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Marine Matters

Bacterial Diversity: A Year in the Western English Channel

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Why look at Bacterial Diversity?

- Surely it's just stamp collecting?
- Stamps are not responsible for the majority of the worlds biogeochemical cycling.
- Understanding the reservoir of bacterial diversity
    - Base line for future change, e.g. climate, ocean acidification.
    - Identification of rare bacteria for the biotech harvest.
- Help to resolve ecological models through identification and interrogation of trophic links.

What did we do?

- Pyrosequencing of 16 x V6-16s-tag samples.
- Current study:
  - 12 time points,
  - February – December 2007
  - 11,327 to 17,339 reads per sample
  - 182,560 reads in total

- Sample site:
  - Western English Channel, L4 site.
  - Unique historical dataset (Southward et al, 2005).
  - Boundary between several bodies of water, including the gulf stream.
  - Busiest shipping lane in the world.

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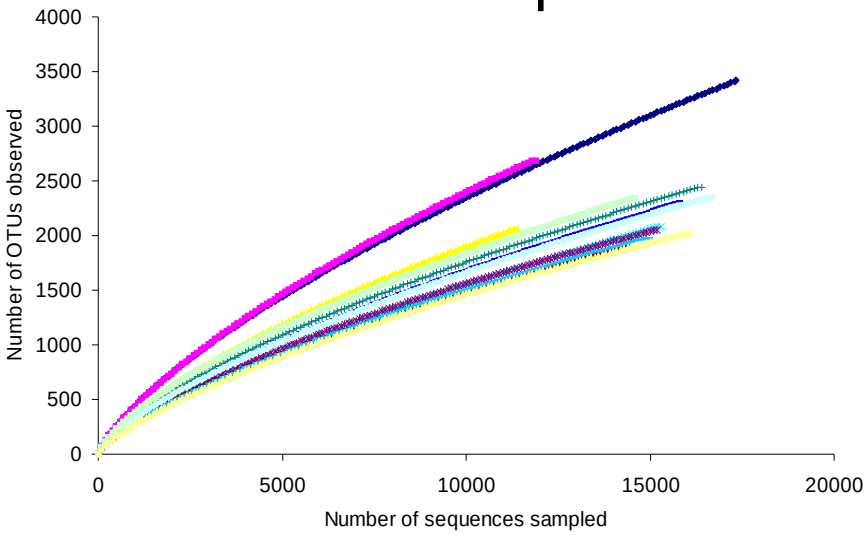
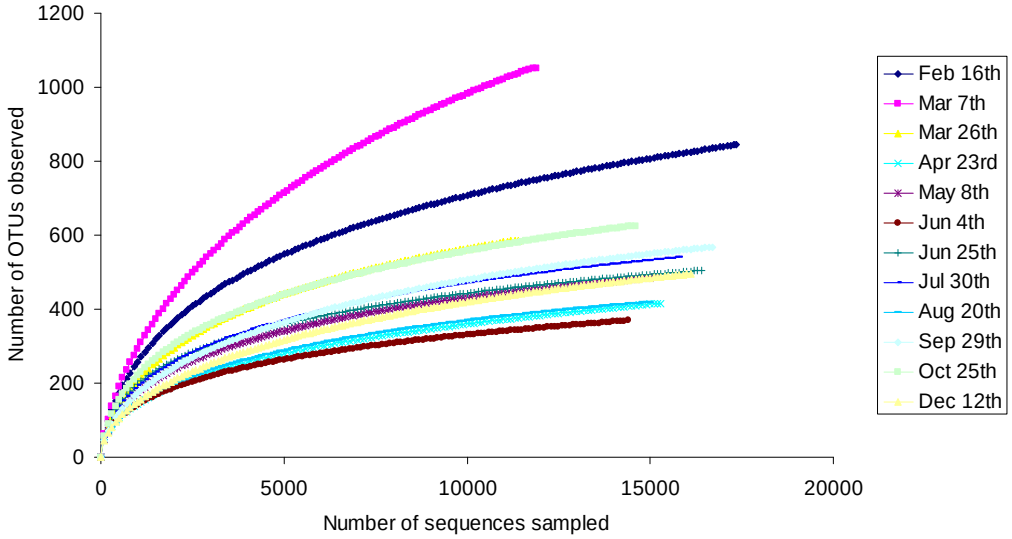
High Diversity – ubiquitous taxa account for 54% of the sequences

- 182,560 reads in total = 17,673 unique operational taxonomic units (OTUs)
- 0.5 % found at all 12 time points
  - 54 % of all reads

Unique



90 % identity



Random Re-sampling to standardise comparisons

- Sampling effort was identical.
- Sequencing effort varied as an artefact of pyrosequencing.
- Randomly Re-sampled to smallest dataset
  - Daisy_Chopper v1.0 (http://www.genomics.ceh.ac.uk/GeneSwytch/Tools.html).
  - 11,327 reads.
  - Lost 30% of unique OTUs
  - 12,393 OTUs left.
- Taxa cannot be added when normalizing to a larger sample.

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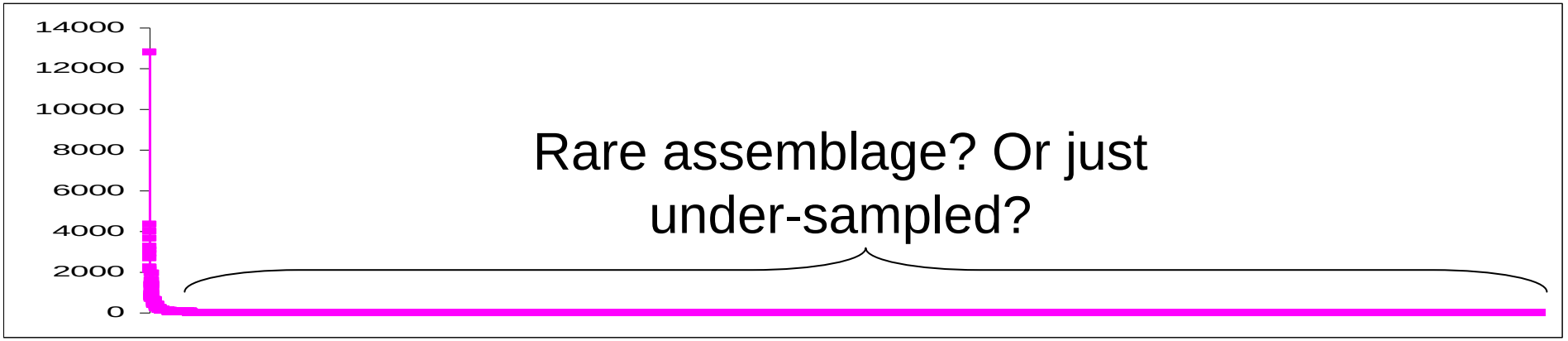
Rare Microbial Assemblage

- Even following re-sampling:
  - 78% of the OTUs were time-point specific
  - 67% of the OTUs were singletons
- Singletons diverged from other tags in the dataset by >2 base pairs – hence not sequencing errors.
- Clustering OTUs at 90 % nucleotide identity:
  - 55 % are still time point specific.



Evidence for seasonality: the presence of 'winter', 'spring', 'summer' and 'fall' communities

- The re-sampled data was cyclical in nature: (RELATE test, R=0.5, p<0.01).
  - Hence seasonal.
- Annotated OTUs through GAST (Huse et al. 2008).
- 83.5% of sequences were bacterial.
- 35 phyla identified – Proteobacteria accounted for 50 % of reads.
- Alphaproteobacteria – 31.7 % of reads.
- SAR11 – 12 % of reads.

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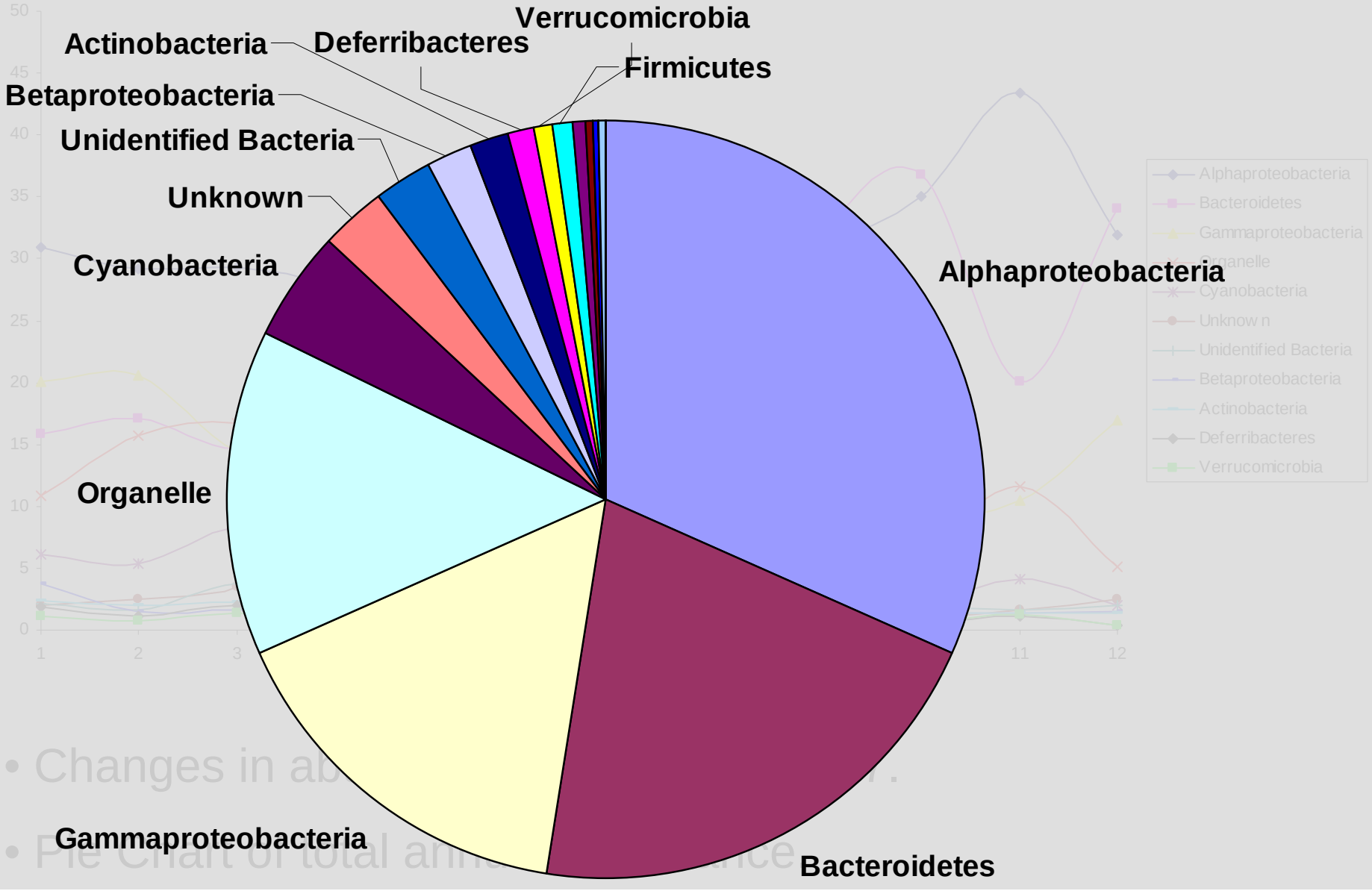

- Changes in ab…
- Pie Chart of total ann… ce

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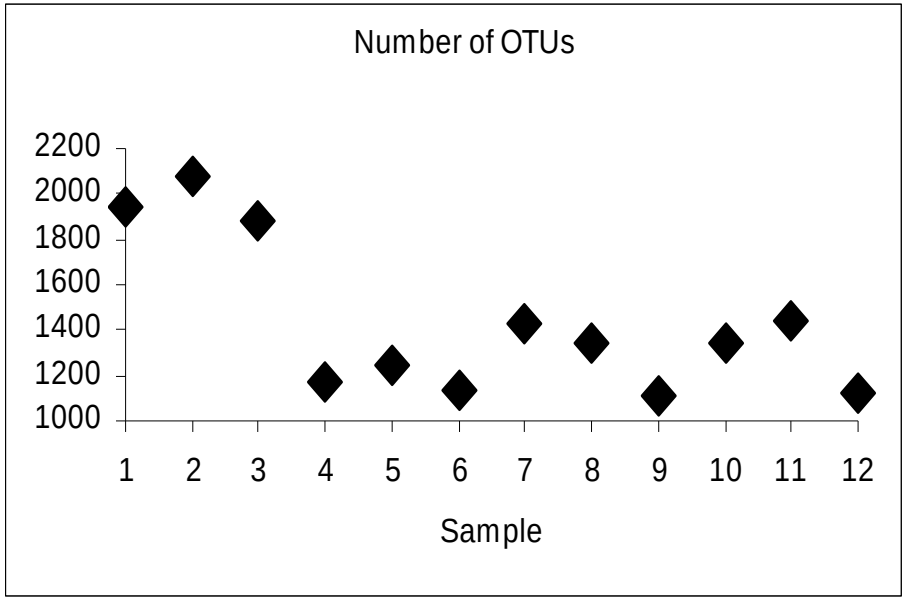
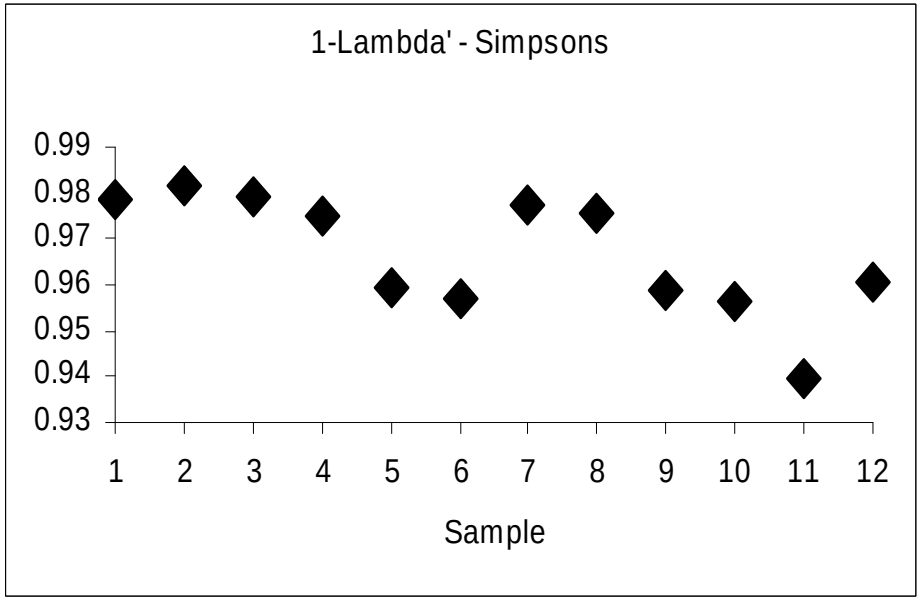
Diversity and Dominance throughout 2007





- Diversity peaks in Feb-Mar, May, Jun-Jul, and Sept-Oct
- Dominance peaks in Feb-Apr, and Jun-Jul.
  - Dominance always high – hence relative few taxa dominate.

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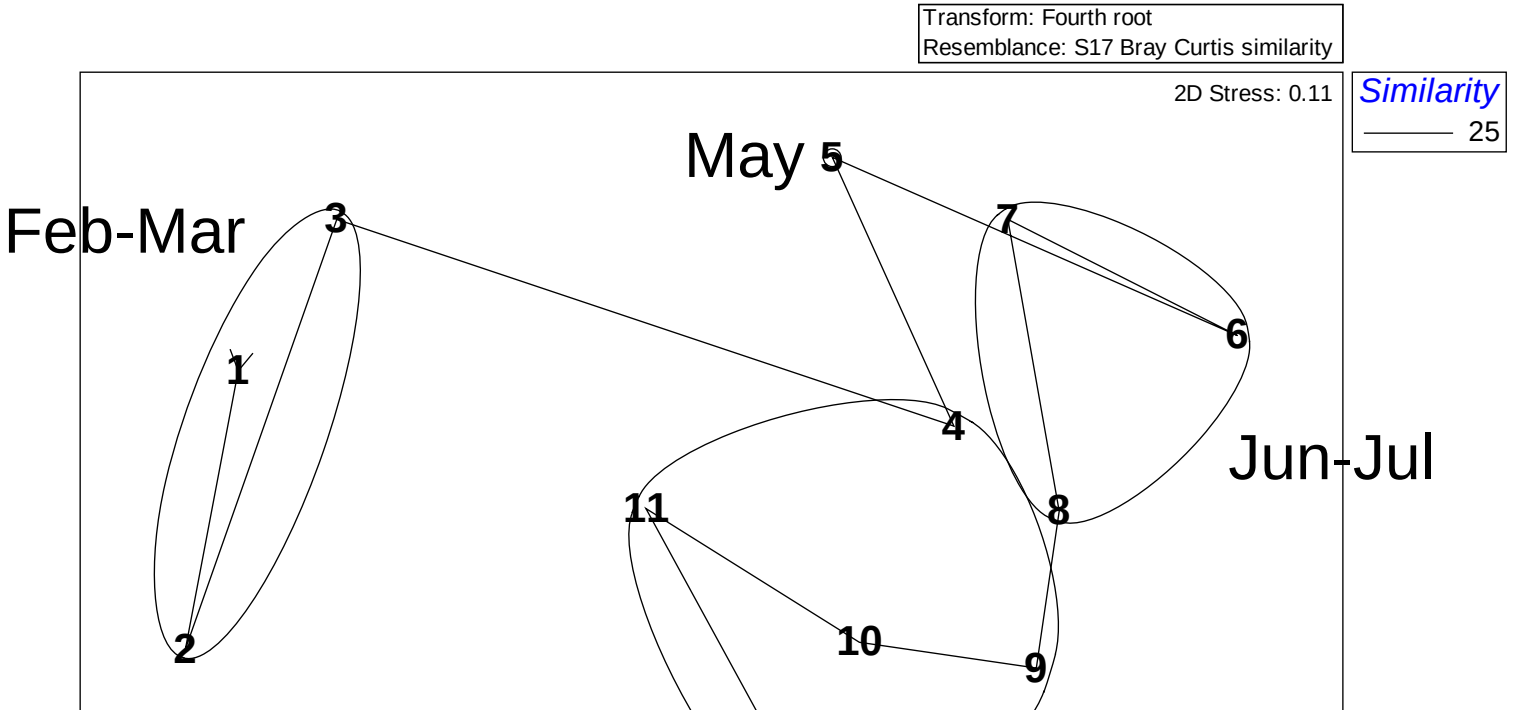
Non-parametric multivariate analysis: Multi-dimensional scaling (MDS)



A test of seriation confirms the short-order temporal autocorrelation demonstrated by MDS (RELATE test, R=0.5, p<0.01).

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Correlation to environmental parameters

	Rho	Variables							
		Temp	Silicate	PO_4	Density	TOC	Nanoeuks	Coccoliths	Dinoflagels
All OTUs	0.73	■	■	■					
Gammaproteobacteria	0.75	■		■					
Alphaproteobacteria	0.73	■		■					
Bacteroidetes	0.72	■		■					
Actinobacteria	0.63	■		■					
Cyanobacteria	0.57	■	■	■	■	■	■		
Betaproteobacteria	0.52	■						■	■

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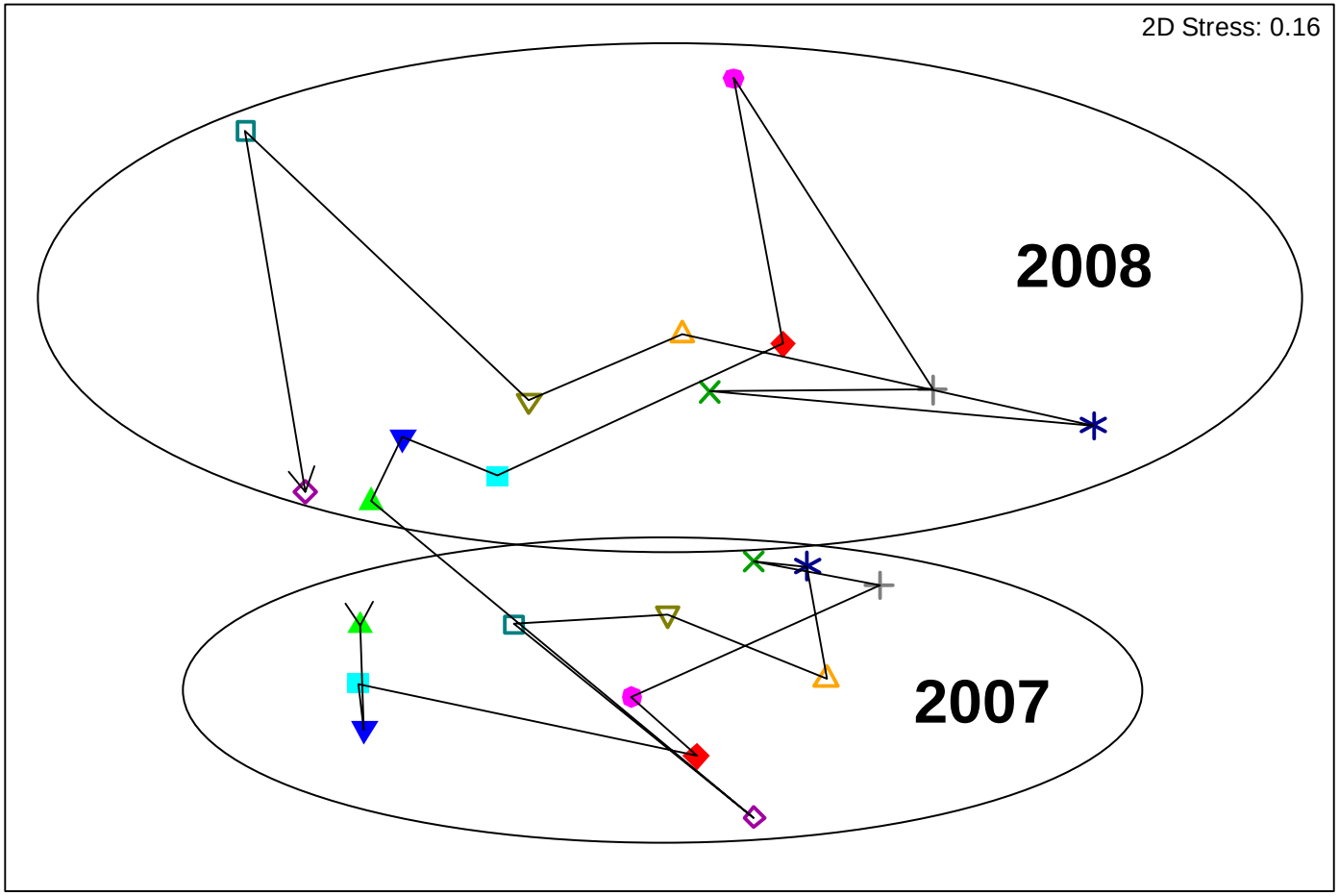
2007-2008

2007-2008 alldomains resampled

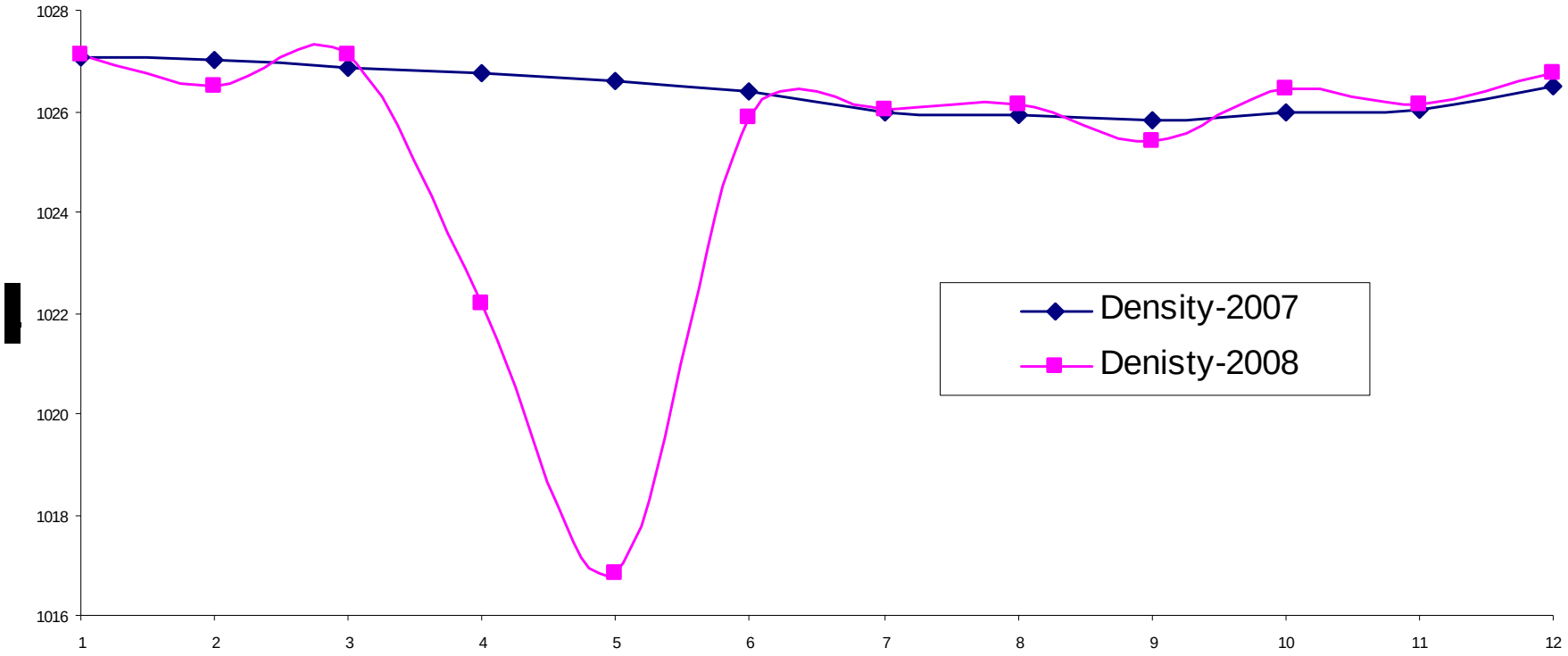
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity



BEST analysis

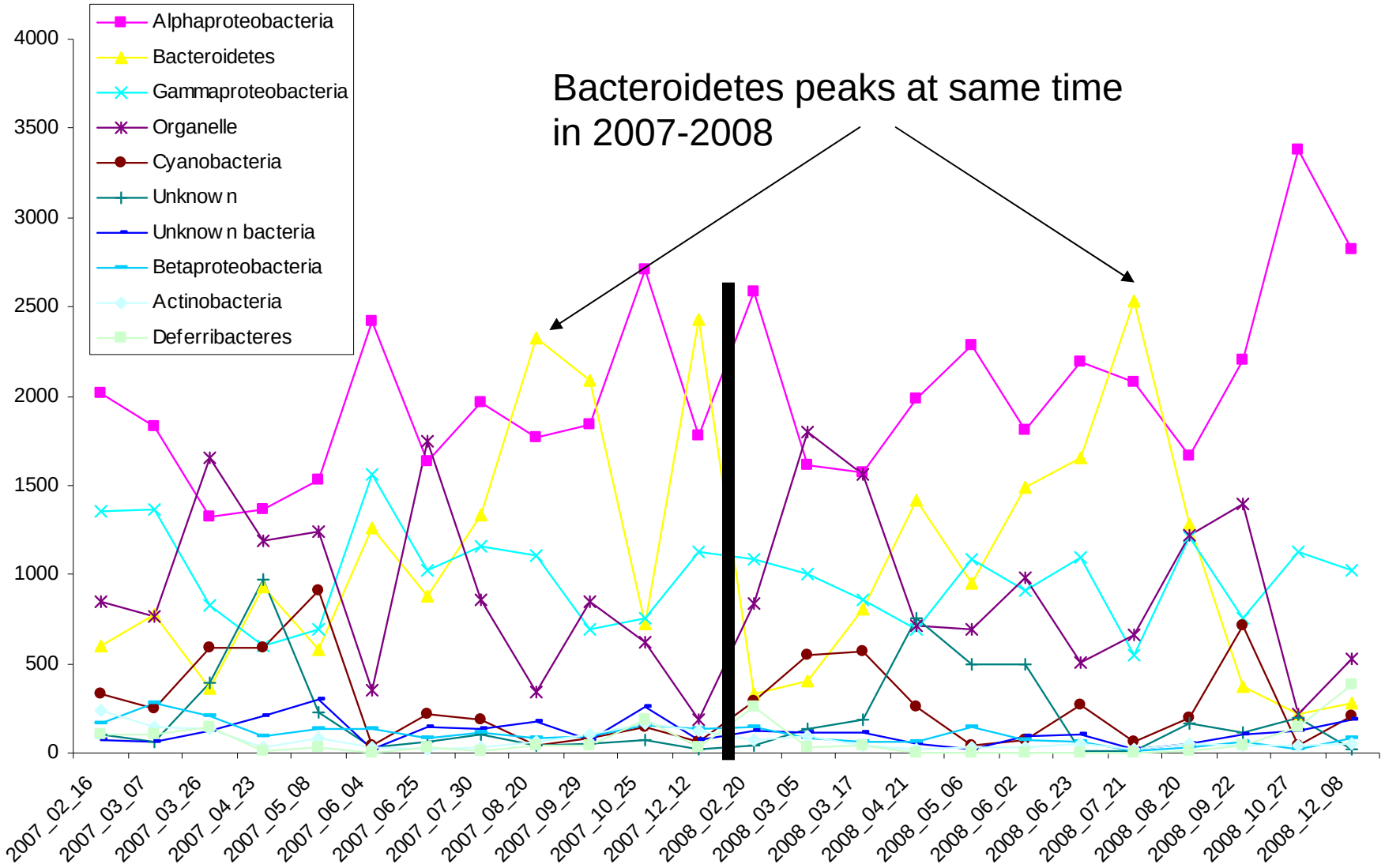
- 2007-2008 overall:
  - Temp, silicate, nitrate, density and phosphorus (R = 0.63, $p<0.01$)
- 2007 only:
  - Temp, Silicate and Phosphorus (R = 0.684, $p<0.01$)
- 2008 only:
  - Silicate, nitrate, salinity, density, cryptophyte abundance.
  - Salinity and Density not a surprise due to the serious depression in salinity during April-May

Densisty and Salinity in 2008



While the primary correlation has shifted from temperature, nutrients are still important and specific factors (density, salinity) must overwhelm the temperature effect.

Top 10 most abundant bacterial phyla



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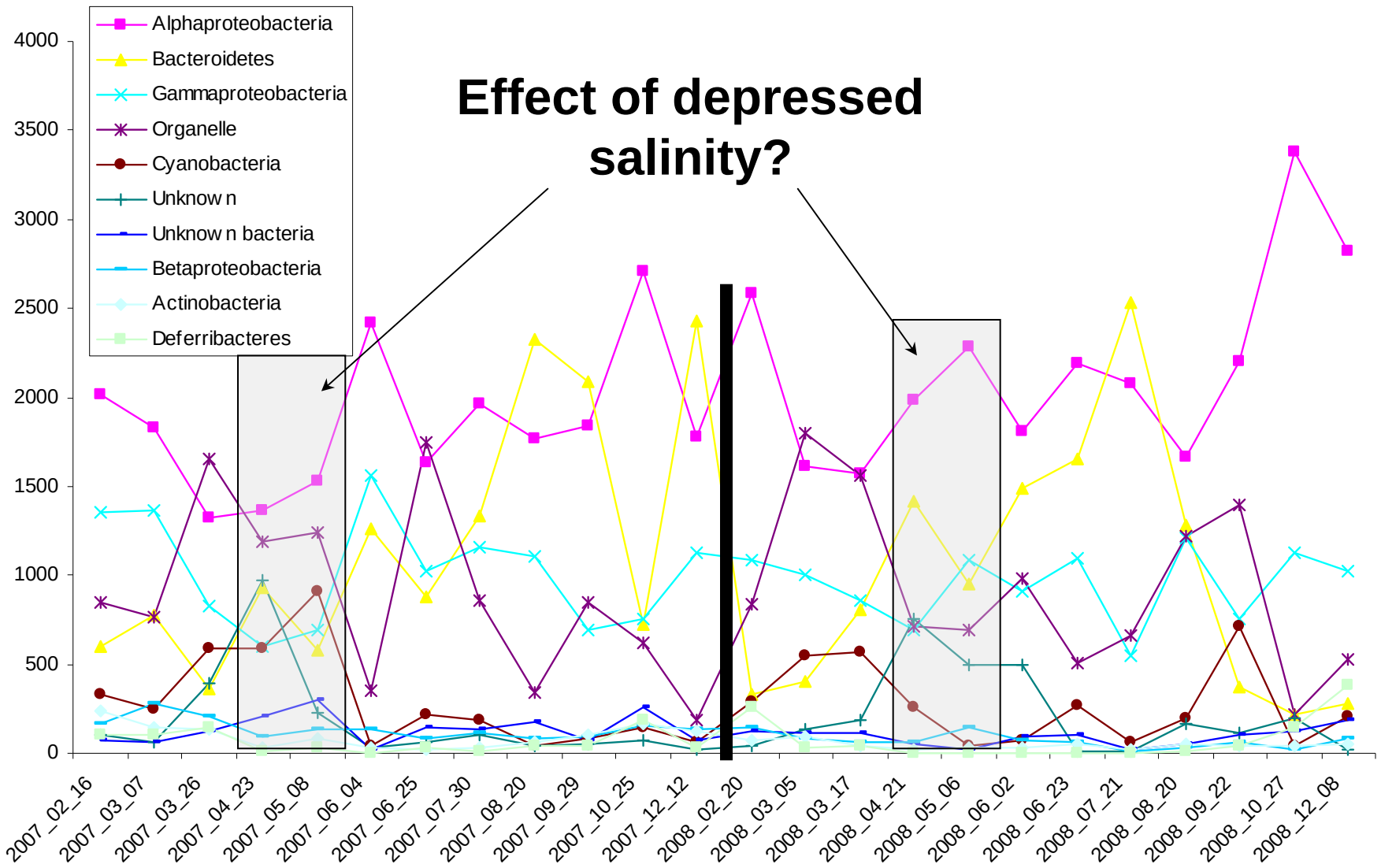

Conclusions

- Large sequencing effort but no plateau on rarefaction.
- Majority of unique species are rare, i.e. occurring only once in the year or numerically rare.
- Community structure of individual phyla showed correlative relationships to specific environmental parameters.
- Overall abundance of OTUs was primarily driven by temperature and nutrient concentration.
- Communities differ between 2007-2008 but show similar trends.
- Specific climatic events have important and measurable ramifications on microbial diversity.

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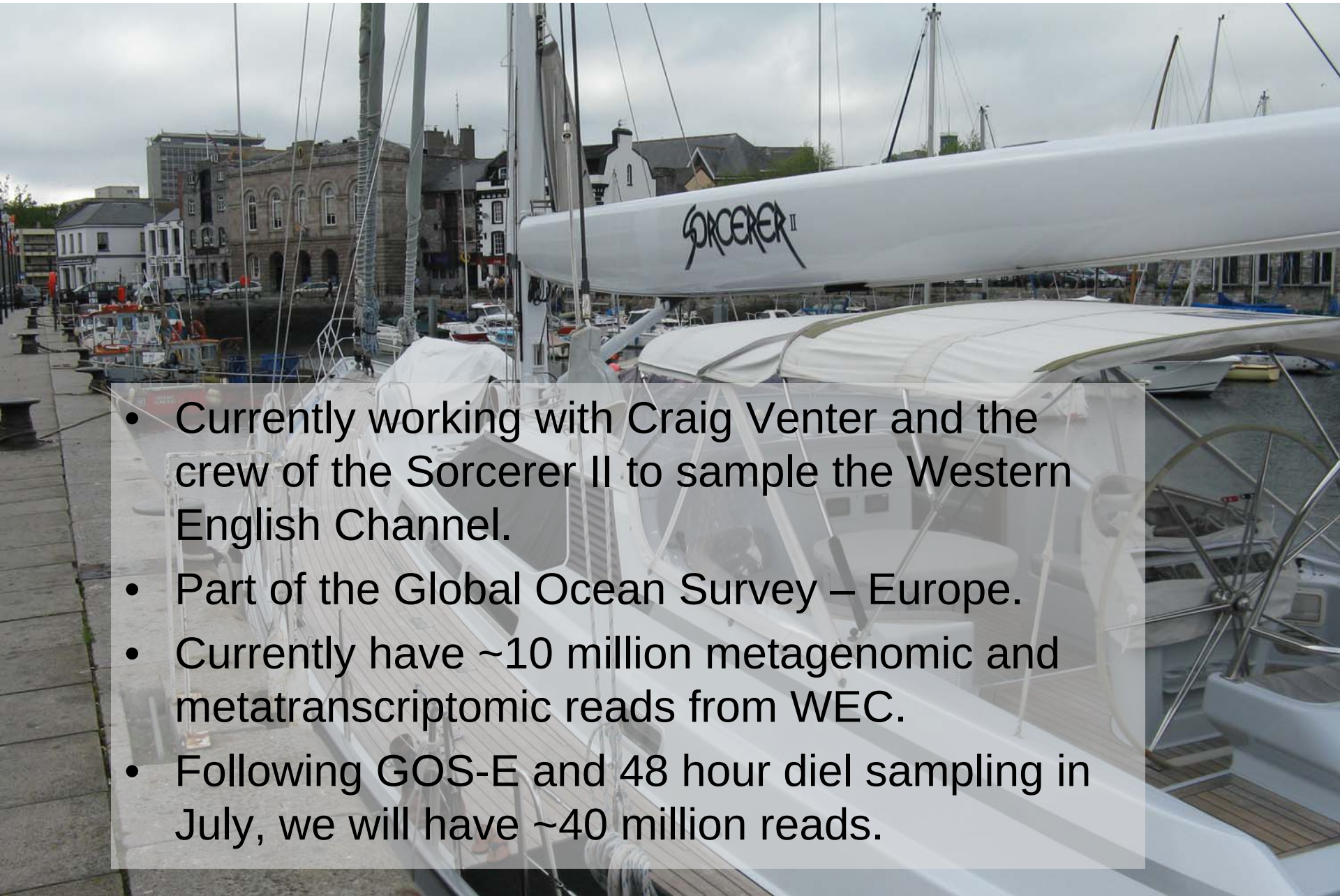

- Currently working with Craig Venter and the crew of the Sorcerer II to sample the Western English Channel.
- Part of the Global Ocean Survey – Europe.
- Currently have ~10 million metagenomic and metatranscriptomic reads from WEC.
- Following GOS-E and 48 hour diel sampling in July, we will have ~40 million reads.

Acknowledgements

- ICoMM and NERC for funding
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Questions Please