

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table 1: Sampling Metadata for 2012 Puget Sound Locations	2
Table 2: DNA Quantity Assessment of 2012 Puget Sound Locations.....	2
Table 3: Sampling Metadata for 2010 Puget Sound Locations	2
Table 4: Antibiotic Resistance Database Index Results.....	3

SUPPLEMENTARY FIGURES

Figure 1: Rarefaction Curves of 2012 Sampling Locations.....	4
Figure 2: Class Distribution of 2012 Sampling Locations	4
Figure 3: Order Distribution of 2012 Sampling Locations.....	5
Figure 4: Phylum Level Annotation using the 16S rRNA Gene	6
Figure 5: Class Level Annotation using the 16S rRNA Gene.....	7
Figure 6: Public Health Relevant Functional Distribution of 2012 Samples.....	8
Figure 7: Functional Drivers of Public Health Relevant Functions of 2012 Samples.....	9
Figure 8: Actinobacteria Abundance versus Salinity	10
Figure 9: Firmicutes Abundance versus Salinity.....	11
Figure 10: Proteobacteria Abundance versus Salinity	11
Figure 11: Bacteroidetes Abundance versus Temperature.....	12
Figure 12: Firmicutes Abundance versus Temperature	13
Figure 13: Proteobacteria Abundance versus Bacteroidetes Abundance.....	14

Supplementary Tables

Sampling Metadata	P28 2011	P32 2011	HHP	Hoodsport	JBP	Marina 2012	WWTP 2012
Latitude	47.700556	47.333333	47.561024	47.403256	47.584796	47.686743	47.661667
Longitude	-122.450556	-122.434722	-122.350121	-123.141155	-122.368733	-122.403775	-122.433333
Collected	10/10/11	10/10/11	7/10/12	7/11/12	7/9/12	7/12/12	8/1/12
Depth [m]	5.00	5.00	5.00	5.00	5.00	0.50	NA
Temperature [°C]	11.89	12.41	15.21	18.85	13.44	15.02	20.26
pH	na	na	7.89	8.16	8.03	8.26	7.01
mV [pH]	na	na	-51.41	-80.94	-66.62	-78.03	-3.55
ORP [mV]	na	na	1146.77	1133.39	1117.01	1143.33	1132.87
Conductivity [µS/cm]	na	na	24513.00	35719.50	39224.00	36890.50	867.35
EC Abs. [µS/cm]	na	na	19951.00	31549.00	30615.50	29891.00	789.15
Resistivity [Ohm-cm]	na	na	40.80	28.00	25.30	27.00	1152.85
TDS [ppm]	na	na	12253.50	17857.00	19610.00	18443.00	433.50
Salinity [psu]	30.03	29.28	14.97	22.61	25.04	23.42	0.43
Density [Sigma T]	na	na	10.55	15.61	18.61	17.07	0.00
Pressure [psi]	na	na	14.92	15.00	14.85	14.89	14.91
Dissolved Oxygen [% sat]	169.06	206.88	136.59	107.24	125.08	95.79	121.50
Dissolved Oxygen [ppm]	5.53	6.77	12.41	8.73	10.74	8.16	11.12
Turbidity [fnu]	na	na	2.65	1.92	1.03	0.74	15.72
Enterococcus [mpn]	na	na	25.00	<10	<10	25.00	>2419.6

Supplementary Table 1. Sampling Metadata for 2012 Puget Sound Locations

Location	Nanodrop (ng/µl)	Picogreen (ng/µl)
P28 2011	131	81
P32 2011	177	151
Herring's House Park	127	107
Hoodsport	124	95
Jack Block Park	122	98
Marina 2012	112	98
WWTP 2012	174	157

Supplementary Table 2. DNA Quantity Assessment of 2012 Puget Sound Locations.

Sampling Metadata	P28 2010	P32 2010	Marina 2010	WWTP 2011	P1	P5	P26
Latitude	47.700556	47.333333	47.686743	47.661667	48.016667	47.883333	48.368056
Longitude	-122.450556	-122.434722	-122.403775	-122.433333	122.300556	-122.366667	122.716667
Collected	10/29/10	10/30/10	12/20/10	1/31/11	10/29/10	10/29/10	10/29/10
Depth [m]	5	5	0.5	NA	5	5	5
Temperature [°C]	11.51	11.48	8.6*	14.9	11.5	11.53	9.5
Salinity [psu]	32.2	30.15	18.7*	<0.5	30.02	30	30.6

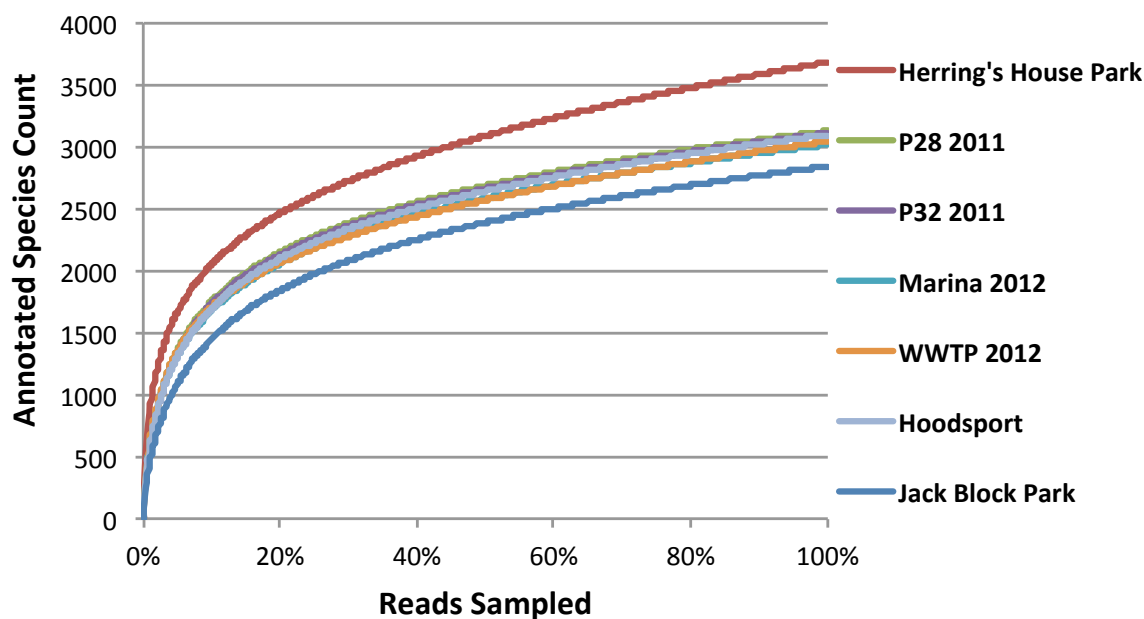
*Estimates based on nearby measurements

Supplementary Table 3. Sampling Metadata for 2010-2011 Puget Sound Locations.

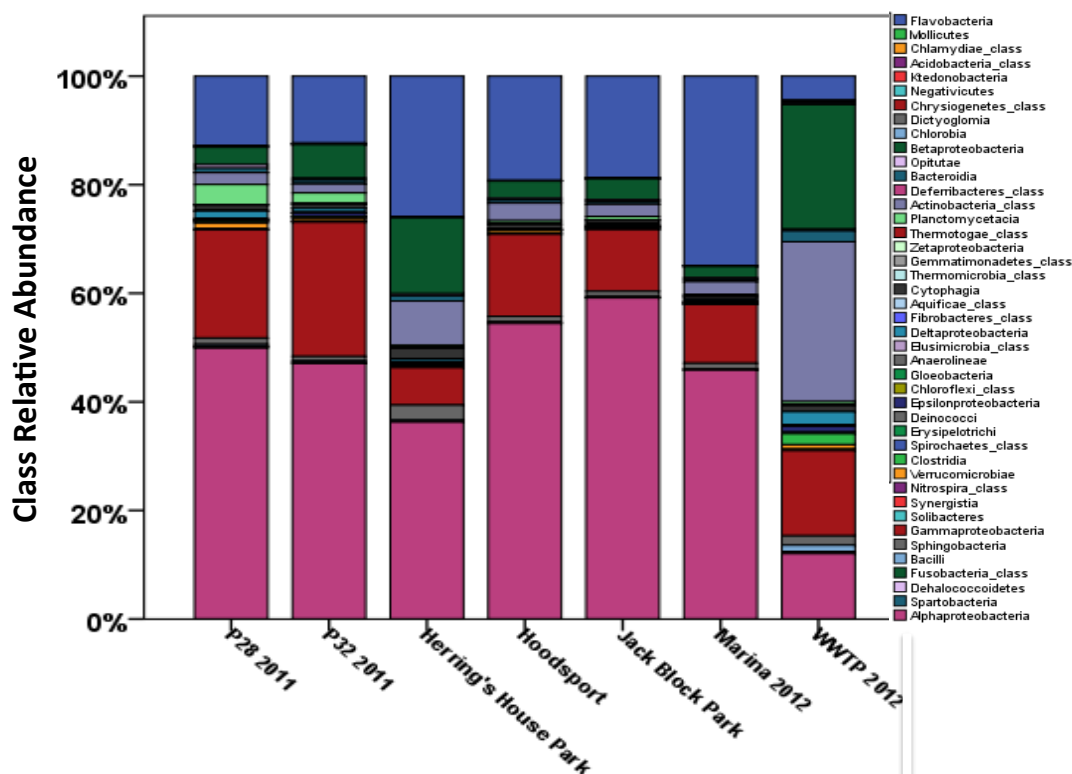
Location	Sample	ARGs	MRGs	Plasmids	TEs	Phages	Pathogens
Open Sound	P1	0.0000%	0.14%	0.01%	0.11%	0.58%	0.0000%
	P5	0.0000%	0.09%	0.01%	0.11%	0.61%	0.0000%
	P26	0.0008%	0.09%	0.00%	0.07%	2.33%	0.0000%
	P28 2010	0.0000%	0.15%	0.01%	0.07%	0.44%	0.0020%
	p28 2011	0.0008%	0.60%	0.01%	0.09%	0.75%	0.0025%
	P32 2010	0.0000%	0.09%	0.01%	0.08%	1.05%	0.0000%
	p32 2011	0.0000%	0.59%	0.01%	0.12%	1.05%	0.0033%
Nearshore	HHP	0.0021%	0.63%	0.02%	0.17%	0.53%	0.0024%
	Hoodsport	0.0000%	0.58%	0.02%	0.11%	2.69%	0.0020%
	JBP	0.0000%	0.59%	0.03%	0.18%	1.22%	0.0000%
	Marina 2010	0.0020%	0.26%	0.02%	0.15%	0.63%	0.0025%
	Marina 2012	0.0010%	0.74%	0.03%	0.20%	0.94%	0.0076%
WWTP	WWTP 2011	0.0158%	0.52%	0.31%	0.60%	0.45%	0.0279%
	WWTP 2012	0.0131%	0.70%	0.42%	0.54%	0.73%	0.0156%

Supplementary Table 4. Relative abundances of Antibiotic Resistance Determinant (ARD) Index per sample. Samples were grouped according to their location (Open Sound, Nearshore, WWTP).

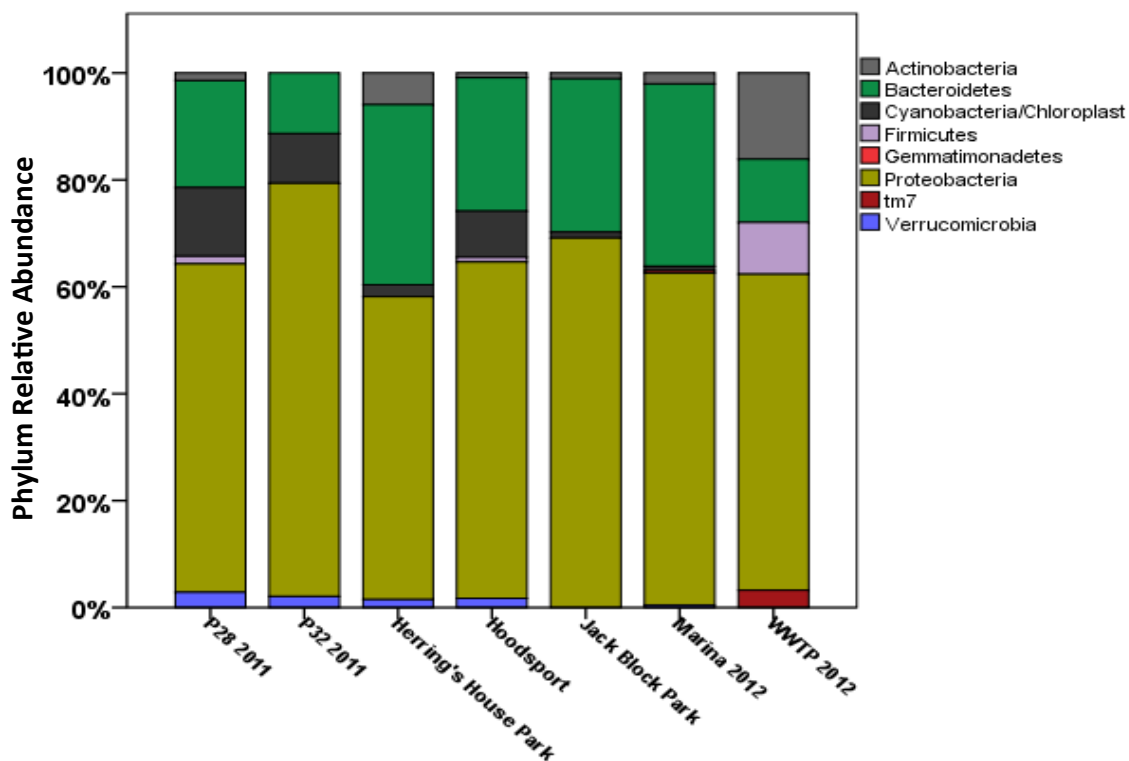
Supplementary Figures



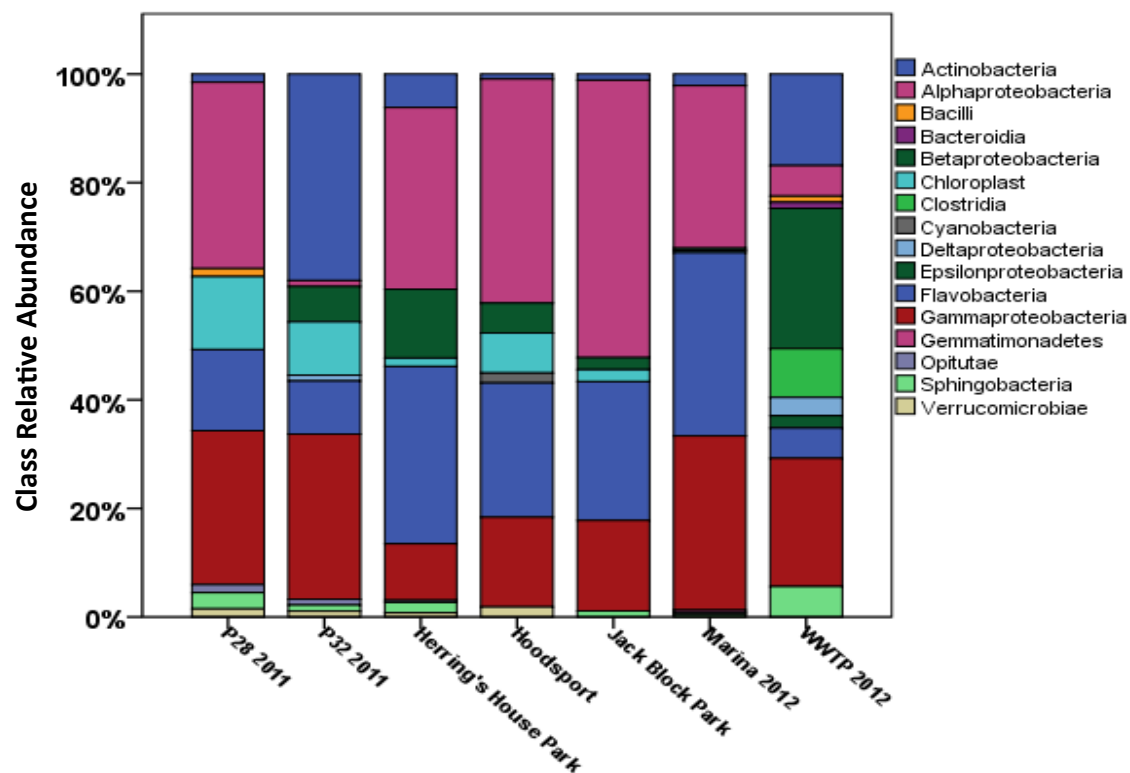
Supplementary Figure 1. Rarefaction Curves (Species Accumulation Curves) of 2012 Samples using total percentage of reads and annotated species richness.



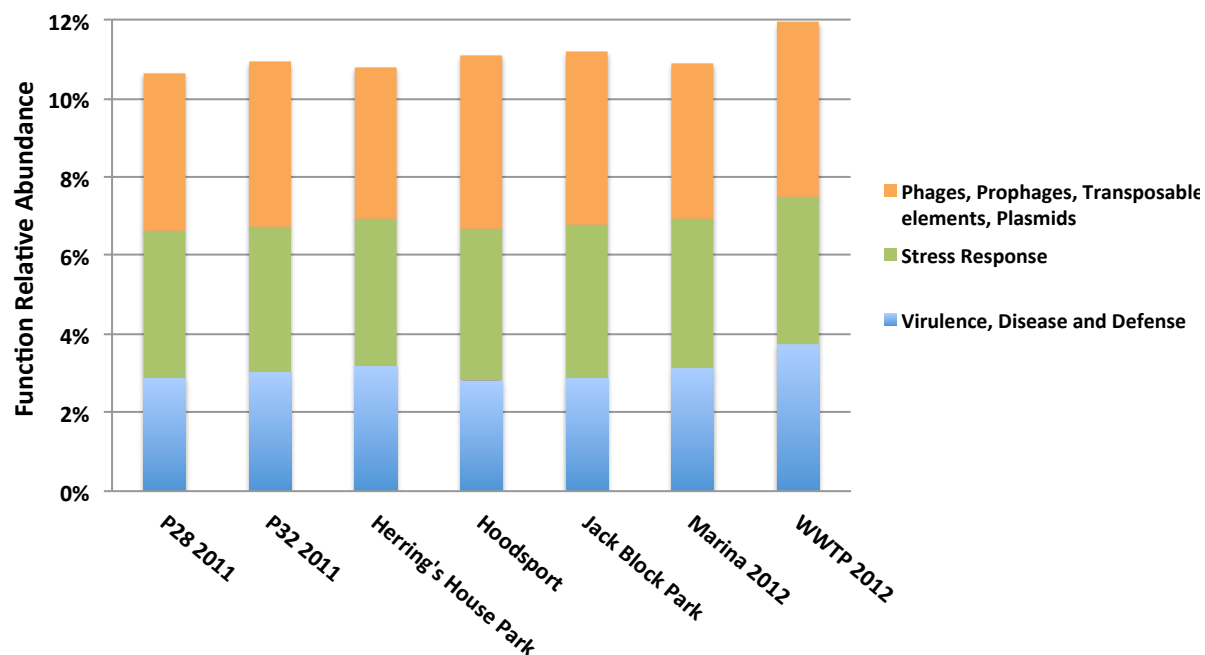
Supplementary Figure 2. Class Distribution of 2012 Samples with LCA annotation using the MG-RAST Pipeline. Class abundance counts were normalized to the total number of Class abundance counts per sample.



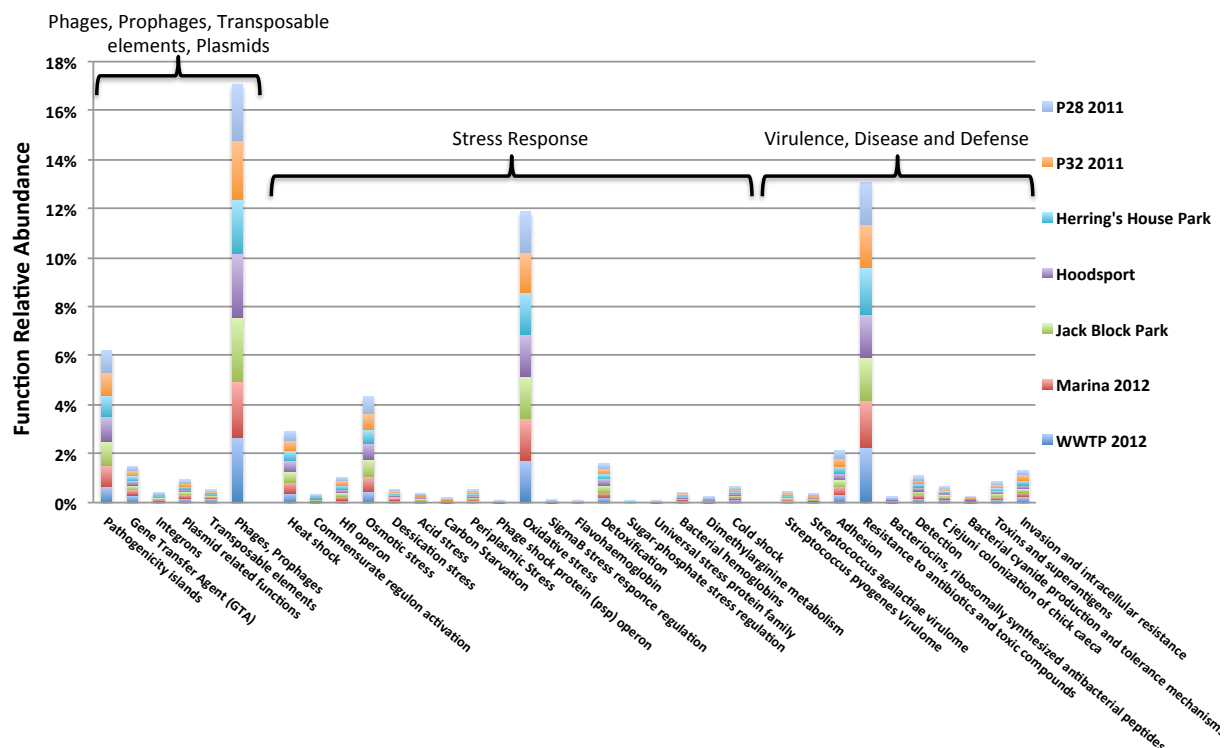
Supplementary Figure 4. 16S rRNA Gene Phylum Distribution. Phylum Distribution of 2012 Samples using the 16S rRNA Gene annotation from the Ribosomal Database Project Classifier. Phylum abundance counts were normalized to the total number of Phylum abundance counts per sample.



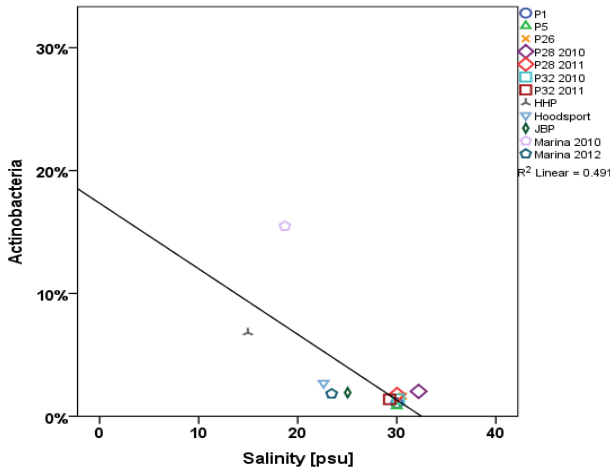
Supplementary Figure 5. 16S rRNA Gene Class Distribution. Class Distribution of 2012 Samples using the 16S rRNA Gene annotations from the Ribosomal Database Project Classifier. Class abundance counts were normalized to the total number of Class abundance counts per sample.



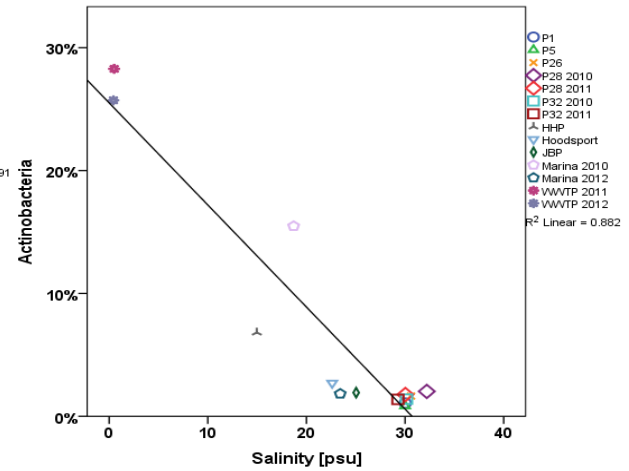
Supplementary Figure 6. Public Health Relevant Functional Distribution of 2012 Samples with SEED subsystems Level 1 annotation using the MG-RAST Pipeline. Functional abundance counts were normalized to the total number of Level 1 abundance counts per sample.



Supplementary Figure 7. Functional Drivers of Public Health Relevant Functions of 2012 Samples with SEED subsystems Level 2 annotation using the MG-RAST Pipeline. Functional abundance counts were normalized to the total number of Level 2 abundance counts per sample.

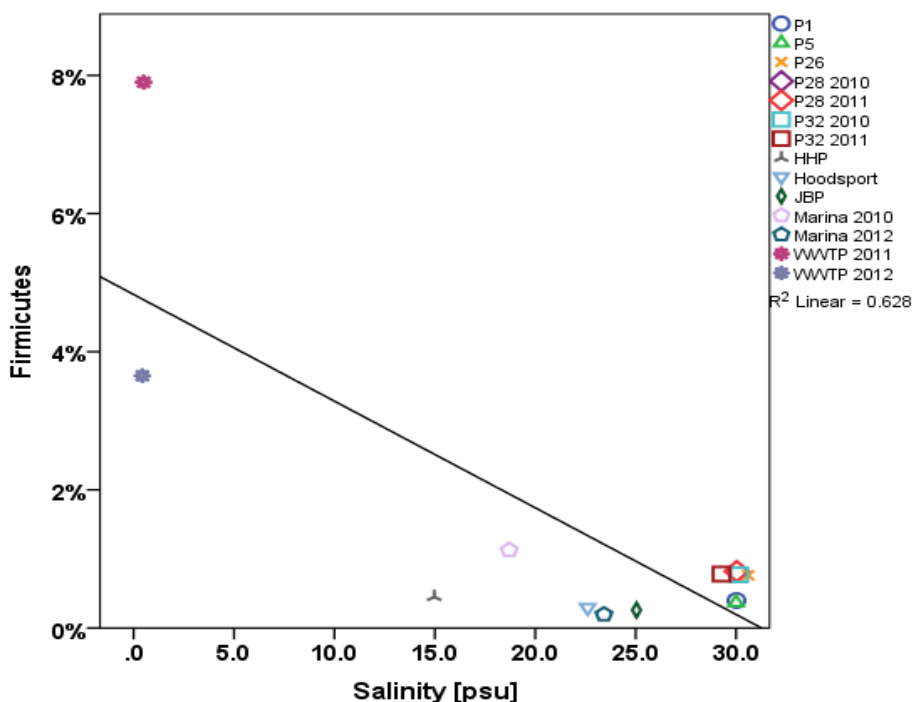


a. Without WWTP Effluents

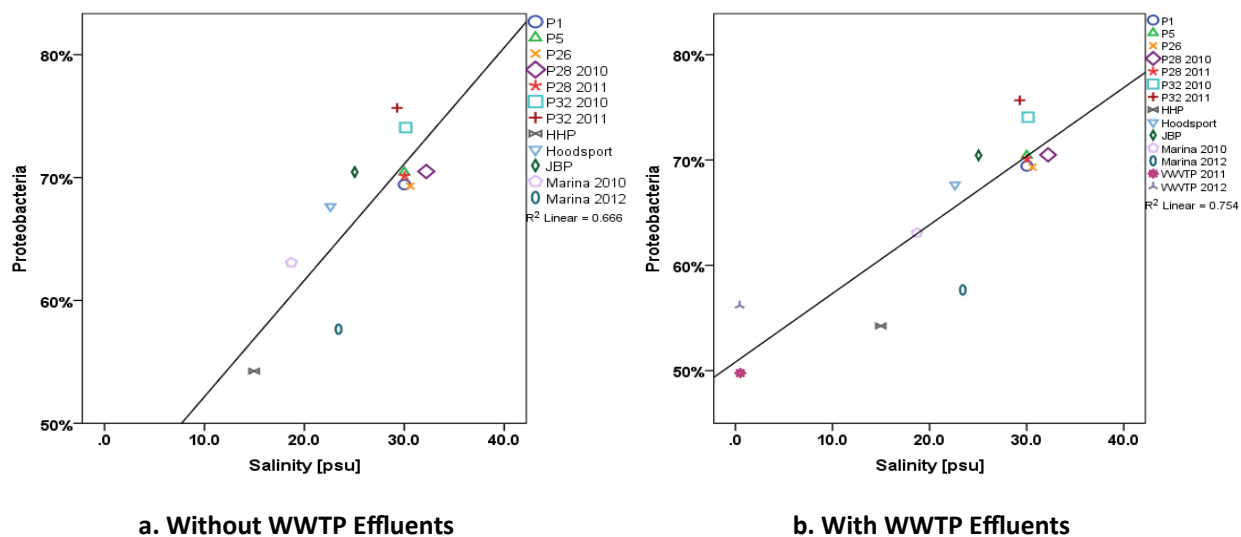


b. With WWTP Effluents

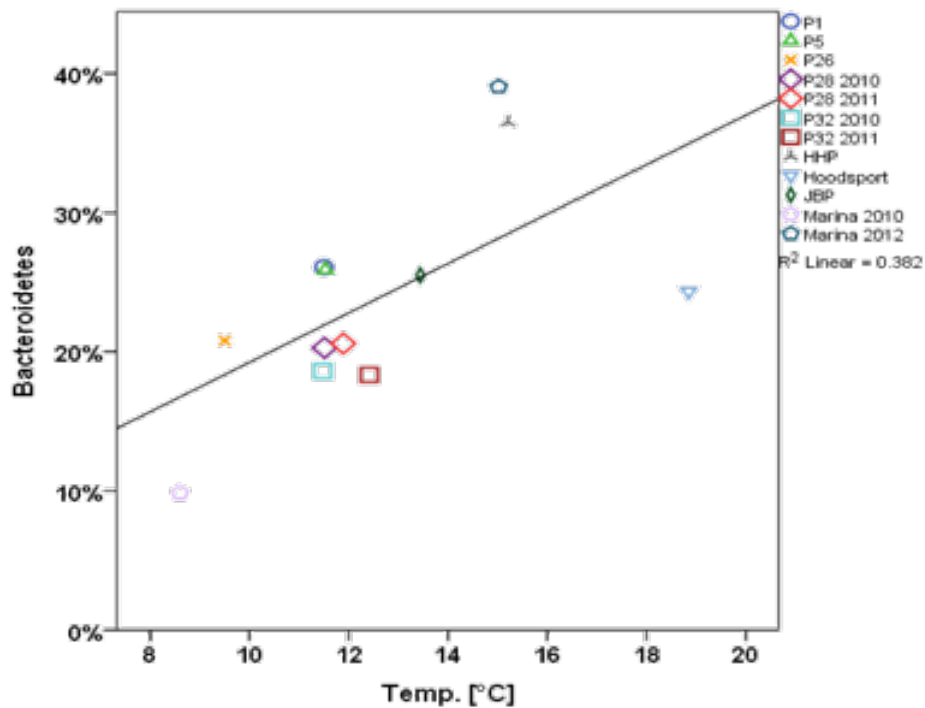
Supplementary Figure 8. Actinobacteria Abundance versus the Salinity. Relationship between the relative abundance of Actinobacteria and Salinity. A statistically significant negative correlation was observed between the relative abundances of Actinobacteria and Salinity in a. all Puget Sound samples not including WWTP effluents and b. All Puget Sound samples including WWTP effluents.



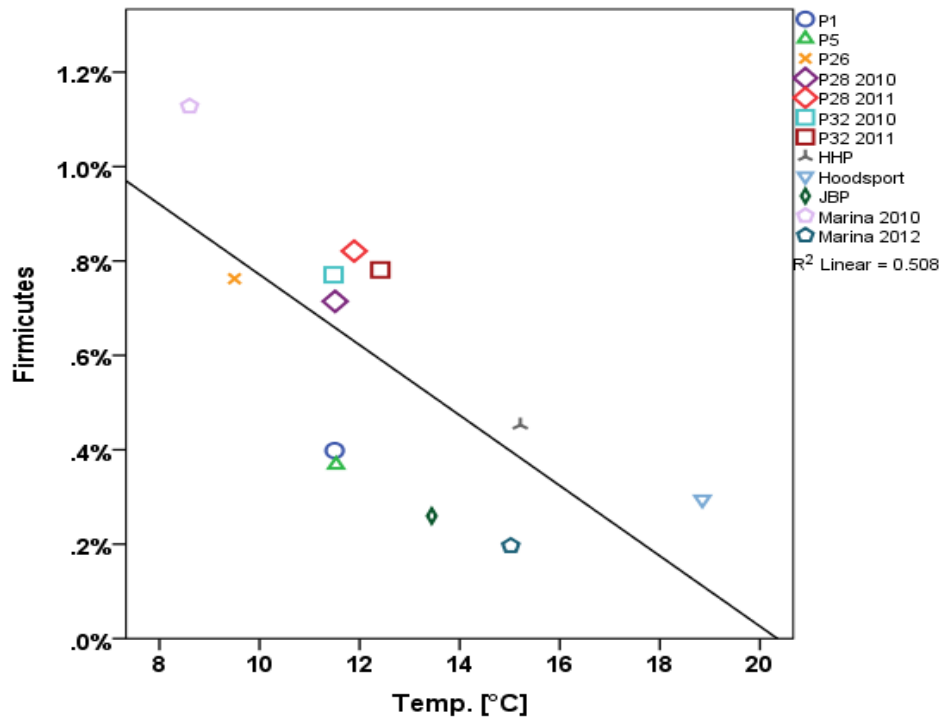
Supplementary Figure 9. Relationship between the relative abundance of Firmicutes and Salinity. A statistically significant negative correlation was observed between the relative abundances of Firmicutes and Salinity in all Puget Sound samples including the WWTP effluents.



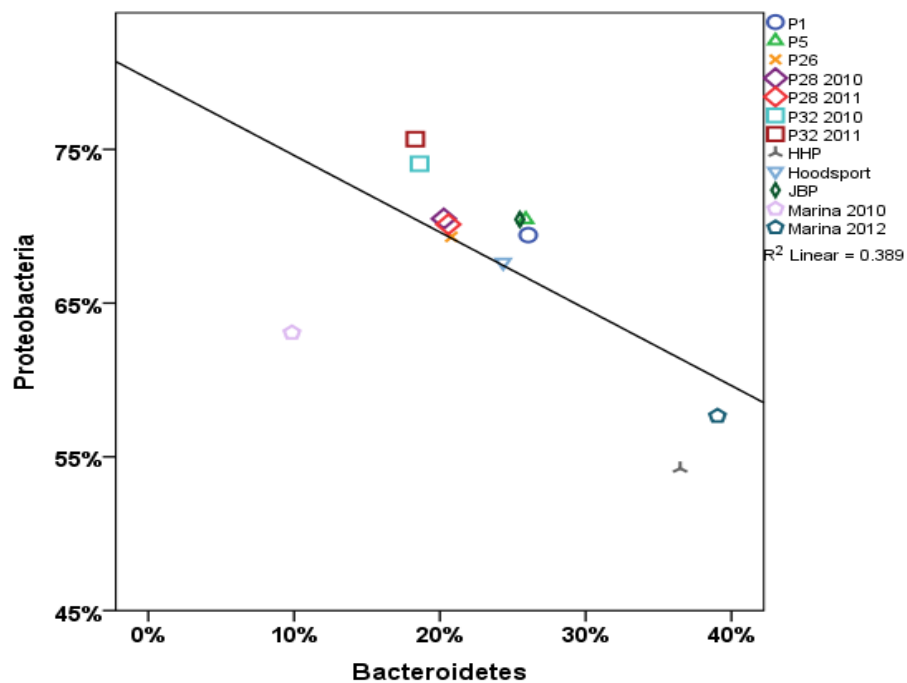
Supplementary Figure 10. Relationship between the relative abundance of Proteobacteria and Salinity. A statistically significant positive correlation was observed between the relative abundances of Proteobacteria and Salinity in a. all Puget Sound samples not including WWTP effluents and b. All Puget Sound samples including WWTP effluents.



Supplementary Figure 11. Bacteroidetes Abundance versus Temperature. Relationship between the relative abundance of Bacteroidetes and Temperature. A statistically significant positive correlation was observed between the relative abundances of Bacteroidetes and Temperature in all Puget Sound samples not including WWTP effluents.



Supplementary Figure 12. Firmicutes Abundance versus Temperature. Relationship between the relative abundance of Firmicutes and Temperature. A statistically significant negative correlation was observed between the relative abundances of Firmicutes and Temperature in all Puget Sound samples not including WWTP effluents.



Supplementary Figure 13. Proteobacteria Abundance versus Bacteroidetes Abundance. A statistically significant negative correlation was observed between the relative abundances of Proteobacteria and Bacteroidetes in all Puget Sound samples not including WWTP effluents.