the more eye-catching color makes me focus on the main messages

Community Dynamics of Marine Microbes at High Genetic, Spatial, and Temporal Resolution

[redacted]

[1]MIT Microbiology Program, [2]Universidad de Granada, [3]MIT-Woods Hole Oceanographic Institute Joint Program, [4]MIT Department of Civil & Environmental Engineering

Motivation & Questions

- Marine microbes have significant impacts on biogeochemical cycles and human and animal health, but understanding their dynamics is challenging given their complex, variable habitat.
- Time-series studies that pair microbial diversity data with environmental metadata allow correlation of microbial dynamics with particular ecological conditions.
- Microbes are typically resolved to the genus level – but great genetic diversity & hence varying ecological preferences exist below the levels of both genus & species. Greater genetic resolution is needed to reveal fine-scale diversity of microbial dynamics in marine habitats.

Approach: Analysis of a 93-day daily coastal time-series

1. Resolving daily dynamics of previously defined Vibrio ecological populations, via marker-gene sequencing…
2. …and correlating their dynamics with abiotic & biotic environmental variables.

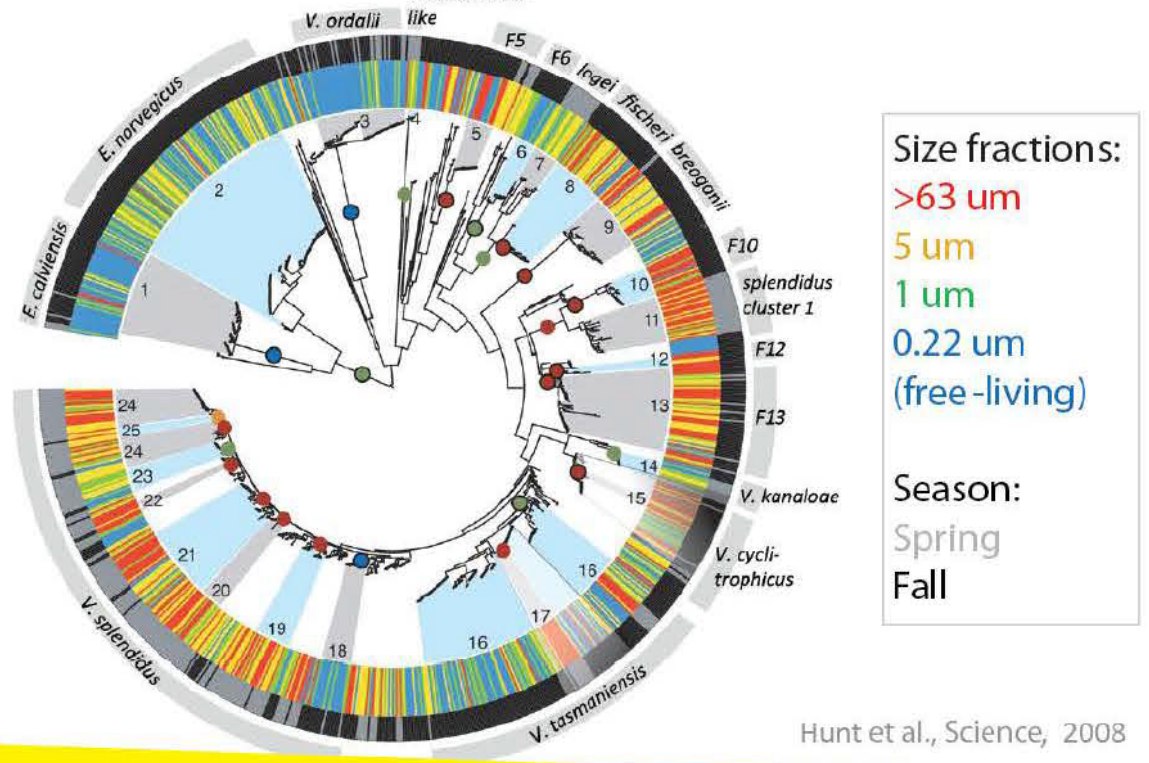
Questions: How stable are dominant taxa? Do we see shifts in ecological lifestyles? Which taxa are responsible for bloom events? Which environmental factors are major controls?

Background: Wild Vibrio as a model for microbial population ecology

Vibrionaceae family: Metabolically diverse, culturable marine heterotrophs. Include human & animal pathogens.

Population = co-occurring members of the same species. The unit of ecology & evolution.

Spatially/temporally defined Vibrio sampling
+
phylogeny
= predicted ecological populations
(AdaptML model)



Hunt et al., Science, 2008

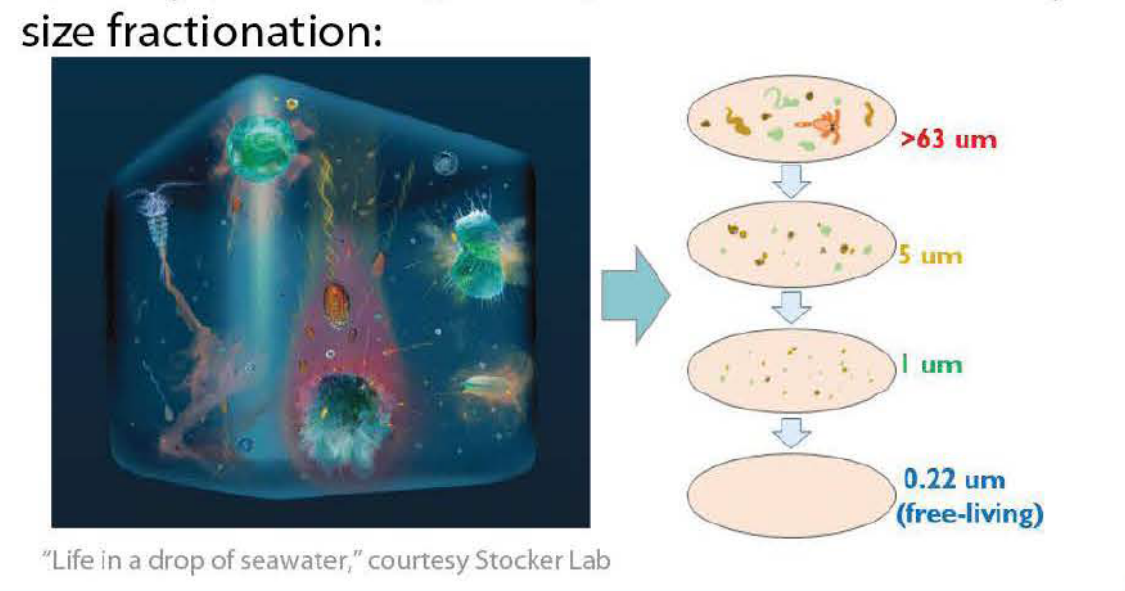
most “boxes” have are at least 50% pictures

Dataset: The Nahant Time-Series

93-day daily coastal time-series: July 23 – Oct. 23, 2010

- Bacteria (16S rRNA data)
- Eukaryotes (18S rRNA data): nutrient sources, predators
- Abiotic: T, salinity, N, P, cell & viral counts

Spatially partitioning a complex marine community via size fractionation:



“Life in a drop of seawater,” courtesy Stocker Lab

Methods: Quantifying Vibrio diversity via hsp60 amplicon libraries & phylogenetic placement

As a marker gene, hsp60 affords population-level taxonomic resolution.

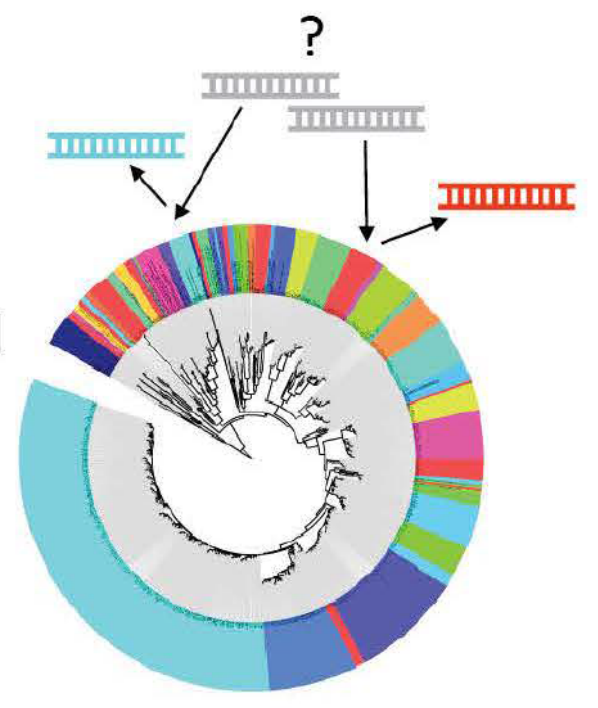
One 16S rRNA sequence	**One 213-bp *hsp*60 amplicon**
= up to **13 populations, 2 genera**	= **1-4 populations**

1. Amplify from community DNA with Vibrio-targeted degenerate primers
2. Illumina HiSeq sequencing
3. Filter out sequences with no BLAST hits to Vibrio
4. Assign taxonomy via phylogenetic placement (pplacer tool, Matsen et al. 2010)

Methods, cont’d

Phylogenetic placement assigns taxonomy by asking, “Within which named clade does a query sequence fall?”

- Far more accurate than BLAST for closely related sequences
- Reference library:
  - ~750 Vibrionaceae hsp60 sequences with curated taxonomic labels
  - 50 previously named taxa, 20 unnamed
  - 10 populations (=5 out of the 70 final taxa) can’t be distinguished with this amplicon
    - collapsed into “supergroups”
    - e.g., “V. kanaloae aestuarianus”



Mock community of known composition revealed no need for correction for PCR bias. (Data not shown.)

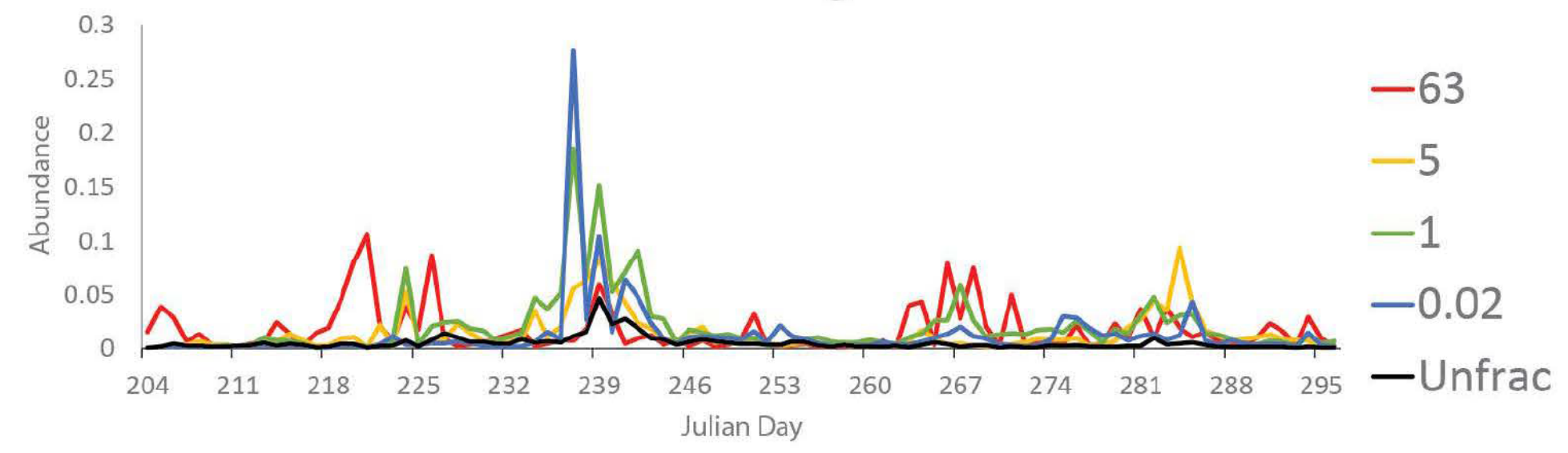
- Cocktail of gDNA from 87 Vibrio strains, 21 diff. populations
- Subjected to same pipeline as real samples

1. Community composition is not affected by # amplification cycles.
2. Observed abundances vary from expected by 1 order of magnitude.

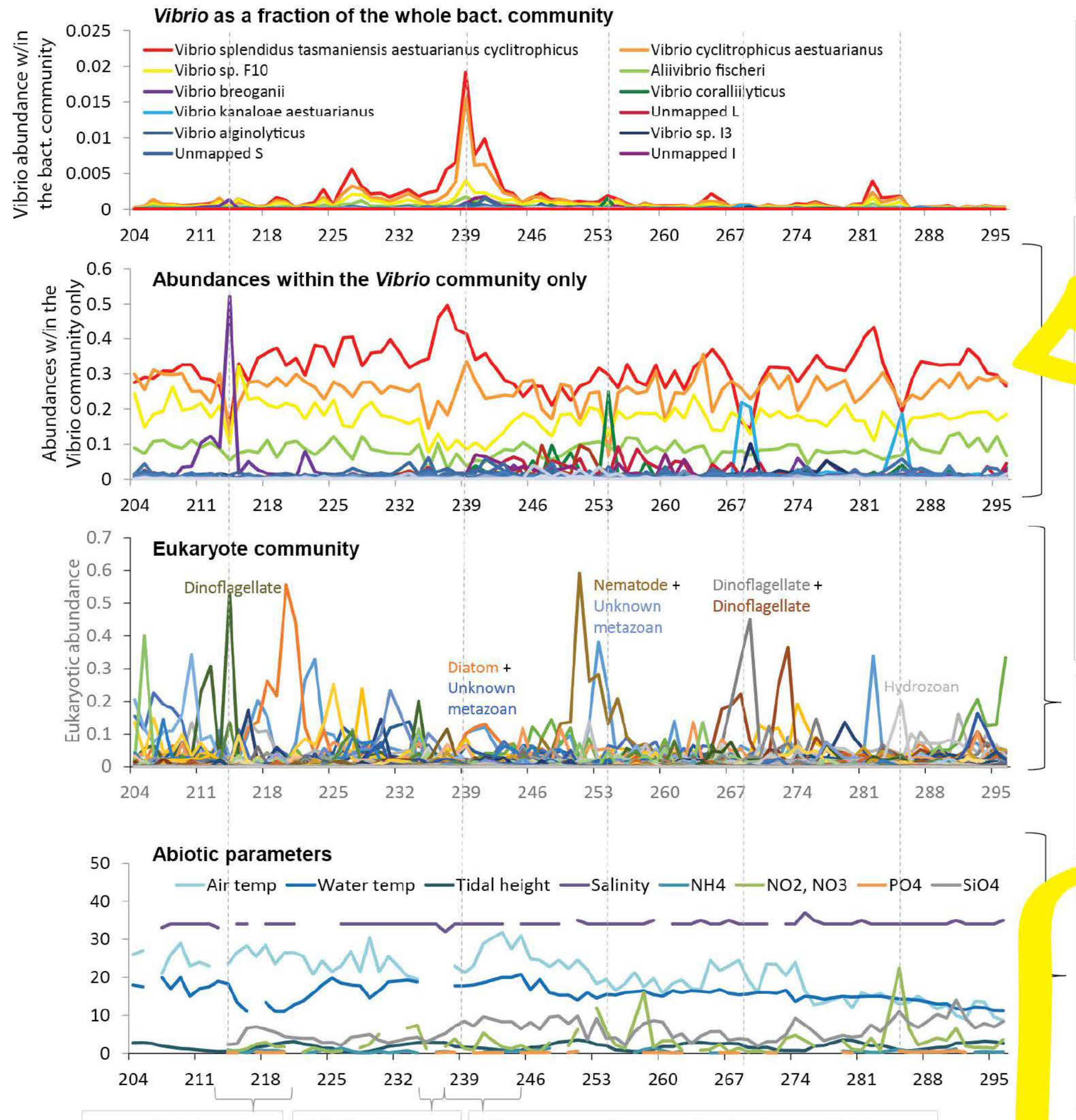
Results

Overview of Vibrio dynamics among size fractions, via 16s rRNA data

Vibrio are typically low-abundance, but can bloom dramatically, and have different behavior among the size fractions.



Population-level resolution of Vibrio in the Unfractionated samples, via hsp60 data



Cold-water mass moves in

Major storm stirs up algal material

Warm weather + algal accumulation = ideal Vibrio conditions

The Vibrio community is highly dominated by V. splendidus supergroup (ecological generalists), cyclitrophicus/aestuarianus, F10 (animal specialist), and fischeri.

Focusing on abundances within the Vibrio community only:

- splend. & cycl. dominate the Day 239 bloom.
  - F10 becomes less significant.
- breoganii (plant specialist), coralliilyticus, & kanaloae/aestuarianus experience brief, dramatic blooms
- Community composition is otherwise stable.

Comparison with eukaryotic community data reveals coincidence of Vibrio & eukaryotic blooms, incl. diatoms & multiple dinoflagellate taxa.

Comparison with abiotic data suggests correlation of bloom events with turnover of water mass (making algal material available), and occasionally with nitrogen concentration.

Results, cont’d

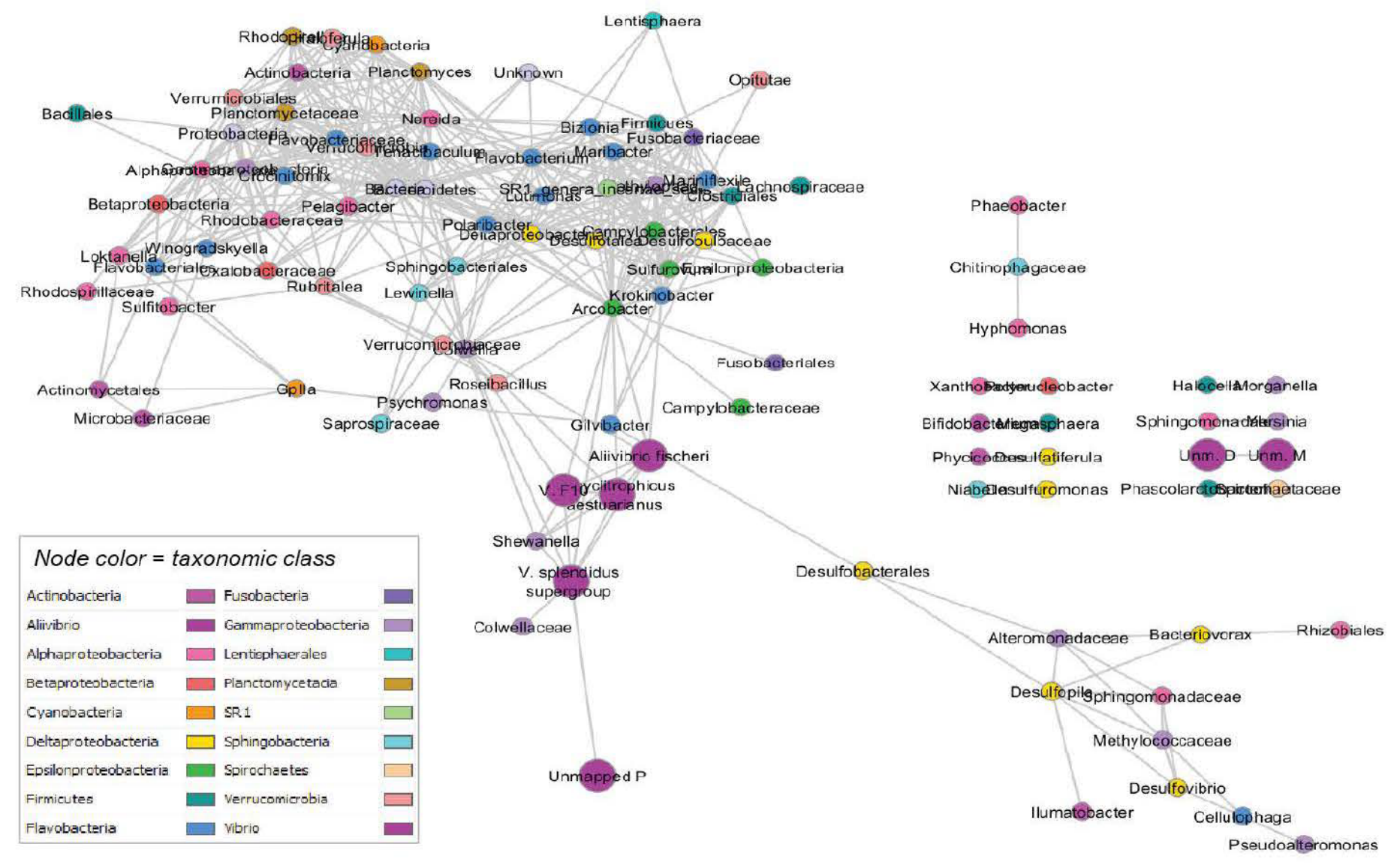
Correlation analysis allows quantitative identification of taxa with similar ecological preferences

where possible, headers are complete thoughts that help me interpret the data

Vibrio taxa (large purple nodes) are strongly correlated with other Gammaproteobacteria (Shewanella, Colwellia), Gilvibacter, Roseibacillus, and Desulfobacterales.

Vibrio hsp60 taxa + other bacterial 16s rRNA taxa correlated via SparCC (Friedman et al., PLOS Comput Biol., 2012).

Results filtered to include only correlation coefficients ≥ 0.6. Network generated in Cytoscape.



Future Directions

- Complete hsp60 analysis for other size fractions.
- Identify quantitatively significant associations with eukaryotes & abiotic parameters via further correlation analyses.
- Identify biological interactions (i.e., competing bacterial taxa) via wavelet dissimilarity analysis.

matching colors in the figure and text help make a complex data set more accessible

Conclusions

- Targeted hsp60 sequencing resolves dozens of ecologically distinct Vibrio taxa
  - Compare to ~10 taxa resolvable by 16s
- Vibrio community composition is stable except for transient, dramatic bloom events by 1-2 taxa at a time
  - Reveals population-specific ecology: exploitation of phytoplankton or zooplankton nutrients
  - Consistent with previous, single-timepoint observations: robust, predictable taxa vs. highly skewed taxa

Acknowledgements

Funding from the NSF GRFP.

almost all text is in bullets. each bullet is one sentence.