

Supplementary Information

Metagenomes of Mediterranean Coastal Lagoons

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Supplementary Figure S1: Sampling locations

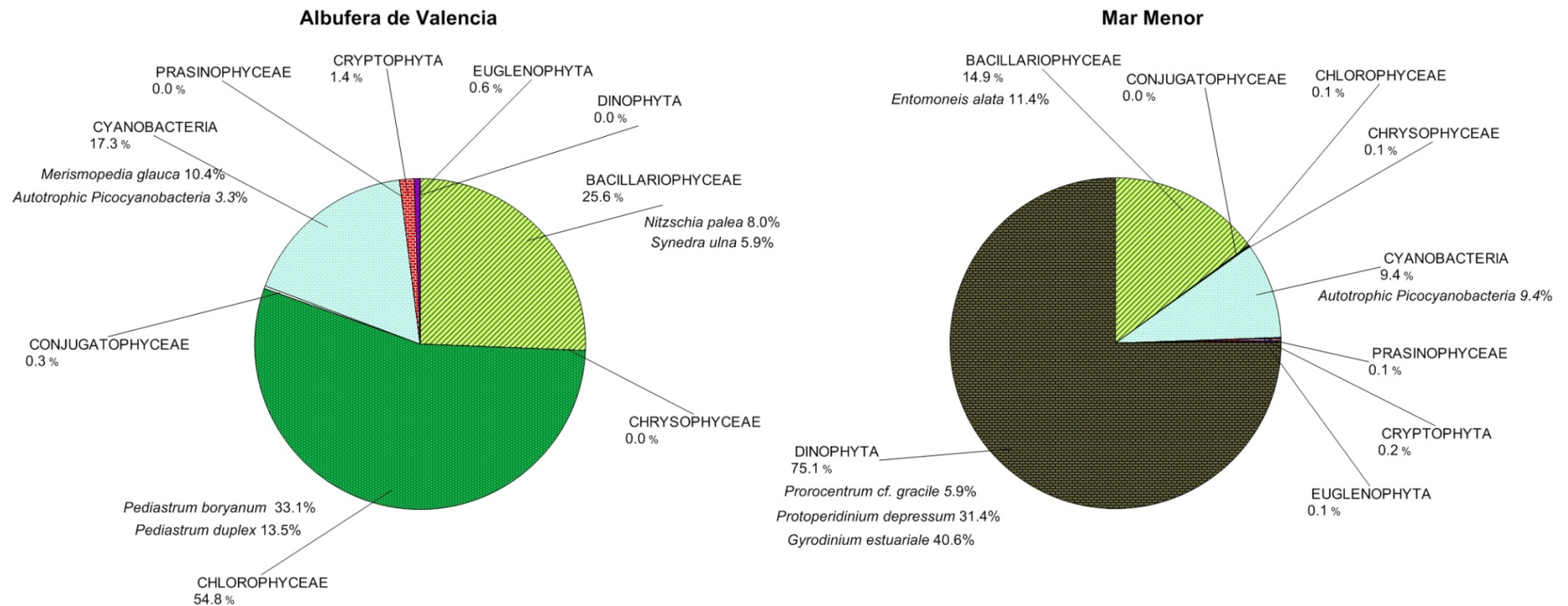
Albufera



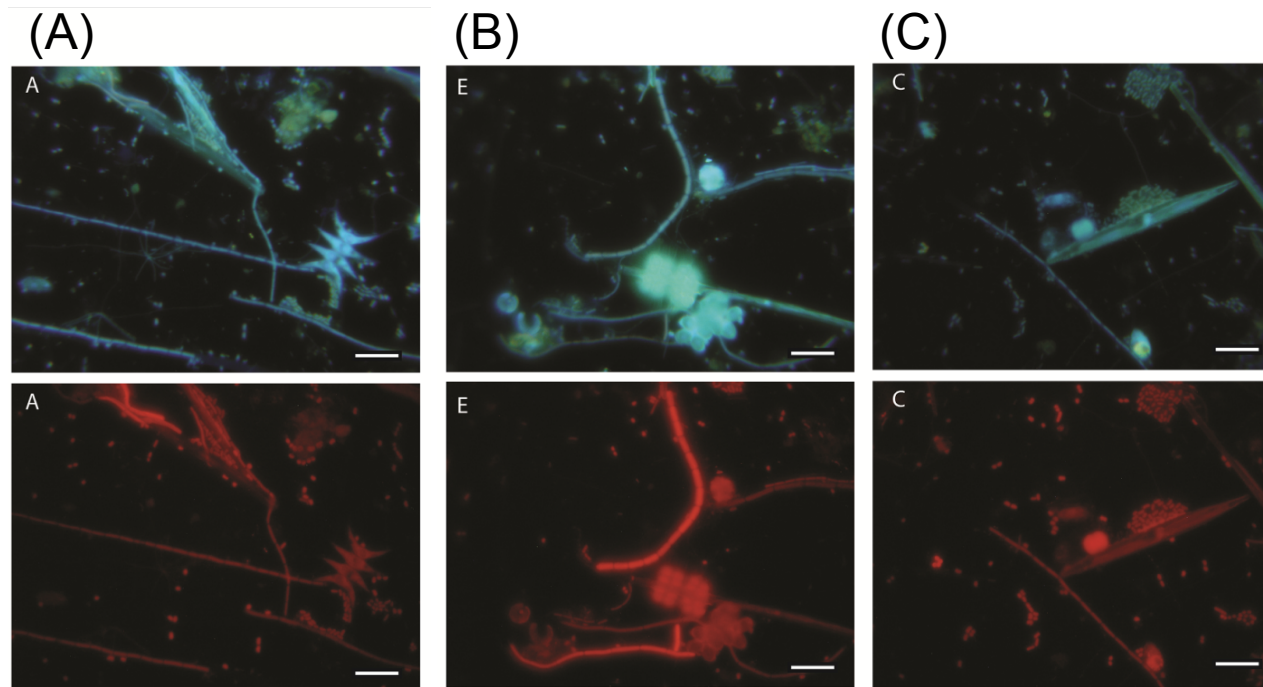
Mar Menor



Supplementary Figure S2. Percent contribution to total biovolume (equivalent to biomass) of the different groups of phytoplankton identified using inverted microscopy. Species accounting for more than 5 % of the biovolume are highlighted.



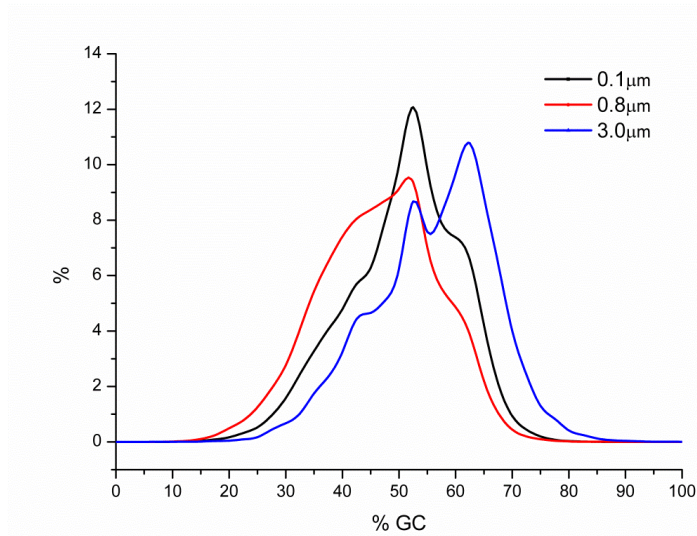
Supplementary Figure S3: Pairs of microphotographs, DAPI stain (blue, up) and photosynthetic pigment autofluorescence (red, down) of samples from Albufera showing different microorganisms. A) the chlorophyte *Scenedesmus* sp. B) several filamentous and colonial (*Merismopedia*) cyanobacteria. C) the pennated diatom *Nitzschia* sp. White bar is equivalent to 10 μ m in all pictures.



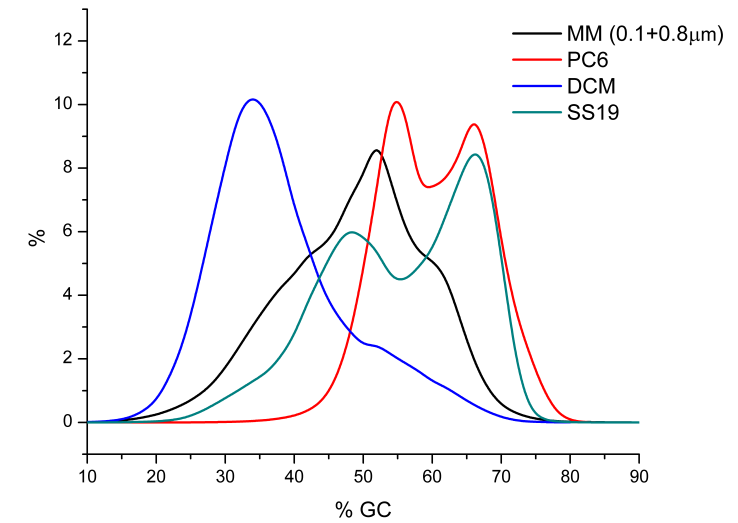
Supplementary Figure S4: GC% profiles of Mar menor and Albufera metagenomic datasets

A) Mar Menor, all three filter sizes B) Mar Menor, 0.1+0.8 μ m and three reference saline metagenomic datasets C) Albufera, all three filter sizes D) Albufera 0.1+0.8 μ m and three reference freshwater metagenomic datasets

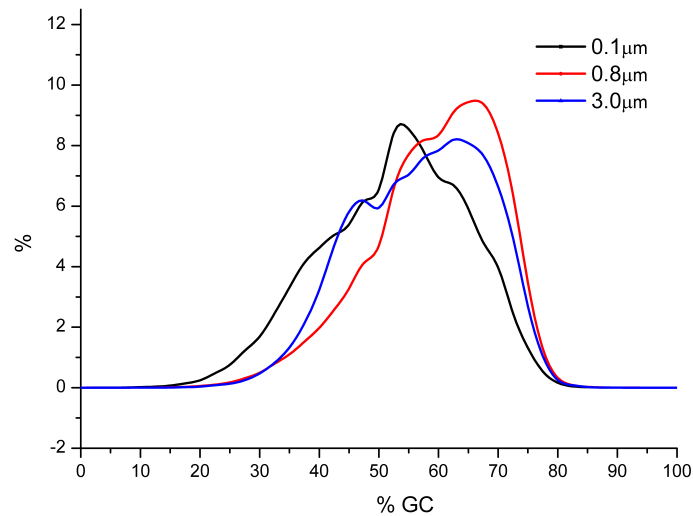
(A)



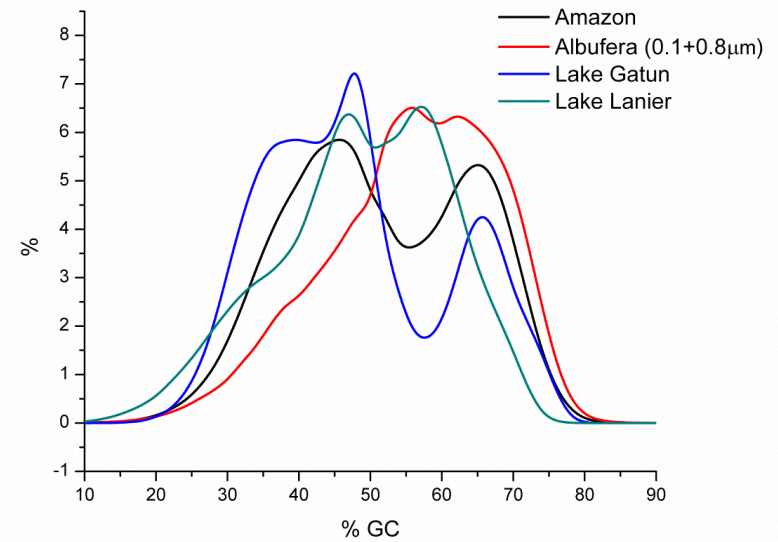
(B)



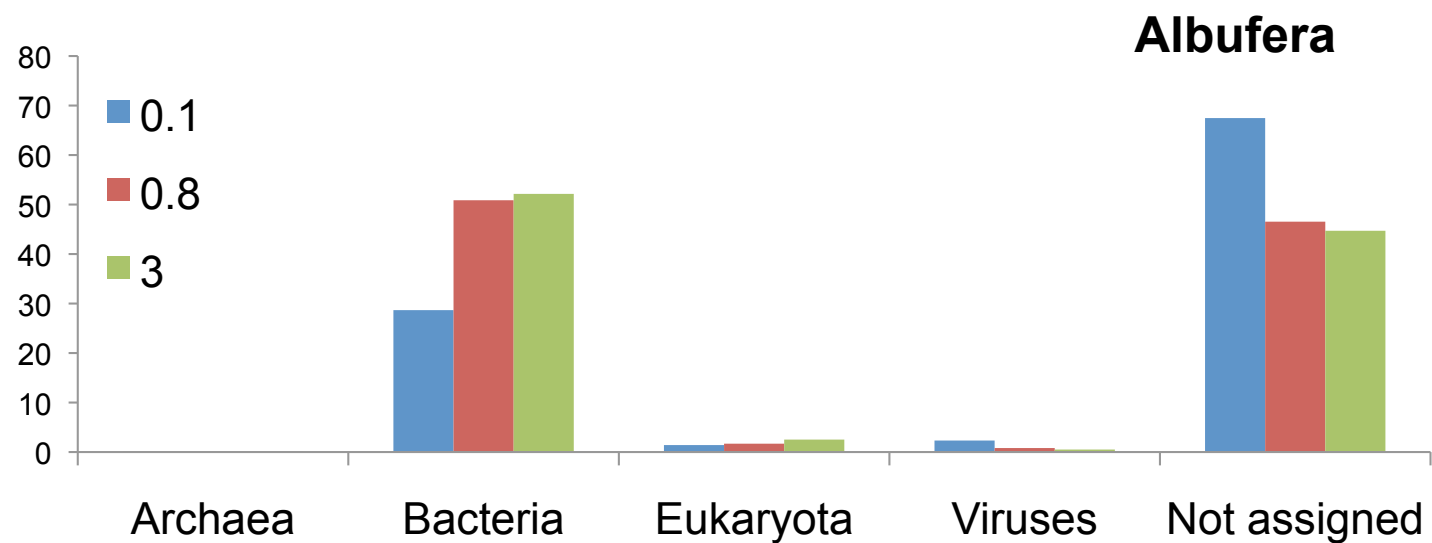
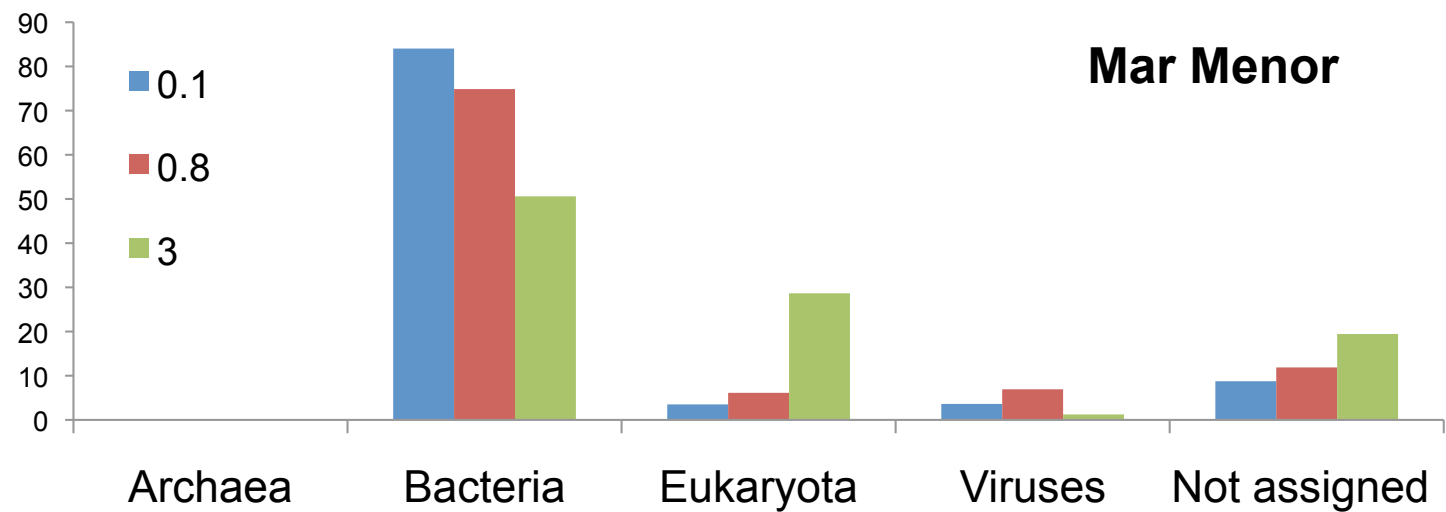
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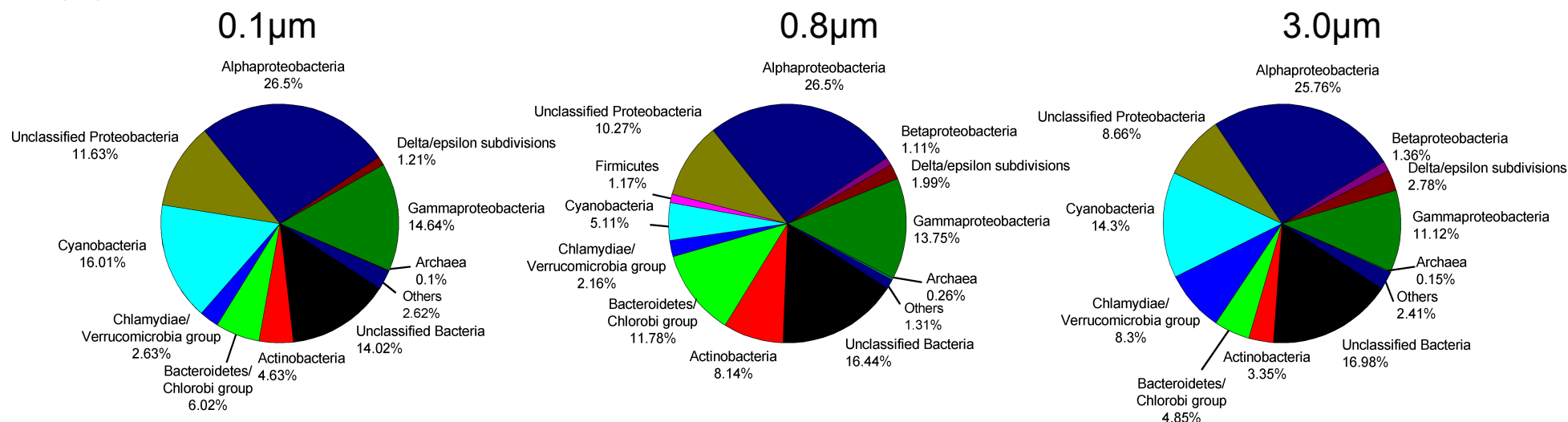
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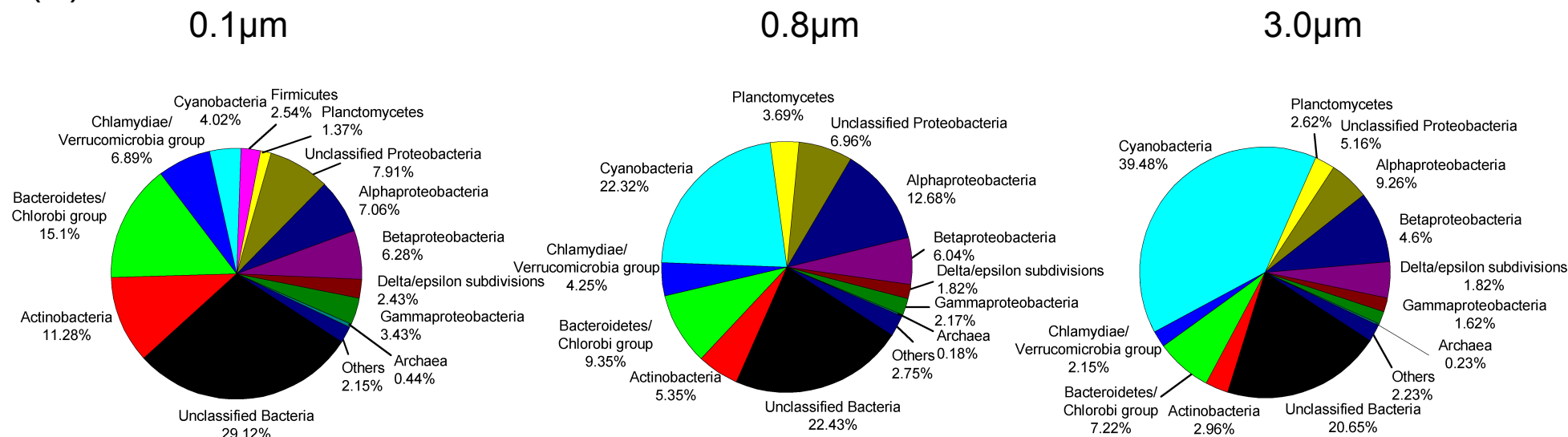
Supplementary Figure S5: Domain level classification of all metagenomic reads from each filter size



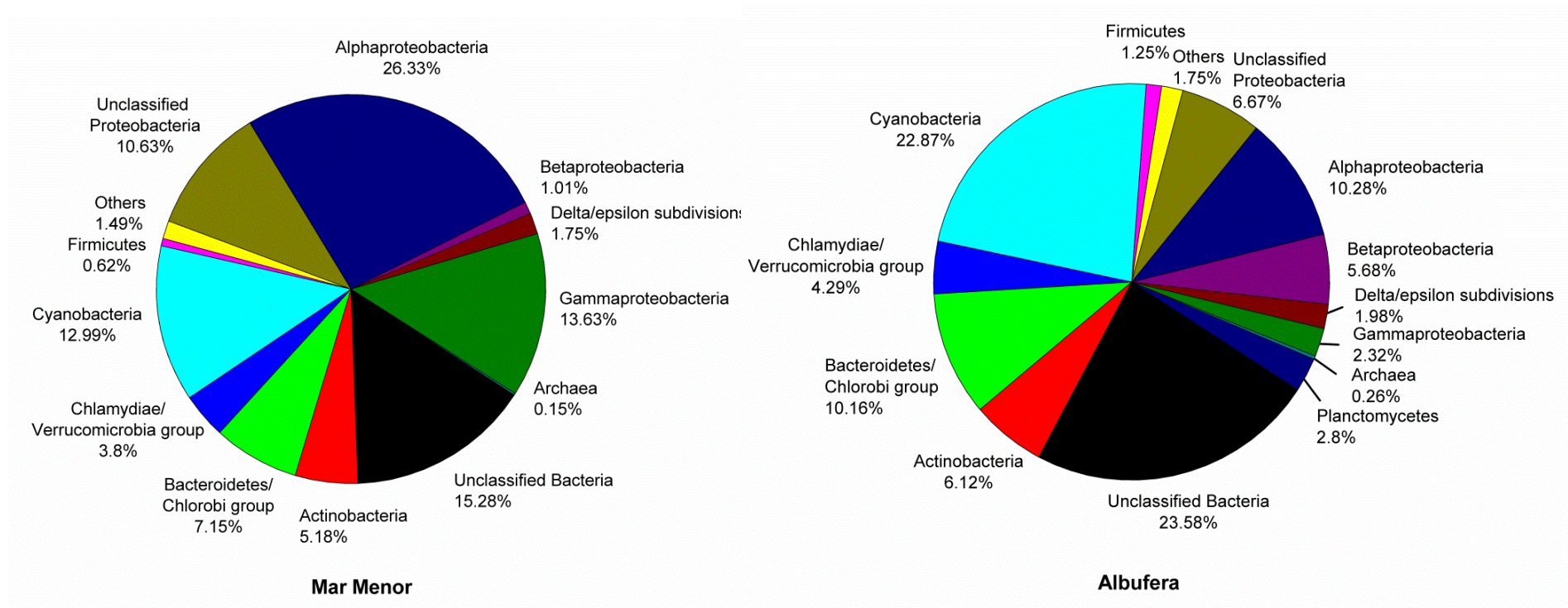
Supplementary Figure S6: Marmenor and Albufera all metagenomic reads classification (all filters) by MEGAN
(A) Mar Menor



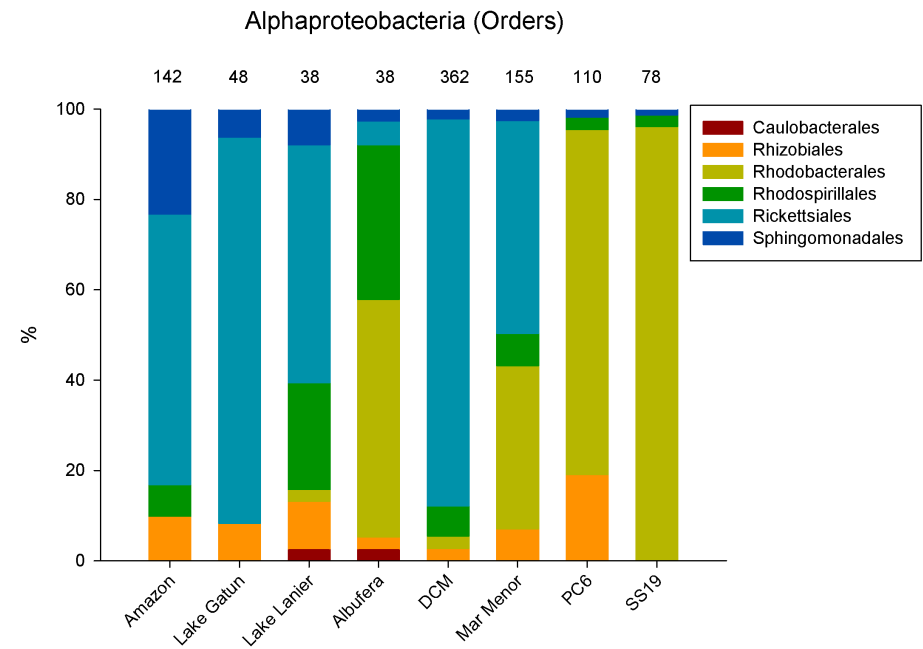
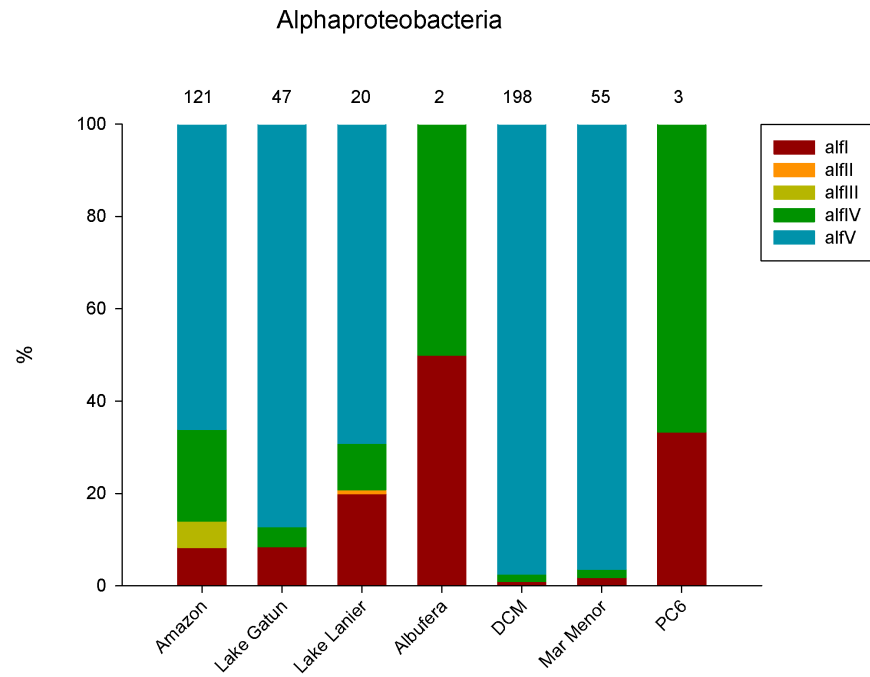
(B) Albufera



Supplementary Figure S7: Comparison of MarMenor and Albufera metagenomic reads to the NCBI NR database using BLASTX. (only archaeal and bacterial reads are shown)

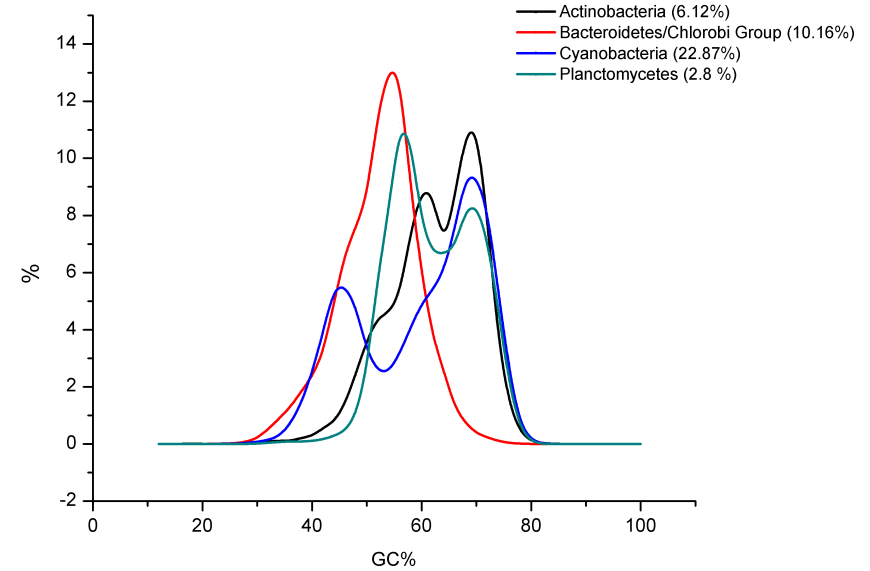
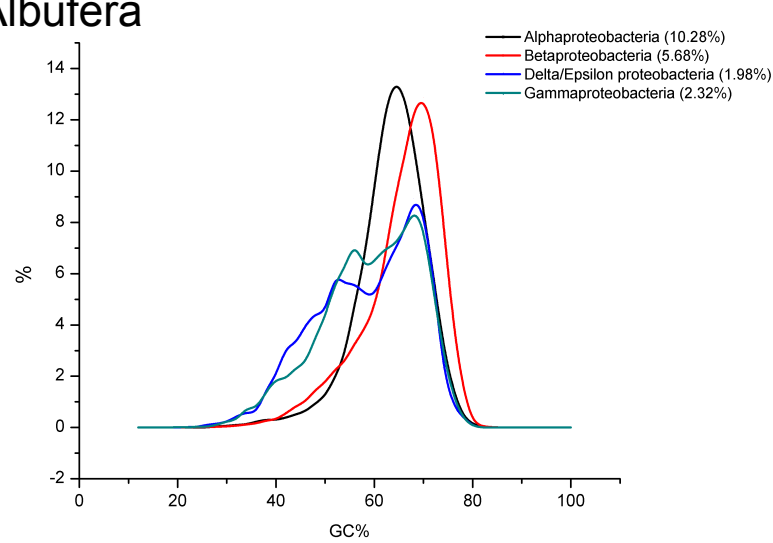


Supplementary Figure S8: Classification of Alphaproteobacterial 16S Reads into known lineages

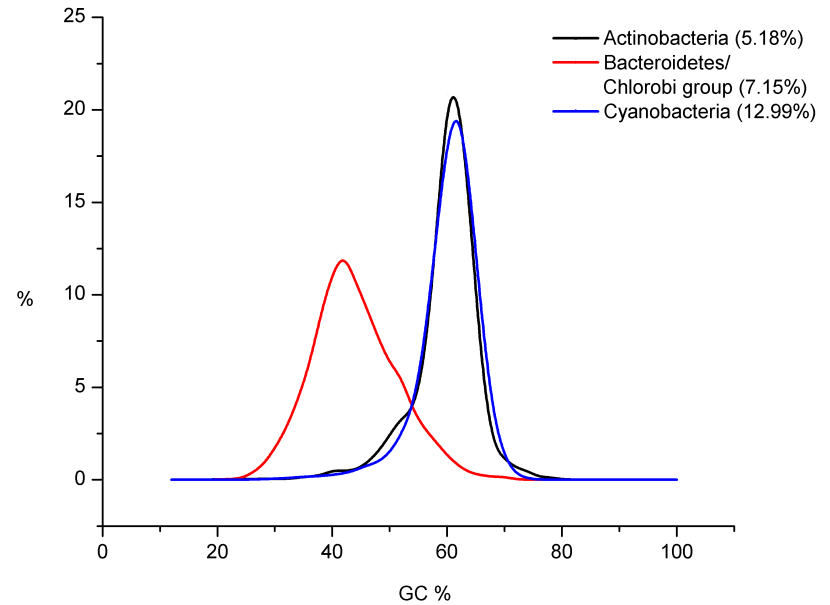
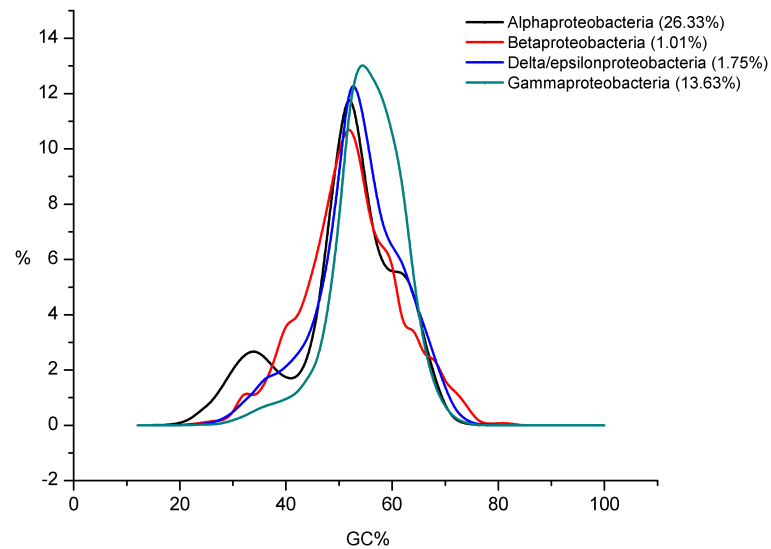


Supplementary Figure S9: GC% profiles of major taxa in Albufera and Mar Menor Metagenomes

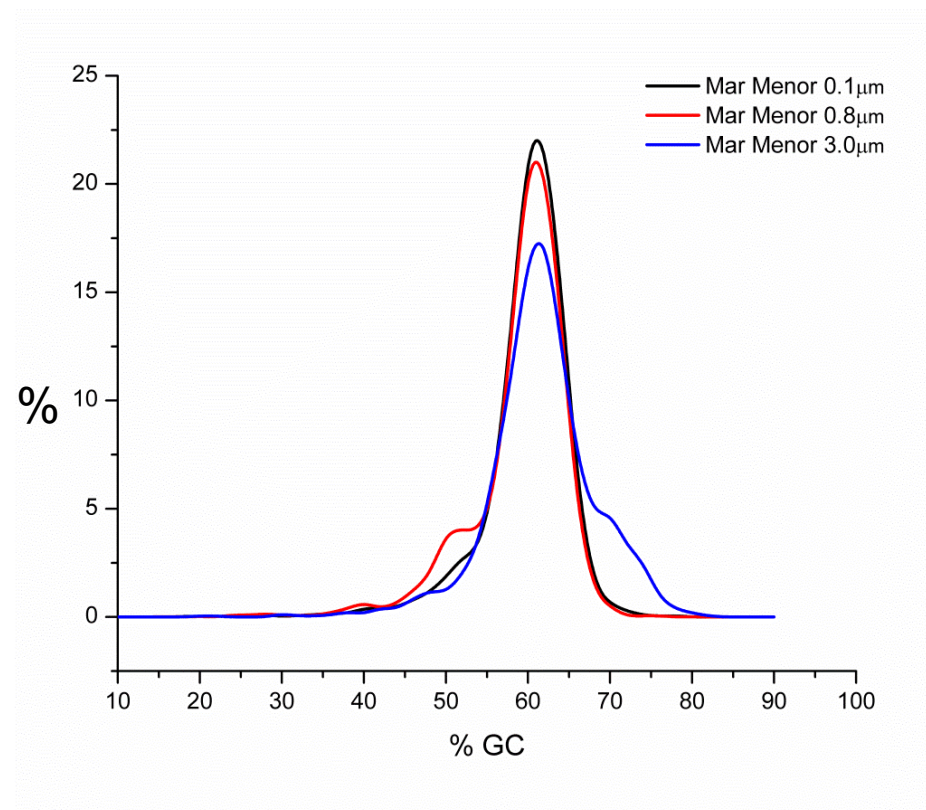
A) Albufera



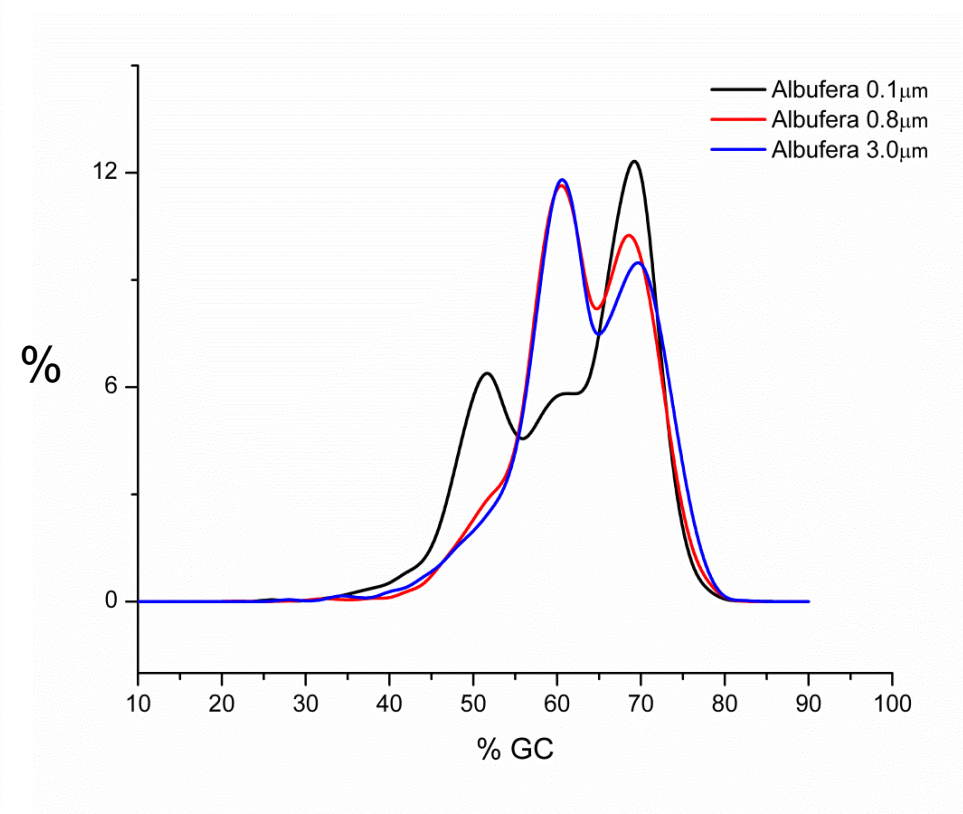
B) Mar Menor



Supplementary Figure S10: GC% profiles of actinobacterial reads in Mar Menor and Albufera

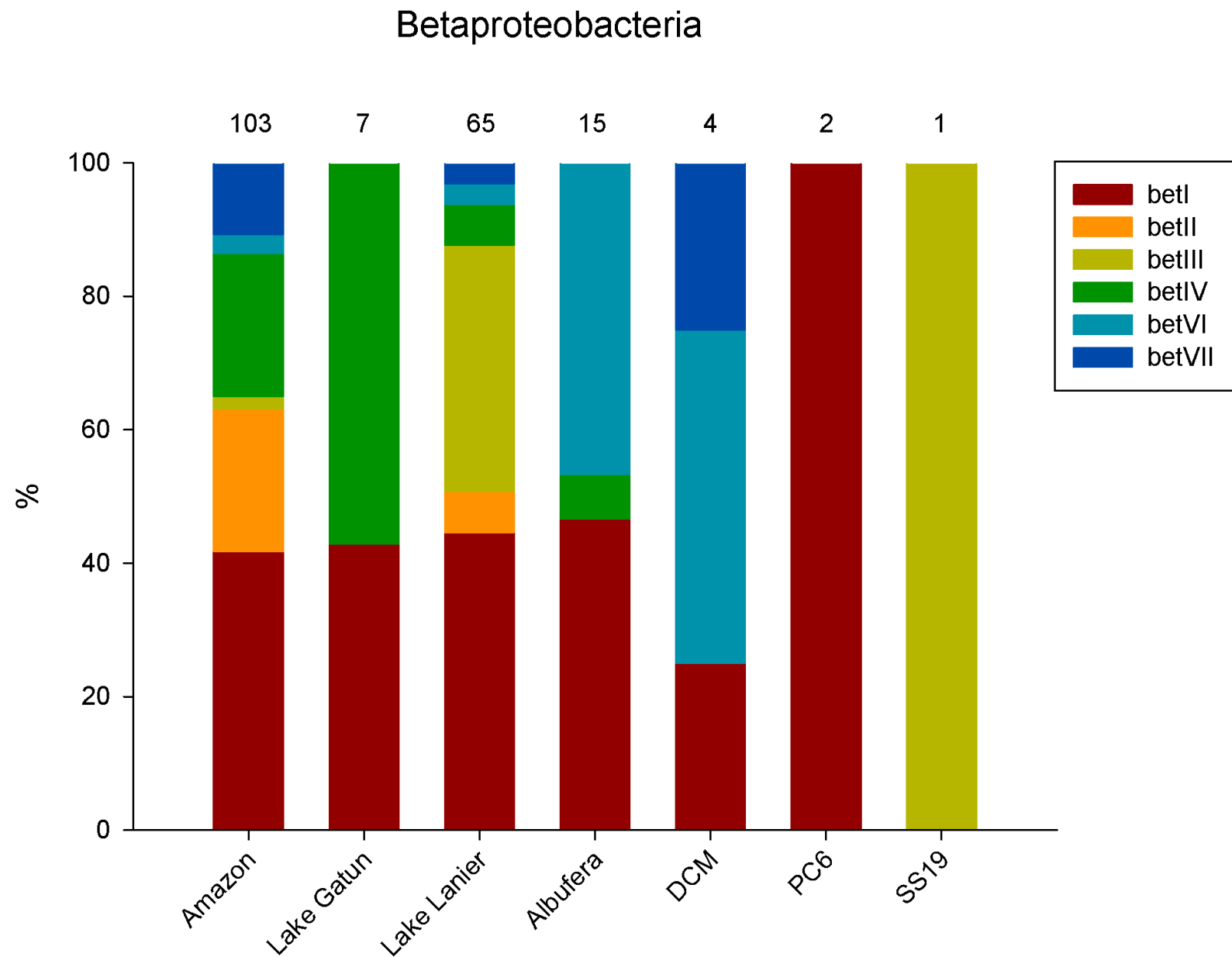


Mar Menor



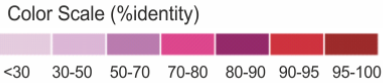
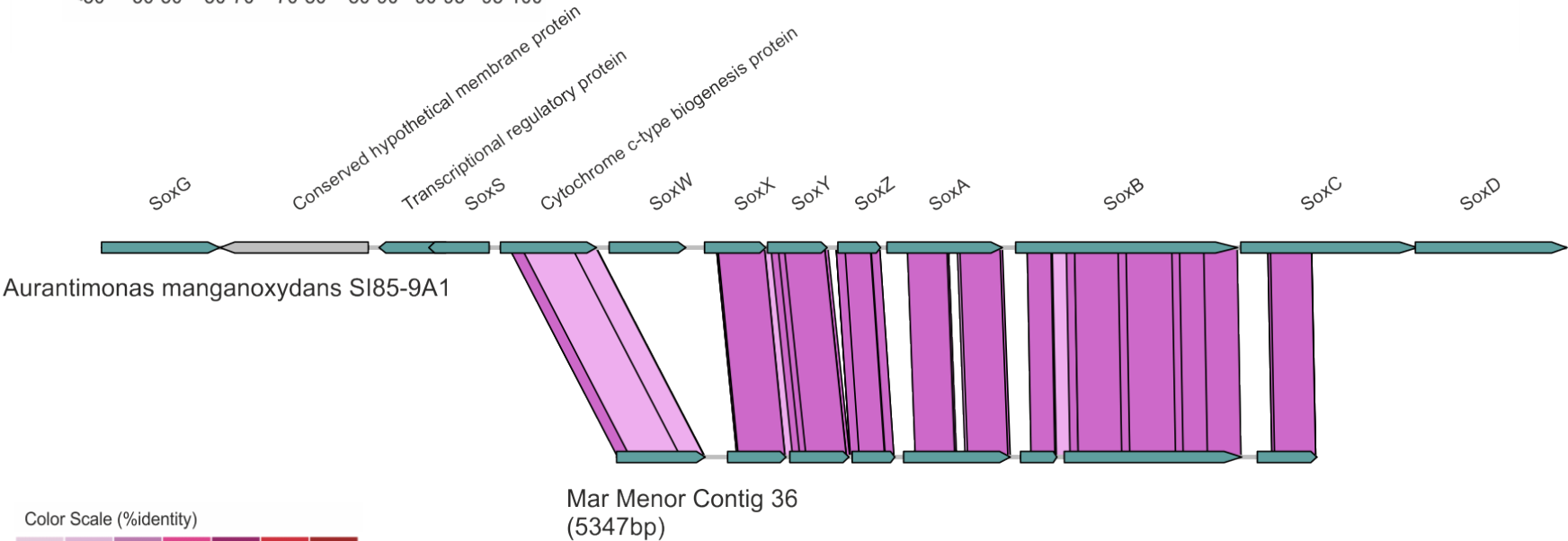
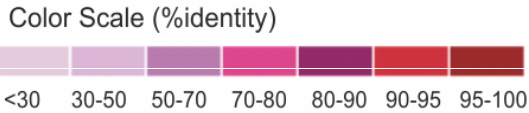
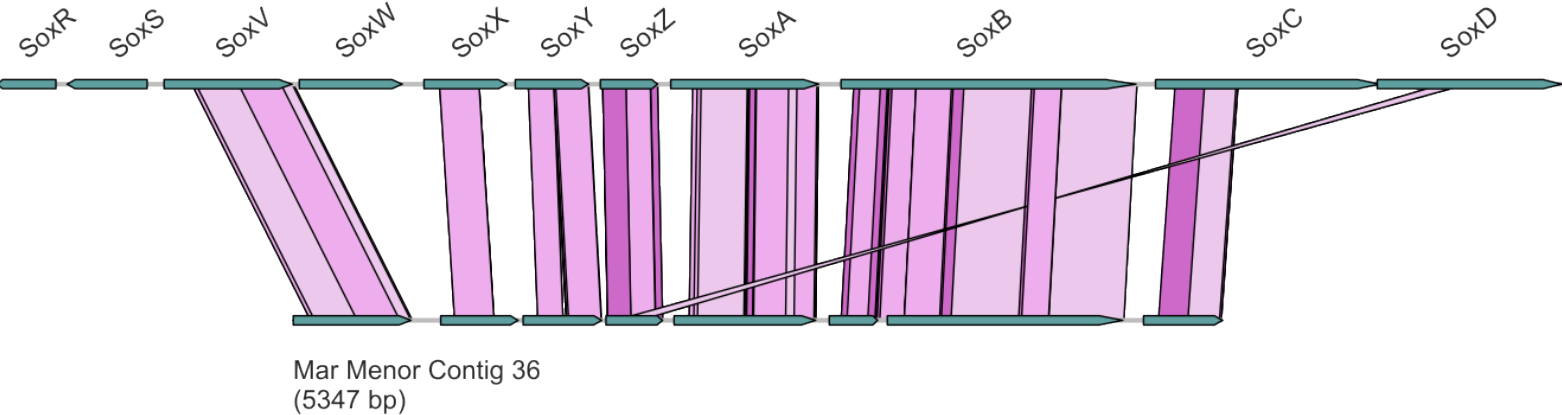
Albufera

Supplementary Figure S11: Classification of Betaproteobacterial 16S Reads into known lineages



Supplementary Figure S12: Sox Genes Cluster Roseobacter sp. MED193 and of Aurantimonas manganoxydans compared to assembled Contig_36 from the MarMenor metagenome. The comparison is done using TBLASTX

Roseobacter sp. MED193
(genomic region 1180751..1189756, 9006 bp)



Supplementary Table S1. Physical and chemical data, bacterioplankton and APP abundances, and major pigments found in waters of Mar Menor and Albufera de Valencia at the time of sampling. The phytoplankton groups for which the different pigments are taxa-specific are indicated in brackets.

		units	Mar Menor	Albufera
Parameters	Temperature	°C	19	19.5
	Electrical conductivity	mS cm ⁻¹	64.1	2.8
	pH		8.40	7.69
	Nitrate	mM	9.1	68.1
	Ammonia	mM	23.7	0.1
	Total Nitrogen	mM	44.4	102.3
	Soluble reactive phosphorus (SRP)	mM	9.6	2.5
	Total Phosphorus	mM	38.7	93.4
	Alkalinity	meq l ⁻¹	2.1	1.3
	Chloride	g l ⁻¹	25.04	0.57
	CO ₃ H ⁻	mg l ⁻¹	102.77	64.86
Cells	Bacterioplankton	cells ml ⁻¹	4.25E+06	5.33E+06
	Autotrophic picocyanobacteria (APP)	cells ml ⁻¹	5.47E+05	9.49E+06
pigments (HPLC)	Chlorophyll <i>a</i> (All groups)	mg l ⁻¹	3.94	271.31
	Chlorophyll <i>b</i> (<i>Chlorophyceae</i>)	mg l ⁻¹	0.071	2,735
	Chlorophyll <i>c</i> (<i>Bacillariophyceae</i>)	mg l ⁻¹	0.098	2,808
	Lutein (<i>Chlorophyceae</i>)	mg l ⁻¹	0.028	1.460
	Neoxanthin (<i>Chlorophyceae</i>)	mg l ⁻¹	0.004	0.513
	Fucoxanthin (<i>Bacillariophyceae</i>)	mg l ⁻¹	0.247	23.76
	Zeaxanthin (<i>Cyanobacteria</i>)	mg l ⁻¹	0.021	14.14
	Myxoxanthophyll (<i>Filamentous Cyanobacteria</i>)	mg l ⁻¹	0.057	1.252
	Peridinin (<i>Dinophyta</i>)	mg l ⁻¹	0.168	0.040
	Alloxanthin (<i>Cryptophyceae</i>)	mg l ⁻¹	0.011	0.151
	Bacteriochlorophyll <i>a</i>	mg l ⁻¹	0.010	0.149
	Bacteriochlorophyll <i>c</i> & <i>d</i>	mg l ⁻¹	< 0.001	< 0.001

Supplementary Table S2: Number of reads obtained from each filter for both metagenomes

	Filter size (μ m)	# reads	Total Length (Mb)	Average read length (bp)
Albufera	0.1	253260	87.99	347
	0.8	262320	98.92	377
	3.0	166180	62.33	375
	Total	681760	249.24	
Mar Menor	0.1	286637	108.46	378
	0.8	182301	66.95	367
	3.0	262059	67.92	259
	Total	730997	243.32	

Supplementary Table S3: Organisms with maximum hits in NR database(Mar Menor), V-Viruses ; B-Bacteria; E- Eukarya

Mar Menor filter size	Domain	Taxon	Genus	# hits	Genome size (mb)	#hits / G.size (kb)	%GC
0.1µm	V	dsDNA, no RNA stage	<i>Roseobacter phage SIO1</i>	592	0.04	14.8	46.2
	B	a-Proteobacteria	<i>Alpha proteobacterium HIMB114</i>	2253	1.29	1.75	29.7
	B	Cyanobacteria	<i>Synechococcus sp. WH 7803</i>	2774	2.37	1.17	60.2
	B	a-Proteobacteria	<i>Candidatus Puniceispirillum marinum IMCC1322</i>	2914	2.75	1.06	48.9
	B	Verrucomicrobia	<i>Coralimargarita akajimensis DSM 45221</i>	2454	3.75	0.65	53.6
	B	Cyanobacteria	<i>Synechococcus sp. WH 7805</i>	1320	2.62	0.50	57.6
	B	g-Proteobacteria	<i>Marine gamma proteobacterium HTCC2080</i>	1729	3.58	0.48	51.8
	B	Bacteroidetes	<i>Flavobacteria bacterium MS024-2A</i>	666	1.91	0.35	35.7
	B	g-Proteobacteria	<i>Gamma proteobacterium NOR51-B</i>	850	3.25	0.26	56.7
	B	d-Proteobacteria	<i>Bacteriovorax marinus SJ</i>	607	3.44	0.18	36.7
0.8µm	V	dsDNA, no RNA stage	<i>Roseobacter phage SIO1</i>	329	0.04	8.23	46.2
	B	a-Proteobacteria	<i>Alpha proteobacterium HIMB114</i>	3536	1.29	2.74	29.7
	B	Bacteroidetes	<i>Flavobacteria bacterium MS024-2A</i>	998	1.91	0.52	35.7
	B	a-Proteobacteria	<i>Candidatus Puniceispirillum marinum IMCC1322</i>	1096	2.75	0.40	48.9
	B	Verrucomicrobia	<i>Coralimargarita akajimensis DSM 45221</i>	917	3.75	0.24	53.6
	B	g-Proteobacteria	<i>Marine gamma proteobacterium HTCC2080</i>	775	3.58	0.22	51.8
	B	a-Proteobacteria	<i>Candidatus Pelagibacter sp. HTCC7211</i>	275	1.46	0.19	29
	B	d-Proteobacteria	<i>Bacteriovorax marinus SJ</i>	583	3.44	0.17	36.7
	B	Cyanobacteria	<i>Synechococcus sp. WH 7803</i>	360	2.37	0.15	60.2
	B	g-Proteobacteria	<i>Gamma proteobacterium NOR51-B</i>	393	3.25	0.12	56.7
3.0µm	B	Verrucomicrobia	<i>Coralimargarita akajimensis DSM 45221</i>	1902	3.75	0.51	53.6
	B	Cyanobacteria	<i>Synechococcus sp. WH 7803</i>	982	2.37	0.41	60.2
	B	a-Proteobacteria	<i>Maricaulis maris MCS10</i>	684	3.37	0.20	62.7
	B	Cyanobacteria	<i>Synechococcus sp. WH 7805</i>	512	2.62	0.20	57.6
	B	g-Proteobacteria	<i>Marine gamma proteobacterium HTCC2080</i>	686	3.58	0.19	51.8
	B	Verrucomicrobia	<i>Akkermansia muciniphila ATCC BAA-835</i>	476	2.66	0.18	55.8
	B	Bacteroidetes	<i>Haliscomenobacter hydrossis DSM 1100</i>	774	8.37	0.09	47.1
	B	Verrucomicrobia	<i>Verrucomicrobium spinosum DSM 4136</i>	492	8.22	0.06	60.3
	E	Ants	<i>Harpegnathos saltator</i>	557		0	
	E	Starlet sea anemone	<i>Nematostella vectensis</i>	473		0	

Supplementary Table S4: Organisms with maximum hits in NR database(Albufera), V-Viruses ; B-Bacteria; E- Eukarya

Albufera filter size	Domain	Taxon	Genus	# hits	Genome size (mb)	#hits/ G.size (kb)	%GC
0.1µm	V	dsDNA, no RNA stage	<i>Prochlorococcus phage P-SSM2</i>	253	0.25	1.01	35.5
	B	Verrucomicrobia	<i>Chthoniobacter flavus Ellin428</i>	1525	7.84	0.19	61.1
	B	Bacteroidetes	<i>Robiginitalea biformata HTCC2501</i>	508	3.53	0.14	55.3
	B	d-Proteobacteria	<i>Bdellovibrio bacteriovorus HD100</i>	274	3.78	0.07	50.6
	B	Bacteroidetes	<i>Chitinophaga pinensis DSM 2588</i>	604	9.13	0.07	45.2
	B	a-Proteobacteria	<i>Novosphingobium aromaticivorans DSM 12444</i>	232	3.56	0.07	65.2
	B	a-Proteobacteria	<i>Hyphomonas neptunium ATCC 15444</i>	238	3.71	0.06	61.9
	B	Verrucomicrobia	<i>Verrucomicrobium spinosum DSM 4136</i>	401	8.22	0.05	60.2
	B	Verrucomicrobia	<i>Bacterium Ellin514</i>	255	7.41	0.03	52.6
	B	Bacteroidetes	<i>Haliscomenobacter hydrossis DSM 1100</i>	244	8.37	0.03	47.1
0.8µm	B	Cyanobacteria	<i>Cyanobium sp. PCC 7001</i>	1917	2.83	0.68	68.7
	B	Cyanobacteria	<i>Synechococcus sp. WH 5701</i>	1817	3.04	0.60	65.4
	B	Cyanobacteria	<i>Synechococcus sp. RS9917</i>	1484	2.58	0.58	64.5
	B	a-Proteobacteria	<i>Roseomonas cervicalis ATCC 49957</i>	1774	4.44	0.40	72
	B	Cyanobacteria	<i>Synechococcus sp. CB0101</i>	822	2.69	0.31	
	B	a-Proteobacteria	<i>Hyphomonas neptunium ATCC 15444</i>	866	3.71	0.23	61.9
	B	Verrucomicrobia	<i>Chthoniobacter flavus Ellin428</i>	1399	7.84	0.18	61.1
	B	Bacteroidetes	<i>Haliscomenobacter hydrossis DSM 1100</i>	953	8.37	0.11	47.1
	B	Planctomycetes	<i>Rhodopirellula baltica SH 1</i>	615	7.15	0.09	55.4
	B	Verrucomicrobia	<i>Bacterium Ellin514</i>	535	7.41	0.07	52.6
3.0µm	B	Cyanobacteria	<i>Synechococcus sp. WH 5701</i>	967	3.04	0.32	65.4
	B	Cyanobacteria	<i>Cyanobium sp. PCC 7001</i>	900	2.83	0.32	68.7
	B	Cyanobacteria	<i>Synechococcus sp. RS9917</i>	772	2.58	0.30	64.5
	B	Bacteroidetes	<i>Haliscomenobacter hydrossis DSM 1100</i>	2233	8.37	0.27	47.1
	B	a-Proteobacteria	<i>Hyphomonas neptunium ATCC 15444</i>	694	3.71	0.19	61.9
	B	Cyanobacteria	<i>Synechococcus sp. CB0101</i>	423	2.69	0.16	
	B	a-Proteobacteria	<i>Roseomonas cervicalis ATCC 49957</i>	433	4.44	0.10	72
	B	Cyanobacteria	<i>Synechococcus sp. PCC 7335</i>	396	5.96	0.07	48.2
	B	Planctomycetes	<i>Rhodopirellula baltica SH 1</i>	380	7.15	0.05	55.4
	E	Diatoms	<i>Phaeodactylum tricornutum CCAP 1055/1</i>	479		0	

Supplementary Table S5: 16s rRNA sequences assigned to a genus

Albufera				Mar Menor			
Taxa	Genus	#hits	%*	Taxa	Genus	#hits	%*
Cyanobacteria	<i>Cyanobium</i>	42	28.8	Alphaproteobacteria	<i>Pelagibacter</i>	69	43.6
Cyanobacteria	<i>Synechococcus</i>	28	19.2	Verrucomicrobia	<i>Haloferula</i>	18	11.4
Alphaproteobacteria	<i>Roseomonas</i>	11	7.5	Cyanobacteria	<i>Synechococcus</i>	14	8.8
Cyanobacteria	<i>Pseudanabaena</i>	10	6.8	Verrucomicrobia	<i>Coralimargarita</i>	10	6.3
Cyanobacteria	<i>Merismopedia</i>	7	4.8	Actinobacteria	<i>Yonghaparkia</i>	8	5.1
Bacteroidetes	<i>Haliscomenobacter</i>	4	2.7	Alphaproteobacteria	<i>Marivita</i>	6	3.8
Planctomycetes	<i>Pirellula</i>	4	2.7	Bacteroidetes	<i>Flavobacterium</i>	6	3.8
Actinobacteria	<i>Microcella</i>	4	2.7	Alphaproteobacteria	<i>Aurantimonas</i>	4	2.5
Alphaproteobacteria	<i>Rhodobacter</i>	3	2.0	Alphaproteobacteria	<i>Maricaulis</i>	3	1.9
Betaproteobacteria	<i>Methylibium</i>	3	2.0	Alphaproteobacteria	<i>Sulfitobacter</i>	3	1.9

*% based in sequences with genus assigned for Albufera (n=146) and for Mar Menor (n=158) from 342 and 431 sequences with %identity > 95 and length > 100bp respectively

Supplementary Table S6- Organisms and metagenomic samples with maximum hits (using BLAST against inhouse JCVI database)

Mar Menor 0.1µm					Albufera 0.1µm			
Domain	Taxon	Species/Sample	#Hits		Domain	Taxon	Species/Sample	#Hits
Viruses	Marine	UDEL CHESAPEAKE VIROPLANKTON SMPL	5634		Viruses	Marine	UDEL CHESAPEAKE VIROPLANKTON SMPL	3687
Bacteria	α-Proteobacteria	Candidatus Pelagibacter sp. IMCC9063	3328		Bacteria	Bacteroidetes	Chitinophaga pinensis DSM 2588	1712
Bacteria	Cyanobacteria	Synechococcus sp. WH 7803	2428		Bacteria	Bacteroidetes	Rhodothermus marinus DSM 4252	1187
Bacteria	α-Proteobacteria	Candidatus Puniceispirillum marinum IMCC1322	2085		Bacteria	Verrucomicrobia	Akkermansia muciniphila ATCC BAA-835	938
Viruses	Marine	SDSU SCRIPPS PIER MARINE VIRUS SMPL	1671		Bacteria	Verrucomicrobia	Opitutus terrae PB90-1	913
Bacteria	α-Proteobacteria	Candidatus Pelagibacter ubique HTCC1062	1264		Bacteria	Bacteroidetes	Haliscomenobacter hydrossis DSM 1100	789
Bacteria	Verrucomicrobia	Coralimargarita akajimensis DSM 45221	1081		Bacteria	Bacteroidetes	Robiginitalea biformata HTCC2501	722
Viruses	Marine	SDSU MARINE SEDIMENT VIRUS SMPL	1079		Viruses	Marine	SDSU SCRIPPS PIER MARINE VIRUS SMPL	667
Bacteria	α-Proteobacteria	alpha proteobacterium HIMB114	982		Bacteria	Verrucomicrobia	Methylocidiphilum infernorum V4	653
Bacteria	Actinobacteria	Clavibacter michiganensis subsp. michiganensis NCPPB 382	942		Bacteria	Verrucomicrobia	Chthoniobacter flavus Ellin428	599
Mar Menor 0.8µm					Albufera 0.8µm			
Domain	Taxon	Specie	#Hits		Domain	Taxon	Species	#Hits
Bacteria	Cyanobacteria	Synechococcus sp. WH 7803	19136		Bacteria	Cyanobacteria	Synechococcus sp. RCC307	3729
Bacteria	α-Proteobacteria	Candidatus Puniceispirillum marinum IMCC1322	5591		Bacteria	Cyanobacteria	Synechococcus sp. WH 7803	2573
Viruses	Marine	UDEL CHESAPEAKE VIROPLANKTON SMPL	5280		Bacteria	Planctomycetes	Pirellula staleyi DSM 6068	2398
Bacteria	α-Proteobacteria	Ruegeria pomeroyi DSS-3	3502		Bacteria	Bacteroidetes	Haliscomenobacter hydrossis DSM 1100	1997
Bacteria	Verrucomicrobia	Coralimargarita akajimensis DSM 45221	2960		Bacteria	Cyanobacteria	Synechococcus sp. CC9311	1970
Bacteria	α-Proteobacteria	Ruegeria sp. TM1040	2330		Bacteria	Cyanobacteria	Synechococcus sp. CC9605	1897
Bacteria	α-Proteobacteria	Candidatus Pelagibacter sp. IMCC9063	2208		Bacteria	Cyanobacteria	Synechococcus sp. WH 8102	1890
Bacteria	α-Proteobacteria	Dinoroseobacter shibae DFL 12	2190		Bacteria	Planctomycetes	Rhodopirellula baltica SH 1	1871
Bacteria	α-Proteobacteria	Roseobacter denitrificans OCh 114	1985		Bacteria	Cyanobacteria	Prochlorococcus marinus str. MIT 9303	1699
Bacteria	α-Proteobacteria	Roseobacter litoralis Och 149	1651		Bacteria	β-Proteobacteria	Methylibium petroleiphilum PM1	1655
Mar Menor 3.0µm					Albufera 3.0µm			
Domain	Taxon	Specie	#Hits		Domain	Taxon	Species	#Hits
Bacteria	Cyanobacteria	Synechococcus sp. WH 7803	6985		Bacteria	Bacteroidetes	Haliscomenobacter hydrossis DSM 1100	3432
Bacteria	Verrucomicrobia	Coralimargarita akajimensis DSM 45221	2511		Bacteria	Cyanobacteria	Cyanothece sp. PCC 7425	2662
Bacteria	α-Proteobacteria	Ruegeria pomeroyi DSS-3	1805		Bacteria	Cyanobacteria	Nostoc punctiforme PCC 73102	2619
Bacteria	Verrucomicrobia	Akkermansia muciniphila ATCC BAA-835	1422		Bacteria	Cyanobacteria	Acaryochloris marina MBIC11017	2185
Bacteria	Bacteroidetes	Haliscomenobacter hydrossis DSM 1100	1303		Bacteria	Cyanobacteria	Nostoc sp. PCC 7120	2165
Bacteria	α-Proteobacteria	Dinoroseobacter shibae DFL 12	1228		Bacteria	Cyanobacteria	Synechococcus sp. RCC307	2107
Bacteria	α-Proteobacteria	Ruegeria sp. TM1040	1205		Bacteria	Cyanobacteria	Anabaena variabilis ATCC 29413	1723
Bacteria	α-Proteobacteria	Maricaulis maris MCS10	1191		Bacteria	Cyanobacteria	Microcystis aeruginosa NIES-843	1616
Bacteria	α-Proteobacteria	Roseobacter denitrificans OCh 114	1182		Bacteria	Cyanobacteria	Cyanothece sp. PCC 7822	1484
Viruses	Marine	UDEL CHESAPEAKE VIROPLANKTON SMPL	1096		Bacteria	Cyanobacteria	Synechococcus sp. WH 7803	1419