Microbial genomics for de-risking offshore oil and gas exploration in Nova Scotia

Casey Hubert

UNIVERSITY OF CALGARY

CANADA 1842 GEOLOGICAL SURVEY - COMMISSION GÉOLOGIQUE

apt APPLIED PETROLEUM TECHNOLOGY

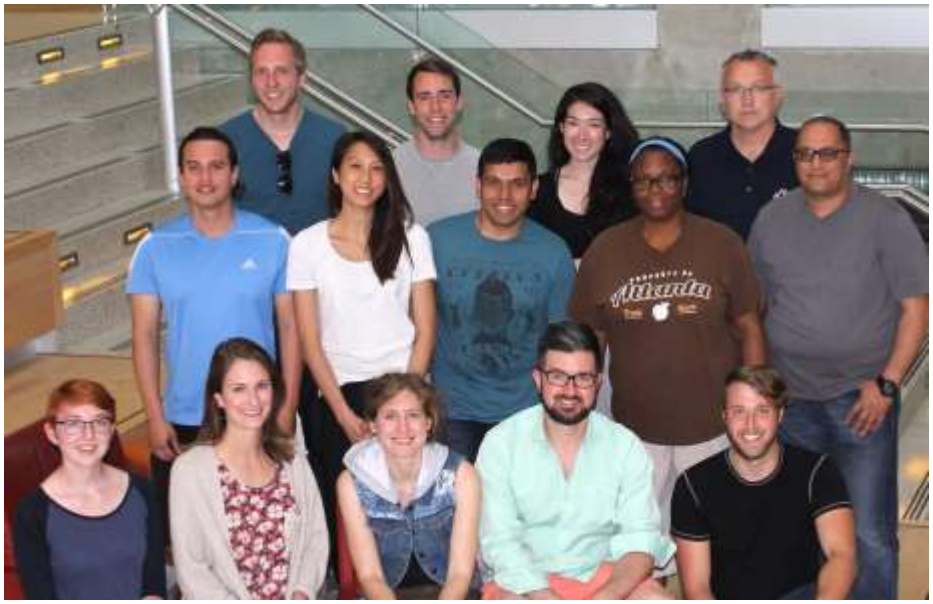
NOVA SCOTIA CANADA

GenomeCanada

GenomeAtlantic

GenomeAlberta

NSERC CRSNG



Research Funding

Alberta Government

GenomeCanada

INNOVATION.CA
CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

NSERC CRSNG

ArcticNet
ᐅᑭᐅᖅᑕᖅᑐᒥᒃ ᑐᑭᓯᓂᐊᖅᑎᑦᑕ

Geomicrobiology Group

- marine oil spill bioremediation
- establishing environmental baselines
- **seabed hydrocarbon seep microbiology**
- deep biosphere microbial ecology
- extremophiles
- bacterial endospores
- oil reservoir microbiology
- food microbiology (coffee, sourdough)

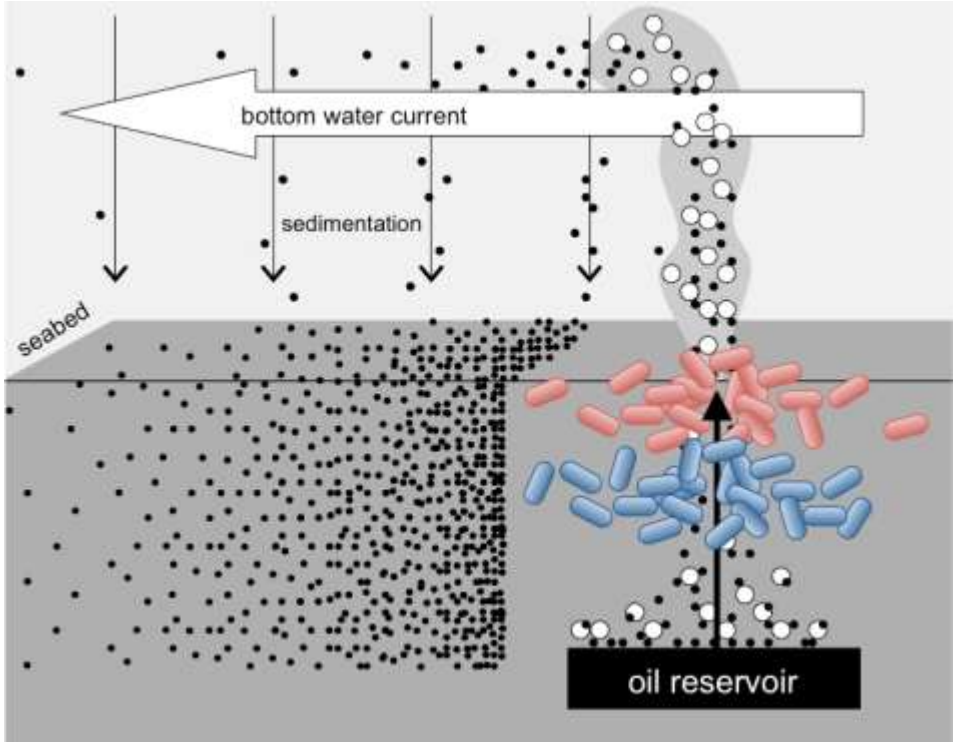
MEOPAR
MARINE ENVIRONMENTAL OBSERVATION PREDICTION & RESPONSE NETWORK

ExxonMobil

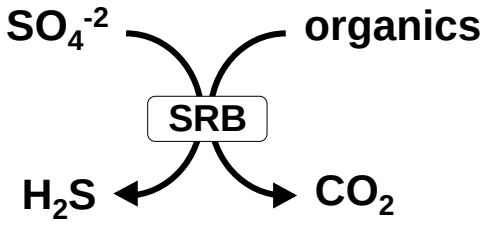
MitJacs

Outline

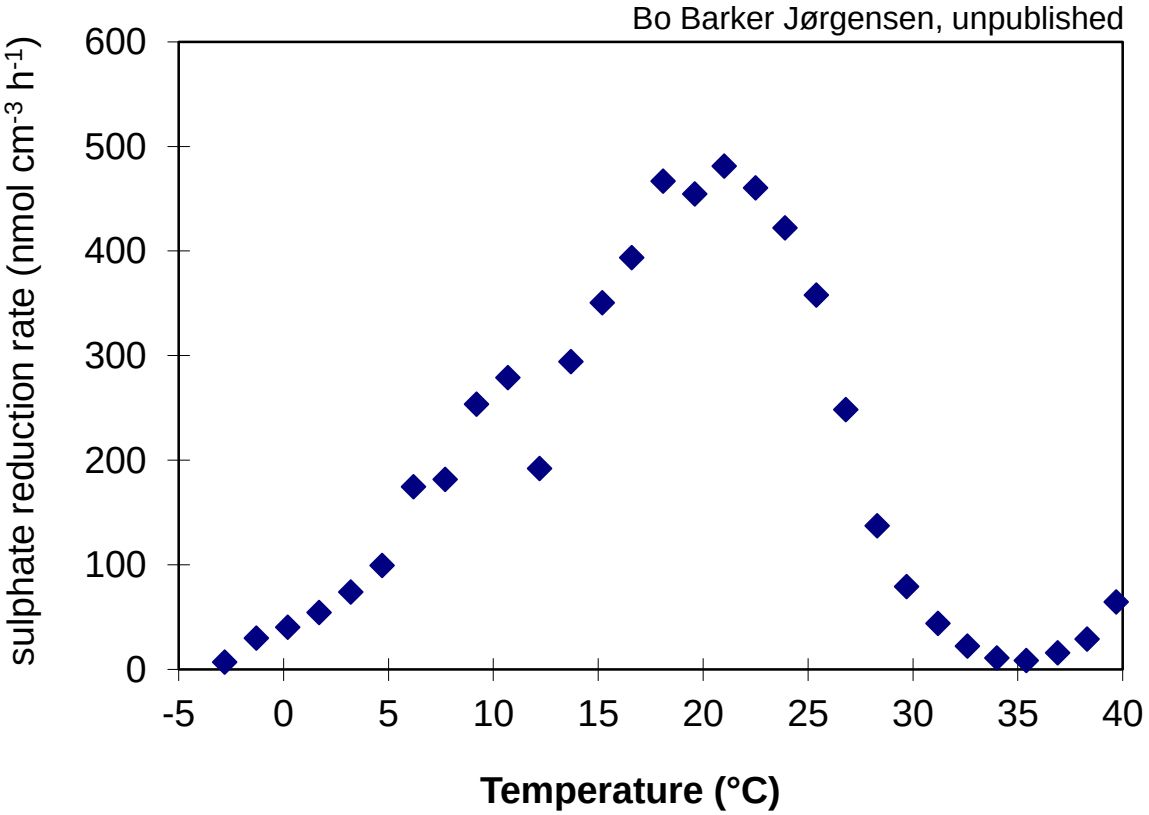
1. original observations and interest in hydrocarbon seeps
2. using cold-adapted seabed microbes for prospecting
3. using dormant thermophilic bacterial spores for prospecting



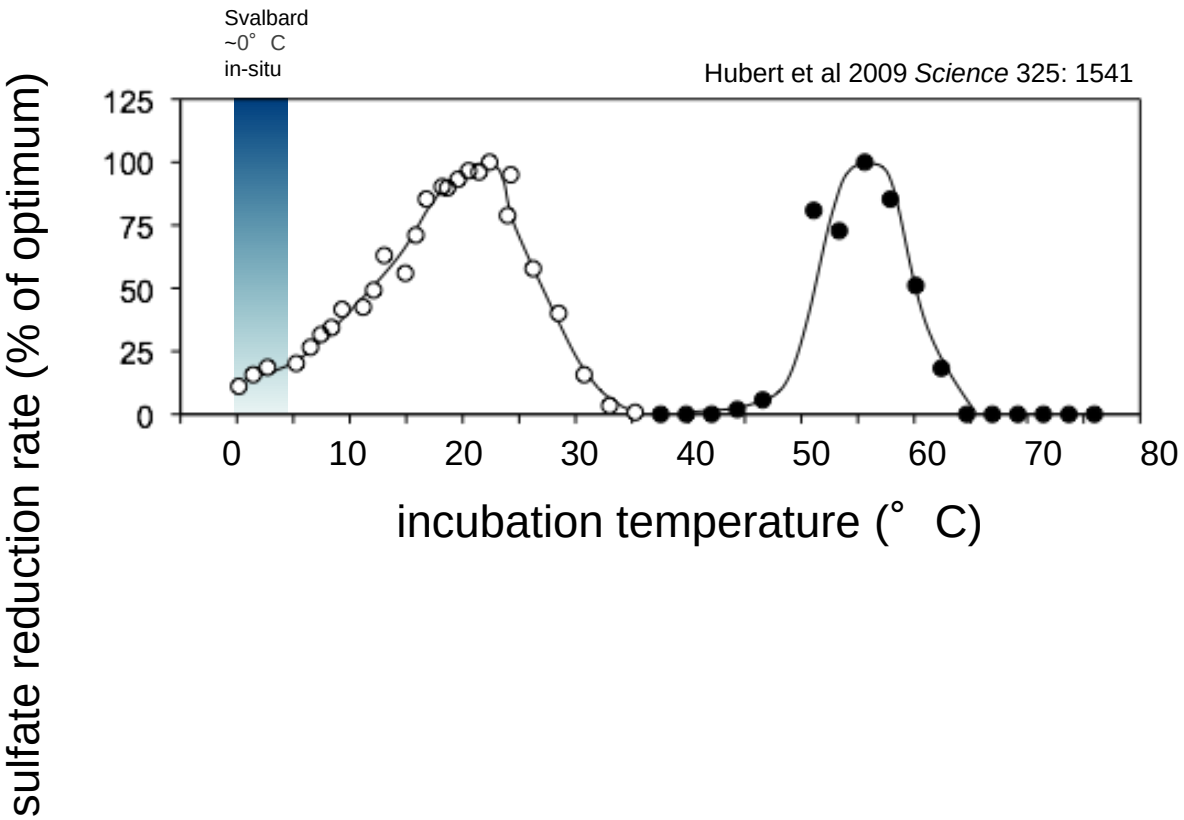

Smeerenburgfjorden, Svalbard, 80° North



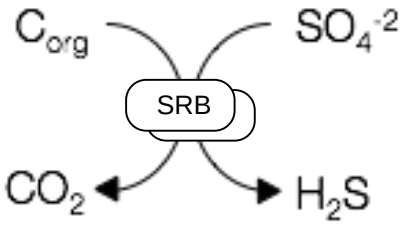
Svalbard marine sediment incubated from -2 to +40°C



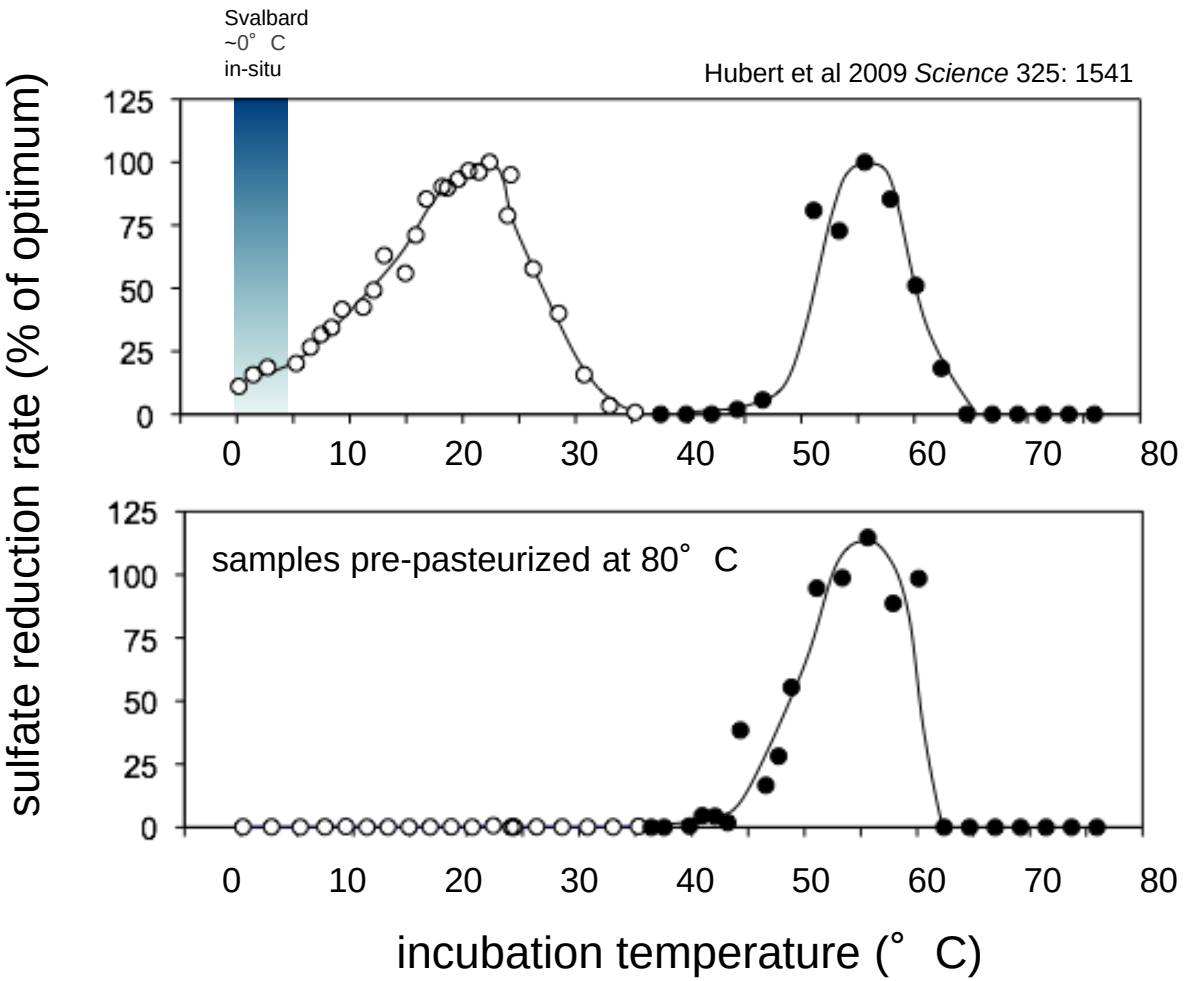
Thermophilic Bacteria in Arctic Sediments



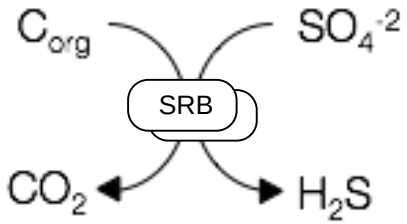
sulfate-reducing bacteria

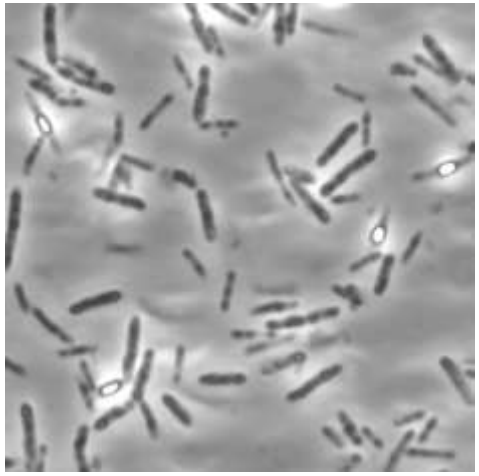


Thermophilic Bacteria in Arctic Sediments

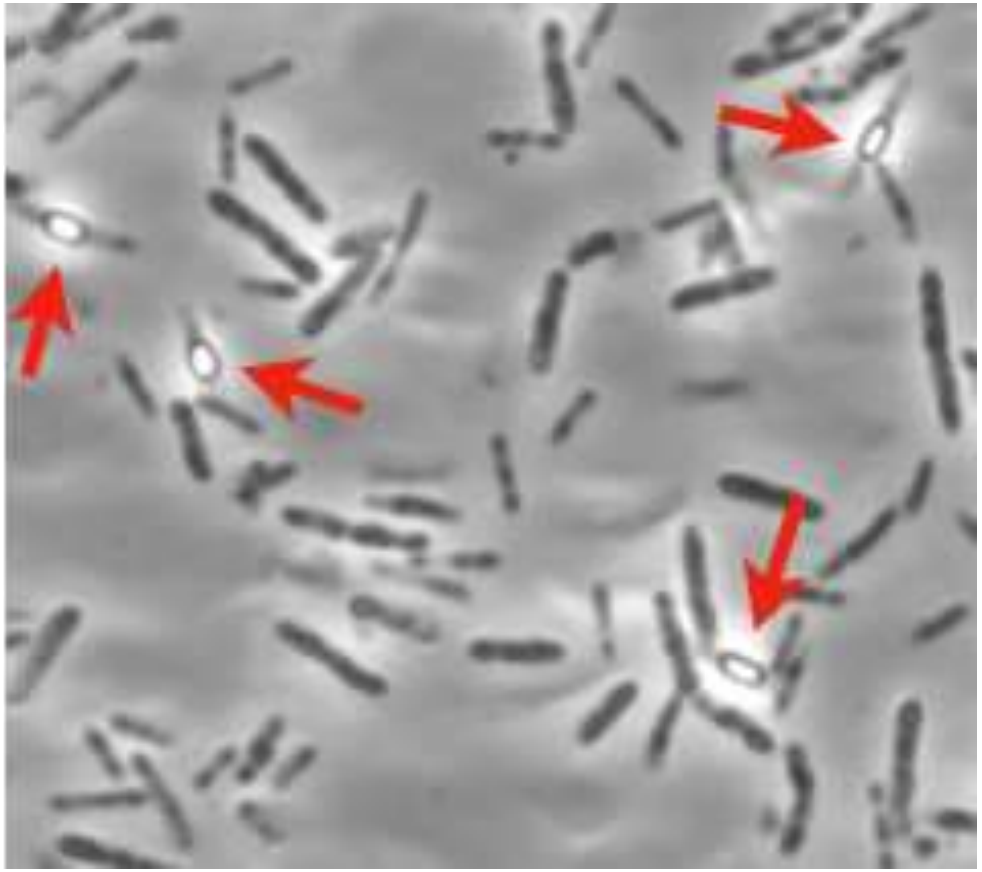


sulfate-reducing bacteria





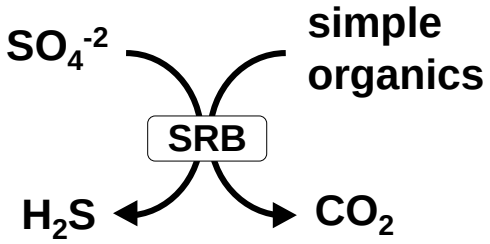
Desulfotomaculum endospores



Flemming Mønsted Christensen, M.Sc. thesis, 2009

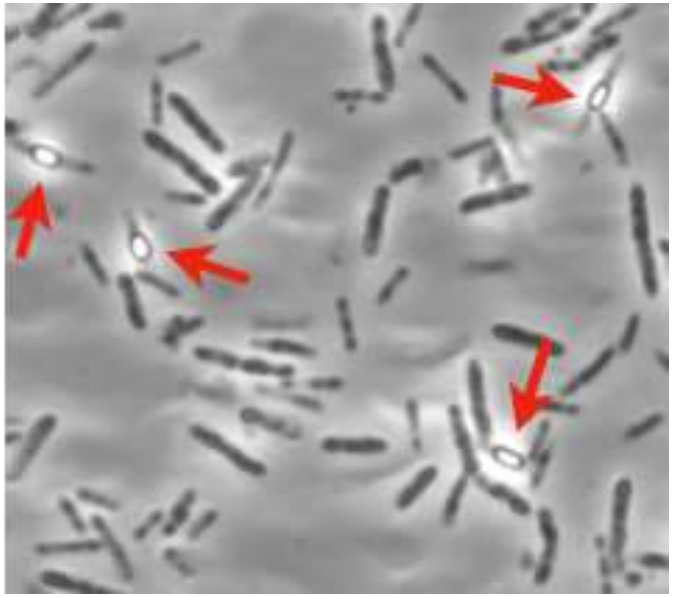
genus *Desulfotomaculum*

- sulfate reducers
- endospore formers
- thermophiles



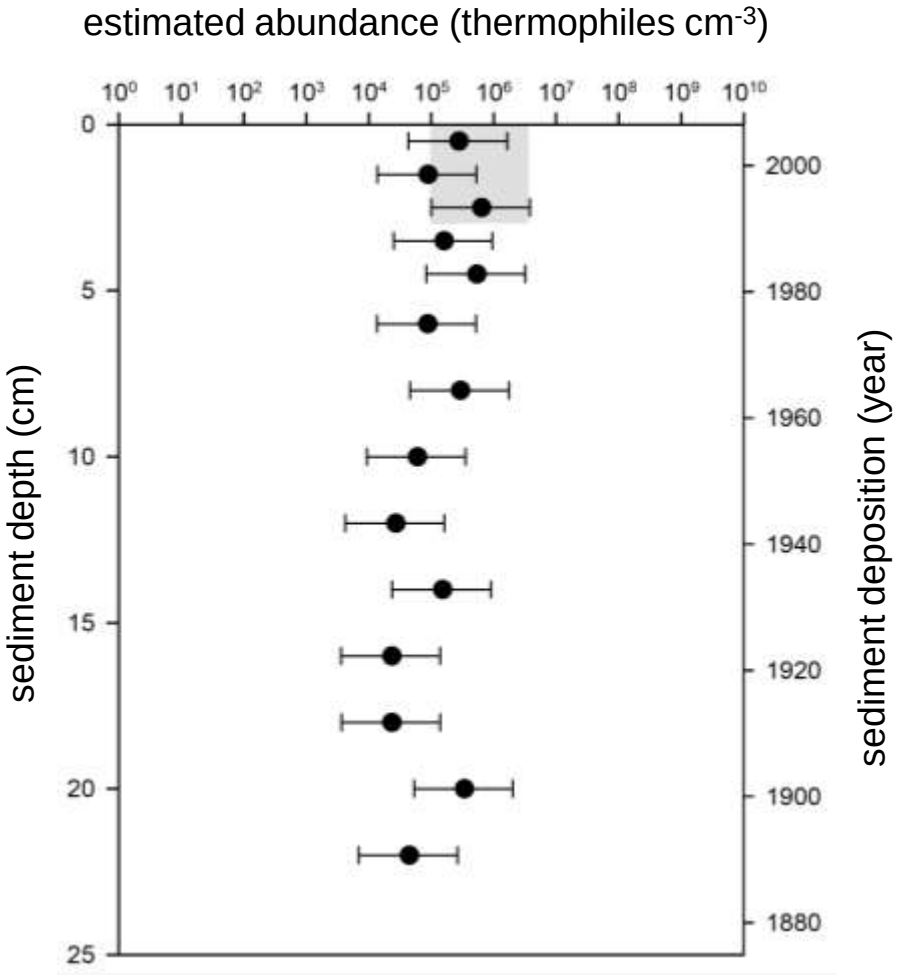
"sulfate reduction"

Desulfotomaculum endospores in Arctic marine sediment



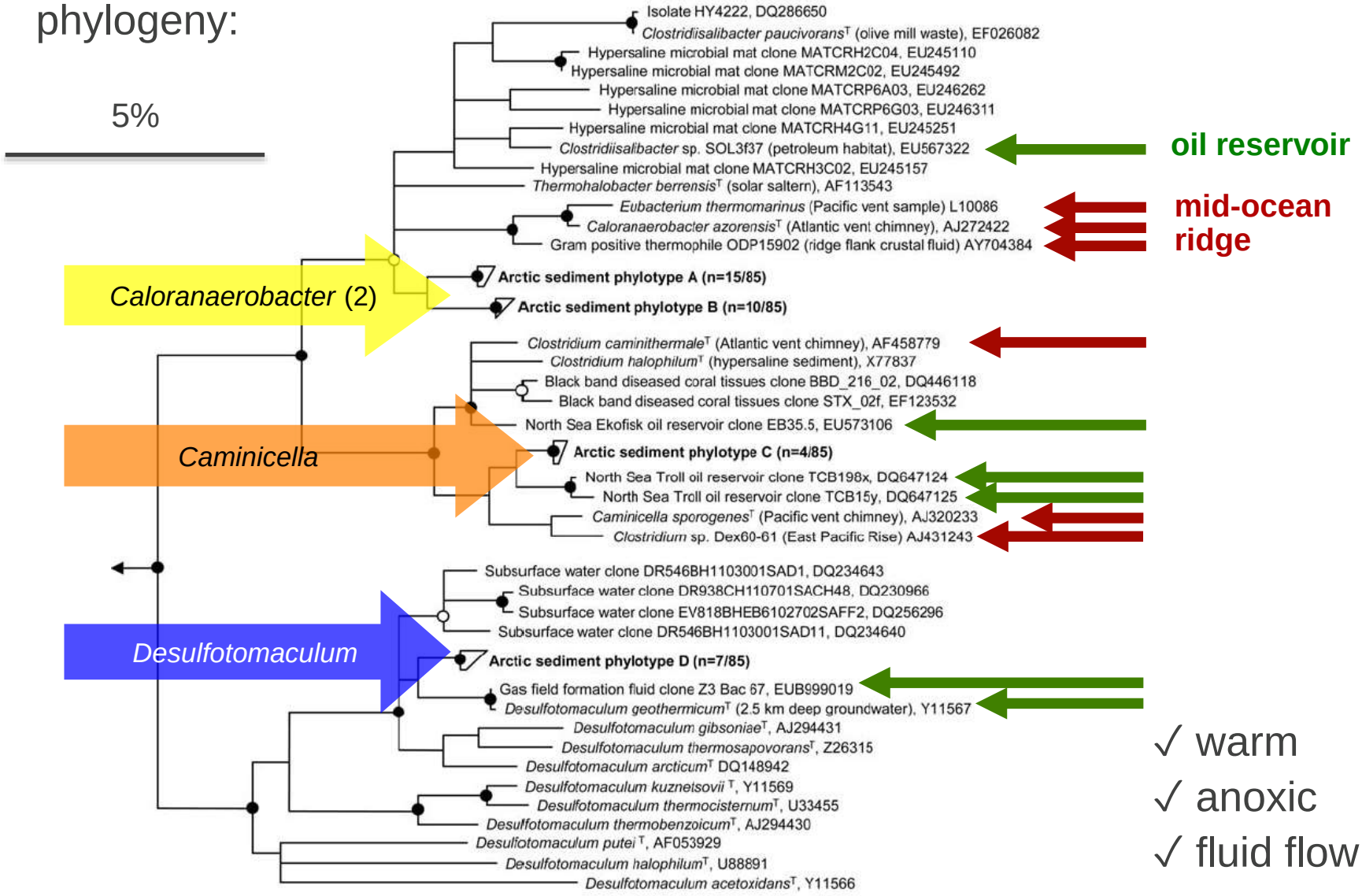
Flemming Mønsted Christensen, M.Sc. thesis, 2009

- 10^5 per gram of sediment
- annual influx 10^8 m^{-2}
- where are they coming from?



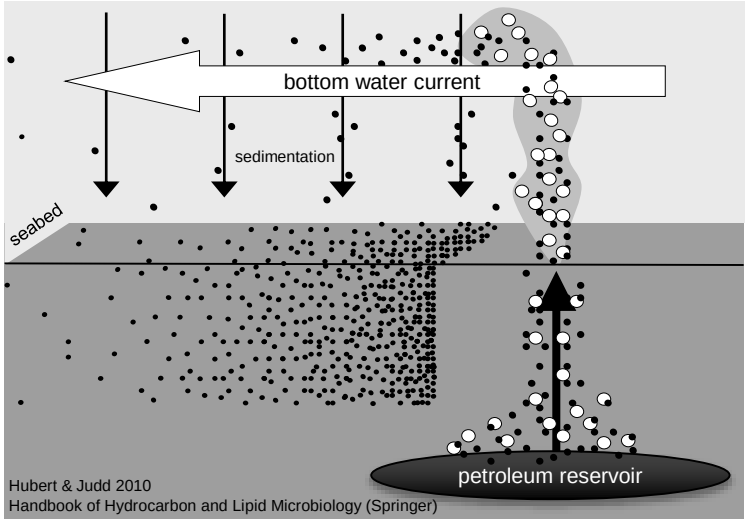
Thermospores… where are they coming from?

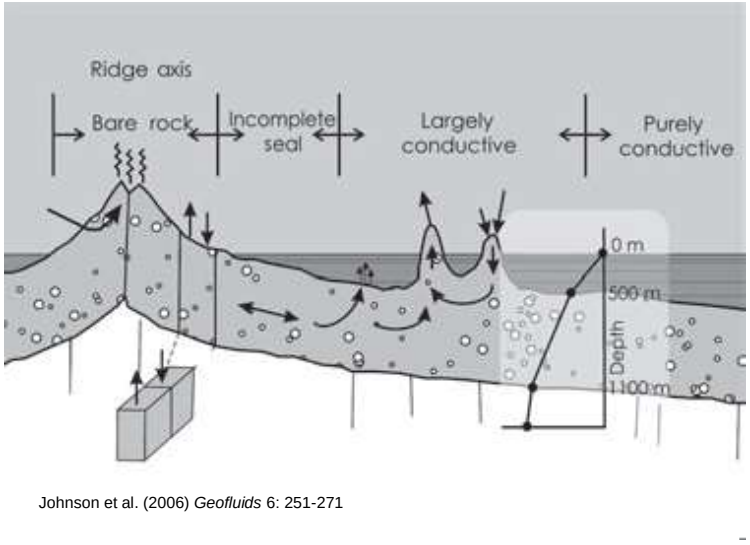
rRNA gene phylogeny:



✓ warm
✓ anoxic
✓ fluid flow

seabed fluid flow ~ an unappreciated microbial dispersal vector?





Johnson et al. (2006) *Geofluids* 6: 251-271

Hydrocarbon seeps

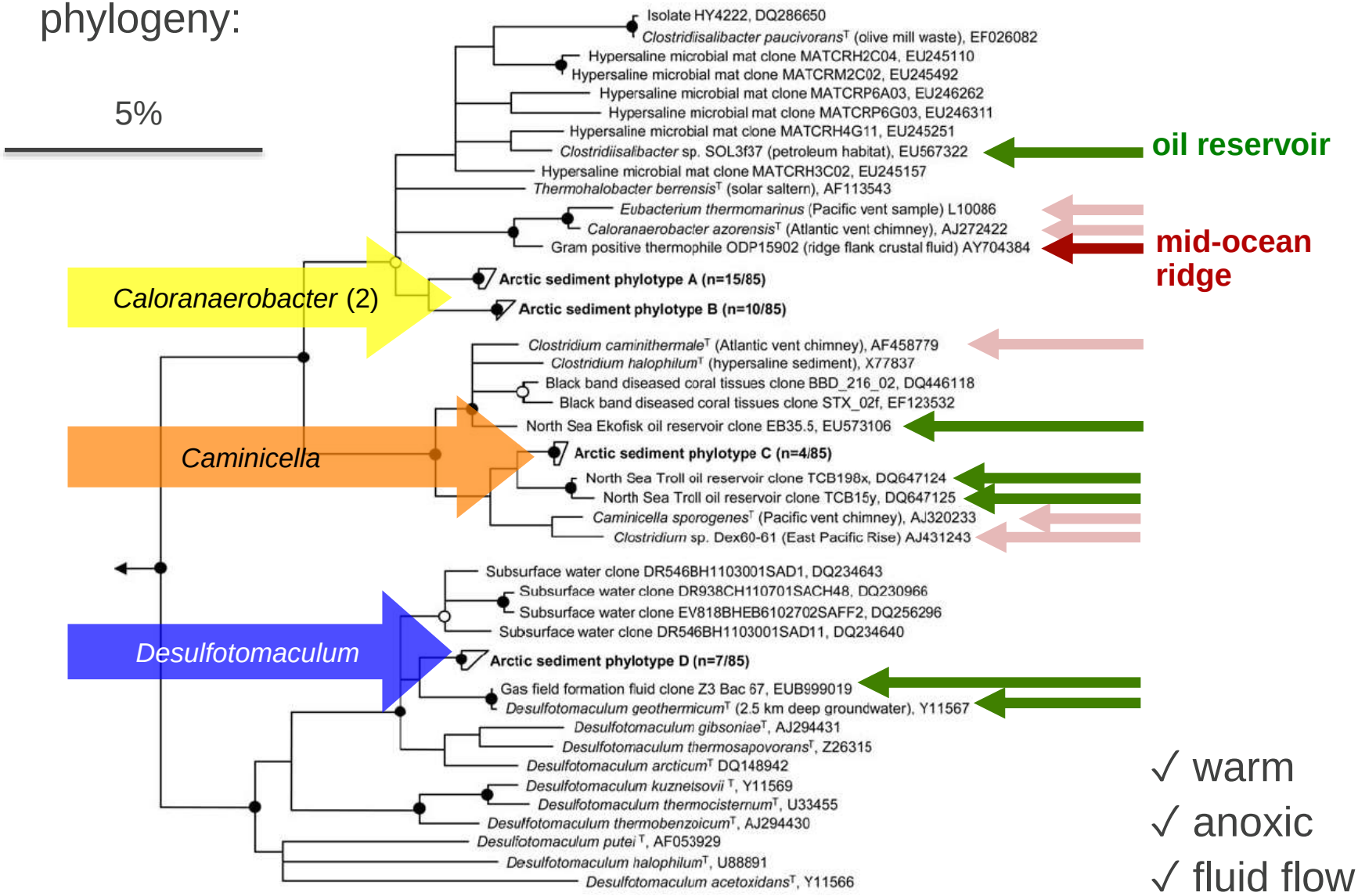
- widespread in the ocean
- fluid seepage from depth
- depth influences in situ T
- thermospores have many rRNA gene database hits to oil fields

Hydrothermal circulation

- worldwide network of ocean ridges
- lateral (axis) influence on in situ T
- thermospores have some rRNA gene database hits to mid ocean ridge systems

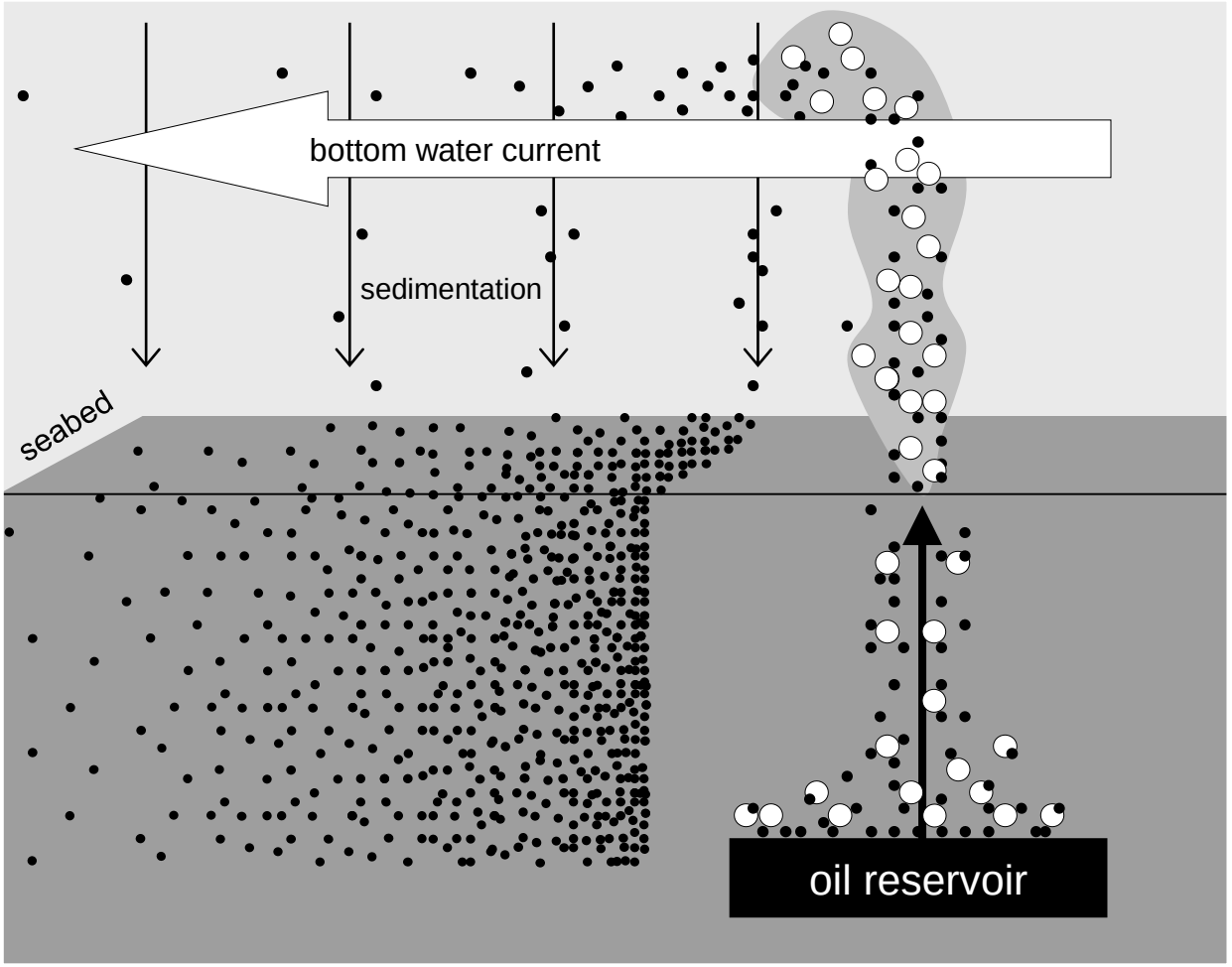
seabed fluid flow ~ an unappreciated microbial dispersal vector?

rRNA gene phylogeny:

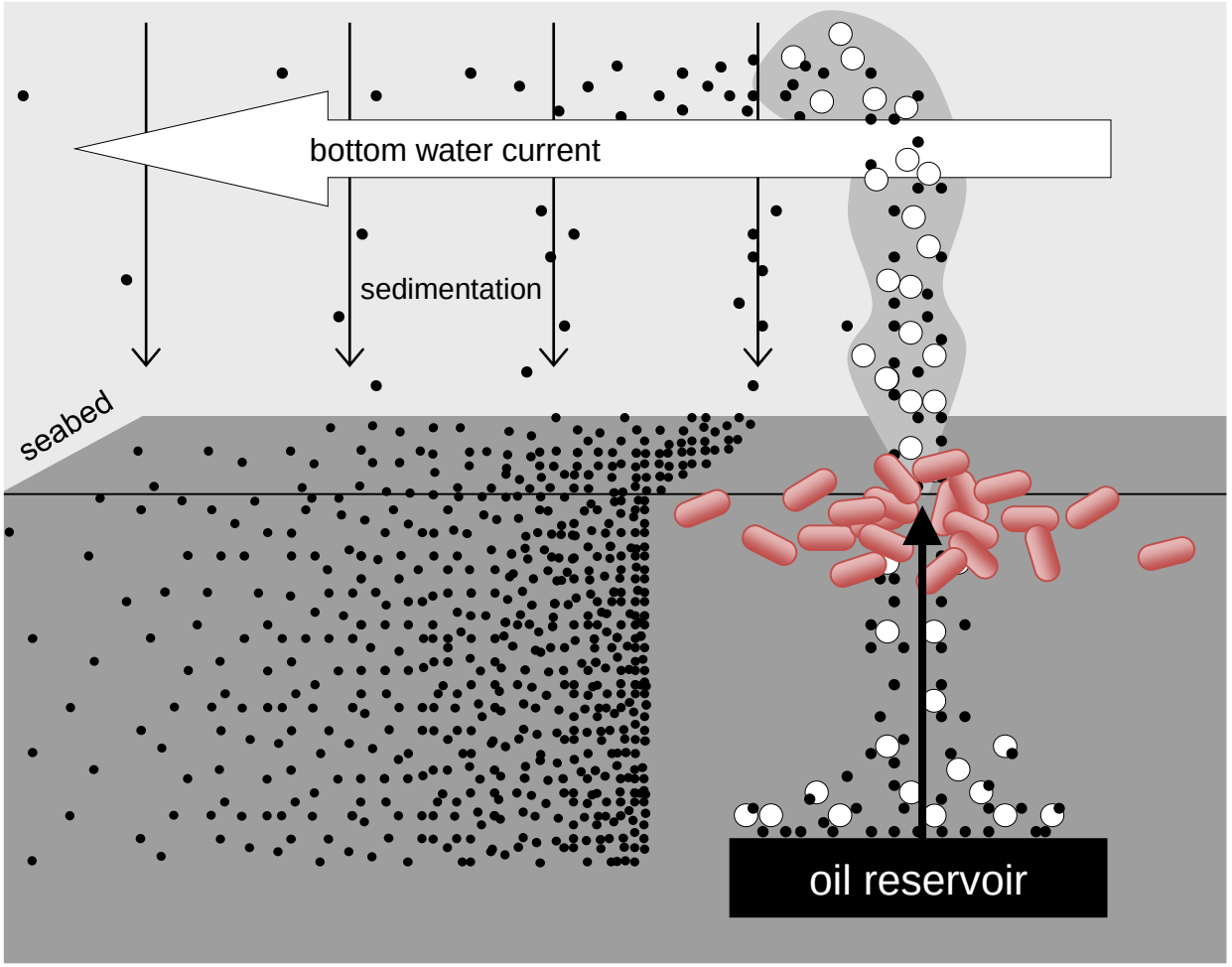


✓ warm
✓ anoxic
✓ fluid flow

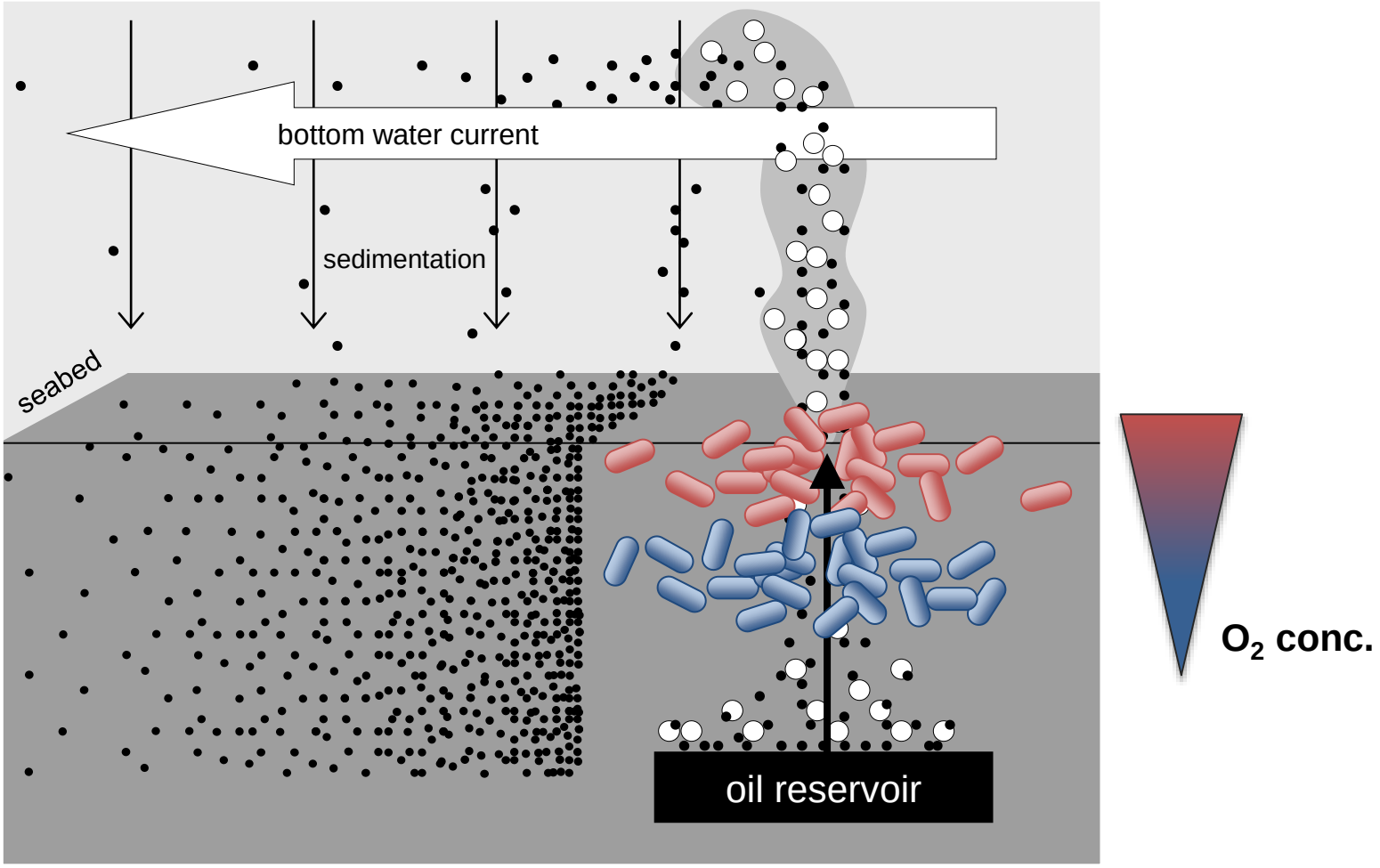
Microbiology-based prospecting for seabed hydrocarbon seeps



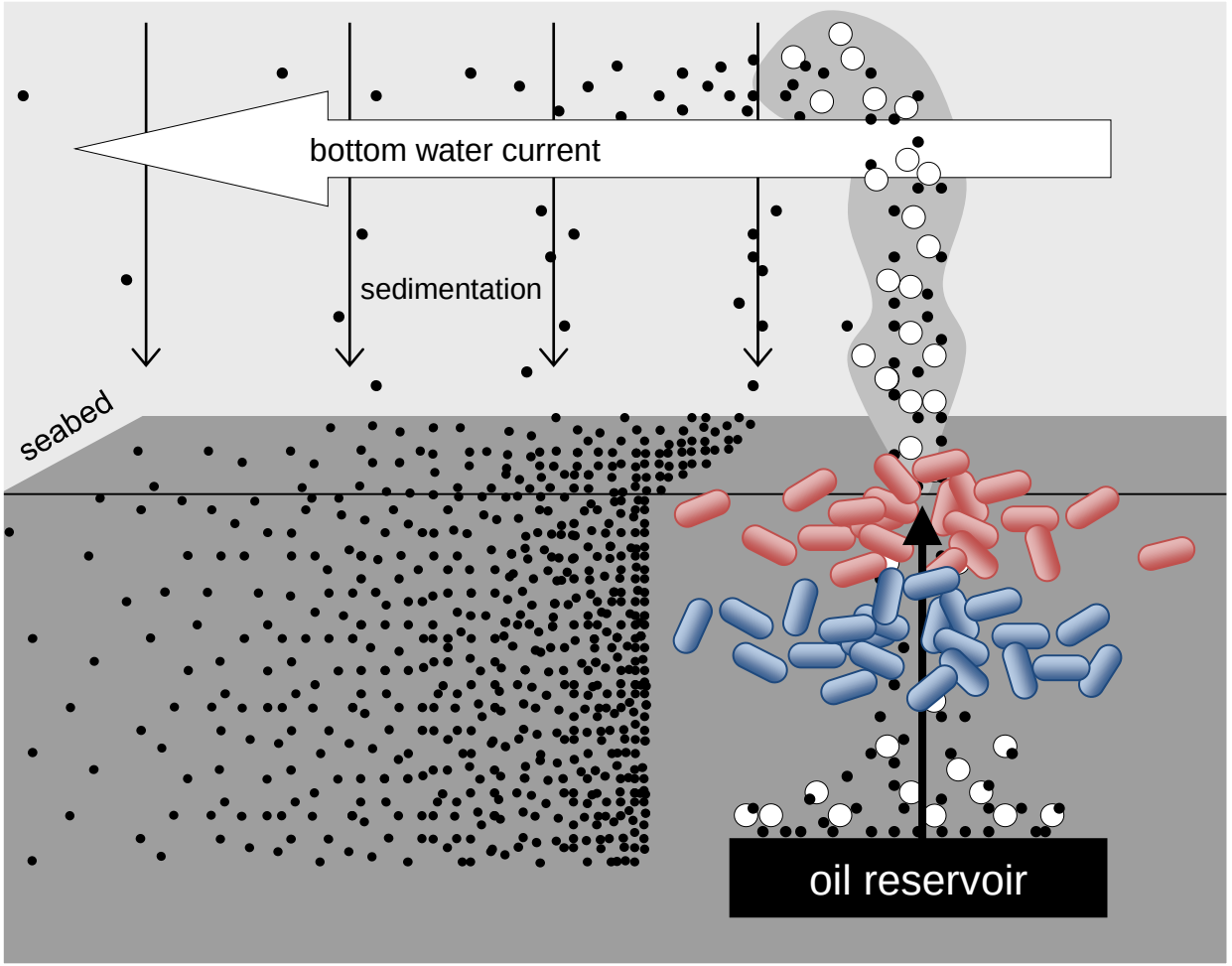
Microbiology-based prospecting for seabed hydrocarbon seeps



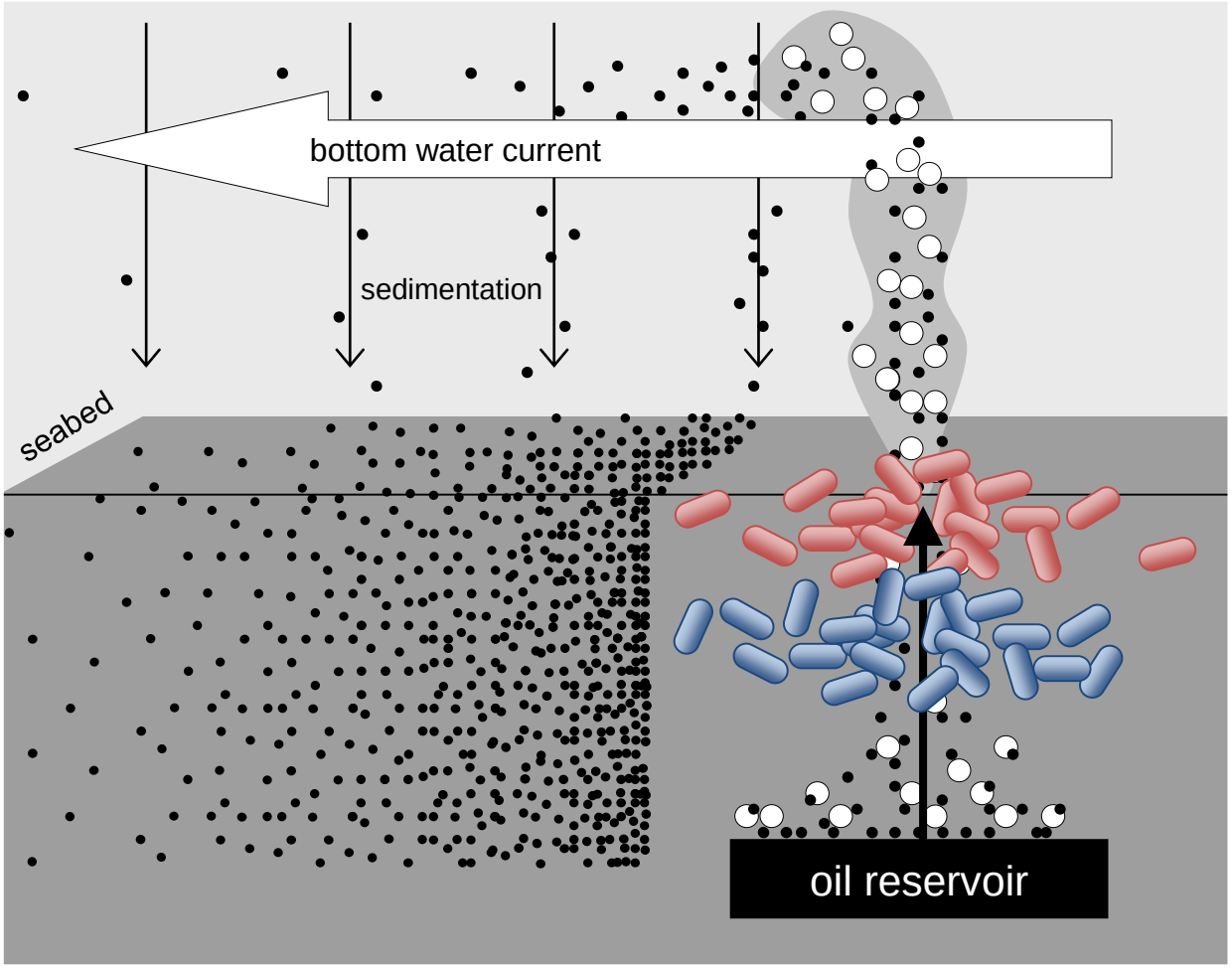
Microbiology-based prospecting for seabed hydrocarbon seeps



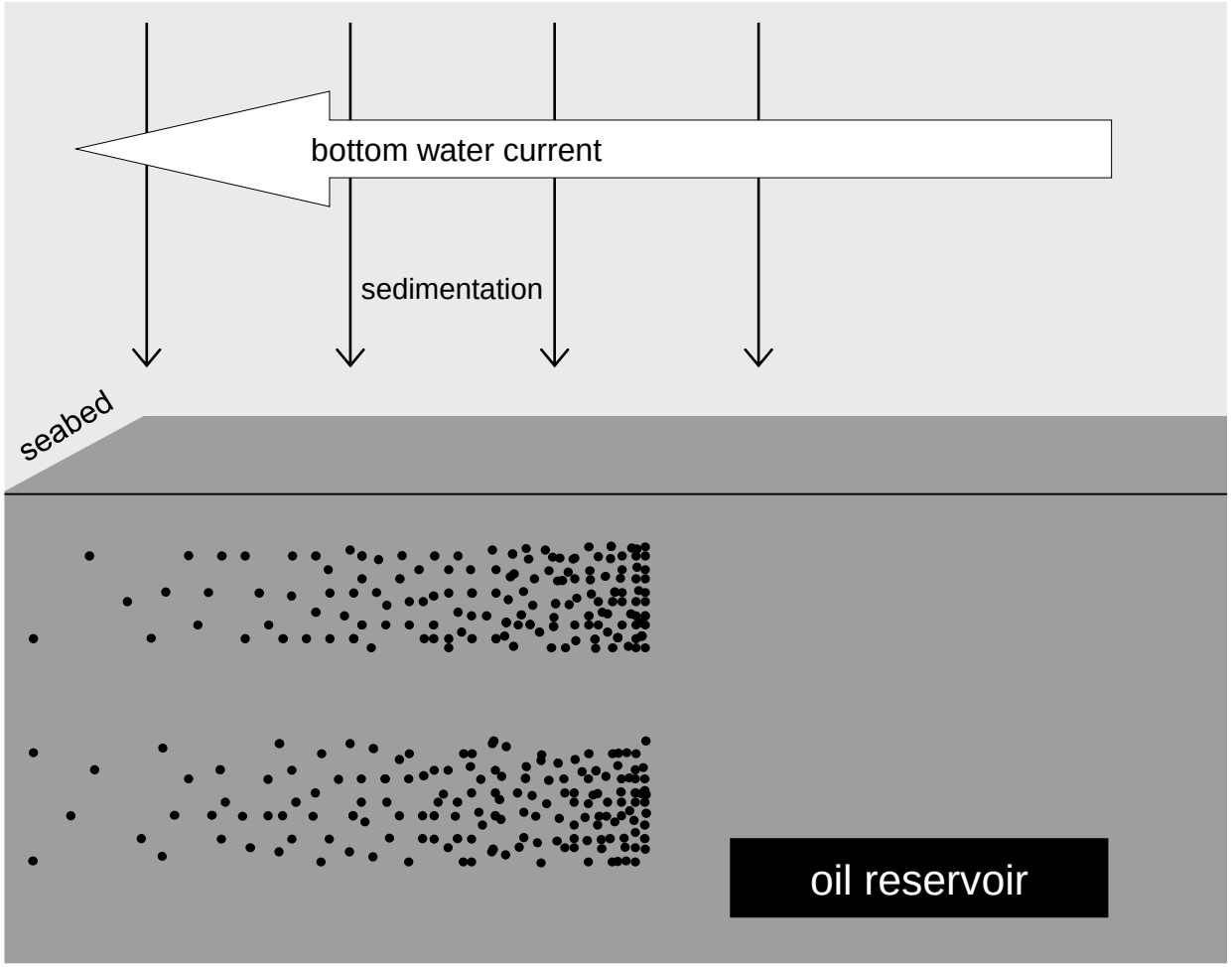
Hypothesis: thermophilic spores [⁘] and cold-adapted oil-degrading bacteria [🦠] have different distributions at seeps



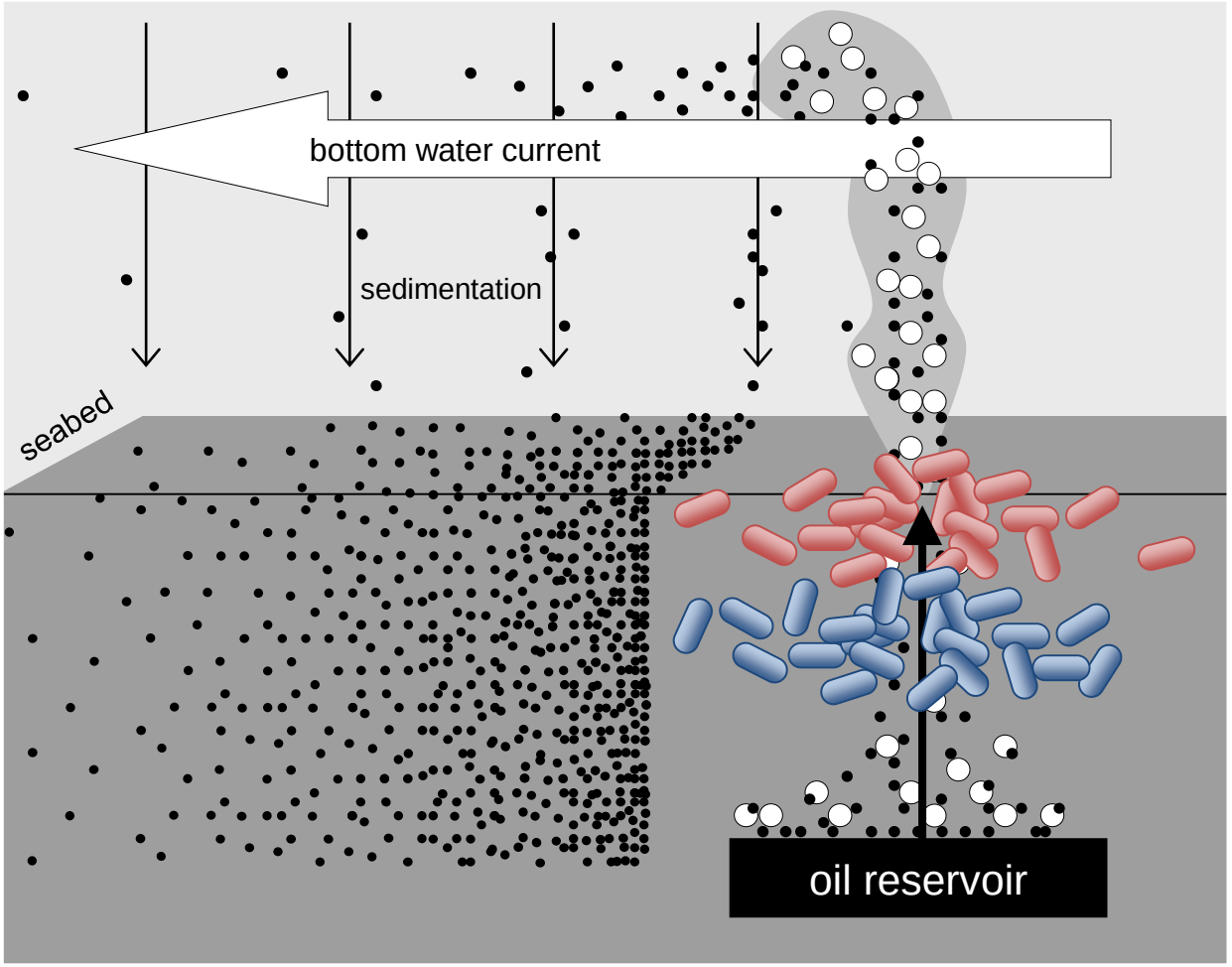
Prospecting advantage using dormant bacterial spores compared to active bacteria, or hydrocarbons…?



Prospecting advantage using dormant bacterial spores compared to active bacteria, or hydrocarbons…?

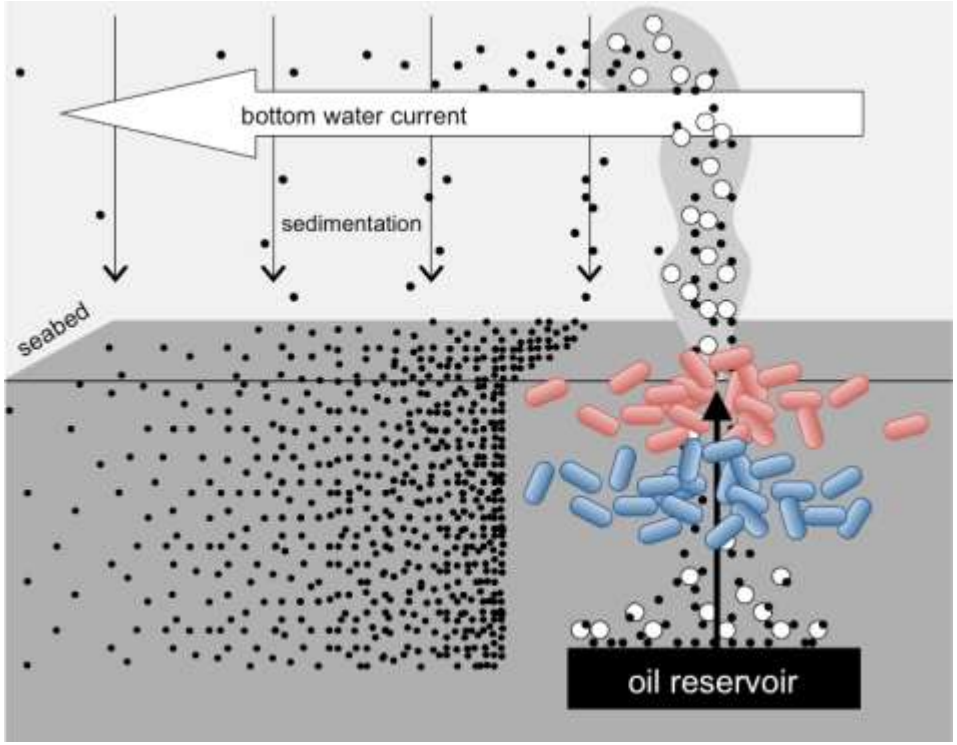


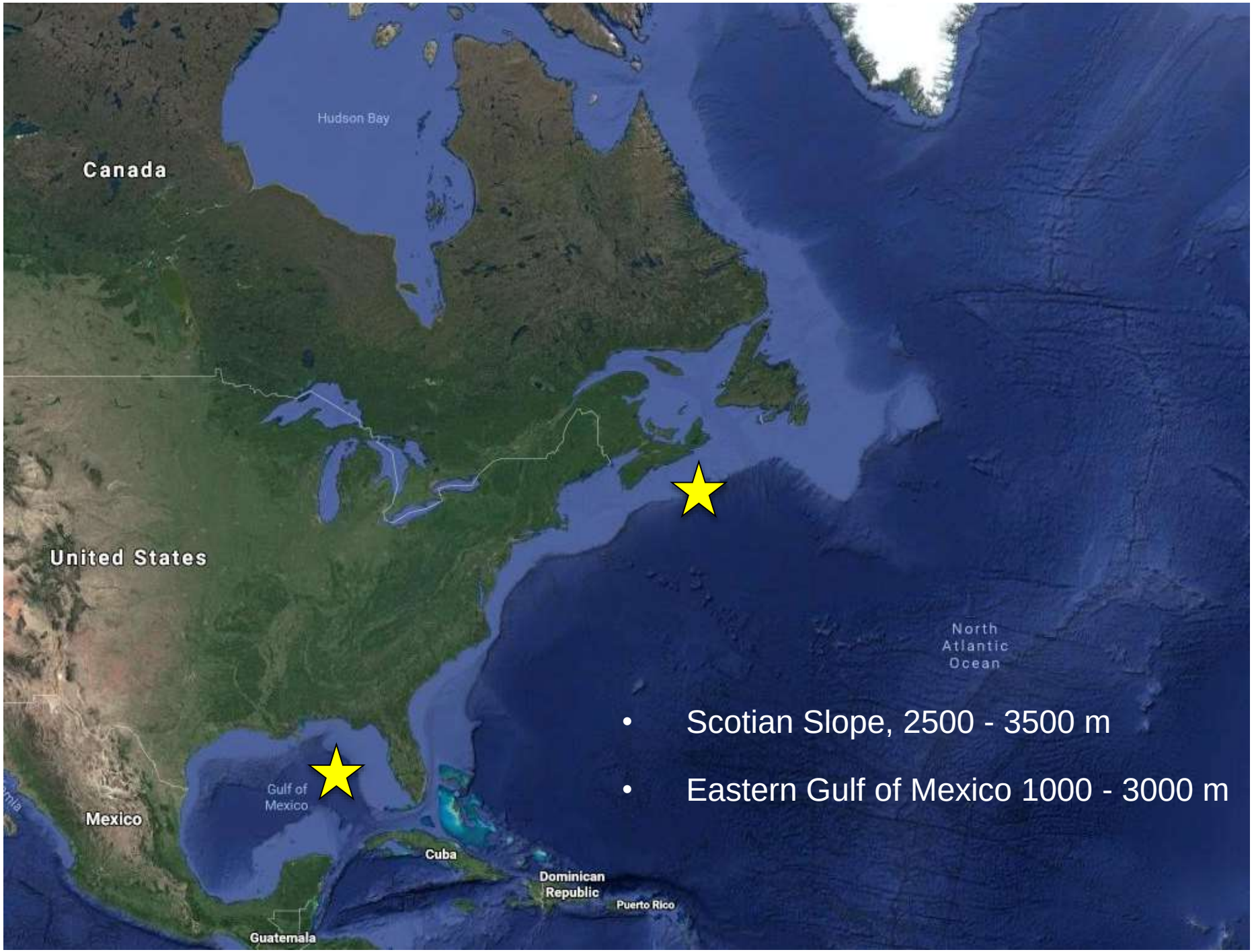
Multiple approaches to exploration risk assessment



Outline

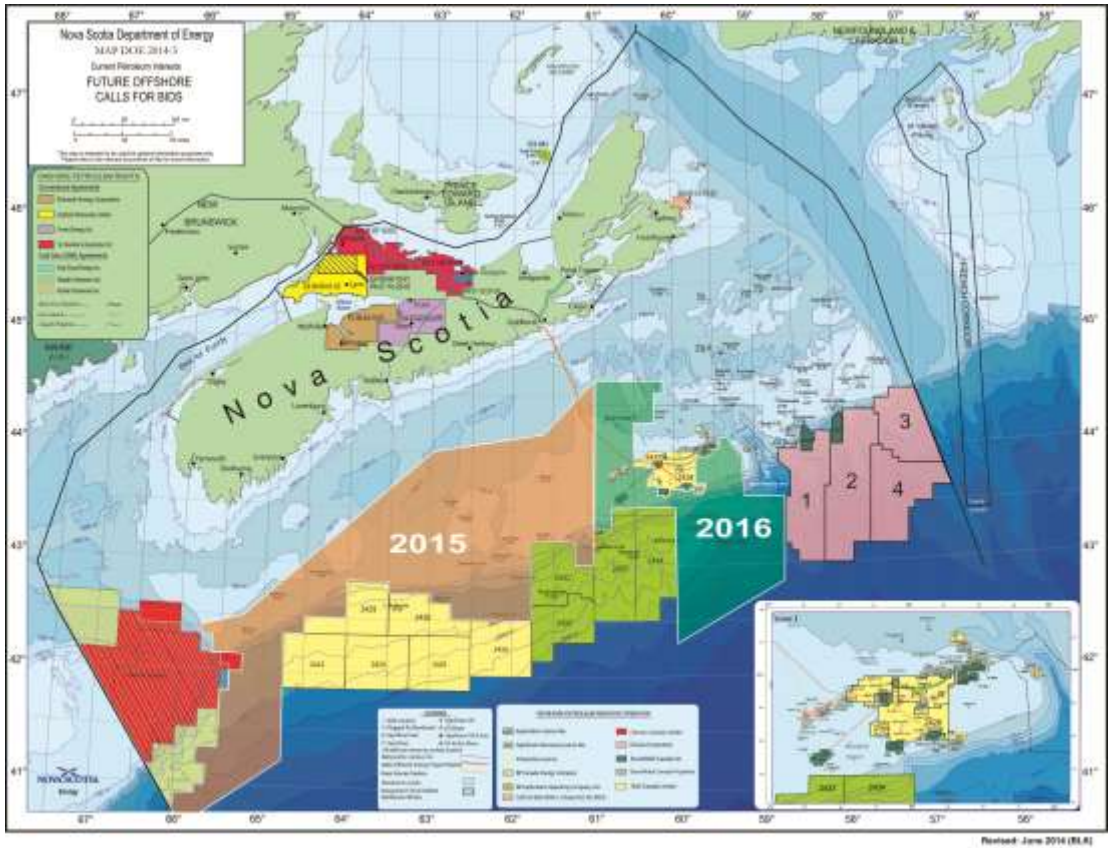
1. original observations and interest in hydrocarbon seeps
2. using cold-adapted seabed microbes for prospecting
3. using dormant thermophilic bacterial spores for prospecting




Hudson Bay
Canada
United States
North Atlantic Ocean
Gulf of Mexico
Mexico
Cuba
Dominican Republic
Puerto Rico
Guatemala
• Scotian Slope, 2500 - 3500 m
• Eastern Gulf of Mexico 1000 - 3000 m

Nova Scotia Department of Energy's Play Fairway Analysis

- $15M investment by NS → interdisciplinary geoscience mapping
- >$2B in exploration commitments (Shell, BP, Statoil)
- Front-end science to characterize the deep seabed → reduced risk



Fisheries and Oceans Canada

Canadian Coast Guard Ship: *Hudson*

UNIVERSITY OF CALGARY

summer 2015 & 2016

Scotian Slope – Piston Coring

>60 sites sampled

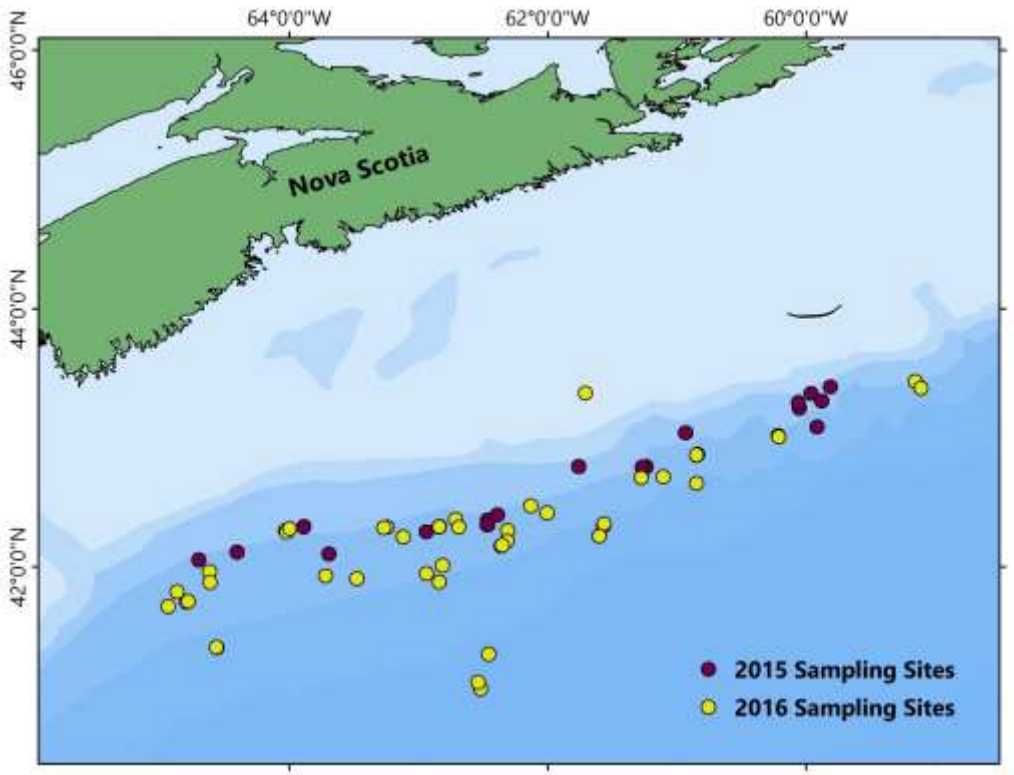
- 1750 to 3450 m water depth
- 0 to 10 mbsf sediment depth

Geochemical analyses (APT)

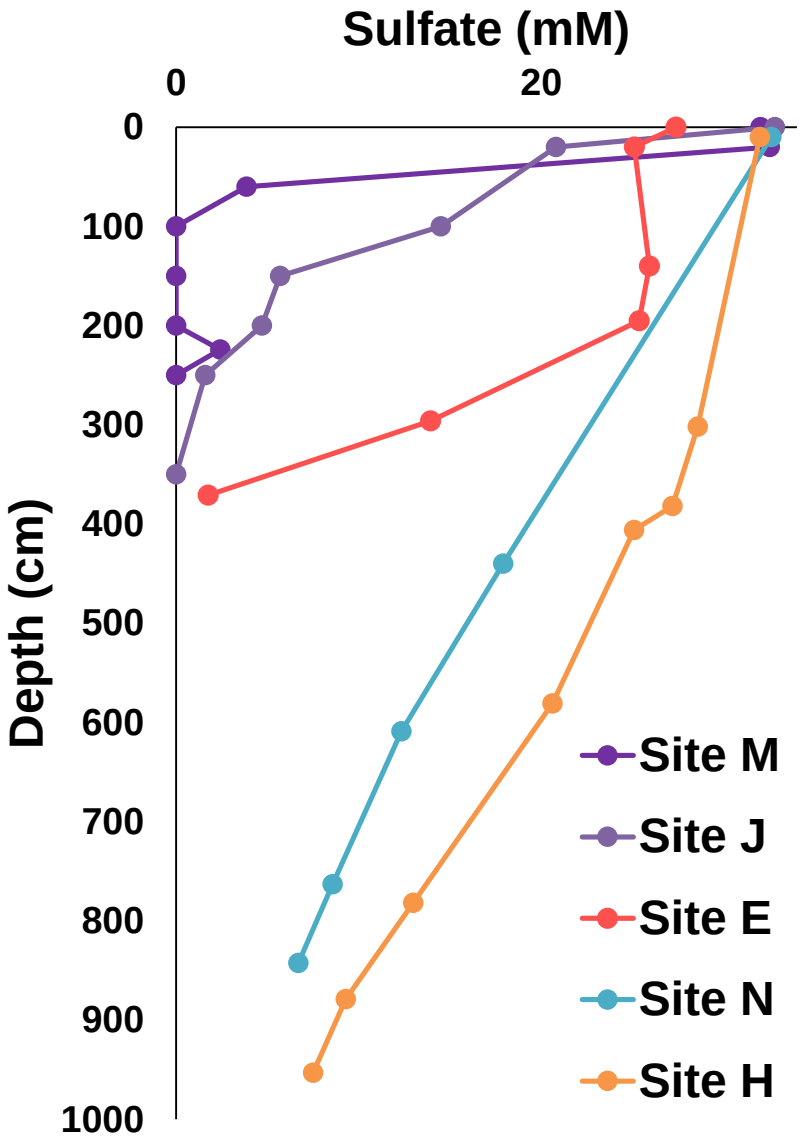
- Organic carbon content
- Gas wetness
- Isotopes ($\delta^{13}C$, δD)
- Sulfate depth profiles (UofC)

Microbial analysis

- >400 samples
- bacterial community composition

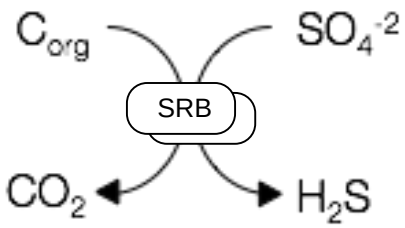


Distinct sulfate profiles for Sites M, J, E



↑ Hydrocarbons = ↑ sulfate reduction

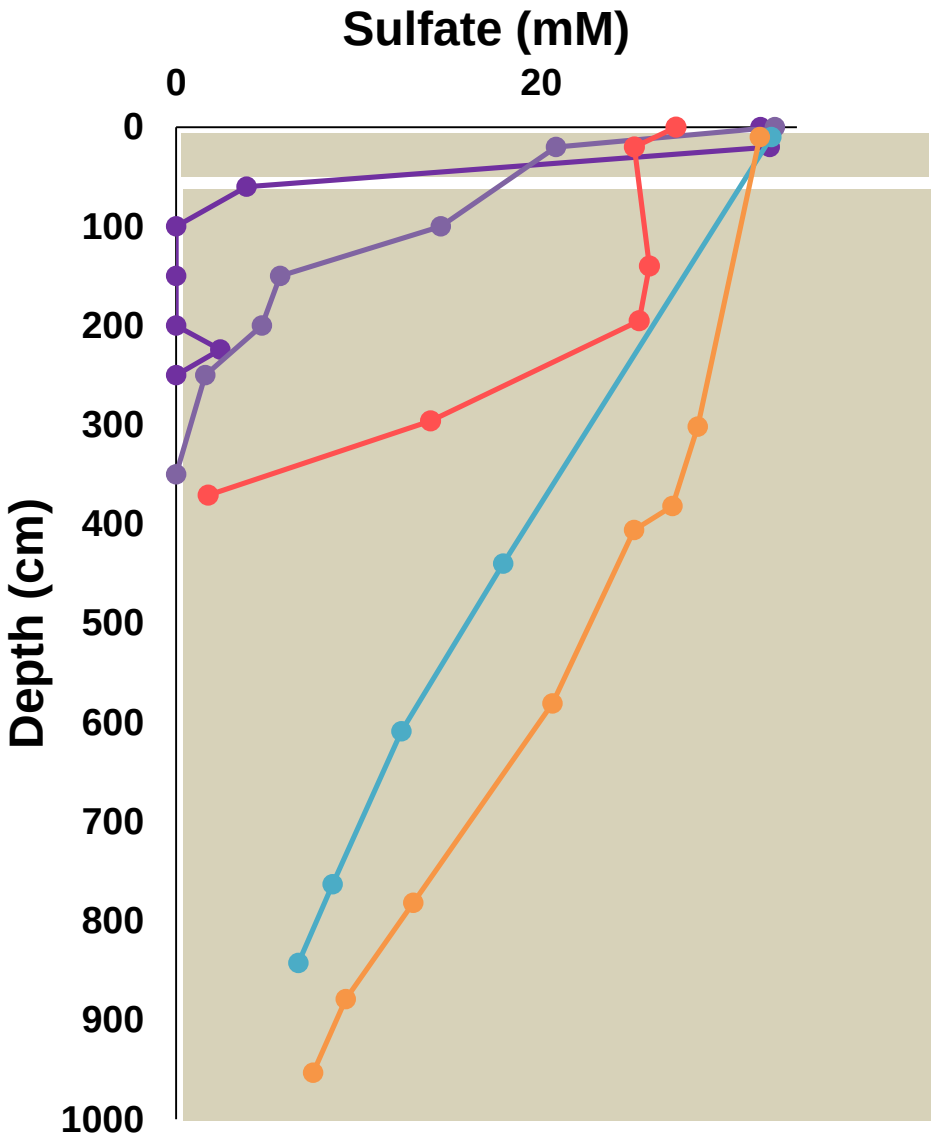
sulfate-reducing bacteria



	Geochem (APT)
Site M	+
Site J	+
Site E	+
Site N	–
Site H	–

Oye Adebayo

Surface and Subsurface samples for DNA



Surface: 0 cm & 20 cm (trigger weight core and piston core)

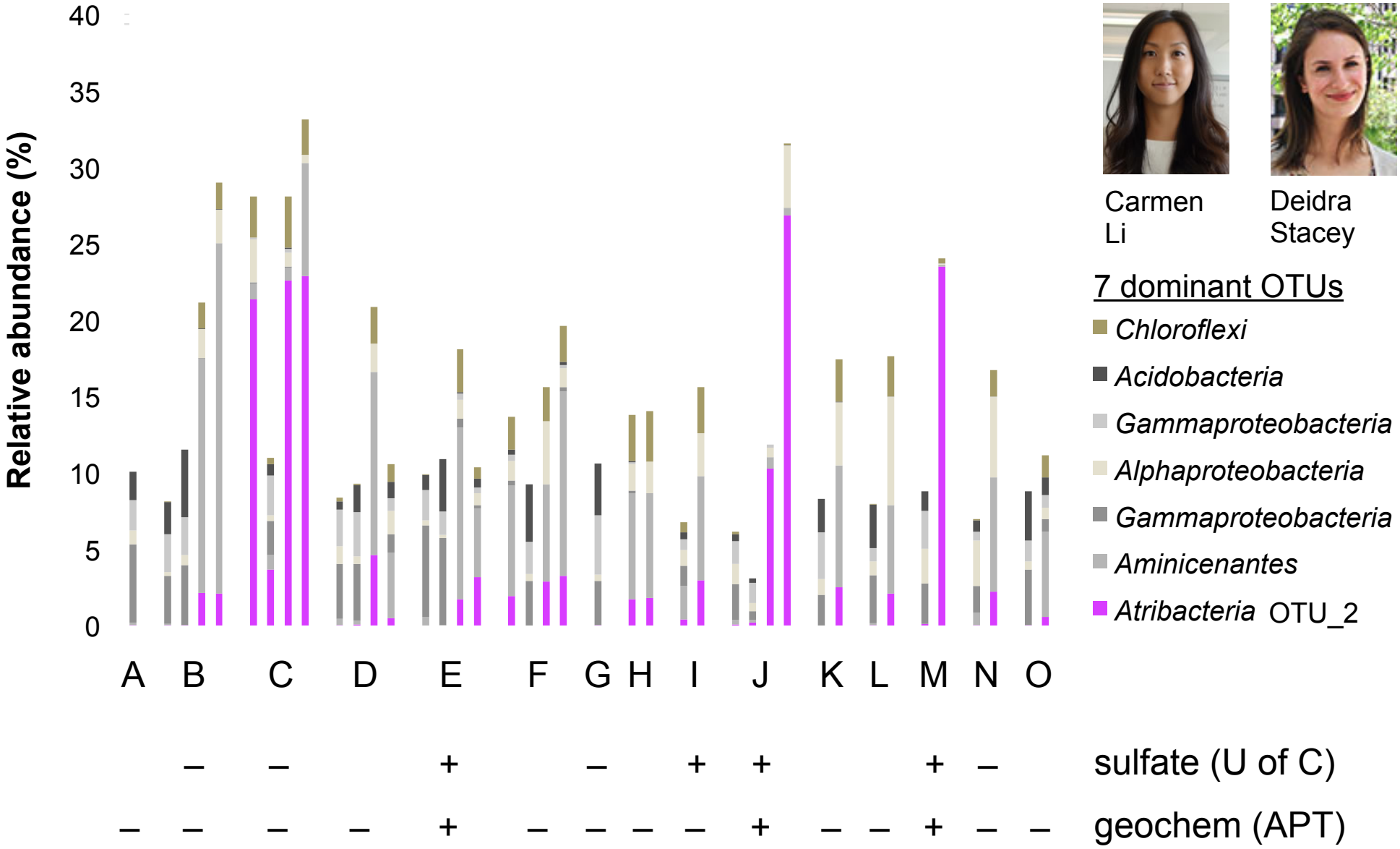
Subsurface: >20 cm (only piston core)

subsurface (>20 cm) *Atribacteria* at 14 sites

Carmen Li

Deidra Stacey

surface (<20 cm) *Atribacteria* at 15 sites



surface (<20 cm) *Atribacteria* at 15 sites

Carmen Li

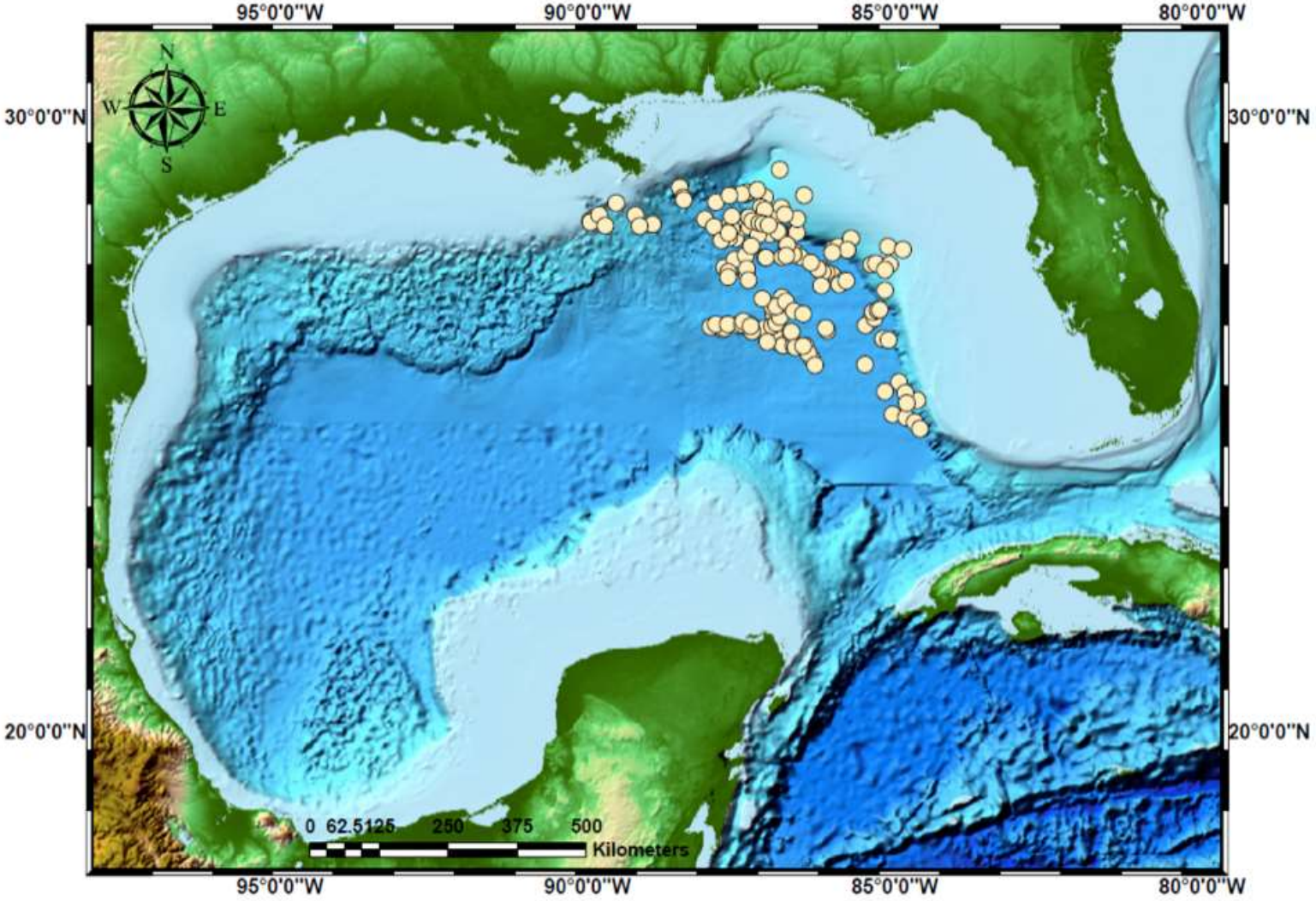
Deidra Stacey

- 173 surface sediments (0-20 cm) stored at -20°C
- petroleum geochemistry analysis (TDI)
- bacterial 16S rRNA gene amplicon libraries (UCalgary)

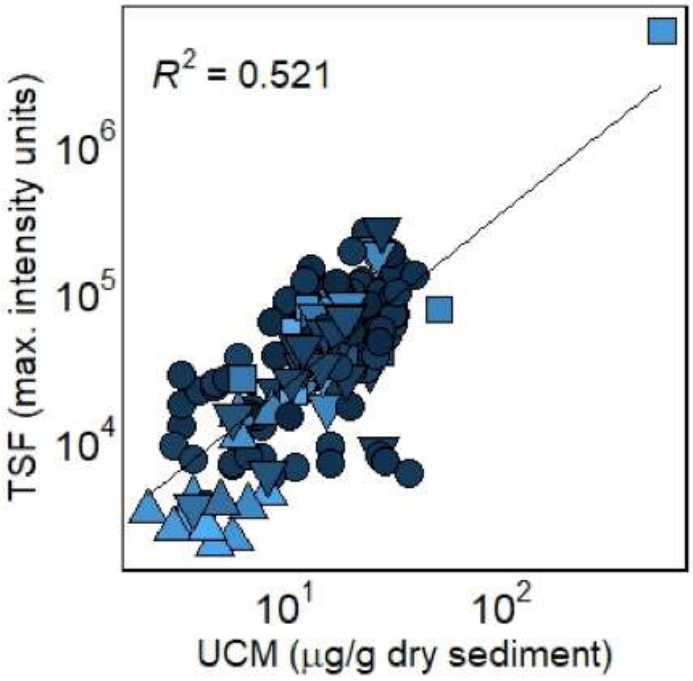
Bernie Bernard

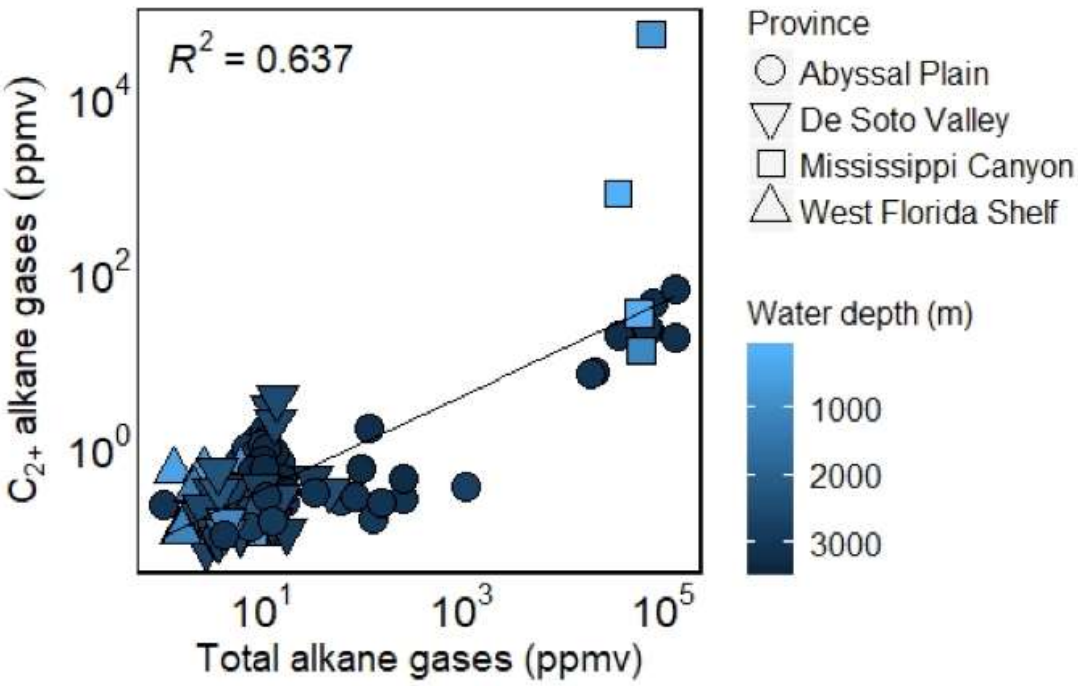
Jim Brooks

Anirban Chakraborty



Oil and Gas parameters in 173 sediment cores





oil positive, gas positive	n = 15	"OG"
oil positive, gas negative	n = 118	"O"
oil negative, gas positive	n = 0	
oil negative, gas negative	n = 40	"N"

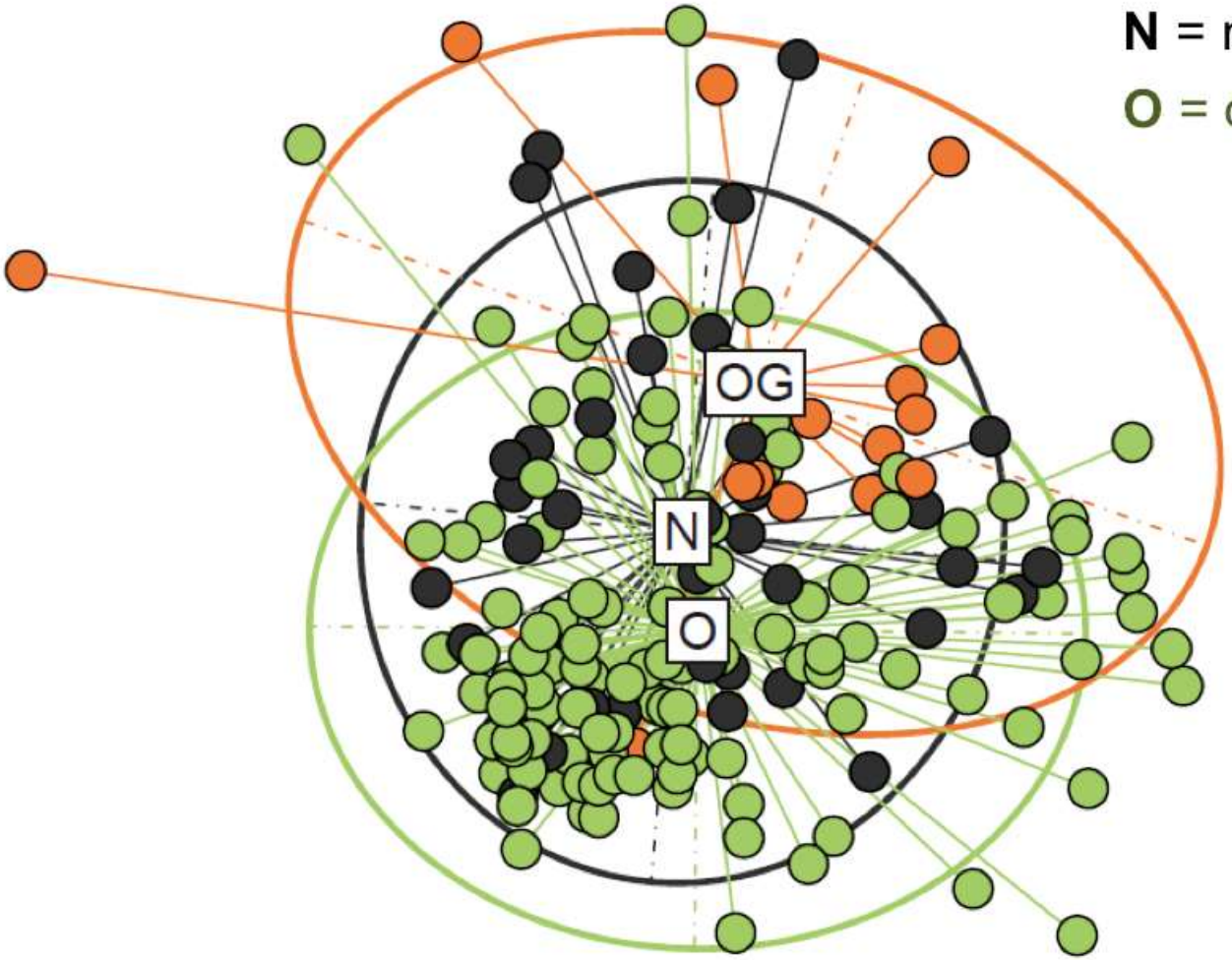
Comparing 173 bacterial communities (NMDS)

(5971 reads per amplicon library)

OG = oil & gas positive (15)

N = negative (40)

O = oil positive (118)

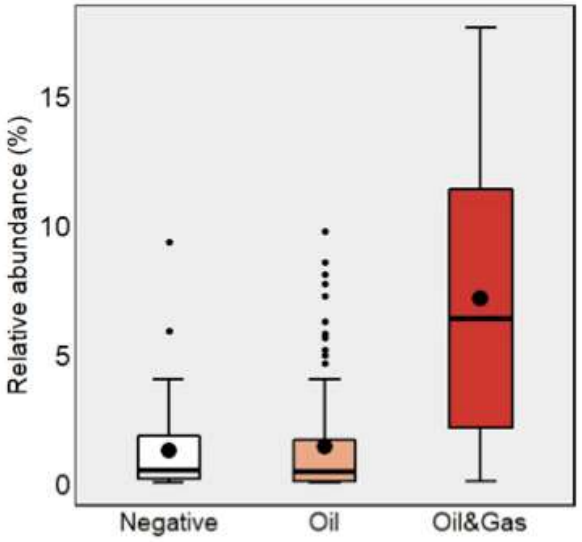


2d stress = 0.18

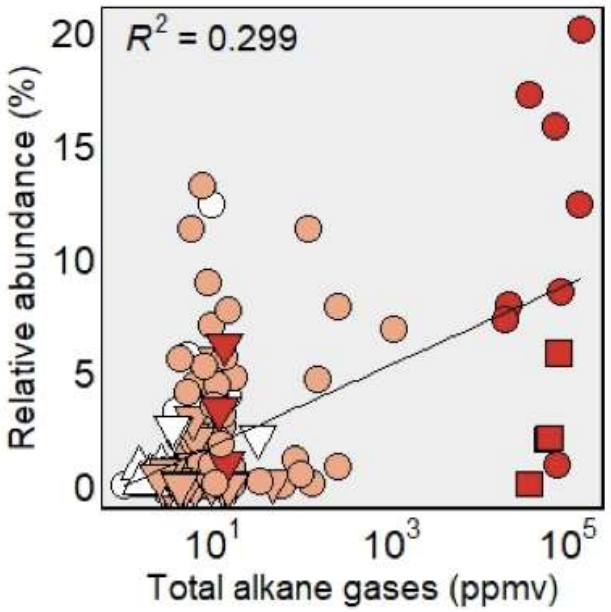
pairwise Permanova

OG vs **O**	p <0.001
OG vs **N**	p <0.001
O vs **N**	p >0.05

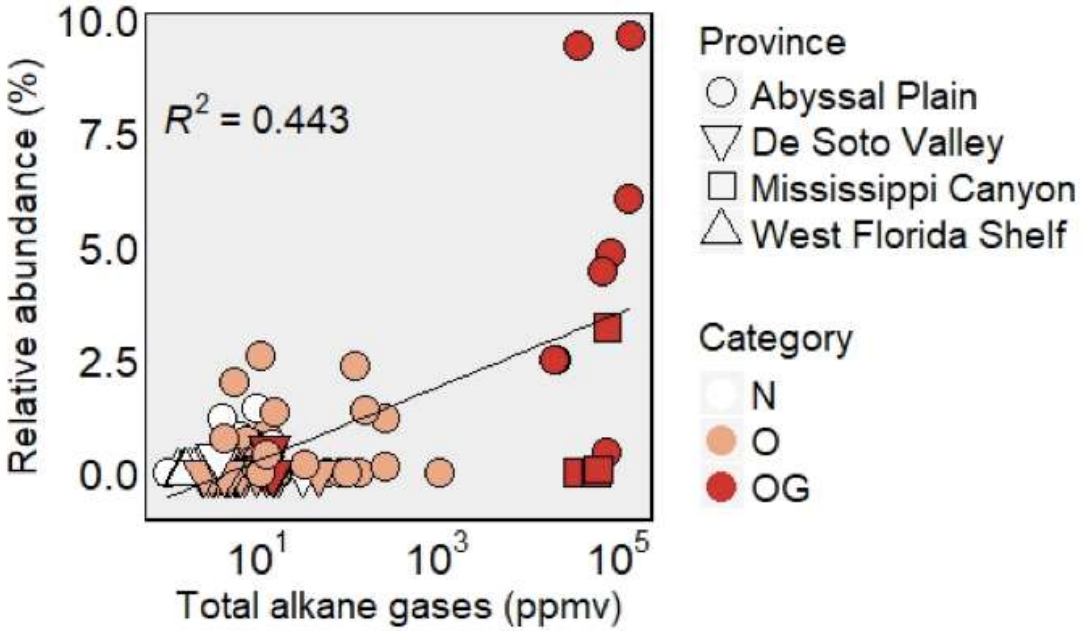
Gulf of Mexico *Atribacteria* (amplicon sequence variants)



(all *Atribacteria*)

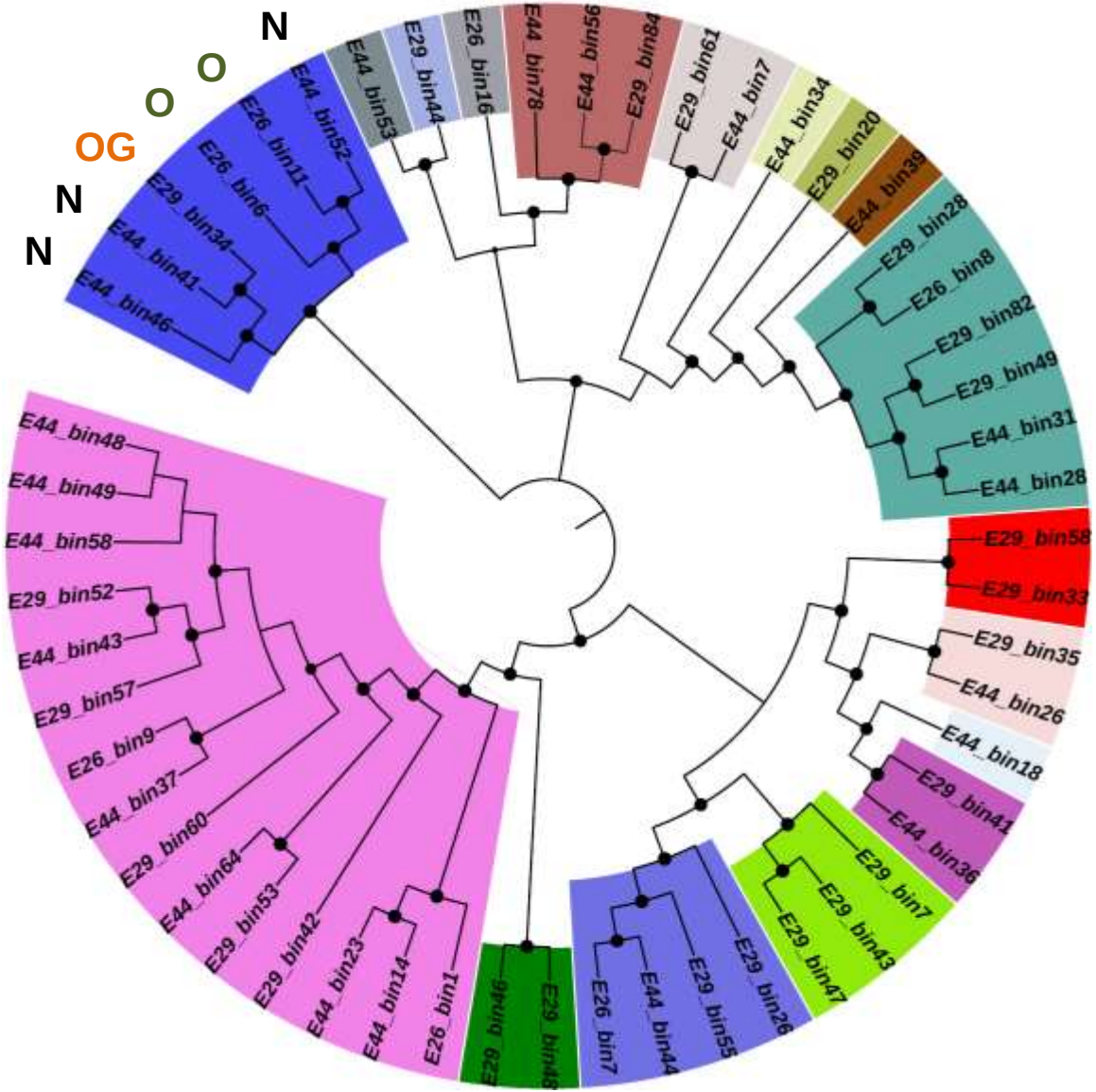


(5 individual *Atribacteria*)



What is the functional role of uncultivated *Atribacteria*? → **Metagenomics**

Xiyang Dong



3 metagenomes

site 26 (O)

site 29 (OG)

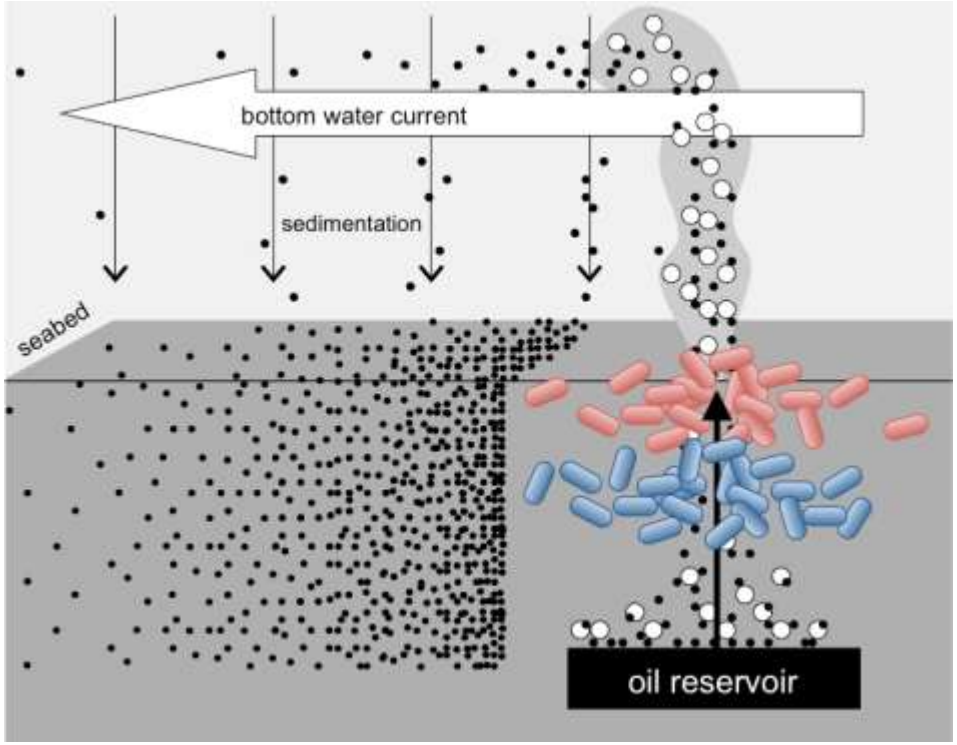
site 44 (N)

- **58 draft genomes from three samples**
- **6 *Atribacteria***

preliminary evidence reveals hydrocarbon degradation genes in 5/6 *Atribacteria*

Outline

1. original observations and interest in hydrocarbon seeps
2. using cold-adapted seabed microbes for prospecting
3. using dormant thermophilic bacterial spores for prospecting



seep-associated *Atribacteria* in both Nova Scotia and Gulf of Mexico sediments may be gas or oil associated, and may be capable of hydrocarbon biodegradation

ongoing detailed analysis of genomic and oil geochemistry datasets

And! using genomics for exploration automatically provides an environmental baseline

MARINE SCIENCE

Deepwater Horizon, 5 years on

Baseline environmental data are crucial for understanding the impacts of oil spills

By Samantha B. Joye

On 20 April 2010, an explosion on the Deepwater Horizon drilling unit initiated an uncontrolled release of oil and gas from the Macondo seafloor well into the Gulf of Mexico that lasted for 87 days. Documenting and tracking the ecological, environmental, and ejected hydrocarbons at a rapid rate, leading to dispersion of oil into droplets and facilitating the formation of deep-water plumes enriched in oil, dissolved gas, and gas hydrate at depths between 900 and 1200 m (*2*, *6*). Plumes were detected with sophisticated chemical sensors lowered through or introduced into the affected waters. Best estimates now suggest that all discharged gas

The Deepwat
resented an ur
discharge into o
historical becaus
cation of chemic
sea surface and d
discharging well
cal dispersants
biodegradation
slicks into smalle
debated (*10*, *11*).
the benefits of
creased dissoluti
reduction of oil
presumed facilit
tion—offset the
ronmental impa
uncertainties, ex

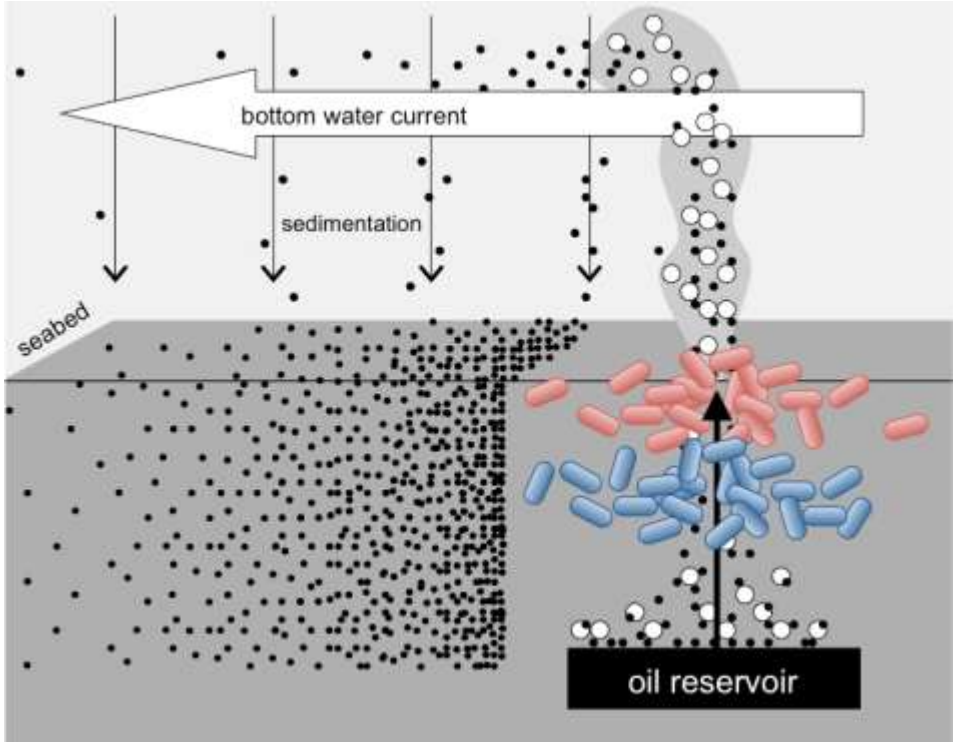
Deidra Stacey

optimizing conditions for baseline data collection

2 Nova Scotia cores × triplicate sediment aliquots × 3 DNA extraction protocoss × 3 PCR primer pairs

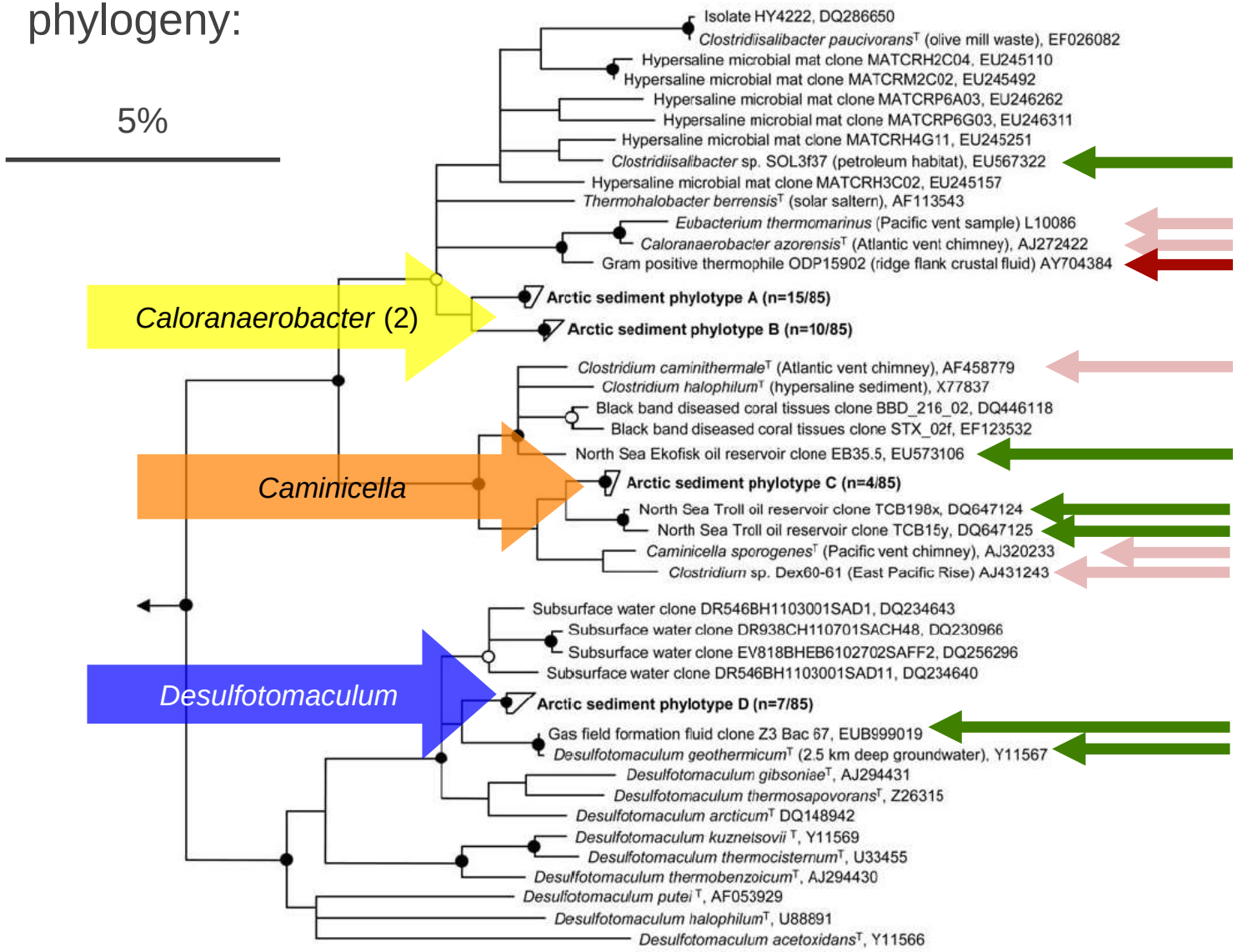
Outline

1. original observations and interest in hydrocarbon seeps
2. using cold-adapted seabed microbes for prospecting
3. using dormant thermophilic bacterial spores for prospecting



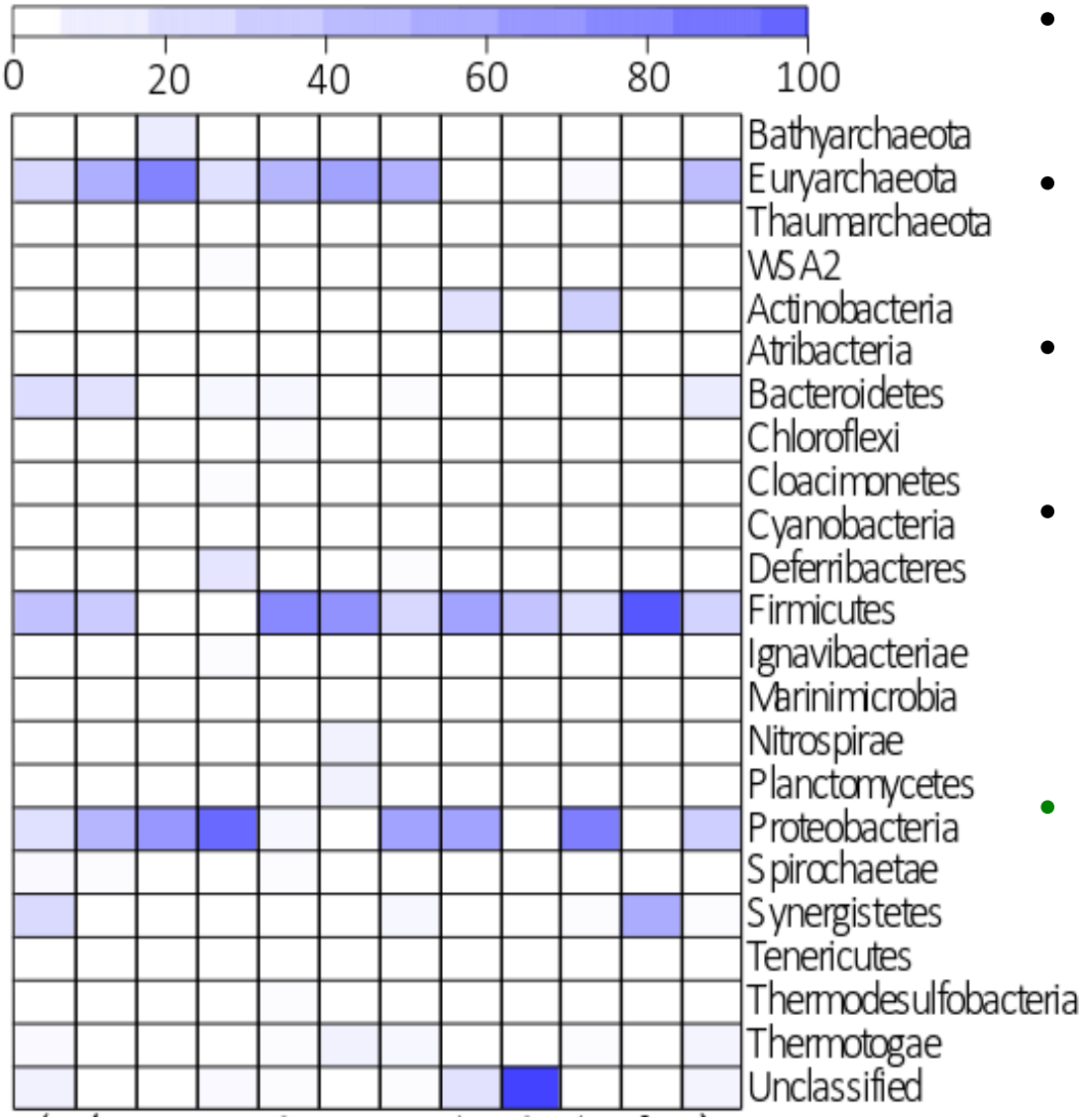
Are spore-forming *Firmicutes* really prevalent in oil reservoirs?

rRNA gene phylogeny:



✓ warm
✓ anoxic
✓ fluid flow

Are spore-forming *Firmicutes* really prevalent in oil reservoirs?

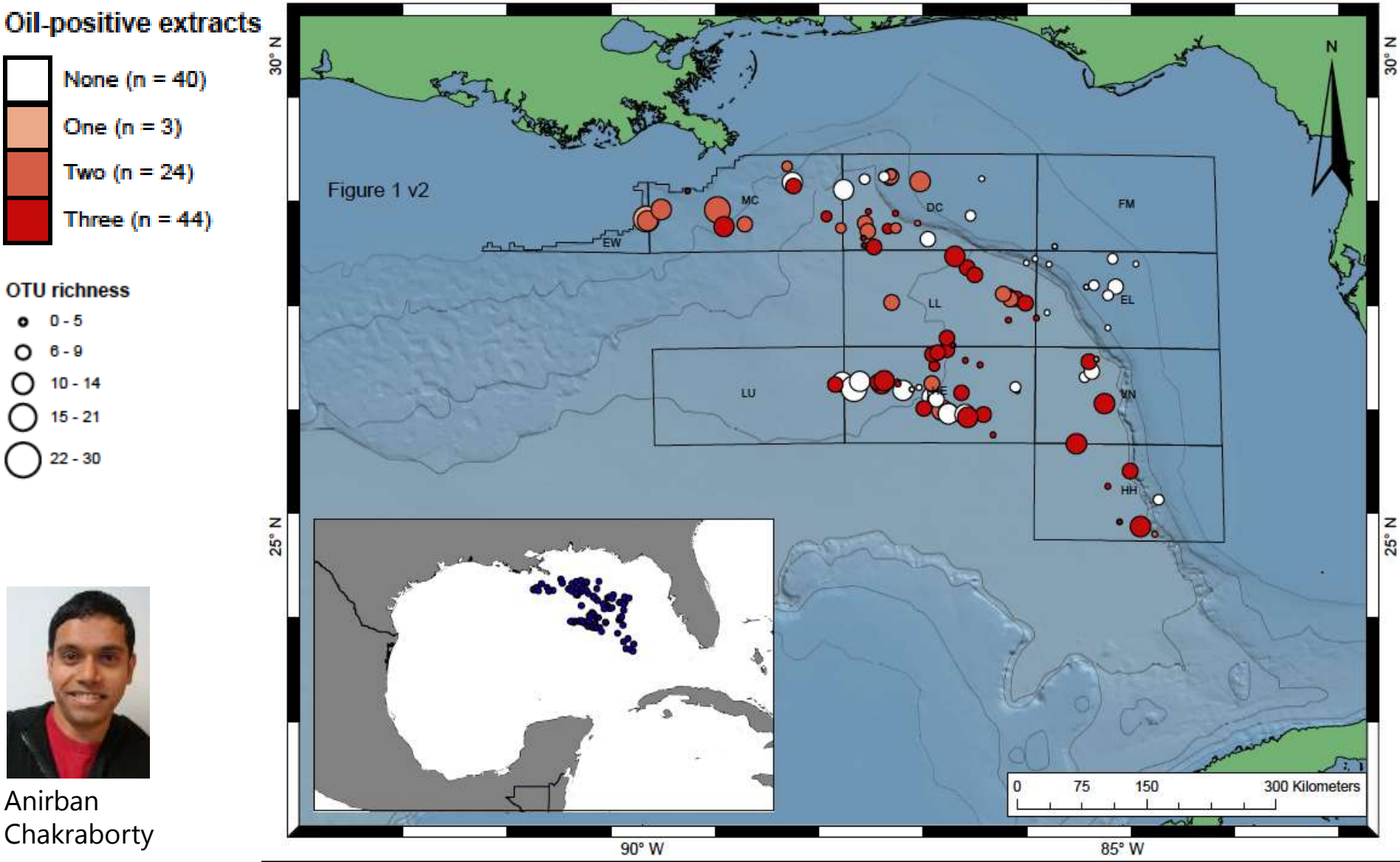


- ~75 studies with amplicon libraries from oil reservoirs
- only 12 from formation water (no water injection)
- therefore truly indigenous communities analysed
- *Firmicutes* indeed prevalent

- **next step: expand the study to include *produced water* samples as well as formation waters**

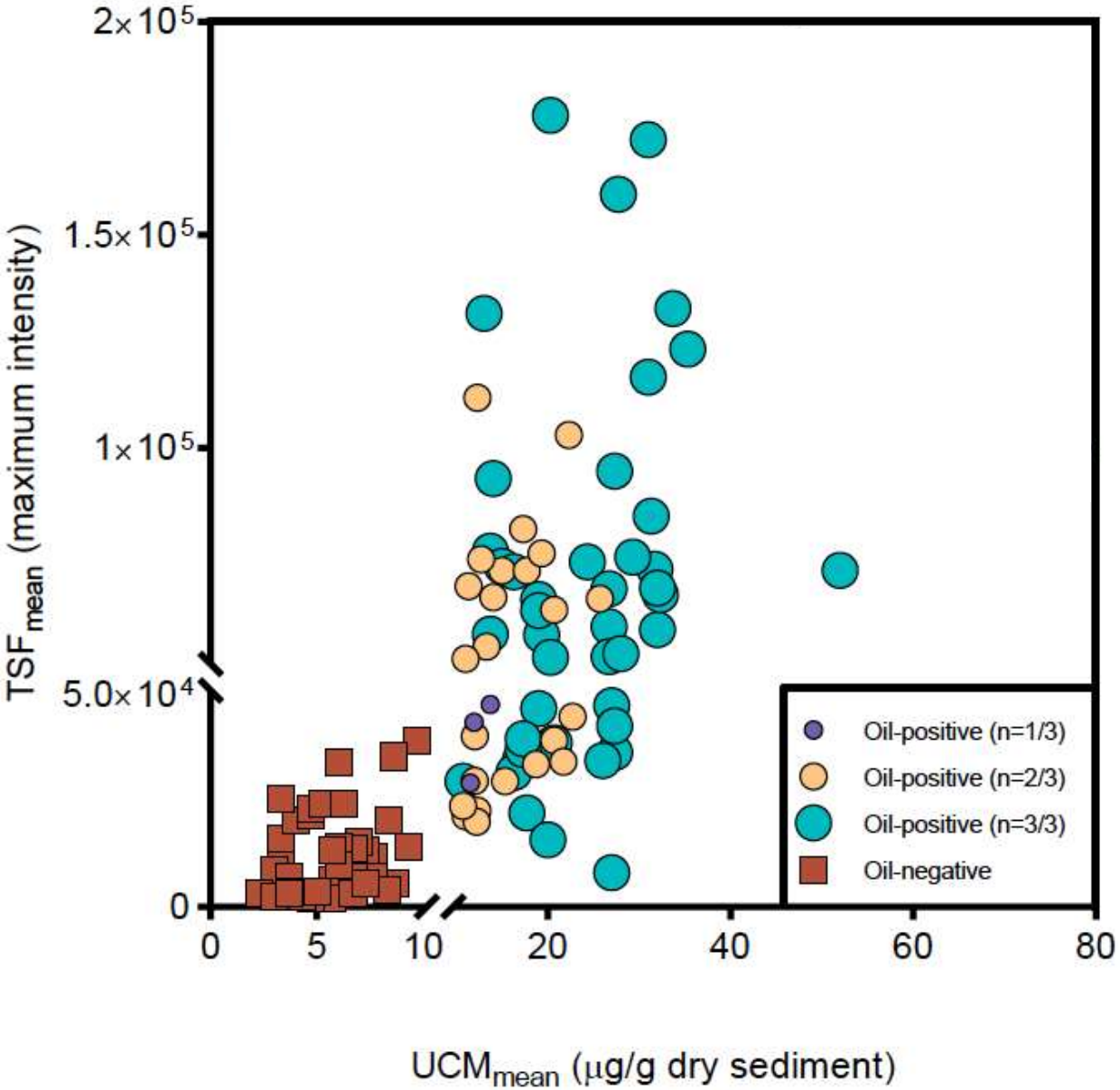
Daniel Gittins

111 surface sediments heated to 50˚C

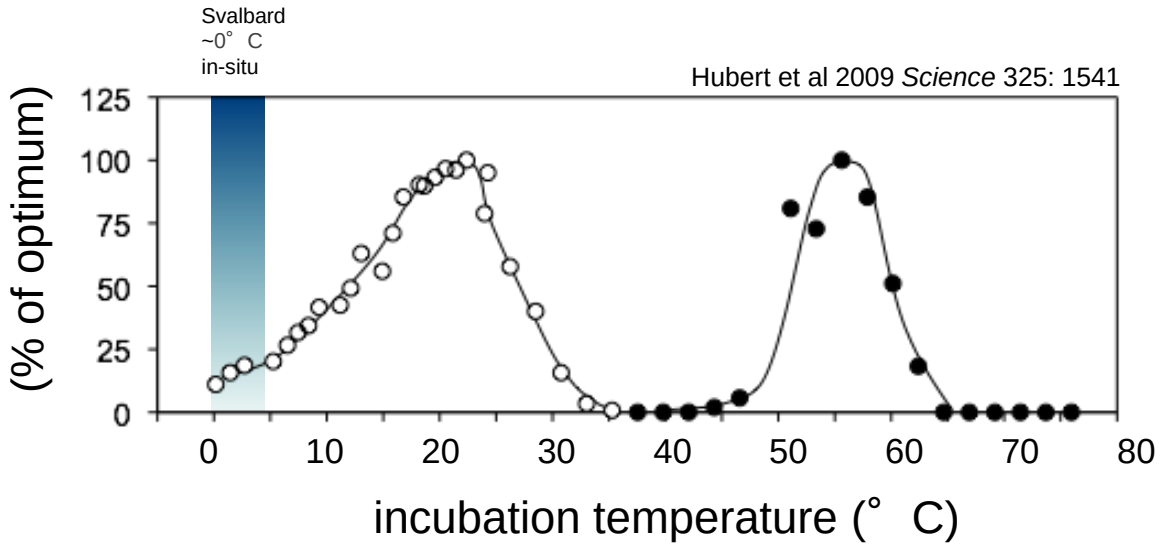


Anirban Chakraborty

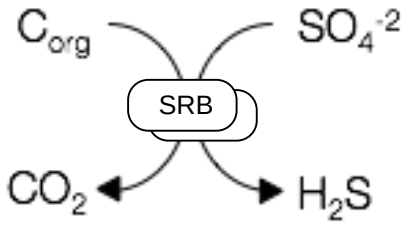
71 oil-positive and 40 oil-negative

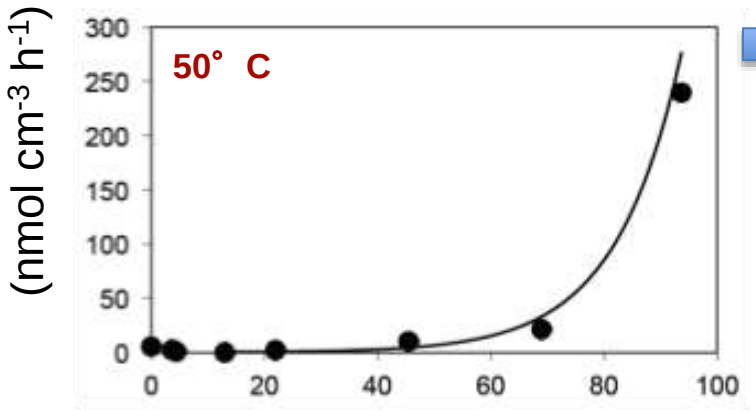


Thermophilic Bacteria in cold sediments



sulfate-reducing bacteria

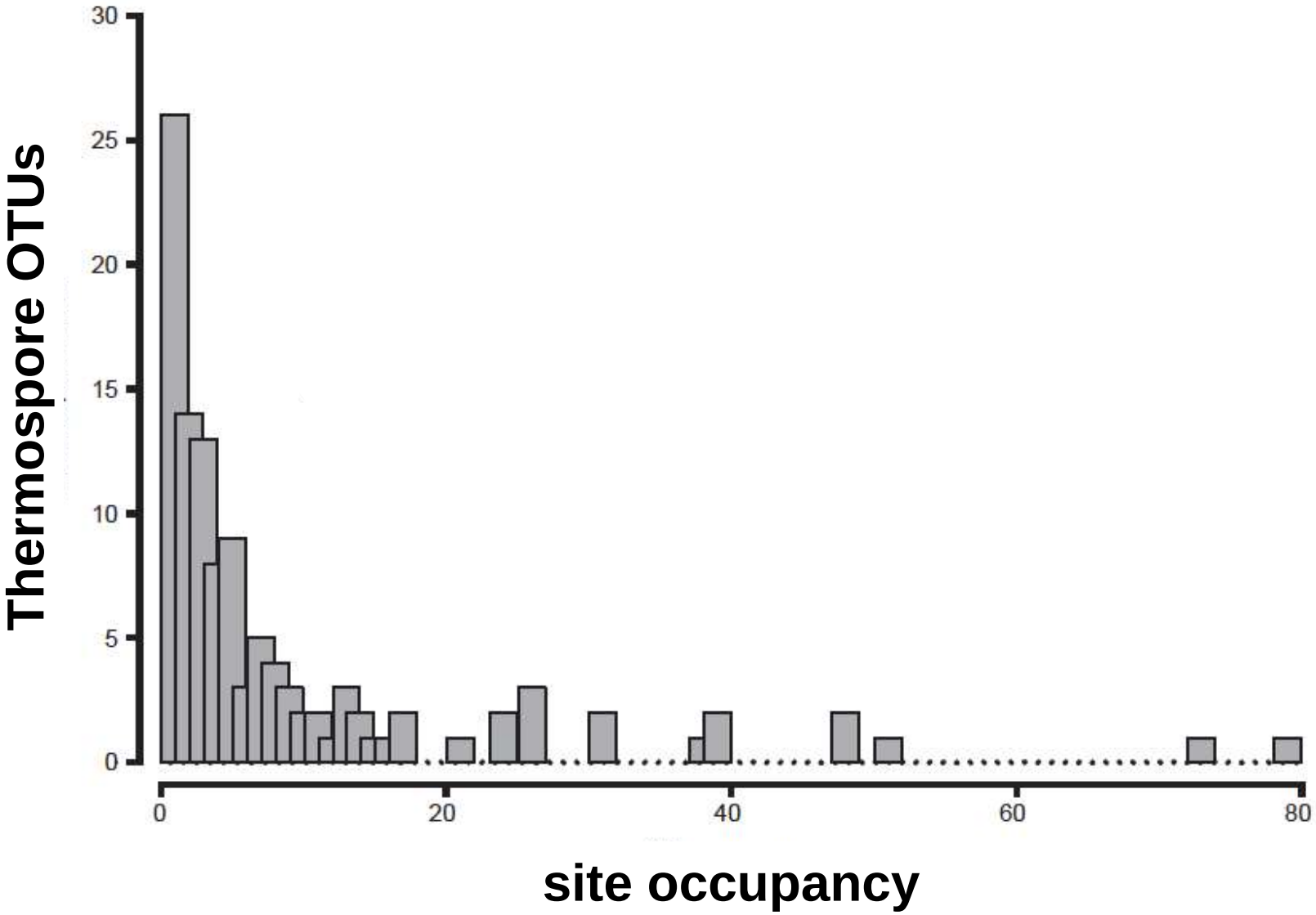


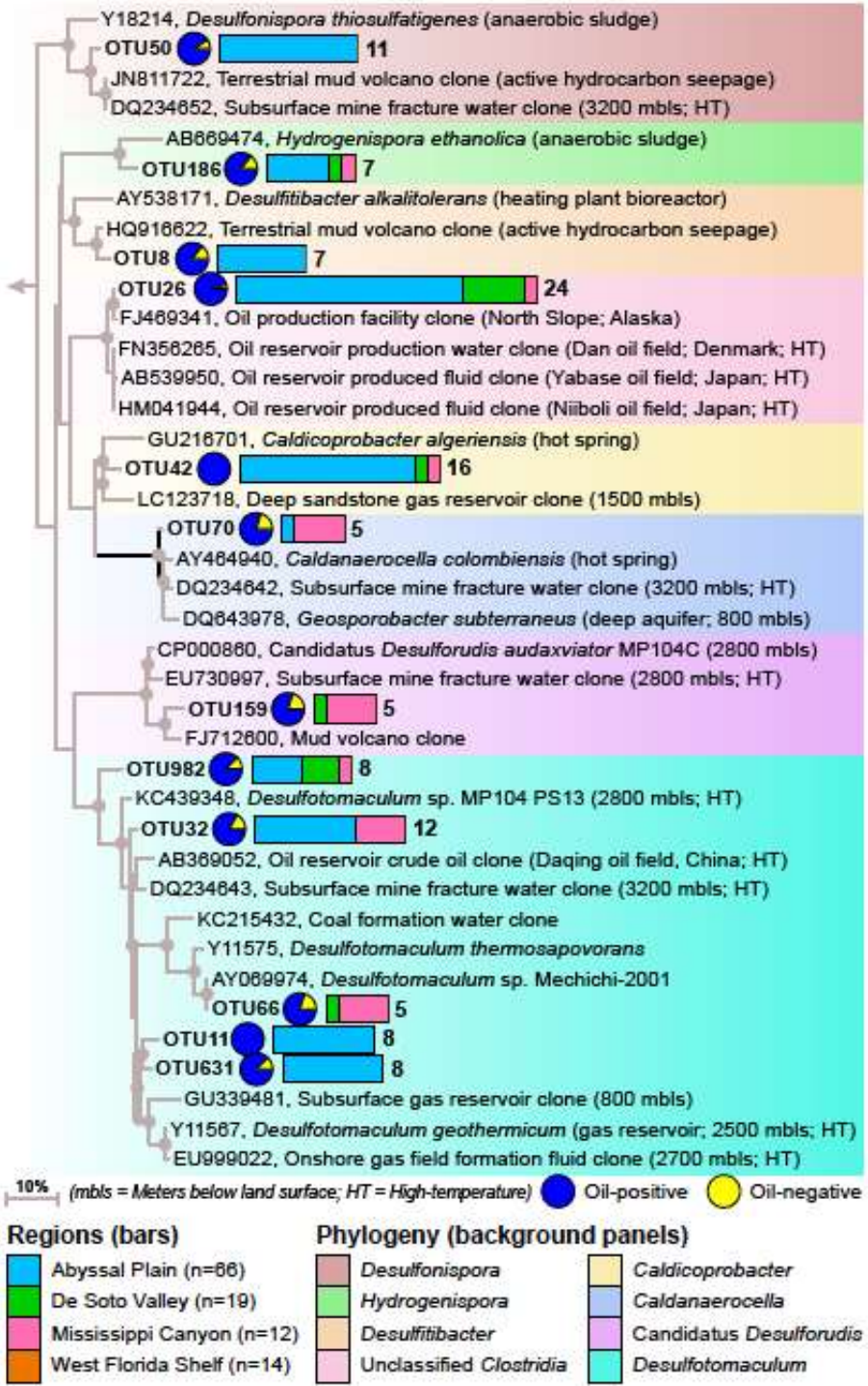


DNA sequencing to characterize the microbial community after several days at 50° C

1-30 thermospore OTUs per sediment

115 thermospores detected @ 50˚ C



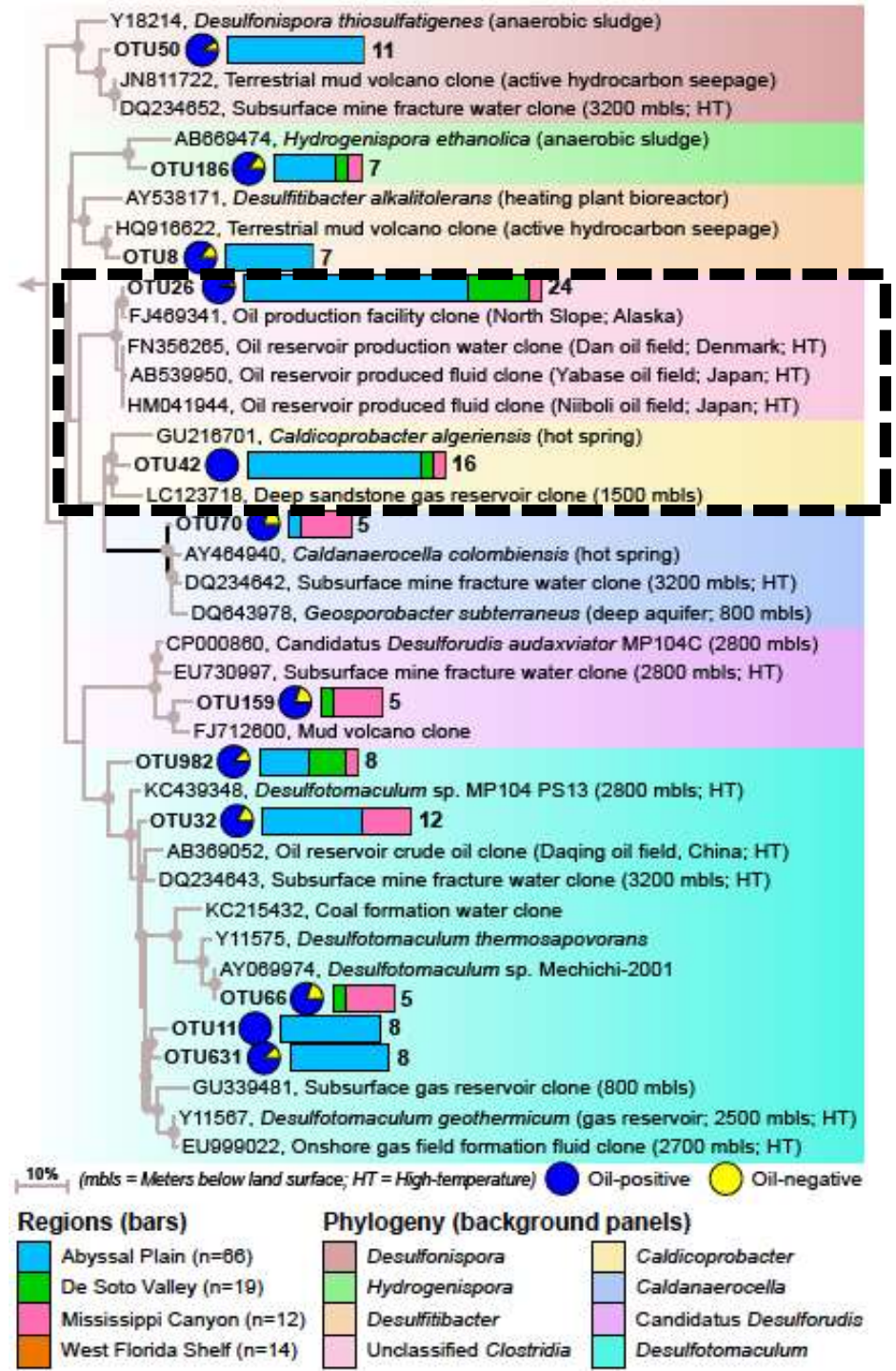


top 12 oil-associated thermospores in EGoM

- ≥80% detection in oil-positive locations

oil-positive

oil-negative

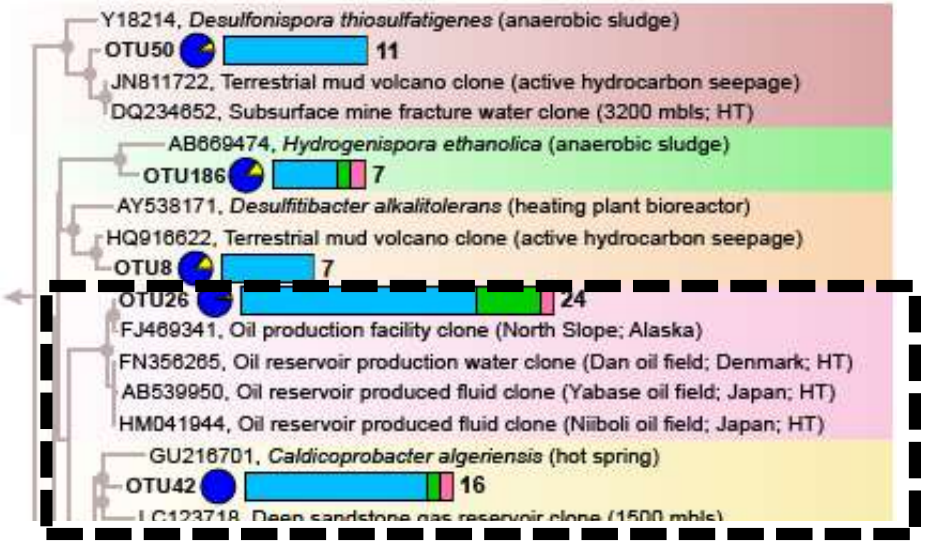


top 12 oil-associated thermospores in EGoM

- ≥80% detection in oil-positive locations

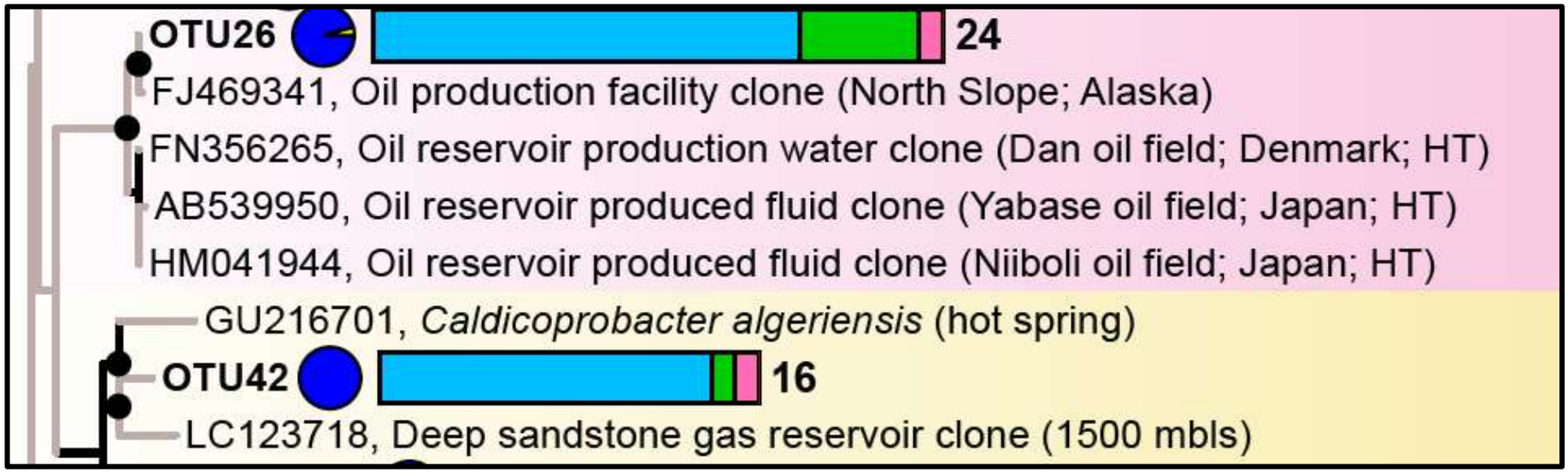
oil-positive

oil-negative



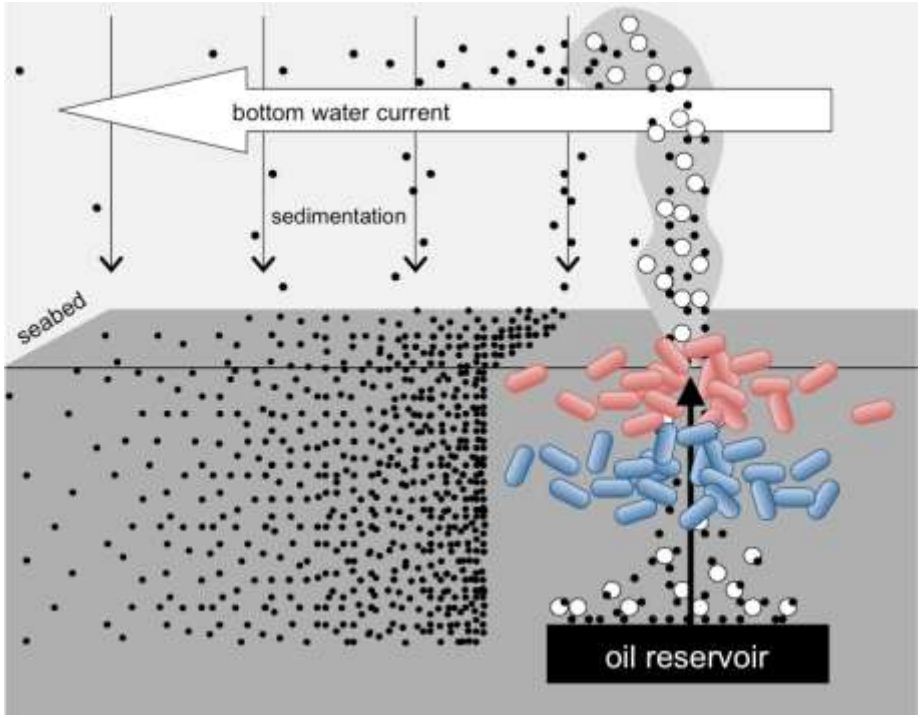
top 12 oil-associated thermospores in EGoM

- ≥80% detection in oil-positive locations



Outline

1. original observations and interest in hydrocarbon seeps
2. using cold-adapted seabed microbes for prospecting
3. using dormant thermophilic bacterial spores for prospecting



Conclusion: mutually exclusive strategies for seep detection are showing promising results

other applications?

thermospores as messengers from the reservoir?