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SUPPLEMENTARY INFORMATION

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Correction: Author correction

Surface properties of SAR11 bacteria facilitate grazing avoidance

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Surface properties of SAR11 bacteria facilitate grazing avoidance

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Supplementary information

Supplementary Tables

Table 1. Summary of feeding experiments.

Sampling date	Sampling method	Location	Class sampled	Taxa sampled	Total N
May 2015	InEx - *In situ* (*VacuSIP*)	Gulf of Aqaba, Israel	Ascidiacea	*Polycarpa mytiligera*	8
May 2012		East Mediterranean Sea Israel		*Microcosmus exasperatus*	4
August 2012					4
September 2014					15
April 2014	InEx - Lab (*VacuSIP*)	NW Mediterranean Sea France	Ascidiacea	*Styela plicata*	3
				Phallusia mammillata	6
				Ciona intestinalis	4
				*Microcosmus sp.**	3
				*Ascidiella aspersa?**	2
				*Unidentified **	3
April 2014	Incubation - *In situ*	NW Mediterranean Sea France	Larvacea	*Oikopleura albicans*	15

*Specimens sent for expert identification

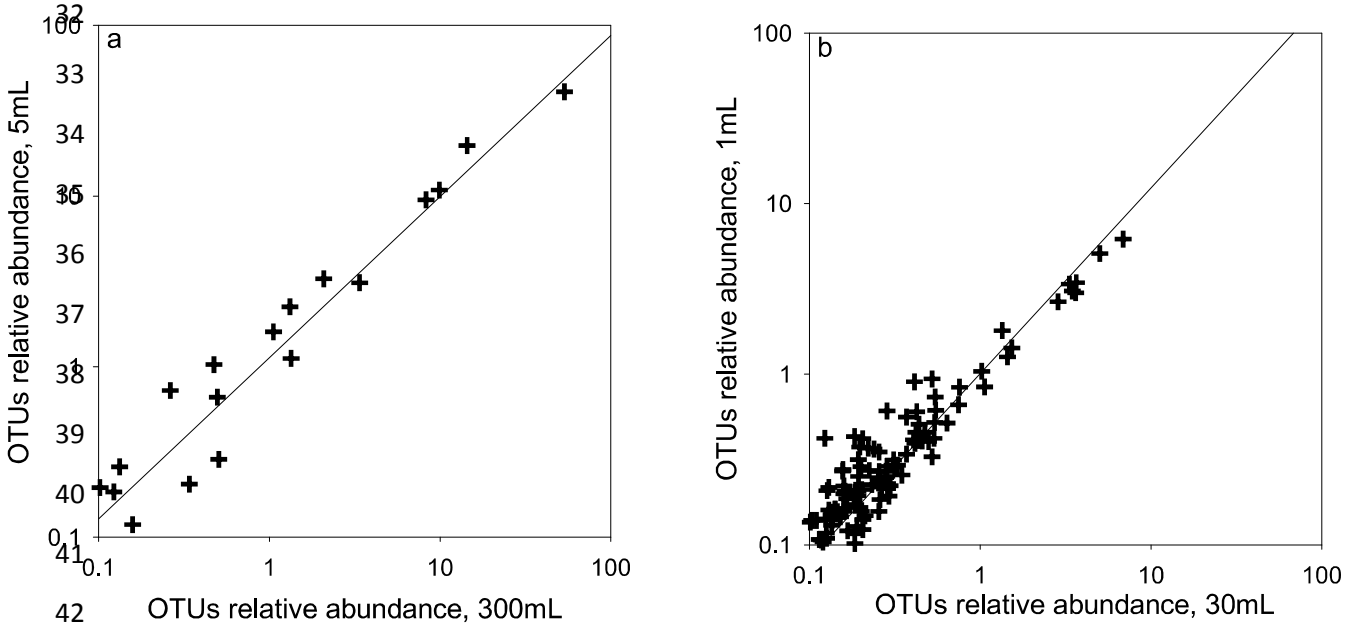
Table 2. Summary of p-values for post hoc pairwise comparisons (Wilcoxon test with sequential Bonferroni correction) between the Chesson selectivity index (α_i) of all phylotypes presented in figure 1c. P-values <0.05 are highlighted in red.

	Syn-Pro	SAR11_I	SAR86	SAR11_II	Prochlorococus	SAR11	Rhodobacteraceae	No Relative	NS5	SAR116	NS4	Candidatus Actinomarina	NS2b	SAR324	Owenweeksia	SAR406	Delta	Rickettsiales S25-593	OM60/NOR5
Syn-Pro																			
SAR11_II	0.000																		
SAR86	0.000	0.003																	
SAR11_II	0.000	0.323	0.020																
Prochlorococus	0.035	0.000	0.005	0.002															
SAR11	0.000	0.231	0.241	0.743	0.002														
Rhodobacteraceae	0.001	0.000	0.000	0.000	0.083	0.003													
No Relative	0.026	0.000	0.000	0.000	0.804	0.000	0.055												
NS5	0.000	0.046	0.132	0.463	0.002	0.938	0.001	0.000											
SAR116	0.000	0.473	0.161	1.000	0.000	0.938	0.000	0.000	0.642										
NS4	0.000	0.001	0.005	0.001	0.152	0.003	0.855	0.055	0.001	0.001									
Candidatus Actinomarina	0.000	0.231	0.054	0.473	0.001	0.690	0.000	0.000	0.473	1.000	0.001								
NS2b	0.107	0.000	0.000	0.000	0.002	0.000	0.000	0.005	0.000	0.000	0.000	0.000							
SAR324	0.000	0.004	0.326	0.012	0.015	0.017	0.026	0.000	0.058	0.005	0.058	0.011	0.000						
Owenweeksia	0.000	0.001	0.058	0.005	0.095	0.003	0.421	0.035	0.011	0.001	0.670	0.006	0.000	0.303					
SAR406	0.000	0.152	0.577	0.315	0.002	0.735	0.001	0.001	0.497	0.198	0.005	0.302	0.000	0.094	0.040				
Delta	0.000	0.161	0.465	0.493	0.001	0.625	0.022	0.000	0.765	0.463	0.017	0.577	0.000	0.147	0.010	0.922			
Rickettsiales S25-593	0.000	0.380	0.054	0.473	0.001	0.946	0.002	0.000	0.946	0.946	0.004	0.692	0.000	0.017	0.017	0.446	0.696		
OM60/NOR5	0.135	0.000	0.000	0.000	0.890	0.001	0.004	0.890	0.001	0.000	0.022	0.000	0.010	0.007	0.104	0.000	0.002	0.001	
NS9	0.001	0.000	0.000	0.000	0.978	0.000	0.048	0.978	0.000	0.000	0.107	0.000	0.000	0.001	0.005	0.000	0.000	0.000	1.000

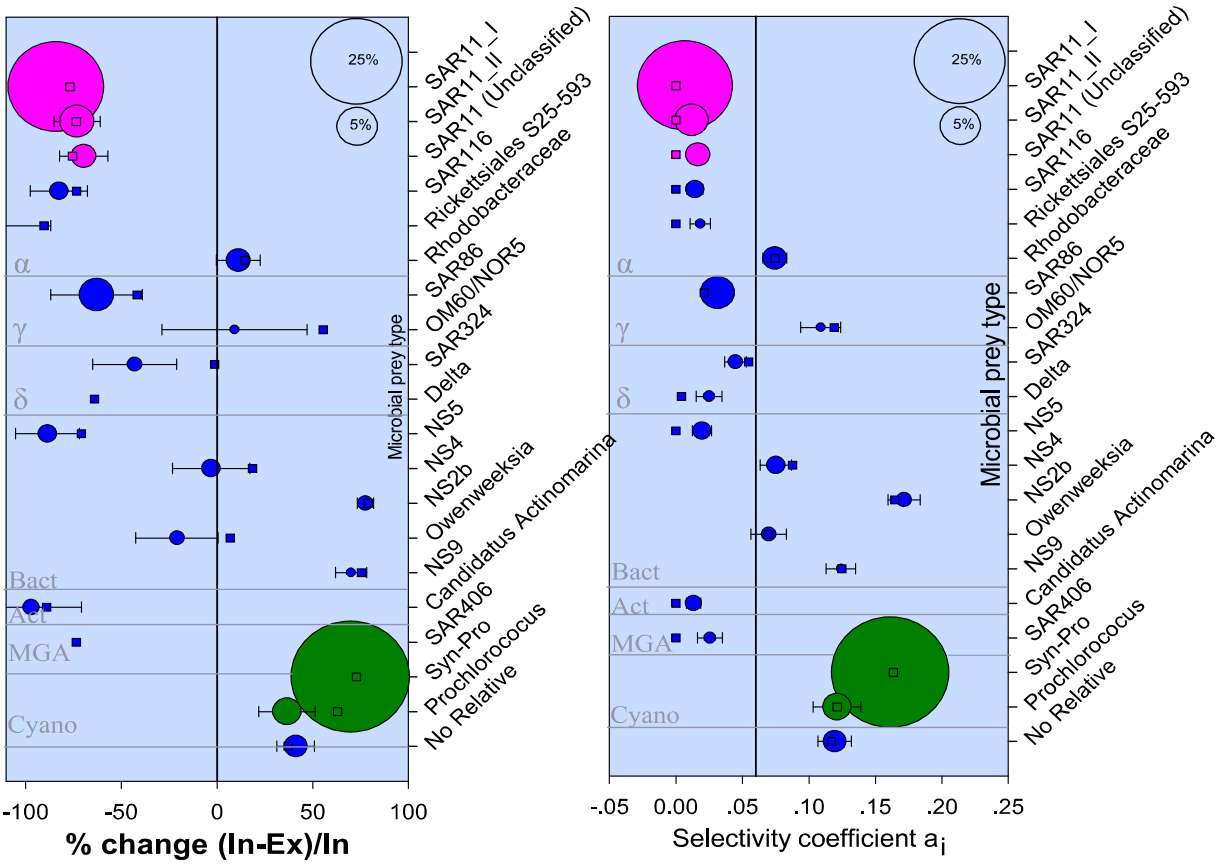
Table 3. Summary of p-values for post hoc pairwise comparisons (Wilcoxon test with sequential Bonferroni correction) between the Chesson selectivity index (α_i) of all phylotypes presented in figure 2c. P-values <0.05 are highlighted in red.

	SAR11-I	Syn-Pro	SAR11-II	SAR116	SAR86	Prochlorococcus	Roseobacter OCT	SAR11	Pseudoalteromonas	NS5	SAR11-IV	NS4	No Relative	Rhodobacteraceae	Ralstonia	Candidatus Actinomarina	OM60/NOR5	Balneola	S25-593
SAR11-I																			
Syn-Pro	0.025																		
SAR11-II	0.085	0.004																	
SAR116	0.765	0.013	0.166																
SAR86	0.052	0.358	0.005	0.068															
Prochlorococcus	0.091	0.626	0.007	0.017	0.426														
Roseobacter OCT	0.093	0.296	0.025	0.102	0.638	0.244													
SAR11	0.625	0.058	0.735	0.497	0.054	0.017	0.019												
Pseudoalteromonas	0.381	0.022	0.922	0.520	0.110	0.022	0.130	0.557											
NS5	1.000	0.030	0.041	0.846	0.085	0.017	0.102	0.653	0.376										
SAR11-IV	0.204	0.463	0.054	0.241	0.465	0.104	0.966	0.148	0.094	0.068									
NS4	0.381	0.013	0.068	0.465	0.520	0.068	0.569	0.465	0.276	0.696	0.234								
No Relative	0.970	0.042	0.321	0.846	0.054	0.003	0.233	0.696	0.323	0.899	0.175	0.831							
Rhodobacteraceae	0.040	0.583	0.002	0.027	0.569	0.542	0.216	0.017	0.022	0.007	0.340	0.064	0.110						
Ralstonia	0.244	0.735	0.027	0.177	0.677	1.000	0.569	0.177	0.014	0.148	0.588	0.321	0.470	0.893					
Candidatus Actinomarina	0.465	0.358	0.020	0.366	0.677	0.168	0.700	0.194	0.194	0.276	0.734	0.831	0.831	0.216	0.465				
OM60/NOR5	0.022	0.952	0.001	0.004	0.413	0.670	0.204	0.027	0.034	0.010	0.204	0.042	0.027	0.970	0.734	0.266			
Balneola	0.520	0.119	0.041	0.571	0.206	0.079	0.700	0.233	0.465	0.770	0.465	0.846	0.922	0.008	0.301	1.000	0.110		
S25-593	0.625	0.033	0.463	0.571	0.102	0.020	0.175	0.821	0.821	0.735	0.054	0.376	0.493	0.014	0.083	0.233	0.043	0.642	
KI89A clade	0.102	0.903	0.003	0.020	0.366	0.715	0.321	0.028	0.093	0.011	0.241	0.042	0.124	0.946	0.850	0.321	0.910	0.085	0.020

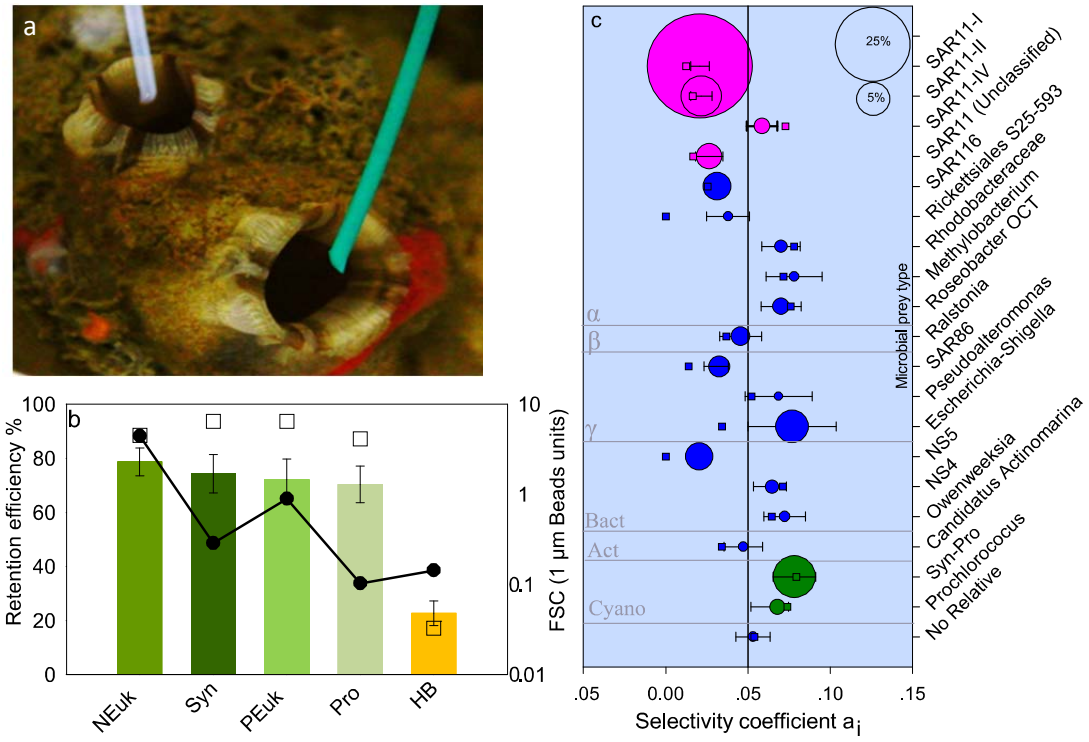
Supplementary Figures



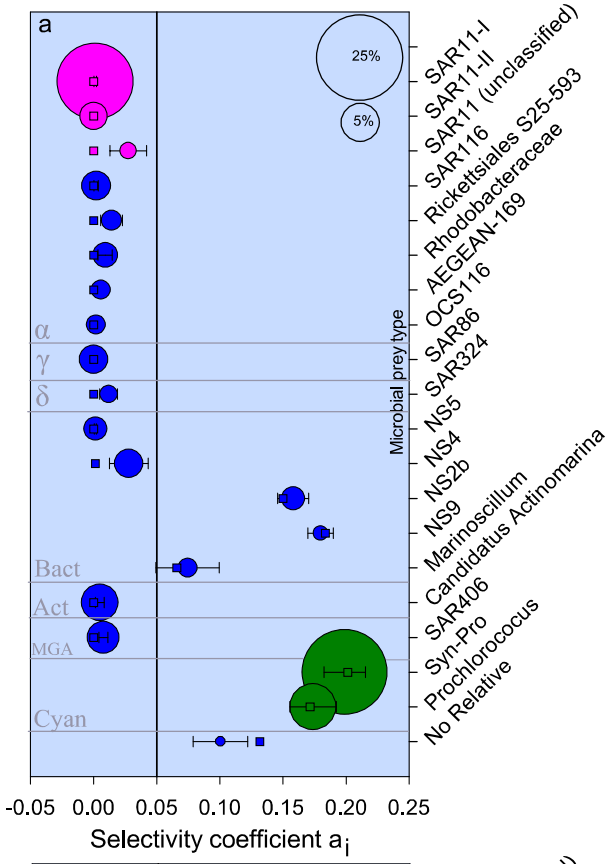
Supplementary figure 1: Effect of sample volume on the relative abundance (% of total) of reads belonging to the dominant OTUs in DNA extracts from the same seawater sample (a) 5 mL versus 300 mL of seawater using Illumina sequencing. (b) 1 mL versus 30 mL of seawater using 454 pyrosequencing.

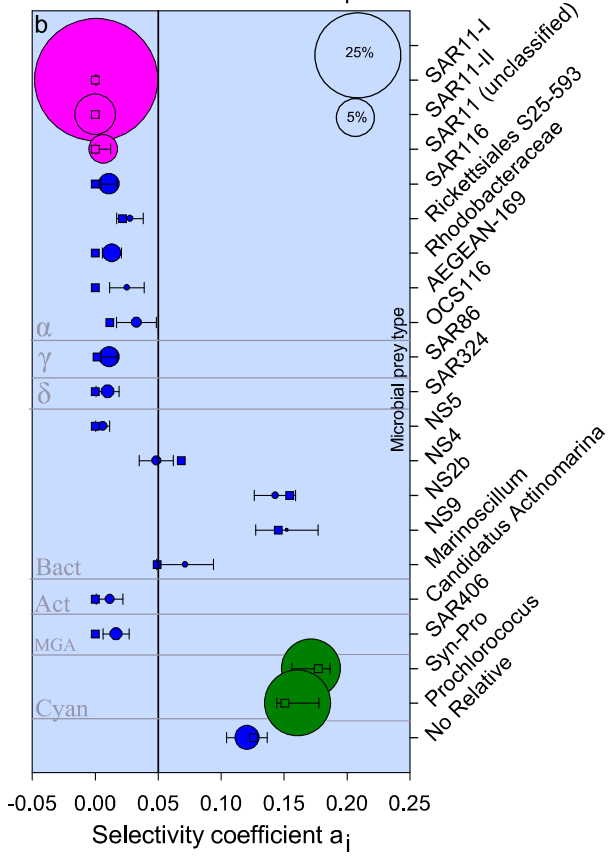


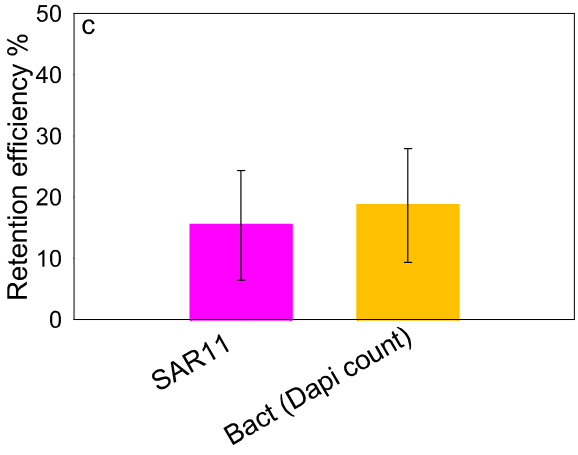
Supplementary figure 2: Comparison of different methods to calculate the differential retention of marine microbes. The same data presented in figure 1c for the ascidian *M. exasperatus* (n=15, Eastern Mediterranean Sea, September 2014) are plotted as (a) Average % change of the 20 most abundant OTUs in the relative frequency of each phylotype (from Illumina MiSeq sequencing) after the passage through the ascidians filtration system (calculated as (%In-%Ex) $(\%In)^{-1}$). Negative values show higher abundance in the exhaled waters and thus indicate lower retention. This analysis is independent of the flow cytometry counts. Grey lines divide OTUs into taxonomic categories; α, Alphaproteobacteria, γ, Gammaproteobacteria, δ, Deltaproteobacteria, Bact, Bacteroidetes, Act, Actinobacteria, MGA, Marine group A, Cyan, Cyanobacteria. Pink-members of the SAR11 clade, Green- autotrophs, Blue-other non-photosynthetic bacteria. Size of circles represents relative abundance in the inhaled water during sampling (Circles in the upper right shows scale for 5 and 25% of total reads). Error bars are SEM, squares represent medians. (b) Microbe specific selectivity coefficient (Chesson α_i), vertical line represents the expected α assuming equal retention probability for all cells.

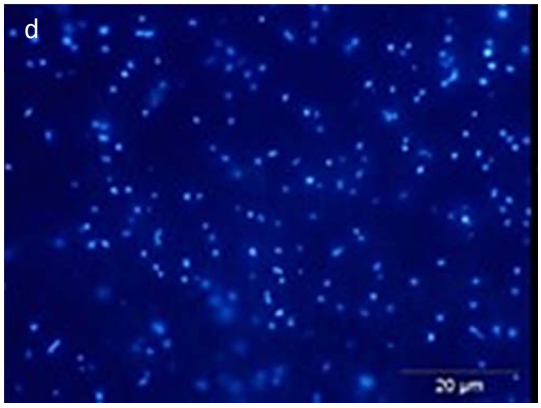


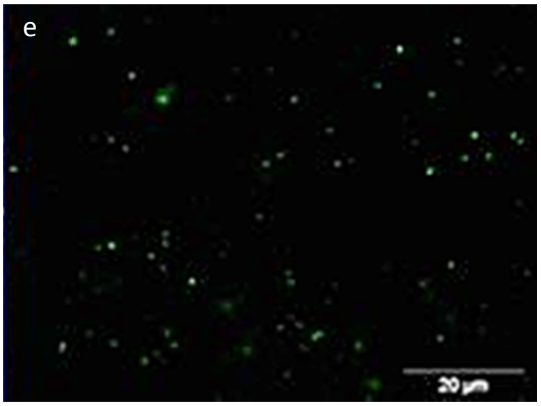
Supplementary figure 3: Mean differential retention of marine microbes by individuals belonging to 6 ascidian species from NW Mediterranean, April 2014, n=21. See Table 1 above for more details. (a) In-Ex sampling of the water inhaled and exhaled by ascidians using the VacuSIP method under controlled laboratory conditions (running seawater system). (b) Mean retention efficiency (%) of different prey types counted by flow cytometry (Error bars are SEM, squares represent medians). Black circles are the median forward scatter (proxy of cell size) of each prey type normalized to the forward scatter of 1 µm reference beads. NEuk, Nano eukaryotic algae, Syn, Synechococcus, PEuk, Pico eukaryotic algae, Pro, Prochlorococcus, HB, non-photosynthetic bacteria (c) Microbe specific selectivity coefficient (α_i) of the 20 most abundant OTUs in the water. Grey lines divide OTUs into taxonomic categories; α, Alphaproteobacteria, β, Betaproteobacteria, γ, Gammaproteobacteria, Bact, Bacteroidetes, Act, Actinobacteria, Cyan, Cyanobacteria. Pink-members of the SAR11 clade, Green- autotrophs, Blue-other non-photosynthetic bacteria. Vertical line represents the expected α assuming equal capture probability for all cells. Size of circles represents relative abundance in the inhaled water during sampling (Circles in the upper right shows scale for 5 and 25% of total reads). Error bars are SEM, squares represent medians.


a
25%
5%
SAR11-I
SAR11-II
SAR11 (unclassified)
SAR116
Rickettsiales S25-593
Rhodobacteraceae
AEGEAN-169
OCS116
SAR86
SAR324
NS5
NS4
NS2b
NS9
Marinoscillum
Candidatus Actinomarina
SAR406
Syn-Pro
Prochlorococcus
No Relative
Microbial prey type
α
γ
δ
Bact
Act
MGA
Cyan
-0.05 0.00 0.05 0.10 0.15 0.20 0.25
Selectivity coefficient a_i


b
25%
5%
SAR11-I
SAR11-II
SAR11 (unclassified)
SAR116
Rickettsiales S25-593
Rhodobacteraceae
AEGEAN-169
OCS116
SAR86
SAR324
NS5
NS4
NS2b
NS9
Marinoscillum
Candidatus Actinomarina
SAR406
Syn-Pro
Prochlorococcus
No Relative
Microbial prey type
α
γ
δ
Bact
Act
MGA
Cyan
-0.05 0.00 0.05 0.10 0.15 0.20 0.25
Selectivity coefficient a_i

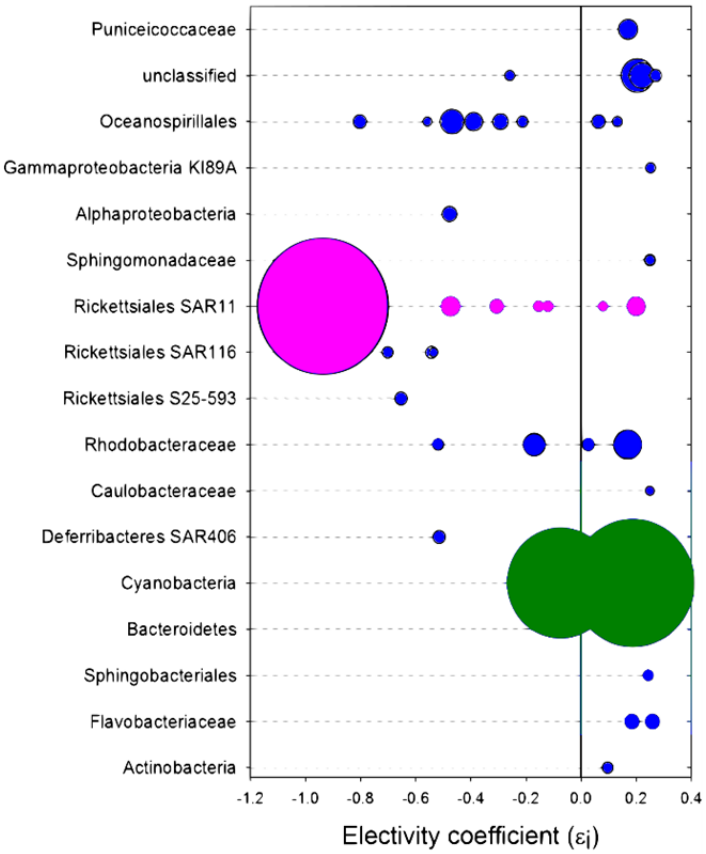

c
Retention efficiency %
50
40
30
20
10
0
SAR11
Bact (Dapi count)


d
20 µm

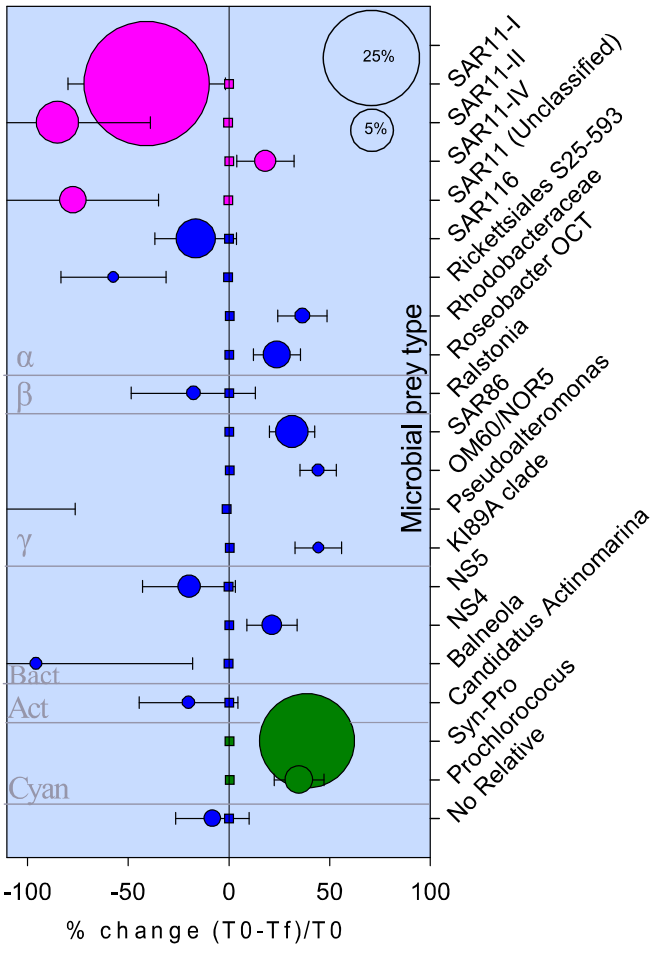

e
20 µm

88

Supplementary figure 4: Comparison of the different molecular methods (two primer sets and CARD-FISH) on the results of in situ InEx experiments. Differential retention of marine microbes measured in situ for the ascidian *Polycarpa mytiligera* at 6 m depth in the Gulf of Aqaba (n=8, May 2015). InEx samples were collected and extracted as described above and the DNA extracted from each sample was then split and analyzed with Illumina MiSeq using two different primer sets and the microbe specific selectivity coefficient (αi) of the 20 most abundant OTUs in the water were calculated and plotted: (a) With primer set 515F-Y-926R (Parada et al 2015), 65 prey items account for 95% of reads. (b) With primer set 28F- 519R, 17 prey items account for 95% of reads. (c) CARD-FISH analysis used to calculate average retention efficiency of SAR11 clade and total bacteria. (d) An example of DAPI staining to quantify the abundance of total bacteria (e) CARD-FISH used to quantify the abundance of SAR11 bacteria. (d and e are identical fields of view).



Supplementary figure 5: Microbe specific electivity coefficient (ε_i, Chesson 1983) for ascidians (*M. exasperates*) sampled *in situ* during May and August 2012, n=8. DNA was extracted using the filtration method described by Bostrom et al. (2004) and sequenced using 454 Pyrosequencing. Vertical line (0) represents the expected ε_i assuming equal retention probability for all cells, ε_i=0 represents null retention and ε_i=1 indicates that only prey type *i* was filtered. Size of circles represents relative abundance of the respective OTU in ambient water. Pink - members of the SAR11 clade, Green - autotrophs, Blue - other bacteria.



Supplementary figure 6: Differential clearance rate of marine microbes by the appendicularian *Oikopleura albicans* measured by in situ incubations of in the NW Mediterranean Sea during April 2014, n= 15. The same data presented in figure 2c are plotted as average % change of the 20 most abundant OTUs in the relative frequency of each phylotype (from Illumina MiSeq sequencing) after the passage through the appendicularian filtration system (calculated as (%T0-%Tf) $(\%T0)^{-1}$). This analysis is independent of the flow cytometry counts. Negative values show higher abundance in the water at the end of the incubation and thus indicate lower retention. This analysis is independent of the flow cytometry counts. Grey lines divide OTUs into taxonomic categories; α, Alphaproteobacteria, β, Betaproteobacteria, γ, Gammaproteobacteria, Bact, Bacteroidetes, Act, Actinobacteria, Cyan, Cyanobacteria. Pink-members of SAR11 clade, Green- autotrophs, Blue-other non-photosynthetic bacteria. Size of circles represents relative abundance in the inhaled water during sampling (Circles in the upper right shows scale for 5 and 25% of total reads). Error bars are SEM, squares represent medians.

Supplementary Reference list:

1. Parada, A. E., Needham, D. M. & Fuhrman, J. A. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* **18,** 1403–1414 (2016).

2. Boström Kjärstin H. , Simu Karin , Hagström Åke , Riemann Lasse , (2004), Optimization of DNA extraction for quantitative marine bacterioplankton community analysis, Limnol. Oceanogr. Methods, 2, doi:10.4319/lom.2004.2.365.