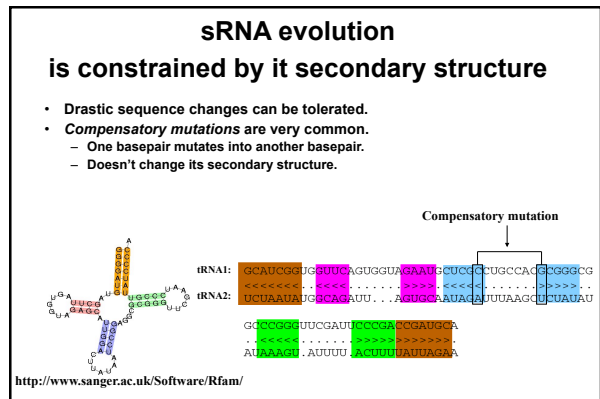
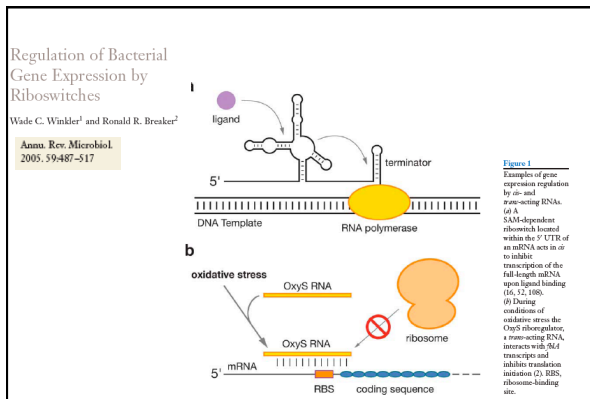
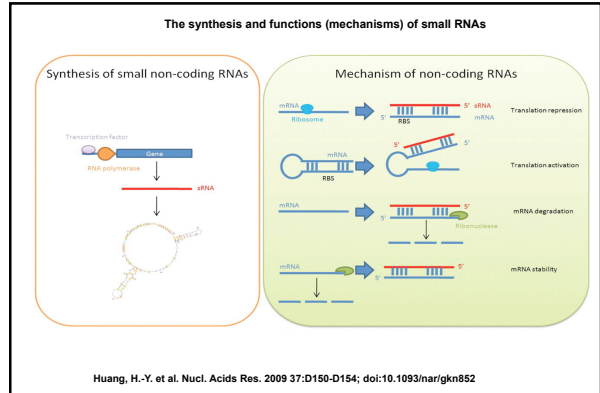




There are many unknown but functional ncRNAs.
 Many ncRNAs may play important regulatory roles.



How many known sRNA families in our data sets?

RNA families database of alignments and CMs

query RNA structure

```

  C A A
  G C G
  G C C
  G C C
  
```

A: structural homolog

```

  A A
  G A
  C G
  C G
  U A
  A U
  
```

B: nonhomologous

```

  G A
  G A
  A x G
  C x A
  C x U
  
```

primary sequence alignment scoring:

```

  query: GGGGGCAAACCCC
        xxxxxixixxxxx
  A: AUCCGAAAAGGAU
        -6
  query: GGGGGCAAACCCC
        xxxxxixixxxxx
  B: CCUAGAAAAGGAU
        -6
  
```

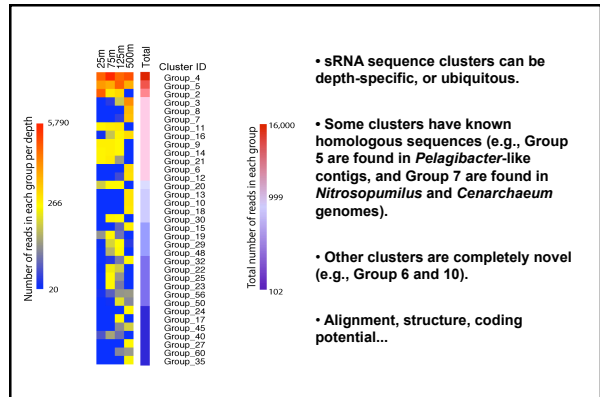
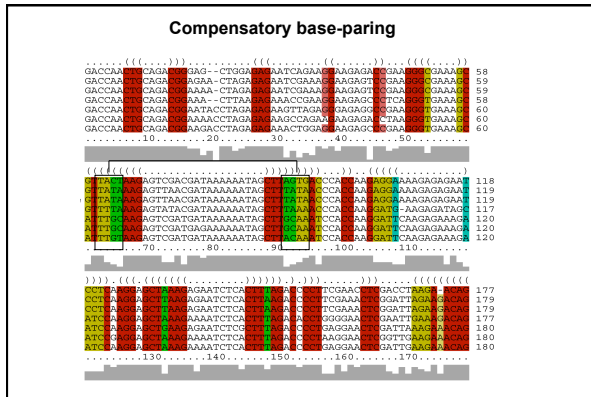
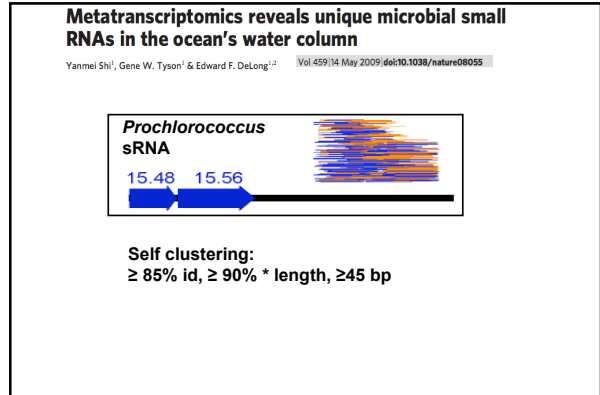
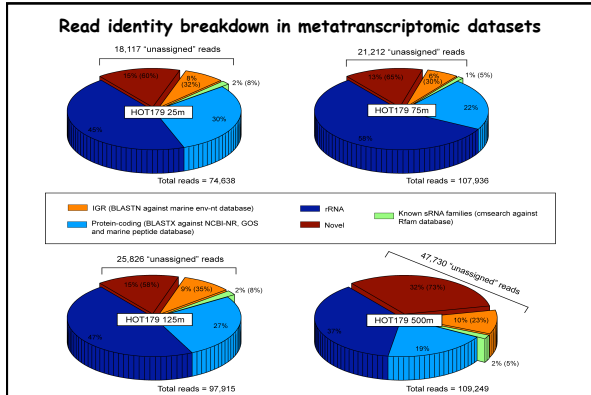
structure + sequence alignment scoring:

```

  query: GGGGGCAAACCCC
        xxxxxixixxxxx
  A: AUCCGAAAAGGAU
        +11
  query: GGGGGCAAACCCC
        xxxxxixixxxxx
  B: CCUAGAAAAGGAU
        -6
  
```

Known sRNA families in our data sets

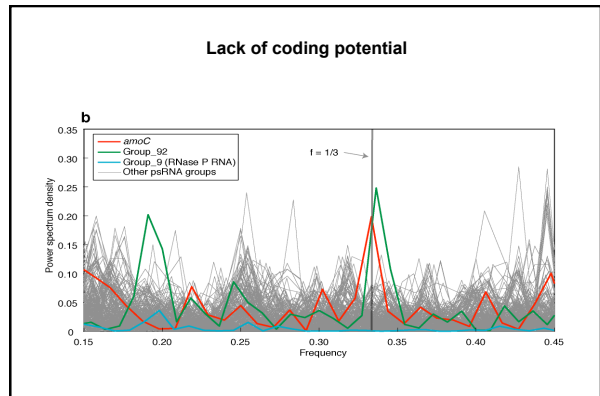
Rfam ID	Function	Total count	cDNA DNA				
			25m	75m	125m	500m	
RF00162.SAM	Riboswitch, methionine/cysteine biosynthesis	4 0	0 0	0 0	0 0	4 0	
RF00029.Intron_gpII	Self-splicing ribozyme	2 0	0 0	0 0	0 0	2 0	
RF00016.SNORD14	Cleavage of eukaryotic precursor rRNA	2 0	0 0	1 0	1 0	0 0	
SRP	RF00169.SRP_bact	Translation and targeting of proteins to cell membranes	474 30	101 9	94 11	148 4	131 6
	RF00004.L2	Pre-mRNA splicing in eukaryotes	14 1	1 1	5 0	1 0	7 0
RNaseP	RF00010.RNaseP_bact_a	Generation of mature rRNA	833 63	238 27	267 21	184 12	134 3
mRNA	RF00023.sRNA	Rescue of stalled ribosomes; cell cycle regulation	1961 200	242 38	413 49	539 50	796 63
	RF00013.6S	Gene regulation during stationary phase	71 18	12 7	23 10	33 1	3 0
	RF00054.Glycine	Riboswitch, glycine metabolism	20 17	17 3	6 3	5 7	1 4
tRNA	RF00005.tRNA	Protein synthesis	1036 874	175 214	138 259	490 265	232 198
	RF00059.TPP	Riboswitch; gene regulation	7 15	1 2	0 4	4 0	2 9
	RF00174.Cobalamin	Riboswitch; gene regulation	2 6	1 2	1 1	0 1	0 2
	RF00017.SRP_euk_arch	Translation and targeting of proteins to cell membranes	4 43	1 19	1 5	1 3	1 16
	RF00161.suB5	Putative sRNA with unknown function	0 2	0 0	0 0	0 0	0 2
	RF00095.L7	Pre-mRNA splicing in eukaryotes	0 5	0 1	0 0	0 3	0 11
	RF00552.SCARN14	small nuclear RNA in eukaryotes	0 8	0 3	0 0	0 2	0 3
	RF00521.SAM_alpha	Riboswitch; methionine biosynthesis in	0 10	0 2	0 0	0 2	0 6

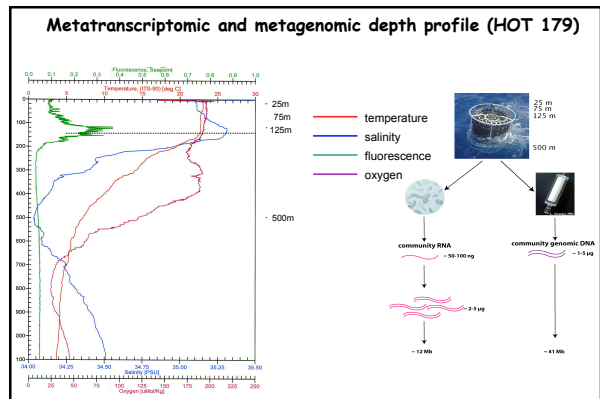
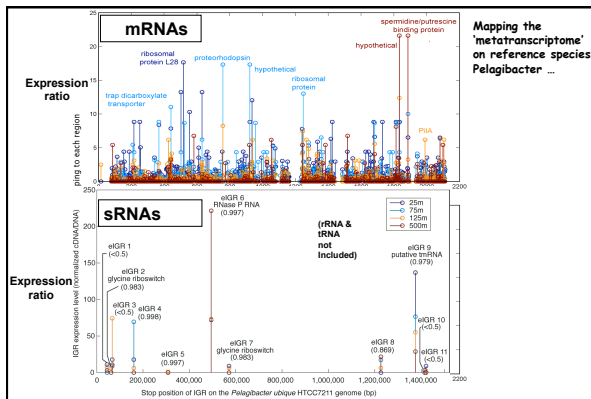
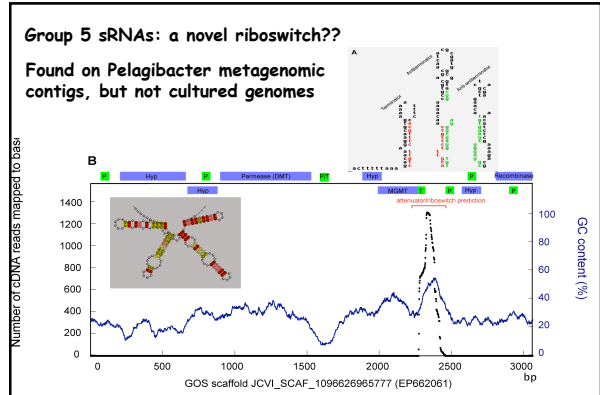
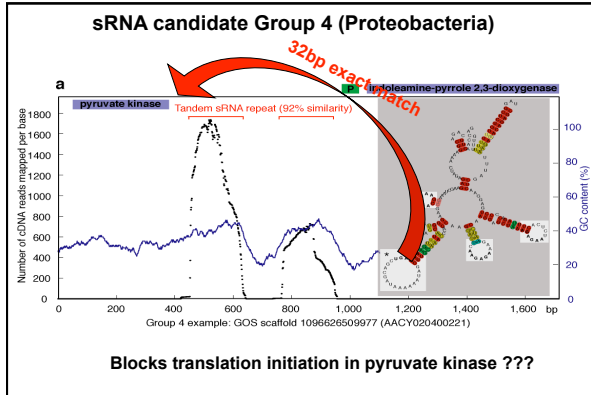


Categories of clustered small RNA sequence groups identified in depth profile

Depth Profile	Group ID	Avg. sRNA length (nt)	SVL probability	Avg. length	sRNA location	Flanking ORF annotations	Putative taxonomy of environmental fragments
25m	Group 1	121.51	0.961	122 bp	5'	COG0026: protein kinase COG0027: protein kinase COG0028: protein kinase	Bacteria: Proteobacteria, Alphaproteobacteria/Carnegie/Prochlorococcus
	Group 2	93.89	0.942	94 bp	5'	COG0026: protein kinase COG0027: protein kinase COG0028: protein kinase	Bacteria: Cyanobacteria, Prochlorococcus, Pfla0103: Green Sulfur
75m	Group 3	95.44	0.846	100 bp	7'	COG0020: ATP/ADP phosphatase	Bacteria: Proteobacteria, Alphaproteobacteria/Carnegie/Prochlorococcus
	Group 4	94.76	0.877	137 bp	5'	hypothetical protein	Bacteria: Proteobacteria, Carnegia/Prochlorococcus
125m	Group 5	95.27	0.808	207 bp	5'	COG1294: Acylphosphatase	Archaea: Oceanobasillus
	Group 11	96.34	0.978	106 bp	5'	COG0077: Proteinase inhibitor	Bacteria: Proteobacteria, Cellulifer/Prochlorococcus/Carnegie/Prochlorococcus
500m	Group 19	94.43	0.989	137 bp	7'	COG0134: Phosphatase	Bacteria: Spirochaetes
	Group 29	95.42	0.908	162 bp	7'	COG0071: omega-specific ribonuclease	Bacteria: Cyanobacteria, Prochlorococcus
25m	Group 14	95.84	0.995	227 bp	5'	COG0114: Proteinase inhibitor hypothetical protein	Bacteria: Cyanobacteria, Prochlorococcus
	Group 22	94.24	0.993	142 bp	5'	COG0180: Tyrosyl-tRNA synthetase	Bacteria: Proteobacteria, Carnegia/Prochlorococcus
75m	Group 8	95.23	0.349	139 bp	Unknown	No hit	Unassigned
	Group 23	95.82	0.943	162 bp	Unknown	No hit	Unassigned
125m	Group 20	96.37	0.888	225 bp	5'	COG0081: hemolysin hemolysin-like toxin COG0082: Pfla0103: Green Sulfur COG0083: Pfla0103: Green Sulfur	Bacteria: Proteobacteria, Alphaproteobacteria/Carnegie/Prochlorococcus
	Group 30	93.55	0.898	193 bp	Unknown	No hit	Unassigned
500m	Group 35	95.8	0.998	133 bp	Unknown	No hit	Unassigned
	Group 36	96.47	0.885	273 bp	5'	COG0033: Amino acid transport	Archaea: Euryarchaeota
25m	Group 30	96.33	0.978	225 bp	5'	COG0086: hemolysin hemolysin-like toxin COG0087: hemolysin hemolysin-like toxin	Bacteria: Cyanobacteria, Prochlorococcus
	Group 38	92.97	0.885	152 bp	5'	COG1491: hemolysin hemolysin-like toxin COG1492: hemolysin hemolysin-like toxin	Archaea: Oceanobasillus
75m	Group 39	94.43	0.974	152 bp	2'	COG0029: Cyclic nucleotide transfer protein, cyclic Pfla0103	Bacteria: Proteobacteria, Carnegia/Prochlorococcus

Group 9 is a member of *Prochlorococcus*-like Group 9 sRNAs.
 Group 11 sRNAs have relative sequence homology to group 11 sRNAs.
 Group 19 contains overlapping sRNAs 19B and 19A identified in *Prochlorococcus* Pfla0103 by Song et al.
 * Number of reads in each group per depth

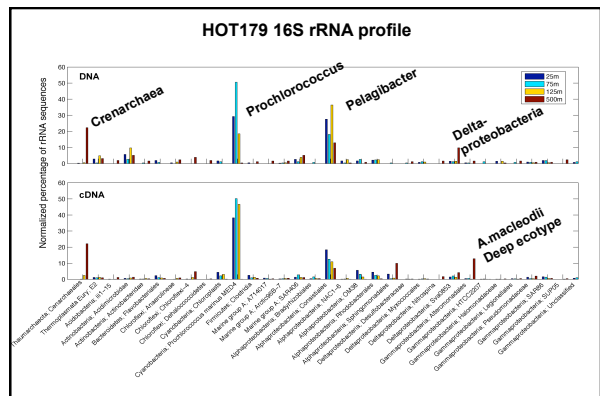


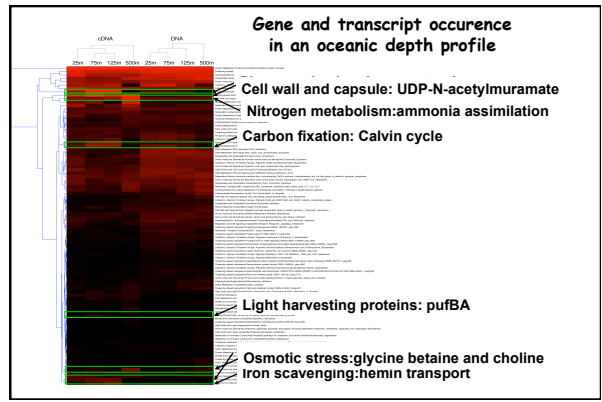
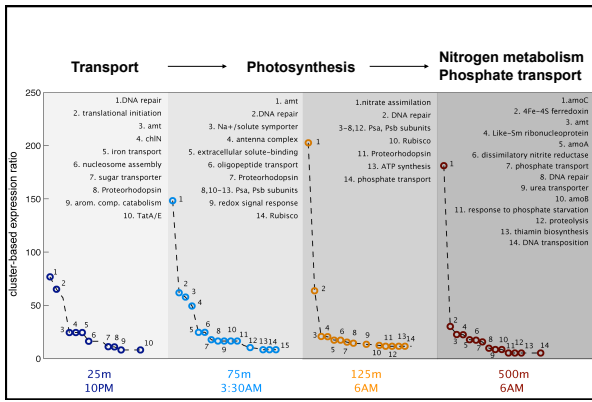
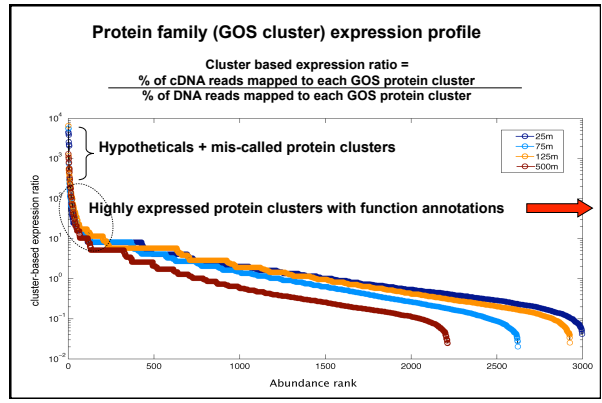
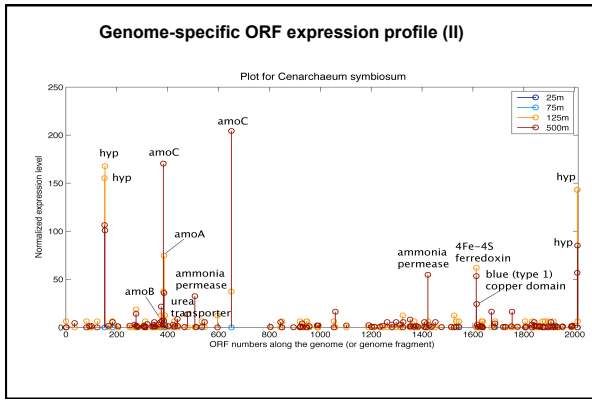
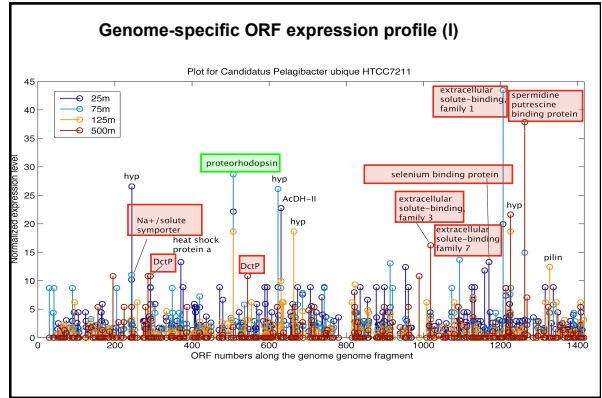
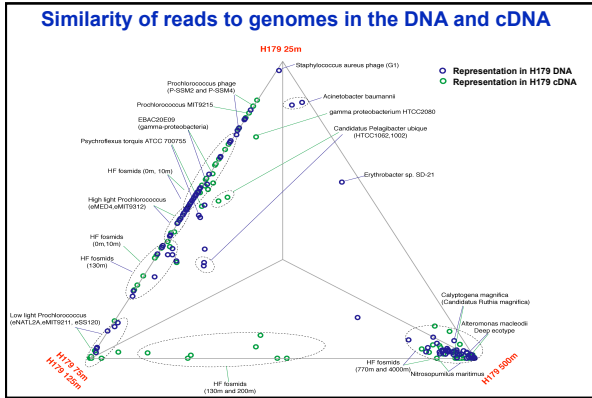


Metatranscriptomics and metagenomics summary (HOT 179)

HOT 179	depth	cleaned reads	average read length	rRNA reads	rRNA%	non rRNA	hit to NR (% of non rRNA)	hit to GOS (% of non rRNA)
cDNA	25m	74638	99	33878	45.4	40760	17.1	45.3
	75m	106936	99	62096	58.1	44840	15.3	49.4
	125m	97915	97	45809	46.8	52106	16.1	46.2
	500m	109249	97	40537	37.1	68712	10.1	26.3
DNA	25m	359665	109	1188	0.3	358477	42.0	63.5
	75m	388652	110	1117	0.3	387535	51.3	71.9
	125m	322751	109	954	0.3	321797	36.3	60.9
	500m	371071	107	1029	0.3	370042	30.5	49.0

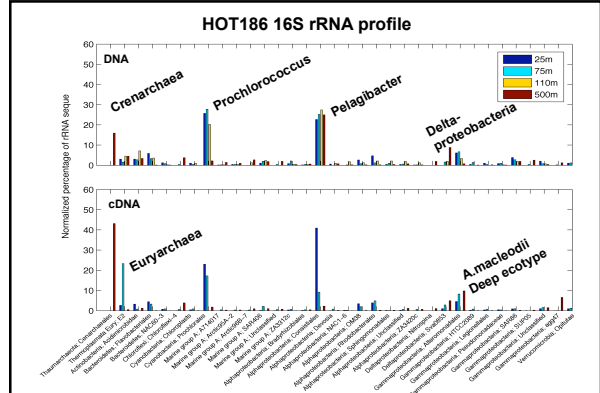
25m: 10 PM 75m: 3:30 AM 125m: 6 AM 500m: 6 AM





Comparison of two depth profiles at station ALOHA

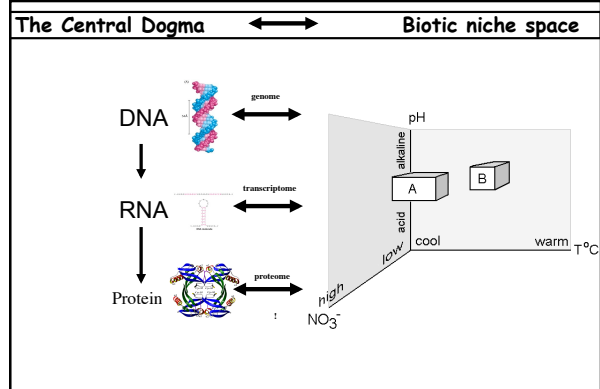
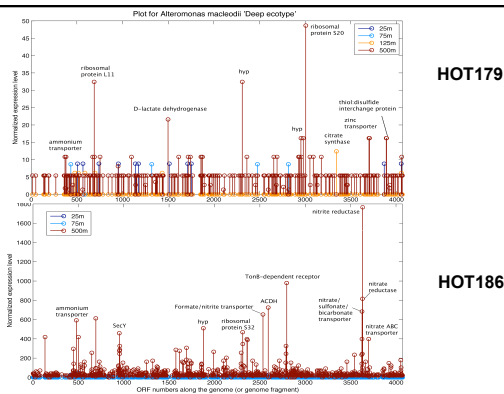
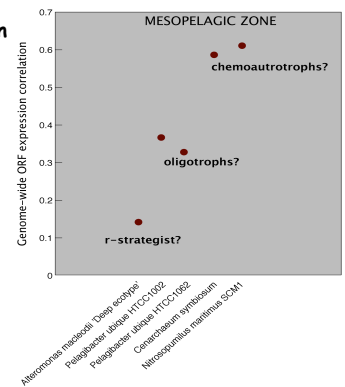
- **HOT 179**
 - March 2006
 - 25m: 22:00
 - 75m: 3:30
 - 125m (DCM): 6:00
 - 500m: 6:00
- **HOT 186**
 - October 2006
 - 25m: 22:00; 13:00
 - 75m: 17:00
 - 110m (DCM): 21:00
 - 500m: 15:30



Comparison of two depth profiles at station ALOHA

- **HOT 179**
 - March 2006
 - 25m: 22:00
 - 75m: 3:30
 - 125m (DCM): 6:00
 - 500m: 6:00
- **HOT 186**
 - October 2006
 - 25m: 22:00; 13:00
 - 75m: 17:00
 - 110m (DCM): 21:00
 - 500m: 15:30

Genome-wide expression correlation in samples taken 6 months apart




Monitoring microbial Community response to environmental change...

Use combined metagenomic, transcriptomic & environmental approaches in field studies.

- Monitor natural variation or perturbations
- Track changes in community gene expression and population structure
- Microcosms, mesocosms, or *in situ*

1. Establish microcosms to monitor transcriptional and population changes



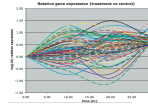
Twenty liter microcosms prepared from natural seawater

2. Subsample microcosms for microbial assemblage response kinetics

Hours	0	6	12	18	24	30
Control	●	●	●	●	●	●
Treatment 1	●	●	●	●	●	●

Legend: T1 - community RNA sample, T2 - community DNA sample

3. Pyrosequence and comparative analyses of subsamples and time points



Experimental community transcriptomics

~2000 L surface seawater

tangential flow ultrafiltration (1 μm membrane) to concentrate HMWDOM

~2 L HMWDOM concentrate

stirred cell ultrafiltration (50 kDa cutoff) to remove viruses

~2 L Virus-free HMWDOM

20 L Mesocosm + ~50x DOM

20 L Mesocosm


Subsamples taken

Time (hrs)	0	6	12	18	24	30
2.0 L - community RNA	●	●	●	●	●	●
0.2 L - flow sorting RNA	●	●	●	●	●	●
10 L - community DNA	●	●	●	●	●	●

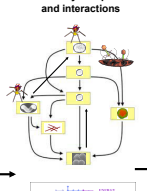
Community DNA encodes network instructions (in part) that specify organism function, community interactions, & ecosystem function

GENOMES

Community genomic and transcriptomic data

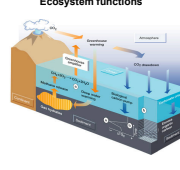


Community composition and interactions

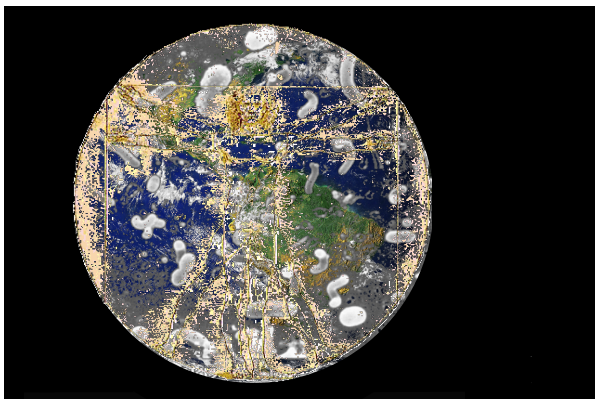


BIOMES

Ecosystem functions



Community metabolism



DeLong Lab

- Justin Buck
- Rachel Barry
- John Eppley
- Sara Lincoln
- Jay McCarren
- Julia Maresca
- Chon Martinez
- Elizabeth Ottesen
- Tzultrim Palden
- Vinh Pham
- Yanmei Shi
- Frank Stewart
- Gene Tyson
- Laure-Anne Ventouras

Collaborators

- MIT - Chisholm Lab
- Penn State - Stephan Schuster
- Dave Karl, CMORE & HOT Teams







