Supplementary Material:

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

Craig E. Nelson, Alice L. Alldredge, Elizabeth A. McCliment, Linda A. Amaral-Zettler , and Craig A. Carlson

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	Temp	Salinity	Chl. a	N+N	SRP	SiO <sub>3</sub>	POC	PON	DOC	Bacteria
						m	°C	PSU	µg L⁻¹	μΜ	μΜ	μΜ	μΜ	μΜ	μΜ	$10^8 L^{-1}$
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 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*.

			In Silico	Measured	
Class UO4/2250		Accession/Genus	TRF	TRF	Notes
Clone_HQ443358		HQ443358	254	253	
Acinetopacter_AB101444.1		Acinetobacter	200	252	2 addt alanaa
	-	HQ443300	255	252	5 addit ciones
	. <u></u>	HQ443390			
	Ð	HO443388			
42 Acinetobacter AV787213.1	5	Acinetobacter	200		
Pequidomonas EE528204.1	g	Pseudomonas	39		
- 10 Clone HO443321	8	HQ443321	39	32	
Clone HO443345	0	HQ443345	225	224	
Alcaligenes AB118220 1	E.	Alcaligenes	226		
Clone HO443342	2	HQ443342	39	32	
48 Pseudoalteromonas AY646155 1	0	Pseudoalteromonas	39		
- Clone HO443323	6	HQ443323	39	32	2 addtl clones
Pseudomonas AB013829 1	Ĕ	Pseudomonas	39		
6 - Clone HQ443339	5	HQ443339	272	272	
Clone HQ443353	g	HQ443353	39	32	
Clone HQ443341	U	HQ443341	39	32	
Marinomonas X67025.1		Marinomonas	322		
r Clone HQ443337		HQ443337	192	191	Fig. 5e
Pseudovibrio AY486423.1		Pseudovibrio			
Isolate HQ443377		HQ443377			
Isolate HQ443405	<u>m</u>	HQ443405			
Clone HQ443356	do la	HQ443356	191	188	
Agrobacterium AY513489.1	E.	Agrobacterium	191		
Pelagibacter_AF510192.1	ac	Pelagibacter			
Clone_HQ443350	õ	HQ443350	117	113	Fig. 5d
98 Clone_HQ443359	0	HQ443359	293	292	Fig. 5a
Clone_HQ443334	te	HQ443334	293		
Roseobacter_DQ659411.1	0	Roseobacter	39		
- Isolate_HQ443396	O.	HQ443396			
66 Clone_HQ443349	σ	HQ443349	39	32	2 addtl clones
Isolate_HQ443373	F	HQ443373			
Paracoccus_EU376960.1	4	Paracoccus			
Clone_HQ443338	A	HQ443338	227	227	
Clone_HQ443325		HQ443325	224	224	2 addtl clones
Nisaea_DQ665838.1		Nisaea	150		
Clone_HQ443357		HQ443357	202	201	
Tenacibaculum_AM990737.1		Tenacibaculum			
Isolate_HQ443367		HQ443367			
4/ Usolate_HQ443372		HQ443372			
Cytophaga_AB073587.2		073587.2	20	22	
26 Clone_HQ443347		HQ443347	39	32	
Roseivirga_EU69/080.1		Roseivirga	39	22	
43 Clone_HQ443324		HQ443324	53	524	
Tomlana AM046640.4	S	Tamlana	39	524	
Talliana_Aw940040.1	Ð	HO443322	00		
	et	HQ443374			
9 Elavohactoriacoao AE402602.1	D	Flavobacteriaceae			
92 Gramella CU207366.1	0	Gramella			
	d)	HQ443371			
Cytophaga AB017046 1	t,	Cytophaga			
39 Clone HQ443340	g	HQ443340	286	285	Fig. 5c
14 - Formosa AY228461 1	Ê	Formosa	39		
Clone HQ443355		HQ443355	410	409	Fig. 5f
Clone HQ443346		HQ443346	39	32	
Clone HQ443330		HQ443330	39	32	
41 Lewinella AB301495.1		Lewinella			
- Isolate_HQ443368		HQ443368			
27 54 Synechococcus_NC_005070.1		Synechococcus			
Prochlorococcus_CP000111.1	-	Prochlorococcus			
Clone_HQ443344	- · ·	HQ443344	290	289	Fig. 5b; 24 addtl clones
Clone_HQ443354	e D	HQ443354	138	136	
Clone_HQ443352	ct a	HQ443352	305		
2B 39 Clone_HQ443327	2 G	HQ443327	305	305	
Mantoniella_X90641.1		Mantoniella			
Clone_HQ443328		HQ443328	004	004	
63 Clone_HQ443329		FIQ443329	231	231	
Plesiocystis_ABCS01000137.1		Fleslocystis	39	20	
74 Clone_HQ443351		HQ443351	39	32	
Clone_HQ443331		HQ443331	213	212	
Cione_HQ443348		Recillus	322	321	
Bacillus_DQ985053.1		Lentisnhaera	309		
95 Lentisphaera_AY390429.1			404	401	
		Pirellula	2401	401	
84 Pirellula_EF589353.1		HO443332	240	240	
45 Clone_HQ443332		Planctomyces	296	240	
Planctomyces_ABCE01000043.1		HQ443333	283	283	
- CIONE HQ443333			200	200	