

Supplementary Material:

Depleted dissolved organic carbon and distinct Bacterial communities in the water column of a rapid-flushing coral reef ecosystem

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ISME Journal 2011

Table S1. Pyrosequencing collection metadata and sequence read information. Samples were collected January 12-14, 2008 at depths ranging from 1-5 m. Reads denotes the number of 16S rRNA gene V6 sequences derived from two pooled replicate libraries collected at each sampling station. OTUs denotes the number of unique operational taxonomic units after sequences were dereplicated according to the RefHVR8 SILVA-derived rRNA reference database (<http://vamps.mbl.edu>). Collection metadata include latitude/longitude (decimal degrees), depth (z), temperature, salinity, and concentrations of chlorophyll *a*, nitrate+nitrite (N+N), soluble reactive phosphorus (SRP), silicate, particulate organic carbon (POC), particulate organic nitrogen (PON), dissolved organic carbon (DOC), and bacterioplankton cell density (Bacteria).

Station	Date	Reads	OTUs	Lat	Lon	z m	Temp °C	Salinity PSU	Chl. a µg L <sup>-1</sup>	N+N µM	SRP µM	SiO <sub>3</sub> µM	POC µM	PON µM	DOC µM	Bacteria 10 <sup>8</sup> L <sup>-1</sup>
Offshore 130 km	13	39024	1542	-16.377	-150.145	2	28.24	35.97	0.030	N/C	N/C	N/C	N/C	N/C	N/C	N/C
Offshore 5 km	14	38192	1355	-17.427	-149.814	5	28.01	35.89	0.055	0.337	0.121	1.538	2.43	0.39	69.8	5.21
Forereef	14	47074	2724	-17.475	-149.837	5	28.15	35.91	0.206	0.634	0.115	0.900	3.65	0.39	71.4	5.13
Bay	12	43356	1871	-17.493	-149.822	5	28.64	35.91	0.434	0.191	0.133	1.777	7.35	0.95	61.7	6.97
Backreef (Lagoon)	12	37712	3477	-17.478	-149.842	1	28.45	35.90	0.093	0.407	0.143	1.448	4.68	0.44	64.4	3.82
Backreef (Fringe)	12	31954	2940	-17.485	-149.834	5	28.23	35.91	0.077	0.339	0.137	1.317	3.11	0.32	64.9	3.61

Fig S1. Concentrations of bacterioplankton and DOC in synoptic surveys conducted Aug-Sept 2008. Depth profiles of DOC and bacteria (a) collected 08/23/08 (Forereef and Offshore) and 08/25/08 (Backreef and Bay; see Fig. 1 for profile locations) show consistent concentrations over depth and spatial patterns which corroborate the 10m depth-averaged time series data in Fig. 3. Two synoptic surface surveys of DOC (b and c) and bacterioplankton (d and e) conducted 1 week apart (09/02/08 and 09/08/08) show temporal stability of spatial trends in the Backreef and Bay habitats corroborating results of the higher resolution 2009 surface synoptic survey (Fig. 2).

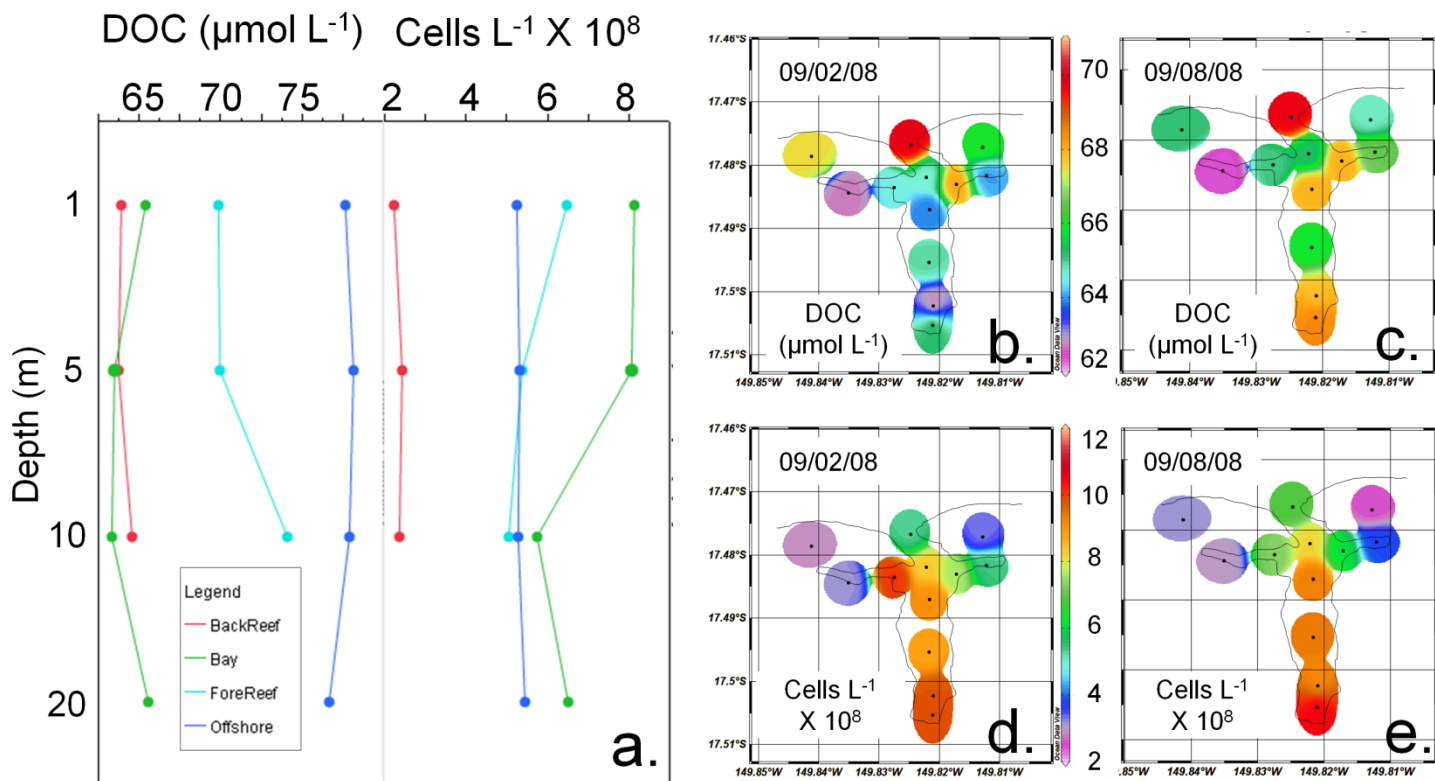


Fig S2. Comparison of ancillary nutrient and particulate organic matter concentrations among sampling habitats averaged over the upper 10m of the water column through time. As noted in the text, significant differences between habitats (assessed via Tukey post hoc test pairwise  $p < 0.05$ ) were found only for nitrate in Winter (Backreef > Offshore) and for particulate measures (Bay higher than other habitats for POC, PON, and Chl. A in both seasons). Abbreviations are as follows: SRP – soluble reactive phosphate, Chl. a – chlorophyll a, POC – particulate organic carbon, PON – particulate organic nitrogen. Particulate variables were log-transformed to approximate the normal distribution in statistical tests.

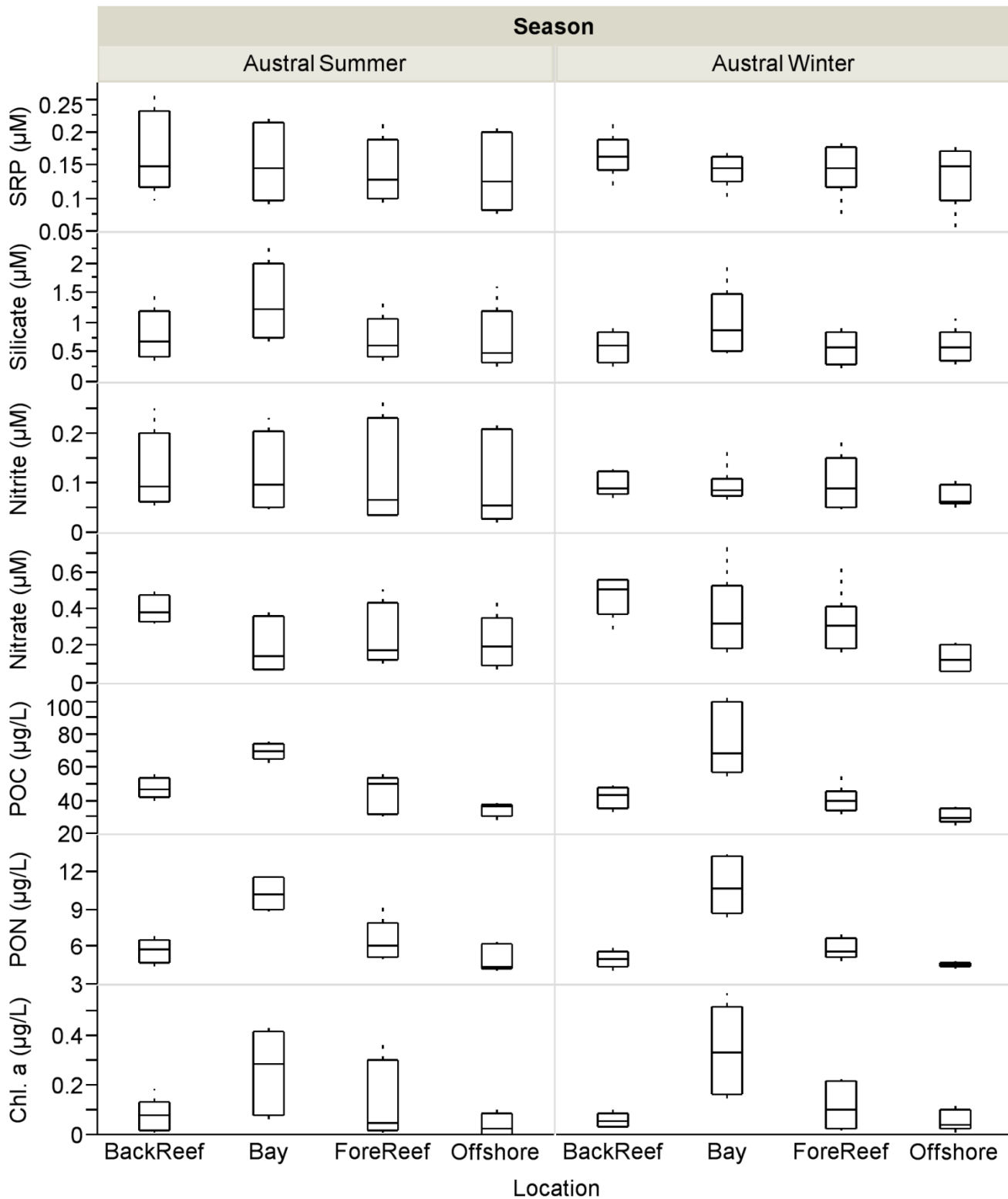


Fig S3. Spatial differentiation of bacterioplankton community composition in synoptic surveys Aug-Sept 2008. DNA samples collected at various locations and depths are symbol/color coded according to 5 community types defined as 70% Bray-Curtis similarity UPGMA clusters of 16S bacterial rRNA TRFLP fingerprints (a). Note that community types were homogenous over depth (b) and through time (b,c,d) but differed consistently by habitat (b,c,d; see Fig 1. for profile locations). Red lines within the dendrogram denote samples not significantly different at 95% confidence level by SIMPROF.

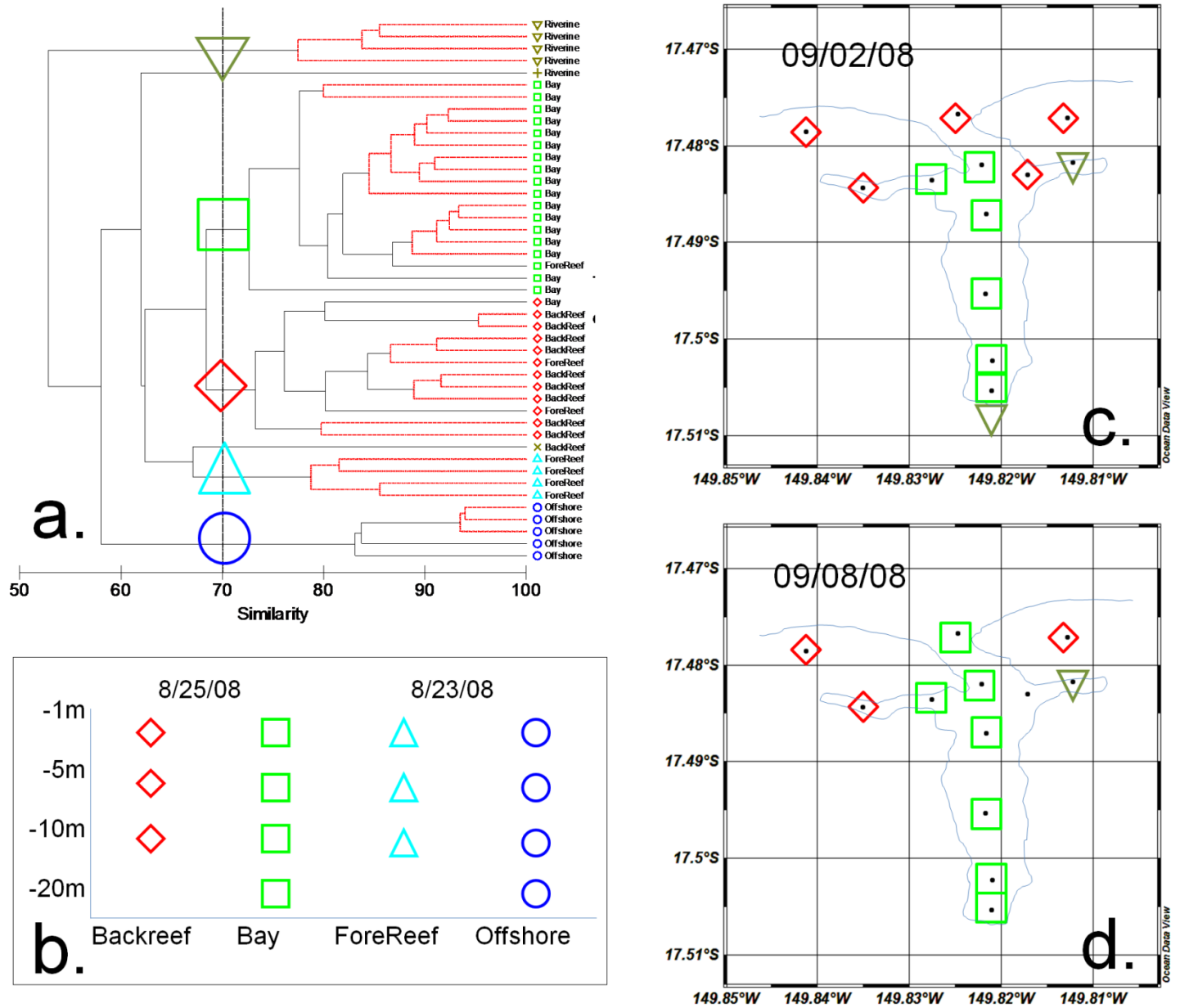


Fig S4. Effect of taxonomic level and binary vs. relativized data analysis on habitat differentiation among bacterioplankton communities. Cluster dendrograms generated from comparing 16S V6 tag pyrosequencing data among samples (Fig. 5) at two taxonomic levels (reference OTU [a,b] and Order [c,d]) and using relative abundance [a,c] or presence/absence [b,d] data to generate the similarity matrices. Note that tree structure is similar across taxonomic levels and that nearshore localities show differences in community structure using presence/absence data, suggesting that species identities, rather than simply abundances, differ between habitats. Red lines within the dendrogram denote samples not significantly different at the 95% confidence level by SIMPROF.

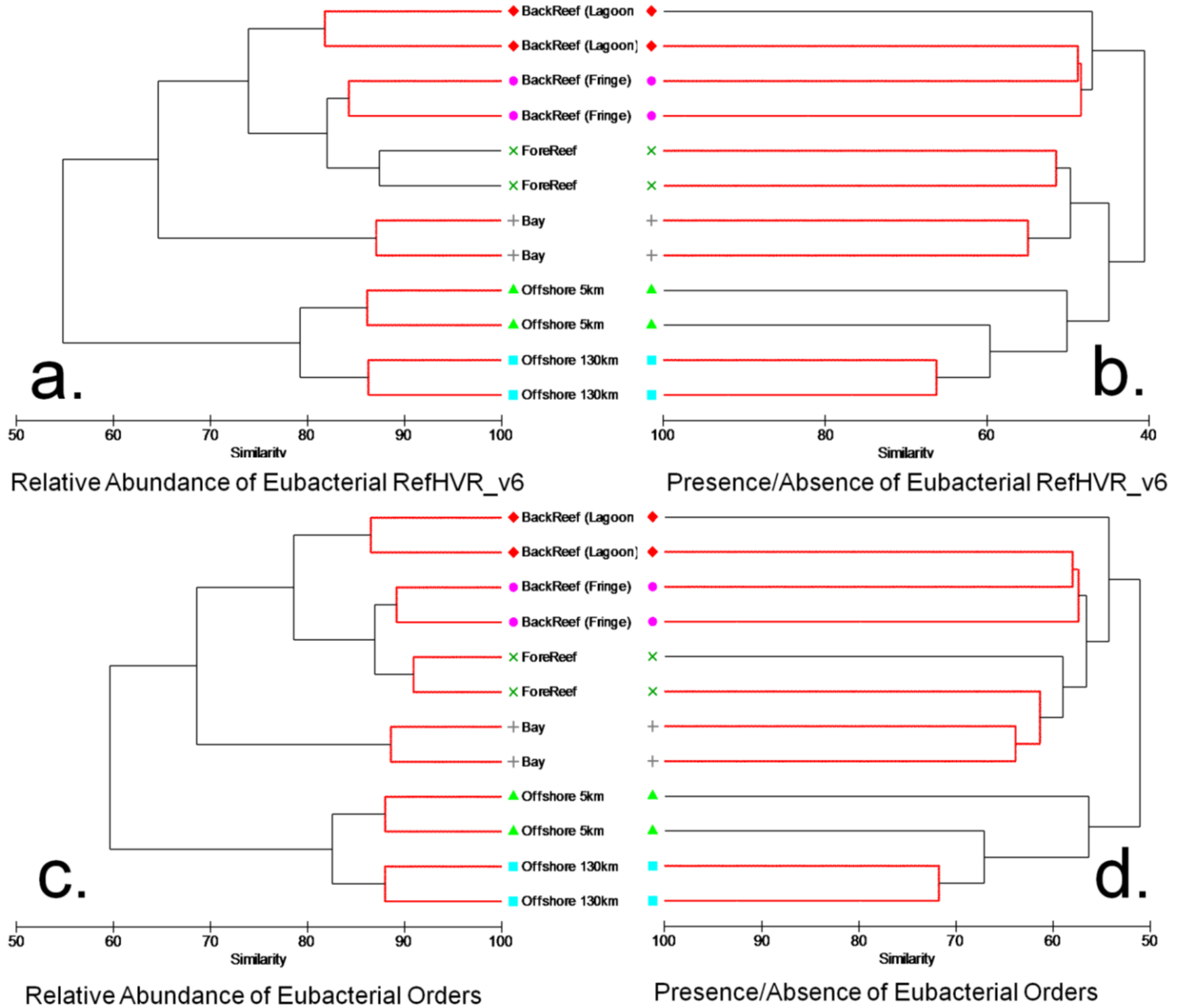


Figure S5. Maximum likelihood tree showing phylogenetic context of 16S rRNA clones generated randomly from a sample of BackReef water collected 3/2007. Sequences were aligned to the greengenes 16S rRNA core alignment via NAST (DeSantis, et al. 2006a, 2006b) and post-curated by GBLOCKS via the Phylogeny.fr pipeline (Dereeper et al 2008). The tree was built from sequences spanning 520 positions using the HKY85 evolution model within the PhyML software package (Guindon and Gascuel 2003) with branch support values displayed as percentages derived from 100 bootstraps and scale bar showing substitutions per base. Nearest neighbor isolate sequences aligned from the greengenes database were used to scaffold the tree and **Clone** and **Isolate** accessions HQ443320-HQ443409 denote the source of the sequence. To the right of each clone and selected isolates are restriction lengths using the HaeIII enzyme and primers 8f and 519r. Both *in silico* (predicted from sequence) and measured lengths (derived from TRFLP of cloned amplicons) are listed, and replicate clones are noted where removed for clarity. Clones used for TRFLP peak identification in Fig. 5 are noted. Note that while the closest isolated relative of clone HQ443344 used in Fig 5b is annotated as *Prochlorococcus*, the top five NCBI RefSeq Blast hits for this clone (genomic sequences assembled without isolation) are in the genus *Synechococcus*.

