Molecular Microbial Tools for Future Oil Spill Monitoring

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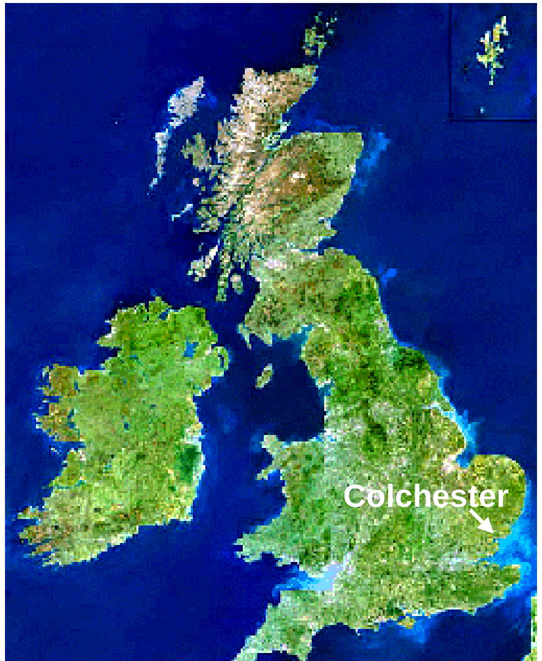
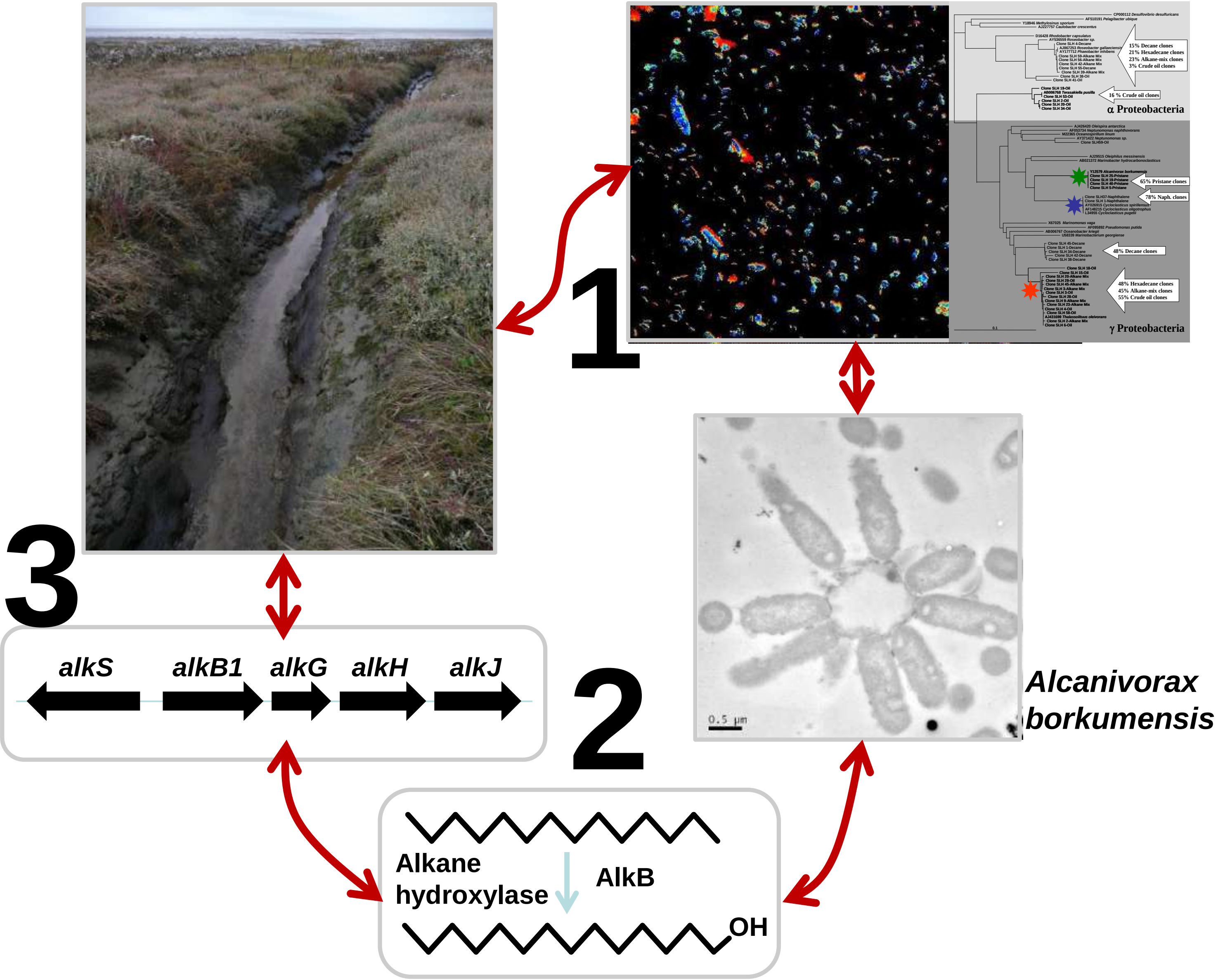
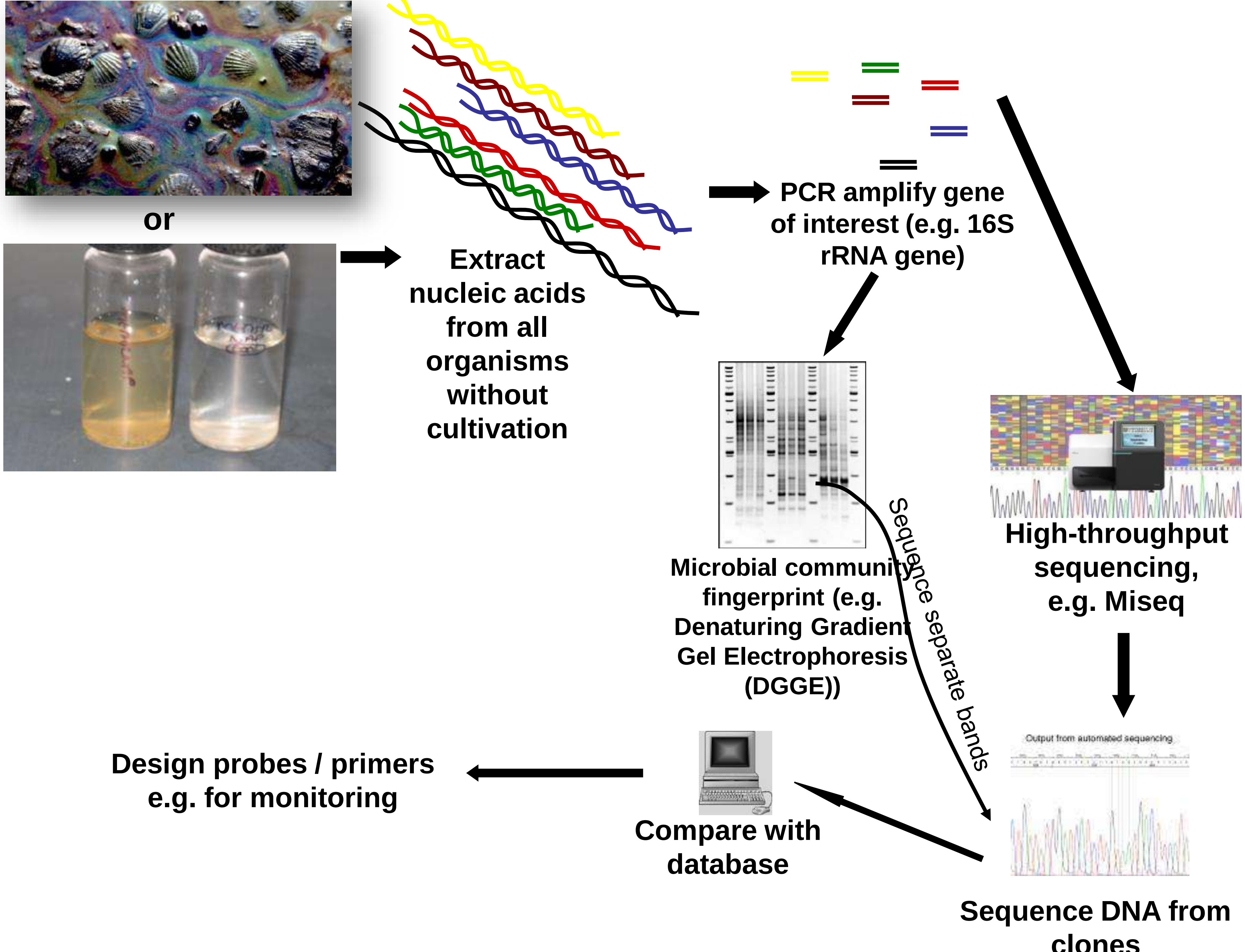


Microbial activity is key to natural attenuation (& genetics / biochemistry for bio-monitoring)



1. Identifying microbial communities and the main hydrocarbon degraders


or
Extract nucleic acids from all organisms without cultivation
PCR amplify gene of interest (e.g. 16S rRNA gene)
Microbial community fingerprint (e.g. Denaturing Gradient Gel Electrophoresis (DGGE))
Sequence separate bands
High-throughput sequencing, e.g. Miseq
Output from automated sequencing
Sequence DNA from clones
Compare with database
Design probes / primers e.g. for monitoring

High-throughput sequencing pipeline at Essex

Fundamental Questions

Which **microbe(s)** are degrading which **hydrocarbons** in which **locations** and at which **times**

What are the effects of **different treatments** on microbial community composition?

Do bottle experiments **scale** to the wider environment?

Increasing environmental complexity

M93

Who does what ?

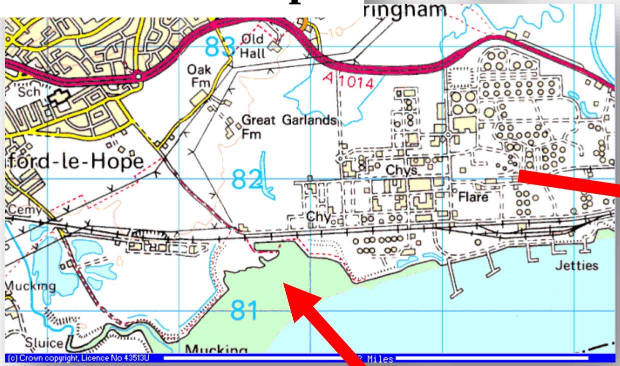

Environmental Microbiology (2007) 9(1), 165–176
doi:10.1111/j.1462-2920.2006.01125.x

Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK

Boyd A. McKew,[1*] Frédéric Coulon,[1]
A. Mark Osborn,[2] Kenneth N. Timmis[3] and
Terry J. McGenity[1]

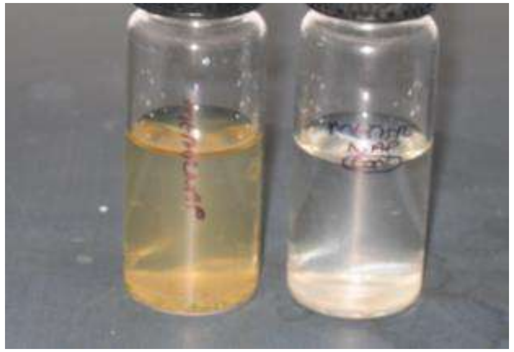
enriched microcosms. The communities in *n*-decane microcosms differed from those in microcosms supplemented with less volatile alkanes, with a phy-

Stanford le Hope



Saltmarsh

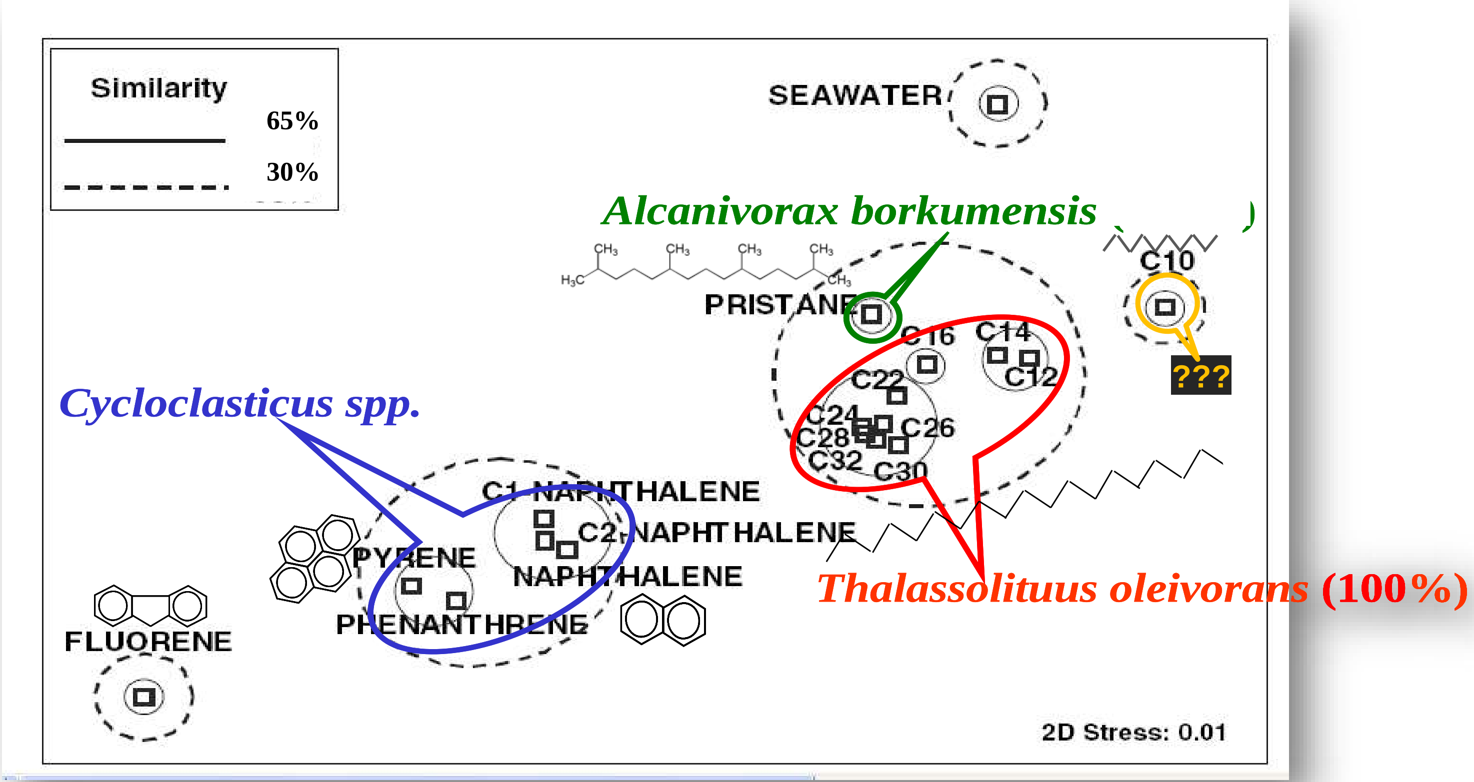
e.g. Methyl-naphthalene



Live

Killed contol

Molecular Tools (DGGE / Clone-Libraries and Sequencing) targetting 16S rRNA genes identifies niche partitioning based on hydrocarbon type



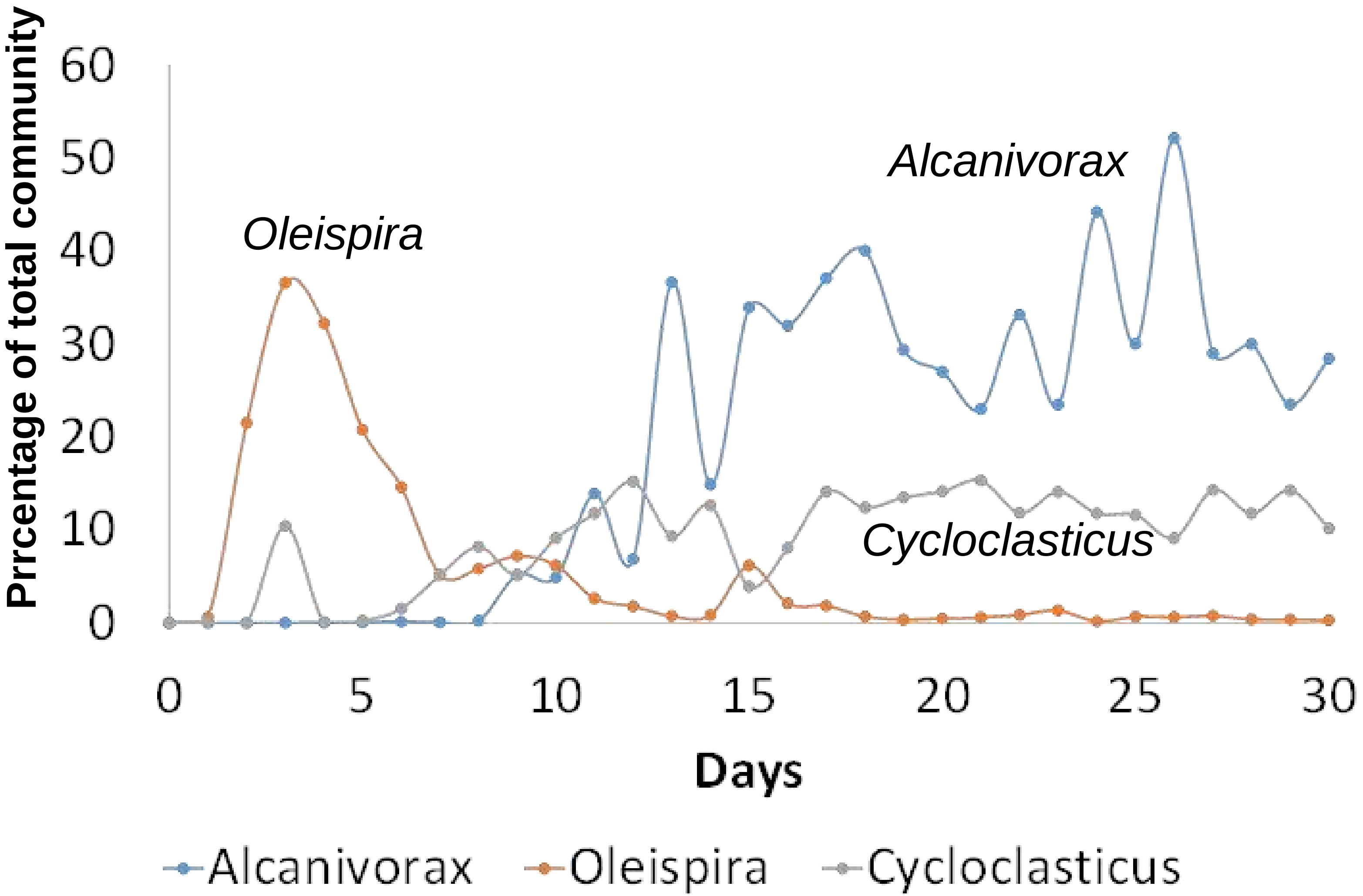
- Straight-chain alkanes – *Thalassolituus*
- Branched alkane – *Alcanivorax*
- PAHs – *Cycloclasticus*

Other genera involved!

- Unknown genera are also important

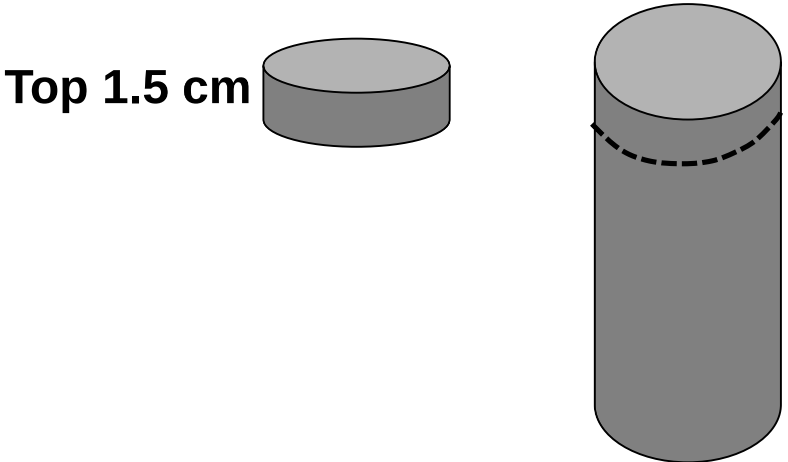
McKew *et al* (2007) *Environ Microbiol*: 165–176

Succession experiments show that *Oleispira* is an early coloniser



Scaling up –

Mudflat cores +/- crude oil were subjected to a tidal cycle, and sub-sampled 7 times over 21 days

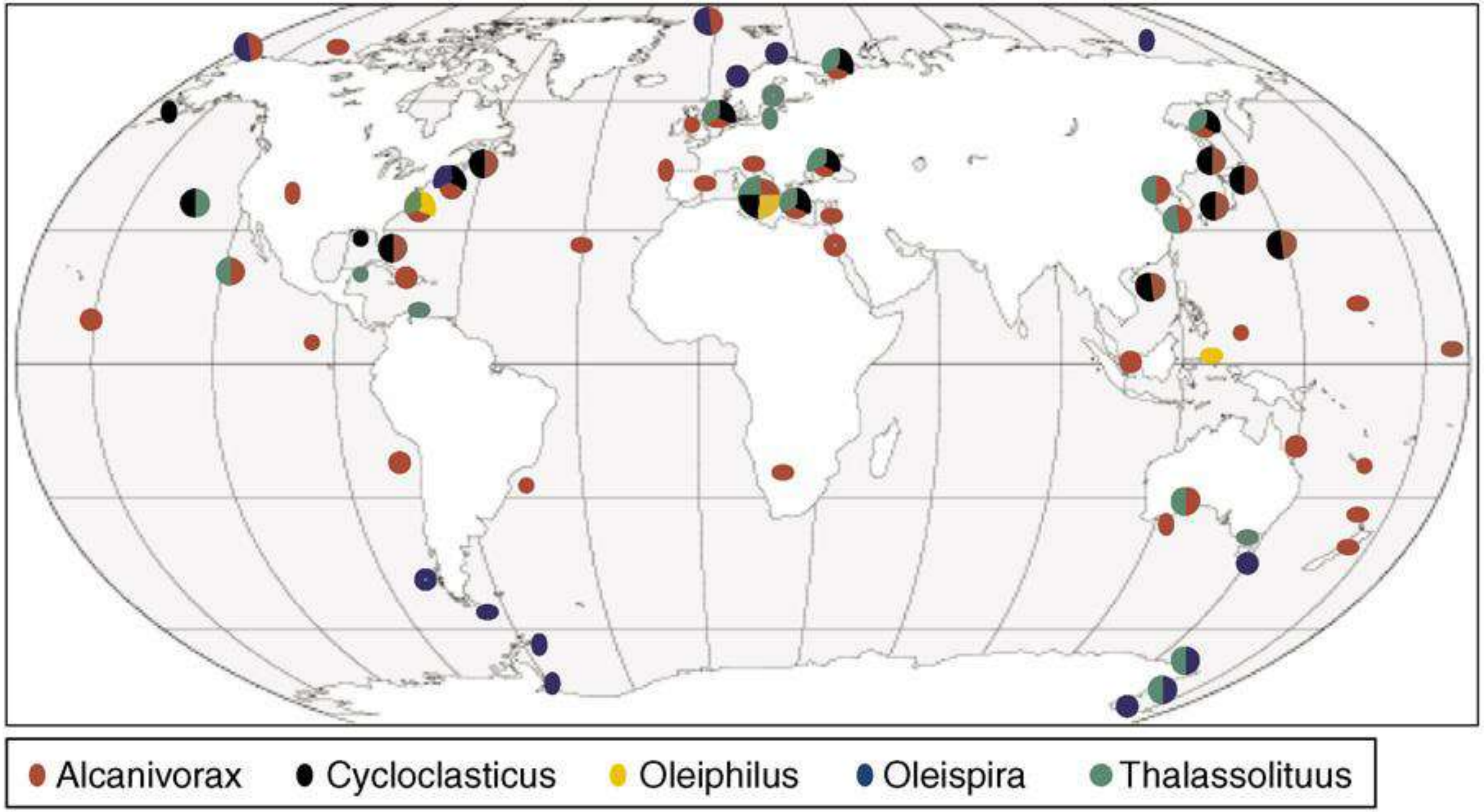


Many of the same genera were detected only in the oiled mudflat sediments

Genus	% sim	CL 2	COL 2	COL 12	CL 21	COL 21
		Percentage sequences from each sample				
Alcanivorax	98	0.11	1.55	2.18	0.00	0.67
Marinobacter	98	0.00	0.00	1.25	0.00	0.13
Oleibacter	95	0.00	0.00	1.87	0.00	0.47
strain ME113	99	0.00	1.55	2.18	0.00	0.87
Cycloclasticus	100	0.00	1.16	1.56	0.00	0.20

Phylogenetic clusters enriched in the upper 1.5 cm of mudflat sediment after oil addition, based on 454 pyrosequencing analysis

Coulon F et al. (2012) *Appl Environ Microbiol* 78: 3638-3648.

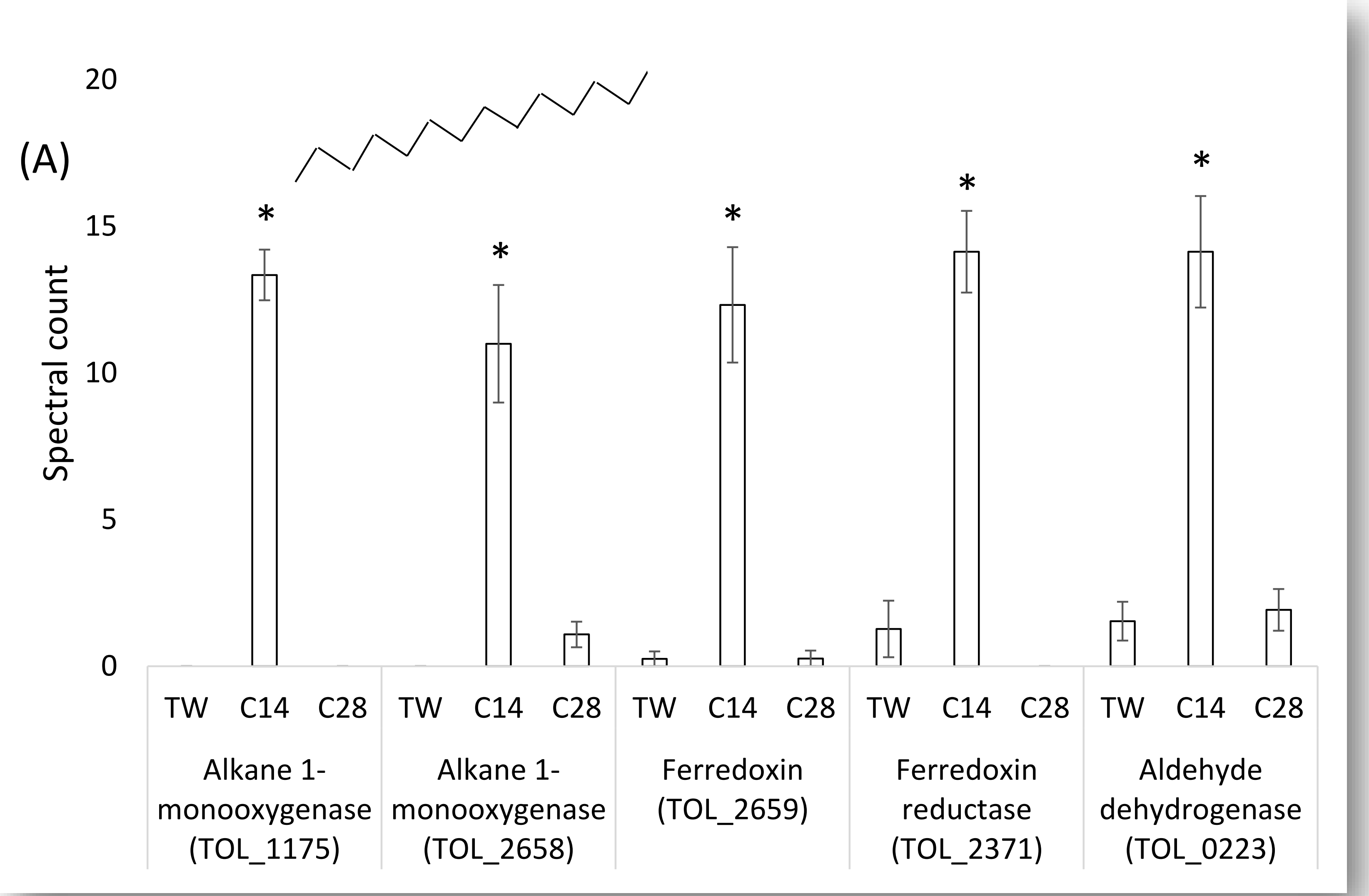


Yakimov et al (2007)

Current Opinion in Biotechnology

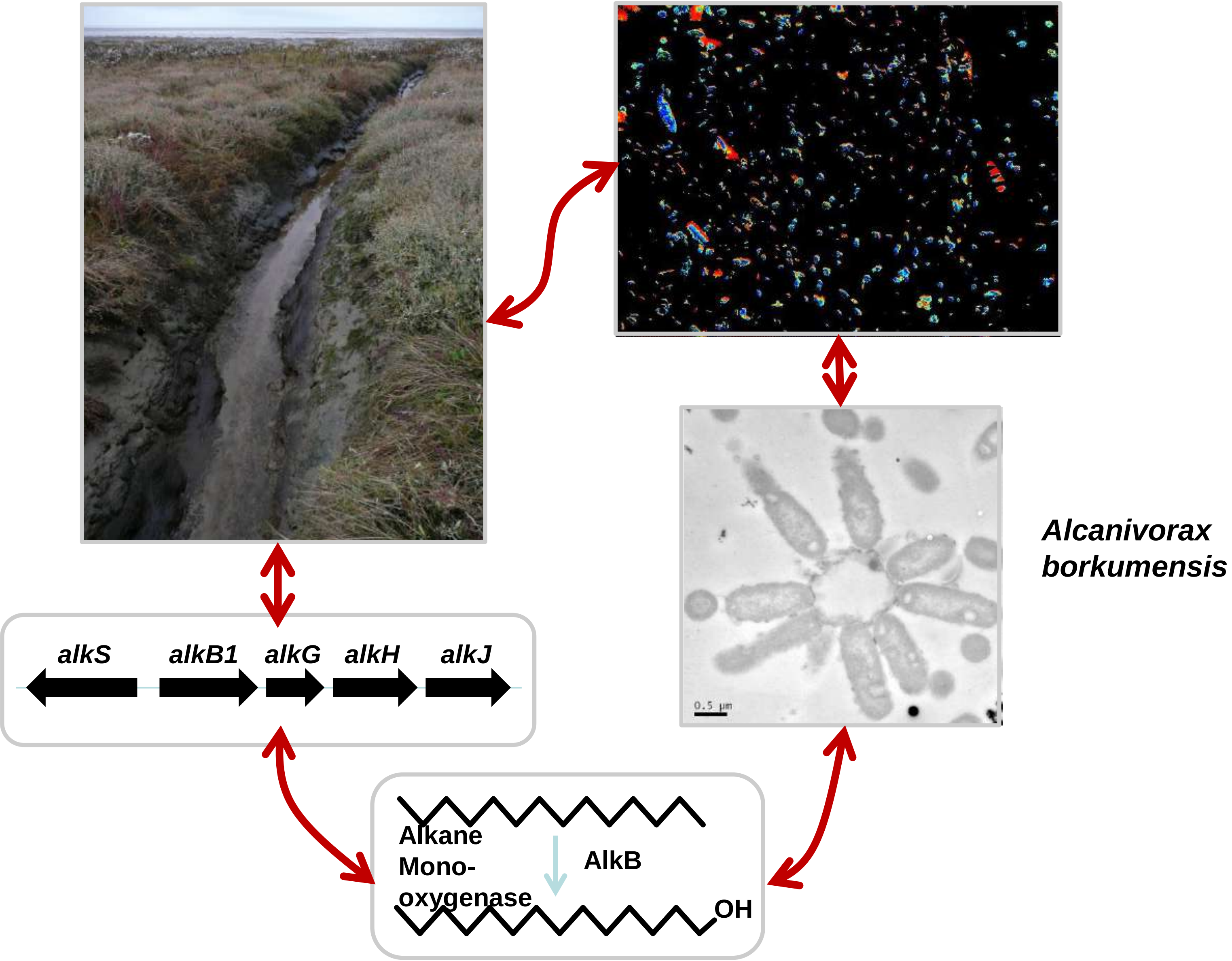
We now know about the ecology of the main hydrocarbon degraders, and are gaining insights into molecular mechanisms of hydrocarbon degradation ?

2. Proteomics to identify proteins preferentially synthesised when *Thalassolituus oleivorans* is grown on medium-chain alkanes



Gregson B & McKew BA unpub.

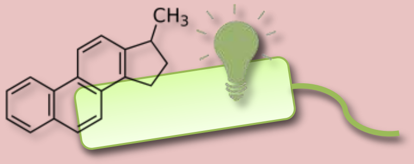
Microbial activity is key to natural attenuation (& genetics / biochemistry for bio-monitoring)



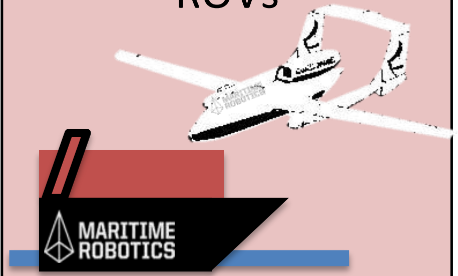
3. Microbial / Molecular Monitoring

Rapid id of sentinel species

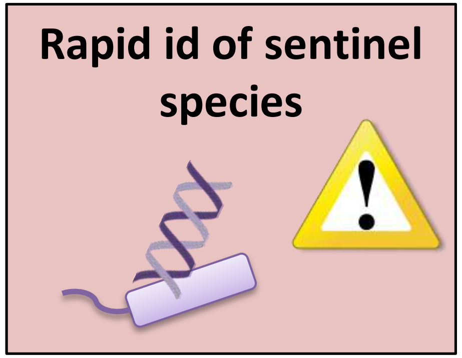
Bioreporters



ROVs



Rapid id of sentinel microbes / communities



Specific positive impacts – focussing on hydrocarbon degraders

- Phylogenetic genes, e.g. *Oleispira* 16S rRNA genes
- Functional genes, e.g. *alkB* coding alkane monooxygenase
- Gene expression may be more informative

e.g. using Quantitative PCR; hybridisation-microarrays

Specific negative impacts– focussing on sensitive species

- Phylogenetic genes, e.g. SAR-11 16S rRNA genes

e.g. using Quantitative PCR

General impacts – community-level analysis

- Converting to a metric, e.g. Lozada et al. (2014), who scored phylotypes according to whether they were from genera that had been shown to include hydrocarbon-degrading species.

e.g. using high-throughput sequencing

Lozada M et al. (2014) *Microbes &Environments* 29: 269–276

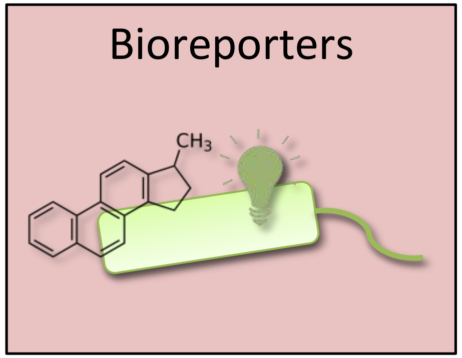
Developing robust, simple, field-applicable methods

Rapid id of sentinel species

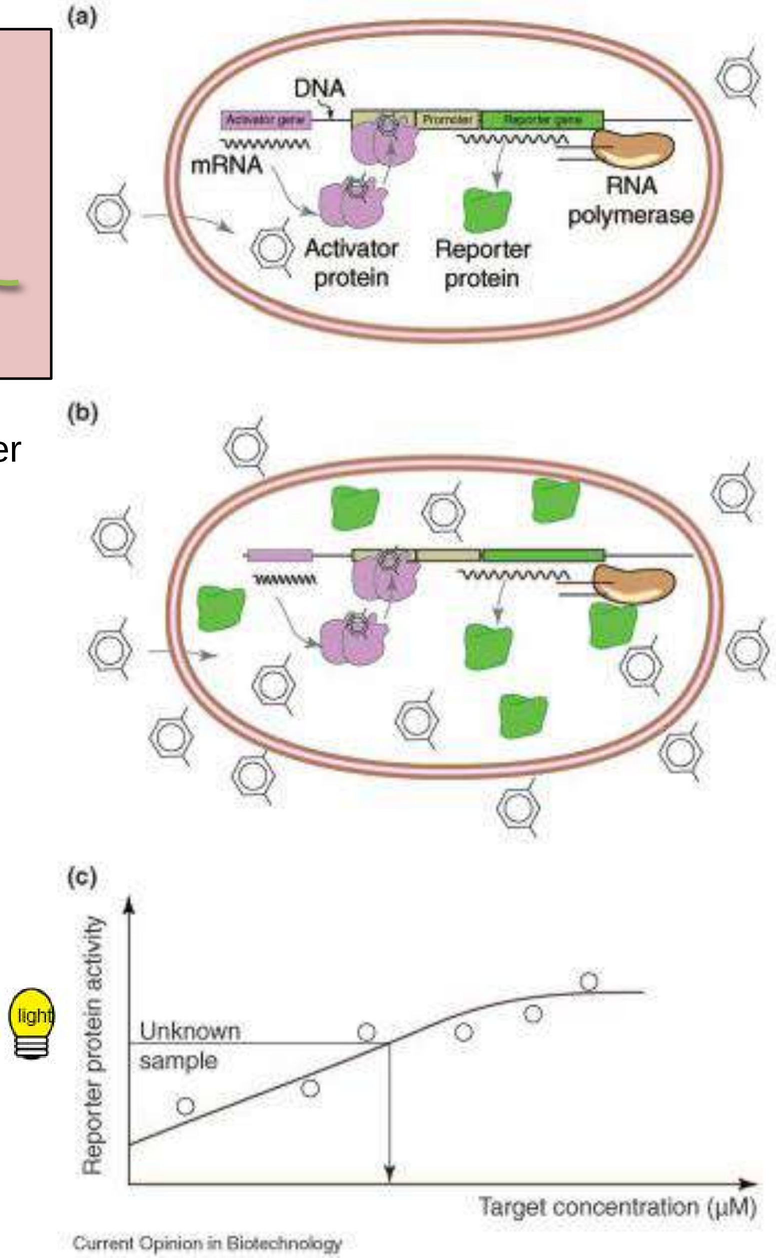
Technologies developed for other fields, e.g. pathogen and harmful-algae detection

- Bead-capture technology with capacitance sensor, e.g. Altratech BeadCAP assay (https://www.youtube.com/watch?v=4fakqJyPrs0)
- Loop-mediated isothermal amplification of DNA/RNA (LAMP) – colorimetric detection
- Nucleic acid sequence-based amplification (NASBA) – also isothermal
- Portable Q-PCR sequencing instruments
- Portable sequencing instrument (MinION)

Developing a specific bioreporter



Tecon R & van der Meer J-R (2006) Information from single-cell biosensors: what is it good for? *Current Opin Biotech* 17: 4-10



- Rapid and sensitive detection and quantification of **bioavailable** hydrocarbons
- Find the genes responsible, e.g. alkane monooxygenase
- Identify regulator, activator / promoter binding site
- Create fusion between a reporter gene and a promoter that exhibits enhanced transcription in response to a target compound.
- Luciferase is a typical reporter
- Signal strength should be proportional to [target compound]

Multi-strain bioreporter for detecting multiple oil fractions

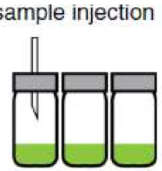
TABLE 1. Bioreporter Assay Specificities[a]

bacterial strain	reporter plasmid	chemicals detected	assay abbreviation	selection marker	reference
E. coli DH5α	pGEC74, pJAMA7	alkanes (C_6–C_{10})	OCT	Ap + Tc	*17*
	pPROBE-LuxAB-TbuT	benzene, toluene, xylene, ethylbenzene	BTEX	Km	this study
	pHYBP109	2-hydroxybiphenyl	HBP	Ap	*25*
	pHYBP103M3	2-hydroxybiphenyl, biphenyl	HBP	Ap	*16*
E. coli MG1655	pJAMA8-cda	DNA-damaging agents	CDA	Ap	this study
B. sartisoli RP007	pPROBE-phn-luxAB	naphthalene, (di)methylnaphthalene, phenanthrene	NAH	Km	this study

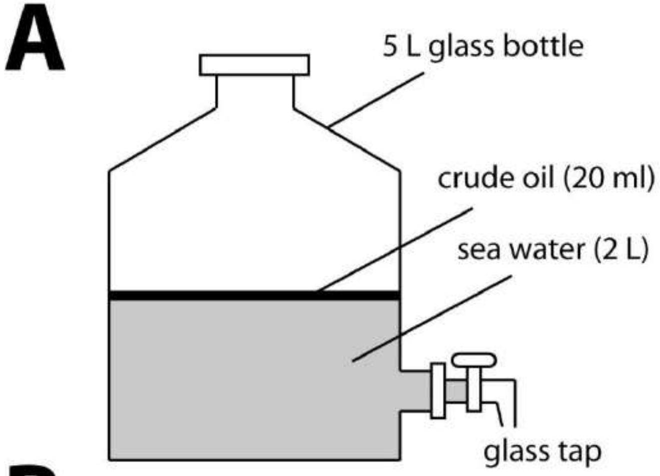
Tecon R et al. (2010) Development of a multiwell bacterial biosensor platform for the monitoring of hydrocarbon contaminants in marine environments. *EST* 44: 1049-1055.

Multi-strain bioreporter for detecting multiple oil fractions

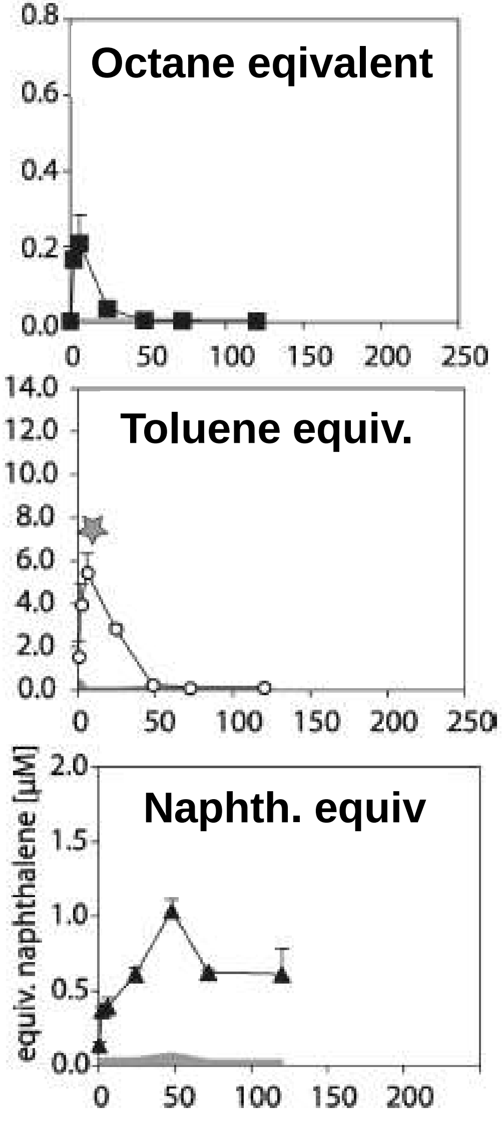
- Designed for:
  - short-chain alkanes
  - BTEX
  - PAHs (up to phenanthrene)
- 96-well glass plates



- Luciferase reporter



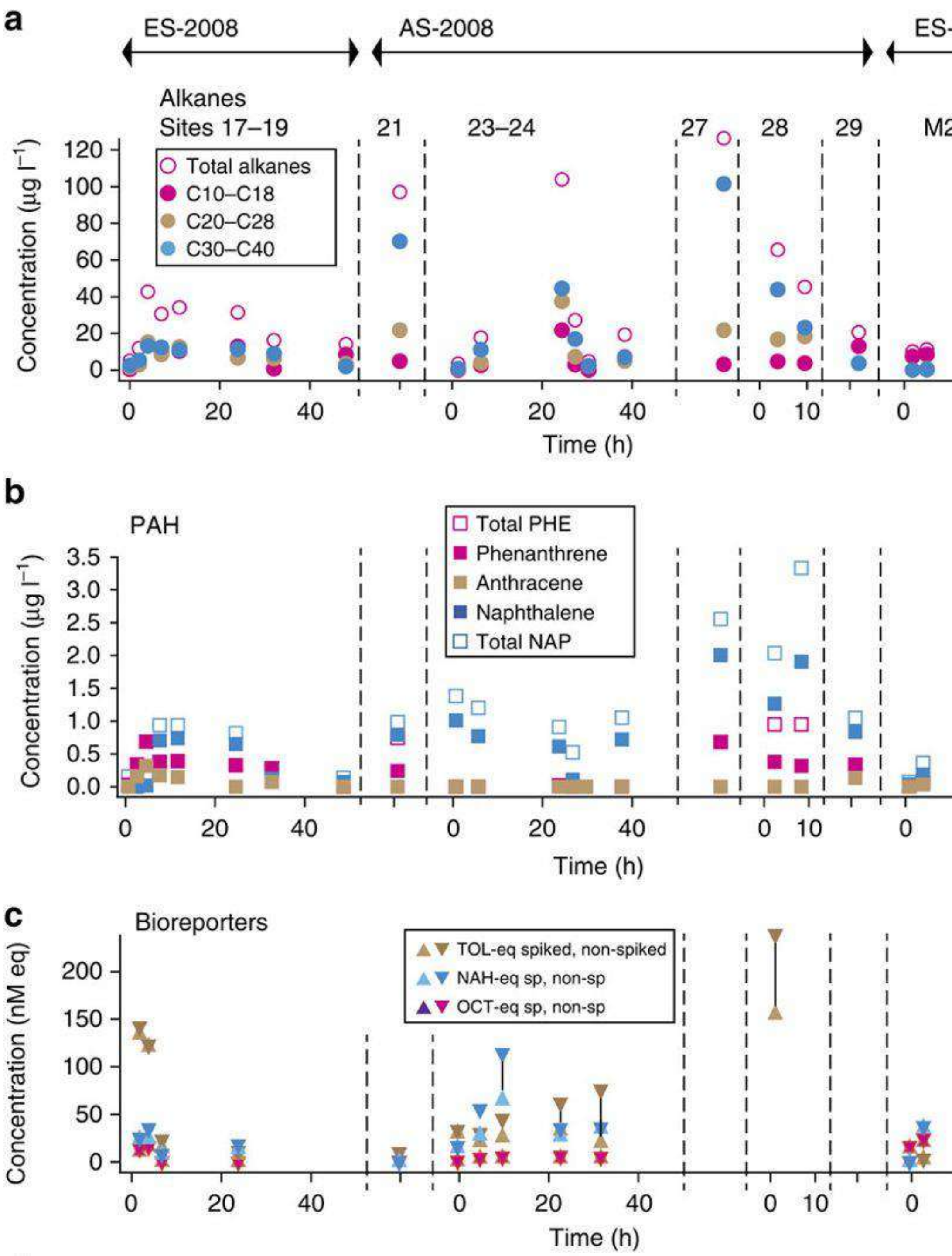
Example of 5 reps



- Rapid, ephemeral detection of short-chain alkanes and BTEX (volatilisation & biodeg)
- PAHs remain for longer
- Potential time indicator for the age of the spill?

Tecon R et al. (2010) Development of a multiwell bacterial biosensor platform for the monitoring of hydrocarbon contaminants in marine environments. *EST* 44: 1049-1055.

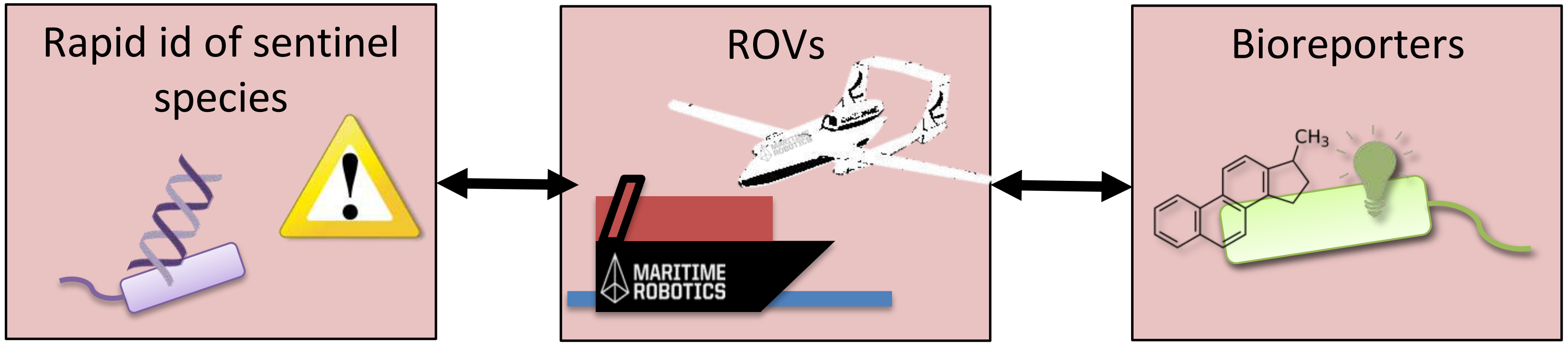
How do bioreporters perform at sea?



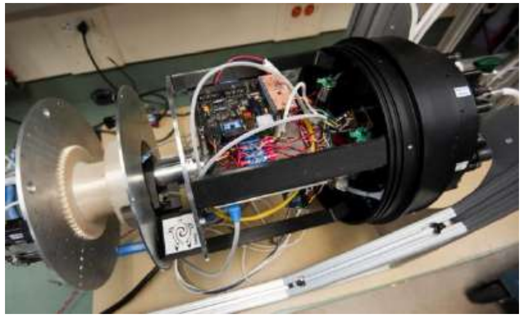
- Quantitatively and qualitatively bioreporters match chemical measurements
- Indicating that dissolved oil components were immediately **available to the biota**

Brussaard CP et al. (2016) Immediate ecotoxicological effects of short-lived oil spills on marine biota. *Nature Comm* 7: 11206.

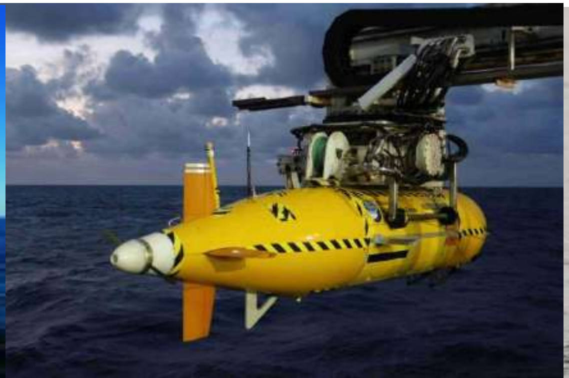
Coupling bio-monitoring tools with mobile and static devices



- Real-time monitoring by strategically positioned mobile and static devices with a variety of (bio)sensors
- Rapid assessment of pollution type, magnitude and effects
- Coupled with satellites to catch the polluter (deterrent)
- More intelligent, rapid response
- Environmental Sample Processor (ESP) AKA Lab-in-a-can



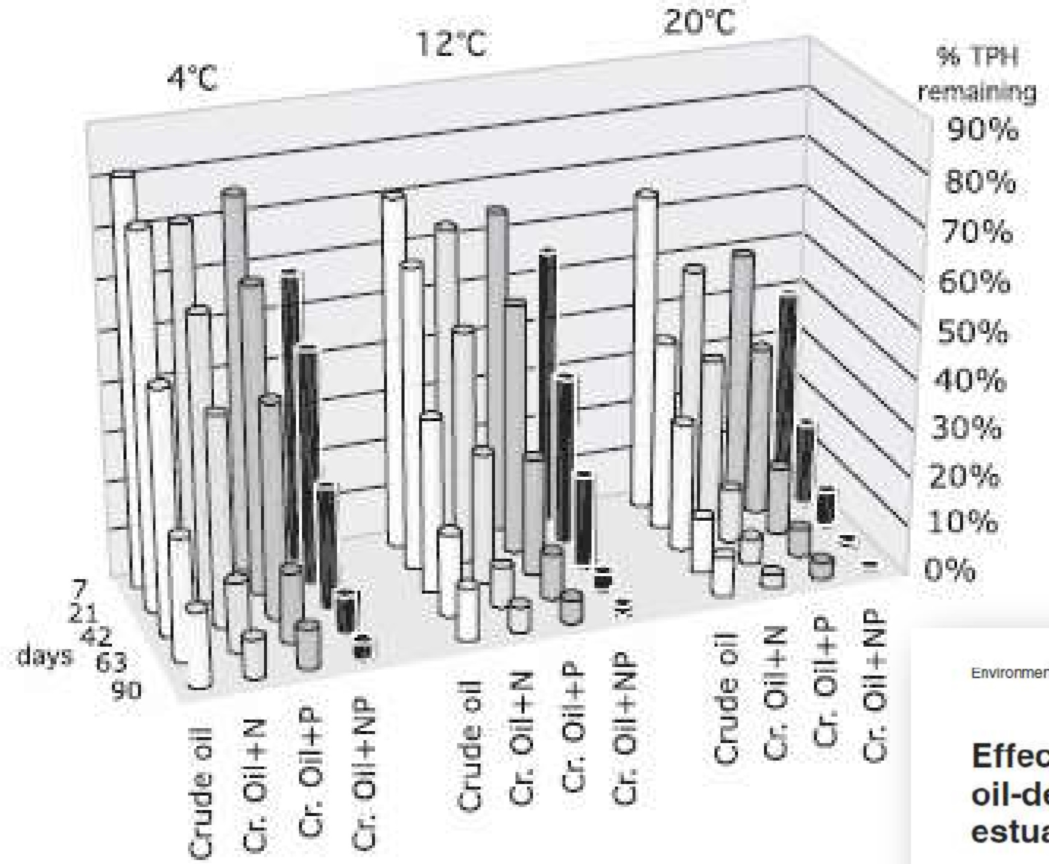
https://www.mbari.org/technology/emerging-current-tools/instruments/environmental-sample-processor-esp/esp-how-it-works/



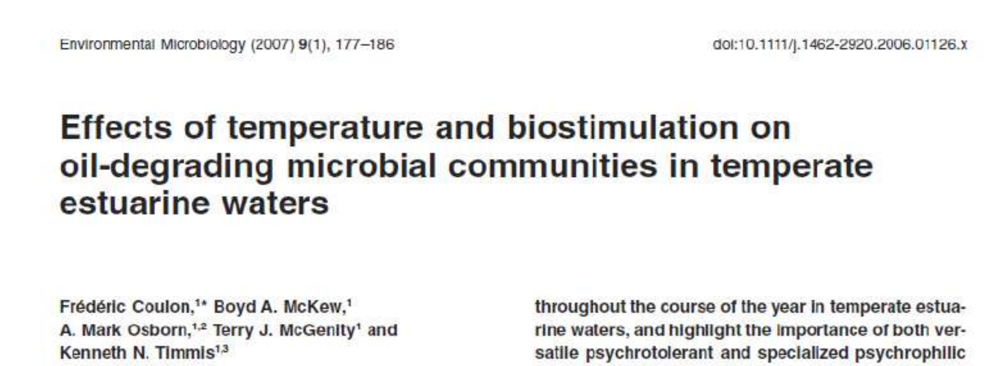
Acknowledgments

Ben Gregson , Gareth Thomas & many more indicated as authors in the papers: Fred Coulon, Ken Timmis, Robin Tecon, Jan-Roelof van der Meer

How are they influenced by temperature / N&P?



- Biodegradation of crude oil is still relatively rapid at 4°C and without added nutrients



Environmental Microbiology (2007) 9(1), 177–186 doi:10.1111/j.1462-2920.2006.01126.x

Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters

Frédéric Coulon,[1*] Boyd A. McKew,[1] A. Mark Osborn,[1,2] Terry J. McGenity[1] and Kenneth N. Timmis[1,3]

throughout the course of the year in temperate estuarine waters, and highlight the importance of both versatile psychrotolerant and specialized psychrophilic

How are they influenced by temperature / N&P?

- But the dominant microbes are different, e.g. ***Oleispira*** dominates in microcosms incubated at 4 and 12 °C