Day-length is central to maintaining consistent seasonal diversity in marine bacterioplankton

Jack A. Gilbert[1], Paul Somerfield[1], Ben Temperton[1], Sue Huse[3], Ian Joint[1], Dawn Field[2]

[1]*Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH, UK*

[2]*NERC Centre for Ecology and Hydrology, CEH Oxford, Mansfield Road, Oxford, OX1 3SR, UK*

[3]*Josephine Bay Paul Centre for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts, USA*

Marine bacterial diversity is vast, but seasonal variation in diversity is poorly understood. Here we present the longest bacterial diversity time series consisting of monthly (72) samples from the western English Channel over a 6 year period (2003-2008) using 747,494 16SrDNA-V6 amplicon-pyrosequences. Although there were characteristic cycles for each phylum, the overall community cycle was remarkably stable year after year. The majority of taxa were not abundant, although on occasion these rare bacteria could dominate the assemblage. Bacterial diversity peaked at the winter solstice and showed remarkable synchronicity with day-length, which had the best explanatory power

compared to a combination of other variables (including temperature and nutrient concentrations). Day-length has not previously been recognised as a major force in structuring microbial communities.

Environmental factors driving the structure and function of microbial community are still poorly understood. Previous efforts to unravel the factors that exert the most significant influence have focused on showing the relative importance of temperature and nutrient concentration in structuring communities (Gilbert et al., 2009; Fuhrman, 2009; Fuhrman et al., 2006; Morris et al., 2005; Kirchman et al., 1995; Cullen, 1991). This is because temperature controls enzyme kinetics (Nedwell and Rutter, 1994) and nutrients drive niche structure through resource partitioning (Church, 2009). Likewise, the global biogeographic distribution of bacteria has recently been shown to follow a latitudinal gradient (primarily driven by temperature) as for other taxa (Fuhrman et al., 2008).

To date, however, despite growing evidence that many phyla of bacteria respond to sunlight (Moran and Zepp, 2000; Béjà et al., 2000; Mopper and Kieber, 2002; Swalbach et al., 2005; Gómez-Consarnau et al., 2007), the role of day-length as a driver of microbial diversity has not been directly considered. Both temperature and nutrient availability lag behind availability of light energy, therefore our hypothesis is that day-length might be a primary factor determining the seasonality of marine bacterial communities. While many areas of the

earth have equal day lengths year-round (around the Equator), more northern or southern latitudes have significant differences between day length between the winter (Dec 20 or 21) and summer (June 20 or 21) solstice, which may drive patterns in microbial diversity.

The Western English Channel provides an ideal model system in which to test for an effect of day-length on bacterial communities. The L4 long-term monitoring site is a coastal water observatory with more than 100 years of data (Southward et al., 2005; Smyth et al., 2009) which exhibits extremely short residence times (>2 months) and hence is being near constantly flushed (Siddorn et al., 2003) which could have an impact on the stability of microbial communities. At latitude of 50.258N (4.2178W), L4 has a winter solstice day length of almost eight hours and a summer solstice day-length of just over 16 hours, giving a maximum difference between winter and summer seasons of eight hours.

We tested whether this difference in day-length explained patterns in the diversity of the L4 bacterial community by generating an additional five years of molecular data to complement our 2007 study of the L4 site (Gilbert et al, 2009). We again determined bacterial diversity using 16S rDNA V6 tag pyrosequencing (Sogin et al., 2006). In addition to day-length, we examined the role of a broad range of biotic and abiotic parameters, including the concentrations of ammonia, nitrate + nitrite, phosphate, silicate, total organic carbon and total organic nitrogen, salinity, chlorophyll, and temperature in determining bacterial diversity. We also explored the difference in temporal abundance between dominant and rare taxa. Here we report the result of this analysis of 72 time points taken over six years of sampling at the L4

site (2003-2009; **Table S1**) and show that day-length is a primary driver of the structure of this marine community and additionally rare bacteria can exhibit irregular blooms of considerable abundance.

Diversity is high, peaks at the winter solstice every year and samples are grouped by season. Overall, 747,496 16S rDNA V6 sequences were identified, including those previously published for the year 2007 (Gilbert et al, 2009). Combined these tags comprised 65,059 unique operational taxonomic units (OTUs) for the complete time-series study. However, due to concerns regarding overestimation of diversity using pyrosequencing (Quince et al., 2009), we further clustering these sequences using a 2% single-linkage pre-clustering methodology followed by an average-linkage clustering based on pair-wise alignments (Huse et al., 2010), resulting in 8794 OTUs. Sequences in the full data set ranged from 4101 to 32,826, with an average of 10,381 per time point. It was therefore necessary to correct for sample-size dependence and allow direct comparison of samples of equal size by randomly re-sampling sequences at each time point to generate subsets of sequences equivalent to the smallest number of sequences for anyone time point (4101) (Gilbert et al., 2009). This resulted in a final data set of 4204 taxa-clusters (295,272 sequences), 2554 of which were singletons (60%). The high proportion of singletons that remain following this reduction process is indicative that a large proportion of the community remains under-sampled, indicating considerable diversity. This is confirmed by rarefaction curves that do not approach plateau for any single sample or

for all samples when pooled (**Fig S1**). Only 12 (0.2 %) of these re-sampled OTU clusters were found at all 72 time points, but these lineages comprised ~45 % of all sequences. The most abundant organism, as seen for the 2007 data at the same station (Gilbert et al, 2009), was a strain of the SAR11 clade bacterium *Pelagibacter ubique*, which comprised 17.4 % of the entire dataset and was the most abundant organism annually.

Richness (S, or number of species) is quite constant across the time-series within a defined range and showing a distinct cyclical patterns of peaks in winter and troughs in summer (**Fig 1**). The mean S per time point is 286, with an average minimum of 194 around the summer solstice (June) and maximum of 352 around the winter solstice (December). This is further confirmed by permutation-based analysis of variance (of S) for all taxa, and also a range of phyla (**Table 1**). This shows that S is more similar at similar times of year across the time series, and that differences between seasons and among years are both highly significant, but seasonal differences tend to be greater than interannual ones (greater pseudo-F values although fewer d.f.). This lack of significant interaction terms suggests that the seasonal cycle is consistent across years.

Nonparametric ordination by multidimensional scaling (NMDS) of resemblances among samples (**Fig. 2**) clearly shows the seasonal nature of variation in the communities, with a clear separation of winter and summer samples and samples from spring and autumn occupying intermediate, but different, positions.

The dominant microbiota exhibit seasonally structured abundance. Overall the trends in microbial diversity were driven by changes in the community composition of the dominant community. The repeatable seasonality of the two most dominant taxa in recorded in this study, SAR11 and Roseobacteriales, are indicative of this (**Fig 3**). Although the abundance of these groups still demonstrated variability, both had distinct seasonal trends, with SAR11 (**Fig 3A**) showing greater abundance in the winter, with a smaller peak in abundance during June/July. Roseobacteriales however demonstrated a much more seasonally separated cycle with first a spring, then a larger summer peak in abundance, followed by a considerably lower abundance during the winter of each year (**Fig 3B**). During 2006 and 2007 SAR11 appeared to show a reduction in the seasonal variability, resulting in a visbale decrease in annual abundance; this interannual variability will need to be explored with the continuation of the sampling in subsequent years to determine the potential causative factors.

***Rare microbiota can exhibit irregular blooms of considerable abundanc*e**. Upon investigation of notable outlying data points in the NMDS plot (**Fig 2**) we discovered that the distant relationship of these samples was due to blooms of otherwise extremely rare taxa. In **figure 2**, point A is dominated by a bloom of the gammaproteobacterial genus *Vibrio* (**Fig 4A**), which constituted ~54% of the community during August 2003, yet for the rest of the time series they

were found at extremely low abundance (0 – 2% of monthly abundance). Interestingly, the presence of these bacteria is correlated with a spike in the abundance (to 1.2 % of total eukaryotic plankton abundance from a background of 0.002 – 0.2 % of total) of the diatom, *Chaetoceros compressus.* This single instance of increased abundance of a specific bacterial genera and a diatom species could support the seed-bank hypothesis, whereby environmental conditions facilitating the bloom of these taxa. Environmental conditions where relatively unusual on this date, with the highest total organic nitrogen and carbon concentrations and second highest chlorophyll A concentration measured between 2003 and 2008 (**Table S1**), which is indicative of a *C. compressus* diatom bloom which may have supported or indeed of been supported by the *Vibrio*. Also a verrucomicrobium *Opitutus* showed extraordinary abundance in spring 2004 and summer 2006 (**Fig 4B**), but was virtually absent at all other time points. However, for these blooms it was not possible to identify correlating eukaryotic plankton abundance or environmental conditions, suggesting that we did not measure the variable which caused this organism to bloom. It is extremely unlikely that these increases in abundance would occur if these taxa were artefactual, hence removal of sequences from analyses because they are observed singletons is likely not an accurate measure of their validity. Blooms of specific microbial species could result in potential human and environmental health risks, especially for the *Vibrio* bloom. Understanding the environmental factors which promote these irregular blooms is therefore a priority for health regulation

agencies. In fact elucidating this relationship could help to improve predictive models for the occurrence of Harmful Algal Blooms (HABs). This study is an excellent example of how time-series can be used to validate singletons as real organisms, and is indicative of the importance of exploring the whole planktonic community to understand ecosystem dynamics.

Observed winter peaks in diversity and seasonality are driven by day-length. Distance-based linear modelling (**Table 2**) shows that for all groups of OTUs there is a strong seasonal cycle in community structure centred on day-length (a cos derived term peaking at the winter solstice – DX1). Adding a second seasonal term centred on the spring equinox (sin derived term – DX2) improves the models significantly (δAIC> -2). The further addition of a linear time trend (D) marginally improves the fit of the resulting models for most groups except Cyanobacteria.

Of all the explanatory variables available the only one that shows a significant trend throughout the time-series is soluble reactive phosphate (SRP) which has tended to decrease slightly (**Table S3**). Detrending SRP and regressing each explanatory variable on the solstice term (DX1) and the equinox term (DX2) (**Table S4**) shows that for the majority of them the major component of variation is explained by the cos-derived solstice term (i.e. day-length), and they peak close to one or other of the solstices. Variables that were exclusively tracking day-length (sin-derived equinox term was not significant) were photo-active radiation (PAR),

mixed-layer depth (MLD), chlorophyll concentration, silicate concentration, and total organic carbon and nitrogen concentration (TOC and TON). Nitrite and nitrate concentration (NOx), and soluble reactive phosphate concentration (SRP), have annual cycles which do not follow day-length, but day-length is still the main component describing their annual cycle. The monthly North Atlantic Oscillation (NAO) peaks nearly half-way between the solstice and the equinox, and temperature peaks closest to the equinox. There is no evidence for a trend or seasonal cycle in salinity. A PCA of these variables (Fig. S2A) shows the seasonal cyclicity in environmental variables at L4.

It is not surprising, therefore, that for the majority of taxonomically defined groups a stepwise multiple linear regression on the explanatory variables, excluding those defining time trends (**Table S4**), selects the variables with the most explanatory power as being PAR (which was the variable which most closely tracked day-length) and temperature (the variable that most closely tracked the sin-derived equinox term) (**Table 2**). In the majority of cases the measured or modelled variables do not fit the biological descriptors quite as well ($\delta AIC \approx 2$) as the artificial descriptors of the seasonal cycle, and without the temporal trend the measured or modelled variables have a significantly poorer fit to the seasonal pattern from all bacterial diversity and the diversity of different phyla (**Table 2**). However, for the Cyanobacteria these relationships are subtly different, in that like phytoplankton (Southward et al., 2005), they tended to peak in diversity during the Spring, and the variables best explaining variation in

cyanobacterial community structure are temperature, then PAR, and then NOx (**Table 2**). As this group are photosynthetic, they tend to respond to the spring conditions, relatively high nutrients (compared to summer) and increasing light availability (compared to winter), and bloom in abundance; hence this trend is entirely expected.

The fact that all relationships in the multivariate space are all highly significant (**Table 2**) it can be difficult to determine their relative importance. The univariate measures selected to describe changes in the assemblage, richness (S) and Simpsons index (1- '), both have significant time trends. For S, this is quadratic in nature (**Table 2**), essentially describing an increase in numbers during the earlier years (2003 and 2004) which then slows and stops (**Fig 1**); while 1- ' shows a weak but significant increasing trend throughout the series. Regressing both measures (following transformation) on the explanatory variables (**Table S4**) shows that the annual cycle in S (**Fig 1**) closely tracks the winter solstice. The combination of the solstice term and serial day describes 66.3 % of the variance in numbers of OTUs. There is a reasonable match between variation in S and environmental conditions (**Fig. S2B**), with the best fit being a combination of PAR, nitrate and nitrite and salinity, and this describes nearly as much of the variance (63.6 %) as the solstice term and serial day combined. This model, however, was a significantly less good fit to the data than the simple solstice term curve with an associated serial day trend. Detrending S, as expected, leaves a variable which is closely fitted by the solstice term alone. Again the closest fit among the explanatory variables is a

combination of PAR, salinity and NOx, but the fit is significantly less good than the solstice term on its own (**Table 2**).

Although there is a weak, but significant increasing trend in evenness this is not fitted by any of the measured or modelled explanatory variables (**Table 2**), either with a trend or detrended (**Table S3** and **S4**). Although not shown, these analyses were repeated on a subset of the data from 2005 onwards. No significant linear temporal trends were detected (either because of a lack of power or because they do not exist) but the cyclical nature of relationships, as described above, was fully retained.

Conclusions

This study demonstrates a strong statistical relationship between the availability of sunlight and the diversity of microbial communities. This system was defined by peaks in diversity in winter and strong seasonal shifts in the composition of the community. Importantly, while bacterial diversity is vast, and different phyla demonstrated different cycles, the overall community cycle is stable year after year. The majority of phyla demonstrated peaks in diversity at the winter solstice, with some subtle variations, notably the Cyanobacteria, which are photosynthetic.

Of the measured and modelled variables most vary with day-length, and there is no evidence that these measurements are better at explaining variation in the community structure

than a simple model based on a seasonal cycle tracking day-length. Reasons for this are probably two-fold. Firstly, bacteria may track this seasonal cycle based on day-length because other variables such as temperature and nutrient concentrations do. However, the community is unlikely to be responding to any of these other variables directly; other options that were not measured during this study are dissolved organic carbon (DOC) concentrations and photo-chemical reactions [Moran and Zepp, 1997]. In the winter, when DOC is lowest many different types of bacteria compete to obtain it, and in the summer, fewer species do well and so diversity decreases. A recent study on the impact of DOC on communities (Mou et al. 2008) demonstrated the potential importance of this on the community dynamic. Secondly, the observed bacterial taxa present on any particular day are the result of the combined responses of many taxa to events over the preceding days or weeks (fast division rates) and are therefore integrating the environmental 'climate', the net effect of many variables changing seasonally, rather than the environmental 'weather', the effects of individual variables operating on the exact day of measurement.

In conclusion, this is the first reported evidence that it is day-length that has the most significant impact on microbial diversity in a well-studied marine habitat. We speculate that this may constitute a general "rule" (e.g. Species-Area: Horner-Devine et al., 2004; Bell et al., 2005; Latitudinal: Fuhrman et al., 2008) that should be tested further using uniform studies of microbial communities across a different latitudes and times of year. While there is some year on year variation, which is occasionally driven by blooms of rare taxa, it is the seasonal cycle

that dominates; so that for all their astonishing variability bacteria seem to show an extraordinarily consistent seasonal cycle.

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Table 1 Permutation-based analysis of variance tests of differences among seasons and years for different taxonomic groupings, using Bray-Curtis similarities calculated from Log(N+1)-transformed abundances.

	Source	df	SS	MS	Pseudo-F	p
All	Season	3	25984	8661.2	6.84	0.001
	Year	5	13742	2748.4	2.17	0.001
	Season × Year	15	18644	1243	0.98	0.592
	Residual	48	60771	1266.1		
	Total	71	121000			
α Proteobacteria	Season	3	24068	8022.8	7.26	0.001
	Year	5	12904	2580.9	2.34	0.001
	Season × Year	15	15895	1059.6	0.96	0.721
	Residual	48	53052	1105.3		
	Total	71	107000			
Bacterioidetes	Season	3	25034	8344.6	6.38	0.001
	Year	5	10595	2119	1.62	0.002
	Season × Year	15	19966	1331.1	1.02	0.392
	Residual	48	62768	1307.7		
	Total	71	120000			
Cyanobacteria	Season	3	15728	5242.6	4.51	0.001
	Year	5	10740	2148	1.85	0.002
	Season × Year	15	23665	1577.6	1.36	0.015
	Residual	48	55768	1161.8		
	Total	71	105000			
Other phyla	Season	3	36883	12294	5.38	0.001
	Year	5	27020	5403.9	2.37	0.001
	Season × Year	15	34248	2283.2	1.00	0.480
	Residual	48	110000	2283.3		
	Total	71	20000			

Table 2. Summary of results from stepwise distance-based linear modeling of inter-sample similarities (or distances for single variables) variables on temporal variables (serial day = D, solstice term = DX1, equinox term = DX2) and measured or modeled variables. S – species richness or number of operational taxonomic units. 1- ' is the Simpsons dominance coefficient, 1-(1-a') refers to the indicative eveness of the community.

	Variables	AIC	SS(trace)	Pseudo-F	p	R^2	Variables	AIC	SS(trace)	Pseudo-F	p	R^2
All	+DX1	523.7	22332	15.9	0.001		+PAR	525.4	19978	13.9	0.001	
	+DX2	520.2	7225	5.5	0.001		+Temp.	522.2	7043	5.2	0.001	22.4
	+D	518.8	4147	3.2	0.001	28.0						
Alphaproteobacteria	+DX1	513.9	21499	17.5	0.001		+PAR	515.9	19165	15.2	0.001	
	+DX2	510.2	6520	5.7	0.001		+Temp.	512.5	6374	5.4	0.001	23.8
	+D	508.7	3840	3.5	0.001	29.7						
Bacterioidetes	+DX1	526.6	17501	12.0	0.001		+PAR	528.0	15526	10.4	0.001	
	+DX2	522.6	8144	6.0	0.001		+Temp.	524.4	7836	5.6	0.001	19.5
	+D	521.7	3785	2.9	0.001	24.6						
Cyanobacteria	+DX1	523.3	7515	5.4	0.001		+Temp.	523.1	7853	5.6	0.001	
	+DX2	520.9	5800	4.4	0.001	12.7	+PAR	520.4	6159	4.7	0.001	
							+Log(NOx)	520.1	2804	2.2	0.019	15.0
All Others	+DX1	566.7	30576	12.0	0.001		+PAR	567.3	29049	11.3	0.001	
	+DX2	564.3	10552	4.3	0.001		+Temp.	565.1	10109	4.1	0.001	18.8
	+D	562. 7	8233	3.5	0.001	23.6						
Log (S)	+DX1	-203.4	5.72	99.1	0.001		+PAR	-195. 7	5.26	82.0	0.001	
	+D	-216.3	0.75	15.8	0.001	66.3	+Log(NOx)	-199. 0	0.32	5.2	0.024	
							+-Log(35.5-Sal)	-202. 4	0.30	5.3	0.020	
							+Log(Sil)	-203. 6	0.17	3.0	0.078	
							+Log(TOC)	-204. 7	0.16	3.0	0.101	63.6
Detrended Log (S)	+DX1	-225.9	5.33	126.2	0.001	64.3	+PAR	-219. 0	5.04	108.5	0.001	
							+-Log(35.5-Sal)	-225. 2	0.35	8.3	0.006	
							+Log(NOx)	-228. 8	0.22	5.6	0.033	
							+NAO	-229. 0	0.08	2.0	0.157	68.6
-Log(1-(1- '))	+D	-128.8	2.90	18.3	0.001		+Temp.	-116. 1	0.76	4.0	0.045	
	+DX2	-130.0	0.49	3.2	0.085	24.5	+-Log(35.5-Sal)	-116. 2	0.38	2.0	0.151	0.08
Detrended -Log(1-(1- '))	+DX2	-132.0	0.49	3.2	0.064	0.04	+Temp.	-133. 6	0.72	4.9	0.026	
							+-Log(35.5-Sal)	-133. 8	0.31	2.1	0.134	
							+Log(Sil)	-134.	0.30	2.1	0.155	

+Log(TON)	0 -134. 8	0.38	2.7	0.111	0.16

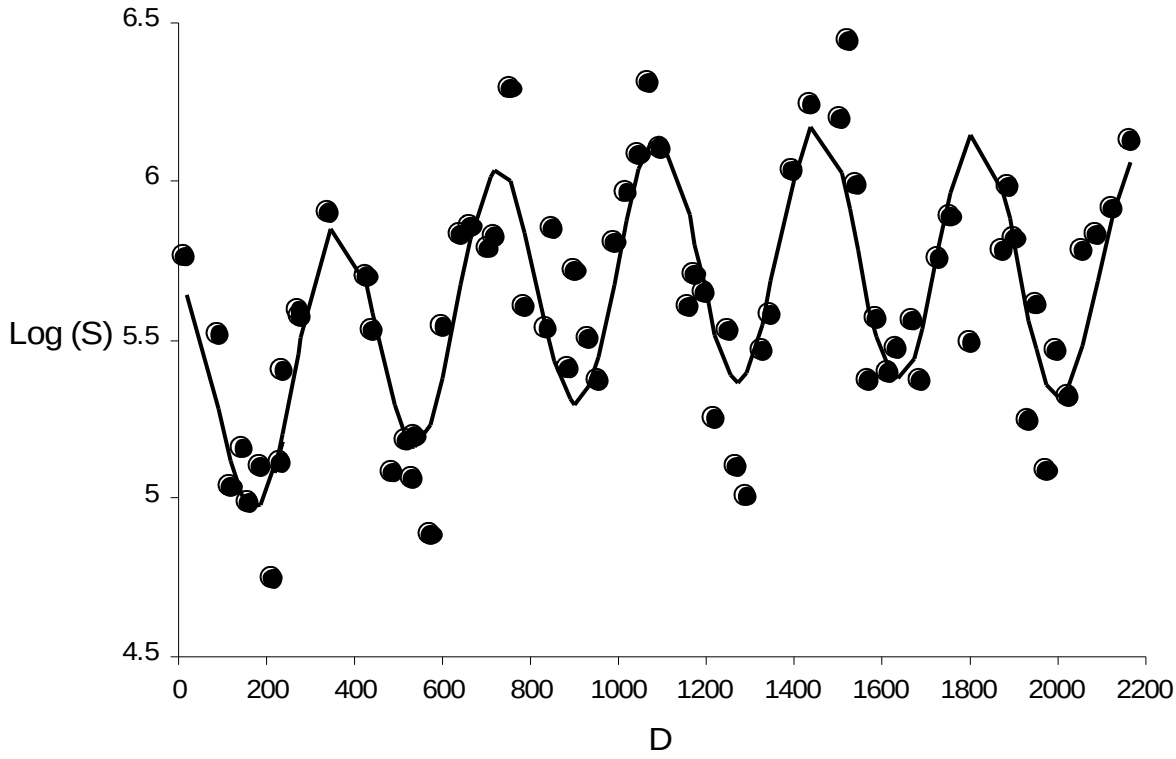


Figure 1 – The log of species richness (S) for each time point as a serial day between Jan 1st 2003 and December 31st 2008.

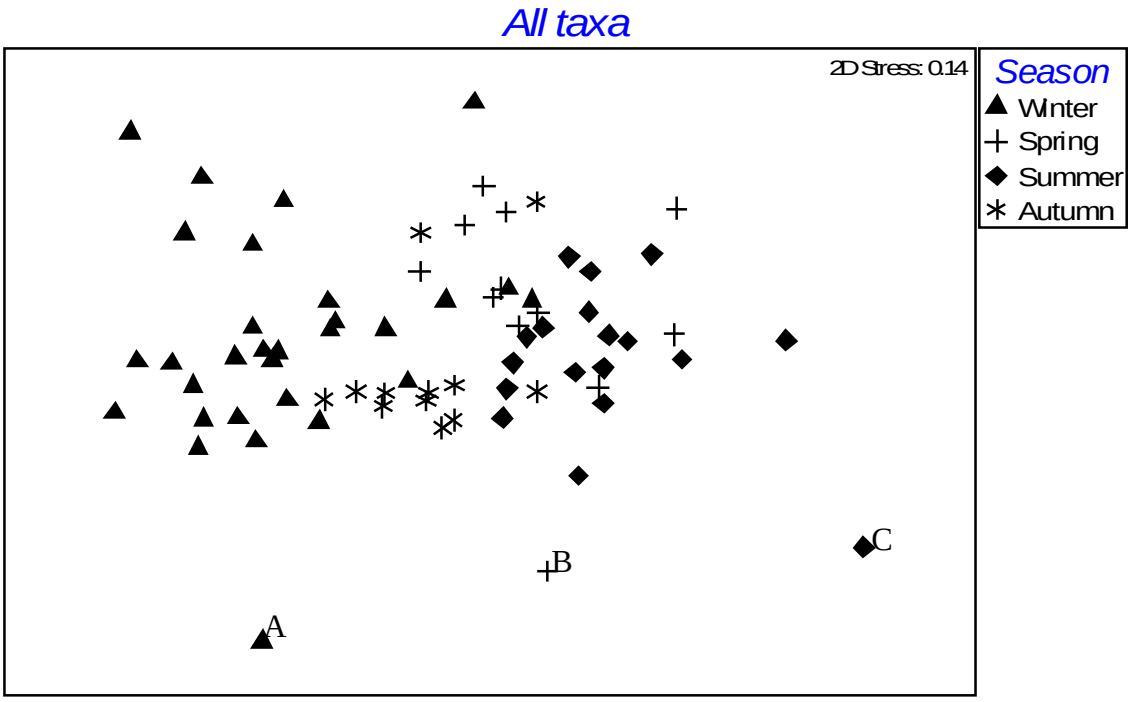
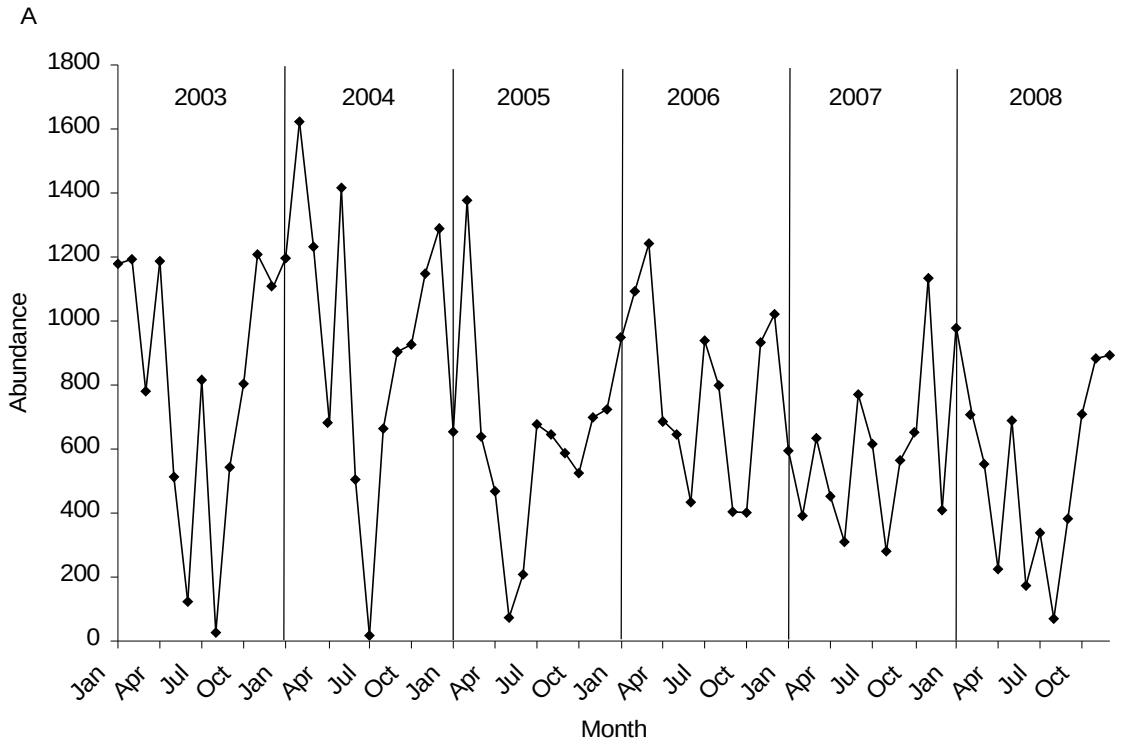


Figure 2 – Non-parametric multi-dimensional scaling (NMDS) ordination of the relative abundance of clustered operational taxonomic units (OTUs) for all 72 time points ordered by season. Considerable outliers to the general trend are labelled (A) which resulted from a *Vibrio* bloom (Fig 4A), and (B) and (C) which result from two separate *Opitutus* blooms (Fig 4B).

Figure 3 – Dominant bacterial taxa, (A) SAR11 and (B) Roseobacteriales, abundance across the 72 time points. All data was randomly resampled by lowest sample abundance, so that each sample has the same sequencing effort.



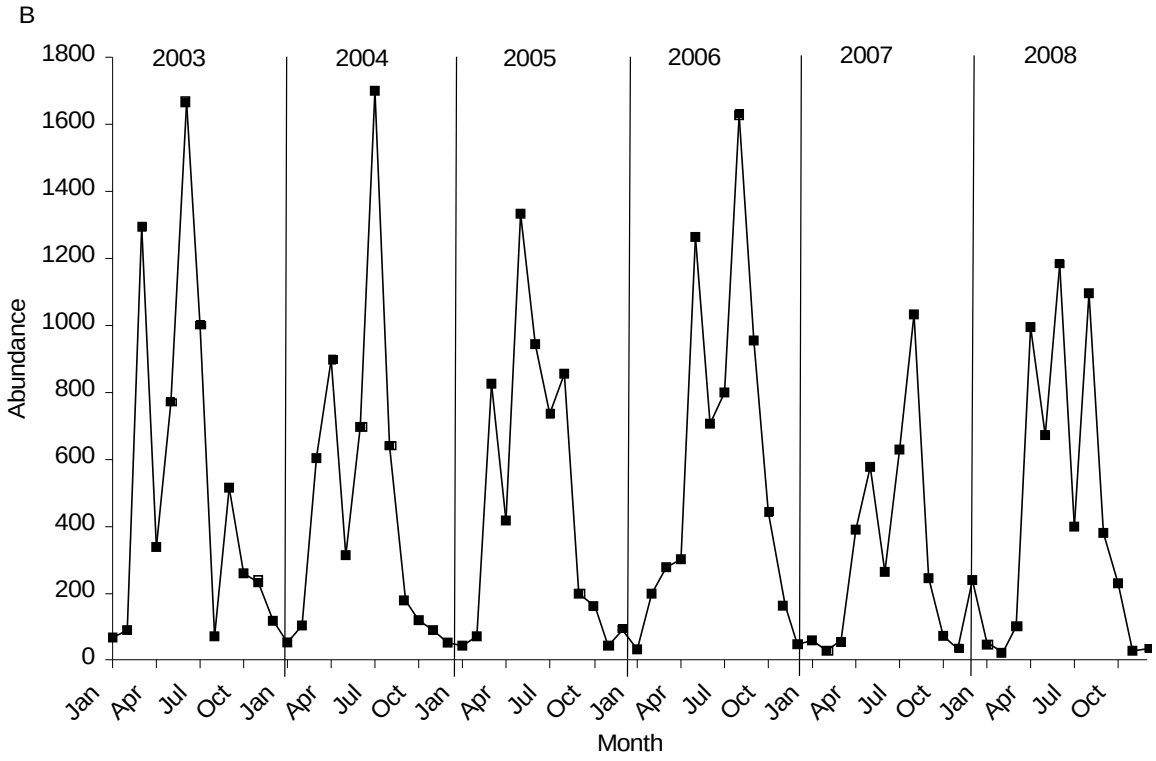
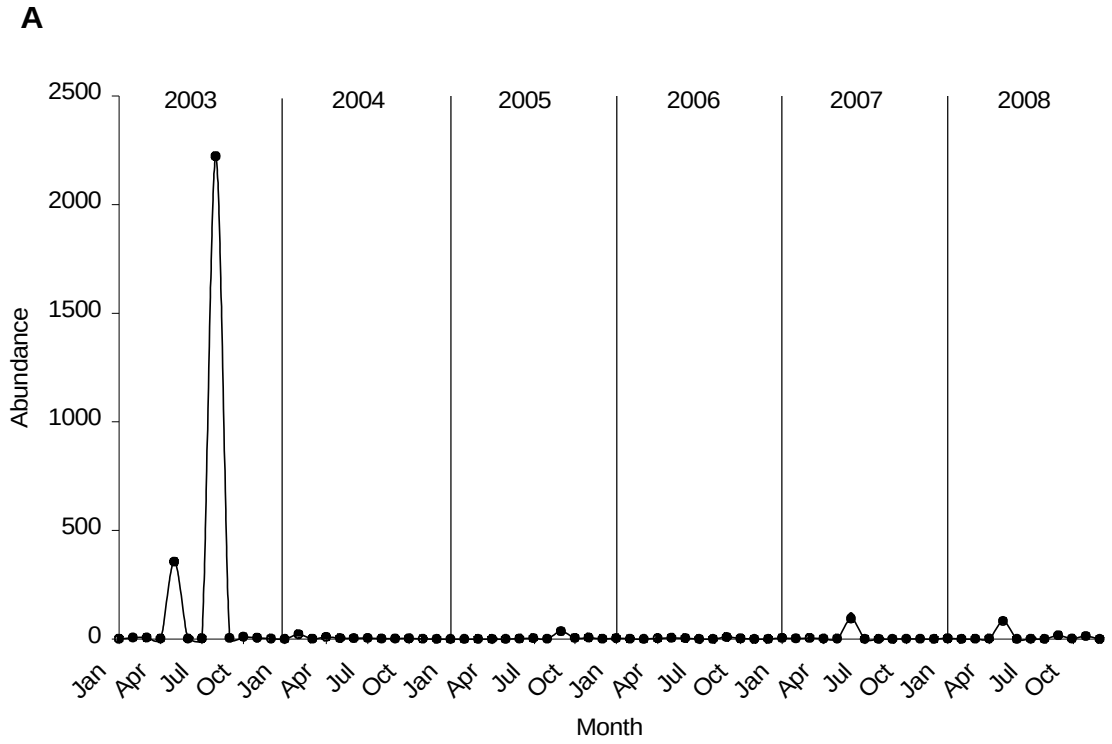
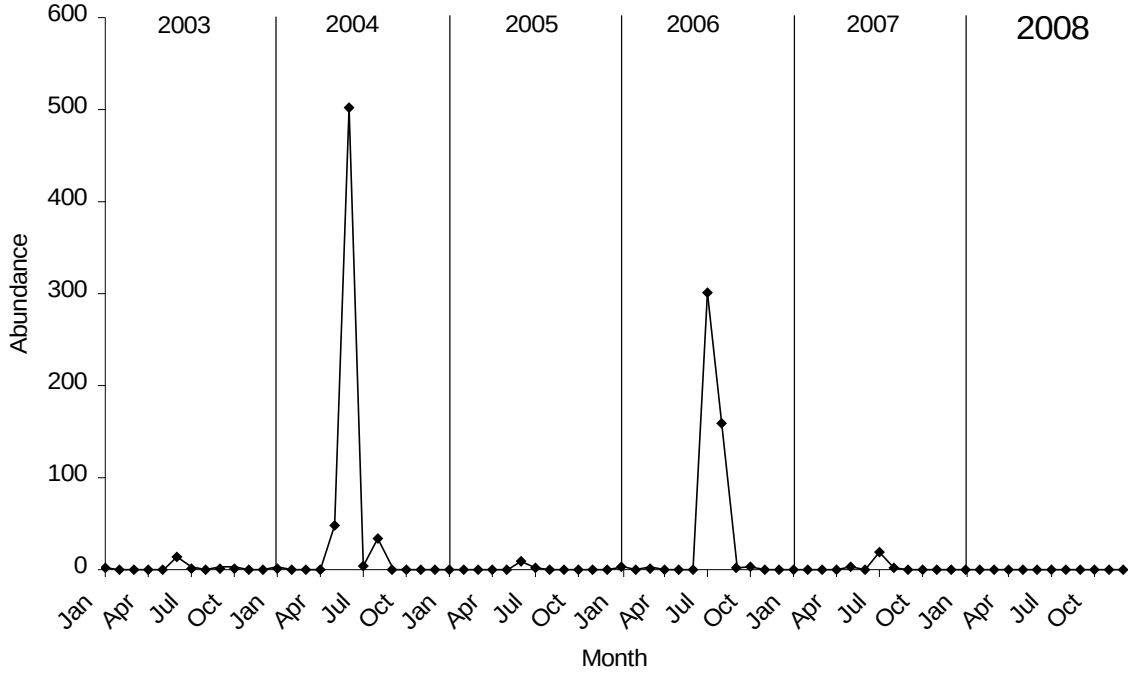


Figure 4 – Rare bacterial taxa, (A) *Vibrio* and (B) *Opitutus,* abundance across the 72 time points. All data base don randomly resampled abundance, so that each sample has the same sequencing effort.



B



Supplementary Methods

Sampling

Seawater samples were collected on 72 occasions from January 2003-December 2008 from the L4 sampling site (50° 15.00' N, 4° 13.02') of the Western Channel Observatory (http://www.westernchannelobservatory.org.uk; **Table S1**). Nineteen environmental variables were obtained from the WCO database, available on the website; these included surface temperature, salinity, water density, silicate concentration, nitrate + nitrite concentration, chlorophyll concentration, total organic nitrogen (TON) concentration, ammonium concentration, soluble reactive phosphorus (SRP) concentration, total organic carbon (TOC) concentration, photo-active radiation (PAR), phytoplankton and zooplankton taxa abundances, etc. Other variables measured were flow cytometric determination of bacterial abundance, microscope counts of cryptophyte, picoeukaryote, nanoeukaryote, coccolithophore and small dinoflagellate abundance. All methods for determining these variables are available on the WCO website (http://www.westernchannelobservatory.org.uk/all_parameters.html).

DNA extraction, 16S rDNA V6 amplification and Pyrosequencing

Nucleic acid was extracted from 5 L seawater, collected from the surface and filtered immediately through a 0.22 µm Sterivex cartridge (Millipore), which was stored at -80 °C.

DNA was isolated from each sample (Neufeld et al., 2007) and then stored at -20 °C. DNA was used as a template for V6-region 16S rRNA amplification using the method of Huber *et al.* (2007) in sets of twelve. Each amplicon in set of twelve was labeled with a unique multiplex identifier (MID) sequence (**Table S2**). Subsequently, amplicon product pools for each year were pyrosequenced on ½ of a 454 pico-titre plate using the GS-flx platform. All sequences have been submitted to the NCBI short reads archive under SRA009436, and registered with the GOLD database (Gm00104). All data submitted are MIENS compliant (Field et al., 2008; Yilmez et al., 2010).

Processing of sequences to generate OTUs and assign taxa

Re-sampling of the 72 samples to identical sequencing depth was done by randomly selecting reads in fasta format using Daisy_chopper v1.0 (http://www.genomics.ceh.ac.uk/GeneSwytch/Tools.html; Gilbert et al., 2009). All sequences were pre-processed using the method of Huse et al (2010). All data was then handled through the Visualization and Analysis of Microbial Population Structure project (VAMPS) website (http://vamps.mbl.edu/index.php). The VAMPS workflow was used to create a profile of the unique sequences, their taxonomic assignment and their abundance in each sample. OTUs in the normalized dataset were annotated with the GAST process (Sogin et al., 2006) and is freely available through the VAMPS website (http://vamps.mbl.edu/index.php).

Statistics

Changes in community structure through time were analysed using nonparametric multivariate methods (Clarke, 1993) implemented in Primer v 6 (Clarke and Gorley, 2006). Resampled abundances of OTUs (however defined) and subsets of OTUs (Proteobacteria, Bacterioidetes, Cyanobacteria, all other phyla combined) were Log(N+1)-transformed, to downweight contributions to intersample resemblances from the few most numerically abundant OTUs. Intersample resemblances (Clarke, Somerfield and Chapman, 2006) were calculated using the Bray-Curtis similarity measure (Somerfield 2008) and the resemblance matrices ordinated using nonmetric multidimensional scaling (MDS). To examine the relative effects of seasonal and interannual variability samples were grouped into seasons (Winter: December, January, February; Spring: March, April, May; Summer: June, July, August; Autumn: September, October, November) and changes in community structure were analysed with 2-way permutation-based analysis of variance (Anderson, Gorley and Clarke, 2008; McArdle and Anderson, 2001) using Season and Year as factors, type III (partial) sums of squares and 999 permutations of residuals under a reduced model.

Two univariate measures of community structure, the number of different OTUs (S) and Simpson's evenness index 1-λ' (Somerfield 2009), were calculated for each sample.

Variation in these indices, and environmental variables, were analysed using multiple linear regression (MLR) to determine temporal patterns. Temporal trends and the form of seasonal cycles were determined by regression on Serial Day (D), the number of days from 01/01/2003, a term representing a seasonal cycle mirroring day-length, peaking in midwinter (DX1 = cos(2 π(d/365)), where d is the number of days from the 20th December, the shortest day in the previous year) and a further orthogonal term to determine the nature of cycles with peaks at other times of the year (DX2 = sin(2π(d/365))). Univariate measures were further regressed on a range of (sometimes transformed) explanatory variables using stepwise MLR. The relative goodness of fit of different regression models was assessed using the Akaike Information Criterion (AIC). When necessary, single variables were transformed to normalise residuals. Changes in community structure were also analysed using stepwise distance-based linear modelling of variation among Bray-Curtis similarities. Analyses were performed using MINITAB v. 13 and the distance-based linear modelling (DistLM) module within PERMANOVA+ v. 1 add-in for PRIMER v. 6 (Anderson, Gorley and Clarke, 2008; McArdle and Anderson, 2001). Inter-seasonal and inter-annual variability among environmental variables were further analysed using correlation-based principal components analysis (PCA).

Supplementary Figures and Tables

Table S1 – Sampling dates, and environmental conditions for each date. Data is MIENS compliant.

Refer to attached excel document.

Table S2 – Primer sequences used for analysis, including A and B 454 adapter sequence and MID sequence.

Refer to attached excel document

Table S3. Results from linear regressions on serial day (D) and D^2 for those variables in which a significant trend was detected.

Response	α	$_1$ (D)	$_2$ (D^2)	R^2	$F_{2,69}$	p	F D	p	F D^2	p
Log(S)	5.2	7×10^{-4}	-2×10^{-7}	15.1	6.12	0.001	8.3	0.001	3.98	0.05
-Log(1-(1-’))	2.3	6×10^{-4}	1×10^{-7}	21.9	9.50	0.001	18.2	0.001	0.78	ns
Log(SRP)	-1.1	-2×10^{-4}	-1×10^{-7}	11.4	4.36	0.020	8.5	0.001	0.17	ns

Table S4. Results from multiple linear regression on solstice term (DX1) and equinox term (DX2), having detrended (*) variables in which there was a significant linear or quadratic trend.

Response	α	$_1$ DX1	$_2$ DX2	R^2	$F_{2,69}$	p	F DX1	p	F DX2	p
PAR	24.3	-22.0	-1.00	84.9	194.5	0.001	388.3	0.001	0.9	ns
Log(NOx)	0.31	2.57	0.61	71.8	87.9	0.001	164.9	0.001	10.5	0.001
MLD	19.3	6.41	2.20	23.1	10.3	0.001	18.3	0.001	2.4	ns
NAO	-0.15	0.61	0.55	15.0	6.1	0.001	6.3	0.001	5.8	0.001
Log(Chl)	0.10	-0.32	-0.10	12.1	4.74	0.012	8.53	0.001	0.9	ns
-Log(35.5-Sal)	0.86	-0.07	-0.05	1.3	0.47	0.628	0.6	ns	0.3	ns
Log(Sil)	0.85	1.16	0.11	44.9	28.1	0.001	55.6	0.001	0.5	ns
Temp	12.7	-1.56	-3.36	89.4	290.5	0.001	79.4	0.001	502.4	0.001
Log(SRP)*	0.13	0.83	0.39	44.3	27.5	0.001	44	0.001	10.9	0.001
Log(TOC)	5.49	-0.28	-0.10	23.0	10.3	0.001	18.1	0.001	2.6	ns
Log(TON)	3.38	-0.31	-0.05	25.3	11.7	0.001	22.7	0.001	0.6	ns
Log(S)*	0.06	0.40	0.03	64.6	63.1	0.001	125.4		0.6	ns

-Log(1-(1-’))*	0.0	-0.02	-0.12	4.5	1.6	0.206	0.03	ns	3.42	ns

Figure S1 – Species accumulation curve for all data from the original dataset of s of 747,494 sequences (S unique), original resampled dataset of 295,272 sequences (S unique resampled), the conservative clustered dataset (S clustered) and the resampled conservative clustered dataset of sequences (S clustered resampled).

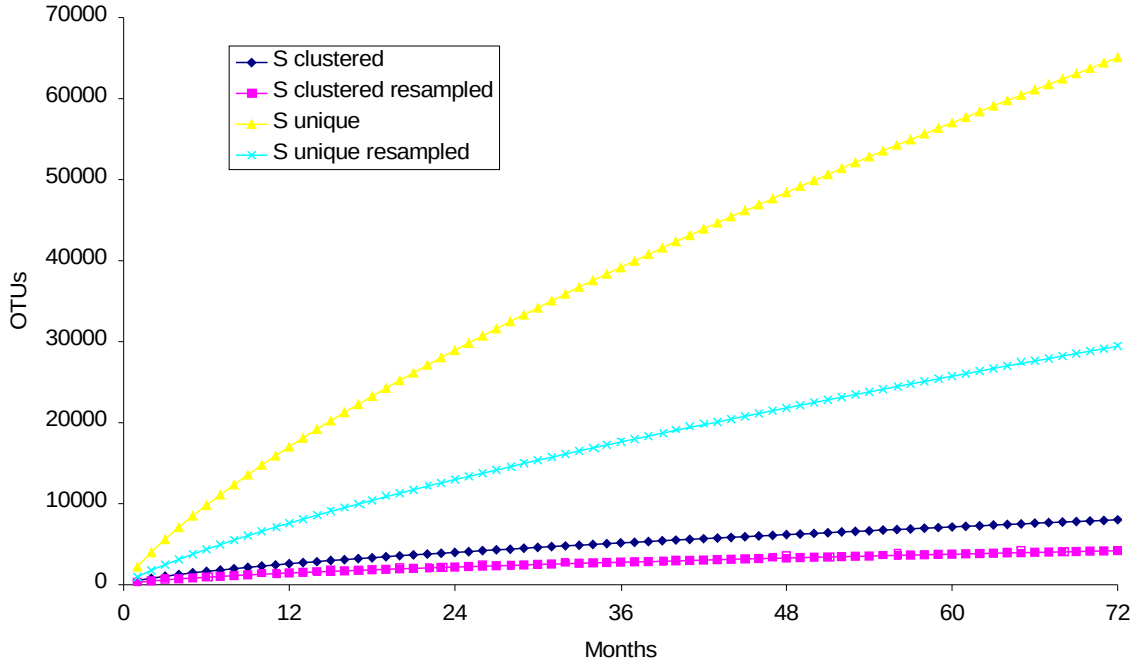


Figure S2– (A) Principle Component Analysis (PCA) of environmental variables and (B) bubble plot of variation in S with time point.