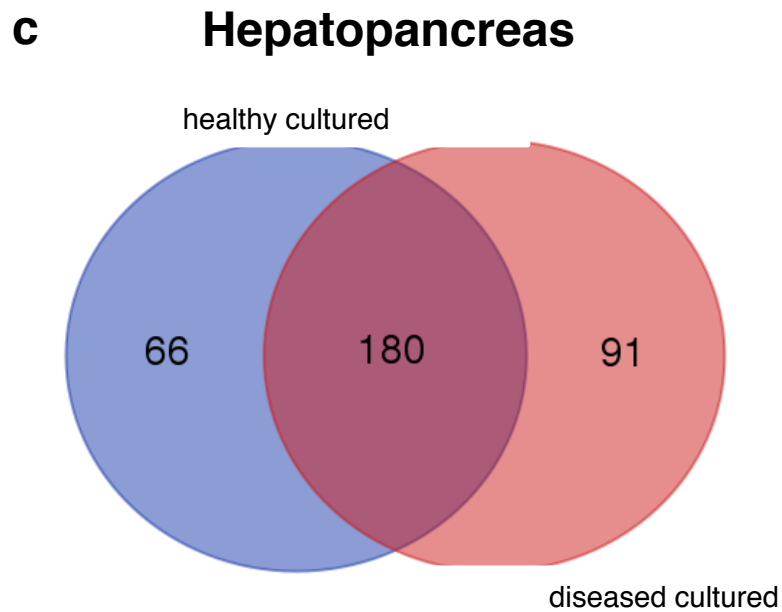
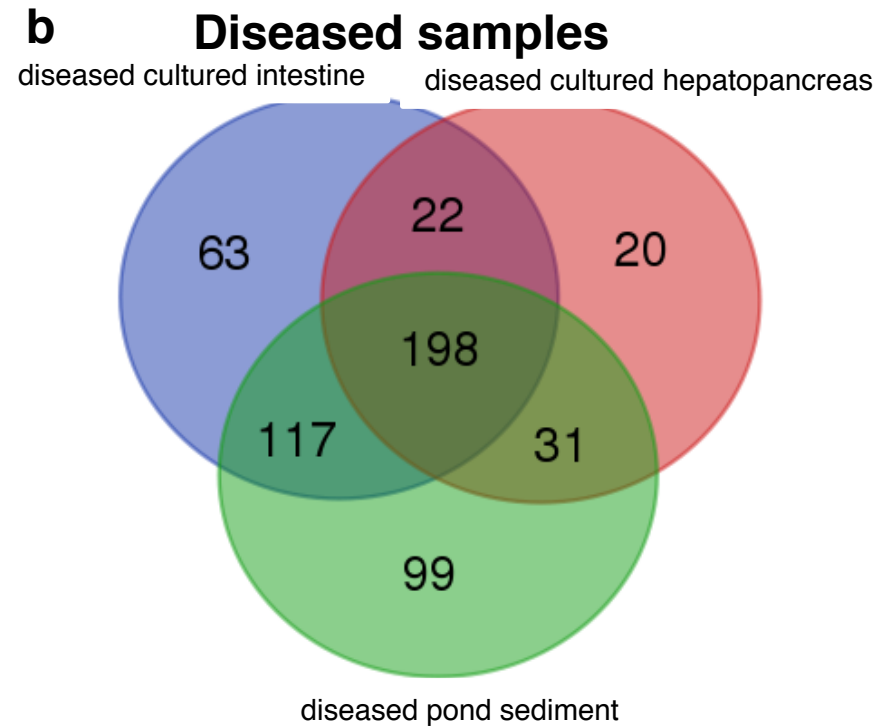
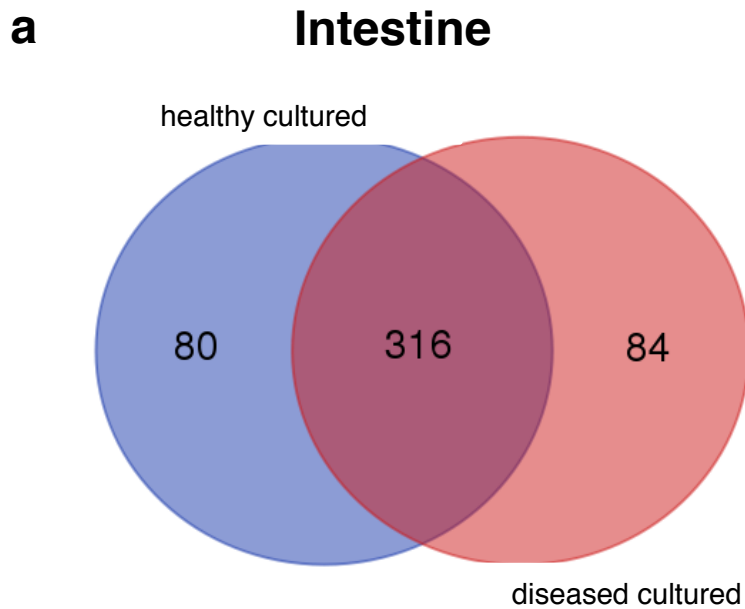
b

Weighted

■ healthy ● diseased

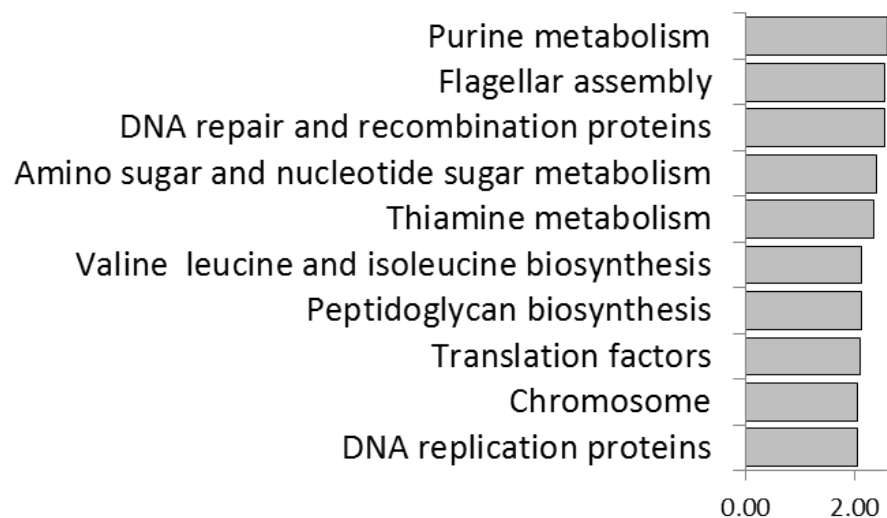
a**b**

Supplementary Fig. S11

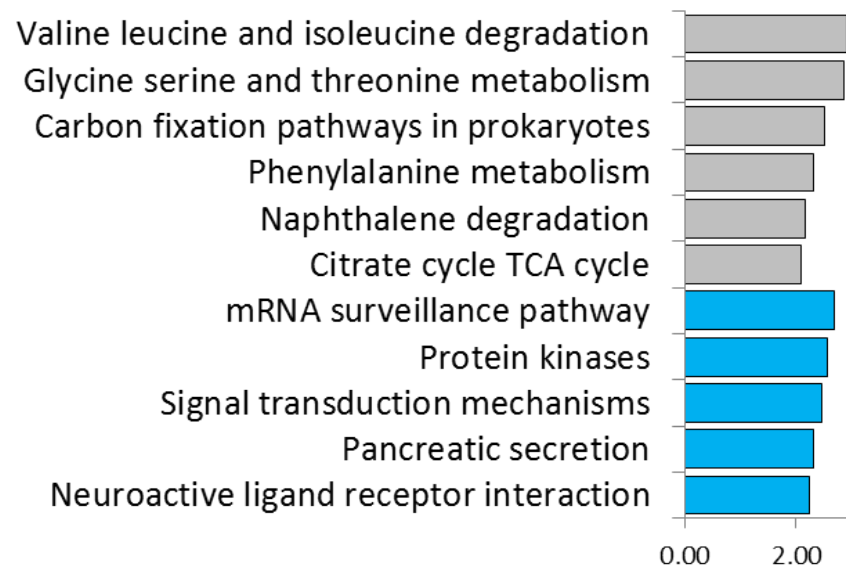


Supplementary Fig. S12

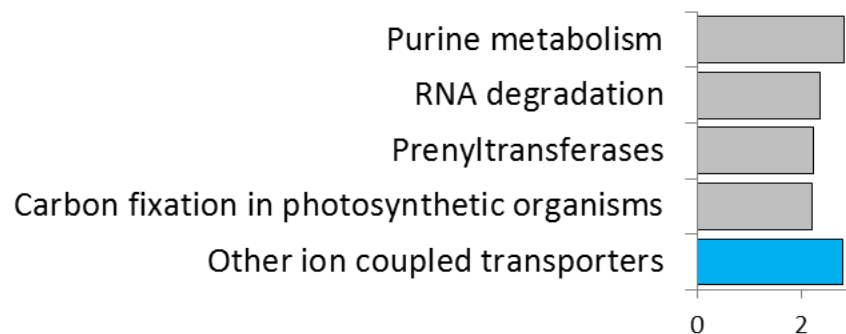
a Hepatopancreas



b Intestine

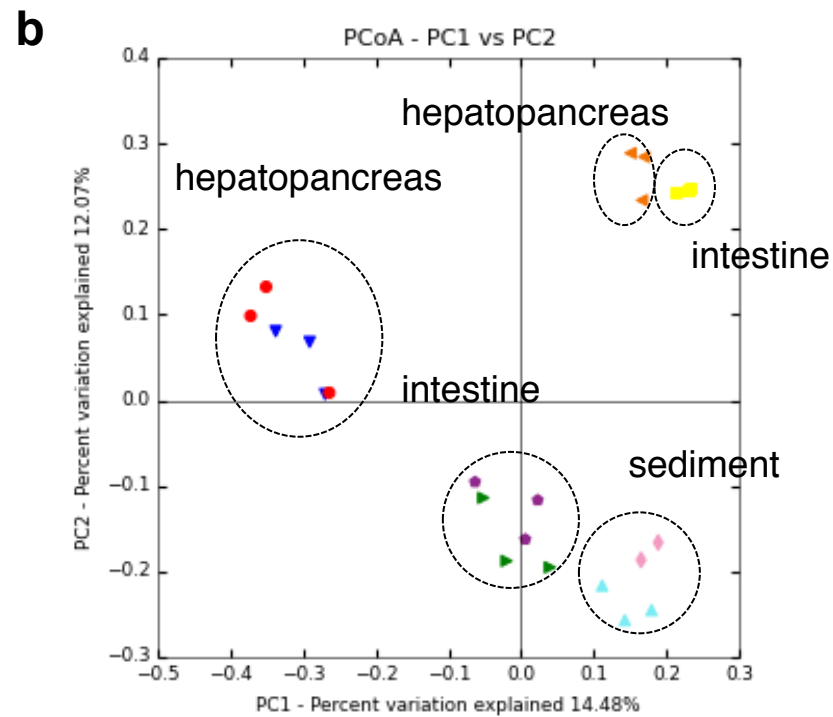
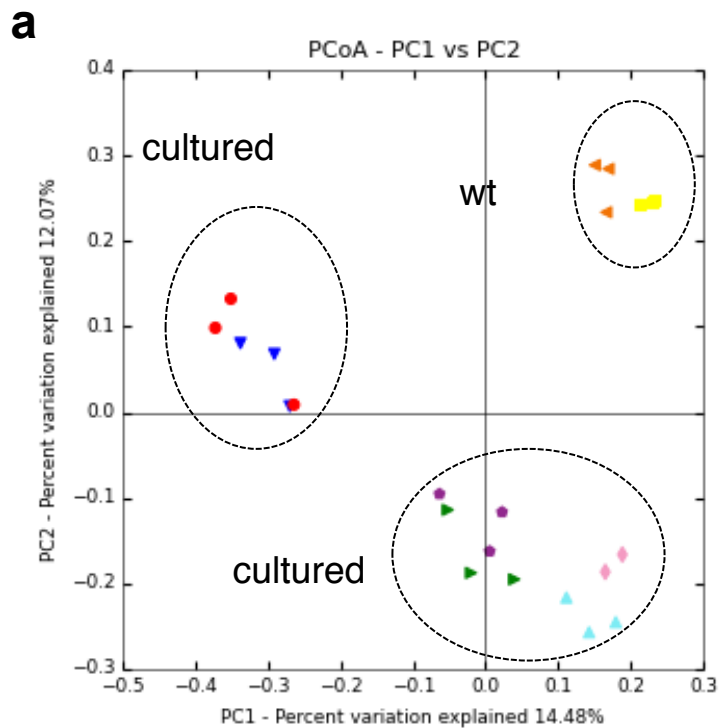


c Pond sediment



■ Diseased

■ Healthy



Unweighted



Healthy cultured shrimp



Diseased cultured shrimp



Wild-type shrimp

Supplementary Table S1.Relative abundance of OTUs with an abundance >0.1% at phylum level.

Phylum	intestine									hepatopancreas									pond sediment					
	wt			cultured healthy			cultured diseased			wt			cultured healthy			cultured diseased			healthy	diseased				
	i 1 wt	i 2 wt	i 3 wt	139-i	68B-i	7A-i	49B-i	27B-i	2B-i	h 1 wt	h 2 wt	h 3 wt	139-H	68B-H	7A-H	49B-H	27B-H	2B-H		139-S	68B-S	7A-S	49B-S	27B-S
Acidobacteria	0.00	0.00	0.00	0.28	0.26	0.26	0.12	0.34	0.24	0.00	0.00	0.00	0.07	0.07	0.17	0.14	0.18	0.27	0.32	0.71	3.86	0.71	3.81	3.80
Actinobacteria	1.35	0.85	3.04	1.59	1.15	0.98	0.79	1.06	0.61	7.45	0.11	2.40	0.57	0.20	0.23	0.37	0.53	0.31	16.73	13.35	3.68	50.53	6.44	3.95
Bacteroidetes	0.00	0.02	0.02	0.56	1.49	0.79	4.90	3.63	13.01	0.00	0.00	0.00	0.03	0.13	0.03	0.06	0.08	0.00	0.62	8.01	0.99	1.12	16.68	8.66
Chloroflexi	0.00	0.00	0.00	0.28	0.28	0.31	0.09	0.19	0.07	0.00	0.00	0.00	0.03	0.10	0.09	0.04	0.08	0.10	14.51	0.71	0.18	0.15	0.27	0.04
Cyanobacteria	0.00	9.09	29.67	4.68	62.47	5.85	9.14	17.35	9.68	0.11	0.17	0.89	0.91	0.75	0.52	0.49	0.96	0.71	1.24	3.91	3.77	2.99	3.61	1.10
Firmicutes	0.02	0.18	10.86	0.00	0.02	0.00	0.00	0.00	0.00	0.18	3.66	2.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gemmatimonadetes	0.00	0.00	0.00	1.78	2.45	1.67	0.82	3.91	1.81	0.00	0.00	0.00	0.64	0.85	1.27	0.49	1.47	1.15	6.65	12.46	44.70	9.39	12.07	34.34
Proteobacteria	98.63	89.86	56.42	90.54	28.19	89.18	74.64	65.83	70.12	92.27	96.06	93.04	97.67	97.75	97.60	98.43	96.63	97.39	59.88	60.50	42.55	34.60	56.60	47.35
Spirochaetes	0.00	0.00	0.00	0.28	0.09	0.16	5.60	6.20	1.44	0.00	0.00	0.00	0.07	0.10	0.06	0.00	0.08	0.00	0.05	0.36	0.27	0.36	0.46	0.77
Tenericutes	0.00	0.00	0.00	0.00	3.61	0.81	3.90	1.47	3.03	0.00	0.00	0.80	0.00	0.07	0.03	0.00	0.00	0.07	0.00	0.00	0.00	0.15	0.07	0.00

Data derived from OUT table with OTUs >0.1% of the total read count to simplify the visualization of the results.

Supplementary Table S2. Average relative abundance of top 5 OTUs with an abundance >0.1% at family and genus level.

Intestine wt				Hepatopancreas wt				Helathy pond sediment			
Family	%	Genus	%	Family	%	Genus	%	Family	%	Genus	%
Vibrionaceae	36.3	Vibrio	20.0	Enterobacteriaceae	44.0	Photobacterium	16.0	Enterobacteriaceae	19.8	Acinetobacter	12.7
Enterobacteriaceae	15.3	Photobacterium	9.0	Vibrionaceae	27.2	Acinetobacter	12.0	Moraxellaceae	12.9	Vibrio	7.1
Moraxellaceae	7.3	Paracoccus	5.1	Moraxellaceae	12.0	Vibrio	7.8	Vibrionaceae	10.9	KSA1	2.7
Rhodobacteraceae	6.5	Fusibacter	3.2	Shewanellaceae	4.0	Shewanella	4.0	Microbacteriaceae	8.0	Enterobacter	2.7
Oxalobacteraceae	5.5	Acinetobacter	3.2	Idiomarinaceae	2.4	Shigella	3.9	koll13	3.0	Pseudomonas	2.6
Healthy cultured intestine				Healthy cultured hepatopancreas				Diseased pond sediment			
Family	%	Genus	%	Family	%	Genus	%	Family	%	Genus	%
Vibrionaceae	35.6	Vibrio	15.1	Enterobacteriaceae	70.2	Pseudomonas	18.1	Microbacteriaceae	19.8	KSA1	5.9
Enterobacteriaceae	20.1	Pseudomonas	3.3	Pseudomonadaceae	18.1	Vibrio	3.0	Enterobacteriaceae	12.0	Vibrio	5.4
Pseudoalteromonadaceae	5.9	Photobacterium	2.3	Vibrionaceae	6.9	Escherichia	2.6	Vibrionaceae	8.7	Pseudidiomarina	2.0
Pseudomonadaceae	3.3	Enterobacter	1.6	Pseudoalteromonadaceae	0.6	Enterobacter	2.6	[Balneolaceae]	5.9	Enterobacter	2.0
Rhodobacteraceae	1.3	Escherichia	1.3	Moraxellaceae	0.5	Shigella	2.4	Desulfovibrionaceae	4.3	Acinetobacter	2.0
Diseased cultured intestine				Diseased cultured hepatopancreas							
Family	%	Genus	%	Family	%	Genus	%				
Vibrionaceae	33.6	Vibrio	15.9	Enterobacteriaceae	77.6	Pseudomonas	10.4				
Enterobacteriaceae	18.6	Photobacterium	3.0	Pseudomonadaceae	10.4	Escherichia	3.0				
Pseudoalteromonadaceae	6.1	Pseudomonas	2.6	Vibrionaceae	7.3	Shigella	2.9				
Marinilabiaceae	4.9	Phaeobacter	1.8	Pseudoalteromonadaceae	0.6	Enterobacter	2.9				
Rhodobacteraceae	4.2	Enterobacter	1.5	Comamonadaceae	0.4	Vibrio	2.5				

Data derived from OUT table with OTUs >0.1% of the total read count to simplify the visualization of the results.

Supplementary Table S3. Spearman correlation test between group triplicates.

		r Spearman	p-value
Intestine wt	i 1 wt vs i 2 wt	0.555	<0.0001
	i 1 wt vs i 3 wt	0.384	<0.0001
	i 2 wt vs i 3 wt	0.871	<0.0001
Cultured healthy intestine	139-i vs 68B-i	0.766	<0.0001
	139-i vs 7A-i	0.864	<0.0001
	68B-i vs 7A-i	0.826	<0.0001
Cultured diseased intestine	49B-i vs 27B-i	0.869	<0.0001
	49B-i vs 2B-i	0.939	<0.0001
	27B-i vs 2B-i	0.866	<0.0001
Hepatopancreas wt	h 1 wt vs h 2 wt	0.829	<0.0001
	h 1 wt vs h 3 wt	0.587	<0.0001
	h 2 wt vs h 3 wt	0.696	<0.0001
Cultured healthy hepatopancreas	139-H vs 68B-H	0.838	<0.0001
	139-H vs 7A-H	0.881	<0.0001
	68B-H vs 7A-H	0.875	<0.0001
Cultured diseased hepatopancreas	49B-H vs 27B-H	0.909	<0.0001
	49B-H vs 2B-H	0.834	<0.0001
	27B-H vs 2B-H	0.797	<0.0001
Helathy pond sediment	139-S vs 68B-S	0.837	<0.0001
	139-S vs 7A-S	0.807	<0.0001
	68B-S vs 7A-S	0.815	<0.0001
Diseased pond sediment	49B-S vs 27B-S	0.873	<0.0001
	49B-S vs 2B-S	0.882	<0.0001
	27B-S vs 2B-S	0.882	<0.0001

Supplementary Table S4. Number of reads, observed OTUs and alpha diversity indices for all the sequenced samples.

	wt intestine			healthy cultured intestine			diseased cultured intestine			wt hepatopancreas			healthy cultured hepatopancreas			diseased cultured hepatopancreas			helathy pond sediment			diseased pond sediment		
	i 1 wt	i 2 wt	i 3 wt	139-i	688-i	7A-i	49B-i	27B-i	2B-i	h 1 wt	h 2 wt	h 3 wt	139-H	688-H	7A-H	49B-H	27B-H	2B-H	139-S	688-S	7A-S	49B-S	27B-S	2B-S
Number of reads	15814	11326	10723	1724	7543	7725	5830	5721	7554	8356	10030	4703	3700	3868	4452	6489	5147	3957	7211	1032	2078	3292	9434	5652
Number of observed OTUs	28.96	26.84	25.25	-	21.26	22.38	23.74	25.15	22.47	25.25	23.40	16.20	13.27	13.54	13.53	12.75	14.97	12.57	26.97	-	27.12	28.16	29.14	24.98
Chao1	8.30	7.93	7.66	-	6.84	7.72	7.75	8.08	7.68	7.56	7.53	5.84	6.20	5.91	6.70	6.57	6.82	6.12	7.33	-	7.58	6.85	8.06	7.61
Phylogenetic diversity (PD)	49.62	45.76	42.84	-	35.68	37.04	39.73	42.22	37.27	42.93	39.27	26.55	20.34	21.17	20.36	18.92	23.11	19.01	46.60	-	46.67	49.47	50.22	42.35
Shannon index	8.30	7.93	7.66	-	6.84	7.72	7.75	8.08	7.68	7.56	7.53	5.84	6.20	5.91	6.70	6.57	6.82	6.12	7.33	-	7.58	6.85	8.06	7.61

Supplementary Table S4. Mean values of number of reads, observed OTUs and alpha diversity indices for all the sequenced samples.

	Intestine			Hepatopancreas			Pond sediment	
	wt	healthy cultured	diseased cultured	wt	healthy cultured	diseased cultured	healthy	diseased
Number of reads	12621.00	5664.00	6368.33	7696.33	4006.67	5197.67	3440.33	6126.00
Number of observed OTUs	27.02	21.82	23.79	21.61	13.45	13.43	27.04	27.43
Chao1	7.96	7.28	7.84	6.98	6.27	6.51	7.45	7.51
Phylogenetic diversity (PD)	46.07	36.36	39.74	36.25	20.62	20.35	46.63	47.35
Shannon index	7.96	7.28	7.84	6.98	6.27	6.51	7.45	7.51

Supplementary Table S5. Good's Coverage of sequenced samples (10,000 iterations at 2,078 sequence depth).

Sequence identity	Intestine									Hepatopancreas									Sediment					
	wt			healthy cultured			diseased cultured			wt			healthy cultured			diseased cultured			healthy		diseased			
	i 1 wt	i 2 wt	i 3 wt	139-i	68B-i	7A-i	49B-i	27B-i	2B-i	h 1 wt	h 2 wt	h 3 wt	139-H	68B-H	7A-H	49B-H	27B-H	2B-H	139-S	68B-S	7A-S	49B-S	27B-S	2B-S
97%	0.814	0.806	0.835	-	0.848	0.846	0.833	0.813	0.85	0.847	0.869	0.902	0.909	0.910	0.909	0.919	0.8946	0.931	0.813	-	0.816	0.808	0.795	0.832

Supplementary Table S5. Mean values for good's coverage of sequenced samples (10,000 iterations at 2,078 sequence depth).

Sequence identity	Intestine						Hepatopancreas						Sediment					
	wt		healthy cultured		diseased cultured		wt		healthy cultured		diseased cultured		healthy		diseased			
97%	0.818		0.85		0.832		0.873		0.909		0.915		0.815		0.812			

Supplementary Table S6. Linear Discriminant Analysis of enriched taxonomies in wt and cultured intestine samples using LEfSe.

Cultured samples		Wild-type samples	
Phyla	LDA	Phyla	LDA
p_Bacteroidetes	4.224	p_Actinobacteria	4.427
p_Spirochaetes	2.768	p_Nitrospirae	2.696
p_Gemmatimonadetes	3.907		
p_Fusobacteria	3.143		
Class	LDA	Class	LDA
c_Bacteroidia	4.041	c_Nitrospira	2.667
c_Gemm_5	3.813	c_RB25	2.763
c_Flavobacteriia	3.571	c_Synechococcophycideae	4.063
c_Gemm_2	3.383	c_Thermoleophila	2.692
c_Fusobacteriia	3.099		
c_TK17	2.980		
c_Rhodothermi	2.976		
c_Nitriiruptoria	2.902		
c_Epsilonproteobacteria	2.824		
c_iii1_8	2.774		
c_Leptospirae	2.764		
Order	LDA	Order	LDA
o_Bacteroidales	4.075	o_Caulobacterales	4.032
o_Flavobacteriales	3.592	o_Rhizobiales	4.006
o_Legionellales	3.294	o_Rhodospirillales	3.481
o_Desulfobacterales	3.292	o_Streptophyta	3.342
o_Fusobacteriales	3.109	o_Thiohalorhabdales	2.842
o_Rhodothermales	2.975	o_Haptophyceae	2.787
o_Nitriiruptorales	2.898	o_Nitrospirales	2.679
o_Campylobacteriales	2.800	o_MWH_UniP1	2.649
o_DS_18	2.796	o_Solirubrobacterales	2.500
o_Leptospirales	2.771		
o_Bifidobacteriales	2.579		
o_SBR1031	2.490		
Family	LDA	Family	LDA
f_Pseudoalteromonadaceae	4.367	f_Moraxellaceae	4.381
f_Marinilabiaceae	3.852	f_Oxalobacteraceae	4.378
f_Flavobacteriaceae	3.558	f_Caulobacteraceae	4.005
f_Alteromonadaceae	3.429	f_Ferrimonadaceae	3.640
f_Lachnospiraceae	3.396	f_Sphingomonadaceae	3.547
f_Desulfobulbaceae	3.302	f_Aurantimonadaceae	3.540
f_Ruminococcaceae	3.236	f_Propionibacteriaceae	3.355
f_Bacteroidaceae	3.144	f_Hyphomicrobiaceae	3.184
f_Legionellaceae	2.993	f_Intrasporangiaceae	2.984
f_Nitriiruptoraceae	2.904	f_Methylocystaceae	2.955
f_Coxiellaceae	2.902	f_Rhizobiaceae	2.903
f_211ds20	2.876	f_Acetobacteraceae	2.865
f_Balneolaceae	2.812	f_Methylobacteriaceae	2.748
f_Psychromonadaceae	2.734	f_EB1017	2.709
f_Helicobacteraceae	2.666	f_Dietziaceae	2.687
f_Rikenellaceae	2.658	f_Phyllobacteriaceae	2.629
f_Bifidobacteriaceae	2.540	f_Streptomycetaceae	2.291
f_Oceanospirillaceae	2.533		
f_Rhodothermaceae	2.502		
f_A4b	2.493		
f_Flammeovirgaceae	2.331		
f_Nannocystaceae	2.313		
		Genus	LDA
		g_Pseudoalteromonas	3.773
		g_Enterobacter	3.722
		g_Escherichia	3.533
		g_Robiginitalea	3.267
		g_Phaeobacter	3.265
		g_Trabulsiella	3.249
		g_Shigella	3.143
		g_Bacteroides	3.123
		g_Agarivorans	3.033
		g_Citrobacter	2.865
		g_Bilophila	2.834
		g_KSA1	2.767
		g_Brenneria	2.747
		g_Aeromonas	2.711
		g_Rhodovulum	2.682
		g_Coproccoccus	2.678
		g_Microbulbifer	2.632
		g_Faecalibacterium	2.601
		g_Fusobacterium	2.584
		g_Bifidobacterium	2.561
		g_Blautia	2.557
		g_Plesiocystis	2.492
		g_Idiomarina	2.483
		g_Clostridiisalibacter	2.476
		g_Providencia	2.391
		g_Butyrvibrio	2.376
		g_Dickeya	2.315
		g_Tolomonas	2.280
		g_Loktanella	2.262
		g_Marinobacter	2.258
		Genus	LDA
		g_Paracoccus	4.165
		g_Rubellimicrobium	3.664
		g_Ferrimonas	3.626
		g_Janthinobacterium	3.556
		g_Propionibacterium	3.395
		g_Mycoplana	3.339
		g_Rhodobacter	3.290
		g_Agrococcus	3.183
		g_Blastococcus	3.152
		g_Ralstonia	3.138
		g_Brevundimonas	3.009
		g_Lysobacter	2.993
		g_Pleomorphomonas	2.923
		g_Nocardioides	2.828
		g_Agrobacterium	2.767
		g_Microbacterium	2.724
		g_Variovorax	2.664
		g_Dietzia	2.659
		g_Methylobacterium	2.548
		g_Microbispora	2.543
		g_Jannaschia	2.500
		g_Leptothrix	2.454
		g_Serinicoccus	2.392
		g_Streptomyces	2.298
		g_Nitrobacteria	2.200
		g_Rothia	2.189
		g_Shinella	2.176
		g_Novosphingobium	2.162

Supplementary Table S7. Relative abundance of KEGG pathways obtained by Picrust analysis.

KEGG_Pathways	Intestine wt										Hepatopancreas wt										Helathy pond sediment									
	11 wt	12 wt	13 wt	139-I	688-I	7A-I	498-I	278-I	28-I	h 1 wt	h 2 wt	h 3 wt	139-H	688-H	7A-H	498-H	278-H	28-H	139-S	688-S	7A-S	498-S	278-S	28-S						
Cellular Processes	0.045	0.054	0.048	0.052	0.039	0.052	0.049	0.043	0.049	0.036	0.037	0.048	0.042	0.041	0.041	0.040	0.042	0.039	0.040	0.039	0.039	0.036	0.046	0.040						
Environmental Information Processing	0.164	0.159	0.152	0.170	0.145	0.168	0.155	0.154	0.157	0.163	0.165	0.164	0.179	0.179	0.179	0.179	0.180	0.178	0.180	0.142	0.153	0.142	0.153	0.132	0.129					
Genetic Information Processing	0.147	0.154	0.159	0.153	0.161	0.154	0.156	0.155	0.156	0.161	0.163	0.157	0.150	0.149	0.151	0.152	0.152	0.152	0.167	0.162	0.175	0.170	0.172	0.173						
Human Diseases	0.013	0.014	0.013	0.014	0.013	0.014	0.014	0.013	0.014	0.011	0.011	0.015	0.012	0.012	0.012	0.012	0.012	0.011	0.010	0.011	0.010	0.011	0.011	0.010						
Metabolism	0.474	0.456	0.471	0.443	0.482	0.443	0.462	0.472	0.457	0.452	0.447	0.450	0.437	0.437	0.433	0.432	0.435	0.431	0.485	0.472	0.473	0.480	0.479	0.481						
None	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Organismal Systems	0.007	0.007	0.008	0.007	0.008	0.007	0.007	0.007	0.007	0.006	0.006	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.007	0.007	0.007	0.007	0.007						
Unclassified	0.147	0.154	0.148	0.160	0.151	0.161	0.154	0.154	0.158	0.169	0.169	0.156	0.174	0.175	0.177	0.177	0.174	0.181	0.147	0.153	0.153	0.141	0.151	0.158						
Level 2																														
KEGG_Pathways	11 wt	12 wt	13 wt	139-I	688-I	7A-I	498-I	278-I	28-I	h 1 wt	h 2 wt	h 3 wt	139-H	688-H	7A-H	498-H	278-H	28-H	139-S	688-S	7A-S	498-S	278-S	28-S						
Amino Acid Metabolism	0.101	0.095	0.095	0.090	0.091	0.090	0.096	0.099	0.092	0.090	0.088	0.092	0.087	0.087	0.085	0.084	0.086	0.082	0.104	0.098	0.100	0.103	0.102	0.103						
Biosynthesis of Other Secondary Metabolites	0.008	0.006	0.007	0.006	0.009	0.006	0.007	0.007	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.008	0.008	0.008	0.009	0.008	0.009						
Cancers	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Carbohydrate Metabolism	0.095	0.090	0.092	0.092	0.090	0.092	0.094	0.094	0.094	0.094	0.096	0.091	0.093	0.093	0.095	0.095	0.094	0.096	0.094	0.096	0.091	0.100	0.092	0.098						
Cardiovascular Diseases	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Cell Communication	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Cell Growth and Death	0.005	0.004	0.005	0.004	0.005	0.004	0.004	0.005	0.004	0.004	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.005	0.004	0.005	0.005	0.005	0.005						
Cell Motility	0.037	0.046	0.040	0.045	0.031	0.046	0.042	0.036	0.042	0.030	0.031	0.041	0.037	0.036	0.036	0.035	0.037	0.034	0.033	0.032	0.032	0.028	0.038	0.032						
Cellular Processes and Signaling	0.043	0.047	0.042	0.049	0.041	0.050	0.046	0.044	0.048	0.052	0.051	0.049	0.054	0.054	0.055	0.055	0.054	0.056	0.043	0.045	0.044	0.040	0.044	0.044						
Circulatory System	0.001	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000						
Digestive System	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Endocrine System	0.003	0.003	0.003	0.002	0.004	0.002	0.003	0.003	0.003	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.004	0.003	0.003	0.003	0.003	0.003						
Energy Metabolism	0.053	0.054	0.064	0.053	0.076	0.053	0.055	0.058	0.057	0.051	0.050	0.053	0.049	0.049	0.048	0.049	0.049	0.048	0.054	0.054	0.055	0.053	0.058	0.055						
Environmental Adaptation	0.001	0.002	0.002	0.002	0.001	0.002	0.002	0.001	0.002	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.002	0.001	0.001						
Enzyme Families	0.019	0.020	0.021	0.020	0.023	0.020	0.019	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.019	0.020	0.022	0.019	0.020	0.021						
Excretory System	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Folding, Sorting and Degradation	0.022	0.024	0.025	0.023	0.026	0.024	0.024	0.023	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.023	0.023	0.025	0.024	0.024	0.025	0.024	0.027						
Genetic Information Processing	0.025	0.025	0.027	0.027	0.027	0.027	0.027	0.027	0.027	0.028	0.027	0.028	0.027	0.027	0.027	0.027	0.027	0.027	0.025	0.026	0.027	0.026	0.028	0.028						
Glycan Biosynthesis and Metabolism	0.018	0.020	0.020	0.023	0.021	0.023	0.022	0.021	0.023	0.024	0.024	0.023	0.025	0.025	0.026	0.025	0.025	0.026	0.021	0.022	0.023	0.023	0.024	0.024						
Immune System	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001						
Immune System Diseases	0.000	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000						
Infectious Diseases	0.005	0.008	0.007	0.008	0.007	0.009	0.008	0.006	0.008	0.006	0.006	0.009	0.007	0.007	0.007	0.007	0.007	0.007	0.005	0.006	0.005	0.005	0.005	0.005						
Lipid Metabolism	0.035	0.034	0.033	0.031	0.030	0.031	0.033	0.033	0.032	0.031	0.031	0.033	0.031	0.031	0.030	0.029	0.030	0.029	0.037	0.035	0.035	0.033	0.035	0.035						
Membrane Transport	0.137	0.128	0.124	0.138	0.119	0.136	0.126	0.127	0.127	0.135	0.138	0.135	0.147	0.147	0.148	0.149	0.147	0.149	0.117	0.127	0.116	0.130	0.107	0.104						
Metabolic Diseases	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Metabolism	0.027	0.026	0.028	0.027	0.027	0.028	0.028	0.028	0.030	0.028	0.030	0.027	0.030	0.032	0.032	0.032	0.031	0.034	0.032	0.032	0.032	0.025	0.030	0.030						
Metabolism of Cofactors and Vitamins	0.041	0.045	0.041	0.040	0.050	0.041	0.042	0.043	0.043	0.041	0.040	0.041	0.039	0.039	0.039	0.039	0.039	0.039	0.043	0.043	0.044	0.045	0.044	0.044						
Metabolism of Other Amino Acids	0.020	0.018	0.017	0.017	0.017	0.017	0.018	0.018	0.017	0.017	0.017	0.018	0.017	0.017	0.017	0.017	0.017	0.018	0.018	0.017	0.018	0.018	0.018	0.018						
Metabolism of Terpenoids and Polyketides	0.020	0.018	0.019	0.016	0.019	0.016	0.018	0.019	0.017	0.017	0.016	0.017	0.015	0.015	0.015	0.015	0.015	0.014	0.021	0.019	0.019	0.020	0.020	0.020						
Nervous System	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Neurodegenerative Diseases	0.004	0.003	0.003	0.003	0.002	0.003	0.003	0.003	0.003	0.002	0.002	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.003	0.003	0.003						
Nucleotide Metabolism	0.031	0.032	0.033	0.032	0.033	0.032	0.033	0.032	0.033	0.033	0.033	0.034	0.030	0.030	0.031	0.031	0.031	0.031	0.031	0.034	0.033	0.035	0.035	0.035						
Poorly Characterized	0.052	0.055	0.053	0.056	0.053	0.057	0.055	0.054	0.055	0.058	0.058	0.053	0.059	0.059	0.059	0.059	0.059	0.059	0.054	0.054	0.055	0.051	0.054	0.056						
Protein Folding and Repair	0.004	0.008	0.005	0.005	0.007	0.006	0.005	0.006	0.007	0.006	0.005	0.006	0.003	0.003	0.004	0.004	0.004	0.004	0.003	0.006	0.004	0.007	0.007	0.007						
Sensory System	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Signal Transduction	0.026	0.029	0.027	0.030	0.025	0.030	0.028	0.026	0.028	0.026	0.026	0.027	0.030	0.030	0.030	0.030	0.030	0.029	0.023	0.025	0.025	0.022	0.023	0.023						
Signaling Molecules and Interaction	0.002	0.002	0.002	0.002	0.																									

Tuberculosis	1.34E-03	1.35E-03	1.69E-03	1.23E-03	2.32E-03	1.25E-03	1.37E-03	1.46E-03	1.42E-03	1.01E-03	1.02E-03	1.29E-03	8.54E-04	8.27E-04	8.45E-04	8.36E-04	8.78E-04	8.31E-04	1.28E-03	1.23E-03	1.34E-03	1.32E-03	1.43E-03	1.25E-03
Two-component system	2.49E-02	2.77E-02	2.51E-02	2.86E-02	2.35E-02	2.87E-02	2.62E-02	2.42E-02	2.70E-02	2.52E-02	2.50E-02	2.63E-02	2.91E-02	2.92E-02	2.86E-02	2.86E-02	2.86E-02	2.82E-02	2.16E-02	2.36E-02	2.33E-02	2.07E-02	2.20E-02	2.21E-02
Type I diabetes mellitus	4.01E-04	5.03E-04	5.43E-04	4.85E-04	5.58E-04	4.91E-04	4.82E-04	4.37E-04	5.12E-04	5.02E-04	4.84E-04	6.22E-04	4.20E-04	4.15E-04	4.34E-04	4.45E-04	4.32E-04	4.34E-04	4.41E-04	4.25E-04	4.49E-04	4.45E-04	4.26E-04	
Type II diabetes mellitus	4.08E-04	4.41E-04	5.18E-04	4.61E-04	6.83E-04	4.71E-04	4.65E-04	4.51E-04	4.90E-04	4.06E-04	4.18E-04	4.84E-04	4.46E-04	4.47E-04	4.46E-04	4.55E-04	4.46E-04	4.50E-04	3.46E-04	3.93E-04	3.16E-04	4.00E-04	3.87E-04	3.21E-04
Tyrosine metabolism	4.53E-03	4.31E-03	4.05E-03	4.13E-03	4.10E-03	4.07E-03	4.17E-03	4.29E-03	4.07E-03	4.06E-03	4.04E-03	3.93E-03	4.45E-03	4.56E-03	4.20E-03	4.15E-03	4.24E-03	4.12E-03	4.33E-03	4.22E-03	3.83E-03	4.01E-03	3.78E-03	4.23E-03
Ubiquinone and other terpenoid-quinone biosynthesis	3.61E-03	4.41E-03	4.60E-03	4.40E-03	5.18E-03	4.50E-03	4.37E-03	4.18E-03	4.42E-03	4.24E-03	3.95E-03	4.54E-03	4.30E-03	4.24E-03	4.39E-03	4.45E-03	4.36E-03	4.48E-03	4.05E-03	4.01E-03	3.75E-03	4.02E-03	4.24E-03	3.81E-03
Ubiquitin system	1.60E-04	2.26E-04	2.73E-04	2.27E-04	2.59E-04	2.32E-04	2.21E-04	2.07E-04	2.12E-04	2.45E-04	2.33E-04	2.59E-04	2.29E-04	2.29E-04	2.30E-04	2.30E-04	2.30E-04	2.32E-04	2.81E-04	2.71E-04	3.06E-04	1.75E-04	2.81E-04	2.67E-04
VEGF signaling pathway	1.16E-06	9.33E-08	1.38E-07	1.02E-06	3.96E-06	1.97E-07	5.24E-07	8.07E-06	2.93E-07	3.35E-08	8.54E-08	0.00E+00	0.00E+00	6.00E-08	5.25E-08	3.60E-08	0.00E+00	0.00E+00	1.36E-07	8.80E-07	0.00E+00	2.06E-07	6.14E-07	0.00E+00
Valine, leucine and isoleucine biosynthesis	6.28E-03	5.78E-03	6.19E-03	5.66E-03	6.59E-03	5.63E-03	6.04E-03	6.39E-03	5.92E-03	6.03E-03	5.98E-03	5.55E-03	5.45E-03	5.48E-03	5.48E-03	5.49E-03	5.52E-03	5.49E-03	6.79E-03	6.32E-03	6.38E-03	6.95E-03	6.50E-03	6.42E-03
Valine, leucine and isoleucine degradation	9.37E-03	7.54E-03	6.76E-03	6.03E-03	5.04E-03	5.91E-03	7.18E-03	7.39E-03	6.10E-03	5.93E-03	5.55E-03	6.36E-03	5.39E-03	5.42E-03	4.86E-03	4.62E-03	5.02E-03	4.36E-03	8.49E-03	7.11E-03	6.23E-03	7.38E-03	7.35E-03	6.89E-03
Various types of N-glycan biosynthesis	4.57E-07	6.46E-06	2.57E-06	8.78E-07	3.34E-07	9.54E-07	5.72E-07	8.47E-07	3.34E-06	6.71E-07	4.56E-07	1.04E-05	1.26E-07	1.80E-07	5.25E-08	7.20E-08	1.84E-07	0.00E+00	4.09E-07	1.17E-06	2.28E-05	1.34E-06	2.31E-06	2.50E-05
Vascular smooth muscle contraction	0.00E+00	2.33E-08	2.76E-08	0.00E+00	0.00E+00	6.58E-08	0.00E+00	0.00E+00	7.34E-08	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	9.09E-08	2.93E-07	0.00E+00	0.00E+00	1.45E-07	0.00E+00
Vasopresin-regulated water reabsorption	2.77E-07	1.38E-06	2.04E-06	5.85E-07	8.34E-07	7.24E-07	8.10E-07	1.15E-06	6.97E-07	1.68E-07	0.00E+00	7.44E-07	6.30E-08	0.00E+00	3.15E-07	1.08E-07	4.60E-08	1.17E-07	2.14E-06	2.05E-06	3.32E-06	1.75E-06	3.25E-06	2.25E-06
Vibrio cholerae infection	2.23E-05	2.84E-04	2.11E-04	2.24E-04	1.12E-04	2.53E-04	2.08E-04	8.86E-05	2.29E-04	5.21E-05	5.05E-05	4.20E-04	5.05E-05	3.28E-05	4.15E-05	4.26E-05	5.36E-05	1.83E-05	8.48E-05	5.46E-05	5.43E-05	7.49E-05	7.80E-05	4.76E-05
Vibrio cholerae pathogenic cycle	1.31E-03	4.45E-03	3.60E-03	4.19E-03	2.74E-03	4.45E-03	3.89E-03	2.29E-03	3.97E-03	1.80E-03	1.82E-03	5.35E-03	2.10E-03	1.83E-03	2.01E-03	2.01E-03	2.17E-03	1.75E-03	2.04E-03	1.72E-03	1.64E-03	1.64E-03	2.05E-03	1.53E-03
Viral myocarditis	2.58E-04	4.24E-05	3.26E-05	4.04E-05	5.05E-05	2.92E-05	9.31E-05	1.59E-04	4.60E-05	1.30E-05	1.16E-05	1.22E-05	2.96E-06	4.02E-06	4.15E-06	3.92E-06	6.02E-06	4.10E-06	3.49E-05	4.70E-05	2.06E-05	7.45E-05	6.91E-05	4.51E-05
Vitamin B6 metabolism	1.71E-03	1.75E-03	1.76E-03	1.85E-03	1.80E-03	1.87E-03	1.85E-03	1.80E-03	1.91E-03	2.02E-03	1.95E-03	1.86E-03	1.95E-03	1.95E-03	1.99E-03	2.01E-03	1.97E-03	2.01E-03	2.09E-03	2.04E-03	2.11E-03	2.11E-03	2.02E-03	2.06E-03
Wnt signaling pathway	1.31E-07	3.27E-07	7.73E-07	2.93E-07	1.25E-07	1.97E-07	7.62E-07	3.99E-07	2.20E-07	3.35E-08	5.69E-08	6.87E-07	2.52E-07	1.20E-07	4.73E-07	2.16E-07	2.30E-07	0.00E+00	7.27E-07	8.80E-07	0.00E+00	6.19E-07	3.97E-07	3.05E-07
Xylene degradation	4.64E-04	4.41E-04	5.10E-04	5.21E-04	5.64E-04	5.41E-04	5.01E-04	4.94E-04	5.19E-04	5.73E-04	6.31E-04	5.26E-04	5.89E-04	5.82E-04	6.64E-04	6.59E-04	6.23E-04	7.71E-04	4.02E-04	5.37E-04	4.92E-04	3.57E-04	3.15E-04	3.89E-04
Zeatin biosynthesis	2.60E-04	2.64E-04	2.95E-04	2.53E-04	3.18E-04	2.53E-04	2.74E-04	2.83E-04	2.98E-04	2.81E-04	2.88E-04	2.67E-04	2.33E-04	2.32E-04	2.33E-04	2.36E-04	2.32E-04	3.29E-04	3.05E-04	3.48E-04	3.43E-04	3.43E-04	3.47E-04	
alpha-Linolenic acid metabolism	4.32E-04	6.12E-04	4.78E-04	5.80E-04	4.14E-04	5.91E-04	5.27E-04	4.28E-04	5.06E-04	5.50E-04	5.38E-04	8.33E-04	5.84E-04	5.71E-04	6.10E-04	6.20E-04	6.05E-04	6.23E-04	6.06E-04	5.58E-04	4.80E-04	6.99E-04	4.75E-04	4.95E-04
beta-Alanine metabolism	5.29E-03	4.52E-03	4.23E-03	3.95E-03	3.35E-03	3.88E-03	4.46E-03	4.59E-03	3.81E-03	4.09E-03	3.96E-03	4.39E-03	3.98E-03	4.00E-03	3.89E-03	3.81E-03	3.89E-03	3.82E-03	4.86E-03	4.50E-03	3.71E-03	4.27E-03	4.34E-03	3.70E-03
beta-Lactam resistance	3.11E-04	2.52E-04	2.13E-04	2.02E-04	2.53E-04	2.03E-04	1.99E-04	1.94E-04	1.92E-04	2.53E-04	2.63E-04	2.30E-04	2.22E-04	2.23E-04	2.22E-04	2.20E-04	2.20E-04	2.24E-04	3.06E-04	2.43E-04	1.98E-04	2.64E-04	2.14E-04	2.08E-04
mRNA surveillance pathway	6.53E-07	3.50E-07	2.48E-07	5.85E-07	5.42E-07	5.27E-07	1.91E-07	2.99E-07	1.10E-07	1.34E-07	2.85E-08	2.86E-07	6.30E-08	1.80E-07	1.58E-07	7.20E-08	1.84E-07	2.34E-07	2.51E-05	1.17E-06	4.98E-07	3.09E-07	4.34E-07	1.83E-07
mTOR signaling pathway	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
p53 signaling pathway	2.60E-04	6.41E-05	4.11E-05	5.98E-05	5.99E-05	5.19E-05	1.21E-04	1.75E-04	6.81E-05	1.43E-05	1.30E-05	1.01E-04	5.36E-06	5.58E-06	6.30E-06	5.94E-06	8.09E-06	5.39E-06	4.04E-05	5.02E-05	2.41E-05	7.66E-05	7.25E-05	5.04E-05

Supplementary Table S8. NSTI mean score for all the sequenced groups.

	intestine			wt	Hepatopancreas		Pond sediment	
	wt	healthy cultured	diseased cultured		healthy cultured	diseased cultured	Healthy	Diseased
NSTI score	0.081 ± 0.035	0.112 ± 0.072	0.1 ± 0.007	0.046 ± 0.005	0.052 ± 0.003	0.05 ± 0.003	0.142 ± 0.027	0.132 ± 0.029
	intestine	Hepatopancreas	Pond sediment					
NSTI score	0.098 ± 0.043	0.0492 ± 0.004	0.137 ± 0.026					

Supplementary Table S9. Linear Discriminant Analysis of enriched taxonomies in wt and cultured hepatopancreas samples using LEfSe.

Cultured samples		Wild-type samples					
Phyla	LDA	Phyla	LDA	Genus	LDA	Genus	LDA
p_Proteobacteria	4.742	p_Actinobacteria	4.646	g_Pseudomonas	4.743	g_Streptococcus	4.136
p_Gemmatimonadetes	3.680	p_Firmicutes	4.597	g_Enterobacter	4.080	g_Shewanella	4.129
p_Spirochaetes	2.621	p_TM7	2.951	g_Trabulsiella	3.891	g_Corynebacterium	3.847
				g_Escherichia	3.820	g_Vibrio	3.807
Class	LDA	Class	LDA	g_Stenotrophomonas	3.425	g_Staphylococcus	3.612
c_Gammaproteobacteria	5.068	c_Actinobacteria	4.596	g_Citrobacter	3.216	g_Haemophilus	3.609
c_TK17	3.484	c_Bacilli	4.406	g_Klebsiella	3.214	g_Rothia	3.534
c_Gemm_5	3.458	c_Clostridia	4.168	g_Bacteroides	3.015	g_Granulicatella	3.296
c_Gemm_2	3.383	c_Alphaproteobacteria	3.511	g_Alcanivorax	2.770	g_Actinobacillus	3.017
c_iii1_8	2.959	c_TM7_3	2.910	g_Pantoea	2.664	g_Brevundimonas	2.933
c_Rhodothermi	2.741	c_RB25	2.663	g_Rummeliibacillus	2.591	g_Kocuria	2.905
c_Leptospirae	2.633	c_Thermoleophilia	2.455	g_Zobellella	2.580	g_Fusibacter	2.856
Order	LDA	Order	LDA	g_Umboniibacter	2.578	g_Anaerococcus	2.758
o_Enterobacteriales	5.308	o_Actinomycetales	4.560	g_KSA1	2.501	g_Micrococcus	2.727
o_Stramenopiles	3.353	o_Alteromonadales	4.364			g_Agrobacterium	2.702
o_Legionellales	2.927	o_Lactobacillales	4.288			g_Paracoccus	2.661
o_Thiohalorhabdales	2.777	o_Clostridiales	4.189			g_Microbacterium	2.655
o_Rhodothermales	2.717	o_Bacillales	3.725			g_Dermabacter	2.497
o_Leptospirales	2.714	o_Aeromonadales	3.660			g_Epulopiscium	2.481
o_Desulfobacterales	2.590	o_Streptophyta	3.265			g_Ferrimonas	2.467
o_DS_18	2.532	o_Rhodospirillales	2.998			g_Nocardioides	2.439
		o_Rhizobiales	2.861				
		o_Caulobacterales	2.857				
Family	LDA	Family	LDA				
f_Enterobacteriaceae	5.323	f_Streptococcaceae	4.205				
f_Pseudomonadaceae	4.767	f_Shewanellaceae	4.158				
f_Coxiellaceae	2.885	f_Corynebacteriaceae	3.798				
f_Thiohalorhabdaceae	2.734	f_Micrococcaceae	3.719				
f_Rhodothermaceae	2.707	f_Staphylococcaceae	3.634				
f_Desulfobulbaceae	2.704	f_Aeromonadaceae	3.628				
f_Bacteroidaceae	2.693	f_Tissierellaceae	3.400				
f_Alcanivoracaceae	2.686	f_Carnobacteriaceae	3.312				
f_Psychromonadaceae	2.570	f_Intrasporangiaceae	3.270				
f_Christensenellaceae	2.554	f_Endozoicimonaceae	3.080				
f_Balneolaceae	2.549	f_Caulobacteraceae	2.872				
f_Piscirickettsiaceae	2.426	f_Acidaminobacteraceae	2.831				
		f_Nocardioidaceae	2.686				
		f_Dermabacteraceae	2.682				
		f_Ferrimonadaceae	2.474				
		f_Rhizobiaceae	2.459				
		f_Exiguobacteraceae	2.447				
		f_Halomonadaceae	2.435				

Supplementary Table S10. Relative frequency of unique genera and species in sequenced wt and cultured samples.

genera_helathy_vs_wt_intestine		Intestine					
Treatment	healthy cultured			wt			
Description	139-i	68B-i	7A-i	i 1 wt	i 2 wt	i 3 wt	
g__Bacteroides	0.00348028	0.00251889	0.00142395		0	0	0
g__Brenneria	0.00232019	0.00013257	0.0007767		0	0	0
g__Bilophila	0.00174014	0.00026515	0.0018123		0	0	0
g__Rhodovulum	0.00174014	0.00013257	0.00012945		0	0	0
g__Robiginitalea	0.00116009	0.00291661	0.00711974		0	0	0
g__Coprococcus	0.00116009	0.00039772	0.00142395		0	0	0
g__KSA1	0.00116009	0.00159088	0.00090615		0	0	0
g__Morganella	0.00116009	0.00013257	0.00064725		0	0	0
g__Microbulbifer	0.00116009	0.00039772	0.0005178		0	0	0
g__Blautia	0.00116009	0.00079544	0.00038835		0	0	0
g__Faecalibacterium	0.00116009	0.00066287	0.00038835		0	0	0
g__Bifidobacterium	0.00058005	0.00013257	0.00064725		0	0	0
g__Butyrivibrio	0.00058005	0.00013257	0.00012945		0	0	0
g__Plesiocystis	0.00058005	0.00013257	0.00012945		0	0	0
g__Tolumonas	0.00058005	0.00013257	0.00012945		0	0	0
g__Rubellimicrobium	0	0	0	0.02668522	0.0011478	0.00037303	
g__Janthinobacterium	0	0	0	0.01846465	0.00264877	0.00018651	
g__Blastococcus	0	0	0	0.00739851	0.00044146	0.00027977	
g__Agrococcus	0	0	0	0.00733527	0.00061805	0.00027977	
g__Lysobacter	0	0	0	0.00505881	8.83E-05	9.33E-05	
g__Pleomorphomonas	0	0	0	0.0048691	8.83E-05	9.33E-05	
g__Pseudomonas	0	0	0	0.0046794	0.00185414	9.33E-05	
g__Brevundimonas	0	0	0	0.00411028	0.00035317	9.33E-05	
g__Crenothrix	0	0	0	0.00284558	8.83E-05	0.00018651	
g__Agrobacterium	0	0	0	0.00259264	0.00026488	0.00018651	
g__Dietzia	0	0	0	0.0023397	8.83E-05	0.00027977	
g__Microbispora	0	0	0	0.00139117	0.00044146	9.33E-05	
g__Serinicoccus	0	0	0	0.00094853	8.83E-05	0.00027977	
g__Ralstonia	0	0	0	0.00088529	0.0081229	0.00018651	
g__Leptothrix	0	0	0	0.00044265	0.00035317	9.33E-05	
g__Streptomyces	0	0	0	0.00031618	0.00017658	0.00027977	
g__Novosphingobium	0	0	0	0.00031618	8.83E-05	9.33E-05	
g__Jannaschia	0	0	0	0.00012647	8.83E-05	0.00037303	
g__Shinella	0	0	0	0.00012647	0.00035317	0.00018651	
g__Bacillus	0	0	0	6.32E-05	8.83E-05	0.00018651	
g__Nitrobacteria	0	0	0	6.32E-05	0.00070634	9.33E-05	
g__Acinetobacter	0	0	0	0.00018971	8.83E-05	9.33E-05	
g__Variovorax	0	0	0	6.32E-05	0.00264877	0.00018651	

Supplementary Table S10. Relative frequency of unique genera and species in sequenced wt and cultured samples.

spp_helathy_vs_wt_intestine		intestine						
Treatment	Treatment	healthy cultured			wt			
Description	Description	139-i	68B-i	7A-i	i 1 wt	i 2 wt	i 3 wt	
g__Agrococcus	s__jenensis	0	0	0	0.00733527	0.00061805	0.00027977	
g__Blastococcus	s__aggregatus	0	0	0	0.00739851	0.00044146	0.00027977	
g__Variovorax	s__paradoxus	0	0	0	6.32E-05	0.00264877	0.00018651	
g__Crenothrix	s__polyspora	0	0	0	0.00284558	8.83E-05	0.00018651	
g__Bacillus	s__flexus	0	0	0	6.32E-05	8.83E-05	0.00018651	
g__Pseudomonas	s__stutzeri	0	0	0	0.0046794	0.00185414	9.33E-05	
g__Nitrobacteria	s__hamadaniensis	0	0	0	6.32E-05	0.00070634	9.33E-05	
g__Microbispora	s__rosea	0	0	0	0.00139117	0.00044146	9.33E-05	
g__Brevundimonas	s__diminuta	0	0	0	0.00411028	0.00035317	9.33E-05	
g__Acinetobacter	s__schindleri	0	0	0	0.00018971	8.83E-05	9.33E-05	
g__Coprococcus	s__eutactus	0.00116009	0.00039772	0.00142395	0	0	0	
g__Brenneria	s__alni	0.00232019	0.00013257	0.0007767	0	0	0	
g__Morganella	s__morganii	0.00116009	0.00013257	0.00064725	0	0	0	
g__Faecalibacterium	s__prausnitzii	0.00116009	0.00066287	0.00038835	0	0	0	
g__Rhodovulum	s__imhoffii	0.00174014	0.00013257	0.00012945	0	0	0	

Supplementary Table S10. Relative frequency of unique genera and species in sequenced wt and cultured samples.

genera_healthy_vs_wt_hepatopancreas		hepatopancreas				
Treatment	healthy cultured			wt		
Description	139-H	68B-H	7A-H	h 1 wt	h 2 wt	h 3 wt
g__Trabulsiella	0.01621622	0.02481903	0.0098832	0	0	0
g__Zobellella	0.00108108	0.00025853	0.00044924	0	0	0
g__Enterobacter	0.00081081	0.00025853	0.00112309	0	0	0
g__Bacteroides	0.00081081	0.00025853	0.00022462	0	0	0
g__Alcanivorax	0.00054054	0.00051706	0.00044924	0	0	0
g__KSA1	0.00054054	0.00077559	0.00022462	0	0	0
g__Haemophilus	0	0	0	0.01244615	0.01186441	0.00042526
g__Granulicatella	0	0	0	0.00430828	0.00937188	0.00042526
g__Rothia	0	0	0	0.00347056	0.01625125	0.00063789
g__Kocuria	0	0	0	0.00191479	0.00139581	0.00106315
g__Staphylococcus	0	0	0	0.00155577	0.00179462	0.00021263
g__Vibrio	0	0	0	0.00143609	0.00089731	0.0323198
g__Anaerococcus	0	0	0	0.00083772	0.00289133	0.00021263
g__Micrococcus	0	0	0	0.00071805	0.00159521	0.00042526
g__Microbacterium	0	0	0	0.00071805	0.00109671	0.00021263
g__Rothia	0	0	0	0.00059837	0.00119641	0.00063789
g__Dermabacter	0	0	0	0.00059837	0.0004985	0.00021263
g__Paracoccus	0	0	0	0.00059837	9.97E-05	0.00021263
g__Rothia	0	0	0	0.00035902	0.0002991	0.00063789
g__Brevundimonas	0	0	0	0.00035902	0.0001994	0.00021263
g__Nocardioides	0	0	0	0.00035902	9.97E-05	0.00021263
g__Fusibacter	0	0	0	0.00023935	0.0003988	0.00297682
g__Marinomonas	0	0	0	0.00011967	0.0003988	0.0021263
g__Epulopiscium	0	0	0	0.00011967	0.0003988	0.00106315
g__Agrobacterium	0	0	0	0.00011967	0.0004985	0.00021263
g__Staphylococcus	0	0	0	0.00813787	0.00418744	0.00085052

Supplementary Table S10. Relative frequency of unique genera and species in sequenced wt and cultured samples.

spp_helathy_vs_wt_hepatopancreas		hepatopancreas				
Treatment	healthy cultured			diseased cultured		
Description	139-H	68B-H	7A-H	h 1 wt	h 2 wt	h 3 wt
Zobellella taiwanensis	0.00108108	0.00025853	0.00044924	0	0	0
Enterobacter cowanii	0.00081081	0.00025853	0.00112309	0	0	0
Haemophilus parainfluenzae	0	0	0	0.01244615	0.01186441	0.00042526
Rothia mucilaginosa	0	0	0	0.00347056	0.01625125	0.00063789
Staphylococcus aureus	0	0	0	0.00155577	0.00179462	0.00021263
Vibrio harveyi	0	0	0	0.00143609	0.00089731	0.0323198
Micrococcus luteus	0	0	0	0.00071805	0.00159521	0.00042526
Rothia dentocariosa	0	0	0	0.00059837	0.00119641	0.00063789
Rothia aeria	0	0	0	0.00035902	0.0002991	0.00063789
Brevundimonas poindexterae	0	0	0	0.00035902	0.0001994	0.00021263
Marinomonas primoryensis	0	0	0	0.00011967	0.0003988	0.0021263

Supplementary Table S11. Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased intestine using LefSe.

Diseased		Healthy	
Phyla	LDA	Phyla	LDA
NS		NS	
Class	LDA	Class	LDA
NS		c_Flavobacteriia	2.382
Order	LDA	Order	LDA
NS		o_Xanthomonadales	2.464
		o_Flavobacteriales	2.349
Family	LDA	Family	LDA
f_Halomonadaceae	2.421	f_Coxiellaceae	2.539
		f_Flavobacteriaceae	2.395
		f_Xanthomonadaceae	2.267
		f_Piscirickettsiaceae	2.234
		f_Rhodothermaceae	2.222
Genus	LDA	Genus	LDA
g_Clostridium	2.147	g_Pantoea	2.526
		g_Umboniibacter	2.233

Supplementary Table S12. Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased hepatopancreas using LefSe.

Diseased Phyla	LDA	Healthy Phyla	LDA	Genus	LDA	Genus	LDA
p_Spirochaetes		N/S		g_Propionigenium	3.243	g_Idiomarina	2.591
p_Fusobacteria				g_Aeromonas	3.097	g_Faecalibacterium	2.537
p_Verrucomicrobia				g_Teredinibacter	2.978	g_Clostridiisalibacter	2.317
				g_Planctomyces	2.858		
Class	LDA	Class	LDA	g_Microbispora	2.839		
c_Leptospirae	4.096	c_TK17	2.826	g_Desulfotalea	2.818		
c_Fusobacteriia	3.650			g_Shinella	2.777		
c_Deltaproteobacteria	3.640			g_Simiduia	2.714		
c_Planctomycetia	2.841			g_Parabacteroides	2.648		
				g_Thiothrix	2.590		
Order	LDA	Order	LDA				
o_Leptospirales	4.111	N/S					
o_Alteromonadales	4.080						
o_Chromatiales	3.828						
o_Fusobacteriales	3.598						
o_Desulfobacterales	3.563						
o_Oceanospirillales	3.319						
o_Aeromonadales	3.082						
o_R76_B128	2.913						
o_Planctomycetales	2.865						
o_NB1_j	2.558						
o_Rickettsiales	2.472						
Family	LDA	Family	LDA				
f_OM60	3.767	f_Microbacteriaceae	3.236				
f_Fusobacteriaceae	3.550	f_Peptococcaceae	2.435				
f_Desulfobulbaceae	3.359						
f_Aeromonadaceae	3.077						
f_Rhizobiaceae	2.882						
f_Planctomycetaceae	2.863						
f_JTB38	2.706						
f_Porphyromonadaceae	2.650						
f_Pelagibacteraceae	2.612						
f_Phylobacteriaceae	2.468						
f_Geobacteraceae	2.396						

Supplementary Table S13.Linear Discriminant Analysis of enriched taxonomies in cultured healthy and diseased sediment usir

Diseased		Healthy	
Phyla	LDA	Phyla	LDA
p_Spirochaetes	2.967	p_Chlamydiae	2.630
p_Verrucomicrobia	2.681		
Class	LDA	Class	LDA
c_Spirochaetes	2.858	c_Acidimicrobiia	4.107
c_Leptospirae	2.803	c_BPC102	3.477
c_OPB41	2.664	c_Gemm_4	2.910
		c_Thermoleophilia	2.823
		c_Chlamydiia	2.709
		c_RB25	2.689
Order	LDA	Order	LDA
o_Desulfobacterales	3.859	o_Pseudomonadales	4.524
o_Myxococcales	3.092	o_Acidimicrobiales	4.135
o_Rickettsiales	2.786	o_B110	3.465
o_Leptospirales	2.779	o_Marinicellales	3.271
		o_Burkholderiales	3.056
		o_Chlamydiales	2.681
Family	LDA	Family	LDA
f_Desulfobulbaceae	3.663	f_Moraxellaceae	4.438
f_Desulfobacteraceae	3.466	f_koll13	3.990
f_Nannocystaceae	3.060	f_Corynebacteriaceae	3.557
f_Sinobacteraceae	2.763	f_Marinicellaceae	3.310
f_Desulfuromonadaceae	2.605	f_Nocardiodaceae	2.844
f_Francisellaceae	2.556	f_Criblamydiaceae	2.542
Genus	LDA	Genus	LDA
g_Sediminimonas	3.087	g_Acinetobacter	4.424
g_Plesiocystis	3.035	g_Microbulbifer	3.682
g_Desulfococcus	2.990	g_Corynebacterium	3.547
g_Flavobacterium	2.943	g_Enhydrobacter	2.736
g_Desulfotignum	2.905	g_Yonghaparkia	2.595
g_Inquilinus	2.804		
g_Halorhodospira	2.667		
g_Desulfobacter	2.609		
g_Acidaminobacter	2.571		
g_Alishewanella	2.554		
g_Salinivibrio	2.524		
g_Salinibacter	2.501		

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples.

genera_healthy_vs_dis_intestine		intestine					
Treatment		healthy cultured			diseased cultured		
Description		139-i	68B-i	7A-i	27B-i	2B-i	49B-i
g__Crenothrix		0.000580046	0.00013257	0.00012945		0	0
g__Microbispora			0	0	0 0.00017479	0.00013238	0.00017153
g__Shinella			0	0	0 0.00017479	0.00013238	0.00017153
g__Teredinibacter			0	0	0 0.00017479	0.00013238	0.00017153

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples.

spp_healthy_vs_dis_intestine		intestine					
Treatment	Treatment		healthy cultured			diseased cultured	
Description	Description	139-i	68B-i	7A-i	27B-i	2B-i	49B-i
g__Microbispora	s__rosea		0	0	0 0.00017479	0.00013238	0.00017153
g__Teredinibacter	s__turnerae		0	0	0 0.00017479	0.00013238	0.00017153

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples.

genera_healthy_vs_dis_hepatopancreas		hepatopancreas					
Treatment		healthy cultured			diseased cultured		
Description		139-H	68B-H	7A-H	27B-H	2B-H	49B-H
g__Escherichia		0	0	0	0.00019429	0.00025272	0.00015411

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased sample

spp_healthy_vs_dis_hepatopancreas		hepatopancreas					
Treatment		healthy cultured			diseased cultured		
Description		139-H	68B-H	7A-H	27B-H	2B-H	49B-H
g__Escherichia blattae		0	0	0	0.00019429	0.00025272	0.00015411

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples.

genera_healthy_vs_dis_sediment		pond sediment					
Treatment		healthy cultured			diseased cultured		
Description		139-S	68B-S	7A-S	27B-S	2B-S	49B-S
g__Pseudomonas		0.00013868	0.00096899	0.00048123	0	0	0
g__Inquilinus		0	0	0	0.00127199	0.00070771	0.0009113
g__Desulfobacter		0	0	0	0.000212	0.00017693	0.00060753
g__Salinibacter		0	0	0	0.000106	0.00053079	0.00060753
g__Flavobacterium		0	0	0	0.000106	0.004954	0.00030377
g__Halothiobacillus		0	0	0	0.000106	0.00053079	0.00030377
g__Acidaminobacter		0	0	0	0.000106	0.00017693	0.00030377
g__Alishewanella		0	0	0	0.000106	0.00017693	0.00030377
g__Salinivibrio		0	0	0	0.000106	0.00017693	0.00030377

Supplementary Table S14. Relative frequency of unique genera and species in sequenced healthy and diseased samples

spp_healthy_vs_dis_sediment		sediment					
Treatment		healthy cultured			diseased cultured		
Description		139-S	68B-S	7A-S	27B-S	2B-S	49B-S
g__Pseudomonas	s__veronii	0.00013868	0.00096899	0.00048123	0	0	0
g__Salinivibrio	s__costicola	0	0	0	0.000106	0.00017693	0.00030377

Supplementary Table S15. AP3 PCR diagnostic results.

Sample		AP3 test
wt intestine	IS	EMS-
	1IS	EMS-
	2IS	EMS-
healthy cultured intestine	7A-I	EMS+
	68B-I	EMS-
	139-I	EMS-
diseased cultured intestine	49B-I	EMS+
	27B-I	EMS+
	2B-I	EMS-
wt hepatopancreas	HS	EMS-
	3HS	EMS-
	1HS	ND
healthy cultured hepatopancreas	7A-H	EMS+
	68B-H	EMS+
	139-H	EMS+
diseased cultured hepatopancreas	49B-H	EMS+
	27B-H	EMS+
	2B-H	EMS+
healthy pond sediment	7A-S	ND
	68B-S	ND
	139-S	ND
diseased pond sediment	27B-S	ND
	49B-S	ND
	2B-S	ND

ND= Not determined because the DNA amount was not sufficient

EMS+ = positive to AP3 PCR test

EMS- = negative to AP3 PCR test

Supplementary Table S16. Relative abundance of 7 age discriminating OTUs present in the sequenced samples.

Constructed from biom file																		
#OTU ID	Intestine wt			healthy cultured intestine			diseased cultured intestine			Hepatopancreas wt			healthy cultured hepatopancreas			diseased cultured hepatopancreas		
	i 1 wt	i 2 wt	i 3 wt	139-I	68B-I	7A-I	49B-I	27B-I	2B-I	h 1 wt	h 2 wt	h 3 wt	139-H	68B-H	7A-H	49B-H	27B-H	2B-H
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__Nautella; s__italica	0	0	0	0	0.00013257	0	0	0.00017479	0	0	0	0	0	0	0	0	0	0
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__S__	0.00094853	0	0.00018651	0	0.00013257	0	0	0.00017479	0	0.00011967	0	0	0	0	0	0	0	0.00353803
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__S__	0	0	0	0	0.00026515	0.0002589	0.00051458	0	0.00026476	0	0	0	0	0	0	0	0	0
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Vibrionales; f__Pseudoalteromonadaceae; g__S__	0	8.82924E-05	0.00027977	0	0.00053029	0.00012945	0	0	0.00039714	0	9.97009E-05	0	0	0	0	0	0	0
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__S__	0.00012647	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__Dinoroseobacter; s__	6.3235E-05	0	0	0	0.00026515	0	0.00102916	0.0033211	0.00026476	0	0	0	0.00027027	0	0.00022462	0	0.00019429	0
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Vibrionales; f__Vibrionaceae; g__Vibrio; s__shilonii	0	0.000264877	0.00018651	0.00058005	0.00092801	0.0005178	0	0	0	0	0	0	0.00027027	0	0	0	0	0

Supplementary Table S17. Weight of the wild-type and cultured shrimps used for the extraction of sequenced intestines and hepatopancreas.

Wild-type shrimps				One-way ANOVA
	Weight (g)	Average weight (g)	Std.Dev	Significant? p<0.05
1 wt	41.4	43.8	2.1	NO
2 wt	43.6			
3 wt	46.5			

Healthy cultured shrimps				One-way ANOVA
	Weight (g)	Average weight (g)	Std.Dev	Significant? p<0.05
139	15.9	17.1	1.2	NO
68B	16.6			
7A	18.7			

Diseased cultured shrimps				One-way ANOVA
	Weight (g)	Average weight (g)	Std.Dev	Significant? p<0.05
49B	15.3	15.3	0.4	NO
27B	15.8			
2B	14.8			