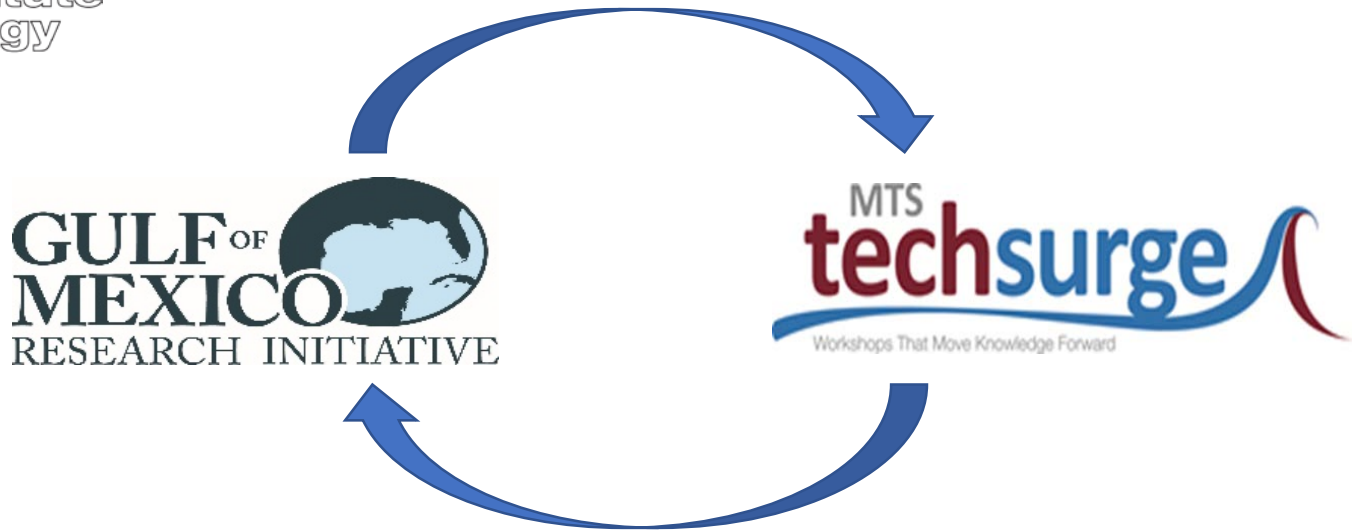
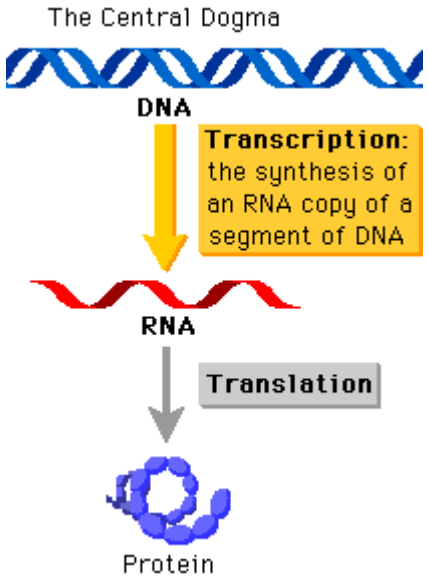
Panel Session 2 – Technology for Detection of Microbes & Their Function

Joel E. Kostka, Georgia Tech

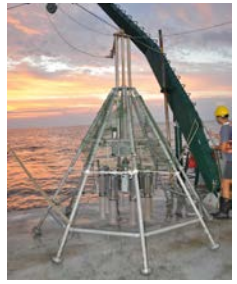




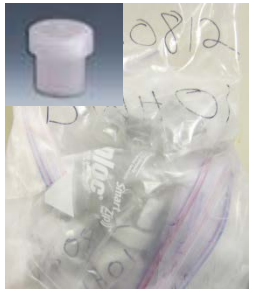
- **GoMRI Research: Detection/ characterization of the structure and function of microbial communities under as close to in situ conditions as possible**
  - Establish baseline metrics for monitoring of ecosystem impacts
  - Quantify impacts to ecosystem services provided by microbes
  - Develop biomarkers or bioindicators for risk assessment and remediation or mitigation potential

- **Challenges**
  - Most methods used in environment target DNA
  - DNA sequence does not provide direct information on function

Next generation sequencing pipeline for the high throughput analysis of microbial communities in the Gulf



Multicoring



Sectioning

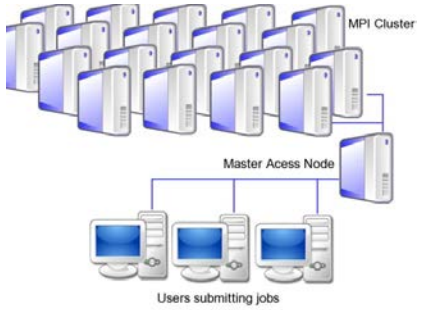
>100 samples / core

Extraction & PCR

MO BIO Laboratories, Inc. Saving You Time For Life

Illumina MiSeq

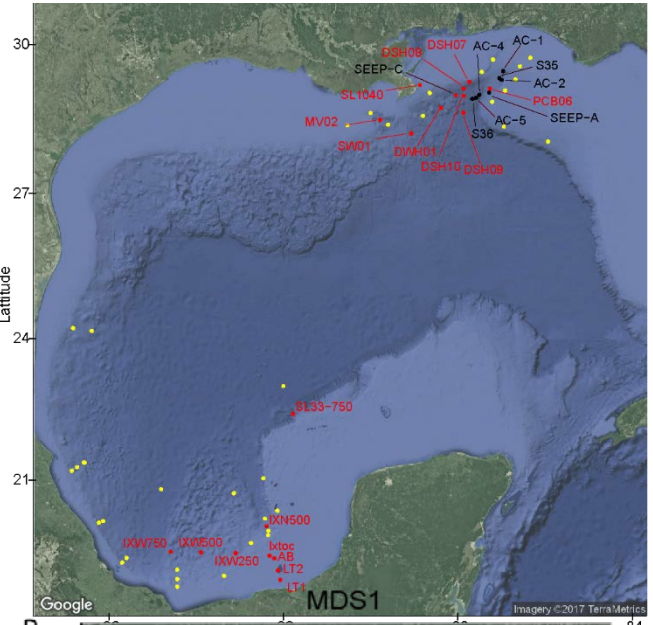
X 3 Million Reads

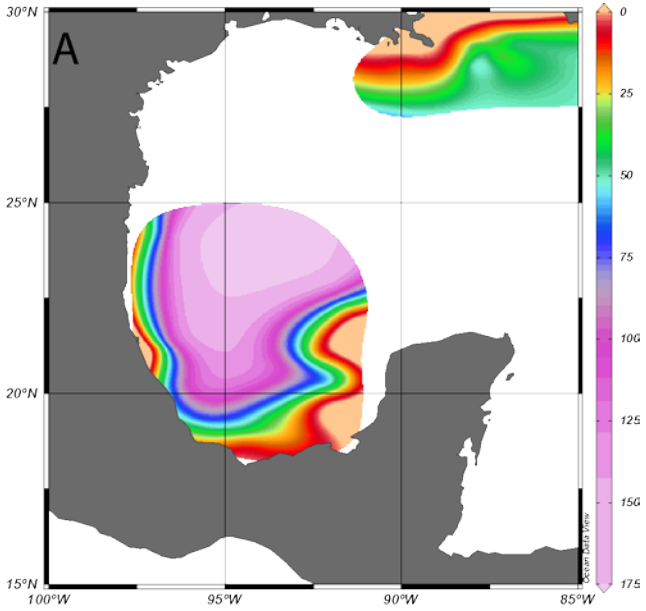


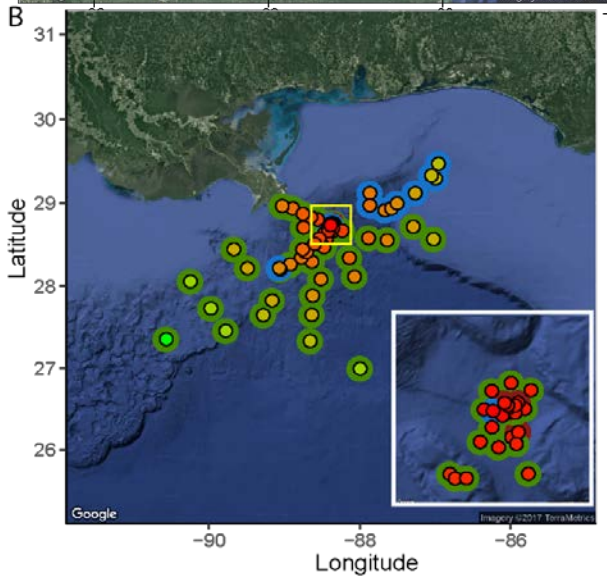
Massively Parallelized QA/QC and Sequence Processing

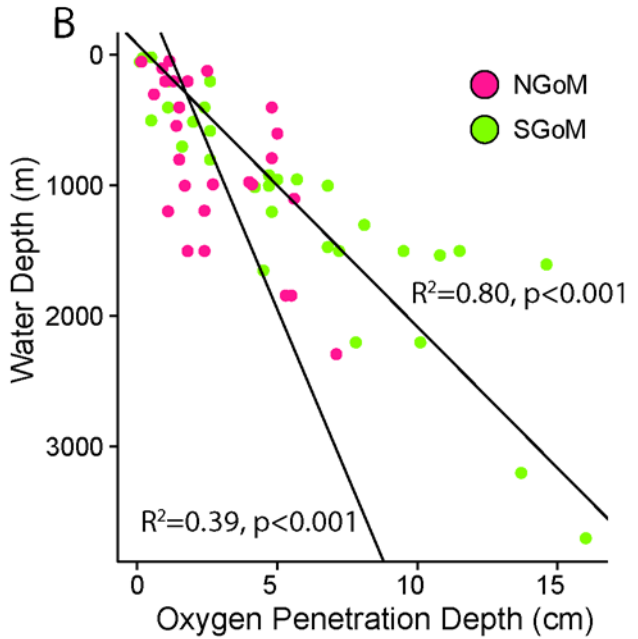
Analyze & Interpret

Case Study: Gulf-wide Distribution of Benthic Microbial Communities





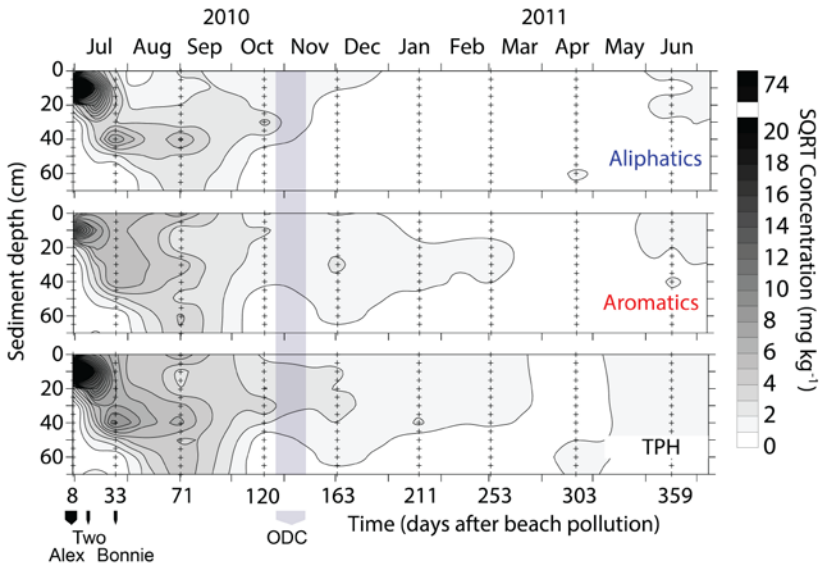


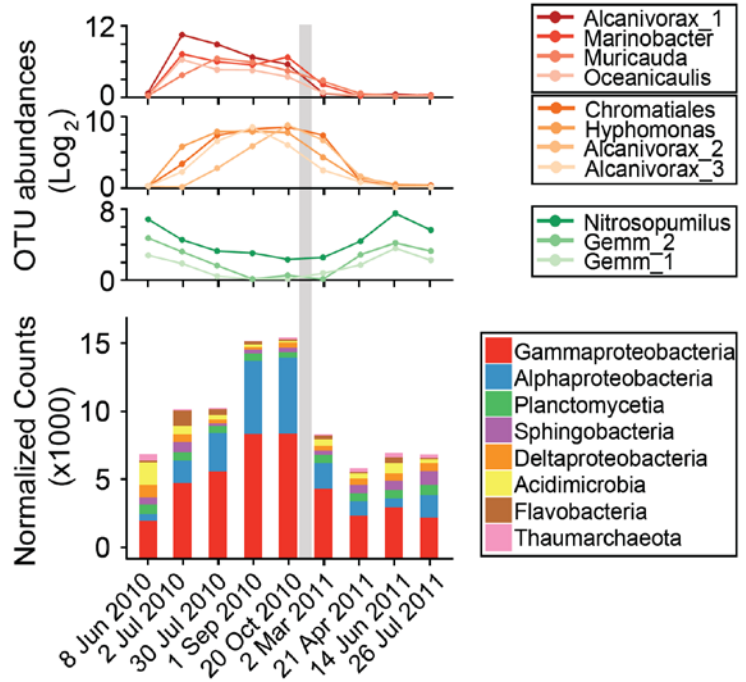


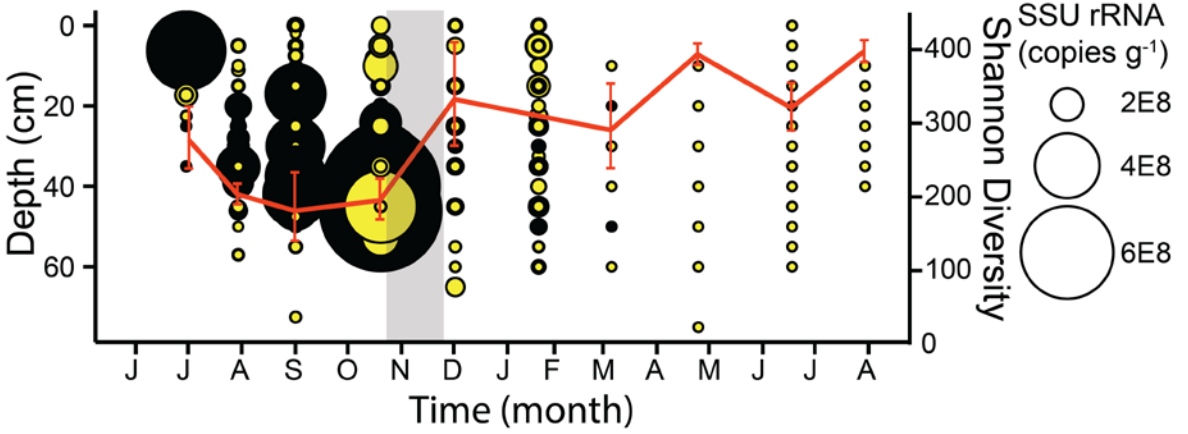
Overholt et al., in. prep.

- Largest dataset available from seafloor: > 120 million gene sequences from > 700 samples, 29 sites, over 4 years
- Random forest model indicates that microbial community structure is linked to oxygen penetration depth and sediment geochemical regime, which are likely controlled through carbon delivery.
- Communities impacted by DWH spill had returned to baseline, or close to baseline, conditions after two years.
- It is now possible to predict community compositions across the Gulf of Mexico and to assess future impacted sediments that may be poorly characterized

Case Study: Oiling of Pensacola Beach



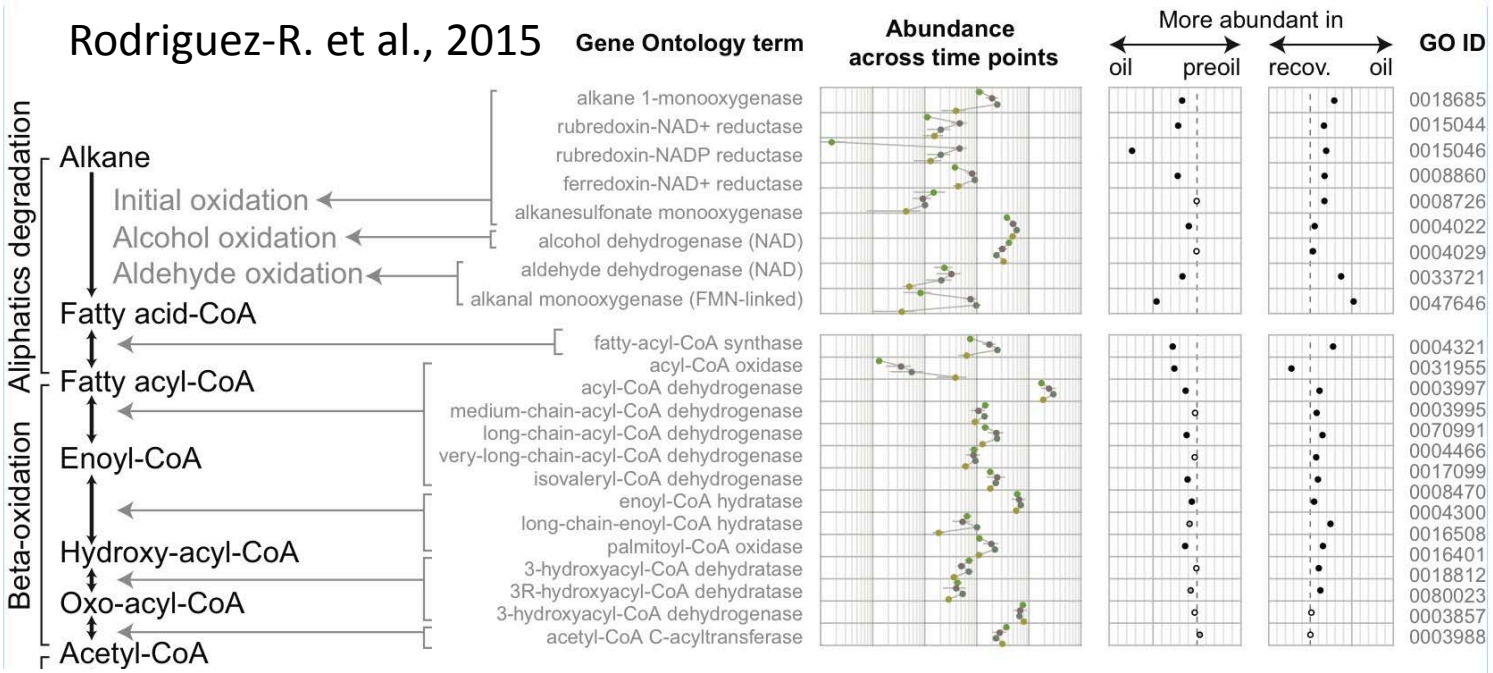


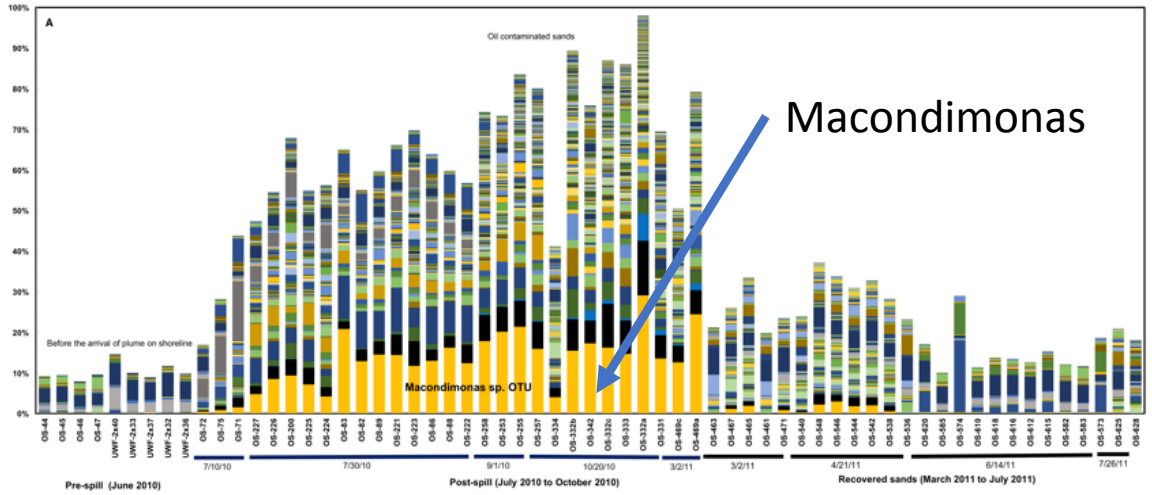


Kostka et al., 2011; Rodriguez-R. et al., 2015; Huettel et al., 2017

- TPH reached background levels in < 1 yr
- Microbial bloom
- Succession of microbial populations paralleled hydrocarbon chemistry

Biomarkers and Bioindicators

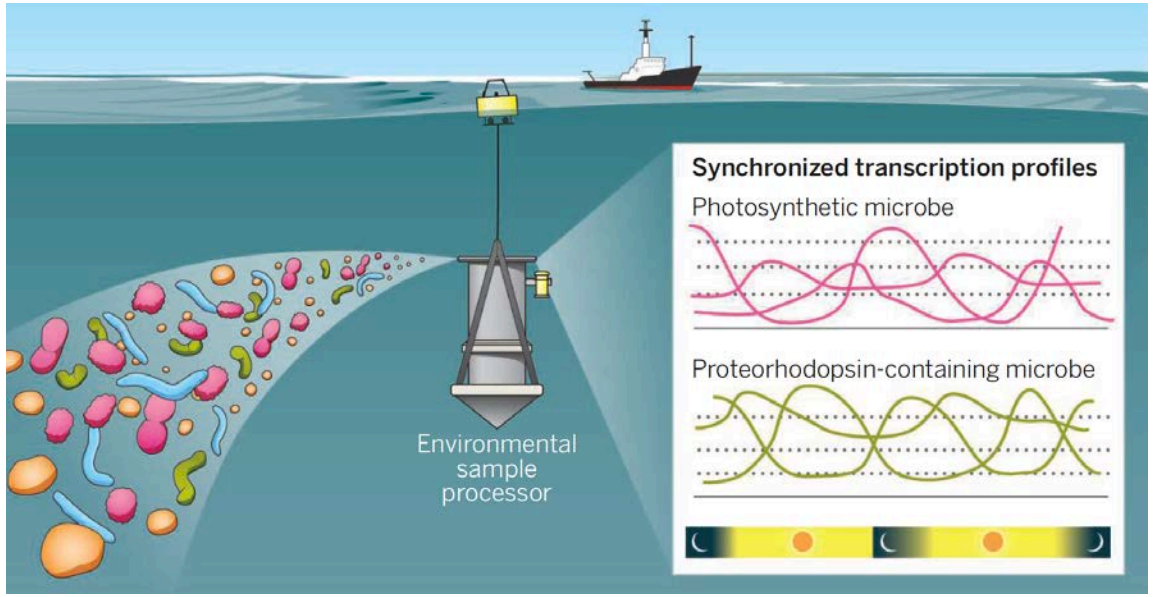




Rodriguez-R. et al., 2015; Karthikeyan et al., in. prep.

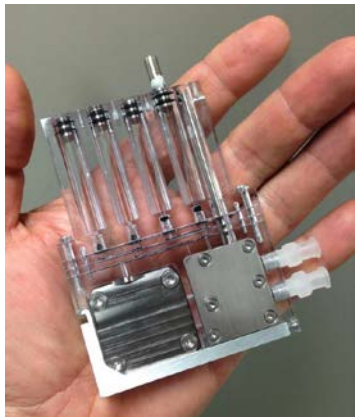
- Validate biomarkers by combining omics with quantification of biodegradation potential
- Metagenomic time series reveals pathways and controls of hydrocarbon degradation
- *Macondimonas* enriched from undectectable levels to ~ 30 % of community in oiled sands
- Ubiquitous in oiled sediments around the world
- Nearly complete genome reveals putative function
- Genome enabled targeted isolation

Needs for Technology Development



Mobile Ecogenomic Sensor
Environmental Sample Processor (ESP)

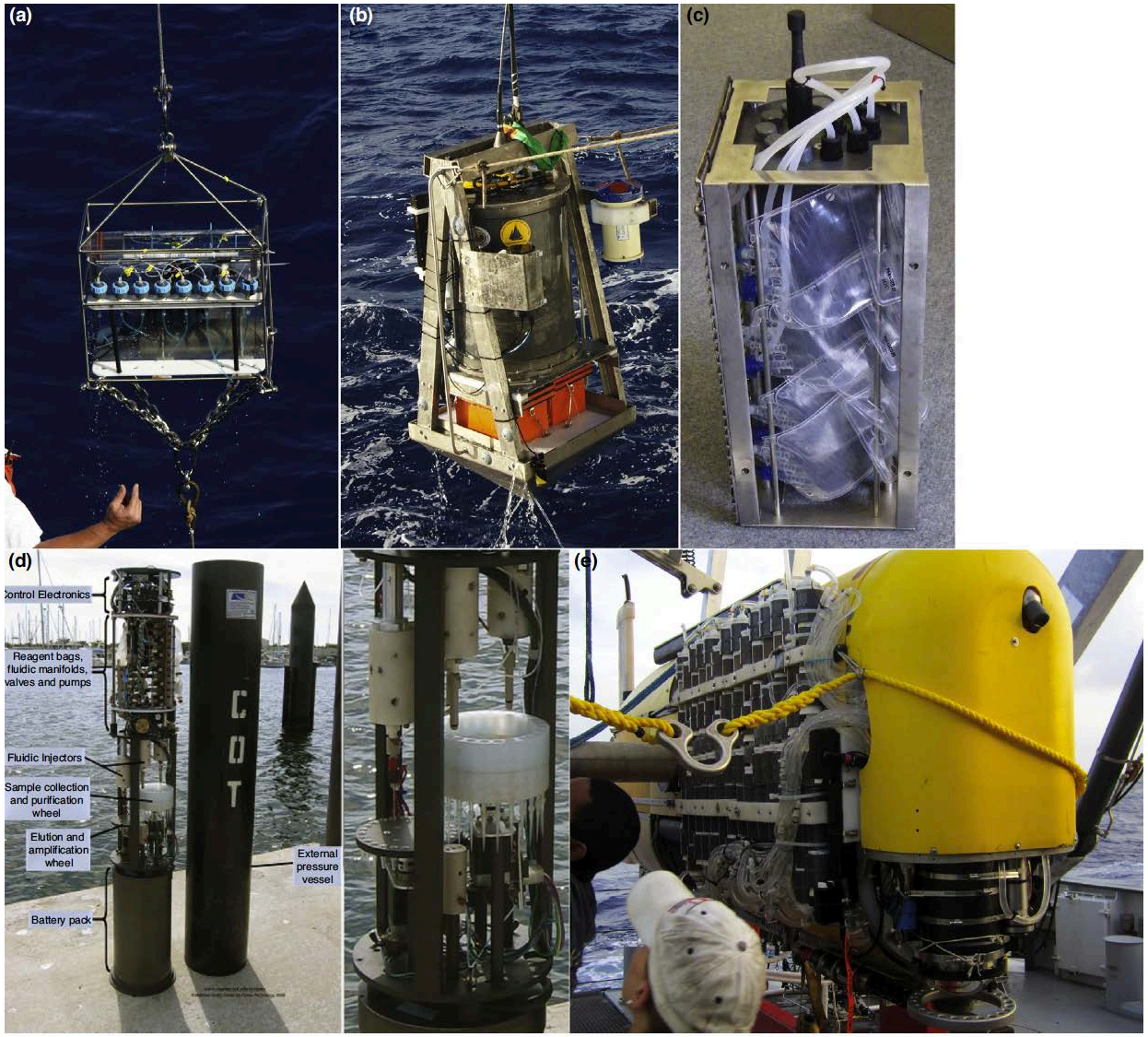
2nd gen,
215 liters



- Automated, in situ sampling/ detection/ analysis of microbial communities or microbial metabolites along with relevant environmental parameters
- Primary challenges: cost of sample acquisition, sample integrity, complexity
- Ecogenomics = genetic along with environmental characterization
- ESP, autonomous molecular biology lab
- Platform for analysis of microbial abundance and community composition (water sampling/ preservation, PCR, qPCR, hybridization)

3rd gen,
40 liters

Future Work



Armbrust, 2014; Pargett et al., 2015; McPartlin et al., 2017; McQuillan et al., 2017

- Many effective in situ samplers available with varying strengths/ weaknesses
- Few provide a platform for in situ sample processing beyond preservation
- ESP tested for 6 mo deployment on a mooring and rated to 4000 m water depth
- Bottom line: in situ sampling is working, in situ analysis and detection ~ 5 years from deployment
- Challenges/ limitations
  - Cost
  - Continued advancements in miniaturization and power efficiency
  - Development of microfluidics and lab on chip technologies
  - Develop RNA and protein detection methods
  - New PCR and qPCR methods
  - Validation and calibration of in situ sensors for monitoring, risk assessment, and determination of ecosystem impacts (genotype-phenotype relationships key)