IRIS

Molecular marker technology for the detection of marine oil pollution

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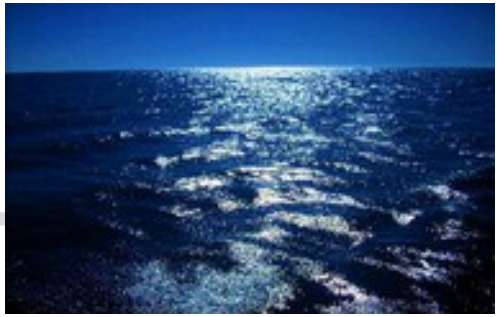
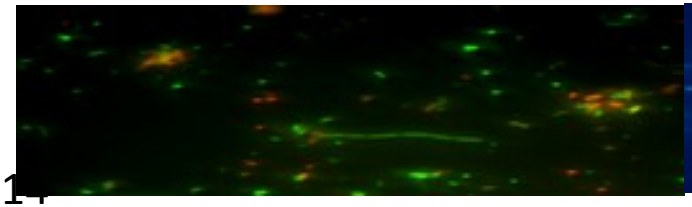
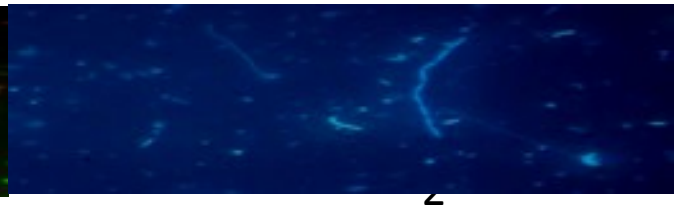
Dominique Durand

Talk outline



- Why build an oil detection system based on microorganisms?
- Identification of microbial molecular markers of oil exposure
- Validation on field samples
- Adaptation of an Environmental Sampling Processor (ESP) for oil detection





Why use microorganims for monitoring the marine environment?

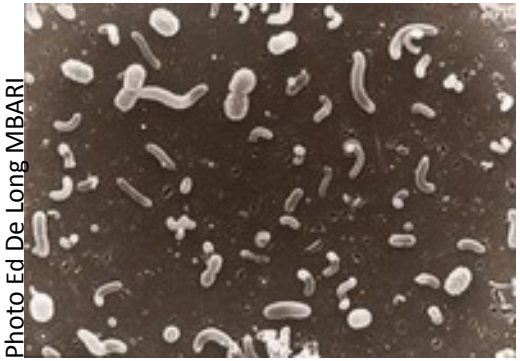
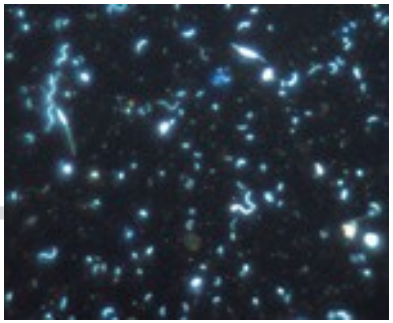


Photo Ed De Long MBARI

- The microbes' response is fast and can be very specific
- Can detect the bioavailable pollutants in the seawater
- Measures the impact on the marine environment
- Intended for monitoring sites which are particularly exposed to oil-related activities (drilling, production templates), or particularly sensitive (Arctic)

Identification of microbial markers of oil exposure



Step 1: Laboratory: Identification of microorganisms specific to oil contaminated seawater

Step 2: Validation of marker species or genes on field samples

Step 3: Fine tuning of targets:

Which markers for which context?

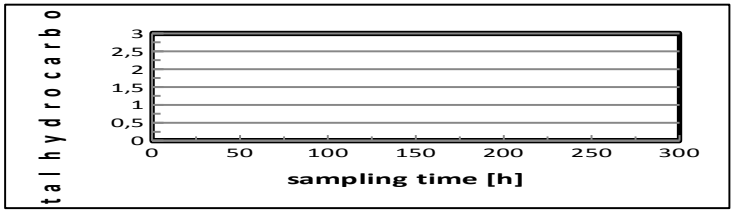
Microbial molecular markers of oil exposure

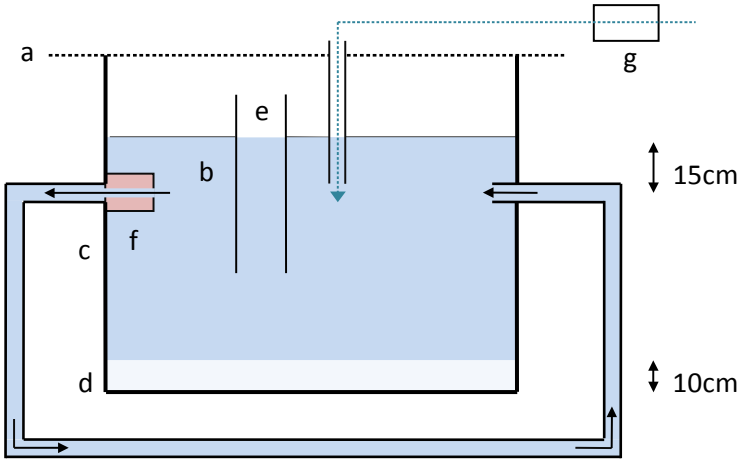
Step 1: Identification of microorganisms specific to oil exposure for different temperatures

- Laboratory seawater petroleum exposures

10 - 200 L tanks containing sediment 13 °C, 8 °C and 4 °C

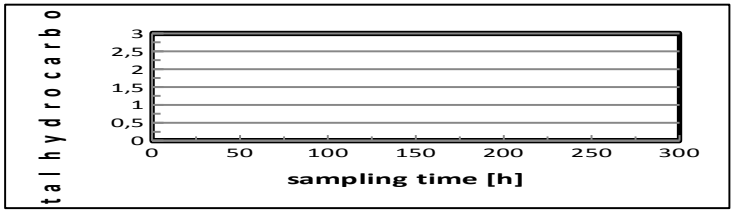
0.1 % to 0.001% light or heavy crude oil





Microbial molecular markers of oil exposure

Step 1: Identification of microorganisms specific to oil exposure for different temperatures



- Laboratory seawater petroleum exposures

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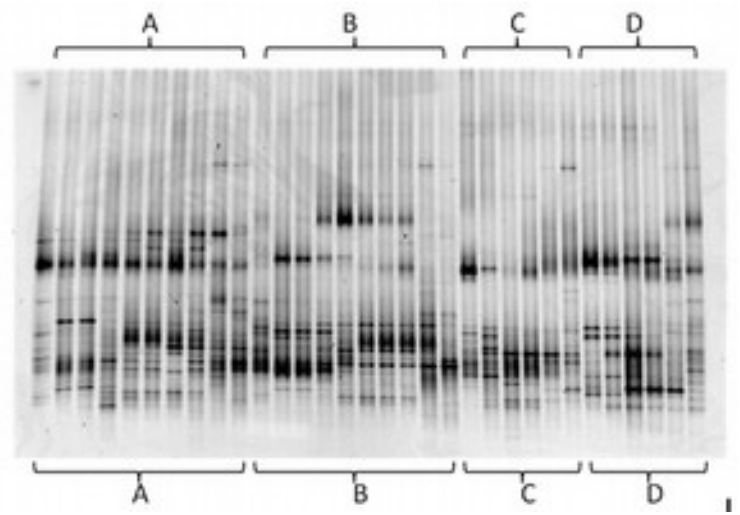
- Microbial community screening using DGGE



Microbial molecular markers of oil exposure

Step 1: Identification of microorganisms specific to oil exposure for different temperatures

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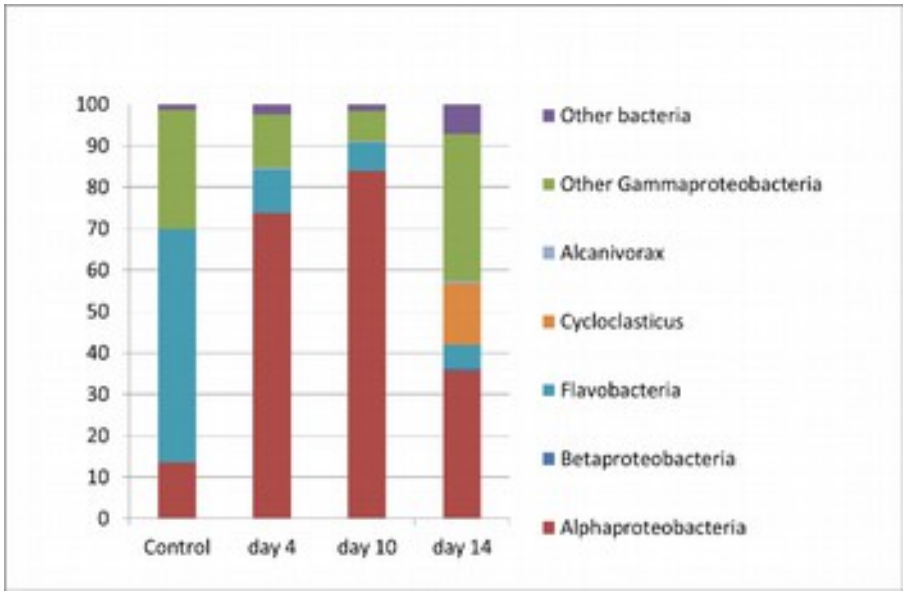
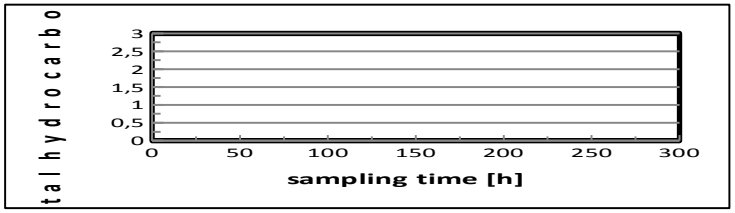
- Microbial community screening using DGGE
- Total microbial analysis with 454 pyrosequencing





- Shift in microbial community and loss of diversity
- Oil degrading organisms came up quickly
- These organisms were not detected in the control areas
- Similar species as in very different geographical locations

Identification of Arctic Oil pollution markers
On site seawater exposure

- Ship settled in East Greenland for 11 months
- Collection of pristine seawater samples at different depth
- On-site oil exposures
- Seasonal changes
- Testing of current markers

Microbial molecular markers of oil exposure

Step 2: Validation of marker species on field samples

- Collection of pristine seawater
- On-site oil exposure

→ Identification of specific markers

→ Validation of markers

Microbial molecular markers of oil exposure

Step 3: Fine tuning of targets

Field test samples:

Oil spill

Chronic contamination

Controlled environment:

Exposed field

Laboratory exposures

- Threshold oil concentration

Exposures down to 30ppb

- Type of oil?

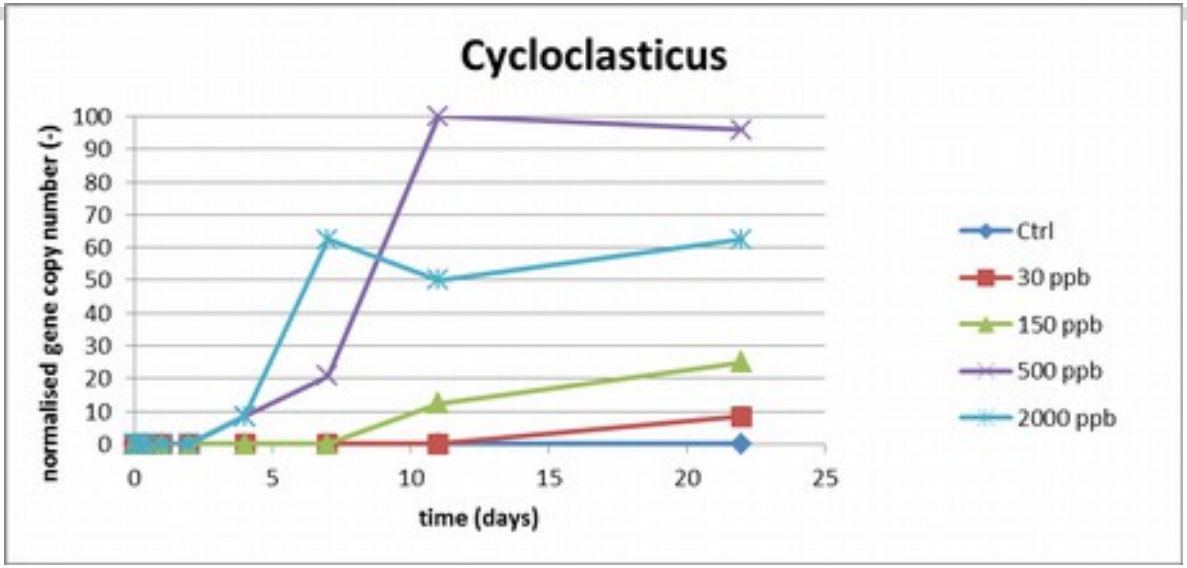
- How fast do the markers respond?

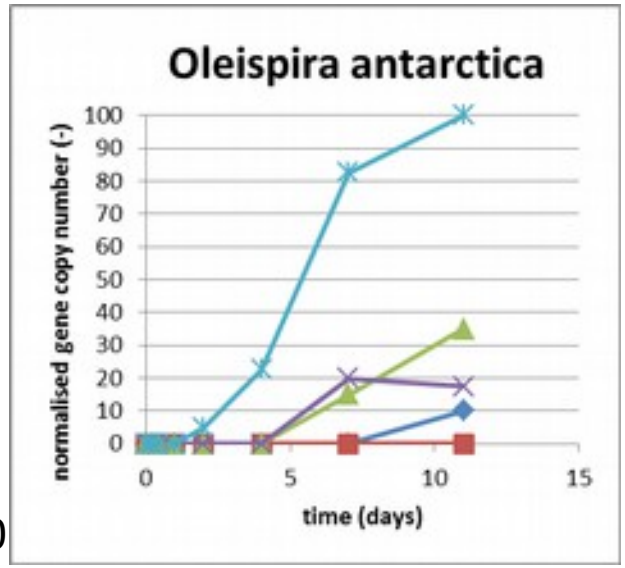
- How long does the response last?

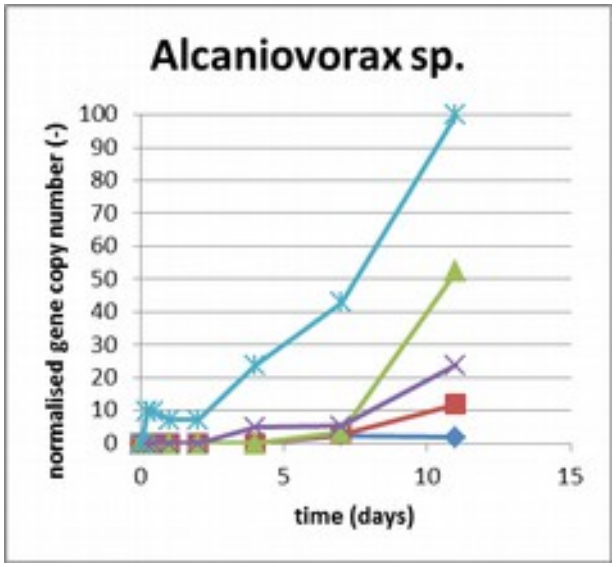
- *Oleispira*
- *Cycloclasticus*
- *Alcaniovorax*
- *Colwelia*
- *Oceanospiralles*
- *Polaribacter*
- *Marinomonas*
- *Pseudomonas*
- *Halomonas*
- *Rhodobacteriaceae*

Photo: Kystverket

Example target species



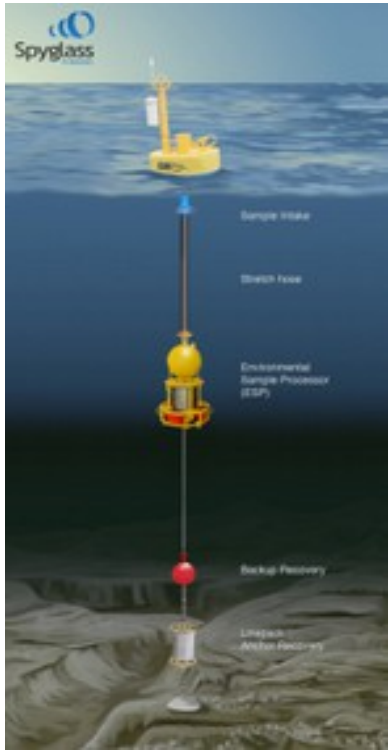




Molecular-based autonomous system for subsurface oil detection

- **Adapt the Environmental Sampling Processor (ESP) developed at Monterey Bay Aquarium Research Institute (MBARI) for real-time detection of oil-degrading bacteria or genes**

1. Identification of marker species and genes
2. Designing appropriate assays for their detection
3. Adapt ESP to perform the necessary assays…..



MOAB Leak detection

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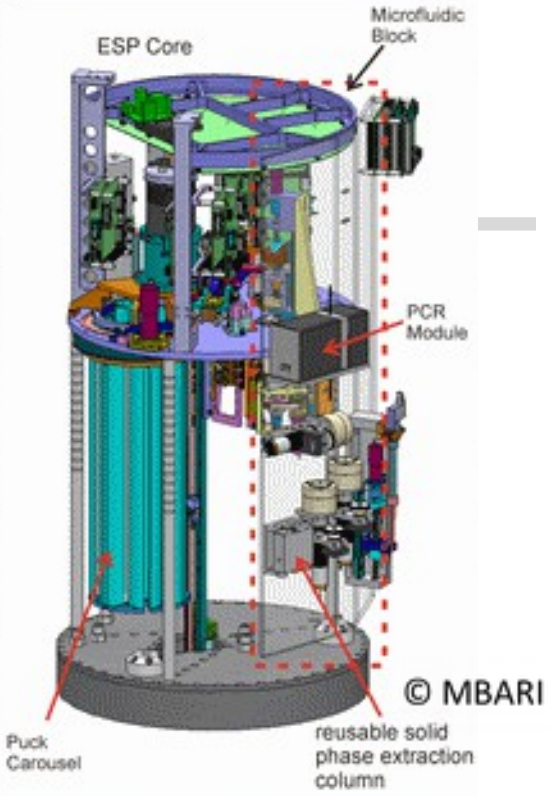
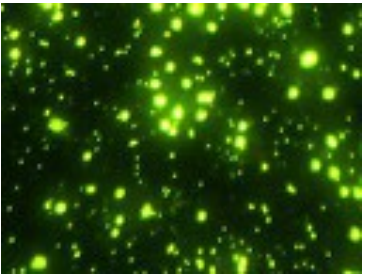
…and bring the lab in the field for real-time detection!

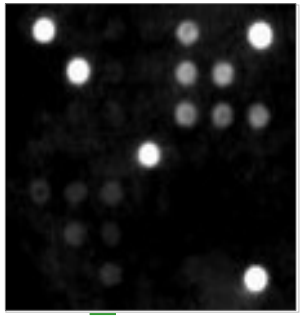
Spyglass ESP Platform

Autonomous Genetic & Protein Analysis

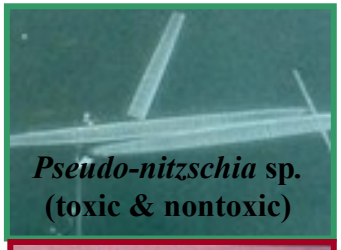


- Collect sample / homogenize / filter DNA
- Real-time analysis
  - DNA probe arrays (Sandwich Hybridization Assay)
  - Protein/Toxin arrays (cELISA)
  - Quantitative PCR (qPCR)
- Broadcast results



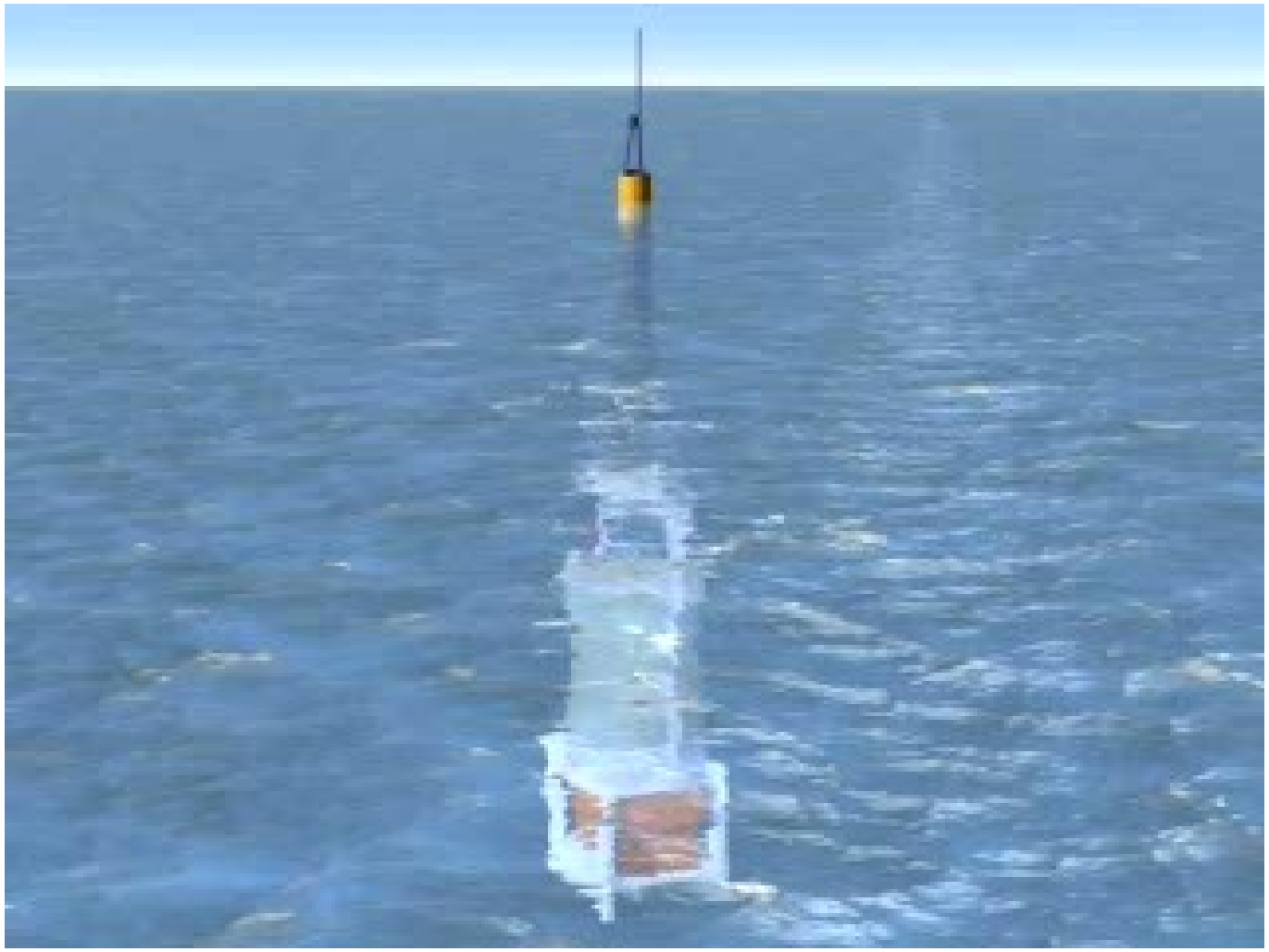


Invertebrate larvae



Harmful algae

How does the ESP work?



http://www.mbari.org/ESP/espworks.htm

Adapting the ESP to detect oil pollution markers

To adapt the analytical modules of the ESP to autonomously detect the presence of specific oil-degrading bacteria around subsea installations.

1) Selection of suitable oil-degrading microbial markers

2) Development of DNA target primers

Highly specialized bacterial genes are the basis for distinguish between contaminated and uncontaminated water

Targets for the markers are hydrocarbonoclastic bacteria which come up in the early stages of oil contamination

Alcanivorax
Cycloclasticus
Marinobacter
Alteromonas
Pseudoalteromonas
Pseudomonas
Neptunomonas
Thalassobius
Oleiphilus
Colwellia

naphthalene 1,2-dioxygenase
alkane 1-monooxygenase
Phosphonoacetate hydrolase A
toluene monooxydase
Cyclic PAH dioxydase

Adapting the ESP to detect oil pollution markers

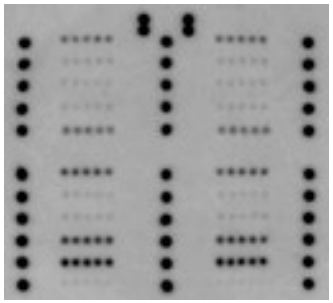
To adapt the analytical modules of the ESP to autonomously detect the presence of specific oil-degrading bacteria around subsea installations.

1) Selection of suitable oil-degrading microbial markers

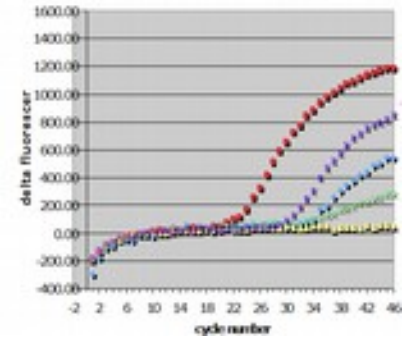
2) Development of DNA target primers

3) Implementation of specific assays onto the ESP instrument

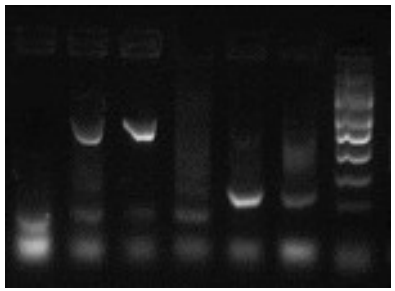
rRNA probe arrays



PCR



qPCR



Calibrating and testing the ESP to detect oil pollution markers

1) Lab testing:
   → relevant leak scenarios: oil types, exposure time, temperature…
   → In realistic exposure set ups for the area of interest

2) Field testing:
   → In different non contaminated environment
   → In situ exposures
   → Deployment in known leakage/contaminated site

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