Supplemental Materials: A global comparison of the bacterial communities of bilge water, boat surfaces and external port water

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Keywords: Microbial community composition, bilge water, vessels, port water microbes.

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Table S1: Description of the samples collected for each site.

Location	**Engine Type**	**Number Port Water Samples (pre-filter)**	**Number Port Water Samples (post-filter)**	**Number Bilge Samples (pre-filter)**	**Number Bilge Samples (post-filter)**	**Number Swab Samples**
Baltimore, MD, U.S.A.	*Outboard*	30	30	0	0	7
Busan, South Korea	*Inboard*	32	31	1	0	5
Charleston, SC, U.S.A.	*Outboard*	31	31	1	1	9
Duluth Fall, MN, U.S.A.	*Outboard*	0	32	0	1	0
Duluth Summer, MN, U.S.A.	*Outboard*	31	30	1	1	4
Galveston, TX, U.S.A.	*Outboard*	26	27	0	0	7
Green Bay Fall, WI, U.S.A.	*Outboard*	0	35	0	1	0
Green Bay Summer, WI, U.S.A.	*Outboard*	32	32	1	1	4
Hong Kong	*Inboard*	34	33	0	0	6
Keweenaw Summer, MI, U.S.A.	*Outboard*	38	37	2	1	5
Keweenaw Fall, MI, U.S.A.	*Outboard*	0	36	0	0	0
Los Angeles, CA, U.S.A.	*Outboard*	31	33	1	1	5
Martigues, France	*Inboard*	31	31	1	1	4

Naples, Italy	*Inboard*	28	29	1	1	6
New Orleans, LA, U.S.A.	*Outboard*	31	31	1	1	4
New York, NY, U.S.A.	*Outboard*	32	32	2	0	6
Norfolk, VA, U.S.A.	*Inboard*	30	30	0	0	8
Oakland, CA, U.S.A.	*Outboard*	29	29	1	1	5
Rotterdam, Netherlands	*Outboard*	31	32	2	2	7
Seattle, WA, U.S.A.	*Inboard*	30	30	3	0	5
Singapore	*Inboard*	32	33	2	2	10
Venice, Italy	*Inboard*	28	28	1	1	8
Wilhelmshaven, Germany	*Inboard*	32	29	0	0	6

Table S2: One-way ANOVA indicating significant difference between bacterial diversity of sample types for both alpha diversity measures (Shannon and Observed ASVs), demonstrating that the bacterial community on the boat is significantly different than the bacterial community of the port water.

Indices	**Degrees of Freedom**	**F Value**	**P Value**
Observed	3	16.68	9.48e-11
Shannon	3	34.96	<2e-16

Table S3: Summary of Tukey HSD (post hoc) test results. P-values for Observed ASVs (upper triangle) and Shannon diversity (lower triangle). These results show a statistically significant difference between each of the sample types for both alpha diversity metrics used. This suggests that the bacterial community in each of our sample types is unique and distinct from the other sample types.

	Port Water	**Bilge**	**Swab**
Port Water		**<0.0001**	**0.0193**
Bilge	**<0.0001**		**0.0003**
Swab	**<0.0001**	**0.0023**	

Table S4 PERMANOVA of sample types shows a statistically significant difference between each pairwise comparison of the sample types. Upper triangle shows p-values, lower triangle describes R^2 values

	Port Water	Bilge	Swab
Port Water		0.001	0.001
Bilge	0.004		0.001
Swab	0.008	0.021	

Table S5 Analysis with DESeq2 shows the enrichment of specific ASVs in swab samples vs. port water samples. This comparison showed 51 ASVs significantly enriched in the port water and 8 ASVs enriched in the swab samples relative to the port water.

Swab Enriched						
log2FoldChange	padj	Phylum	Class	Order	Family	Genus
4.68289	0.00094	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
4.63883	0.00228	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
4.5304	0.00382	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
3.15627	0.00401	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
3.08828	0.00054	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
2.97634	0.00005	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
2.4389	0.00174	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
2.30479	0.0007	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
Open Water Enriched						
log2FoldChange	padj	Phylum	Class	Order	Family	Genus
-3.81304	0.00008	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium
-3.25788	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-3.17197	0.0007	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-3.16064	0.00049	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium

-3.14717	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-3.07668	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-3.01977	0.00039	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-2.98304	0	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.9532	0.00011	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-2.89006	0	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.78538	0.00001	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.76347	0.00003	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.68748	0.00024	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.65892	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.61737	0.00014	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.60301	0.00448	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.57742	0.00263	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.53177	0.0007	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.50445	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-2.49813	0.00449	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-2.49532	0.00577	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NA
-2.49403	0.00104	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	NA

-2.46164	0.00967	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.42637	0.00005	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	Pseudohongiella
-2.42422	0.00011	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Litoricolaceae	Litoricola
-2.40735	0.0063	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.37089	0.00007	Proteobacteria	Alphaproteobacteria	Puniceispirillales	SAR116_clade	Candidatus_Puniceispirillum
-2.36697	0.00263	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aurantivirga
-2.34931	0.00013	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.32545	0.00095	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.31621	0.00008	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-2.30551	0.004	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11
-2.29235	0.00171	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.29012	0.00054	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.26492	0.00059	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.22698	0.00852	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella
-2.22175	0.00027	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.2198	0.007	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
-2.21209	0.00051	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
-2.20949	0.0006	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter

-2.19308	0.00113	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
-2.17369	0.00353	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.1615	0.00094	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.14396	0.00103	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.14061	0.00277	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.13133	0.00342	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.12326	0.00001	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-2.11231	0.00572	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.11214	0.0001	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.04224	0.00277	Proteobacteria	Gammaproteobacteria	SAR86_clade	NA	NA
-2.0357	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA

Table S6 DESeq enrichment table for bilge vs. port water comparison. Analysis with DESeq2 showed the enrichment of specific ASVs in swab samples vs. port water samples. This comparison showed 38 ASVs significantly enriched in the port water and no ASVs enriched in the bilge samples relative to the port water.

Open Water Enriched						
log2FoldChange	padj	Phylum	Class	Order	Family	Genus
-5.50948	0	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-5.11399	0.00001	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-4.77234	0.00004	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-4.56999	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-4.56019	0.00013	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-4.55037	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-4.41666	0.0003	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-4.3832	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-4.36032	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-4.31001	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-4.30388	0.00033	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-4.25212	0.00076	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-4.24254	0.00127	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia

-4.08377	0.00236	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-4.03043	0.00145	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-4.0211	0	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-4.00375	0.009	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-3.99199	0.0044	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-3.95214	0.00096	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-3.92089	0.00131	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-3.9067	0.00001	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-3.89526	0.00169	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-3.84627	0.00013	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.78317	0.00025	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.78044	0.00554	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-3.70614	0.00034	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-3.64932	0.00008	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-3.64651	0.00486	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans
-3.64639	0.00236	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.59221	0.001	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.48276	0.00025	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia

-3.3866	0.00486	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.38517	0.00358	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	Pseudohongiella
-3.25922	0.00997	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Formosa
-3.20628	0.0067	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_II	NA
-3.16597	0.00477	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-3.13174	0.00762	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-3.06206	0.00033	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia

Table S7: Average abundance of phyla which are higher in the bilge than in the port water in specific locations, demonstrating that there are observable differences between bilge and port water when observing specific taxa in specific locations, despite the fact that analysis with DESeq2 did not show any differentially enriched ASVs in the bilge water.

Average Abundance of Phyla in Bilge and Open Water			
Phylum	Location	Bilge	Port Water
Actinobacteria	Duluth Fall	0.063	0.0463
Actinobacteria	Green Bay Fall	0.0528	0.041
Actinobacteria	Keweenaw Summer	0.1742	0.1361
Actinobacteria	Los Angeles	0.2086	0.104
Actinobacteria	Martigues	0.3217	0.0592
Actinobacteria	Naples	0.1807	0.0992
Actinobacteria	Oakland	0.2067	0.0497
Actinobacteria	Rotterdam	0.2473	0.1259
Actinobacteria	Seattle	0.4704	0.0847
Actinobacteria	Singapore	0.3483	0.2085
Bacteroidetes	Duluth Fall	0.3116	0.2892
Bacteroidetes	Duluth Summer	0.2988	0.2777
Bacteroidetes	Green Bay Fall	0.2918	0.2894

Bacteroidetes	Green Bay Summer	0.2693	0.2063
Cyanobacteria	Busan	0.1606	0.0639
Cyanobacteria	Charleston	0.1706	0.0666
Cyanobacteria	Duluth Fall	0.1569	0.1025
Cyanobacteria	Green Bay Fall	0.0968	0.0768
Cyanobacteria	Keweenaw Summer	0.201	0.1833
Cyanobacteria	Los Angeles	0.1508	0.0964
Cyanobacteria	Martigues	0.071	0.0592
Cyanobacteria	Naples	0.518	0.2173
Cyanobacteria	New Orleans	0.2685	0.183
Cyanobacteria	New York	0.2337	0.0762
Cyanobacteria	Oakland	0.1301	0.0495
Cyanobacteria	Rotterdam	0.2893	0.0703
Cyanobacteria	Seattle	0.1056	0.0489
Cyanobacteria	Singapore	0.1802	0.0583
Cyanobacteria	Venice	0.3094	0.136
Firmicutes	New York	0.0334	0.0237
Planctomycetes	Green Bay Fall	0.0367	0.0333

Planctomycetes	Green Bay Summer	0.0462	0.0389
Planctomycetes	Keweenaw Summer	0.0594	0.0499
Planctomycetes	Rotterdam	0.0573	0.0415
Planctomycetes	Venice	0.069	0.0497
Proteobacteria	Busan	0.4753	0.4504
Proteobacteria	Naples	0.3726	0.3491
Proteobacteria	New Orleans	0.3926	0.368
Verrucomicrobia	Charleston	0.0391	0.036
Verrucomicrobia	Duluth Summer	0.0457	0.0403
Verrucomicrobia	Keweenaw Summer	0.0371	0.0303
Verrucomicrobia	Los Angeles	0.0345	0.0293
Verrucomicrobia	New York	0.0286	0.0278

Table S8 Analysis with DESeq2 to look for differentially abundant ASVs between the port water and the boat samples (both bilge and swab samples) showed 9 ASVs enriched in the boat samples relative to 80 ASVs enriched in the port water.

Boat Enriched						
log2FoldChange	padj	Phylum	Class	Order	Family	Genus
1.67520476	0.00396	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
2.117216792	0.00747	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
2.62357256	0.00576	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
2.679533669	0.00002	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
2.683503948	0.00500	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
4.232894371	0.00065	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
4.341155472	0.00051	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
4.36116871	0.00021	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Port Water Enriched						
log2FoldChange	padj	Phylum	Class	Order	Family	Genus
-3.98589338	0.00000	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium
-3.627910891	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-3.476387172	0.00000	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium
-3.328127303	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-3.289391299	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA

-3.286671052	0.00002	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-3.212086509	0.00000	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-3.139714786	0.00001	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-3.058909444	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.98147419	0.00289	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium
-2.946463558	0.00584	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Marinobacterium
-2.837718207	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.833624911	0.00000	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.818891948	0.00006	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.799045339	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-2.789886089	0.00011	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.776870598	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.773995812	0.00071	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.760196656	0.00616	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-2.758050187	0.00079	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.748600763	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.73975402	0.00009	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-2.73780539	0.00000	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307

-2.7226325	0.00019	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NA
-2.607066686	0.00000	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.605000972	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-2.599997519	0.00006	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aurantivirga
-2.599334776	0.00094	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	RS62_marine_group
-2.587357232	0.00001	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.569755334	0.00071	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.537846414	0.00038	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.532716002	0.00189	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.53239314	0.00002	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.489144396	0.00000	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Litoricolaceae	Litoricola
-2.471475622	0.00232	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-2.451510594	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.451158591	0.00000	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	Pseudohongiella
-2.448909412	0.00000	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.445319386	0.00010	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	NA
-2.444002731	0.00000	Proteobacteria	Alphaproteobacteria	Puniceispirillales	SAR116_clade	Candidatus_Puniceispirillum
-2.409252115	0.00001	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307

-2.405969499	0.00277	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NA
-2.400744675	0.00002	Proteobacteria	Gammaproteobacteria	SAR86_clade	NA	NA
-2.390444878	0.00000	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.390359391	0.00616	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-2.377501827	0.00007	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.371587598	0.00274	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.371114186	0.00000	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.362214954	0.00000	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.361438047	0.00012	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group
-2.342636632	0.00969	Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinaceae	Candidatus_Actinomarina
-2.333304181	0.00175	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
-2.332531382	0.00047	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.318014782	0.00000	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.298314289	0.00005	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
-2.290601547	0.00067	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11
-2.281251459	0.00086	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.268941967	0.00010	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.262450743	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina

-2.202111703	0.00008	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-2.196913802	0.00051	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-2.194811609	0.00008	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.190878275	0.00012	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.18874064	0.00022	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.175963376	0.00515	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.171666154	0.00430	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.168415656	0.00747	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-2.163763068	0.00232	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella
-2.134597222	0.00580	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
-2.128474264	0.00001	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-2.127184142	0.00512	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polynucleobacter
-2.110912092	0.00005	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-2.101333343	0.00221	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.095513591	0.00274	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11
-2.081516765	0.00269	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
-2.069929418	0.00082	Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinaceae	Candidatus_Actinomarina
-2.064307881	0.00050	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_II	NA

-2.05662602	0.00068	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.031945474	0.00167	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter
-2.03101215	0.00155	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-2.021819447	0.00127	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	Pseudohongiella
-2.010995555	0.00179	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Candidatus_Aquiluna
-1.999655128	0.00010	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_II	NA
-1.989488314	0.00468	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aurantivirga
-1.987216944	0.00206	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-1.98591858	0.00444	Bacteroidetes	Rhodothermia	Balneolales	Balneolaceae	Balneola
-1.985229021	0.00093	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-1.980786415	0.00078	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Lentibacter
-1.979641353	0.00221	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.925176695	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-1.925175871	0.00047	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-1.922156418	0.00000	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina
-1.913114761	0.00236	Bacteroidetes	Rhodothermia	Balneolales	Balneolaceae	Balneola
-1.894941702	0.00056	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-1.878475279	0.00944	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila

-1.863681232	0.00272	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
-1.855329054	0.00598	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Litoricolaceae	Litoricola
-1.814274145	0.00252	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Lentibacter
-1.789835045	0.00071	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29_marine_group
-1.776904839	0.00657	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	NA
-1.761137284	0.00254	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-1.754477522	0.00616	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophila
-1.752372401	0.00036	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.748942271	0.00304	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-1.742101367	0.00684	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella
-1.712721509	0.00045	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.695787039	0.00400	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	NA
-1.694730648	0.00950	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11
-1.676561497	0.00228	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA
-1.672902705	0.00013	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.667759442	0.00903	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Formosa
-1.655813676	0.00870	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans
-1.633442022	0.00585	Cyanobacteria	Oxyphotobacteria	Chloroplast	NA	NA

-1.56077625	0.00616	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Formosa
-1.55968514	0.00023	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.53354049	0.00005	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_I	Clade_Ia
-1.482313163	0.00576	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans
-1.423779936	0.00537	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29_marine_group

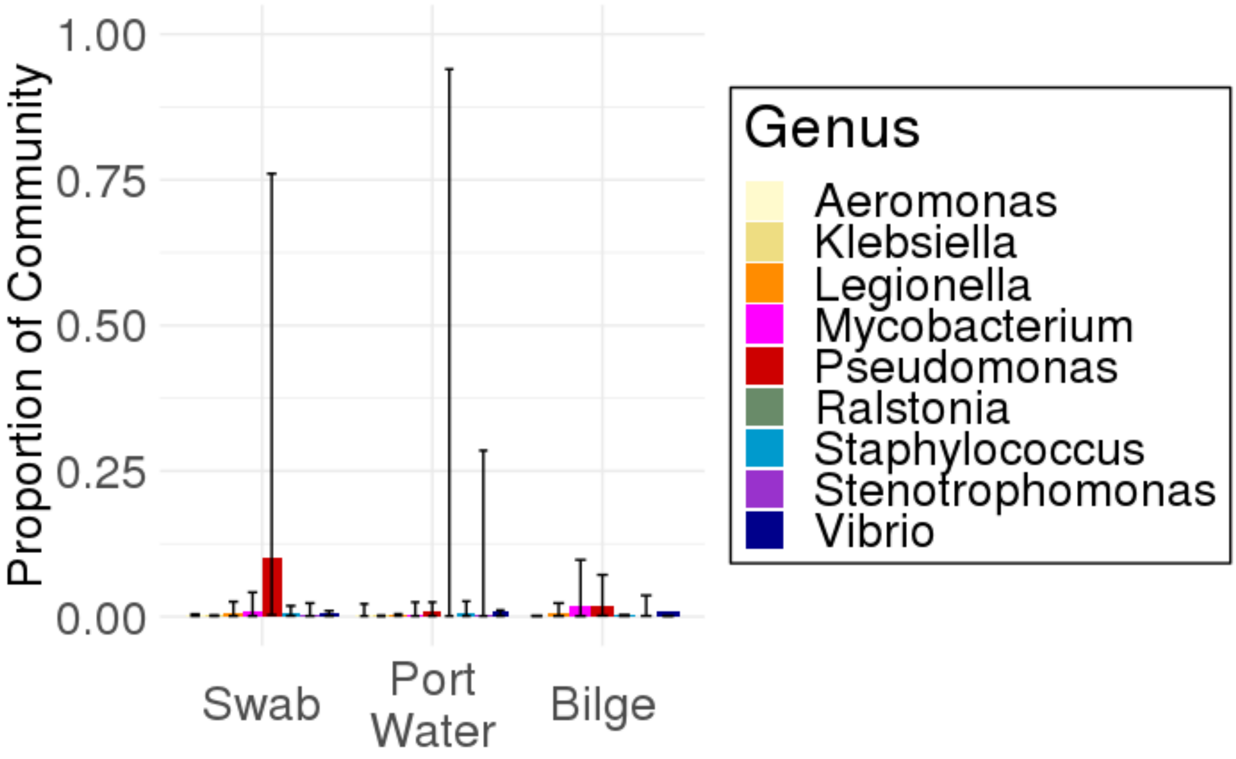

Figure S1 Average abundance of genera associated with pathogens is highly variable across each sample type. Port water samples had ten genera frequently associated with pathogens while swab and bilge samples represented seven and eight genera, respectively. Swab and bilge samples had a higher percentage of reads from genera associated with pathogens (13.4% and 6.0%, respectively) than port water (4.4%).

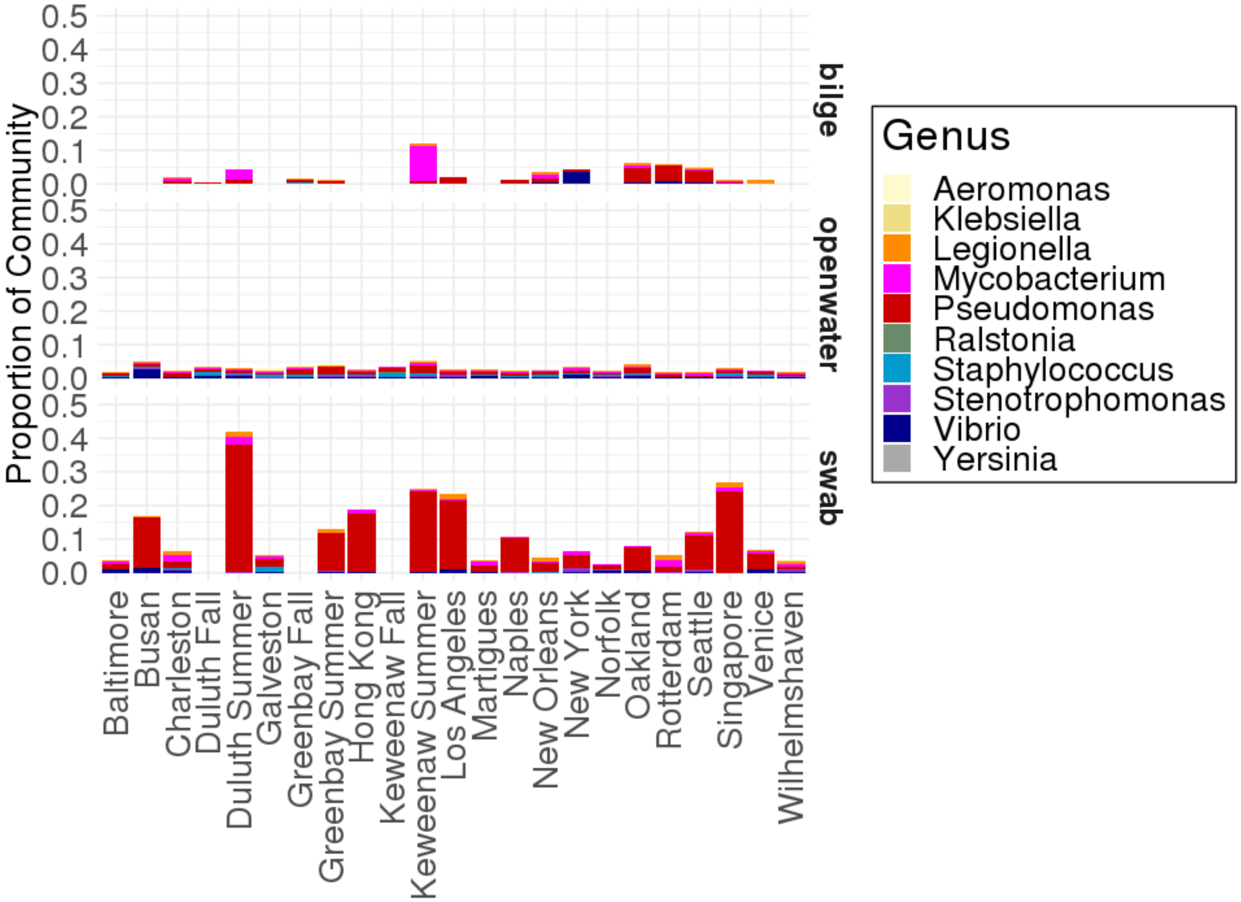

Figure S2 Abundance of genera associated with pathogens showing variance between sample types and location. Swab and bilge samples have higher abundances of these genera on average than the port water samples. Pseudomonas spp. are highly abundant across swab samples from most locations compared to the other sample types.

Table S9 Range of the relative abundance of genera closely associated with common water-borne pathogens found in each sample type. The abundances of these genera were highly variable between sample type and location.

Family	Genus	Port Water	Bilge	Swab
Aeromonadaceae	Aeromonas	0.003±0.019	---	0.002±0.002
Burkholderiaceae	Ralstonia	0.001	---	---
Enterobacteriaceae	Klebsiella	0.003±0.002	0.001	0.002±0.001
Legionellaceae	Legionella	0.004±0.021	0.005±0.018	0.007±0.019
Mycobacteriaceae	Mycobacterium	0.004±0.021	0.017±0.081	0.008±0.033
Pseudomonadaceae	Pseudomonas	0.010±0.930	0.020±0.052	0.100±0.660
Staphylococcaceae	Staphylococcus	0.006±0.020	0.002±0.001	0.007±0.012
Vibrionaceae	Vibrio	0.008±0.277	0.010±0.027	0.005±0.018
Xanthomonadaceae	Stenotrophomonas	0.003±0.008	0.001	0.004±0.006

Table S10 Variance of the average, minimum and maximum relative abundance of Cyanobacteria (phylum) found in each sample type. The presence of Cyanobacteria in the bilge compartment supports our hypothesis that the bacterial community on the boat is strongly influenced by the port water.

Sample Type	**Average**	**Minimum**	**Maximum**
Port water	0.126	0.011	0.762
Bilge	0.081	0.010	0.678
Swab	0.070	0.011	0.585

Table S11 ANOVA of the population of Cyanobacteria in each sample type shows a statistically significant difference between the alpha diversity of Cyanobacteria our different sample types. Significant p-values were given for both Shannon diversity and Observed ASVs.

Diversity Metric	Degrees of Freedom	F-Value	P-value
Shannon	2	24.56	< 0.001
Observed	2	51.31	< 0.001

Table S12: Summary of p-values for Tukey HSD (post hoc) test results for the population of Cyanobacteria found in our samples. Both Observed ASVs (upper triangle) and Shannon diversity (lower triangle) showed statistically significant differences between the population of Cyanobacteria in each of our sample types.

	Port Water	Bilge	Swab
Port Water		< 0.001	< 0.001
Bilge	< 0.001		0.0096
Swab	< 0.001	0.001	

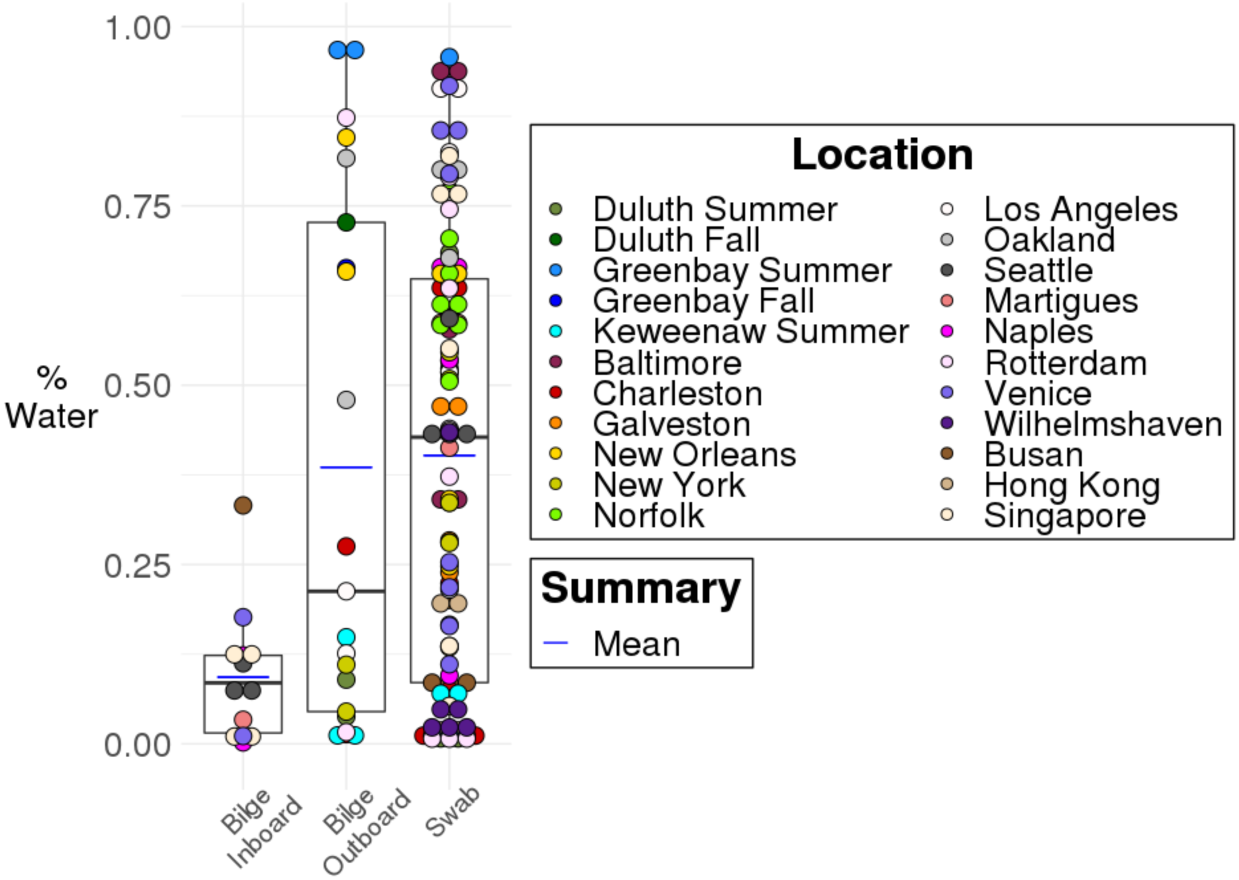

Figure S3 Summary of SourceTracker results showing the similarity of each boat sample to the port water. The bilge samples are divided into two categories to represent boats with inboard and outboard engines since the engine type may affect how much water leaks into the bilge compartment from the port.

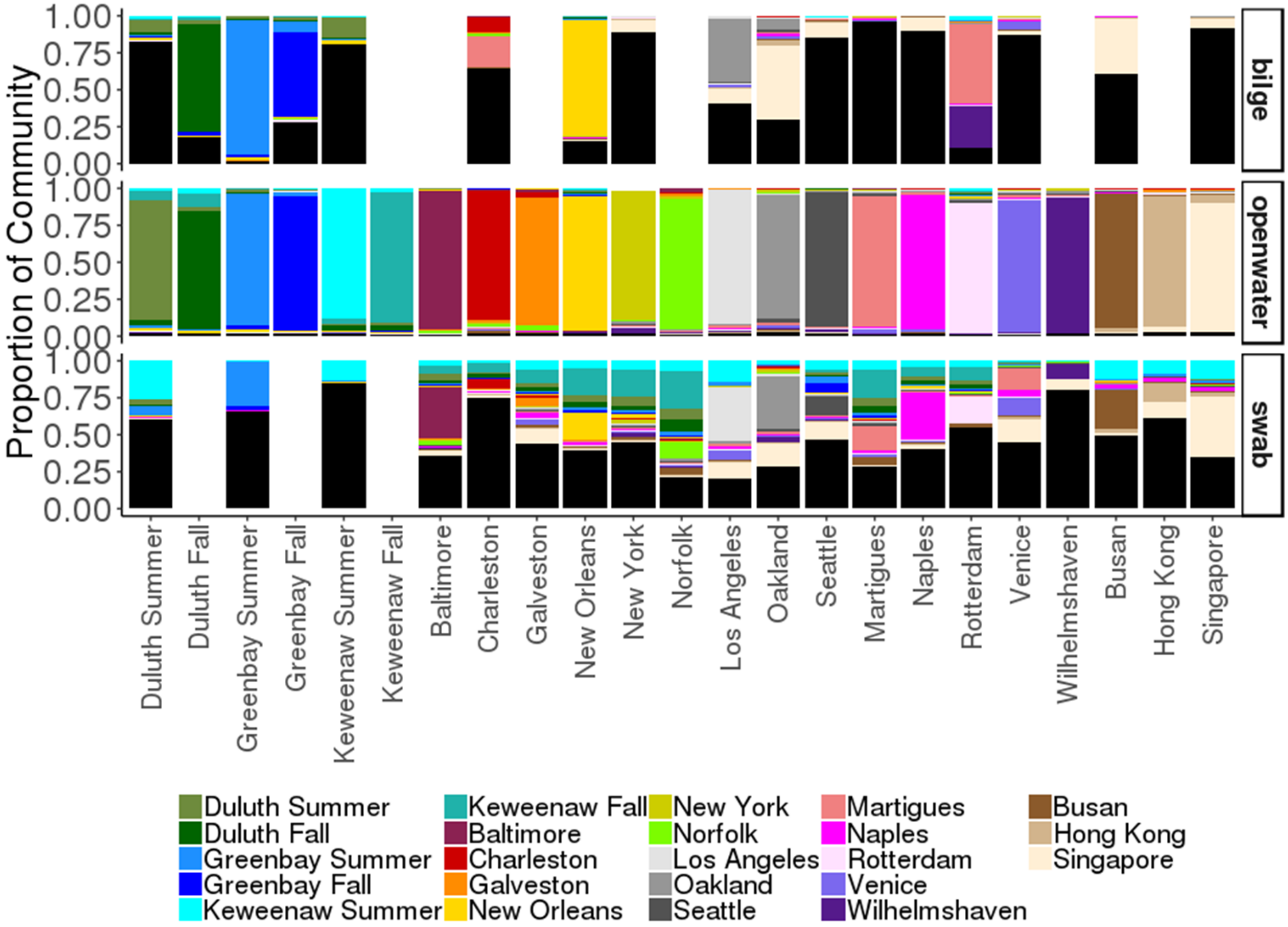

Figure S4 Proportions output by SourceTracker, separated by sample type, location and season (for Great Lakes samples). The proportion of the microbial community classified as the correct home port of the boat was highly variable for the boat samples (swab and bilge). We suggest that this is due to factors other than port water which also influence the bacterial community living on a boat.

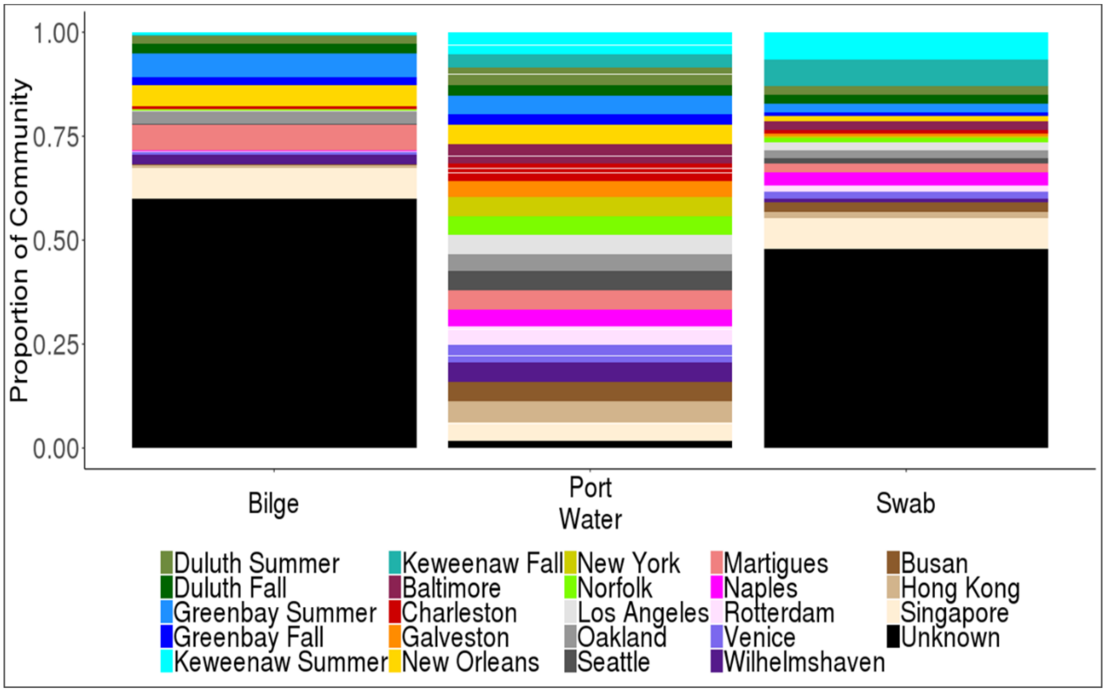

Figure S5 Average proportion of each sample type which was correctly classified as each location by SourceTracker. All of the colored part together represents how much of the microbial community (on average) is classified as water.

Table S13 Average SourceTracker proportions of each sample type described as water (sum of average proportion from each location).

	Bilge	Port Water	Swab
Percent Port Water	40%	92%	52%