tb@iris.no

IRIS

Perspectives with genetic sensors for FerryBox-based data observations

Thierry Baussant IRIS (Norway),
Jim Birch and Chris Scholin MBARI (USA),
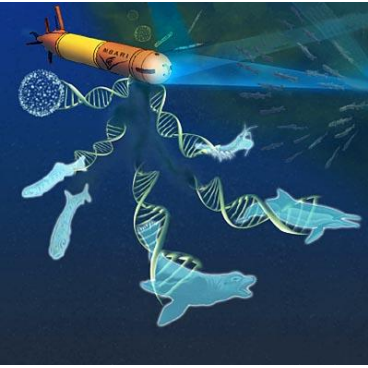Clement Furlong and Scott Soelberg Univ. Washington (USA)

Status

- IRIS and partners involved in research projects financed by RCN with the goal to use eDNA and adapt to genosensing platforms for marine monitoring
  - ✓ Targeted species-specific monitoring related to
    - Petroleum
    - Aquaculture
    - Baseline
  - ✓ What species are present?
  - ✓ How do they respond to alterations in their chemical and physical environment ?
  - ✓ Are there species indicators of these changes ?

Sampling eDNA from microbes to eukaryotes using robotized platforms

ESP 2G and MBARI team at IRIS during mesocosm test in RCN MOAB project
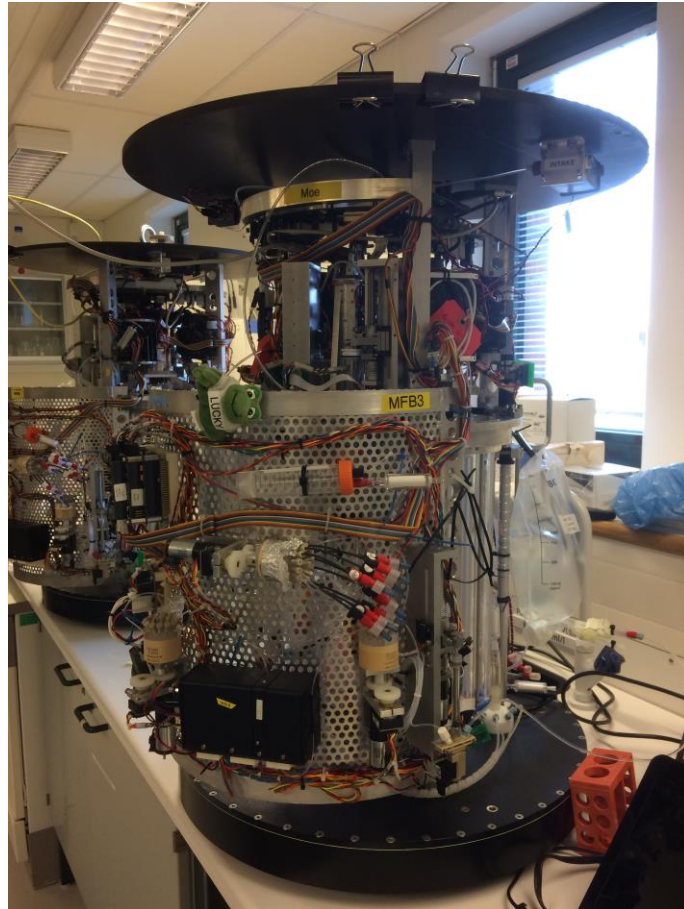
*Use eDNA from microbes to marine mammals using a **single water sample***

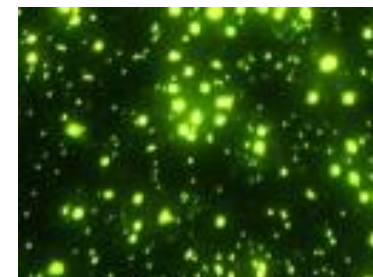*Use the Environmental Sample Processor (ESP) for **eDNA sample analysis***

Example of application
RCN MOAB (Petromaks 2)
2012-2015

Mooring and
2G-ESP
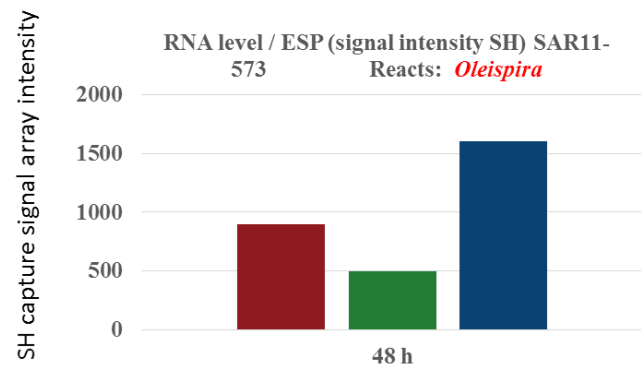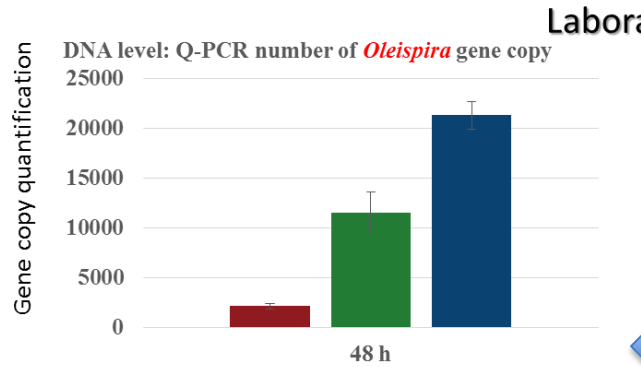©MBARI

What microbial assemblage is there ?
Identification and quantification of specific microbial assemblages
Can microbial diversity change tell us about the presence of
oil in seawater and ESP detect key early changes *in situ* ?

Work flow used for oil-related microbial gene detection with ESP
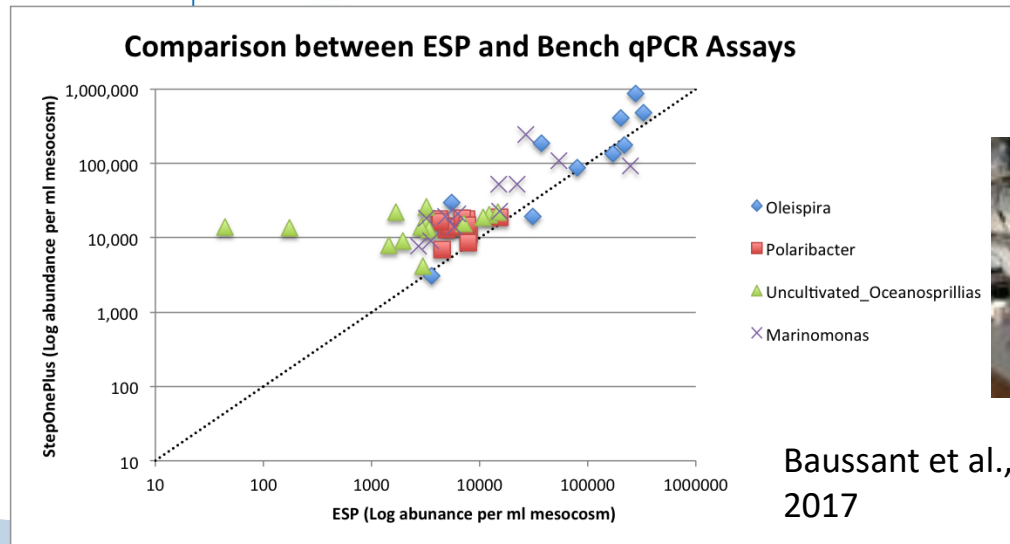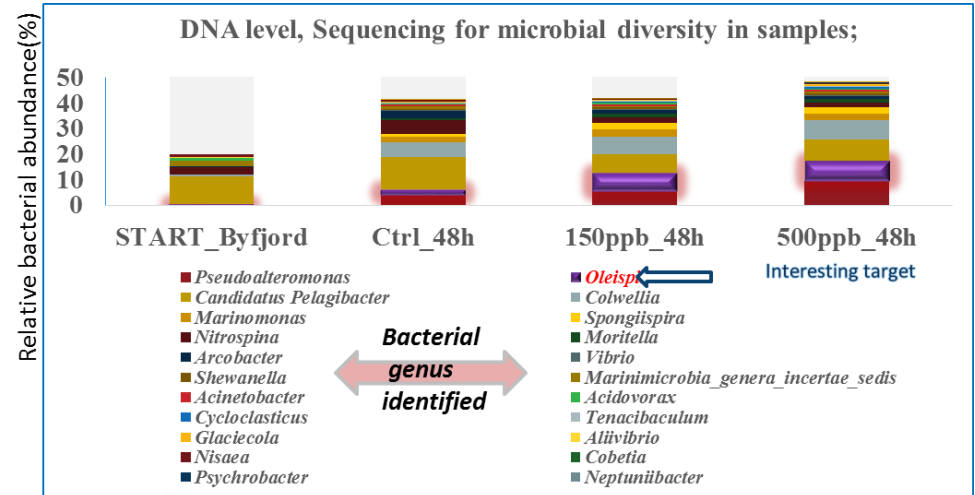
IRIS

ESP compatible targeted asssays

NGS sequencing of oil-incubated samples
Identification of oil gene markers

Laboratory phase

DNA level: Q-PCR number of *Oleispira* gene copy

Gene copy quantification

DNA level, Sequencing for microbial diversity in samples;

Relative bacterial abundance(%)

START_Byfjord   Ctrl_48h   150ppb_48h   500ppb_48h   **Interesting target**

■ *Pseudoalteromonas*   ■ *Oleispira*
■ *Candidatus Pelagibacter*   ■ *Colwellia*
■ *Marinomonas*   ■ *Spongiispira*
■ *Nitrospina*   ■ *Moritella*
■ *Arcobacter*   ■ *Vibrio*
■ *Shewanella*   ■ *Marinimicrobia_genera_incertae_sedis*
■ *Acinetobacter*   ■ *Acidovorax*
■ *Cycloclasticus*   ■ *Tenacibaculum*
■ *Glaciecola*   ■ *Aliivibrio*
■ *Nisaea*   ■ *Cobetia*
■ *Psychrobacter*   ■ *Neptuniibacter*

Bacterial genus identified

48 h

RNA level / ESP (signal intensity SH) SAR11-573   Reacts: *Oleispira*

SH capture signal array intensity

48 h

Dose-response

ESP demo phase

ESP performance evaluation

Comparison between ESP and Bench qPCR Assays

StepOnePlus (Log abundance per ml mesocosm)

◆ Oleispira
■ Polaribacter
▲ Uncultivated_Oceanosprillias
✕ Marinomonas

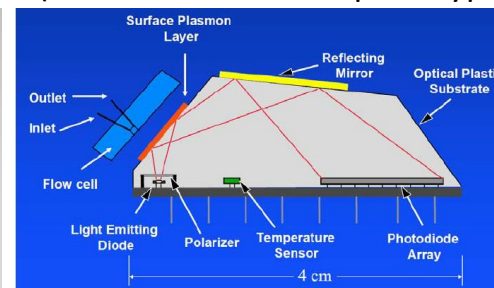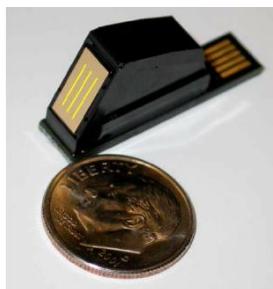ESP (Log abunance per ml mesocosm)

Baussant et al., 2017

RCN GENOMAPE (Petromaks 2)

Surface plasmon resonance (SPR) based genosensing of microbial species of interest, e.g., hydrocarbon degrading bacteria – based on nucleic acid (DNA or RNA) signatures

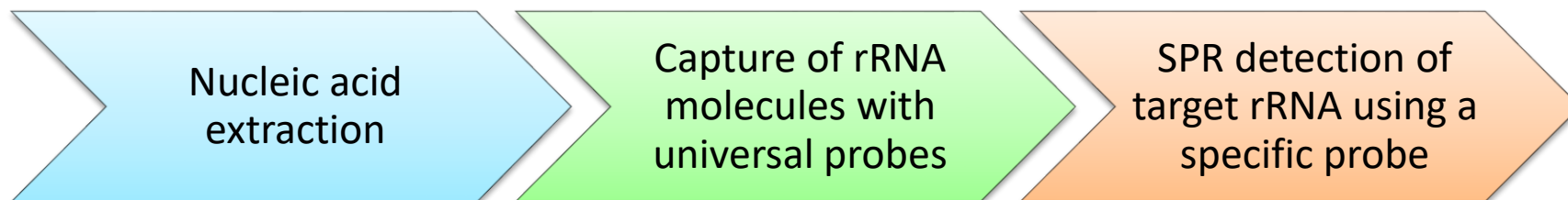SPREETA biosensor[1] (4 sensors in current prototype)

Portable SPR instrument

Highlights

- Label-free & hybridization based direct detection of nucleic acids
- Minimal sample preparation required
- Real-time data acquisition in the form of binding curves
- High specificity of binding between immobilized probe and analyte
- Assay time within minutes

[1] https://www.researchgate.net/publication/258335113_Immunoassays_and_Biosensors_for_the_Detection_of_Cyanobacterial_Toxins_in_Water

Aim: To develop a 16S rRNA based assay for the detection of the cold-adapted obligate alkane degrading bacterium: *Oleispira antarctica* – using a portable surface plasmon resonance instrument
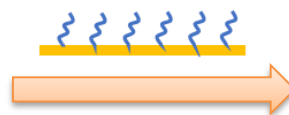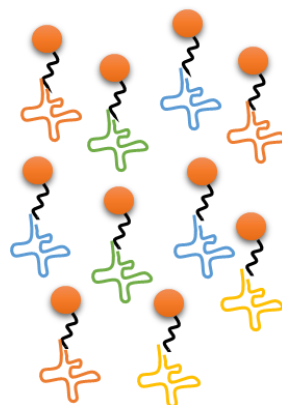

| Nucleic acid extraction	Capture of rRNA molecules with universal probes	SPR detection of target rRNA using a specific probe
Nucleic acid pool	rRNAs captured on magnetic beads	Hybridization of *Oleispira* rRNA onto gold surface
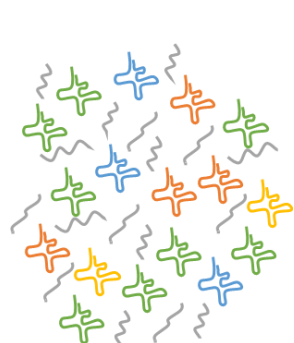
Magnetic beads functionalized with universal probe

Gold surface functionalized with Oleispira specific probe

Binding curves

Signal intensity (RIU)

Time (sec)
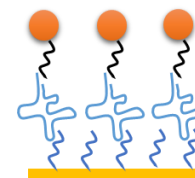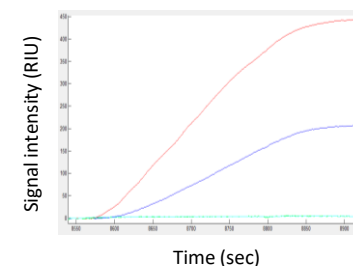
Potential application with FerryBox

- Adaptive sampling

- Archiving

- Near-real time analyis of gene targets from microbes to small plankton

Use of continuous, real-time observations and model simulations to achieve autonomous, adaptive sampling of microbial processes with a robotic sampler

Lydie Herfort,[1] Charles Seaton,[1] Michael Wilkin,[1] Brent Roman,[2] Christina M. Preston,[2] Roman Marin III,[2] Kiley Seitz,[†1] Maria W. Smith,[1] Vena Haynes,[‡1] Christopher A. Scholin,[2] António M. Baptista,[1] Holly M. Simon*[1]
[1]NSF Science & Technology Center for Coastal Margin Observation & Prediction (CMOP) and Institute of Environmental Health, Oregon Health & Science University, Portland, Oregon
[2]Monterey Bay Aquarium Research Institute, Moss Landing, California
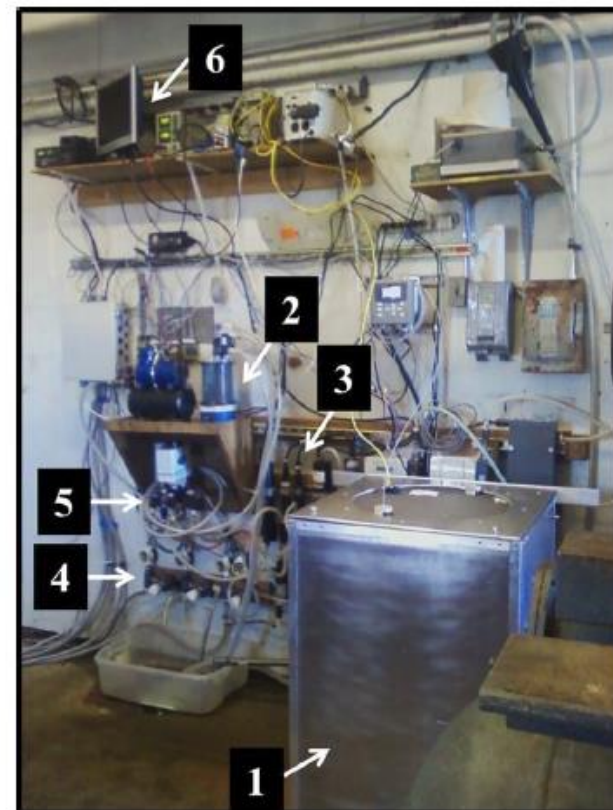
Fig. 2. Inside view of the shed that houses SATURN-03. Several key components are highlighted: aluminum enclosure containing the ESP (1), ESP external sampling reservoir (2), sensors (3), input lines from the three pumping ports (4), manifold receiving water from these lines (5), and the station computer (6).