



The Microbiology of the Sea Surface Microlayer: “the Bacterioneuston”

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surface ocean **solas** lower atmosphere study
20192

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(Previously : Mark Franklin & Tom Frost)

Aims & Objectives

The role of the sea surface microlayer (bacterioneuston) in marine trace gas biogeochemistry

Molecular Ecology

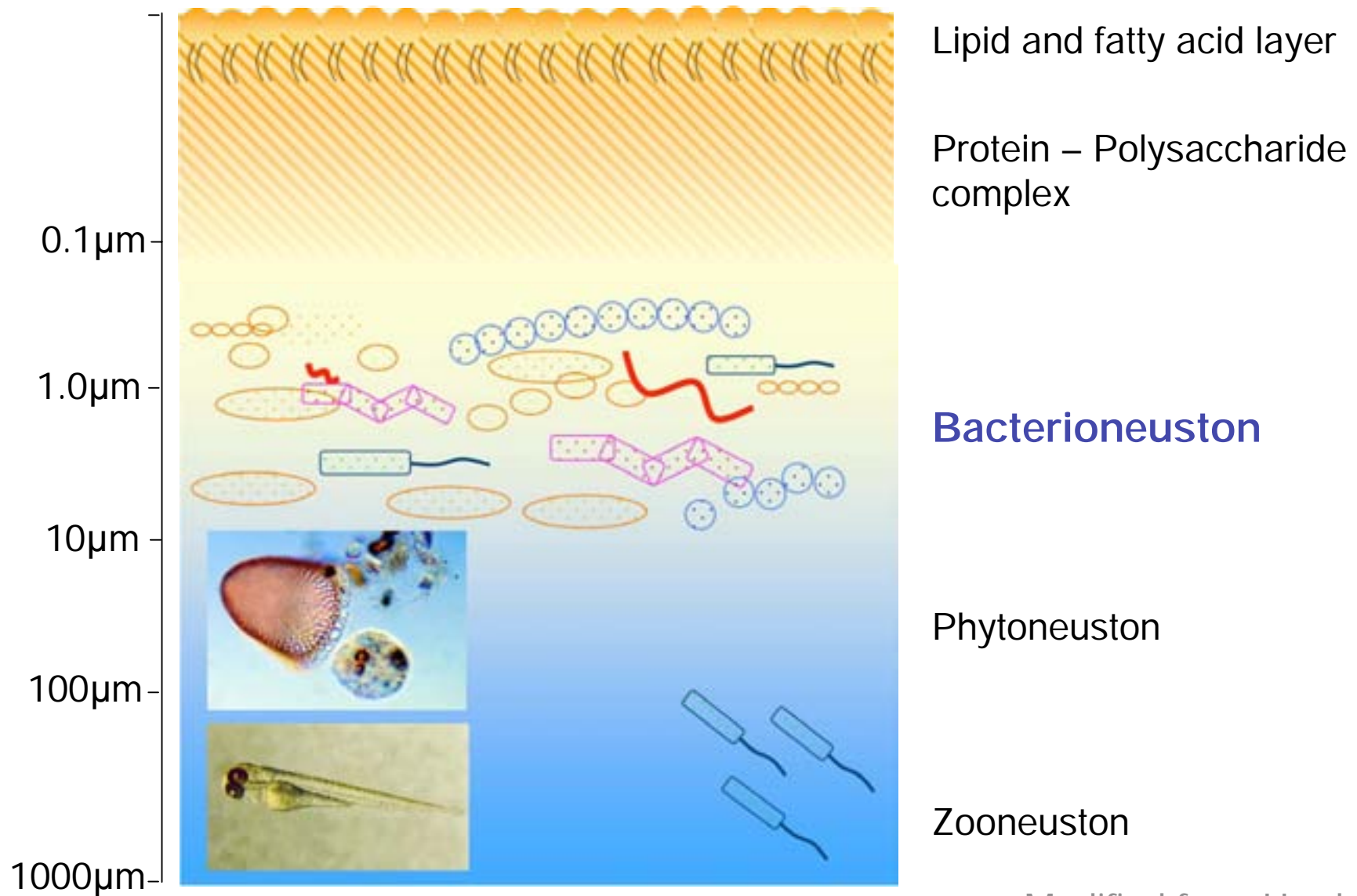
Biogeochemistry

- Assess the community structure of the sea-surface microlayer in comparison to sub-surface waters.
- Assess the **functional** (trace gas utilizing) community structure of the sea-surface microlayer in comparison to sub-surface waters.
- Assess the effect of the sea-surface microlayer on trace gas transfer rates between the ocean and lower atmosphere.

- **The sea surface microlayer- bacterioneuston**

- A source and a sink for materials in the water column and the atmosphere
- Covers 70% of the planet: **poorly understood**
- Enrichment of microorganisms in an organic biofilm of lipid, fatty acids and polysaccharide
- Must be different to subsurface.
- Enrichment of dissolved organics including anthropogenic sources: oils, PAHs, Chlorinated hydrocarbons, metals
- An extreme environment?
- Solar radiation, temperature, toxic compounds, organics, metals (higher nutrient concs)
- THICKNESS: Defined by sampling regimen
- **TRACE GASES GO THROUGH THIS MICROLAYER!**

Conceptual model of the *Sea surface microlayer*



Modified from Hardy (1982)

Sea surface microlayer – sampling defines “thickness”

Rotating Drum

(drum diameter 10cm-1m)



Glass Plate

(plate length 10-15cm)

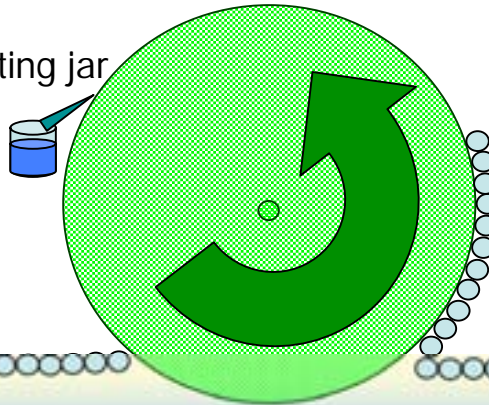


Membrane Filter

(membrane diameter 47mm)

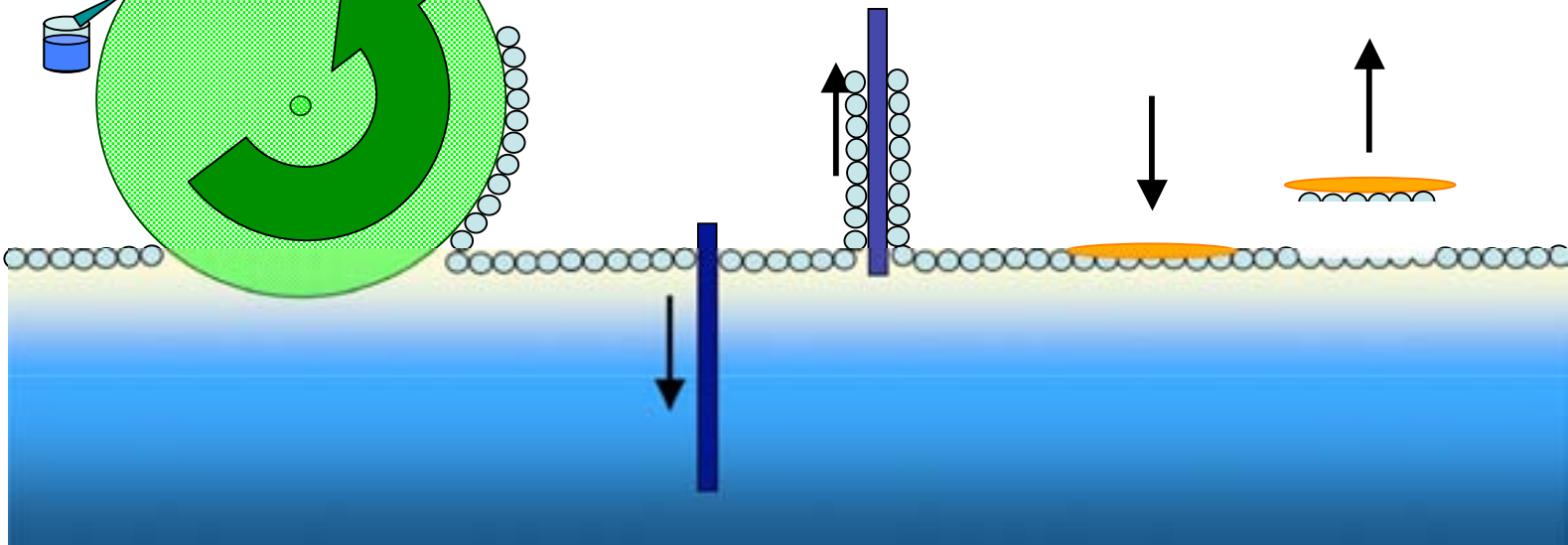


wiper & collecting jar



Surface microlayer

Sub-surface



Sampling the bacterioneuston

Surface microlayer sampling methods:

Sampler	Sample thickness (μm)	Sample collected	References
Freezing probe	1000	Seawater and particles	Hamilton and Clifton (1979)
Mesh screen (Garrett screen)	150–400	Microbiology, lipids and fatty acids	Sieburth (1965); Garrett (1967)
Rotating drum	60–100	Microbiology and organics	Harvey (1966)
Glass plate	20–100	Chemical and microbiological	Harvey and Burzell (1972)
Hydrophilic Nucleopore membrane	4–40	Microbiology and organics	Crow <i>et al.</i> (1975)
Hydrophobic Nucleopore membrane	20–50	Microbiology and organics	Kjelleberg <i>et al.</i> (1979)
Bubble microtome	1	Fractionated chemical and microbiological aerosol over sea surface	MacIntyre (1968)

Tested three membranes;

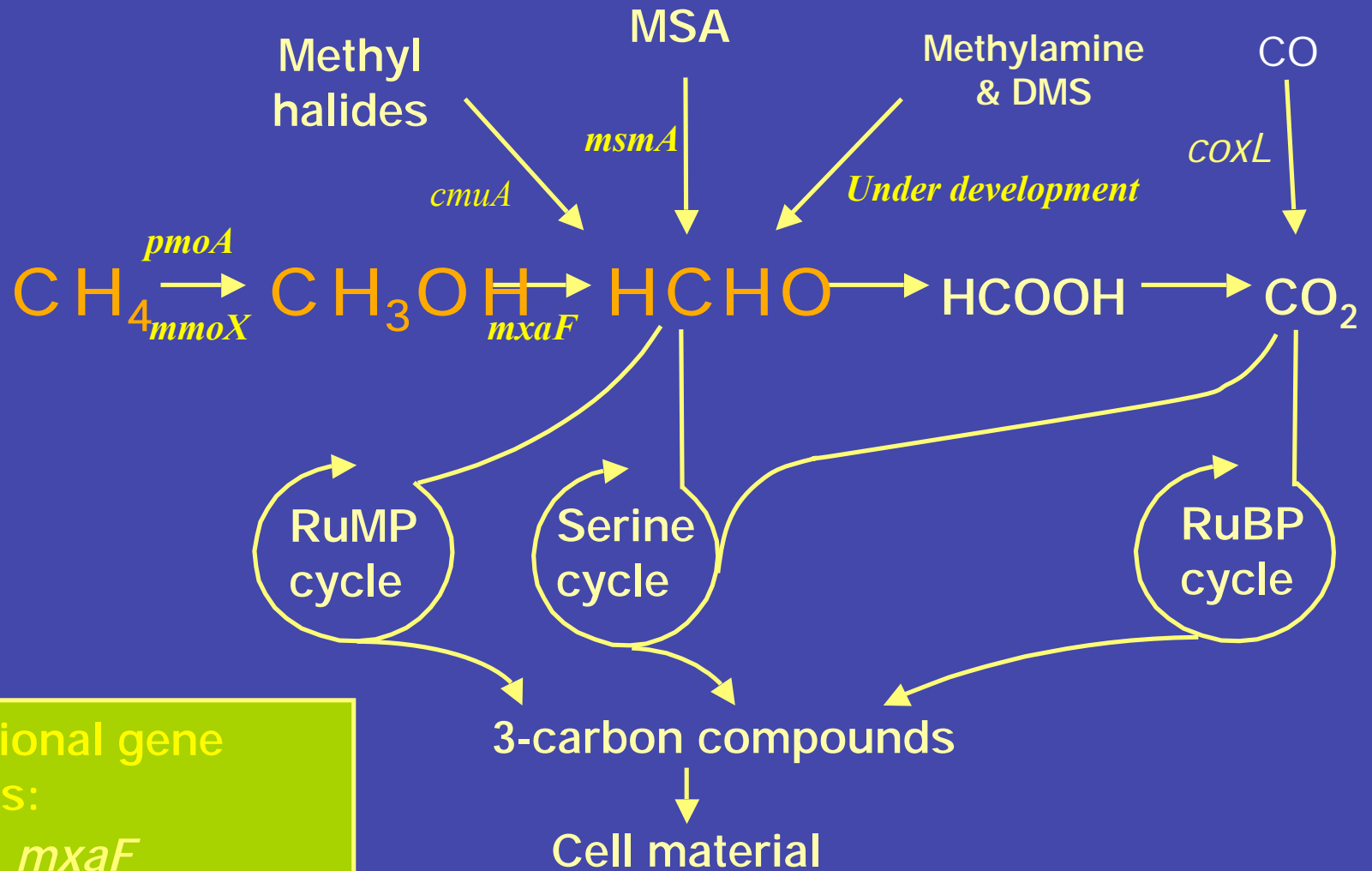
A - PTFE (Teflon)
hydrophobic

B - ISOPORE (polycarbonate) hydrophilic

C - Poretix (polycarbonate) hydrophilic

All three membrane types consistently remove the top ~30-50 μm of the Bacterioneuston. Avoids contamination!

Pathways of Aerobic Methylotrophy (*Proteobacteria*)



Functional gene probes:

MDH: *mxoF*

MMO: *pmoA*, *mmoX*
cmuA, *msmA*, *coxL*

- Trace gas microbiology
- Many important marine biogeochemical cycles are driven by bacteria
- How to study these bacteria?
- **An example: CH₃Br metabolism**

Hendrik Schäfer & Colin Murrell
Phil Nightingale (PML)
(Rich Boden & Josh Neufeld)

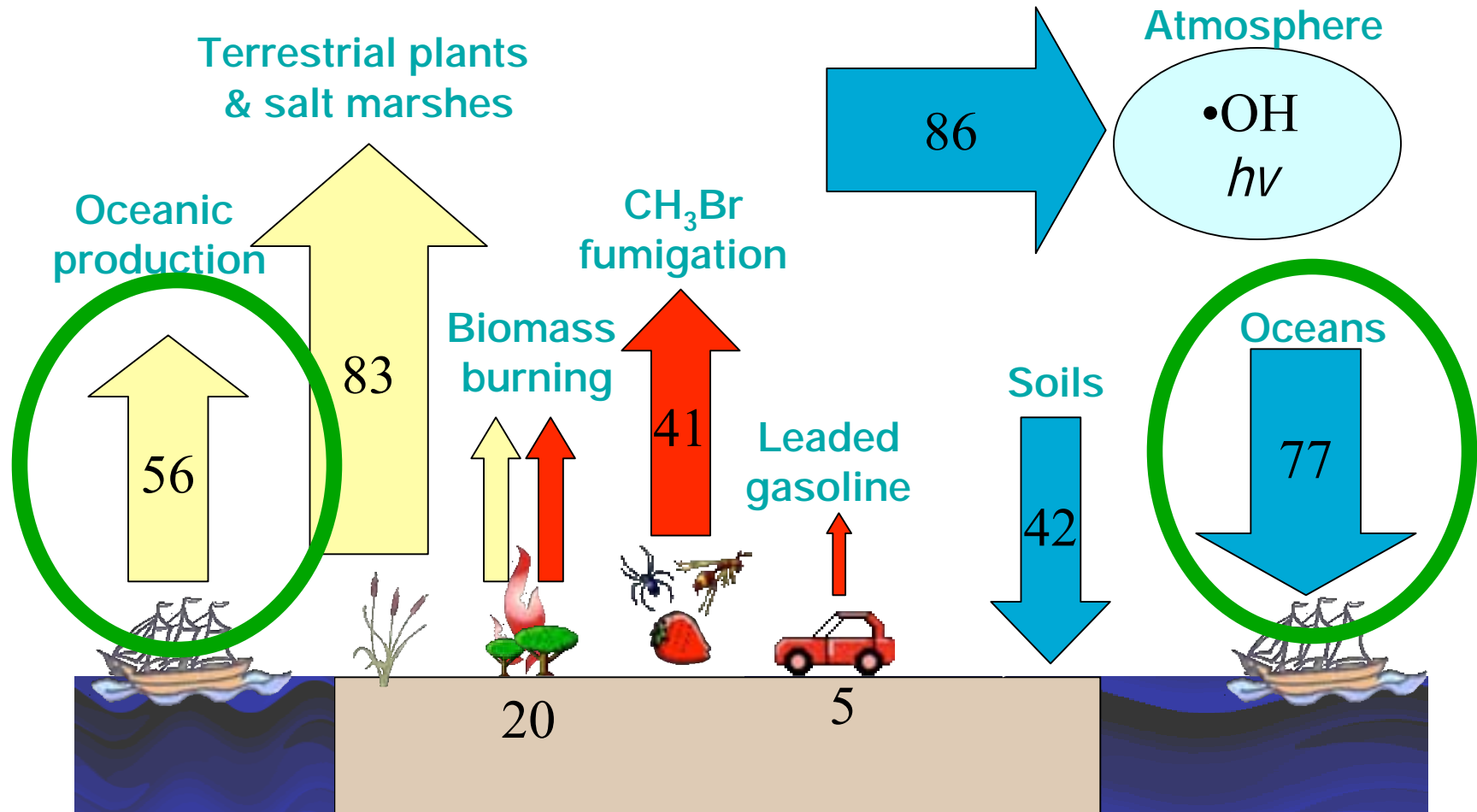
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Marine methylotrophs

- Marine bacteria that use C1 compounds as carbon source
- C1 compounds in the marine environment include
 - Methane
 - Methanol
 - Methylated amines methyl halides
 - Methylated sulfur compounds (dimethyl sulfide, MSA)
- Many of these compounds have implications for climate regulation and/or atmospheric chemistry
- Methylotrophic bacteria are potential sinks for these compounds

Methyl Bromide Budget – Fluxes in Gg yr^{-1}



Thanks to Rob Rhew for this slide

(1 $\text{Gg} = 10^9 \text{ g yr}^{-1}$)

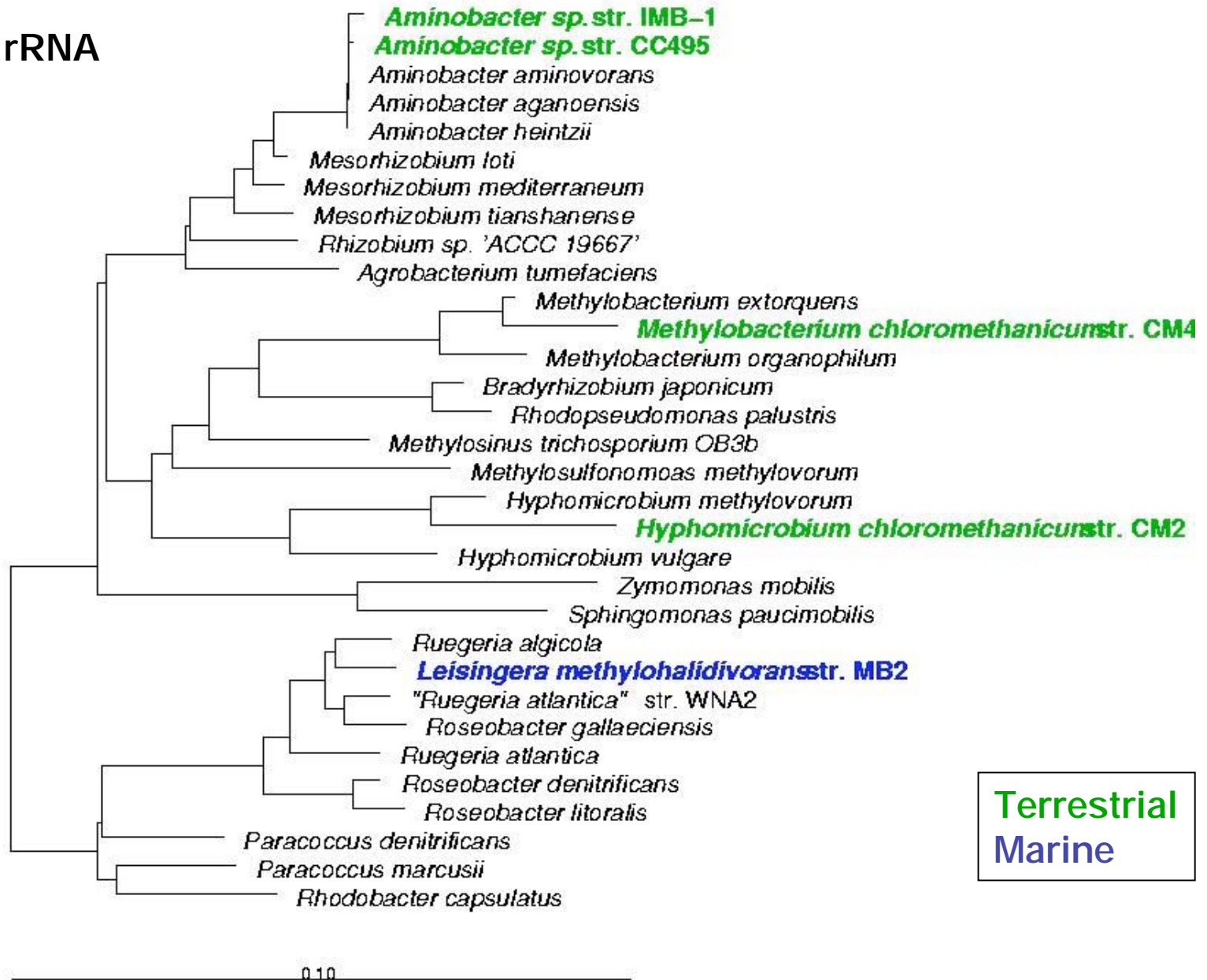
Which bacterial populations and metabolic pathways are responsible for biological methyl halide degradation in the marine environment?

May not be able to cultivate them all in the lab

Need to develop cultivation-independent methods to look at population dynamics, activities and relate to gas flux measurements

Need to develop phylogenetic (16 rRNA) and functional gene markers targeting key metabolic processes in the marine environment

16S rRNA



MeCl metabolism in *Methylobacterium*

Methyltransferase/corrinoid protein **CmuA**

Methyltransferase

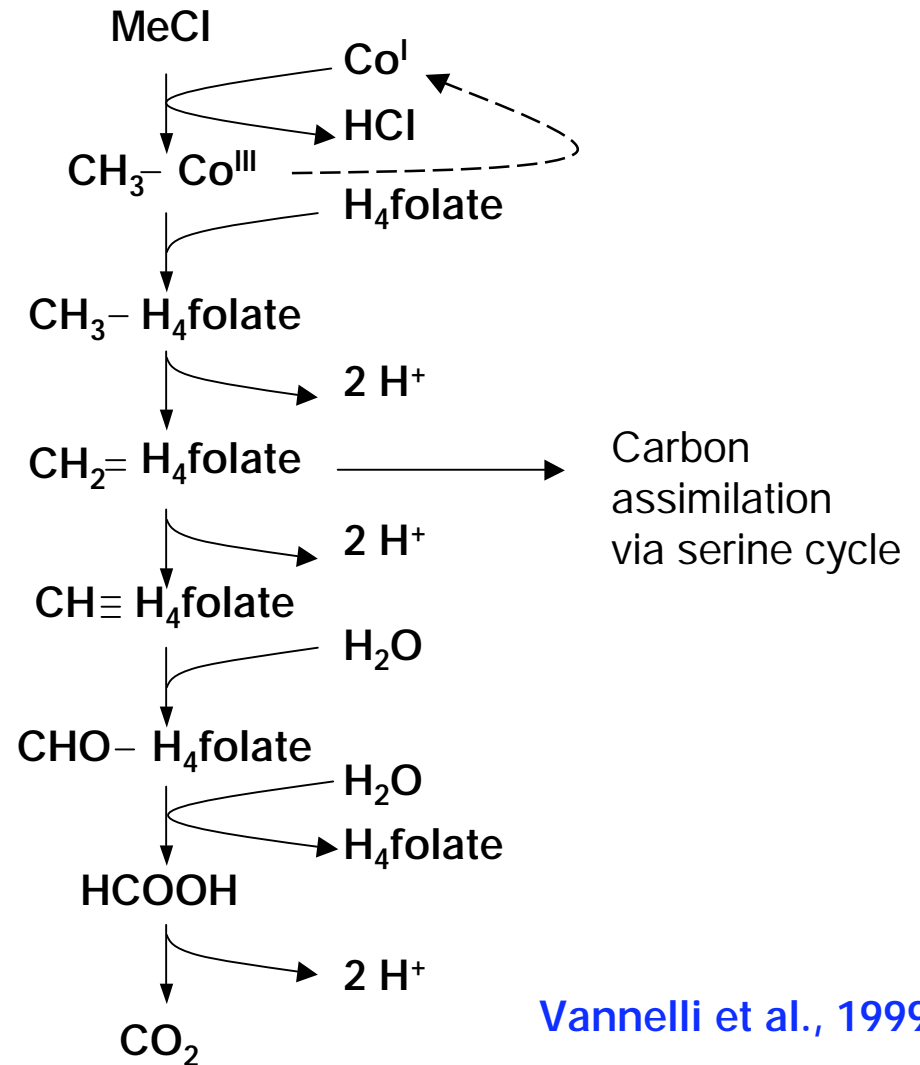
5,10-methylene- H_4 folate reductase

5,10-methylene- H_4 folate dehydrogenase

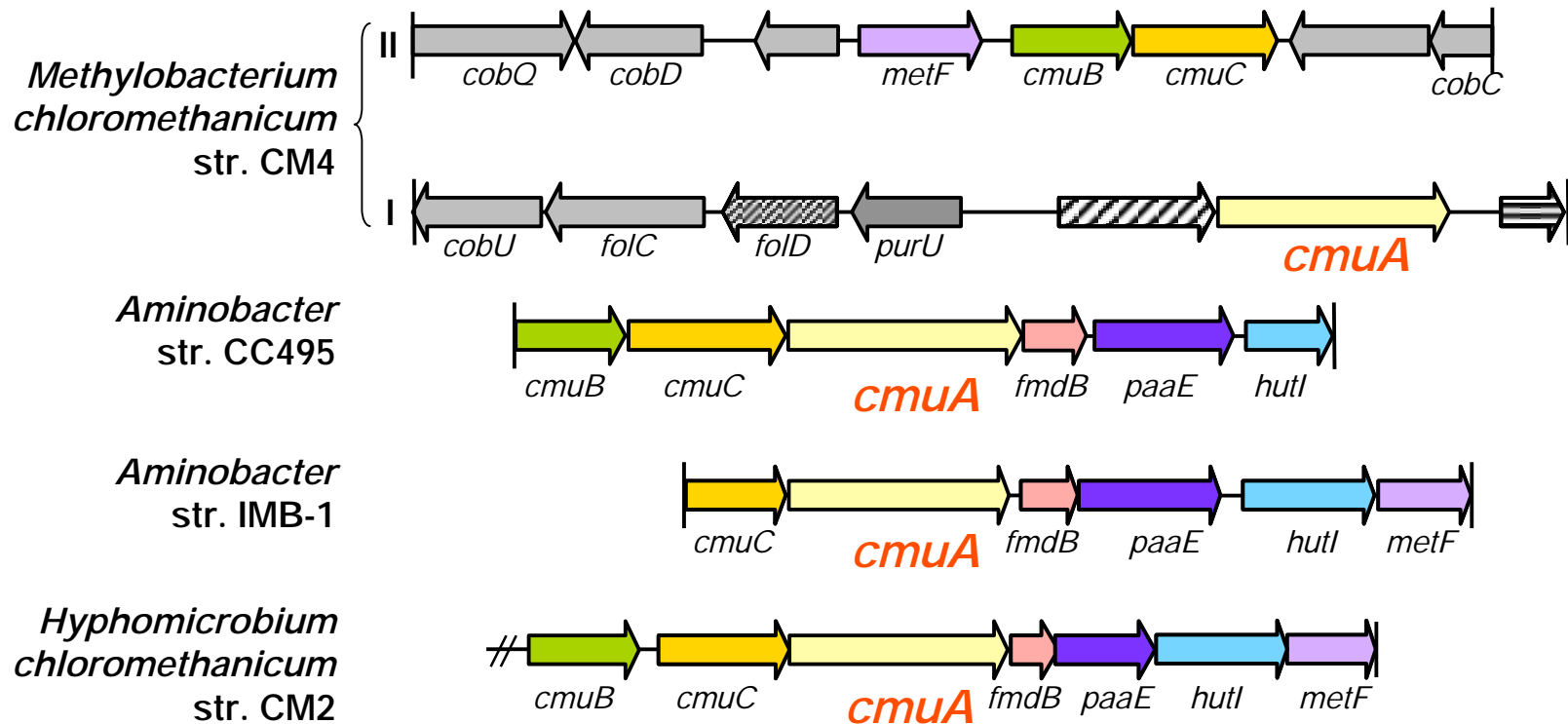
5,10-methylene- H_4 folate cyclohydrolase

10-formyl- H_4 folate hydrolase **PurU**

Formate dehydrogenase **FDH**



Functional genes of methyl halide oxidation



2 kb

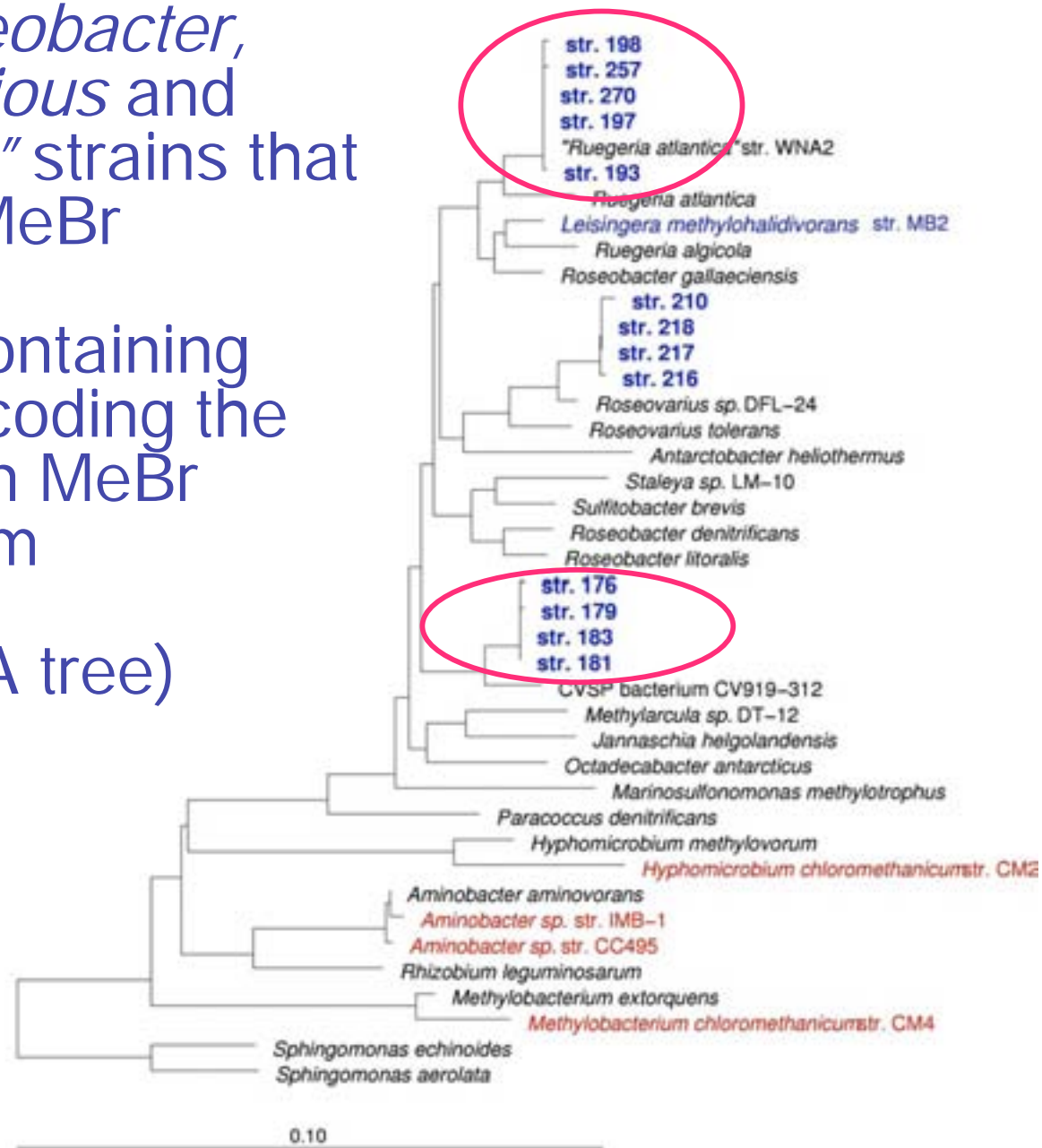
cmuA becomes a functional gene marker for methyl halide degraders

Look for this gene in new isolates and directly (via DNA and PCR) from the environment

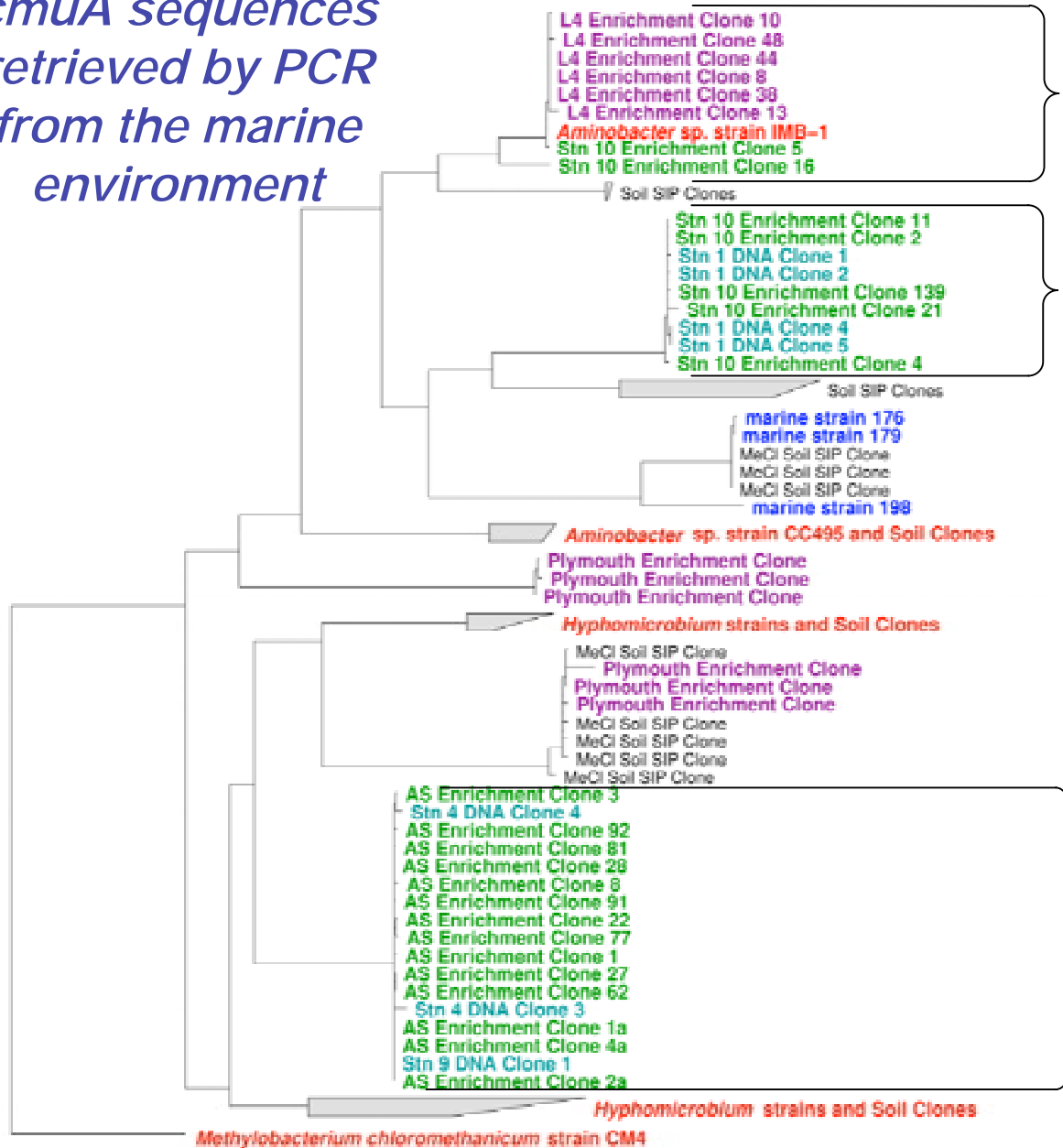
New *Roseobacter*,
Roseovarius and
“*Ruegeria*” strains that
degrade MeBr

Isolates containing
cmuA, encoding the
first step in MeBr
metabolism

(16S rRNA tree)



cmuA sequences
retrieved by PCR
from the marine
environment



Plymouth station L4
enrichments 90 %

Station 1 DNA
clone library 92 %

Station 4 and 9 DNA
clone libraries 90
and 94 %

Conclusion: Marine MeBr degraders exist and *cmuA* is a good marker gene

Sea surface microlayer

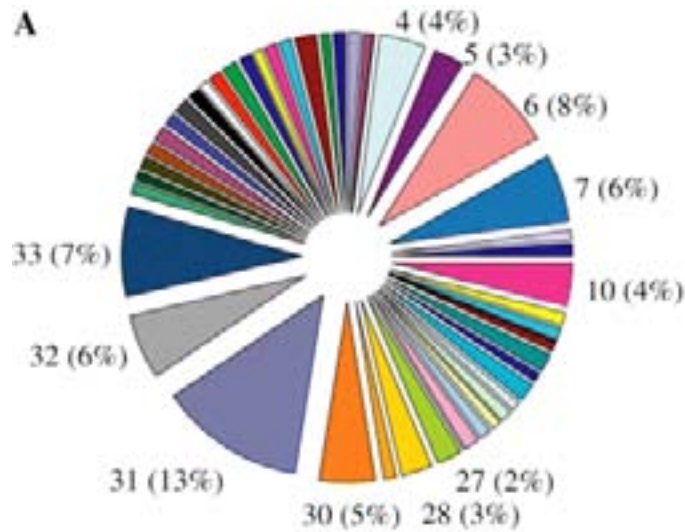
Aims and Objectives

To test the hypothesis that the sea surface microlayer is a distinct ecosystem and plays an important role in influencing the air-sea exchange of atmospheric trace gases



1. Determine microbial community structure of the sea surface microlayer and make comparisons with sub-surface communities
2. Specifically, determine the metabolic potential of bacterial groups in the sea surface microlayer that may influence the air-sea exchange of atmospheric trace gases

Molecular analysis of the bacterioneuston by sampling bacterioneuston, isolating DNA and using PCR to survey the bacterial populations present in the North Sea off the coast of Newcastle

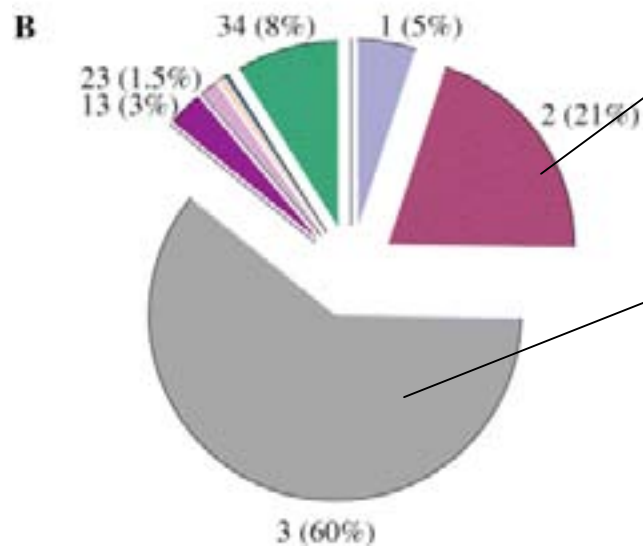


Grouping from 16S rRNA clones

A. Sub-surface sample (1m)

B. Bacterioneuston

Reduced diversity in the bacterioneuston



Vibrio

Pseudoalteromonas

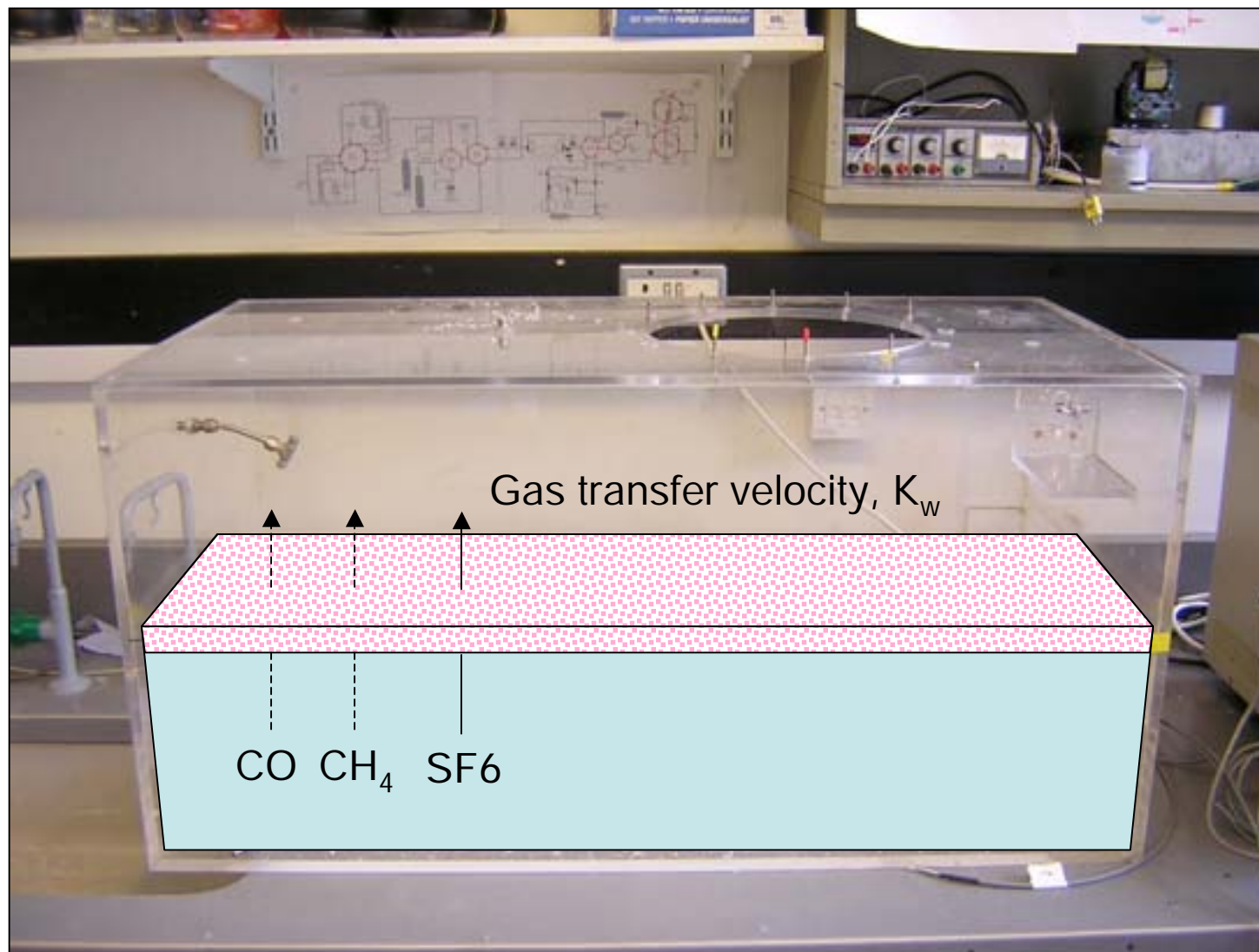
Sampling of top 30-50 microns of bacterioneuston using polycarbonate filters

Franklin *et al.* Environmental Microbiology, 2005

Conclusions

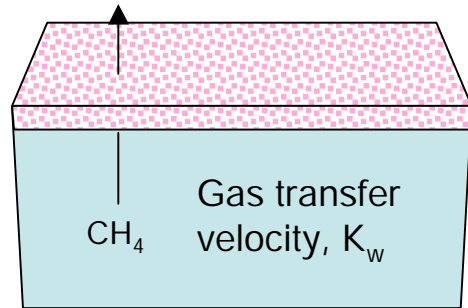
- Sampling the bacterioneuston using polycarbonate membranes is efficient
- DNA analysis shows that there is a distinct bacterioneuston community present
- *Recovery of pmoA* genes by PCR shows that distinct groups of methanotrophs are present in the bacterioneuston and in subsurface waters

The effect of microbial populations on invasive and evasive air-sea gas exchange rates in controlled laboratory tank experiments



The effect of microbial populations on invasive and evasive air-sea gas exchange rates in controlled laboratory tank experiments

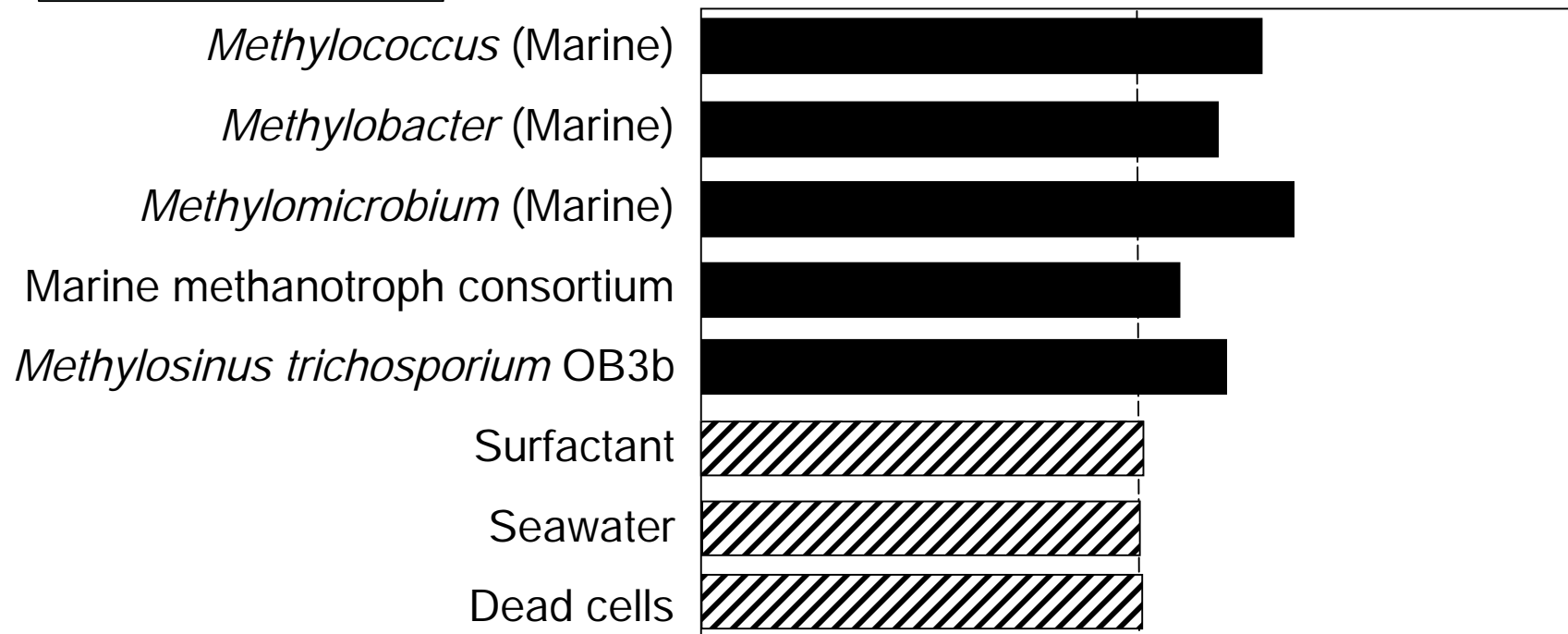
Conclusions: methanotrophs decrease CH_4 exchange



Ratio of $\text{CH}_4 / \text{SF}_6$ for gas exchange

K_w

1.0



Microbial diversity and function in the bacterioneuston

- Two examples
- A coastal estuary Blyth (Nr Newcastle)
- Offshore waters, near Hawaii

- Bacterioneuston sampling plus corresponding sub-surface sample
- Retrieval of DNA
- PCR amplification of gene sequences
- Characterization of gene sequences

Estuarine surface microlayer

Blyth River Estuary, Northumbria, UK.

Two sample sites at the marine end of the estuary.



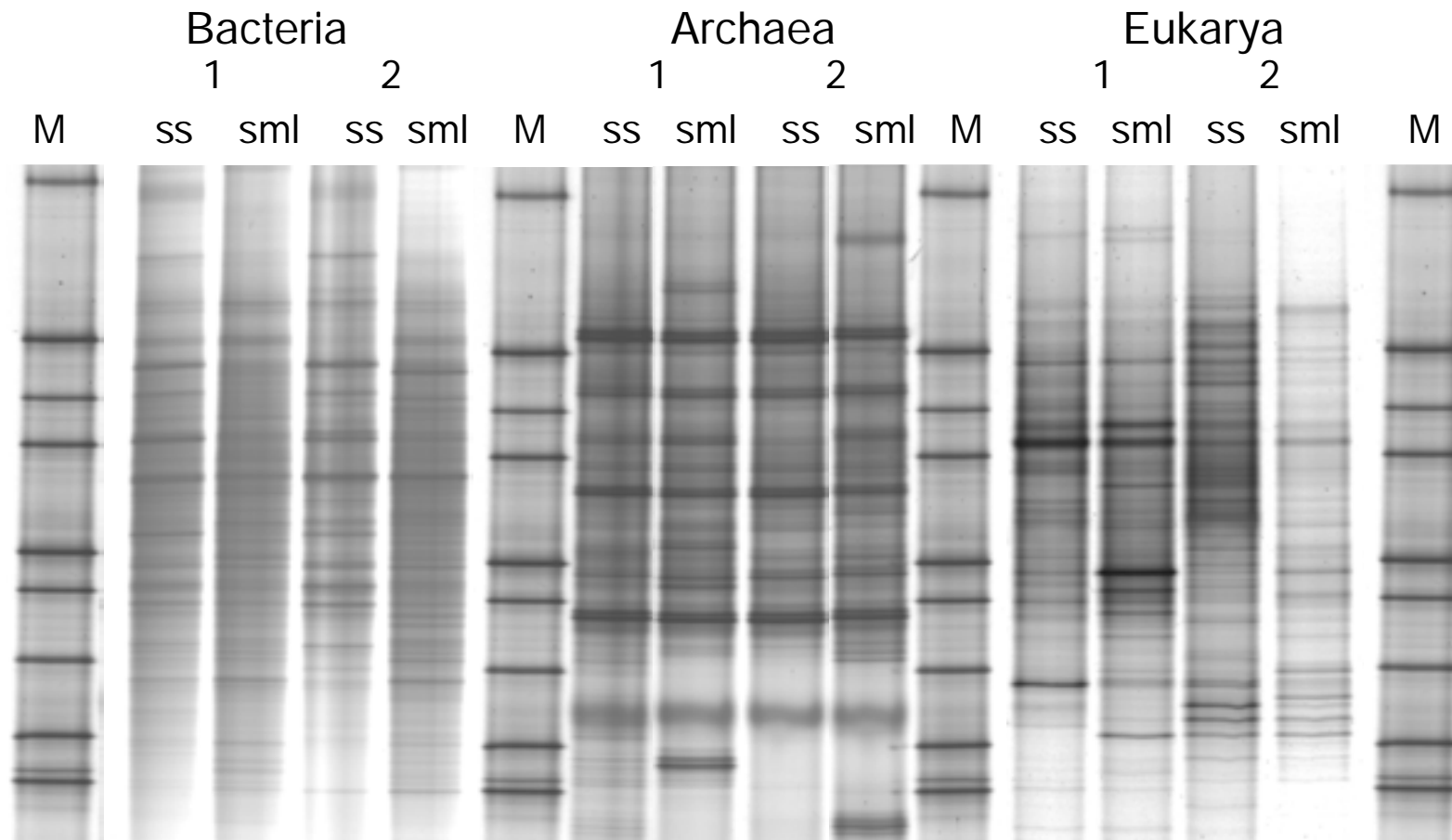
Site 1:
Salinity 21psu

Site 2:
Salinity 31psu

Estuarine surface microlayer

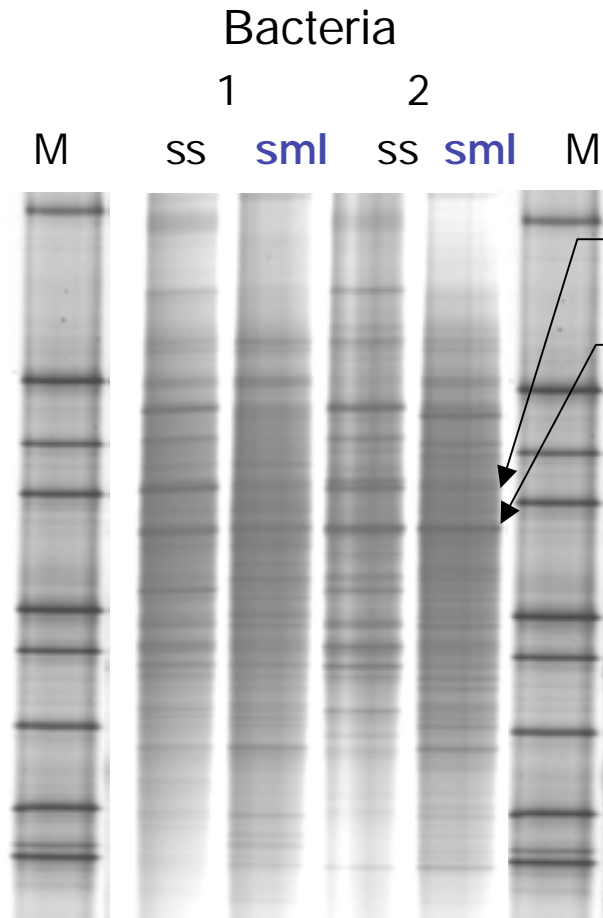
Microbial community structure

- Bacterial and Archaea 16S rRNA gene, Eukarya 18S rRNA
- Denaturing Gradient Gel Electrophoresis (a quick way to profile samples)



Cunliffe., *et al* (2006) In preparation

Estuarine surface microlayer Bacteria community structure



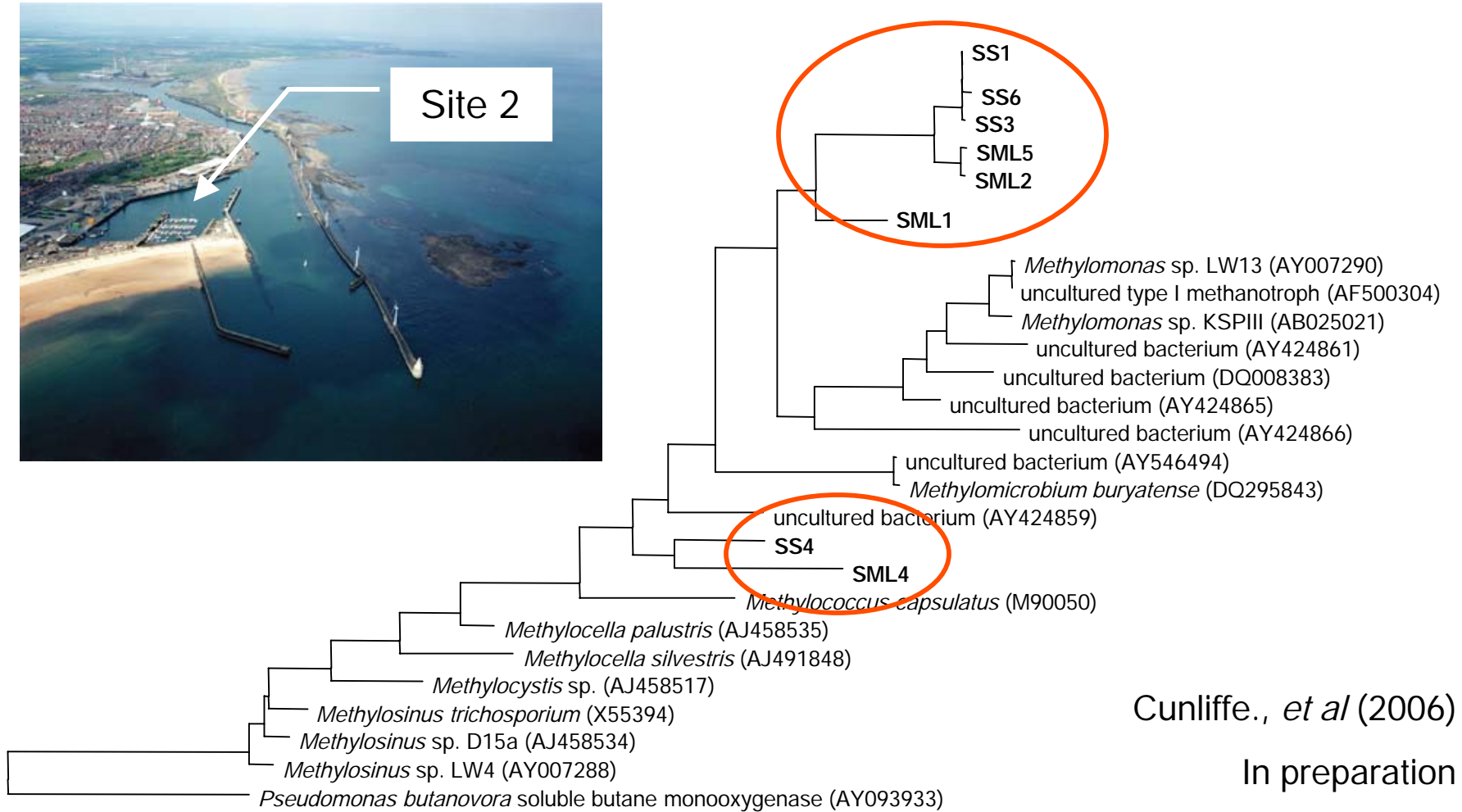
Alteromonas sp. Alteromonadales: Gammaproteobacteria

Glaciecola sp. Alteromonadales: Gammaproteobacteria

- *Alteromonadales* produce and are resistant to inhibitory compounds allowing for antagonistic community interactions
- *Pseudoalteromonas* and *Alteromonas* are resistant to solar radiation. UV impact may be particularly important for microbes inhabiting the SML and surface waters

Estuarine surface microlayer

methane monooxygenase gene diversity (*mmoX*) at site 2



Cunliffe., et al (2006)

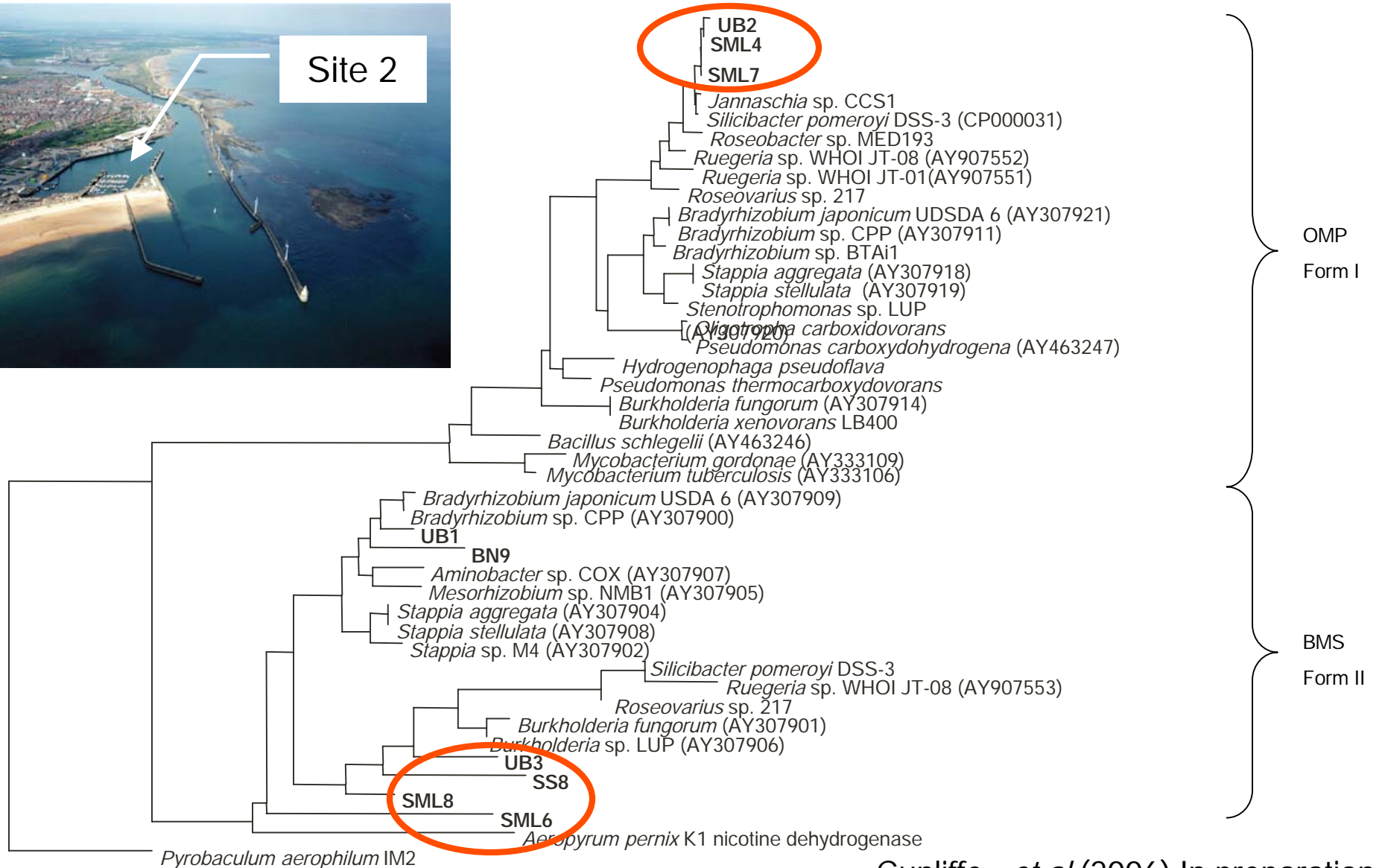
In preparation

0.10

Methanotrophs are present in the bacterioneuston

Estuarine surface microlayer

Carbon monoxide dehydrogenase gene diversity (*coxL*)

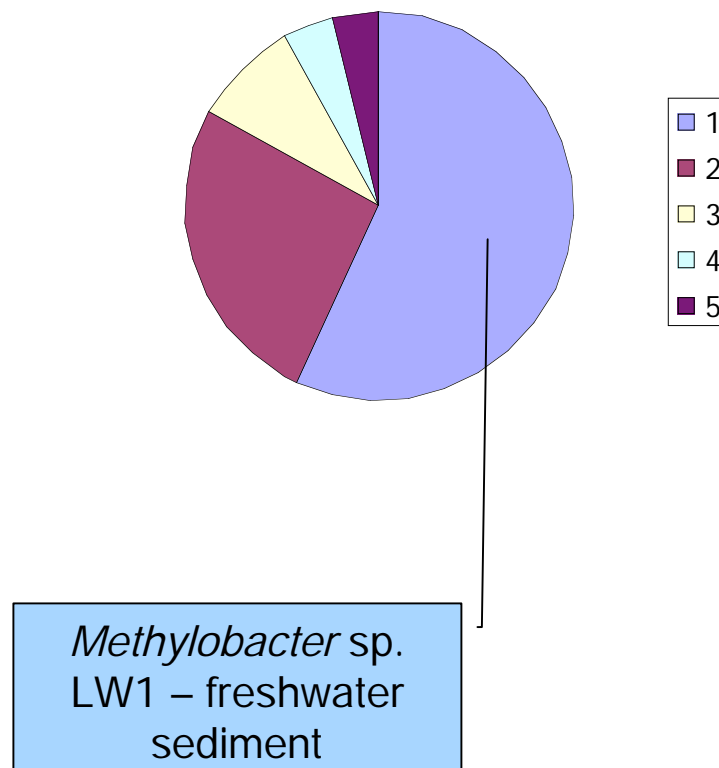


Cunliffe., et al (2006) In preparation

CH₄ oxidiser: Particulate methane monooxygenase *pmoA* clone diversity

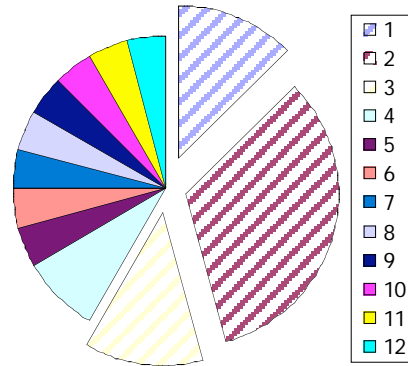
Sub-surface

Low diversity of *pmoA* genes in Blyth estuary at the time of sampling



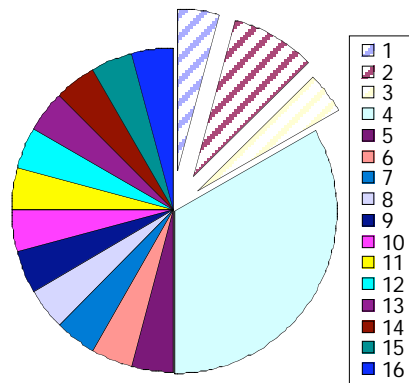
Dominant *pmoA* found had closest match to sequence from a methanotroph isolated from freshwater sediment

CO oxidiser: Carbon monoxide dehydrogenase *coxL* clone diversity



Sub-surface

Three *coxL* sequence types dominant in the SS and present in the SML below.



Surface microlayer

Bacterioneuston contains three *coxL* sequence types present in the sub-surface but most *coxL* sequence types are unique to the SML.

Can conclude that CO degraders are present in the bacterioneuston

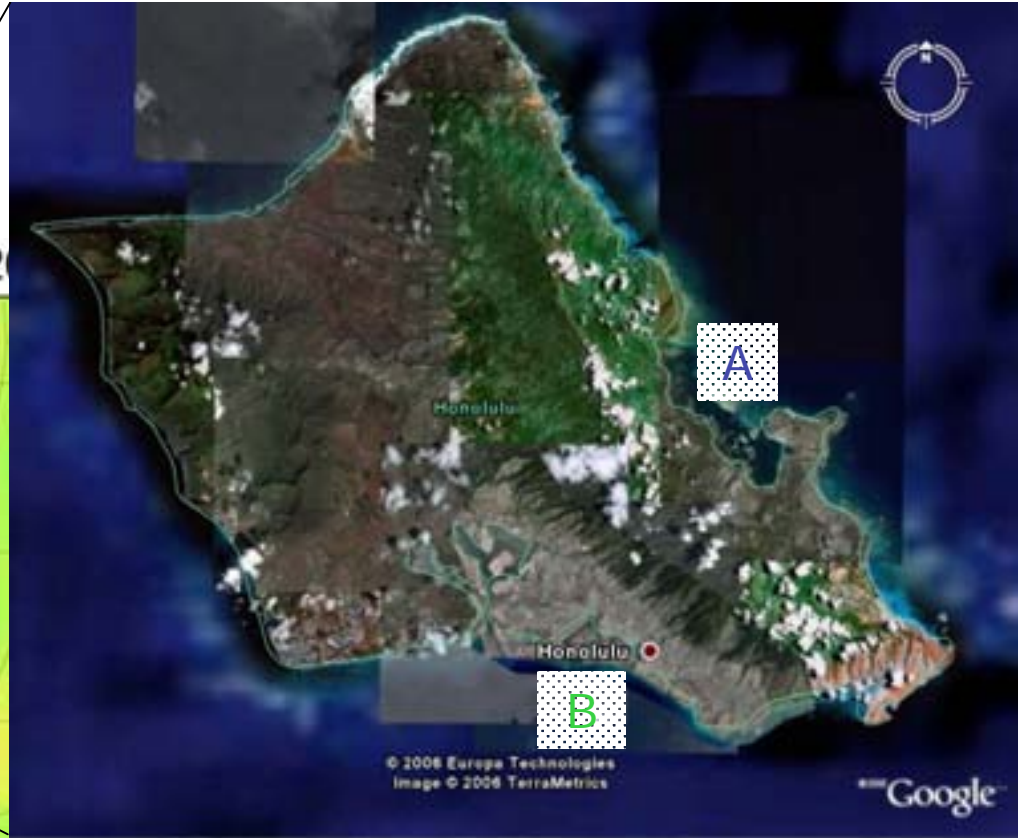
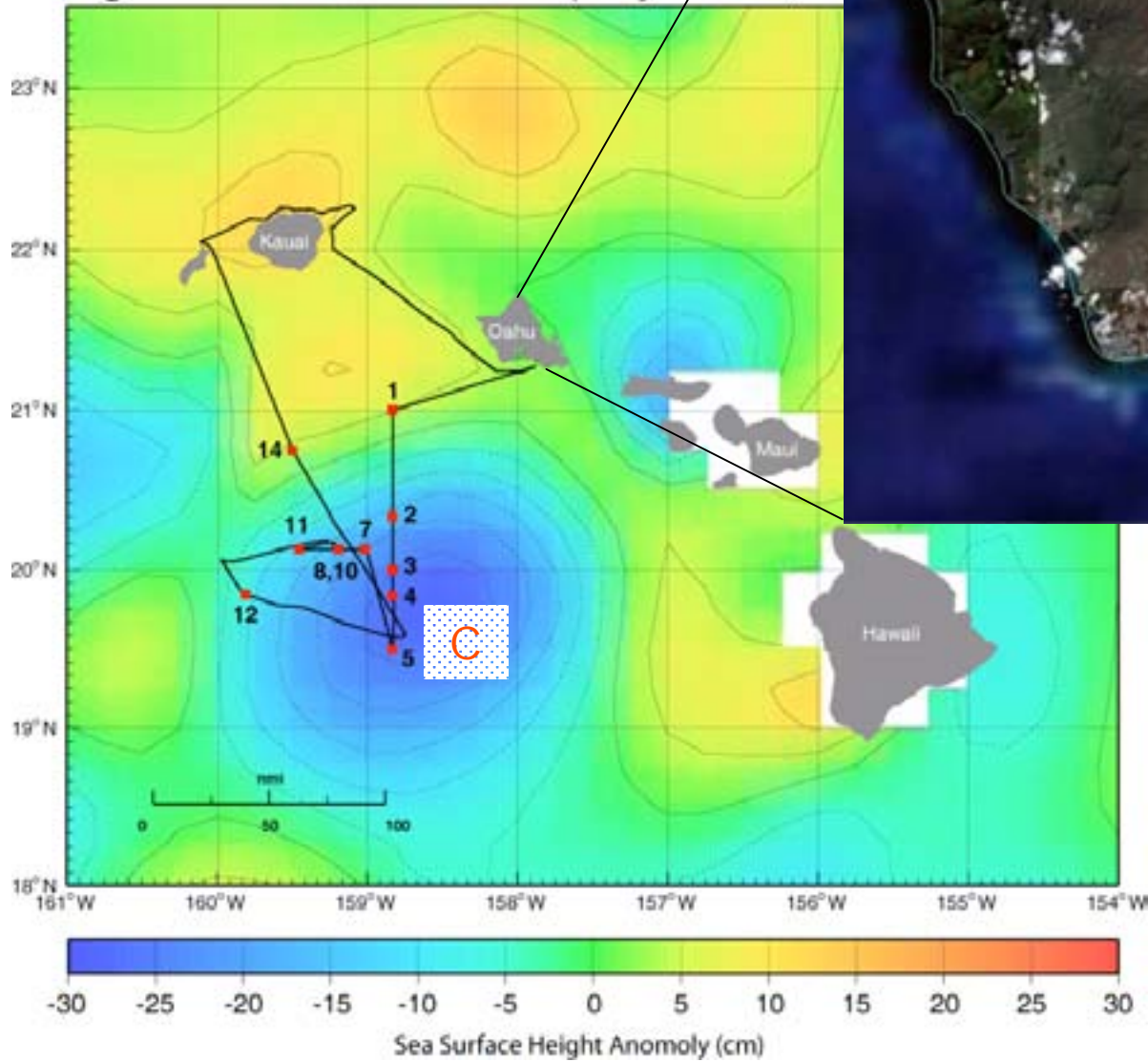
Ongoing work with Blyth samples

- Phylogenetic analysis of 16S rRNA gene sequences
- Analysis of *pmoA* gene libraries
- PCR with other “functional” genes
 - *mxnF* (*methanol utilizers*)
 - *cmuA* (*methyl halide degraders*)

DMS, methylamine utilizers

- Is there a seasonal effect on the bacterioneuston?
 - Sample the sub-surface and surface microlayer at two sites (Newcastle & L4 Plymouth).
 - Over an annual seasonal cycle
 - Examining samples from other SOLAS cruises

Agouron 2006 Cruise 2 (July 16-21, 2006)



A Kaneohe Bay

B Keahi Bay

C Down Welling

“Microbial Oceanography: Genomes to Biomes”

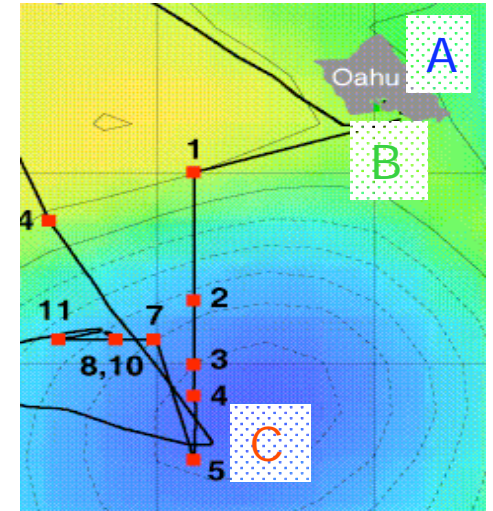
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Sea surface microlayer Bacteria community structure

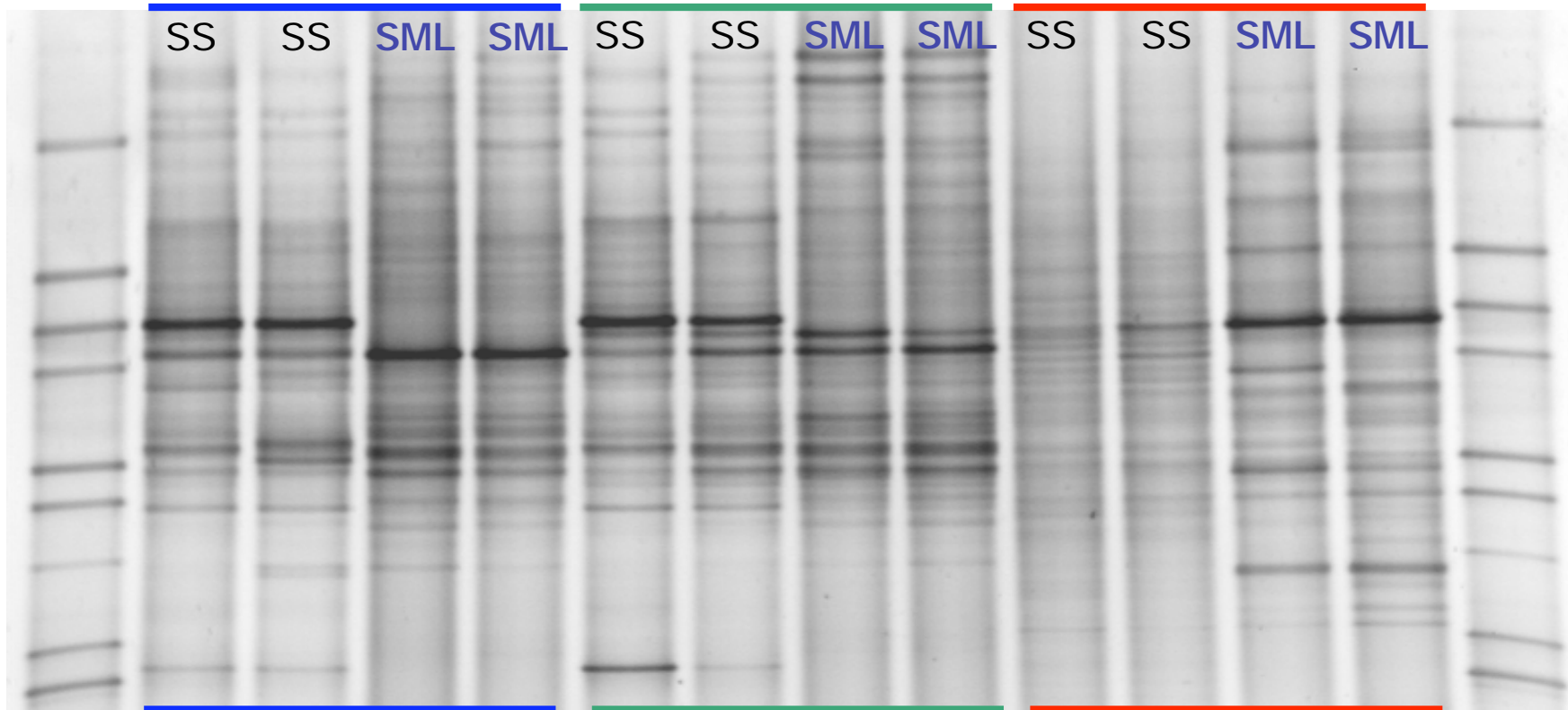
- Bacterial 16S rRNA gene survey
- Denaturing Gradient Gel Electrophoresis analysis



A: Kaneohe Bay

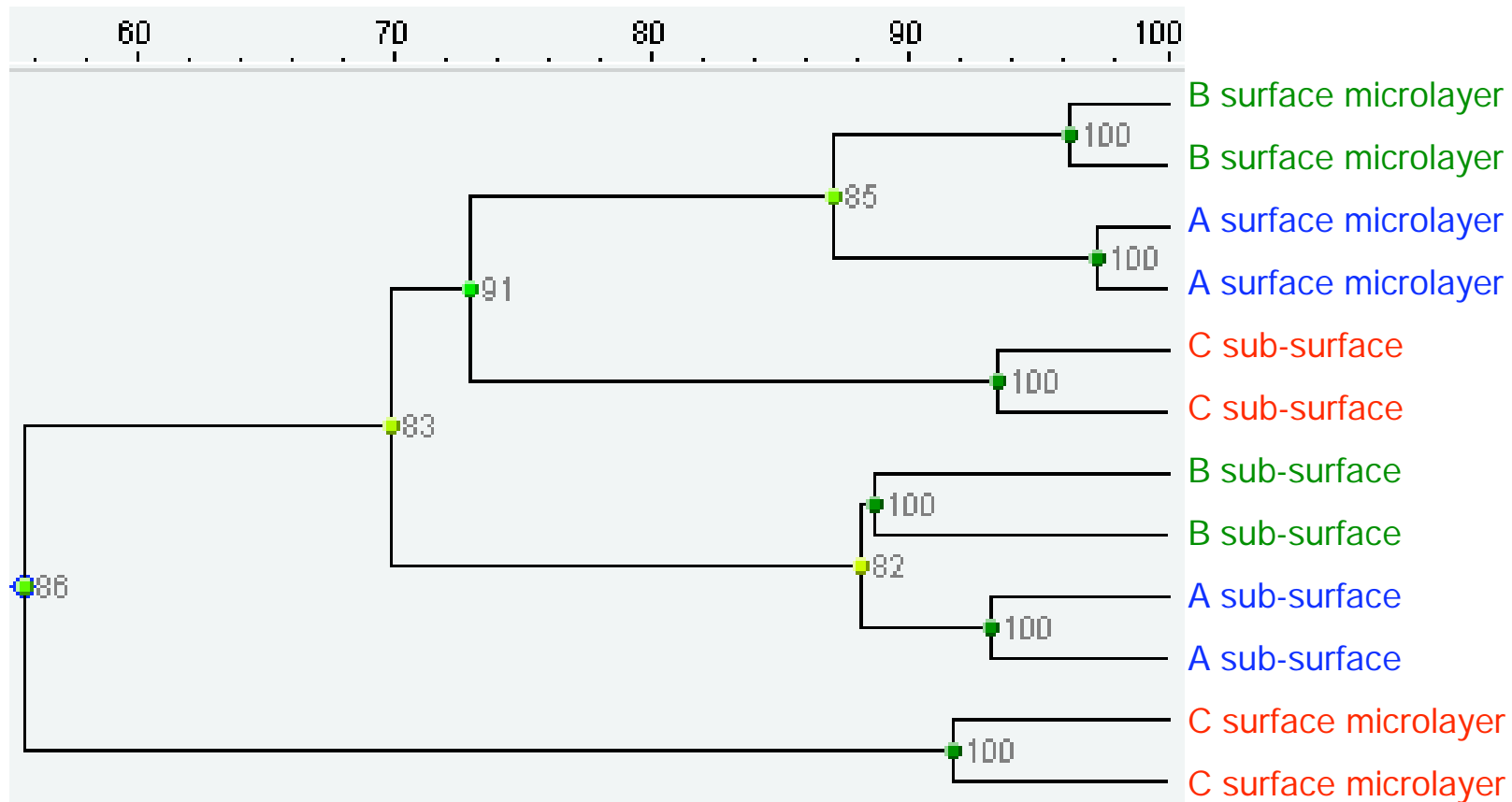
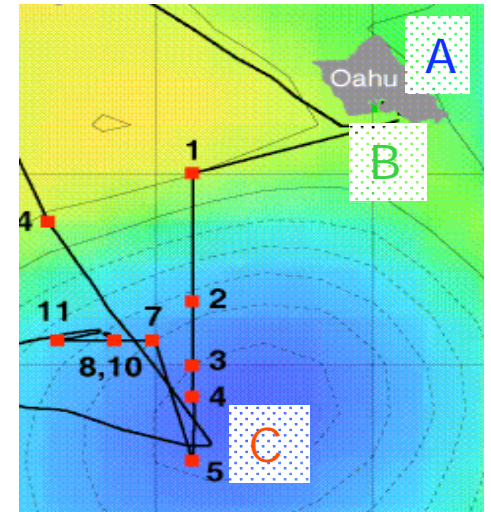
B: Keehi Bay

C: Down Welling



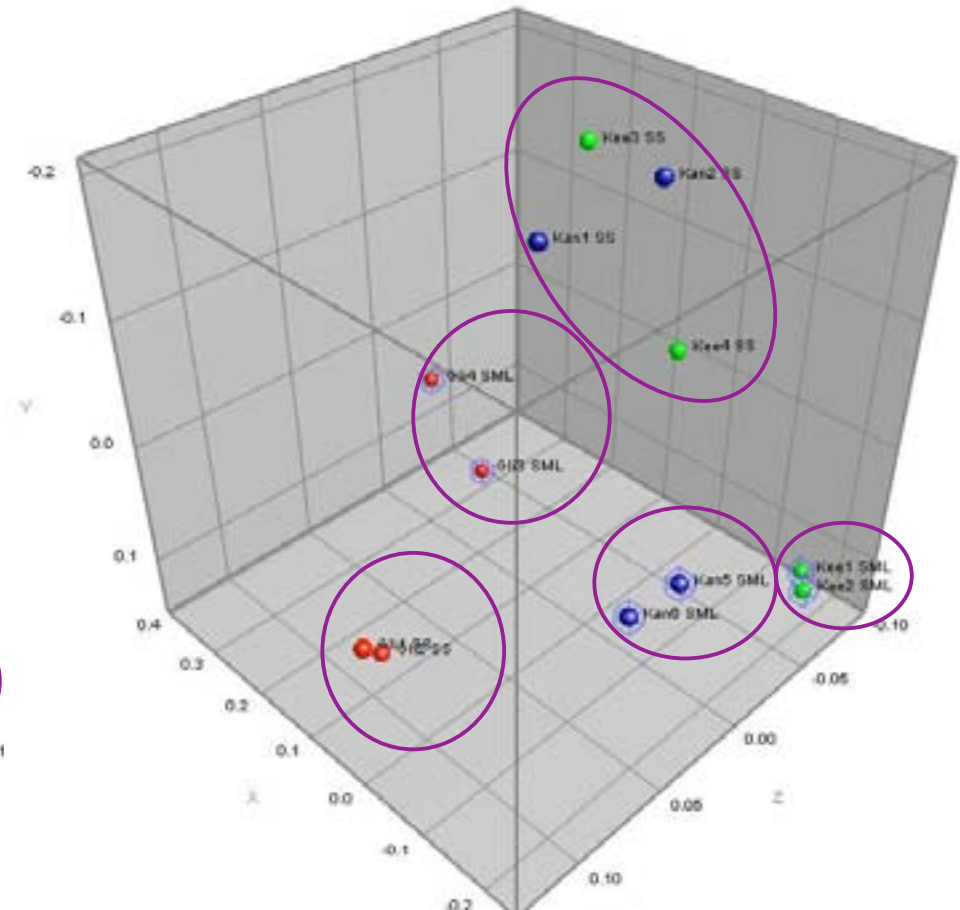
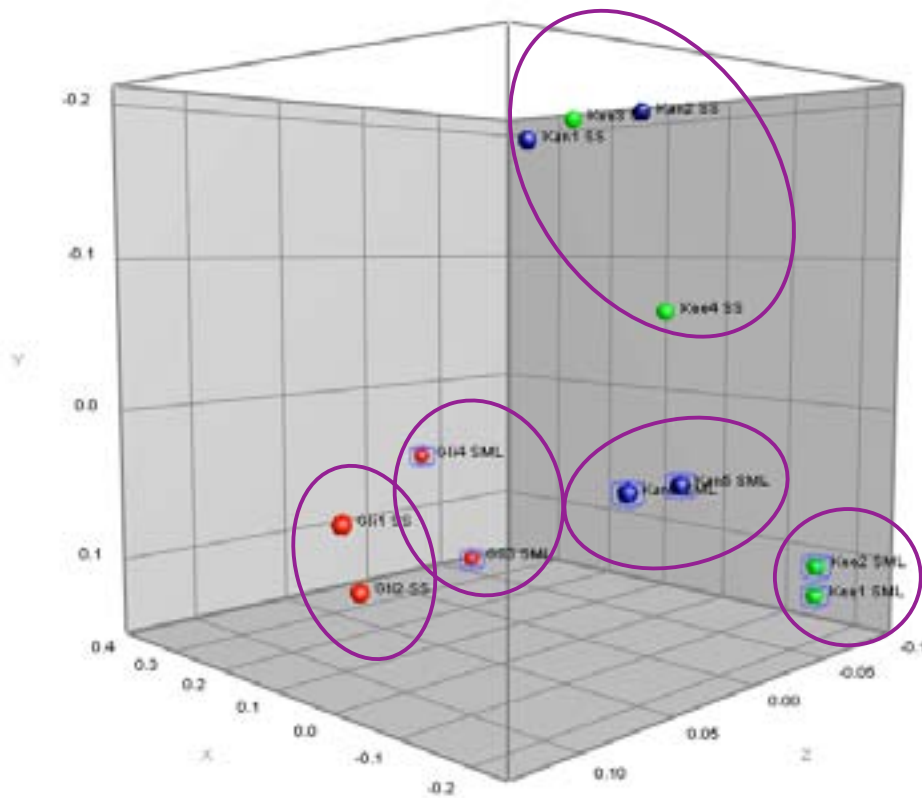
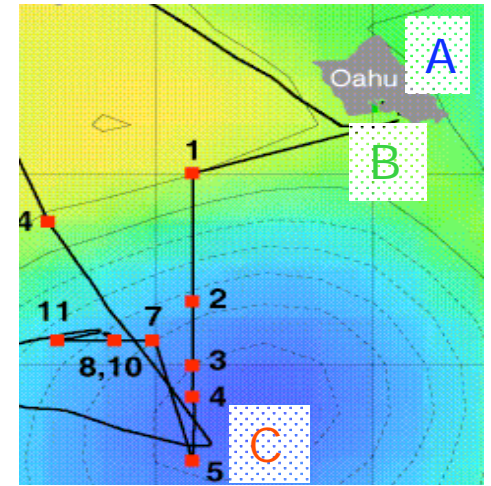
Bacterial community structure of the bacterioneuston and subsurface is distinct

- Bacterial 16S rRNA gene distribution
- Dendrogram from DGGE



Sea surface microlayer Bacterial community structure

- Multidimensional spacing calculated from Pearson Rank correlation
- Clustering of sea surface microlayer and sub-surface samples



The Sea Surface Microlayer has a distinct bacterial community structure compared to the subsurface waters

- Data suggest a “geographical” effect on the specific surface microlayer and sub-surface communities
- Greatest differences between open ocean surface microlayer and sub-surface
 - Higher bacterial diversity in the sub-surface
 - Reduced diversity in the surface microlayer
 - Sampling systems are robust
 - Can now analyse DNA (and RNA) for “functional genes” to detect bacteria involved in trace gas metabolism

Tie in with gas flux measurements and tank studies

So far we are “Just Scratching the Surface”. Still much to be done!

Acknowledgements

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Emma Harrison, Matt Salter, Grant Forster



NERC SOLAS Thematic
Programme



"Microbial Oceanography: Genomes to Biomes"

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Gene probes for the study of bacteria involved in trace gas metabolism

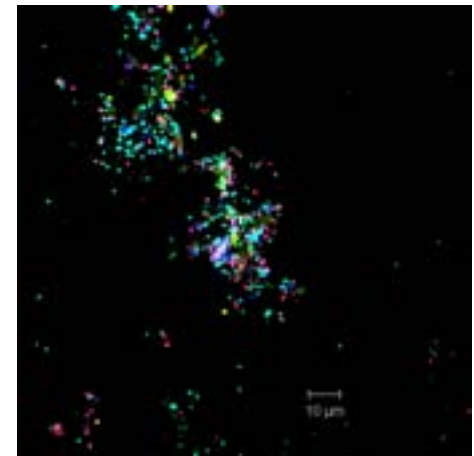
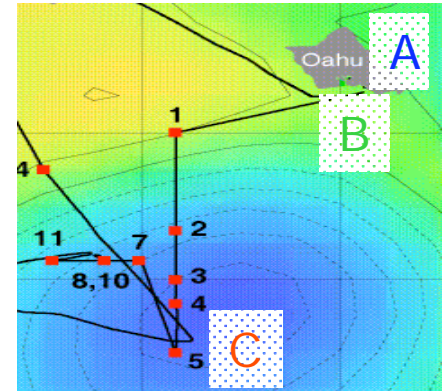
Compound (process)	Bacteria	Key enzyme	16S rRNA primers/probes	Functional gene probe	Reference
Methane (oxidation)	Methanotrophs	Methane monooxygenase (particulate and soluble form)	Probes for: <i>Methylobacter</i> <i>Methylococcus</i> <i>Methylomonas</i> <i>Methylosinus</i>	<i>pmoA</i> <i>mmoX</i>	35, 36, 37
Methane (co-oxidation)	Nitrifiers	Ammonia monooxygenase (Co-oxidation of methane)	Beta ammonium oxidiser probes	<i>amoA</i>	38
Methanol (oxidation)	Methylotrophs	Methanol dehydrogenase	<i>Hyphomicrobium</i> <i>Methylobacterium</i>	<i>mxoF</i>	39
Methyl halides, CH ₃ Br, CH ₃ Cl, CH ₃ I (oxidation)	Methylotrophs	Methyltransferase/corrinoid binding enzyme CmuA	<i>Roseobacter</i> <i>Roseovarius</i>	<i>cmuA</i>	40, Schäfer et al. in press
Methanesulfonate (oxidation)	Methylotrophs	Methanesulfonic acid monooxygenase	<i>Marinosulfonomonas</i>	<i>msmA</i>	41
Carbon monoxide (oxidation)	Carboxidotrophs	Carbon monoxide dehydrogenase	<i>Stappia</i>	<i>coxL</i>	42
DMS (production)	Bacteria & eukaryotic phytoplankton	DMSP lyase	none	None available yet	
DMS (oxidation)	Methylotrophs	DMS monooxygenase	none	In development (Schäfer)	
DMS (oxidation)	Methylotrophs	DMS methyltransferase	none	In development (Schäfer)	
NO (production)	Denitrifiers	Nitrite reductase	none	<i>nirK</i> & <i>nirS</i>	43

C1-functional gene probes

Functional guild	Target enzyme	Gene	site 1		site 2	
			SS	SML	SS	SML
Methanotroph	Particulate methane monooxygenase	<i>pmoA</i>	*	*		*
Methanotroph	Soluble methane monooxygenase	<i>mmoX</i>	*	✓	✓	✓
CO oxidiser	Carbon monoxide dehydrogenase	<i>coxL</i>	✓	✓		
Methyl-halide oxidiser	Methyltransferase	<i>cmuA</i>	*	*	*	*
Total bacteria "house keeping"	RNA polymerase	<i>rpoB</i>	✓	✓	✓	✓

Summary

- Aquatic surface microlayer microbial communities are different compared to microbial communities in sub-surface waters
 - the level of 'difference' between communities varied from the Pacific 'down welling' region to the estuarine communities
 - from the Pacific data there appears to be a **biogeographical** influence in surface microlayer community structure
 - interestingly surface microlayer populations of **Archaea** were detected in the estuary
 - in the surface microlayer of a freshwater pond there are **quantifiable differences** in active bacterial community structure



Summary

- Functional gene diversity in the aquatic surface microlayers is different compared to sub-surface waters
 - all *mmoX* genes detected in the surface microlayer of Blyth estuary were different compared to the sub-surface
 - surface microlayer specific *coxL* sequences were also detected

