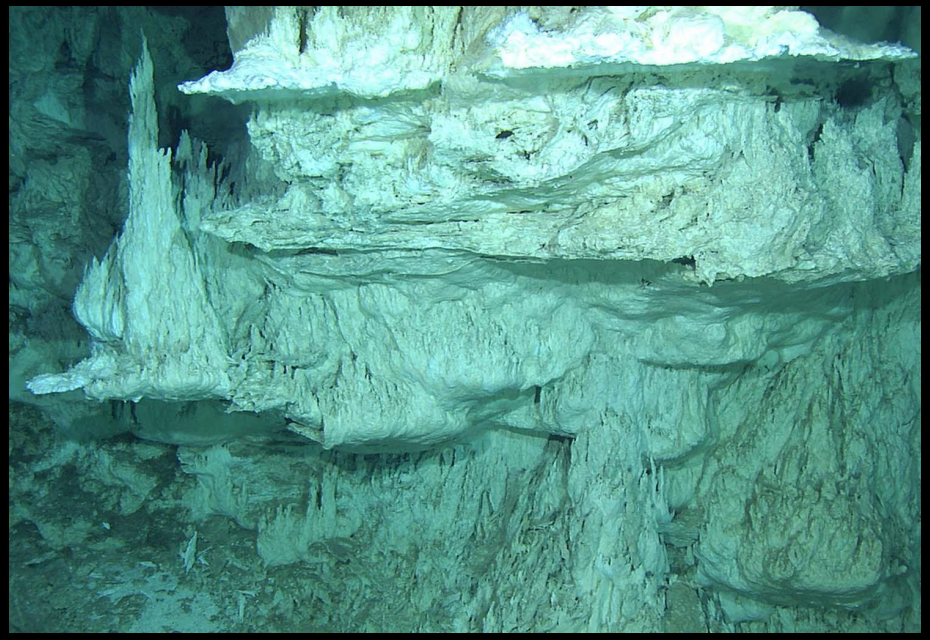
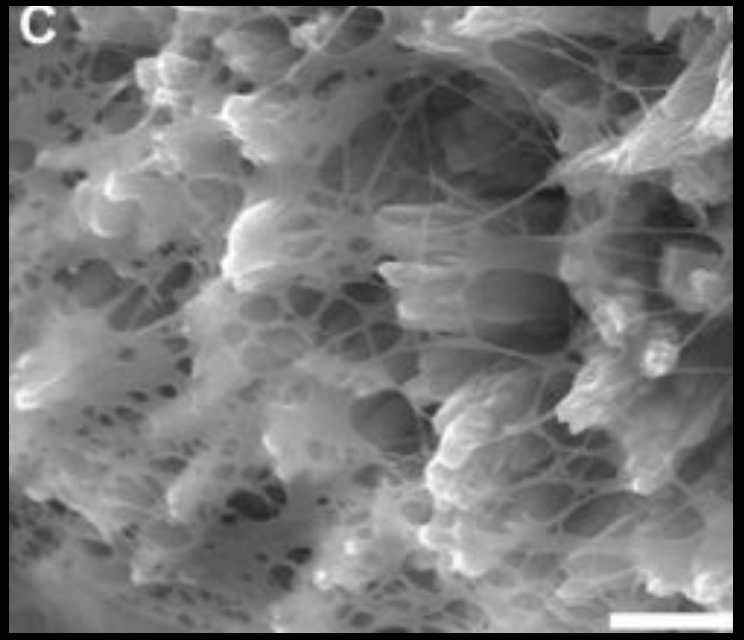
Considerations for Microbiological Sampling of the (Sub)Seafloor

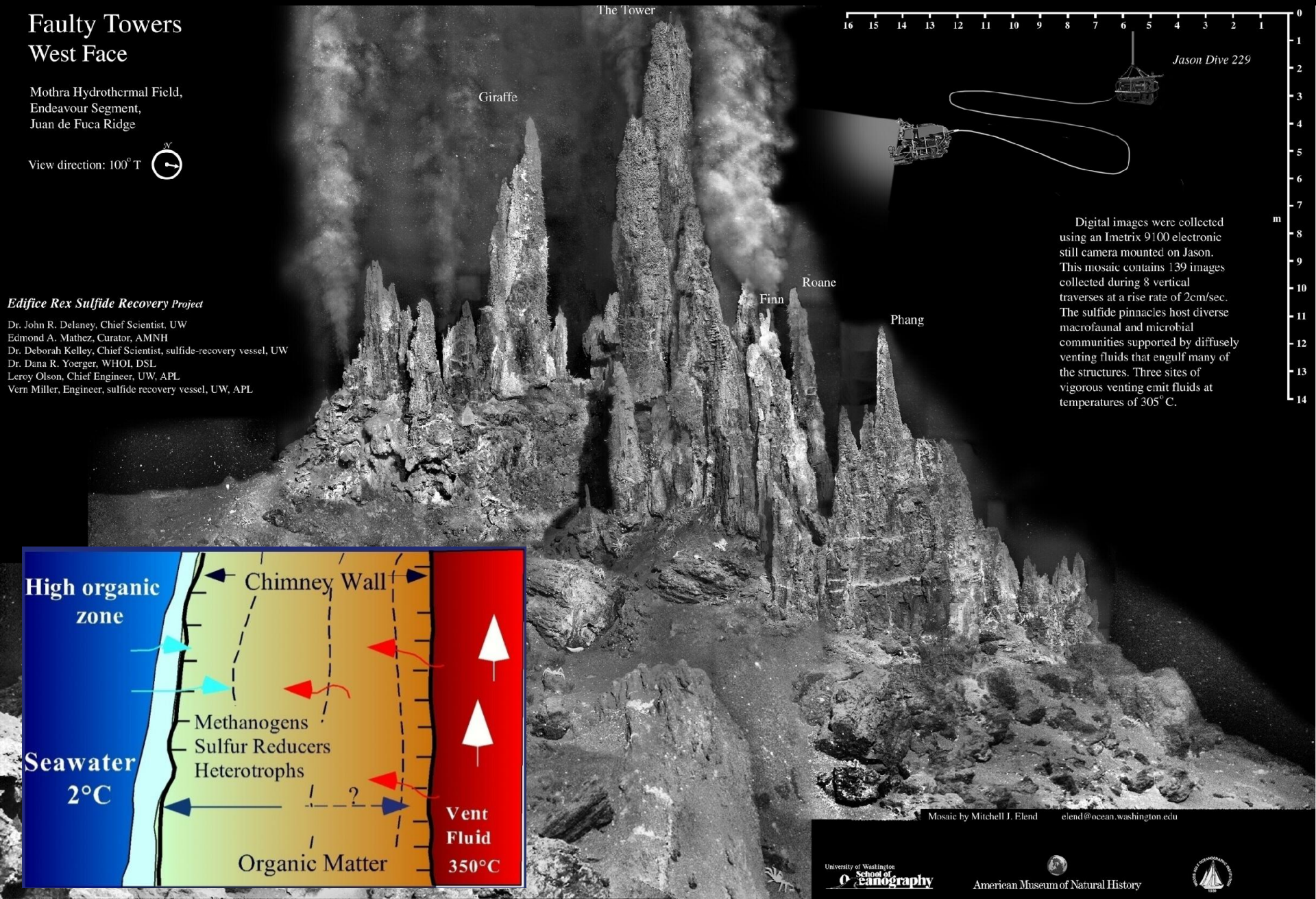
Matt Schrenk

Michigan State University





Mothra Hydrothermal Field, Juan de Fuca Ridge



Overview:

- Taking clean samples
- Measuring *in situ* activities
- Maintaining *in situ* conditions
- CORKs and seafloor observatories
- Biomarkers
- Core repositories

Limiting Contamination (seafloor)

Slurp sampling microbial mats at the Mid-Cayman Rise using ROV Jason

- highly precise sampling
- isolates samples from seawater during ascent

Photo courtesy of Chris German/WHOI/NSF, NASA/ROV Jason 2012, © Woods Hole Oceanographic Institution

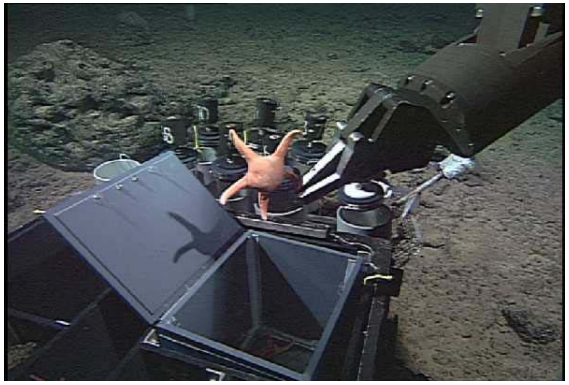


Image courtesy of Davidson Seamount Exploration 2002, NOAA/OER.

Bio-box used with ROV Tiburon at Davidson Seamount

- can accommodate larger samples (e.g., animals, rocks)
- can prevent cross-contamination and limit seawater infiltration during ascent

Limiting Contamination (subseafloor)

Try to maintain sample integrity

- High core recovery or large sample sizes
- Preserving stratification and/or important intervals
- Slowing infiltration into the sample interior

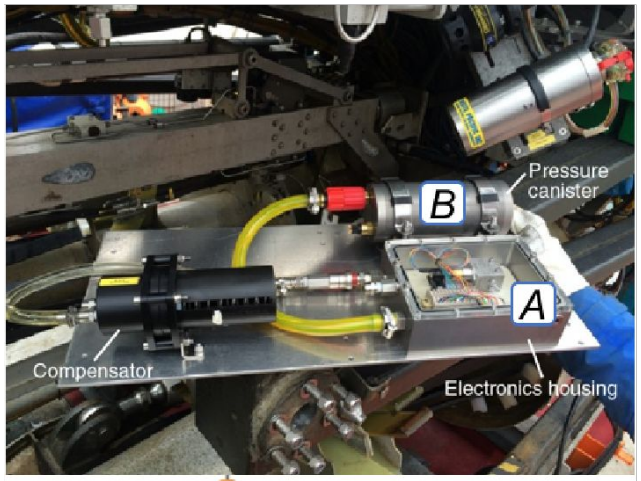
Sediment long corer deployed from the R/V Neil Armstrong in the Puerto Rico Trench to a depth of 8,385 meters
Photo courtesy of Woods Hole Oceanographic Institution

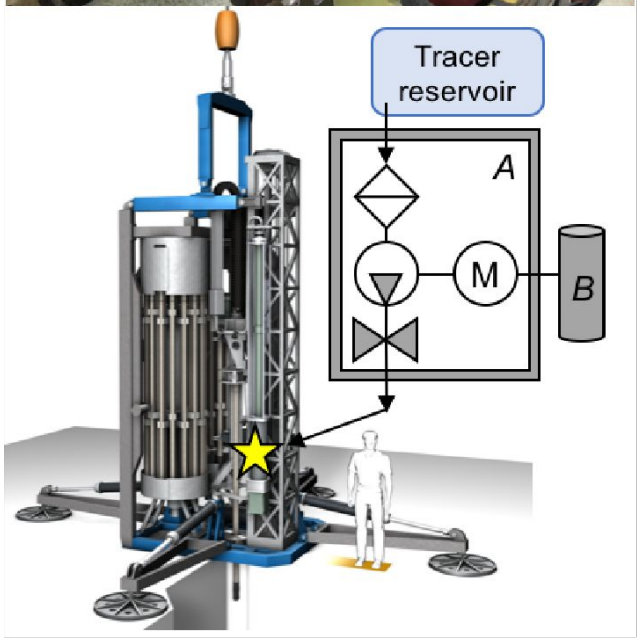
Core recovery during Antarctic drilling, Exp382
https://joidesresolution.org/core-on-deck-14/

Encapsulate cores in liners.
Manage the composition of drilling muds

- Limits exposure of cores to O_2, seawater, and other organisms
- Stabilizers used in drilling muds can stimulate microbial growth, so pro's and con's of drilling fluid (if needed) should be considered

Measuring Contamination

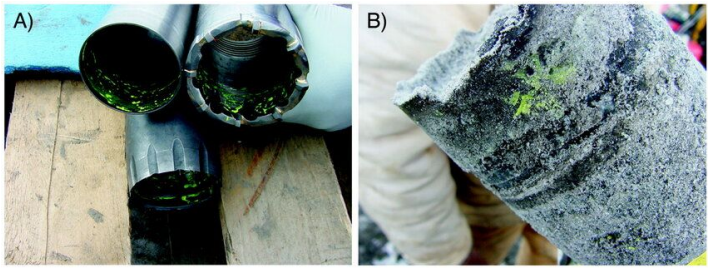




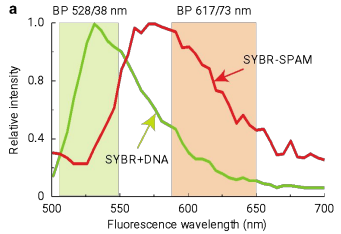
Orcutt*SciDrilling*2017

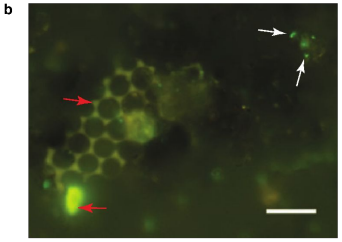
Injection of PFC tracers during drilling operations

- tracks proliferation of chemicals into the sample matrix
- Can be removed by volatilization
- (If you aren't careful, it can get everywhere)



Juck*AEM*2005

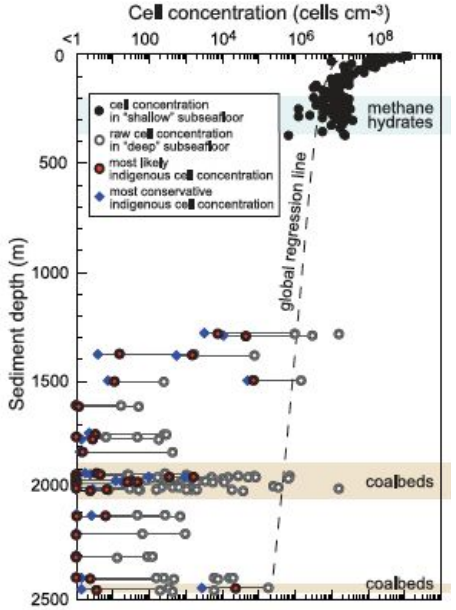




Morono*ISMEJ*2009

Fluorescent microspheres

- Cell-sized particles (~ 1 um)
- Distinct fluorescence spectra
- Hard to control delivery



Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor

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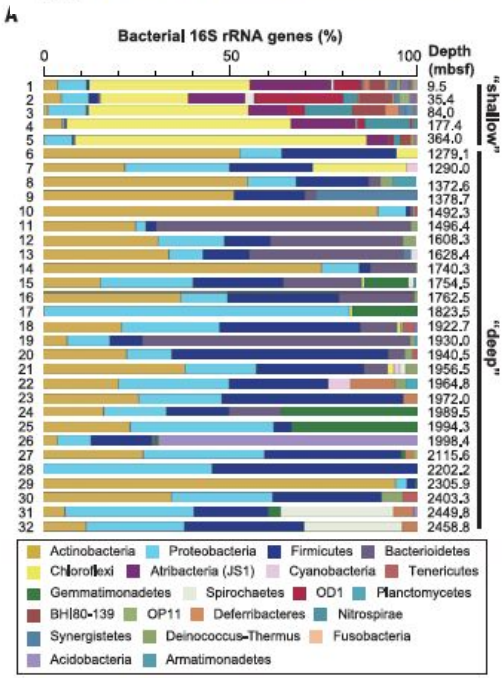
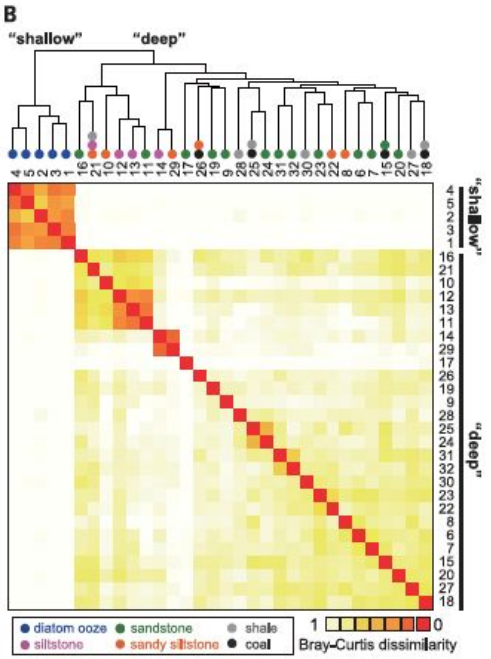
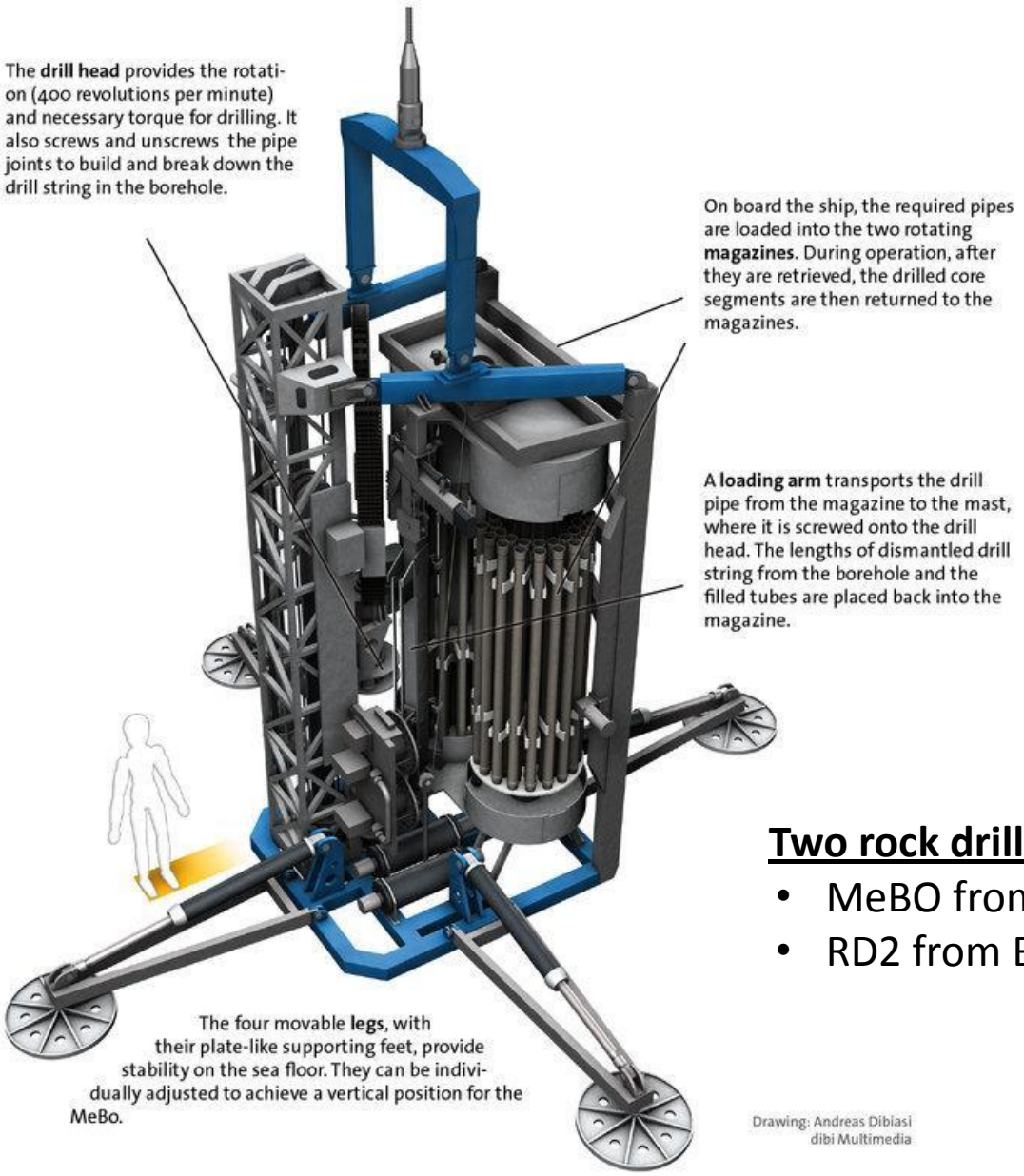


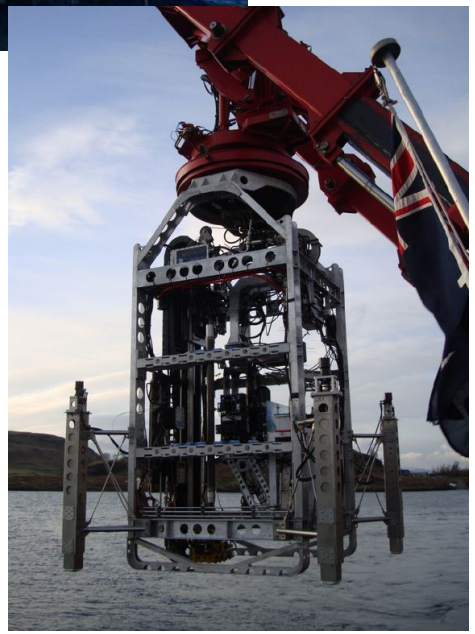


- Biochemical, geochemical, and sequence-based evidence of life deep within the seafloor
- Importantly, *applied multiple, independent lines of inquiry* to address the limits of the biosphere and to understand the mechanisms

Inagaki*Science*2015

X357 used Seabed Rock Drills for the first time in IODP





Two rock drills to be used:

- MeBO from MARUM
- RD2 from BGS

Measuring *In situ* Microbial Activities

Injection of tracers

Injection of preservatives

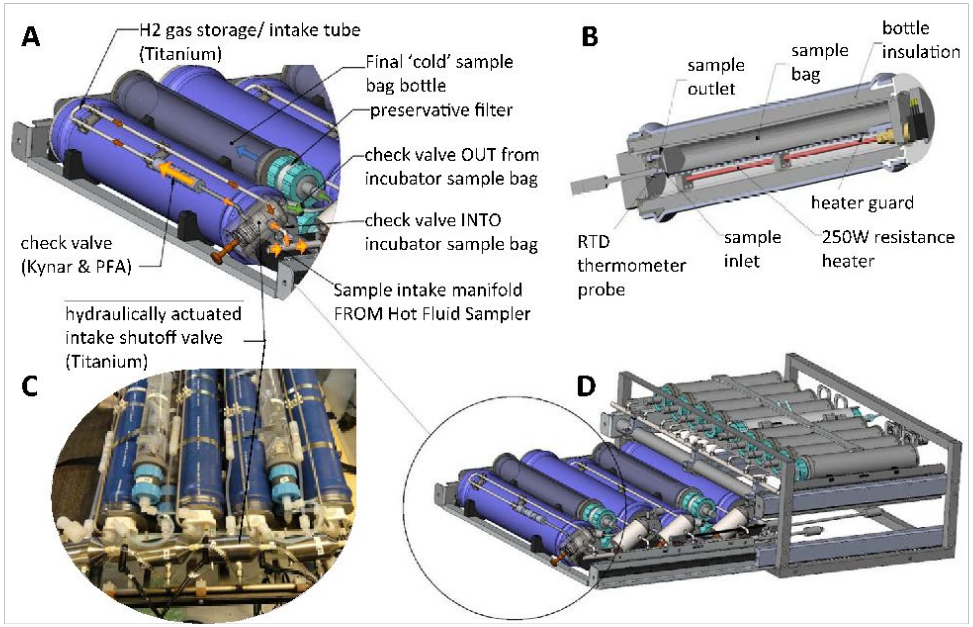


FIG 1 Incubator setup for the *in situ* RNA stable isotope probing (RNA-SIP) experiments. Each of the four incubation chambers was heated to a chosen set point temperature. Fluid was pulled into the insulated incubation chamber from the manifold of the hydrothermal fluid and particle sampler (HFPS) through a custom titanium shutoff valve, pulling hydrogen gas and buffering acid into the chamber as it filled. (A) After the incubation period, the fluid was pulled from the incubation chamber through a 0.22-μm filter with the passive addition of an RNA preservative. (B) A cutaway view of the incubation chamber shows the incubation bag over the heating element, with the RTD used to monitor the chamber temperature near the end of the bag. (C and D) The fully assembled incubator module (as deployed in 2015) (C) slides into the HFPS sample rack (D). Fluid transfer is accomplished with the HFPS sample pump and selection valve. PFA, perfluoroalkoxy.

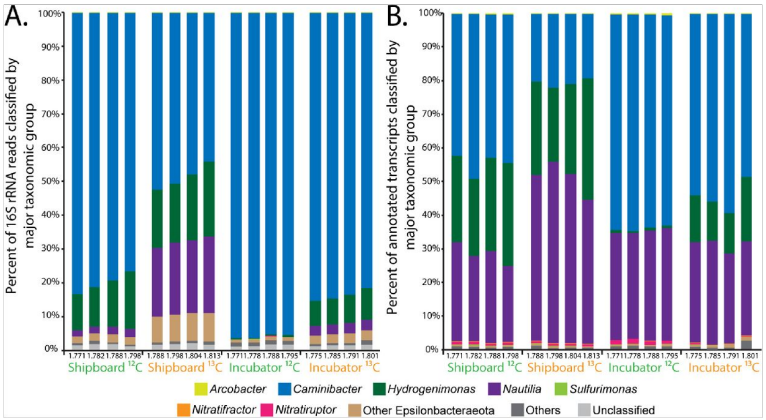


FIG 3 Taxonomic classification of 16S rRNA reads (A) and functionally (KO) annotated non-rRNA transcripts (B) from RNA-SIP metatranscriptomes.

Range of different additions possible:

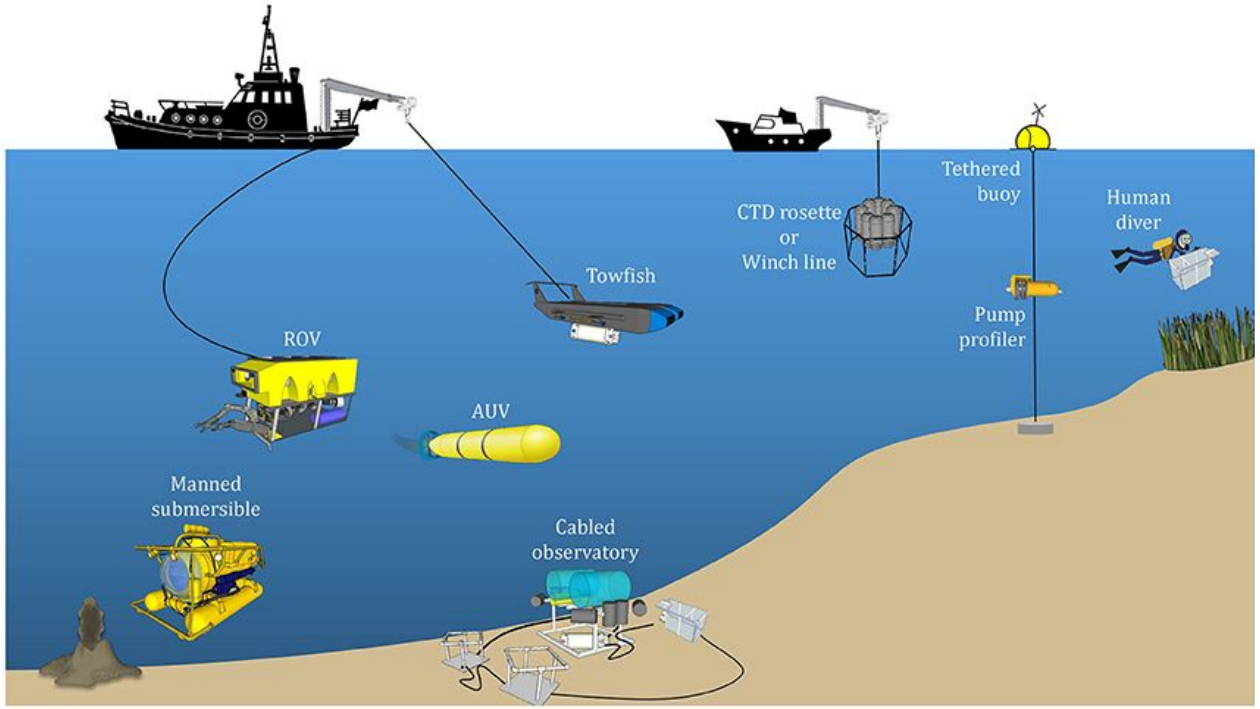
- Stable isotopes
- Radioisotopes
- Amendments

Activity arrested by:

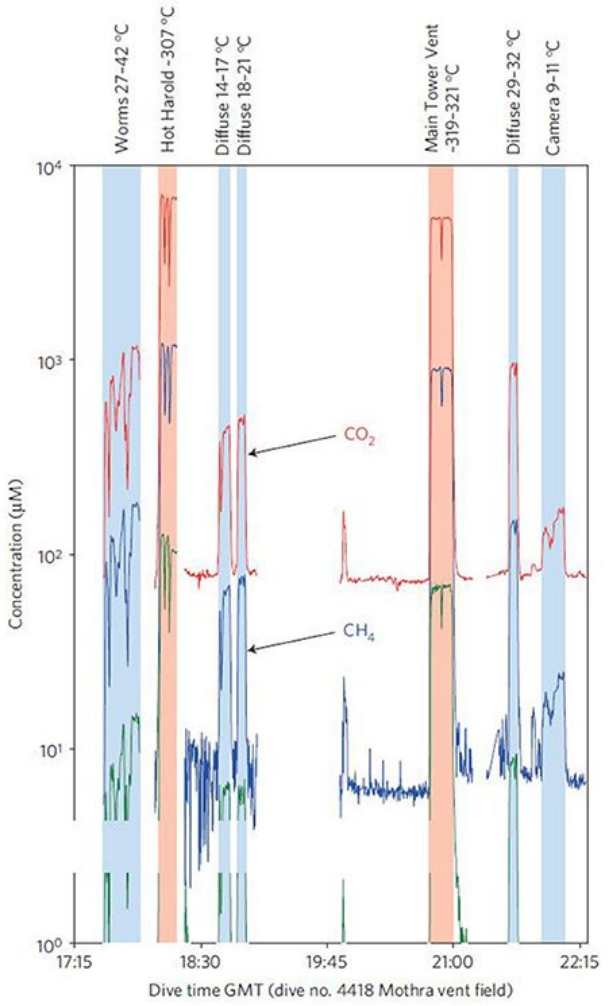
- Fixatives
- Filtration

Measuring *In situ* Microbial Activities

Mass spectrometry at the seafloor



Chua*FrontMarSci*2016



Wankel*NatGeosci*2011

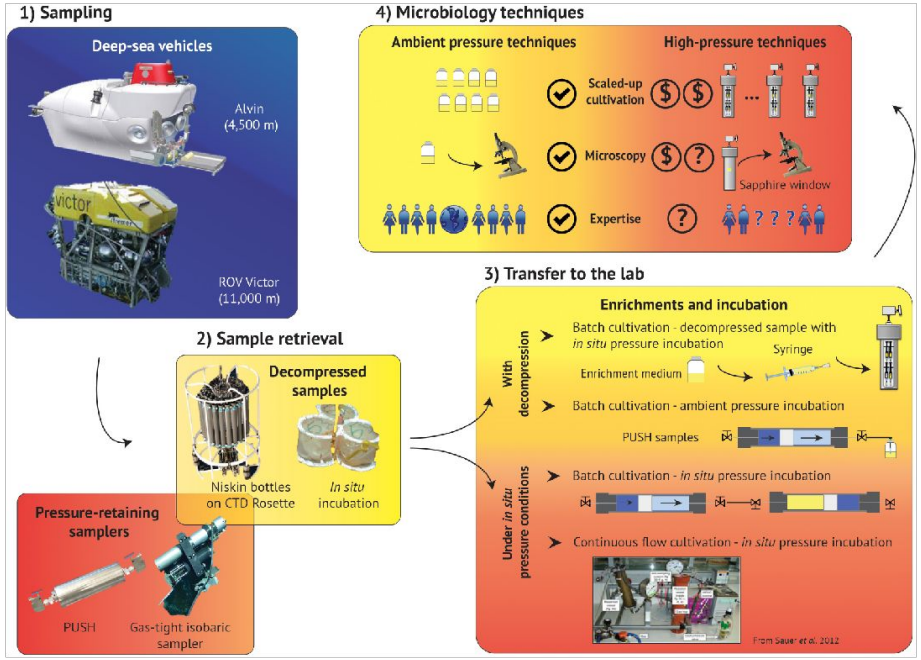
- Deployable from a number of different platforms
- Can be used to measure (bio) geochemical concentrations and fluxes

Cross-section of a black smoker chimney from Juan de Fuca Ridge

2°C seawater

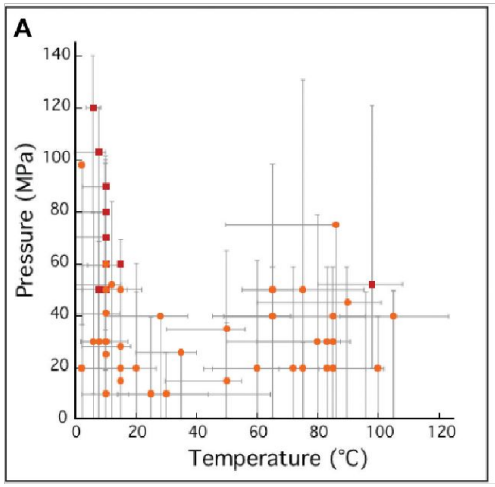
302°C inner conduit

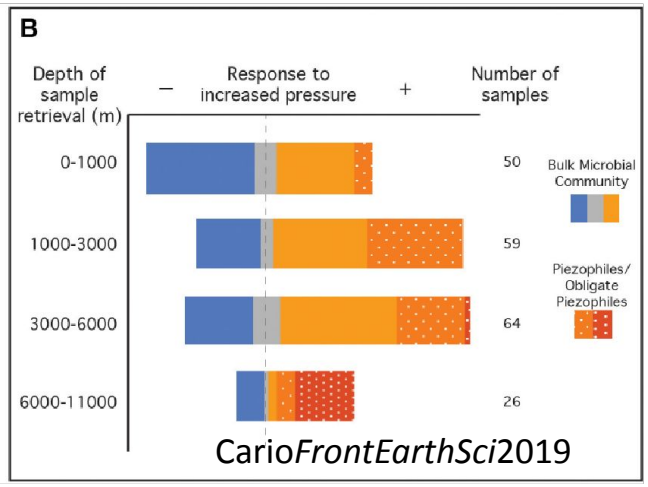
Maintaining *In situ* Conditions



Pressure retaining samplers (PUSH)

Pressure retaining sediment samplers (e.g., associated with gas hydrates)





CarioFrontEarthSci2019

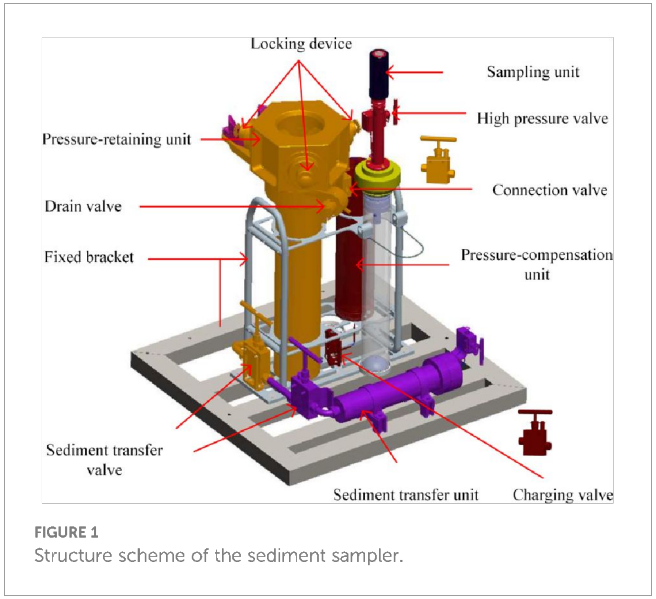
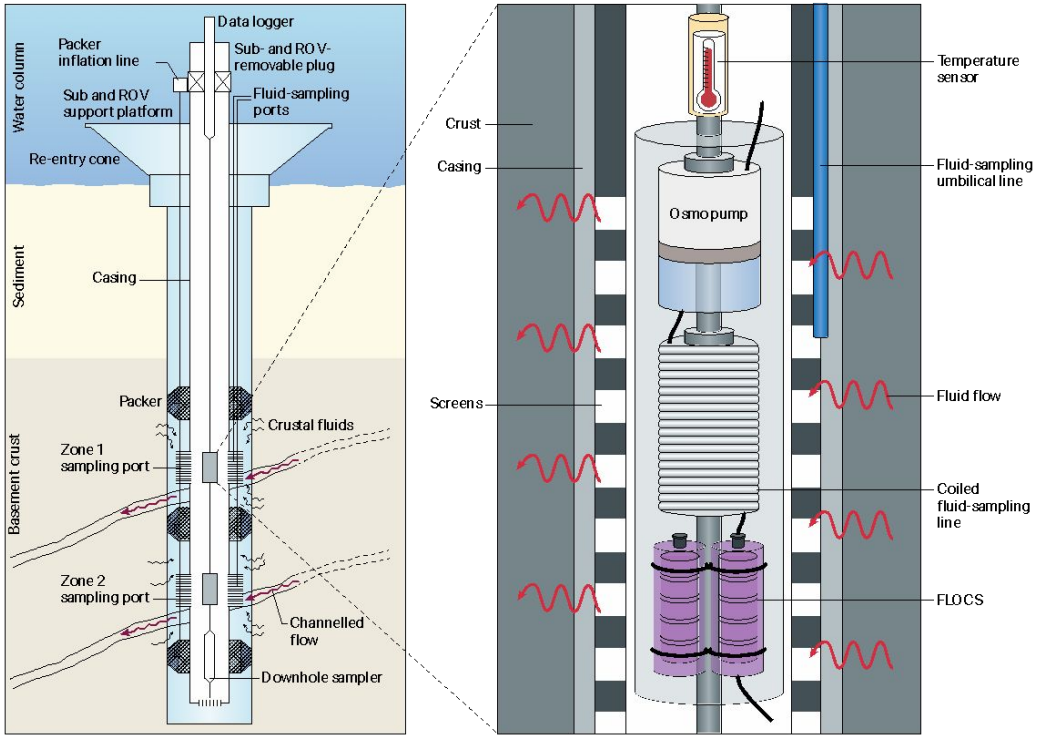


FIGURE 1
Structure scheme of the sediment sampler.

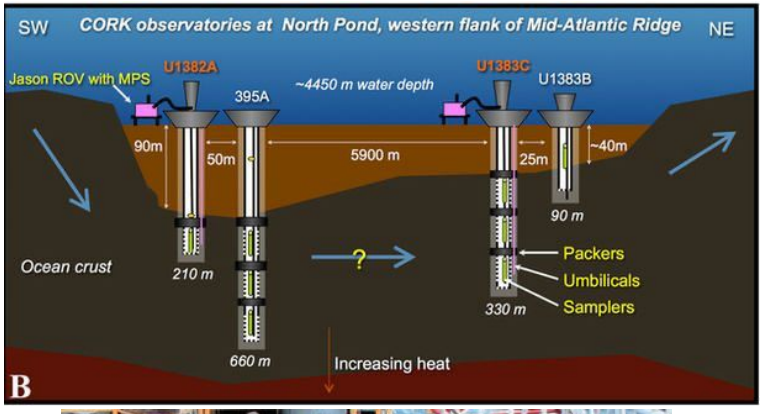
HeFrontMarSci2023

CORK Borehole Observatories



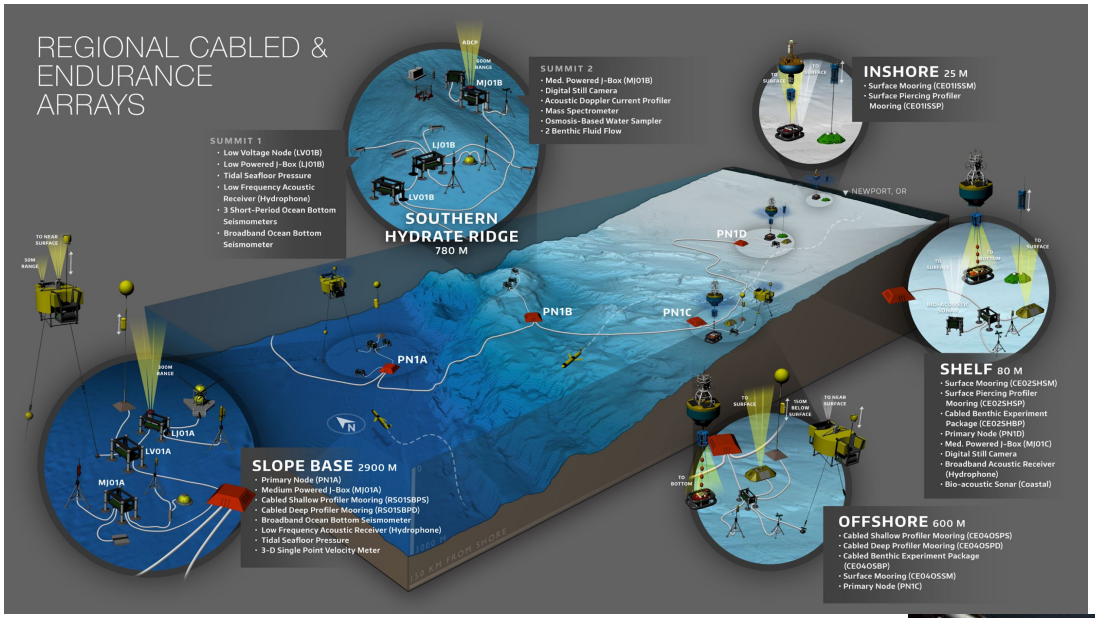
EdwardsNatRevMicro2011

- Really difficult to obtain intact samples from the fractured regions below the seafloor
- Installation of CORK observatories to directly sample the subseafloor aquifer

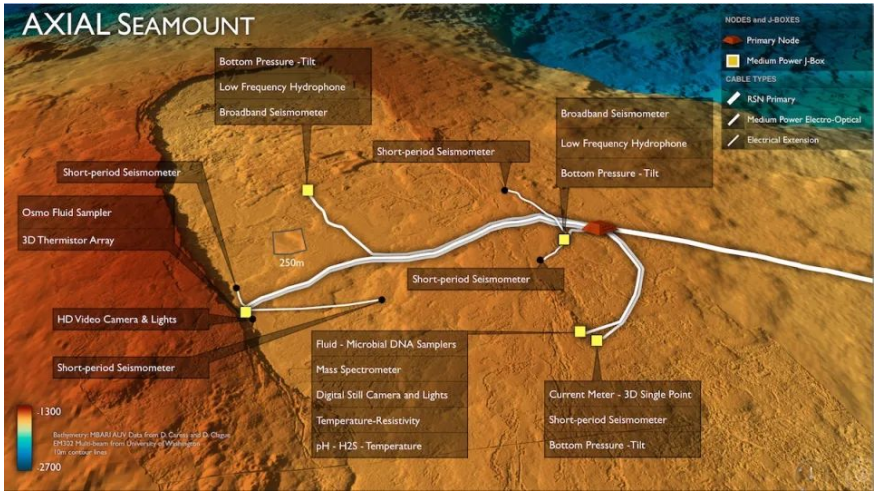


- Enable time-series sampling as well as experimentation on the seafloor
- Can be retro-fitted to existing boreholes

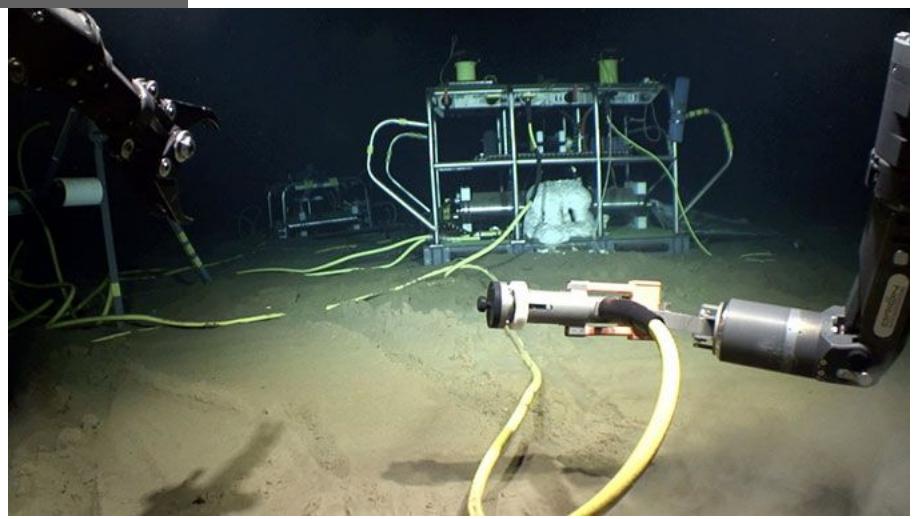
Cabled Seafloor Observatories



- Rapid response time
- Data feeds back to shore
- Power supply and docking capabilities
- Plate scale processes

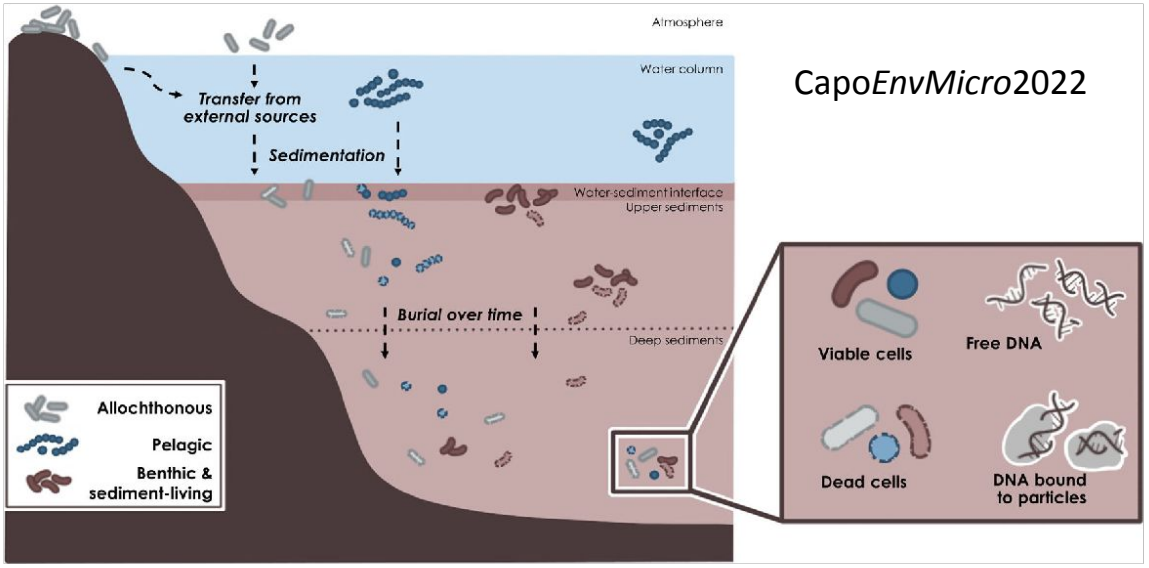


(Photo credit: UW)



(Photo credit: ACO, *Luʻukai*/ UH)

Biomarkers

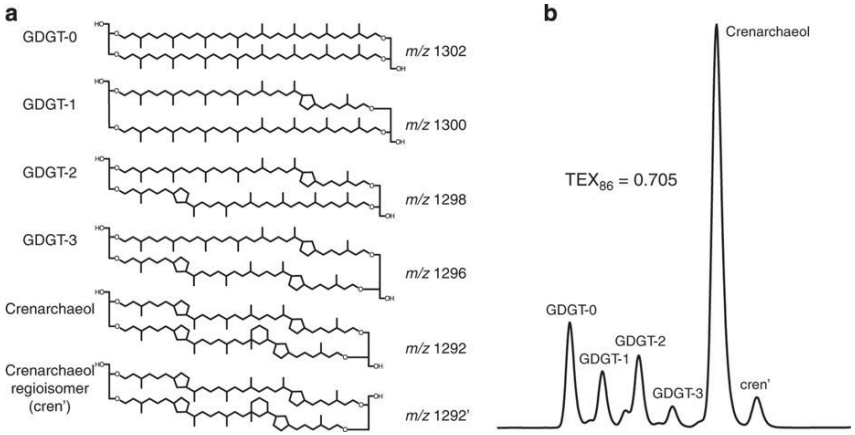


CapoEnvMicro2022

Consider both the living and fossil record or organisms in the seafloor

- Intact Polar Lipids, PLFA and lipid biomarkers
- Other metabolites?
- Stable isotope signatures
- eDNA and sedaDNA

Fig. 2. Composition of the microbial pool in aquatic sediments. The different sources of microbial cells (from external sources, the water column, and the sediments) are depicted with cells with different shapes and colours (see caption in the bottom left part of the figure). The different forms of microbial cells and DNA that can be found in deep sediments are shown at the bottom right part of the figure.



TierneySciData2015

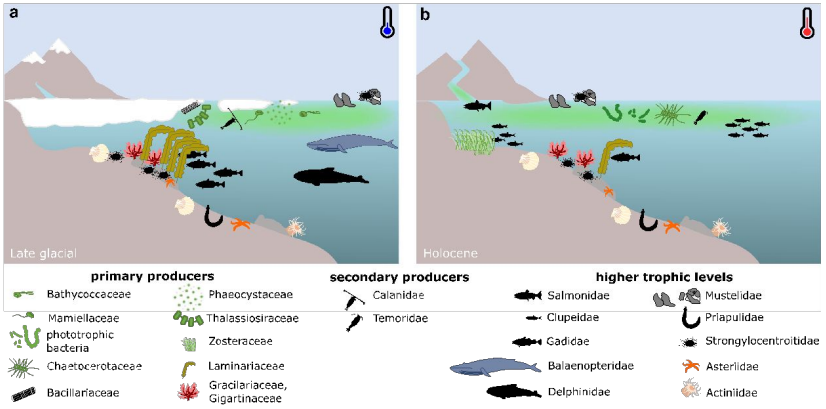


Fig. 5 | Schematic illustrations of ecosystem change after postglacial sea-ice loss. Representatives of functional groups of **a** the seasonal sea-ice ecosystem, which prevailed for most of the late glacial and **b** the ice-free ecosystem, which dominated during the Holocene.

ZimmermannNatComm2023

Opportunities at Core Repositories

- Legacy mbio samples stored at -80 C
- Ultra clean sample curation and processing facilities
- Clean rooms inside of clean rooms with laminar flow
- UV irradiation, static elimination, decontamination solutions, and plenty of blanks
- Cooled stage diamond saws for subsampling materials

Thank you!