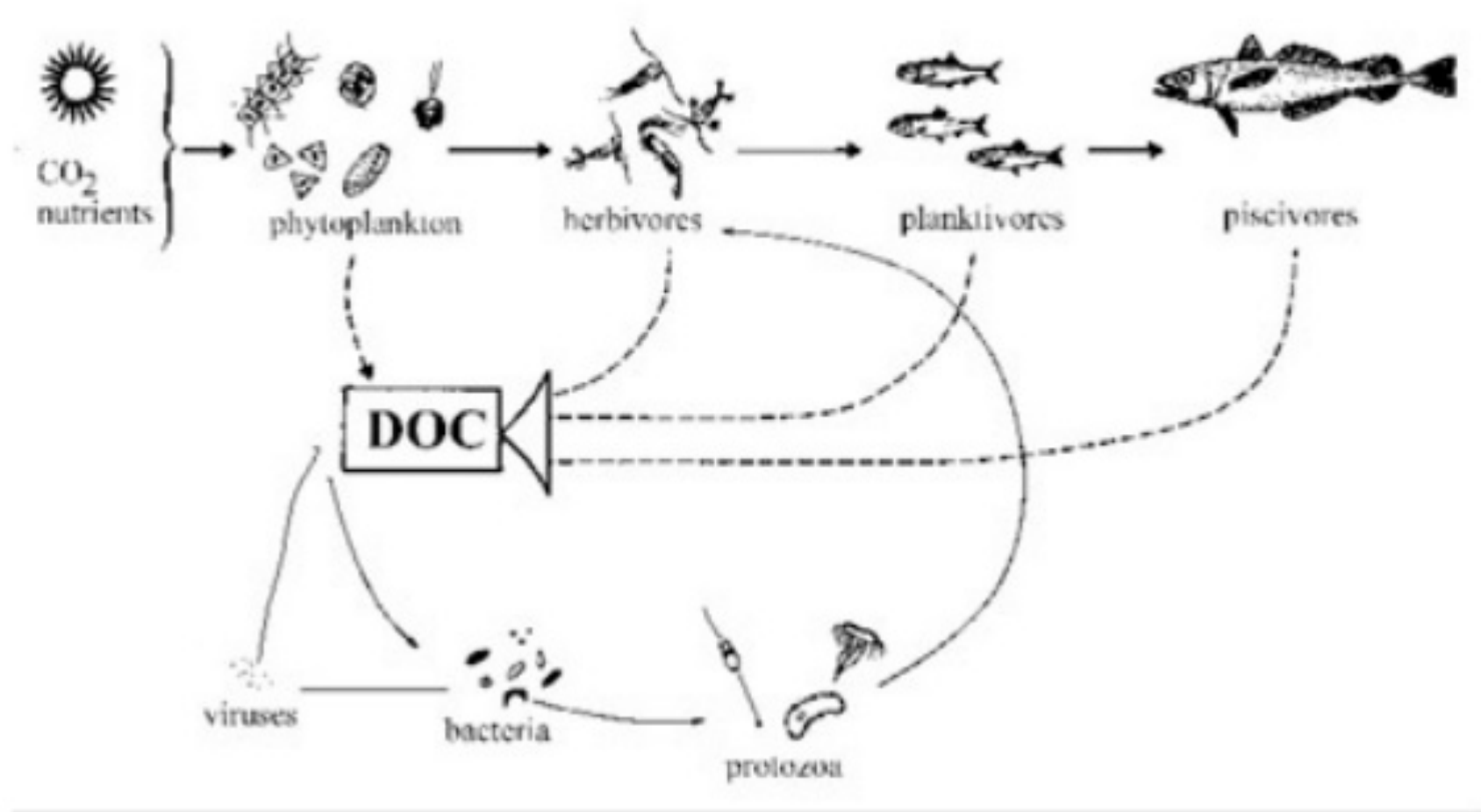


Comparative Transcriptomics and Functional Genes





seaweedindustry.com



www.snipview.com

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<http://www.photomacrography.net/forum/viewtopic.php?p=112737&sid=67d5205987aef5c8d5b6582646add153>

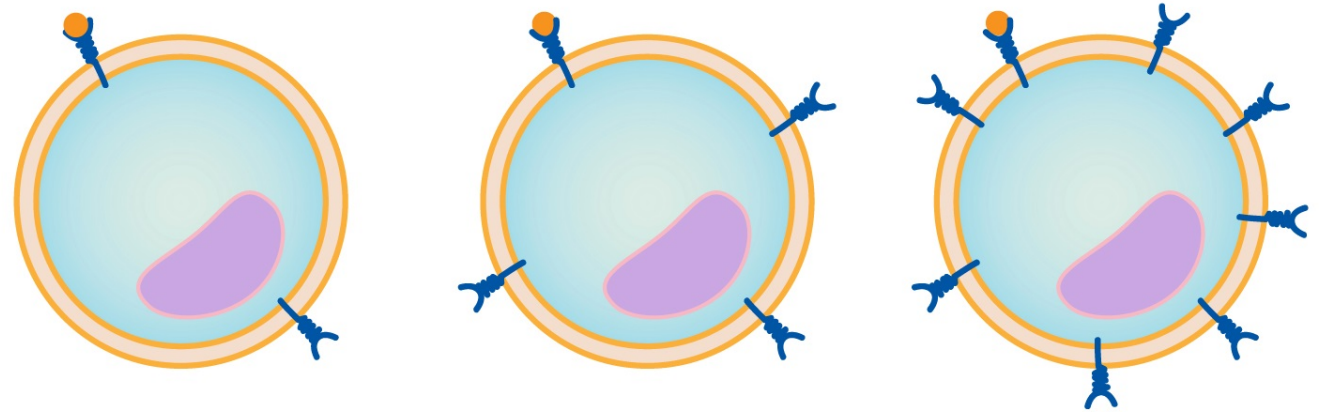
Diatom Blooms

- One opportunity for a comparative study is diatom research
- Diatoms are also heterokonts (brown algae relatives) and produce *some similar* compounds
- Plankton transcriptomics affords the opportunity to selectively remove the diatoms based on size (via filtration), leaving the bacteria for nucleic acid extraction
 - This allows for RNA collection from (*nearly only*) bacteria and archaea –(some small eukaryotic algae may show up...but they will be a small fraction of the community)
 - Problems with this include losing diatom-attached bacteria in the filtration, though these taxa are usually present in a free-floating form, too

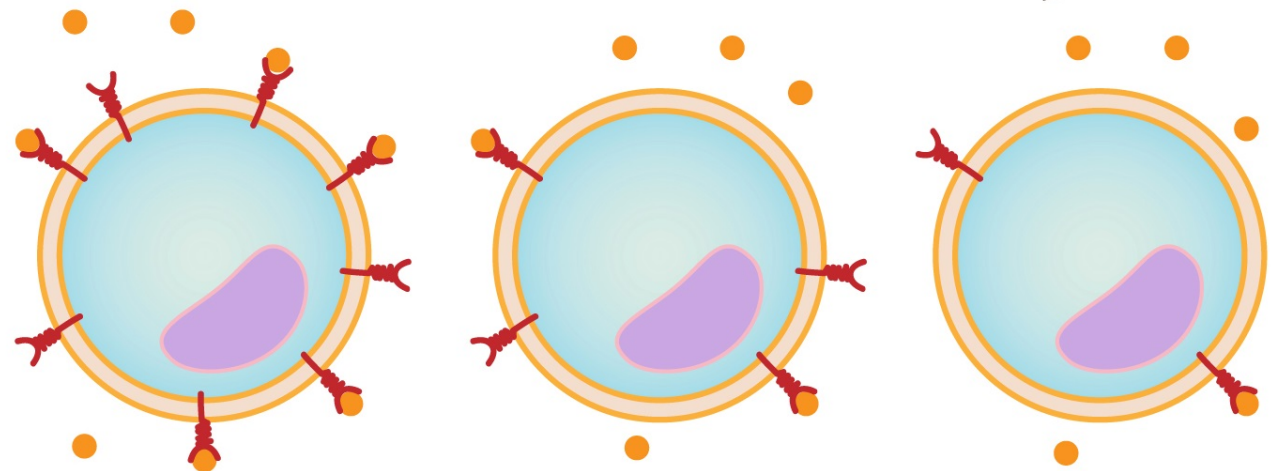
Up/Down Regulation

- Comparative studies need reference
- Use relative expression to 'housekeeping' gene
- Alternatively, spike in RNA for quantitation

upregulation



time →



downregulation

lookfordiagnosis.com

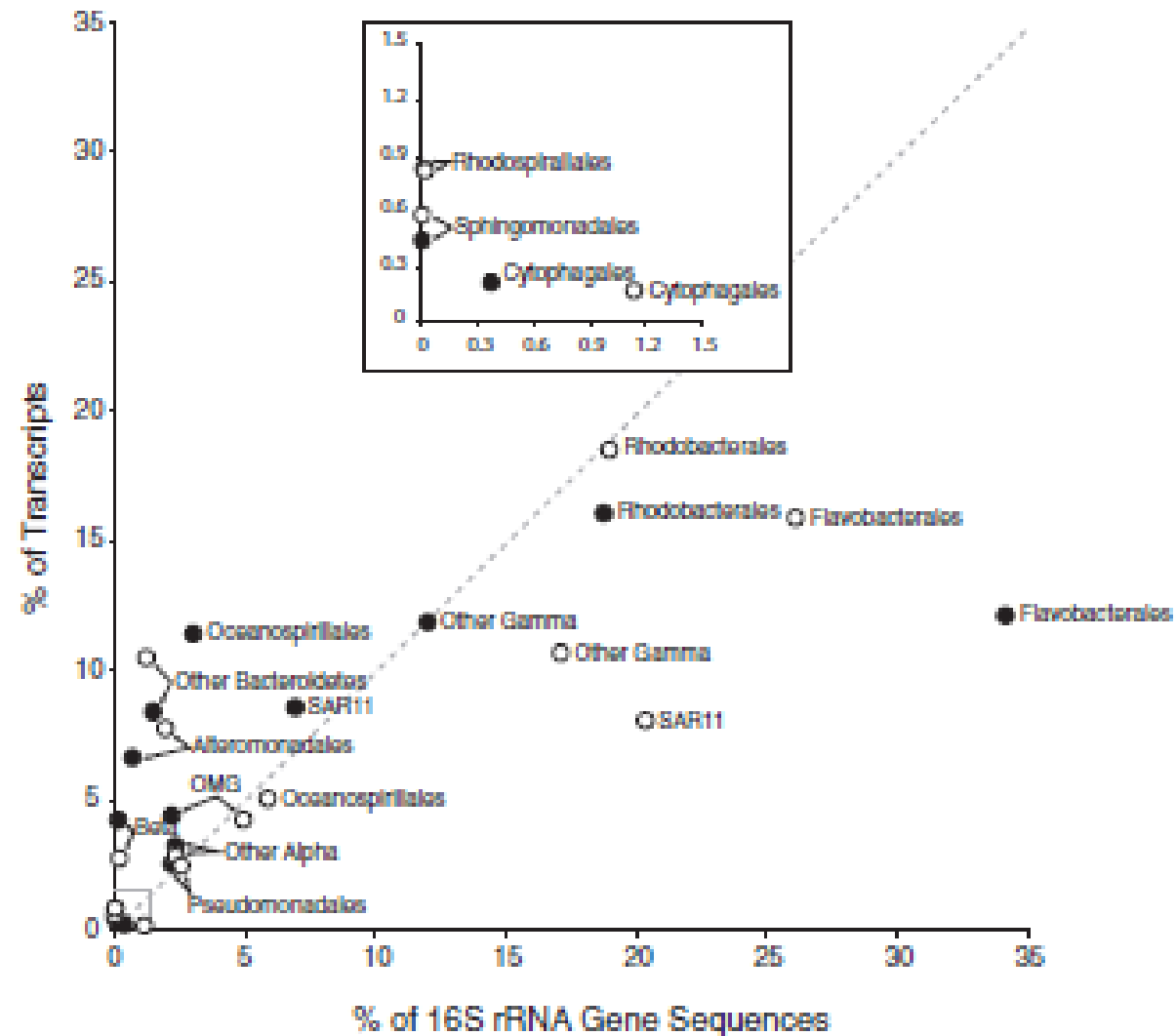


Fig. 2. Relative representation of taxonomic bins in the community transcript pool compared with the 16S rRNA gene amplicon pool. Black circles = bloom microcosms; white circles = control microcosms. The inset shows a magnification of the origin. The 1:1 line is indicated by a dotted line.

- Rinta-Kanto, 2012

which synthesizes ATP from ADP and phosphates. Finally,

in accordance with the pattern found for the full metatranscriptome.

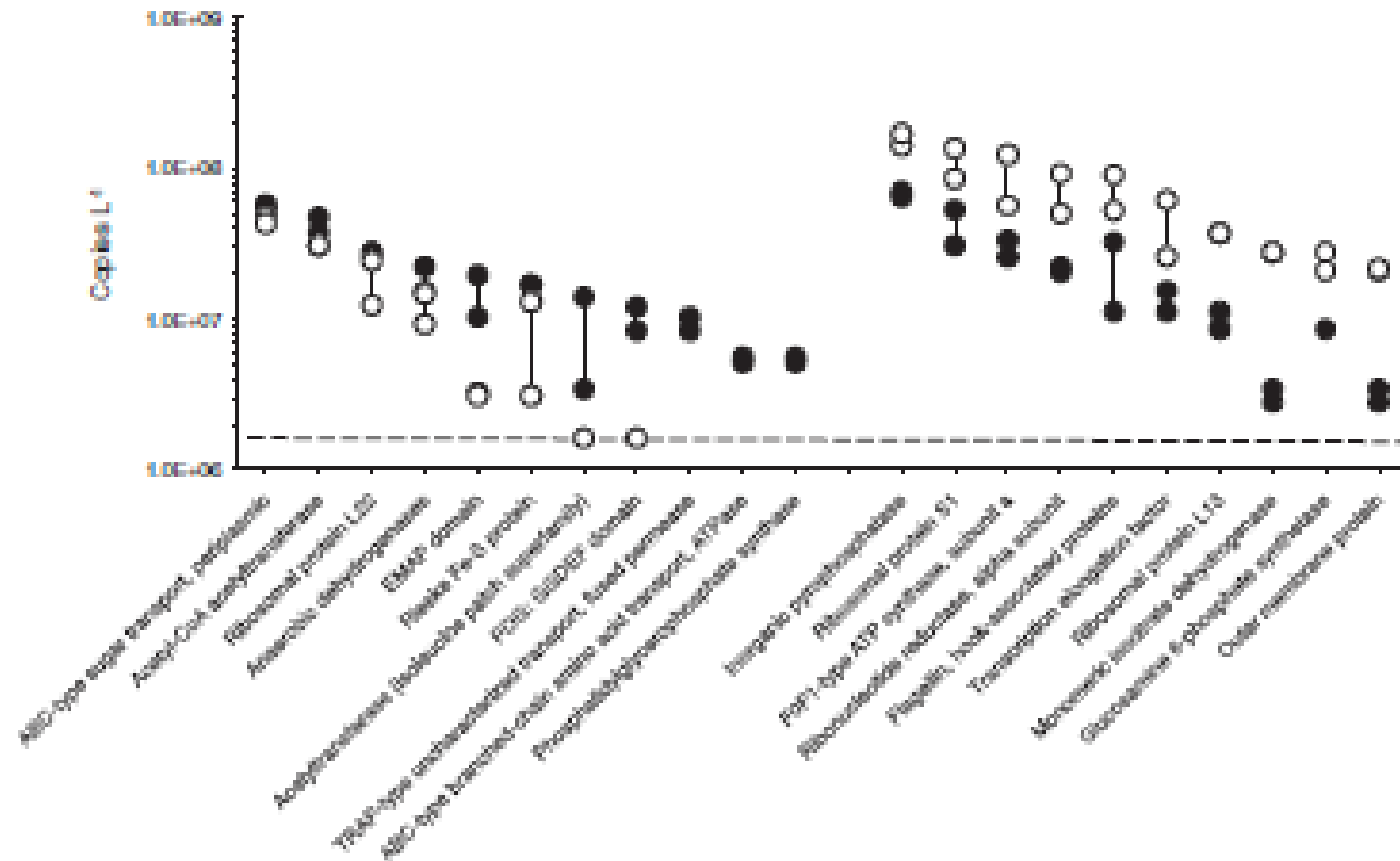


Fig. 3. Statistically significant COG categories ($P < 0.05$) in a comparison of bloom and control metatranscriptomes. Statistical testing was conducted using transcript counts corrected for volume of water filtered and sample sequencing depth (Table 1) but before calculations of per litre copy numbers so as not to artificially inflate statistical power of the tests. The dotted line shows the average limit of detection (that is, where a category containing just one transcript would plot). Black circles = duplicate bloom microcosms; white circles = duplicate control microcosms. For the acyltransferase (isoleucine patch superfamily) and FOG: GGDEF domain COGs, there were zero counts in one of the control microcosms.

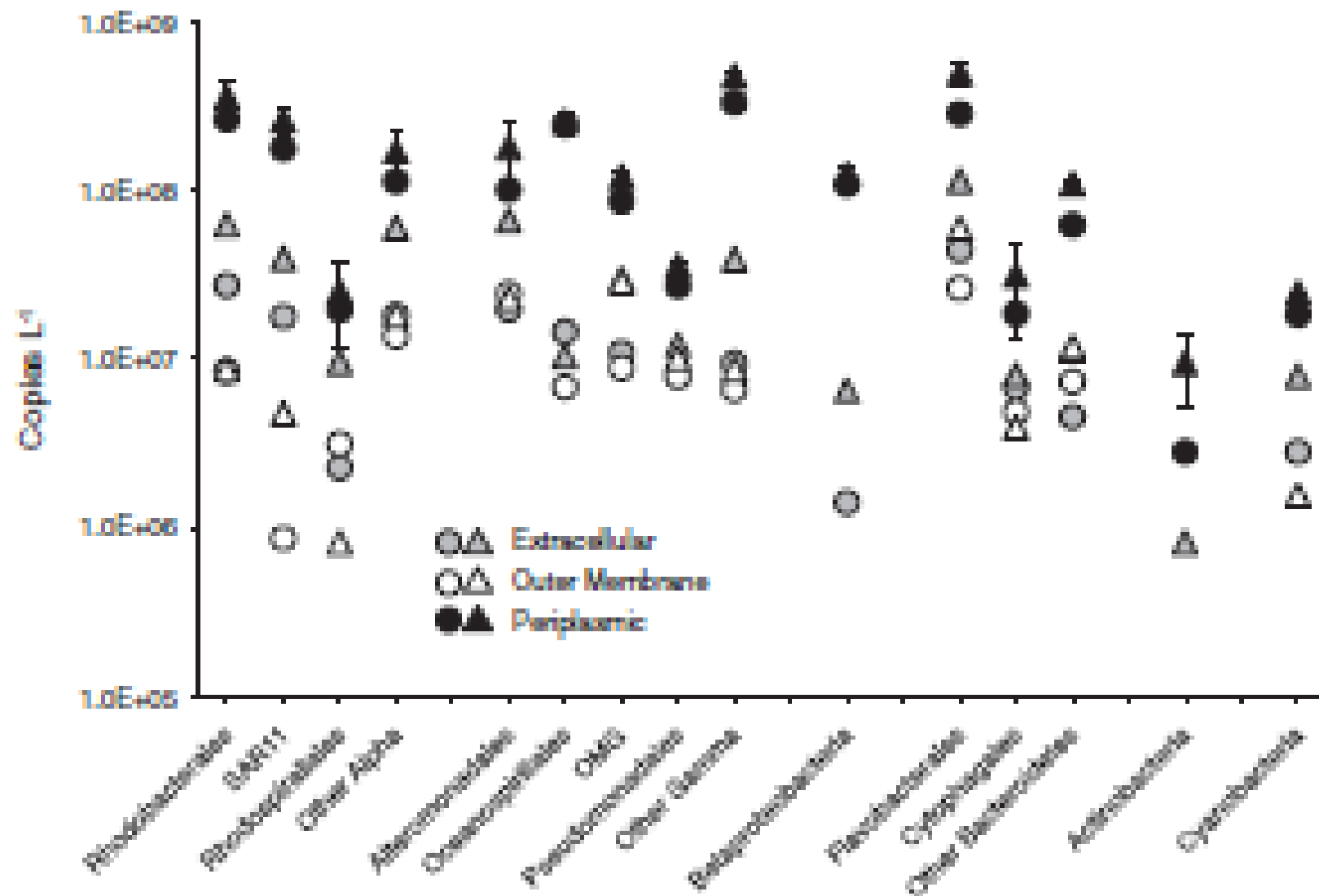


Fig. 5. Abundance of transcripts in three predicted subcellular localization categories by taxonomic bin. Assignments were made using MetaP (Luo et al., 2009). Gray symbols = extracellular; white symbols = outer membrane; black symbols = periplasmic. Circles = bloom microcosms; triangles = control microcosms. Other Alpha = Alphaproteobacteria-like sequences not assigned to the four groups shown; OMG = oligotrophic marine gammaproteobacteria; Other Gamma = Gammaproteobacteria-like sequences not assigned to the four groups shown; Other Bacteroidetes = Bacteroidetes-like sequences not assigned to the two groups shown.

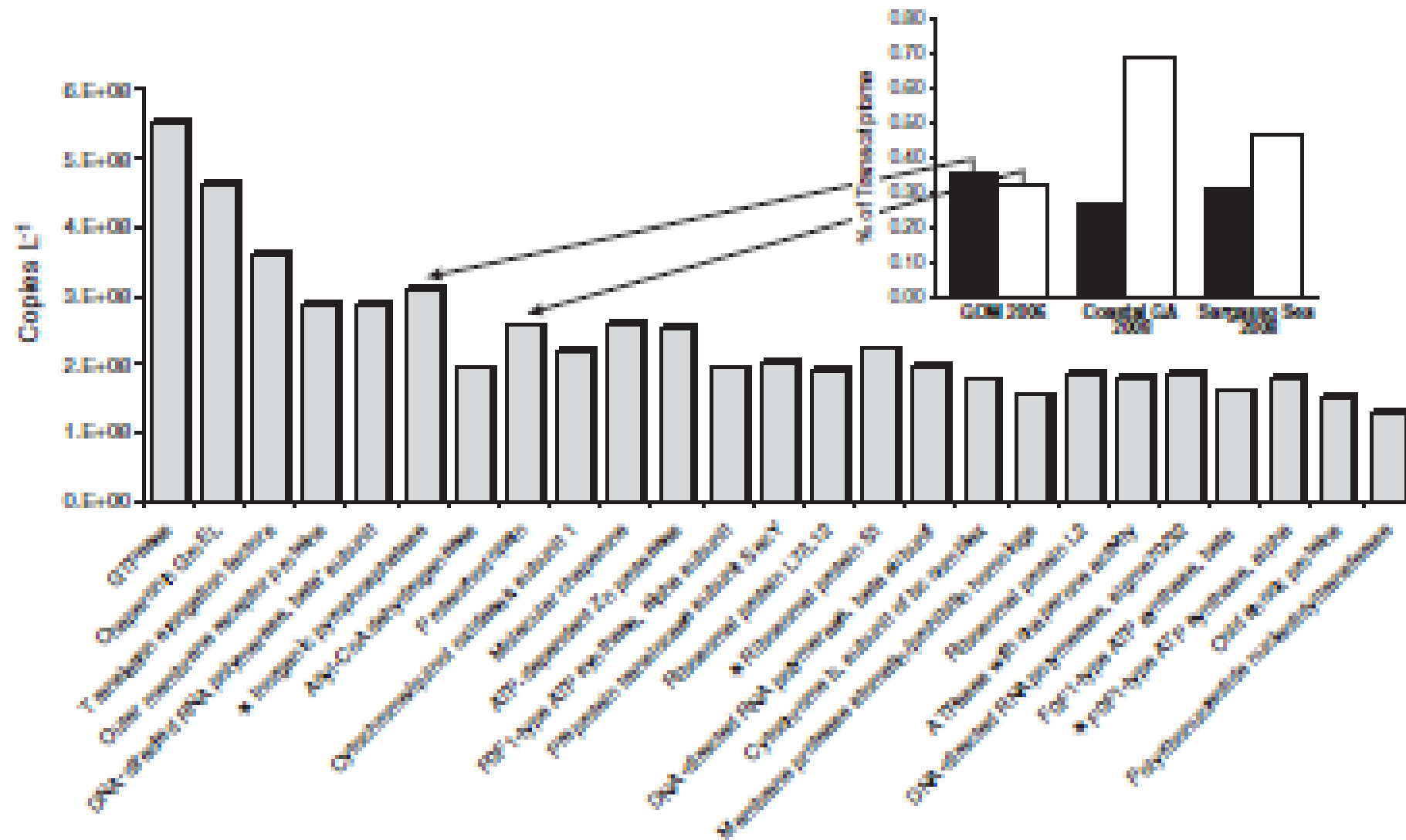
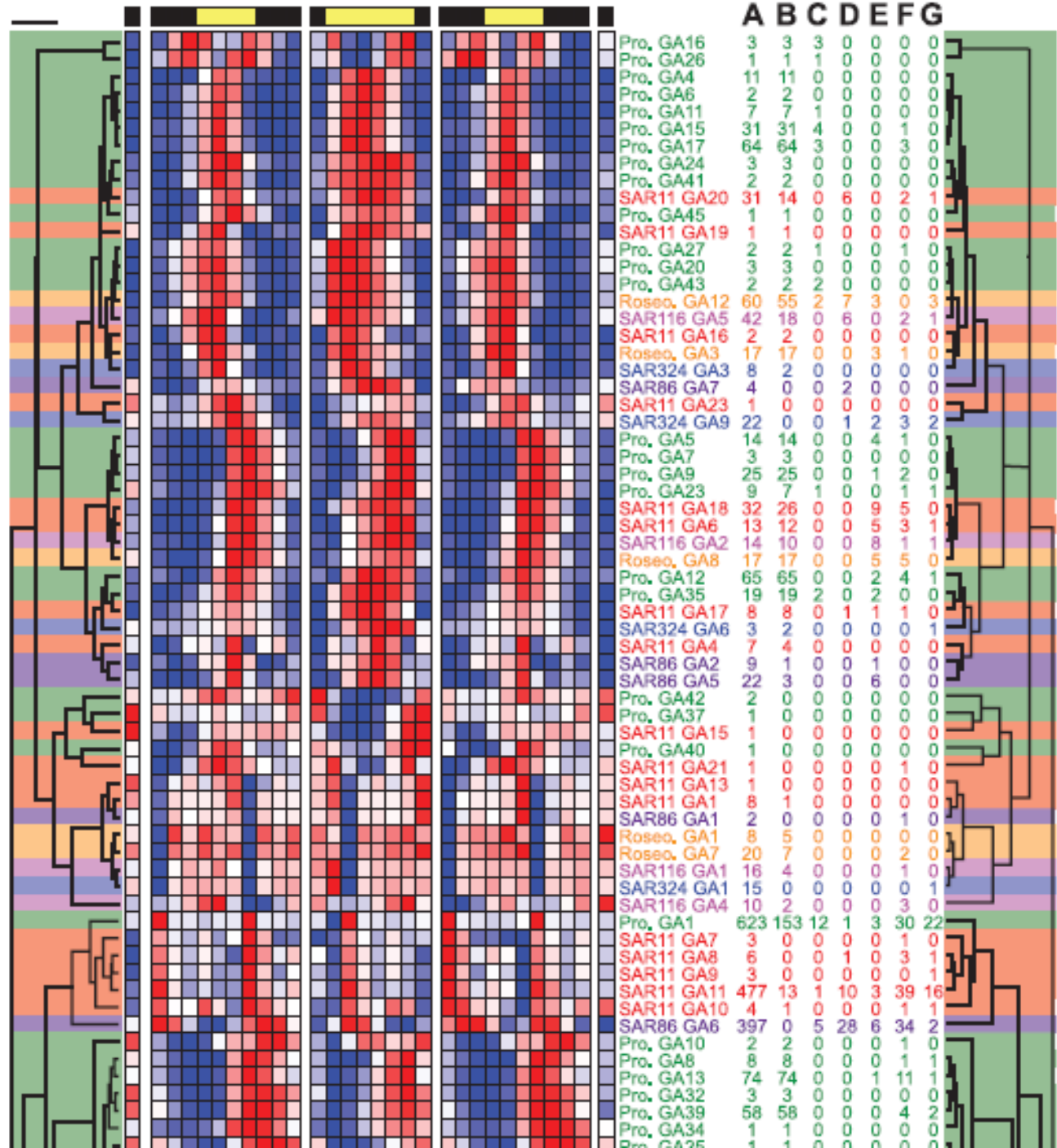


Fig. 6. Membrane-bound inorganic pyrophosphatase transcripts are among the most highly expressed genes in this (main figure) and other (inset) marine metatranscriptomes, with similar expression levels to that of proteorhodopsin. Asterisks indicate significant differences between treatments in this study. Inset legend: black bars = membrane-bound inorganic pyrophosphatase (atpA); white bars = proteorhodopsin. Metatranscriptome datasets used are available in the CAMERA database with project IDs CAM_PROJ_SargassoSea and CAM_PROJ_GOM2005.

Fig. 4. Timing of expression of functional gene clusters among different taxa, clustered by the similarities of their temporal expression patterns. Heat map shows cluster models for all GA clusters, colored by mean-centered relative expression (red, high; blue, low). Black and yellow bars show the daily photoperiod. Each box represents a single sampling event; for sample times, see table S1. Dendrograms show cluster model similarity (Pearson correlations, average linkage clustering, scale bar at upper right represents a correlation of 0.5). The total number of genes (column A), significantly periodic genes (B), and genes associated with photosynthesis (C), ribosome (D), oxidative phosphorylation (E), amino acid metabolism (F), and transport (G) (defined as for Fig. 2), are listed for each cluster. See table S4 for the identities of genes found within any specific GeneARMA (GA) cluster.

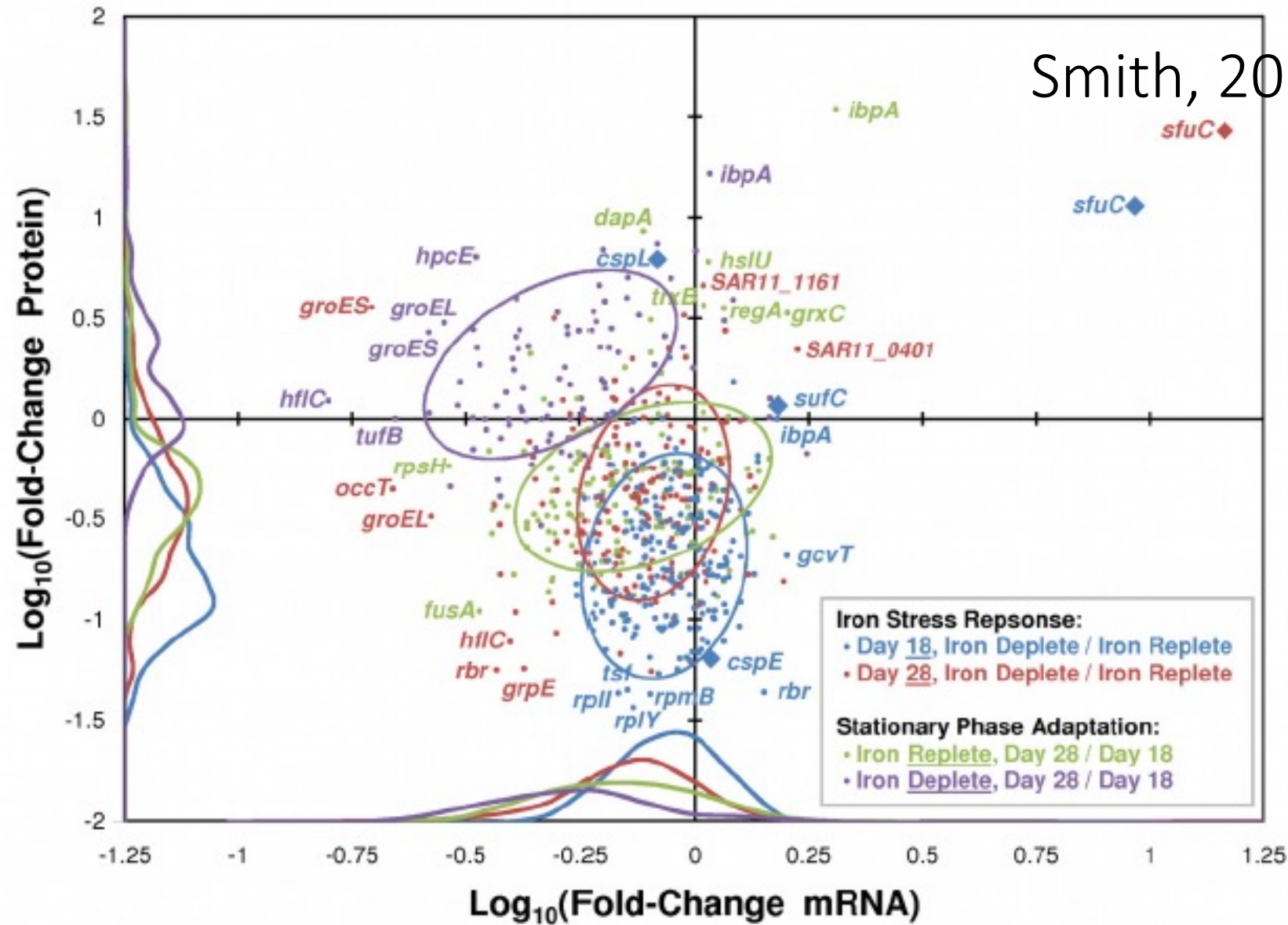


Otteson,
2014

What does it all mean?

- Comparing to diatom blooms isn't perfect
 - They aren't the same species as your brown algae
 - We don't know if the bacteria are responding to changes in diatom physiology (exudates) or if they're responding to the same environmental conditions producing the bloom (elevated nutrients, sunlight)
- Knowing what the diatoms are doing && knowing what the bacteria are doing will help (your literature searches!)
- Gene expression associated with polysaccharide and peptide uptake are most likely responding to algae production

Smith, 2011 (Dissertation OSU)



Important groups-Generalizations (sort of)

- Cyanobacteria-Prochlorococcus&Synechococcus
- Alteromonads-Gamma Proteobacteria
 - Common environmental bacteria, heterotrophic,
- Gamma proteobacteria-SAR86-streamlined genome
- Alpha-proteo bacteria, SAR11, SAR116 & Rhodobacterales-can have bacteriochlorophyll, anoxygenic photosynthesis
 - About SAR116 “*Candidatus Puniceispirillum marinum*” IMCC1322, the first cultured representative of the SAR116 clade in the *Alphaproteobacteria*, is reported here. The genome contains genes for proteorhodopsin, aerobic-type carbon monoxide dehydrogenase, dimethylsulfoniopropionate demethylase, and C₁ compound metabolism. The genome information proposes the SAR116 group to be metabolic generalists in ocean nutrient cycling.”
- Flavobacteria-associates/commensals
- Firmicutes-soil/sediment bacteria

Abbreviations: ED, Entner-Doudoroff; Ehhadh, enoyl CoA hydratase/3-hydroxyacyl CoA dehydrogenase; EMP, Emden-Meyerhof-Parnas; ORF, open reading frame; %GC, percent the genome that is guanine-cytosine rich	<i>Pelagibacteraceae</i>			<i>SAR86 clade</i>			
	<i>HTCC1062</i>	<i>HTCC7211</i>	<i>HTCC1002</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
<i>Characteristics</i>							
Size (Mbp)	1.309	1.457	1.328	1.25	1.7	0.75 ^{a}	0.925 ^{a}
ORFs	1389	1478	1423	1316	1712	859 ^{a}	1111 ^{a}
%GC	29.7	29	29	32.8	32.6	31.2	30.1
%Complete (core gene count)	97.2 (104)	98.1 (105)	97.2 (104)	92.5 (99)	93.4 (100)	54.2 (58)	48.6 (52)
<i>Vitamin/co-factor biosynthesis</i>							
B6	No	No	No	No	No	^{a}	^{a}
B12	No	No	No	No	Yes	^{a}	^{a}
Thiamine	No	No	No	No	No	^{a}	^{a}
Carotene/ retinal/retinol	Yes	Yes	Yes	No	No	^{a}	^{a}
Folate	Yes	Yes	Yes	Yes	Yes	^{a}	^{a}
Biotin	No	No	No	No	No	^{a}	^{a}
Pantothenate	No	No	No	No	No	^{a}	^{a}
<i>Sugar utilization</i>							
Glycolysis	ED	No	ED	EMP	EMP	^{a}	^{a}

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