

*HKUST-KAUST Global Collaborative
Research Program*

**Microbial community structure
and function of two deep-sea
brine pools from the Red Sea**

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Deep-sea hydrothermal systems

- **1977: 1st black smokers at East Pacific Rise (Galapagos Rift) by Jack Corliss of Oregon State U boarded on Alvin of WHOI;**
- **Average depth: 2100 m along Atlantic, Pacific ridges;**
- **Deepest: 5000 m in Caymen trough;**
- **1979: 1st observation of deep sea vent communities by WHOI;**
- **1979: 1st publication on hydrothermal vent life by Peter Lonsdale;**
- **1 ounce of tubeworm contains 285 billion bacteria;**
- **2005: 1st discovery of a phototrophic bacterium at 2500 m in Mexico black smoker**
- **2005: Neptune Resources NL gained right to explore 35000 km² in Kermadec Arc (lead-zinc-copper sulfides)**
- **Clue of origin of life, mineral resources.....**

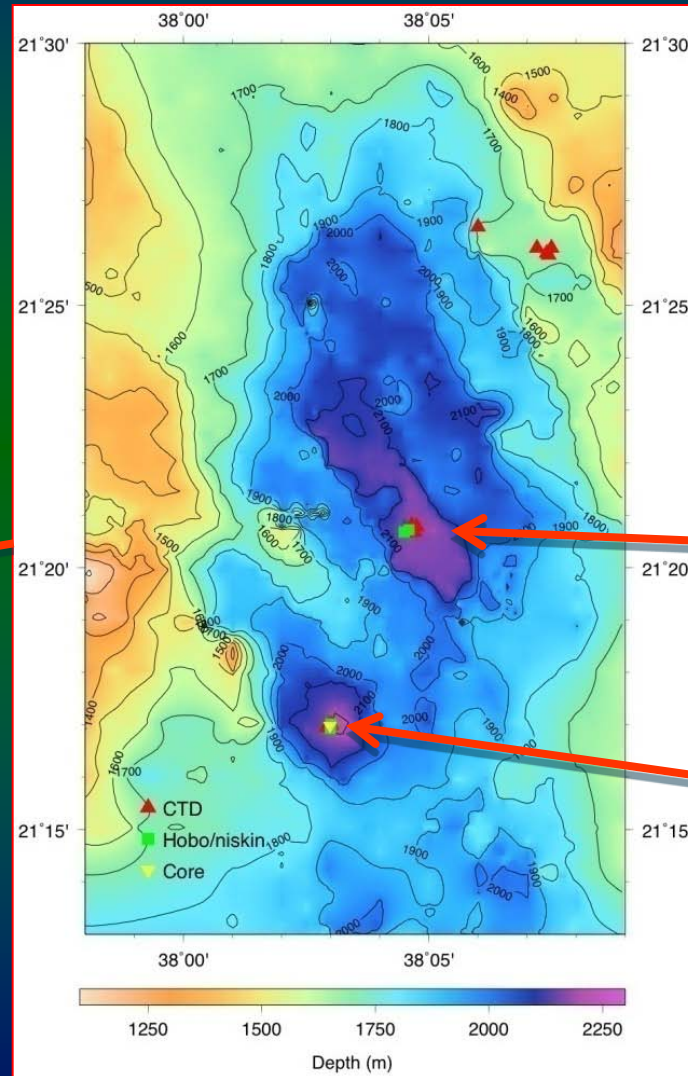
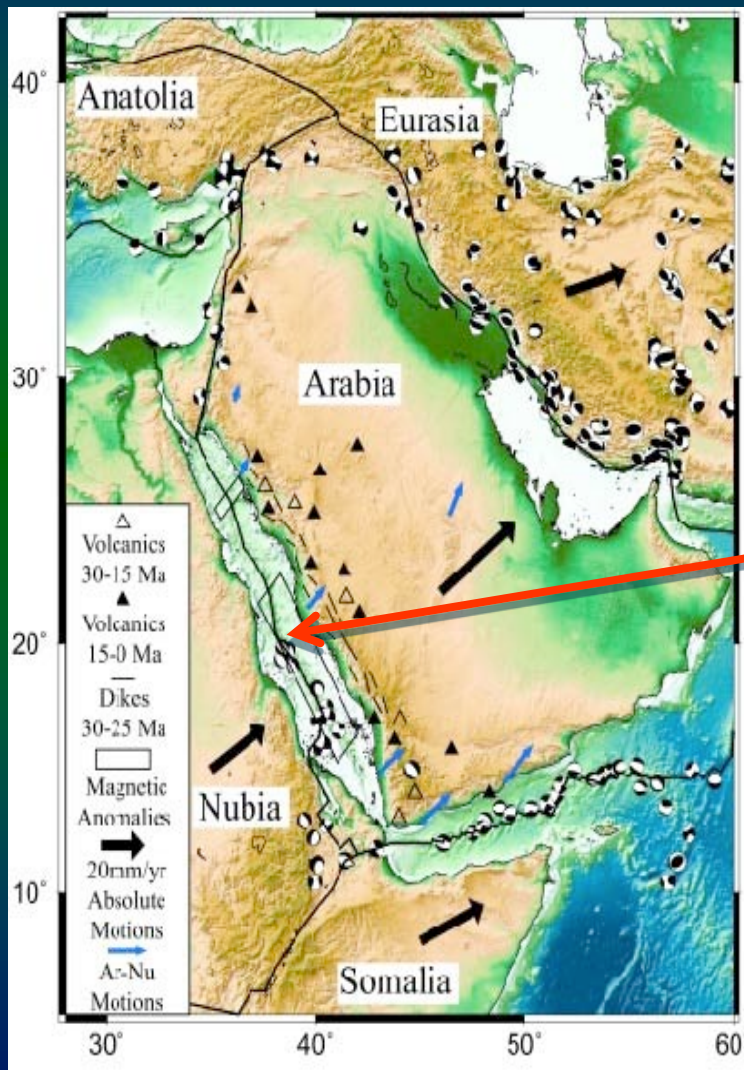
What is the range of global seafloor spreading rate?

	Fast	Half-rate > 5.0 cm/yr
5.0 cm/yr >	Intermediate fast	> 2.5 cm/yr
2.5 cm/yr >	Slow	> 1.0 cm/yr
1.0 cm/yr >	Ultraslow	

(Arctic Ridge; Southwest Indian Ridge;

Red Sea: 0.9 cm/yr at 16° N, 0.5 cm/yr at 26° N)

Deep-sea brine pools in Red Sea



Atlantis II Deep

7.4 km

Discovery Deep

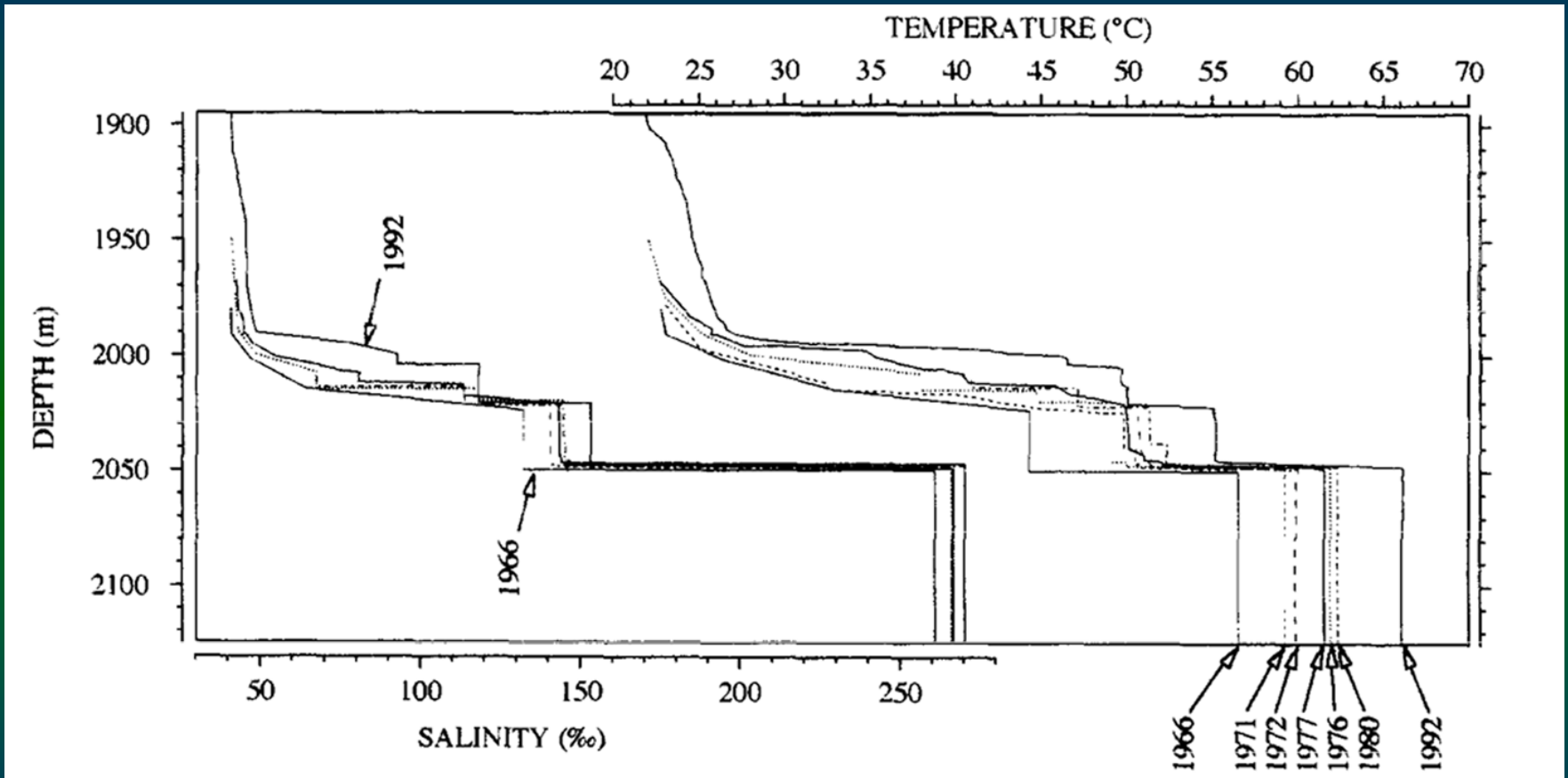
Red Sea Brine Pools

- **1949: 1st discovery of hot brines**
- **1960s: Confirmation**
- **25 brine pools (Degens et al 1969, Pautot et al 1984...)**
- **Numerous geological and geochemical survey of the brine pools since 60's (Faber et al 1998, Swallow and Crease 1965...)**
- **A novel genus had been isolated in Atlantis II brine pool (Fiala et al. 1990)**
- **No information on microbial community structures (particular in-depth analysis)**

Red Sea Brine Pools

- **Two connected pools (brine flow over each other, Neumann & Chave 1965)**
- **Parallel change in anhydrite content in sediment pore water (Monnin & Ramboz 1996)**
- **Separated by a hill at 1950 m (50 m above brine, Ross & Hurt 1969)**
- **ABP temp increased substantially when DBP unchanged (Hartmann et al 1998)**
- **CH₄ in ABP is 4 times higher than in DBP (Faber et al 1998)**
- **Higher Fe, Mn, Li, Zn (Gurvich 2006)**
- **3 Convective layers in ABP but 1 in DBP (Blanc & Auschutz 1995)**

Gradually increasing temperature in Atlantis II lower layer



Little difference in temperature in early 20th century suggests similarity in bacterial communities colonizing the two deeps in the past; temperature increased from 56°C in 1966 to 68°C in 2008

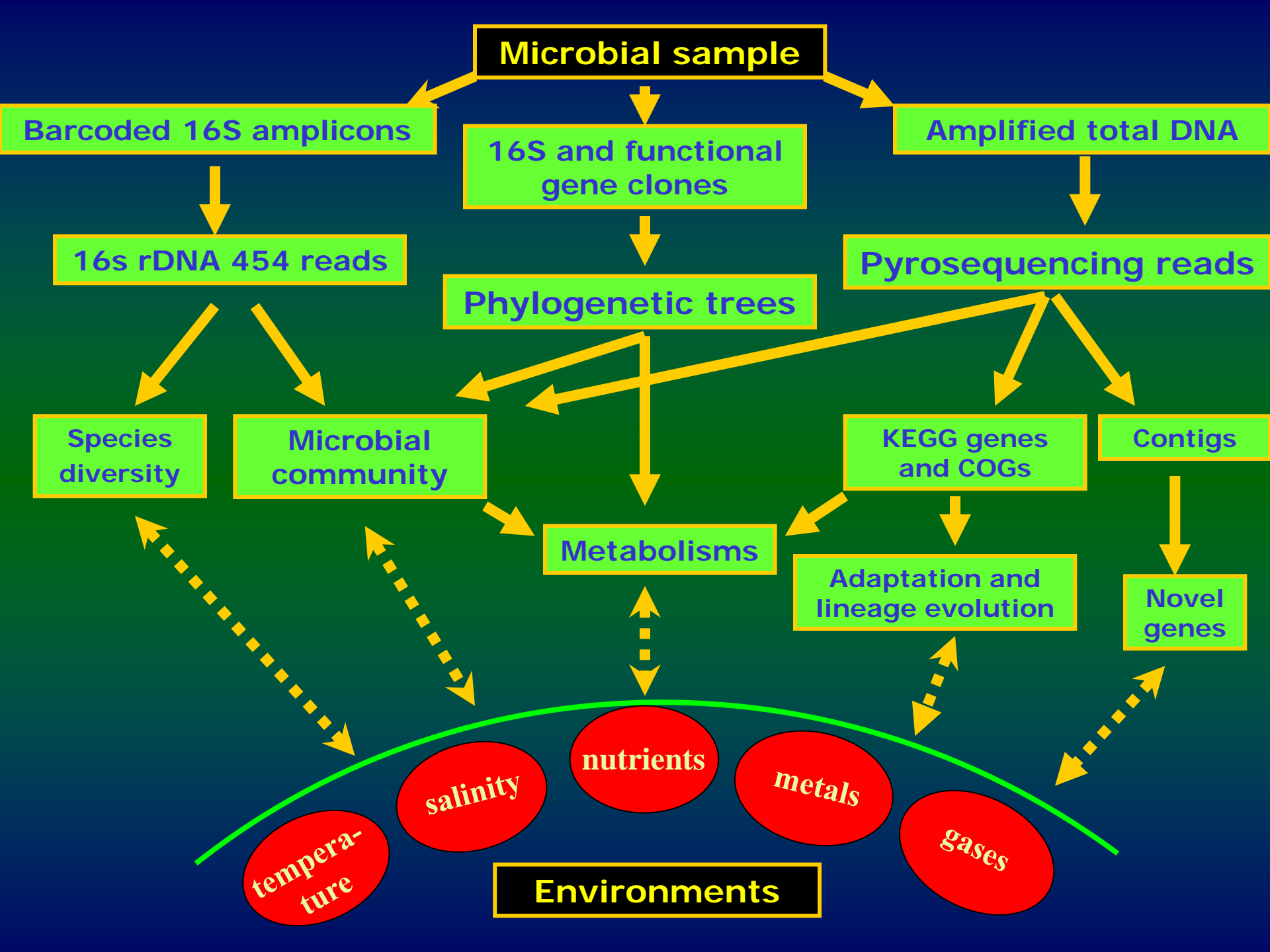
Ecological features Atlantis II and Discovery brine pools

- **Extremely high salinity 255 psu**
- **High temperature (ABP: ~68°C; DBP: ~44 °C)**
- **High metal contents**
- **Low nutrient contents**
- **High ammonia and methane concentrations**
- **Anaerobic**

Objectives

Using pyrosequencing technique to study microbial metagenomics of two brine pools with contrasting environmental conditions

- Determine community diversity in terms of species, genes, and pathways
- Understand the possible functions of microbes in the ecosystems
- Study the adaptive mechanisms of microbes in extreme environment



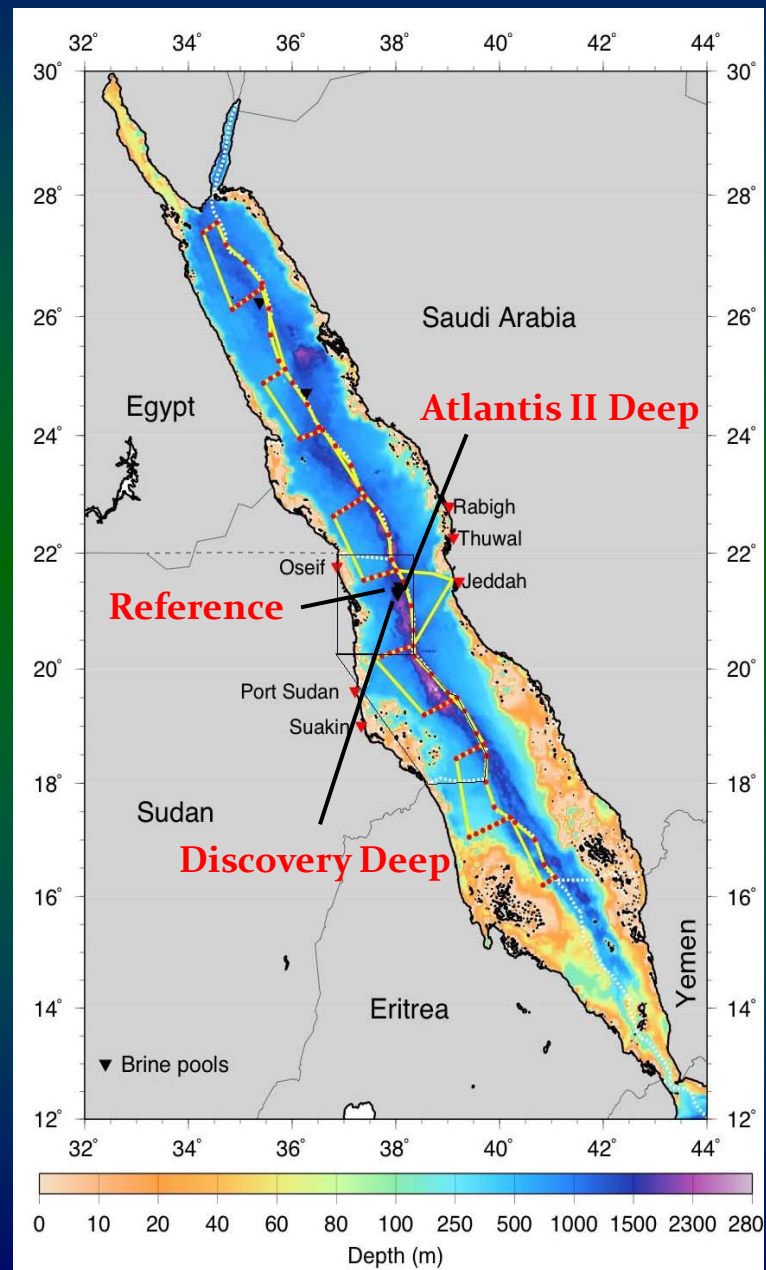
Cruises

- ❖ Oceanus cruise in October 2008
- ❖ Aegaeo cruise in April 2010

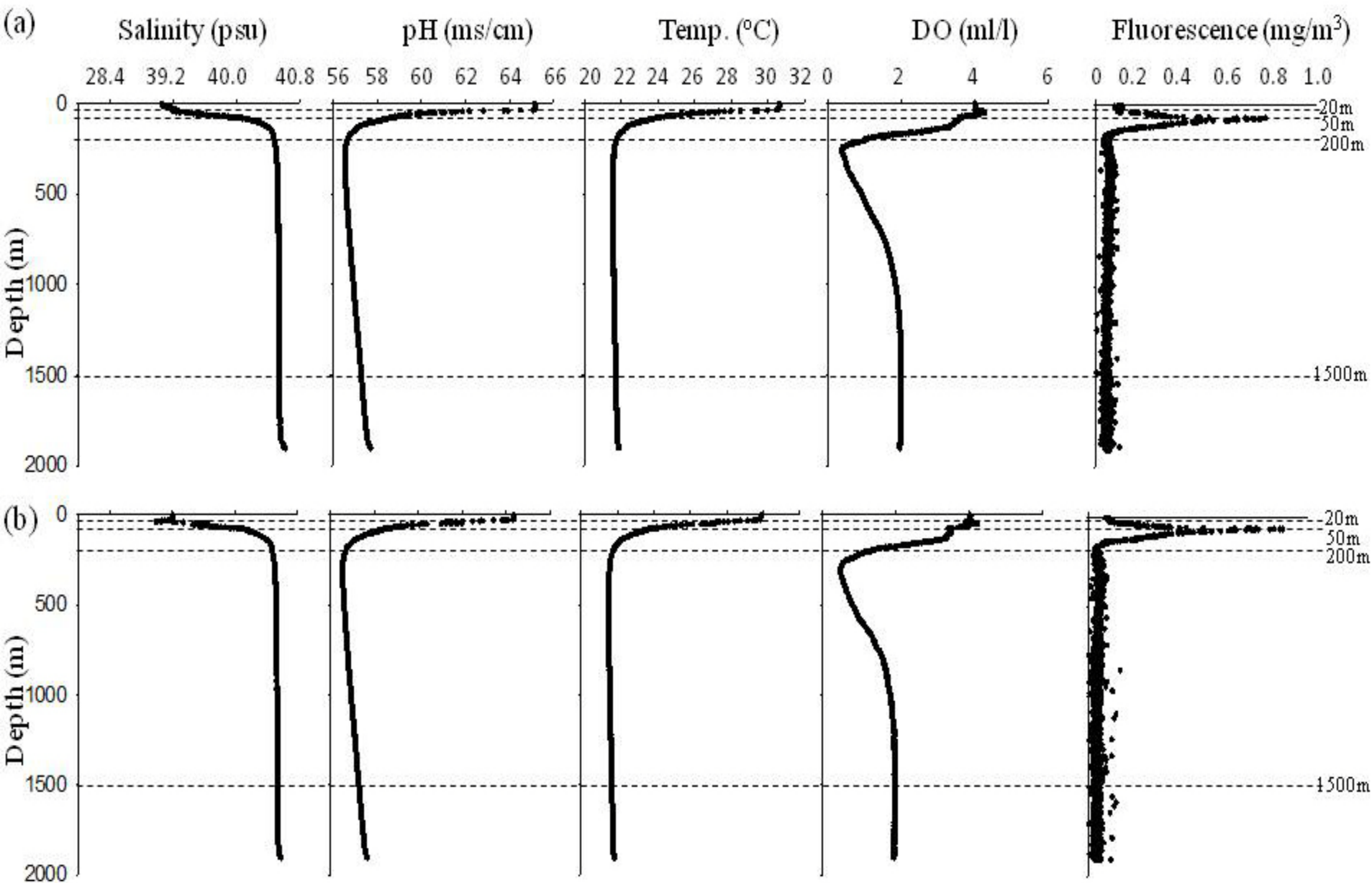


Sampling Sites of first cruise

Location	Depth (m)	Amount collected
Reference site 21° 26.07' N, 38° 07.35' E	50	4L
	1500	4L
	200	4L
	700	4L
	20	4L
Atlantis II 21° 20.63' N, 38° 04.61' E	20 & 50	4L each depth
	1500	4L
	200	4L
	700	4L
	>2100 (brine pool)	100L
	>2100 (brine pool)	20L
	Gravity core	2.25m core
Discovery 21° 16.96' N, 38° 02.97' E	20 & 50	4L each depth
	1500	4L
	200	4L
	700	4L
	>2100 (brine pool)	100L



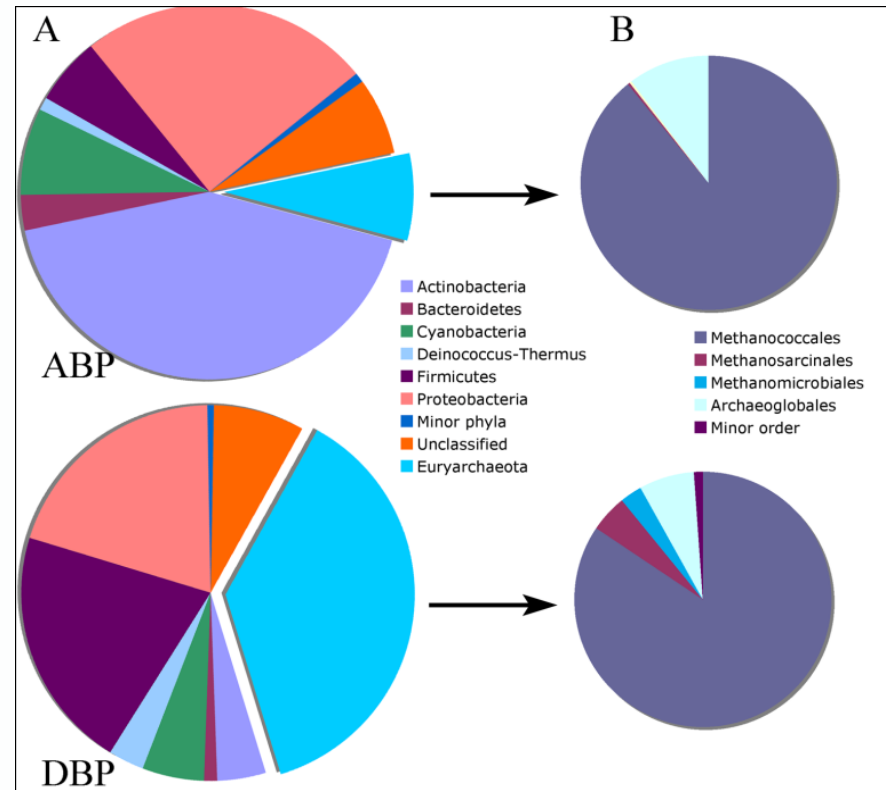
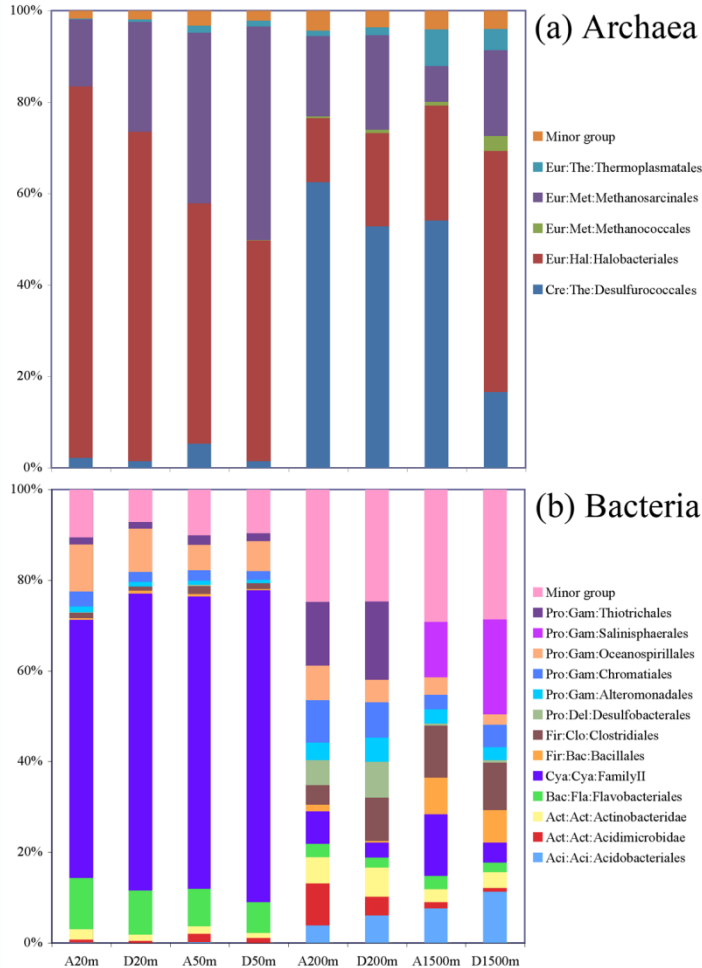
Environmental factors



Microbial communities revealed by pyrosequencing of 16S rDNA amplicons

- Samples included seawater from water columns at different depths and brine water from brine pools
- DNAs were extracted from microbial cells and amplified by universal primers targeting V3 region of bacterial and archaeal 16S rDNA
- Primers for each sample were tagged with a 6-nucleotide barcode, which differentiates different samples in a single run
- Barcoded amplicons were sequenced on a 454 platform
- ~330,000 high quality reads from 454 platform (>92%)

Classification using RDP classifier



Atlantis II and Discovery brine pools

Both dominated by Actinobacteria, Firmicutes, Proteobacteria, Cyanobacteria

Threshold similarity 50%

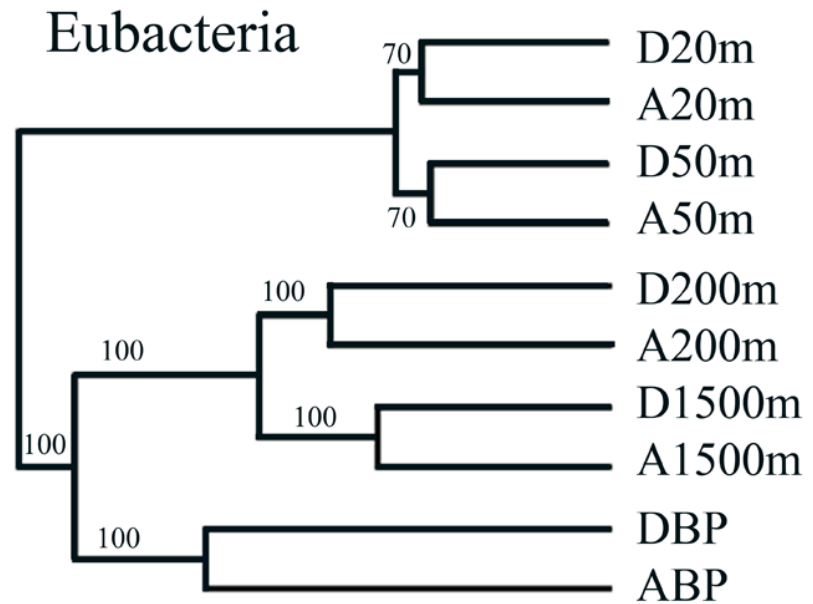
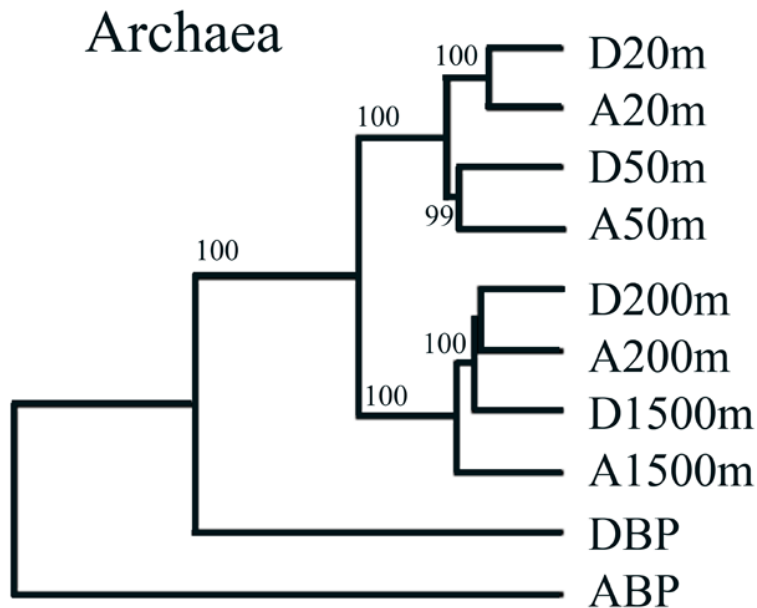
Water column overlying the brine pools

Number of useful reads & diversity index

	Archaea				Bacteria			
	Reads	OTU	ACE	Chao1	Reads	OTU	ACE	Chao1
A20m	13294	966	1919	1866	16822	847	1607	1533
A50m	13293	1078	2280	2073	17486	1087	1663	1685
A200m	4234	384	807	699	10019	684	1034	1026
A1500m	5245	578	1452	1269	6943	646	924	916
D20m	7664	494	1071	1056	11671	341	532	539
D50m	18647	448	855	875	18864	839	1218	1220
D200m	7032	407	798	785	13723	704	993	1022
D1500m	10359	561	1034	977	12418	850	1148	1181
ABP	13968	164	487	382	6208	418	638	600
DBP	6188	502	920	961	6163	438	764	771
Total	99924				120317			

A: Atlantis II; D: Discovery; BP: Brine Pool
OTU, ACE & Chao1 are calculated at 3% dissimilarity

Comparison of similarity of microbial communities among different samples



Key findings

- **Vertical stratification of archaeal and bacterial communities but horizontal homogeneity were observed along the water columns;**
- **The two brine pools harbored diverse archaeal and bacterial communities in which Euryarchaeota, Actinobacteria, Firmicutes and methanogens were dominant;**
- **Cyanobacteria were observed in the deep sea and brine pools of the Red Sea.**

*** Qian et al, ISME J (2011)**

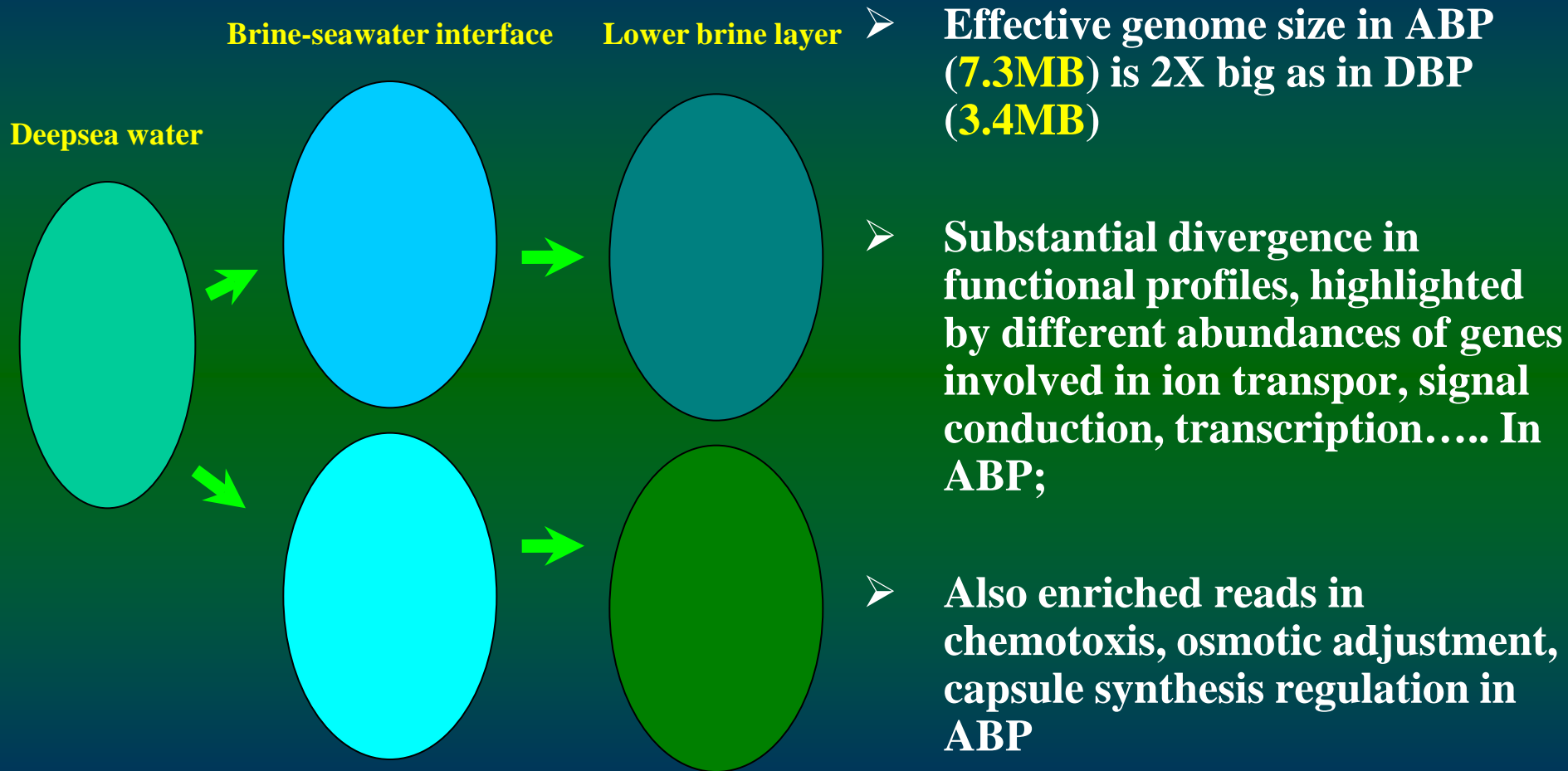
Metagenomic analysis of microbial communities in brine pools

Objectives

- To fully characterize the diversity of microbes in the brine water samples and sediment samples;
- To understand the important ecological functions in these systems

	Atlantis II brine water	Discovery brine water
Raw read (bp)	991,000	915,000
Contigs (bp)	12,003	88,413
Longest contig (kbp)	92.6	30.7

Metagenomes and adaptation strategies



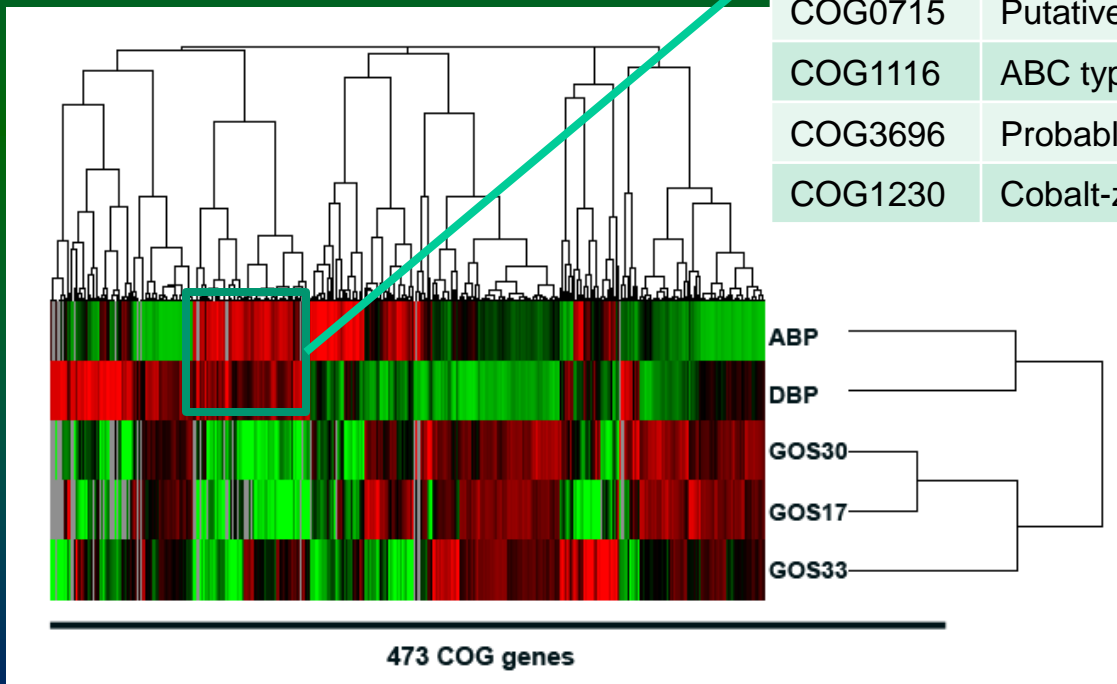
Environmental changes drive compositional shifts of microbial communities and genomic modifications (revealed by 16s)

Comparison of COG genes

- Abundant COG genes in Atlantis II and Discovery Deeps were compared to GOS references

Wang et al, under review

COG ID	Function
COG0370	Ferrous iron transport protein B
COG0474	P-type ATPase, Mg ²⁺ ATPase transport protein
COG2217	Heavy metal translocating P-type ATPase
COG0715	Putative periplasmic protein
COG1116	ABC type transporter ATPase component: NitT family
COG3696	Probable cation efflux system transmembrane protein
COG1230	Cobalt-zinc-cadmium efflux permease



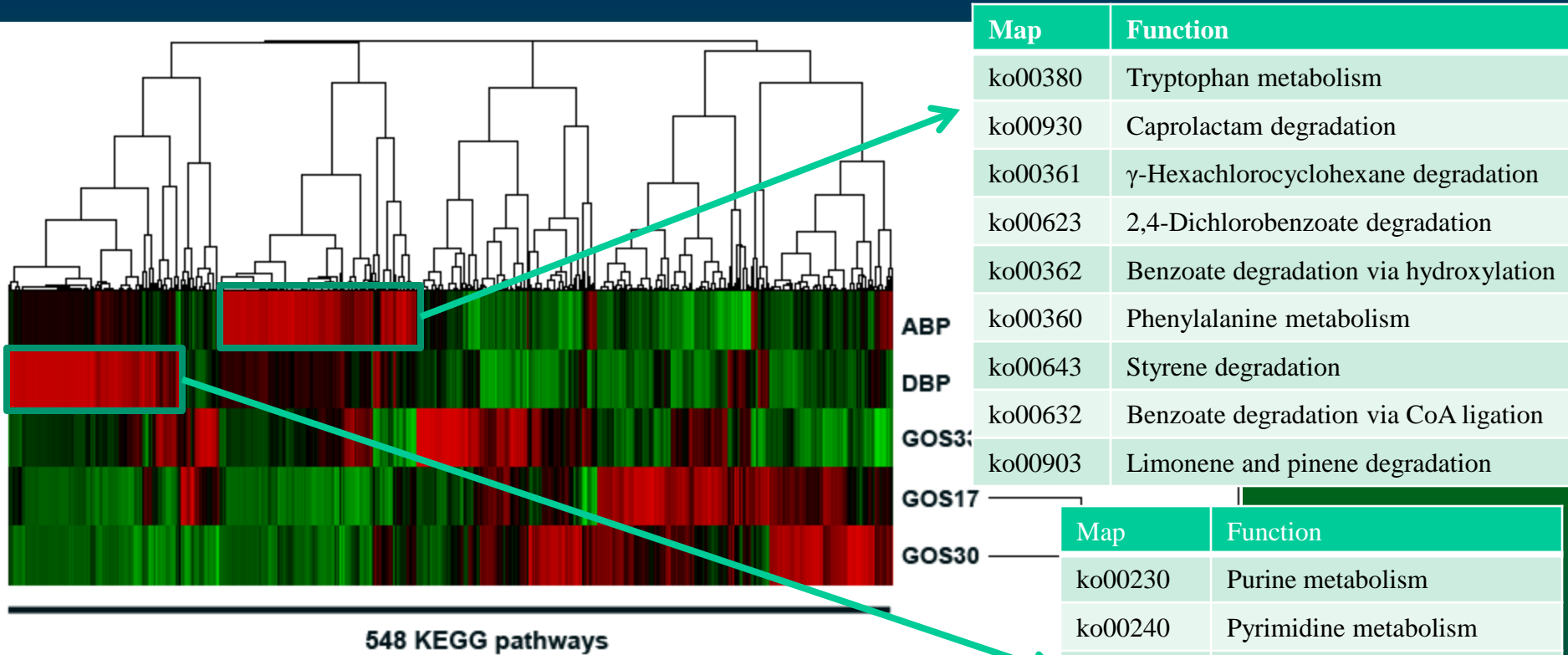
Hot COG genes revealed in ABP and DBP are related with inorganic ion transport and metabolism

GOS33: Surface hypersaline water (37°C), Galapagos island
 GOS17: Caribbean surface sea water (27°C)
 GOS30: Depth 19m, warm seep (27°C), Galapagos island

Number of reads/effective genome for ABC transporter genes

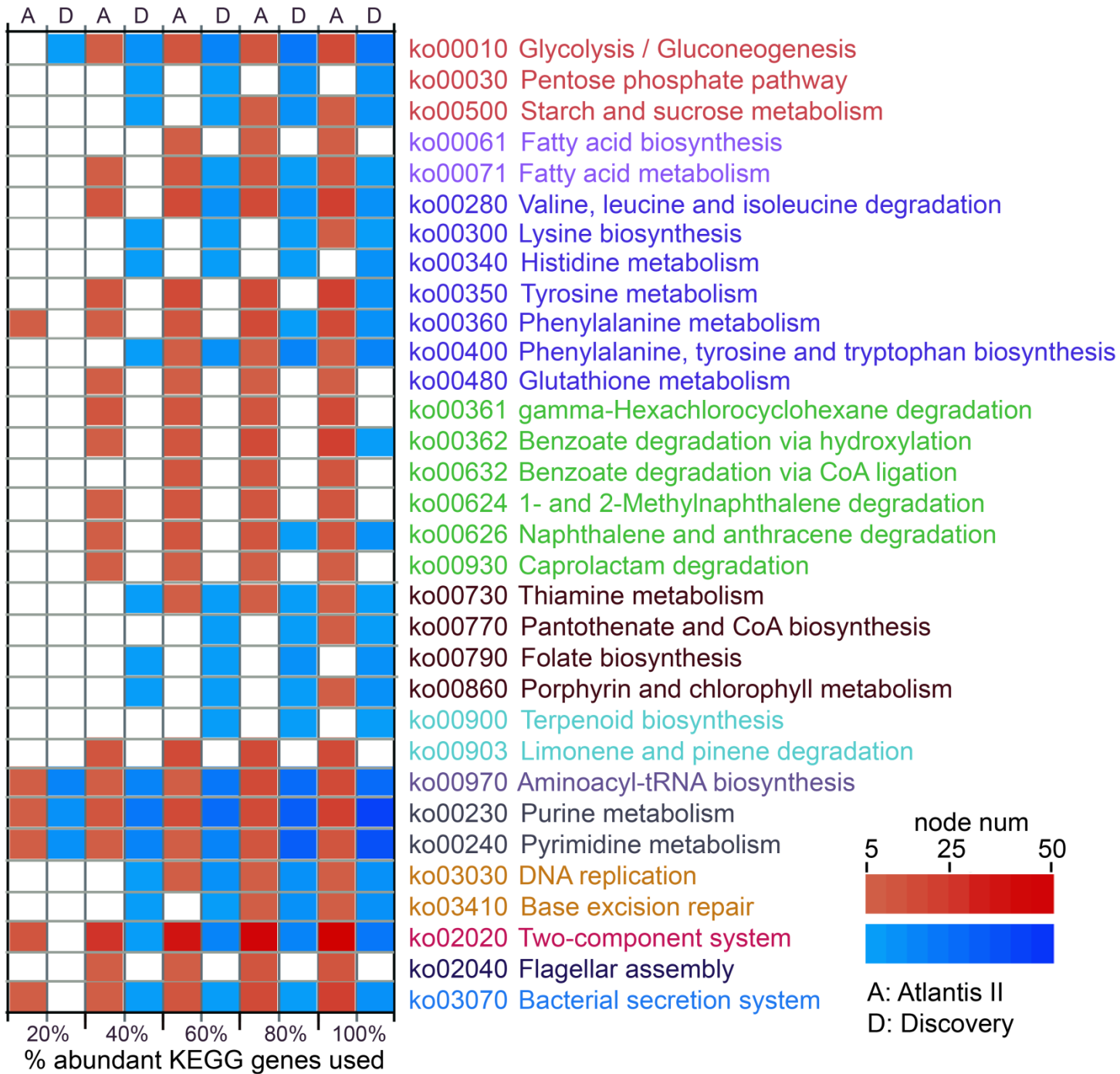
Substance	KEGG ID	Protein	ABP	DBP
Iron(III)	K02012	AfuA	1.50	0.53
	K02011	AfuB	0.59	0.35
	K02010	AfuC	0.31	0.26
Iron complex	K02016	FhuD	0.59	1.23
	K02015	FhuB	0.46	1.12
	K02014	FhuA	11.4	1.1
	K02013	FhuC	0.17	1.34
Nickel	K02008	CbiQ	0.02	0.57
	K02006	CbiO	0.02	0.86
Phosphate	K02040	PstS	0.65	1.06
	K02037	PstC	0.26	0.88
	K02038	PstA	0.19	0.80
	K02036	PstB	0.26	1.16
Sulfate	K02061	Unnamed	0.15	0.79
Sulfonate/nitrate	K02051	SsuA	3.80	1.58
/taurine	K02050	SsuC	3.15	1.08
	K02049	SsuB	3.30	1.26

Comparison of KEGG pathways (Wang et al., ISME J in press)

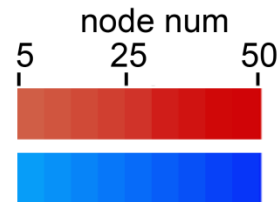


GOS33: Surface hypersaline water (37°C), Galapagos island
 GOS17: Caribbean surface sea water (27°C)
 GOS30: Depth 19m, warm seep (27°C), Galapagos island

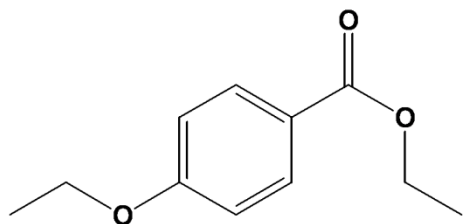
- Hot KEGG maps in ABP are related with aromatic substance degradation while those in DBP are for DNA repair and transposons



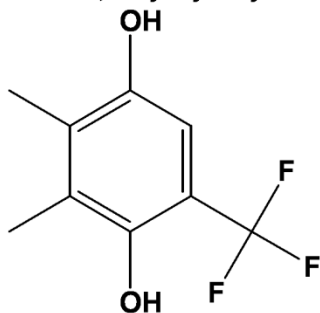
KEGG maps showing a significant difference in completeness



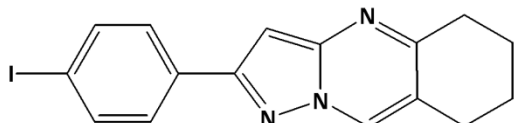
A: Atlantis II
D: Discovery



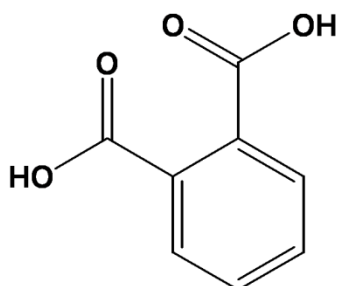
Benzoic acid, ethoxy ethyl ester



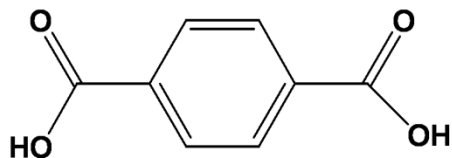
Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-



2-(4-Iodo-phenyl)-5,6,7,8-tetrahydro-pyrazolo[5,1-b]quinazolin-9-ol

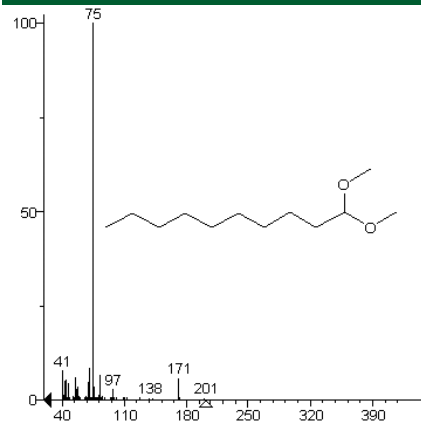


1,2-Benzenedicarboxylic acid

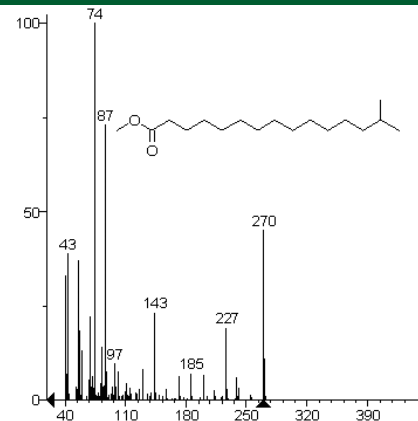


1,4-Benzenedicarboxylic acid

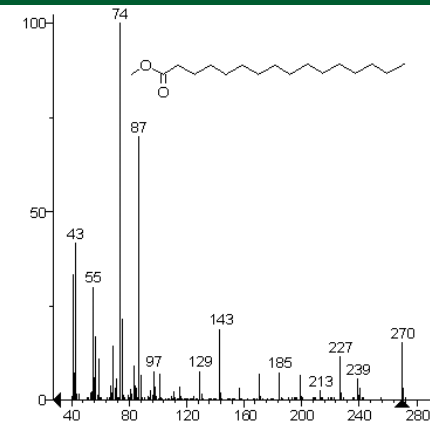
Aromatic compounds identified in the ABP and other compounds identified from the Atlantis II



(replib) Decanal dimethyl acetal



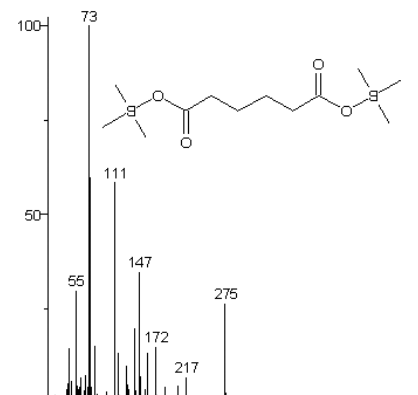
(mainlib) Pentadecanoic acid, 14-methyl-, methyl ester



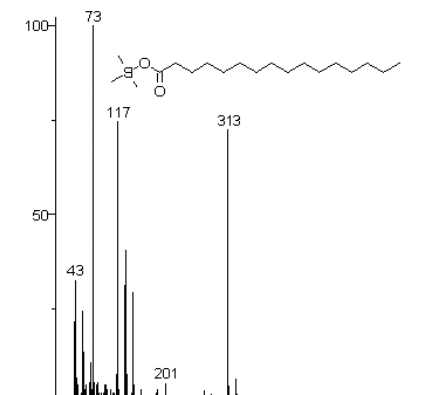
(mainlib) Hexadecanoic acid, methyl ester



(mainlib) Butanedioic acid, bis(trimethylsilyl) ester



(replib) Hexanedioic acid, bis(trimethylsilyl) ester

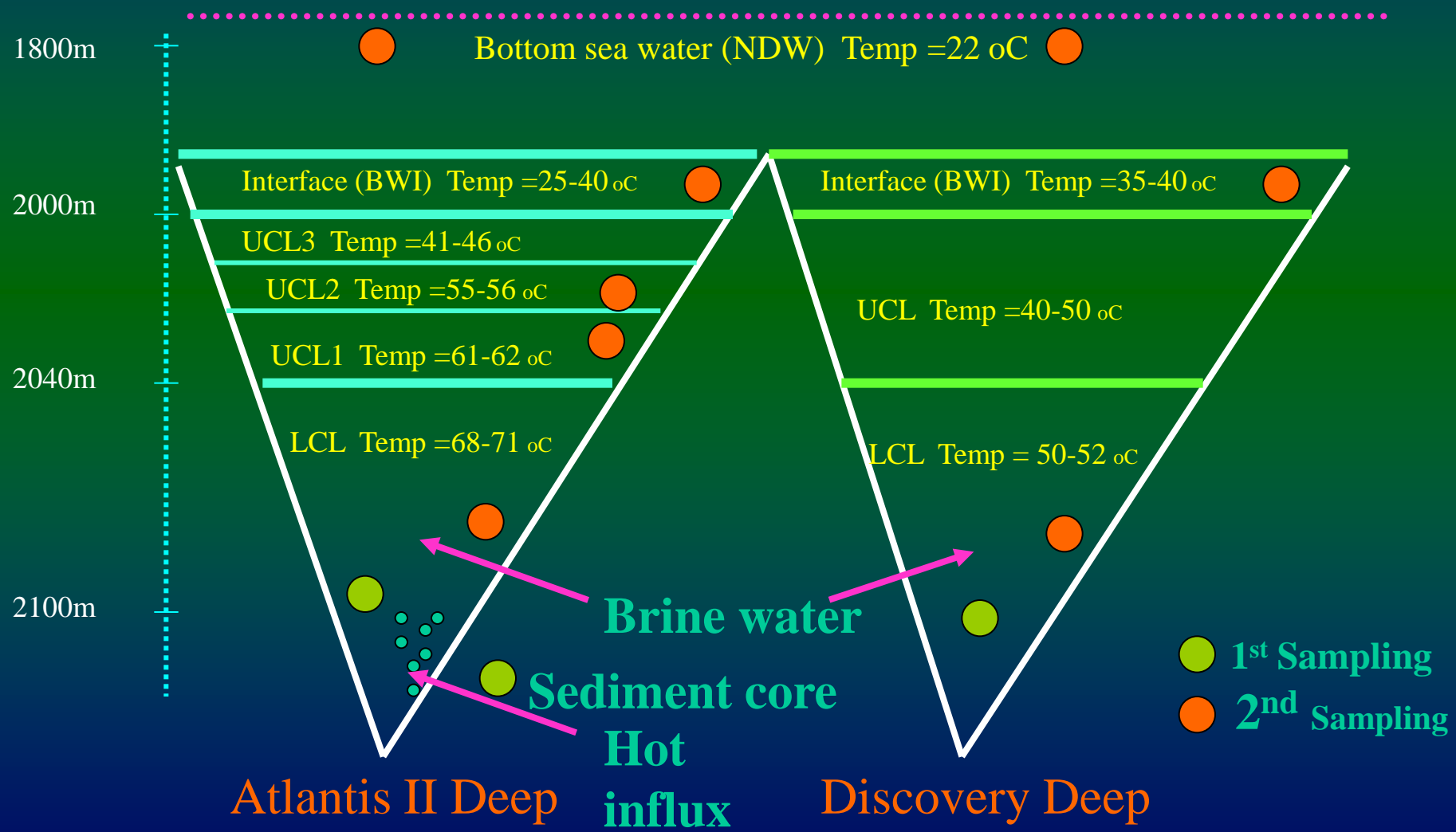


(mainlib) Hexadecanoic acid, trimethylsilyl ester

Key findings

- **Atlantis II and Discovery Deeps displayed unique ecological functions, which were also drastically different from other habitats;**
- **Microbes in Atlantis II brine pool actively involve in consumption of aromatic compounds;**
- **Microbes in the Atlantis II brine pool own more genes responsible for coping with the high metal concentrations;**
- **Better understanding of the ecosystem dynamics, microbial function and evolution required further cruises.**

Second cruise focusing on Atlantis II and Discovery Deeps



Environmental parameters

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Archaeal orders in the Deeps

RDP classification of 16S amplicons:

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Bacterial orders in the Deeps

RDP classification of 16S amplicons:

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Species Diversity

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UniFrac PCA plots of bacterial and archaeal communities

Bacteria

Archaea

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High diversity at the bottom layers of the two brine pools

Carbon and Nitrogen concentrations
in brine water of Atlantics II

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Nitrogen content in Discovery and Atlantis II Deep

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**amoA gene is a popular functional marker for
nitrification**

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Archaea
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Bacteria

amoA gene
phylogeny
tree

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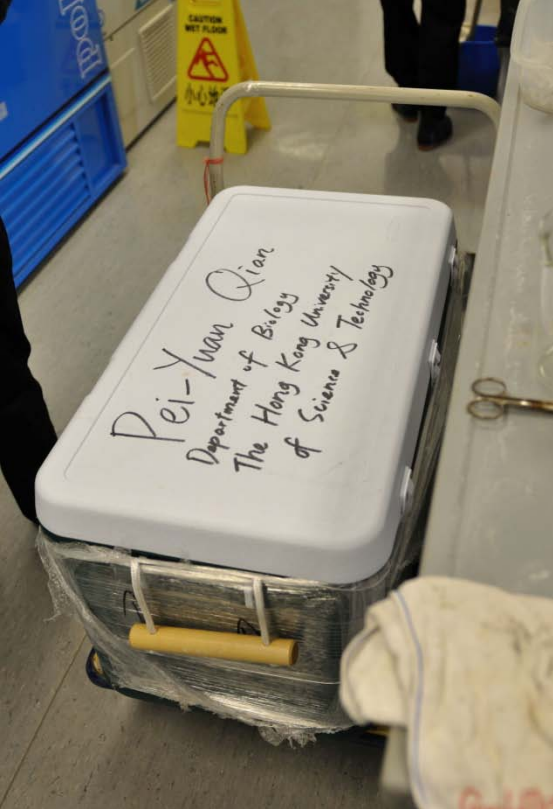
Key findings

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Metagenomic analysis of microbial communities in sediment

- A 2.25m long sediment core was obtained from the Atlantis II Deep
- DNAs from five selected layers (12cm, 63cm, 105cm, 183cm and 222cm) were extracted, amplified with WGA and sequenced on a 454 platform





Metal concentration of sediment from ABP

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Chemical analysis

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Pyrosequencing reads

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Stratified microbial communities and metabolism activities

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Large number of genes with unknown functions in sediments

--A long way to go when it comes to understand this special
ecosystem

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Carbon and Nitrogen concentrations

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Main players of nitrogen cycle

➤ Nitrogen-fixing bacteria.

- Nitrogen gas (N_2) to ammonia (NH_4) (Functional gene: *nifH*)
- *nifH* gene in *Bradyrhizobium* was found in the sediment layers

➤ Nitrifying bacteria

- Ammonium (NH_4) to nitrites (NO_2^-) ; nitrites (NO_2^-) to nitrates (NO_3^-)
- Functional genes: *amoA*; *hao* were not found in the sediments
- Ammonia oxidization mechanism is unknown, possibly involved in metal oxides

➤ Denitrifying bacteria

- Nitrates (NO_3^-) to nitrites (NO_2^-) and then to nitrogen gas (N_2)
- Functional gene: *nirS* was not found; *nirK* gene (Cu-dependent) was identified in the sediments

Key findings

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Conclusions

- **Two brine pools in the Red Sea have drastic differences in environmental setting;**
- **Microbial community (bacteria & archaea) in two brine pools are substantial different from each other, and distinct from overlying water column – strong biological evidence of separation of two brine pools;**
- **Functional groups of microbes are substantially different and appears to reflect adaptive shift to cope with environmental changes.**
- **A thorough understanding of these ecosystems requires substantial future effort.**

Acknowledgment

- **Funding support: KAUST Global Collaborative Research Program**
- **Cruise support: WHOI and HCMR of Greece;**
- **Bench work and data analysis: Drs. OO Lee, Y Wang, JK Yang, F Lafi, GS Zhang, T Wong, G Chung...**

Organization of this conference for invitation

An aerial photograph of a university campus built on a lush green hillside. The campus features numerous modern, multi-story buildings with white and grey facades. In the foreground, a large, circular sports field with a green field and a reddish-brown track is visible. A pink arrow points from the text 'Marine Laboratory' to a small, white building situated on a peninsula that juts into the turquoise water. The background shows rolling green hills and a city skyline under a clear blue sky.

Thank You!

Marine Laboratory