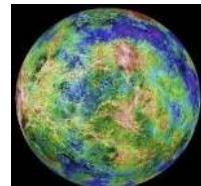
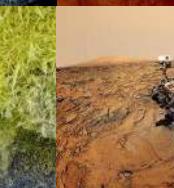
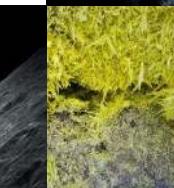
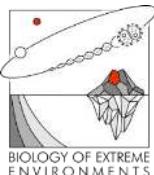
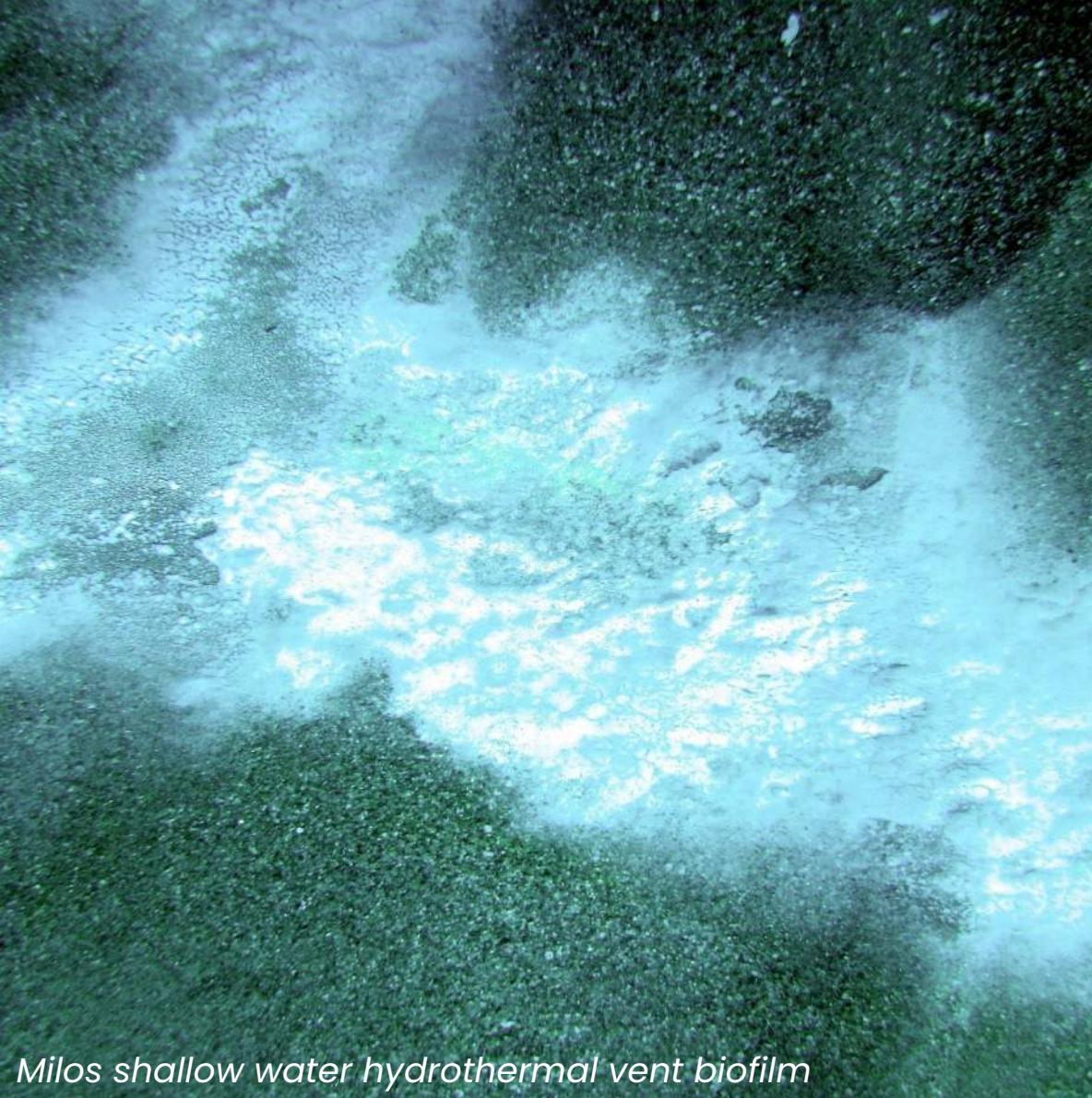


MICROBIOLOGY OF EXTREME ENVIRONMENTS

STUDYING MICROBIAL DIVERSITY IN EXTREME ENVIRONMENTS

Alessia Bastianoni, PhD
Postdoctoral researcher
Giovannelli Lab





Milos shallow water hydrothermal vent biofilm

General (first order) questions in studying microbial diversity

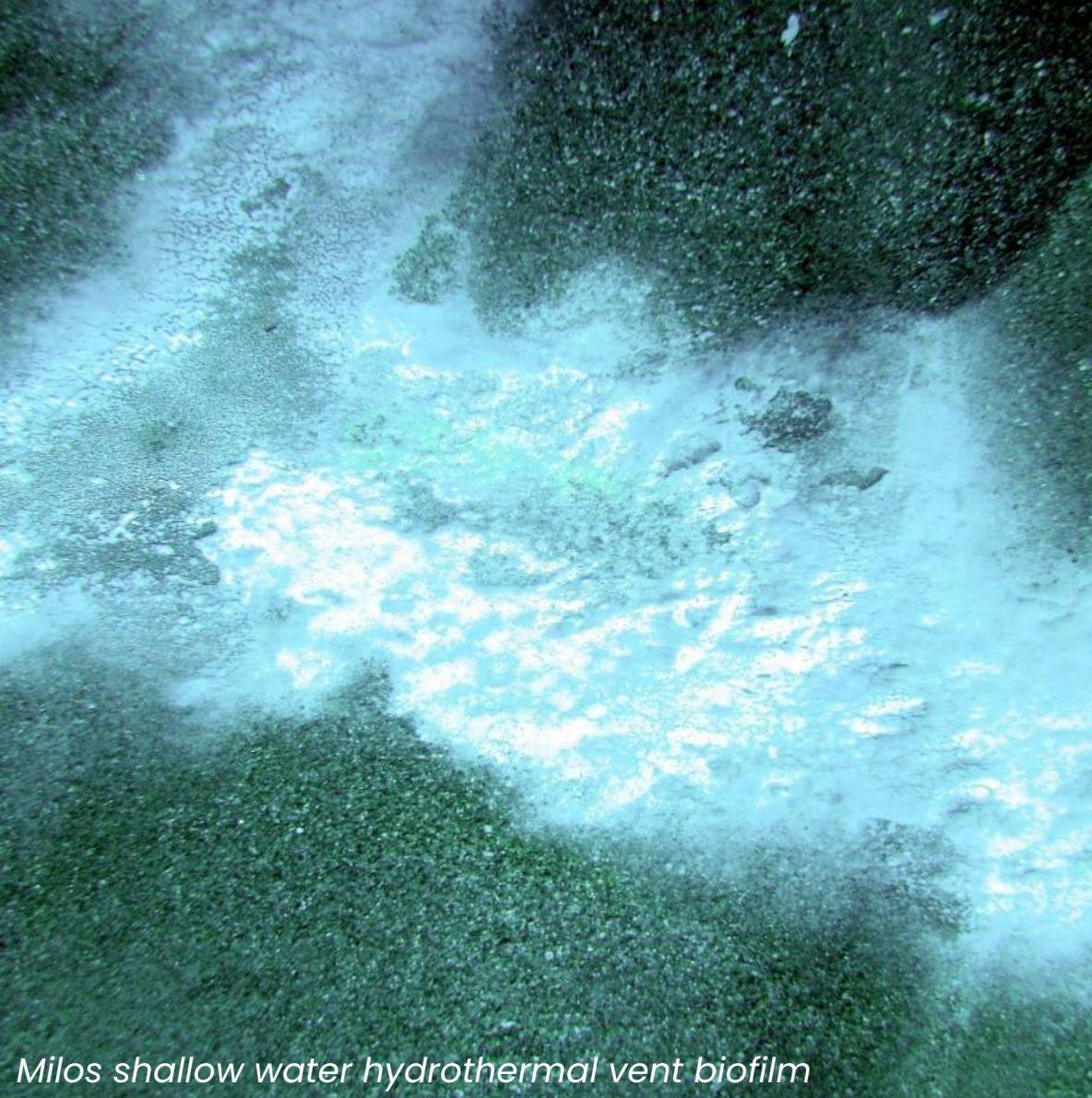
Who's there?

What are they doing?

Who's doing what?

How are they doing it?

To what extent?



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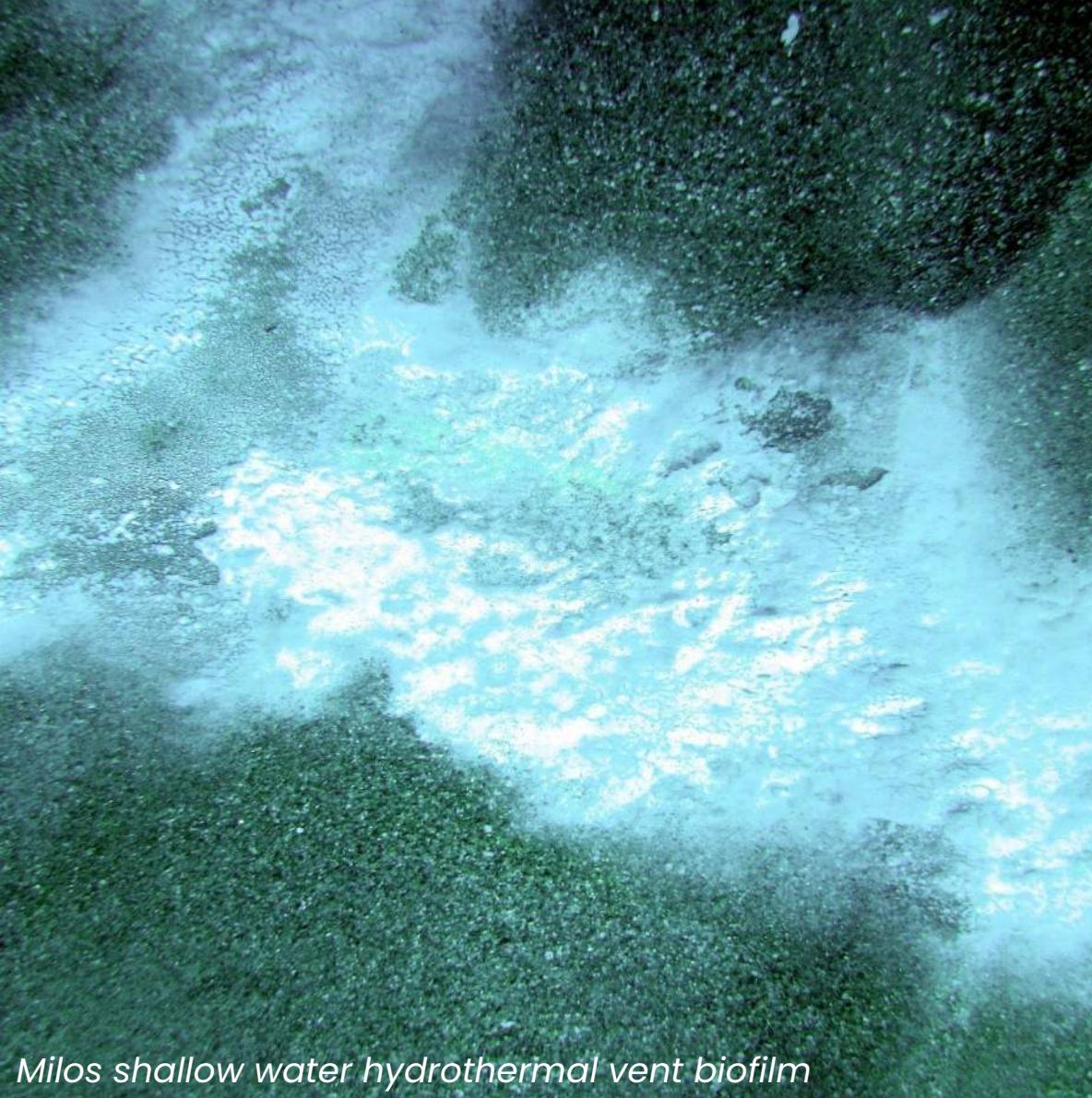
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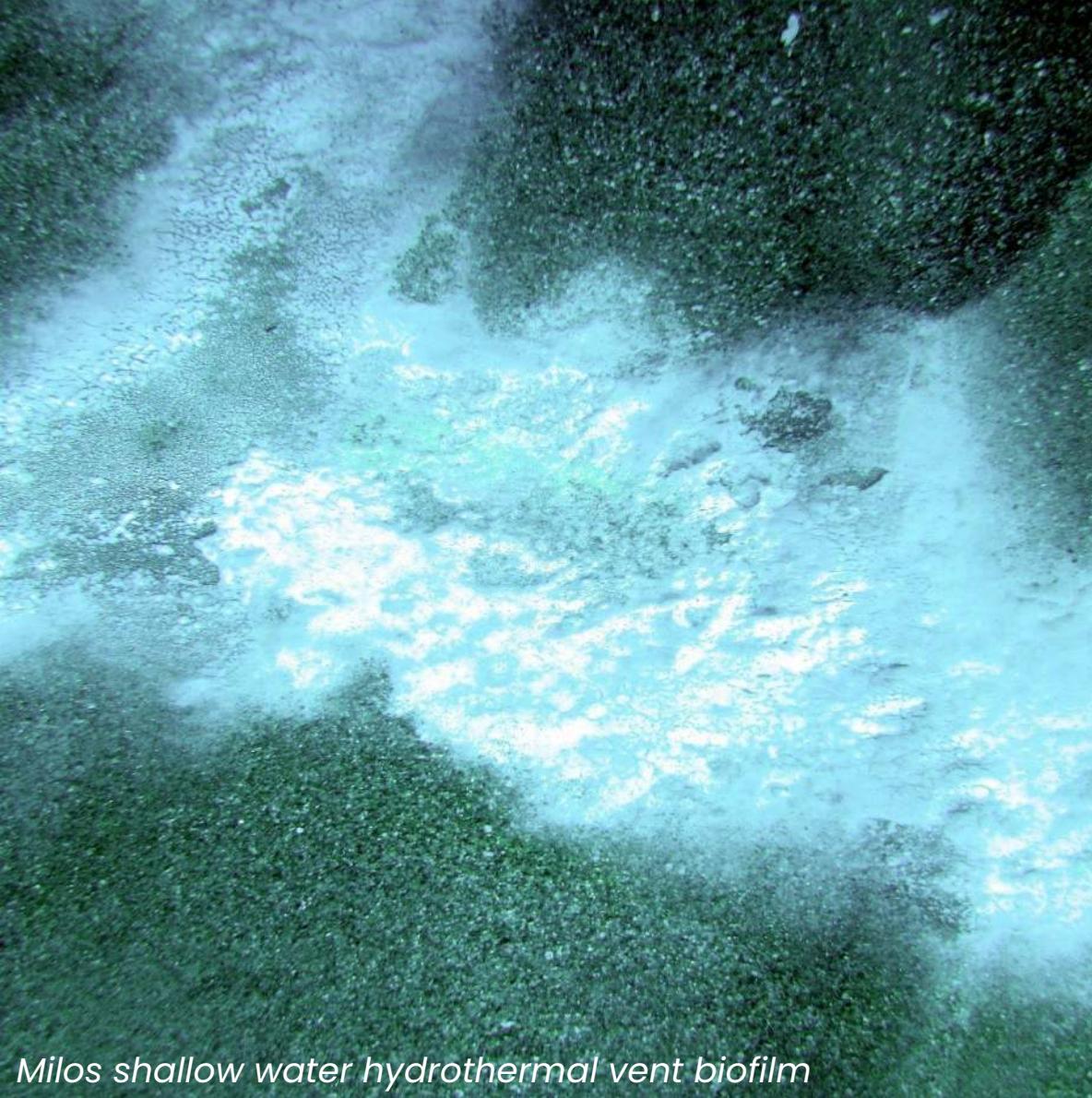
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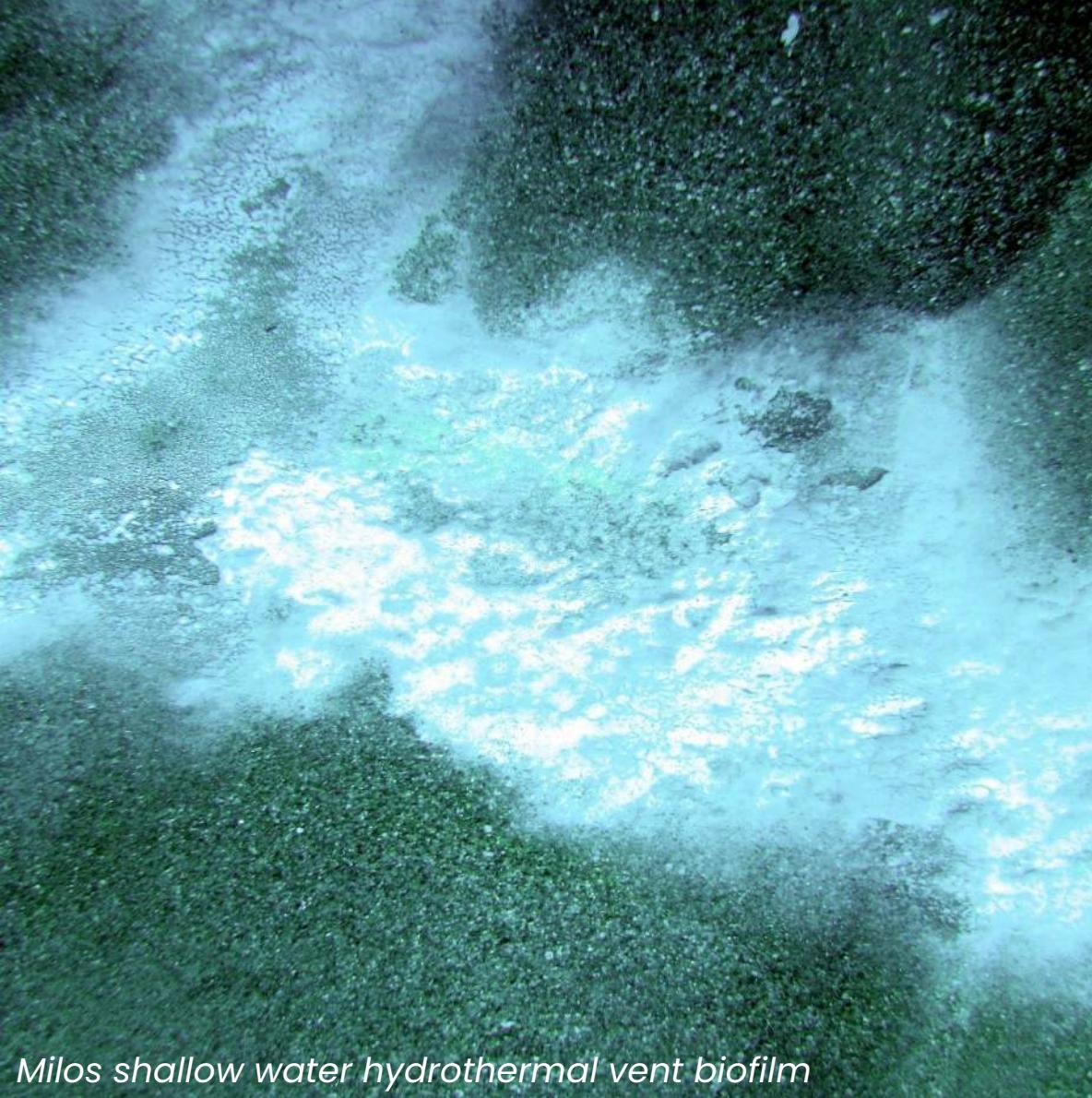
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Milos shallow water hydrothermal vent biofilm

Higher order questions in studying microbial diversity

How is diversity influenced by environmental conditions?

How is functional diversity influenced by environmental conditions?

How is diversity and functioning influenced by species interactions?

How are metabolic rates influenced by environmental conditions and species interactions?

Approaches to Microbial Diversity

Each time we approach the study of microbial diversity we are following a similar workflow generally consisting of:

1. Definition of the study question and study design

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4. Sample processing and data acquisition
5. Data analysis
6. Interpretation of results

Approaches to Microbial Diversity

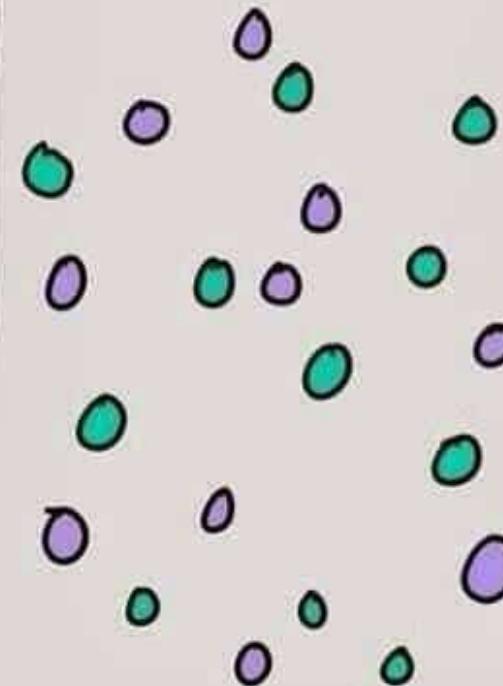


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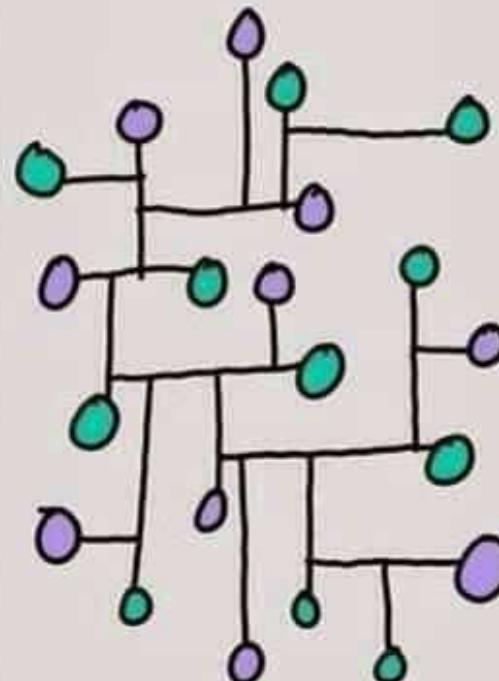
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Approaches to Microbial Diversity

information:



knowledge :



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Observe natural phenomena

Formulate Hypothesis

Modify Hypothesis

Test hypothesis
via rigorous Experiment

Establish Theory
based on repeated validation of results

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Formulate Hypothesis

Test hypothesis via rigorous Experiment

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JORGE CHAM © 2006

Establish Theory based on repeated validation of results

THE ACTUAL METHOD

Make up Theory based on what Funding Agency Manager wants to be true

Modify Theory to fit data

Design minimum experiments that will prove ~~show~~? suggest Theory is true

Publish Paper: rename Theory a "Hypothesis" and pretend you used the Scientific Method

Defend Theory despite all evidence to the contrary

Problems in studying microbial diversity



Problems in studying microbial diversity

Problem	How to address it	Drawbacks
Spatial heterogeneity		



Problems in studying microbial diversity

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Spatial heterogeneity	using several hundreds milliliters of waters or few grams of sediments	

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Technical bias of chosen methods	selection of different and complementary techniques	bias and limitations of each technique, affecting the robustness of results and conclusions

Sampling for Microbial Diversity



Sampling an environment can be done in several **different ways** depending on the scientific question, the environment under investigation and the technique to be used

Generally speaking samples can be collected of (sea)water, specific fluids, sediments/soils, aerosols, biofilms or flora/fauna

The difficulties and technological hurdles associated with collecting these sample largely depend on the environment investigated

The need for **specific sample preservation protocols** might also complicate the sampling procedure



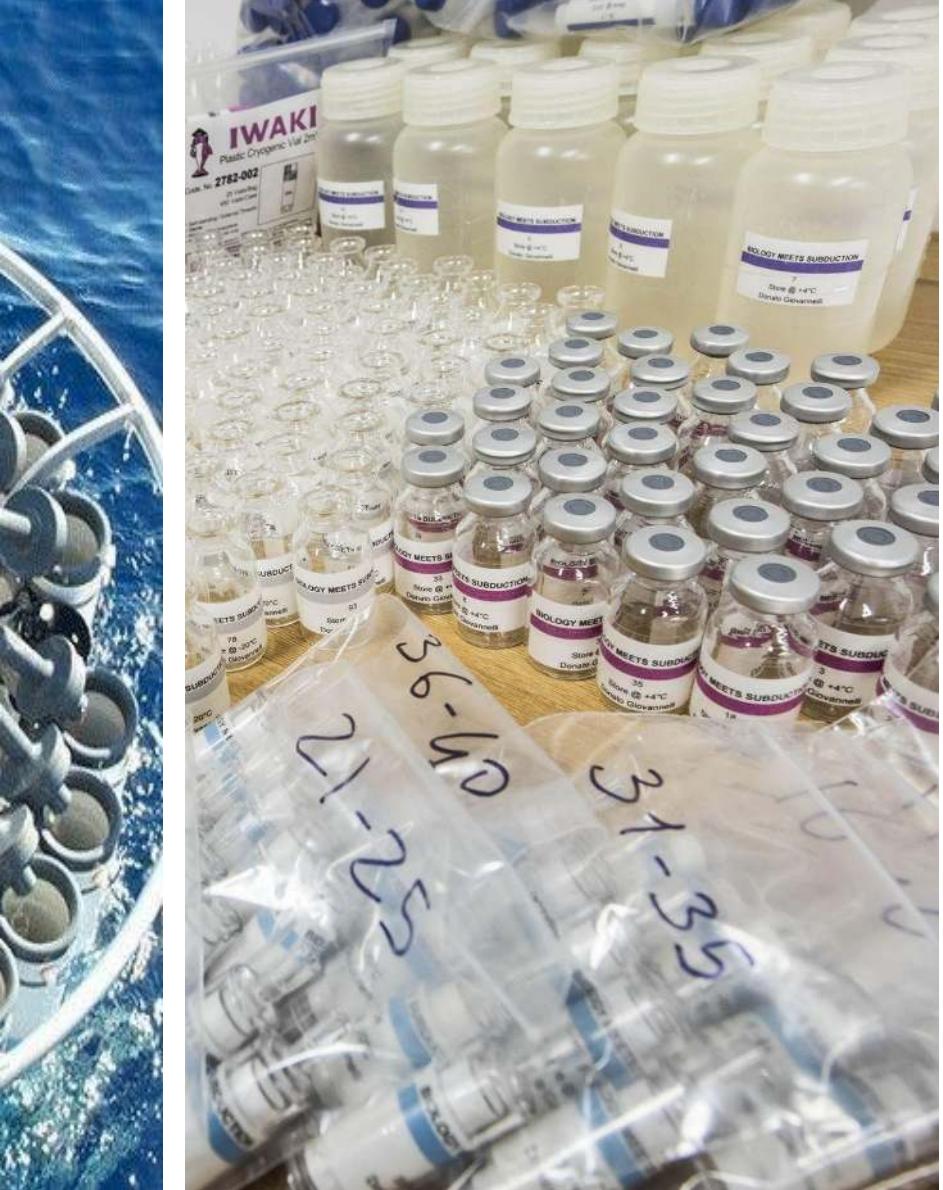
Sample **access** is key!

Accessing remote locations is a continuous challenge, and sampling logistic need to be carefully evaluated, planned and executed

Each specific environment has its own set of challenges, that might include technological, methodological and hazard hurdles

There is no specific training that can get you ready for the field, beside fieldwork itself

Fieldwork and Sampling activities in Extreme Environments
(Prof. Domenico Fulgione – 6 CFU)



Analysis	Code	Sample type	Analyses	Quantity × samples	Vials	Storage	Total samples
Diversity	S F BG	Sediment	Molecular analysis	~40 g	50 mL falcon	-20°C	
		Fluids	Molecular analysis	3x	Sterivex filter 0.22 µm	-20°C	
		Soils	Molecular analysis	~40 g	50 mL falcon	-20°C	
Geochemistry	GEO	Sediments	OM, Isotopes, solid geochemistry, dithionite extractable reactive phases of metals, elemental sulfur	~40 g	50 mL falcon	-20°C	
Geochemistry	Geo F Geo F acid	Fluids	Nutrients and anions	~ 10 mL	50 mL falcon	+4°C	
		Fluids Acid	Cations and metals	~ 10 mL	50 mL falcon (acid-wash)	Acidified, +4°C	
Enrichments	A	Mixed	Chemoautotrophic anaerobic enrichments	~20 g / 100 mL	120 mL anaerobic vials	+4°C	
Isotopes	ISO	Fluids	¹³ C-DIC, ¹³ C-DOC, ¹⁸ O-H ₂ O, ² H-H ₂ O	15 mL	15 mL acid-washed vials	+4°C	
Prokaryotic Counts	PCS PCF	Sediments	Cell counts	1 g	Cryovials pre-loaded 200 µL of 37 % formaldehyde and 800 µL of water	2% formaldehyde, +4°C	
		Fluids	Cell counts	2 mL	Cryovials pre-loaded 121 µL of 37 % formaldehyde	+4°C	
POM		Fluids	Particulate Organic Matter	200 ml	0.47 µm GFF ashed	-20°C	



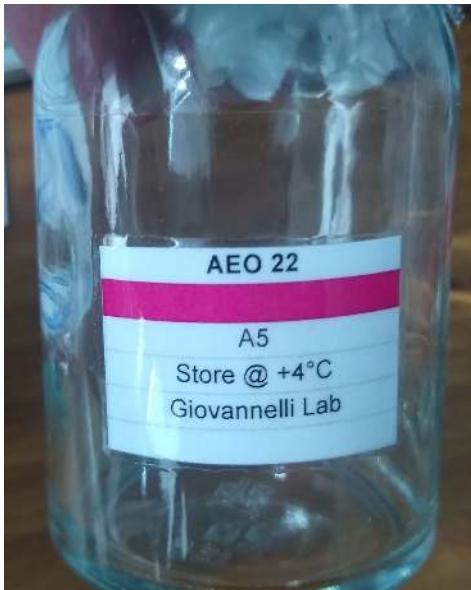
Sterivex Filters (Blue labels with F-#). **Filter up to 2 Liters for each filter** (3 in total), about 20x 60 ml syringes. Remove the excess fluids from the filter at the end by passing some air through the filter. **Store at -20 °C (best option)** upon return at the hotel (or at +4 during travel time). Alternatively add the storage solution provided (1-1.5 ml), cap both ends, tape it close and store at +4 °C.



Falcon 50 mL (pink and orange label marked as Geo F-# and Geo F acid-#). **Fill them with 40 ml of filtered fluids (coming out of the strivex above)** and **store at +4 °C**. Please **allow at least 1 full syringe to go through the filter** before collecting the filtrate.



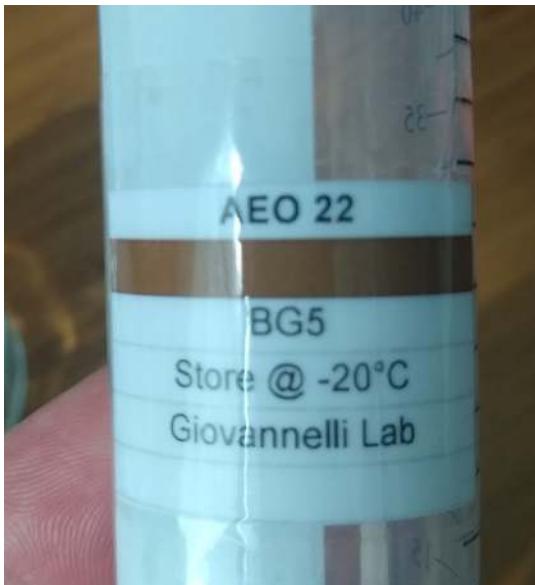
Small Glass vials (teal label marked ISO-#). **Fill it to the rim with filtered fluids (from the sterivex above)** and **cap it avoiding any bubbles**. To be used for **DIC** and **DOC** analysis. **Store at +4 °C**.



Large glass vials (purple label marked A#). Fill it with a **mix of fresh (non filtered) fluids**, about **1/5th of sediments** and **colored biofilm**. Cap it avoiding bubbles using the white rubber cap (flipping the edges). **Store at +4 °C**.



Falcon 50 mL (blue and yellow label marked S-# and Geo-#). **Fill them up to 40 ml with sediments collected as close to the vent as possible.** Avoid if possible excess liquid (or it might break the container expanding) and dead organic matter (twigs, leaves, moss, biofilm). **Store at -20 °C** (at least to kill the microbes).



Falcon 50 mL (brown label marked BG#). **Fill it up to 40 ml with background soils** collected upstream from the springs. **Avoid** if possible **excess organic matter** (twigs, leaves, moss, biofilm). **Store at -20 °C.**

Sample **access** is key!

Driving, off-road driving, hiking, climbing, back-country skiing, mountaineering, scuba diving, boating, and in general outdoor skills can all be important assets in the field and depending on the location

Additionally, the use of advanced technologies might play a key role. This includes drilling rigs, drilling ships, ROVs, AUVs, drones, submarines, oceanographic ships, etc...



A significant portion of the research in extreme environments is devoted to **developing and testing technologies for sampling in remote areas** under challenging conditions (i.e. gas emissions from volcanoes)









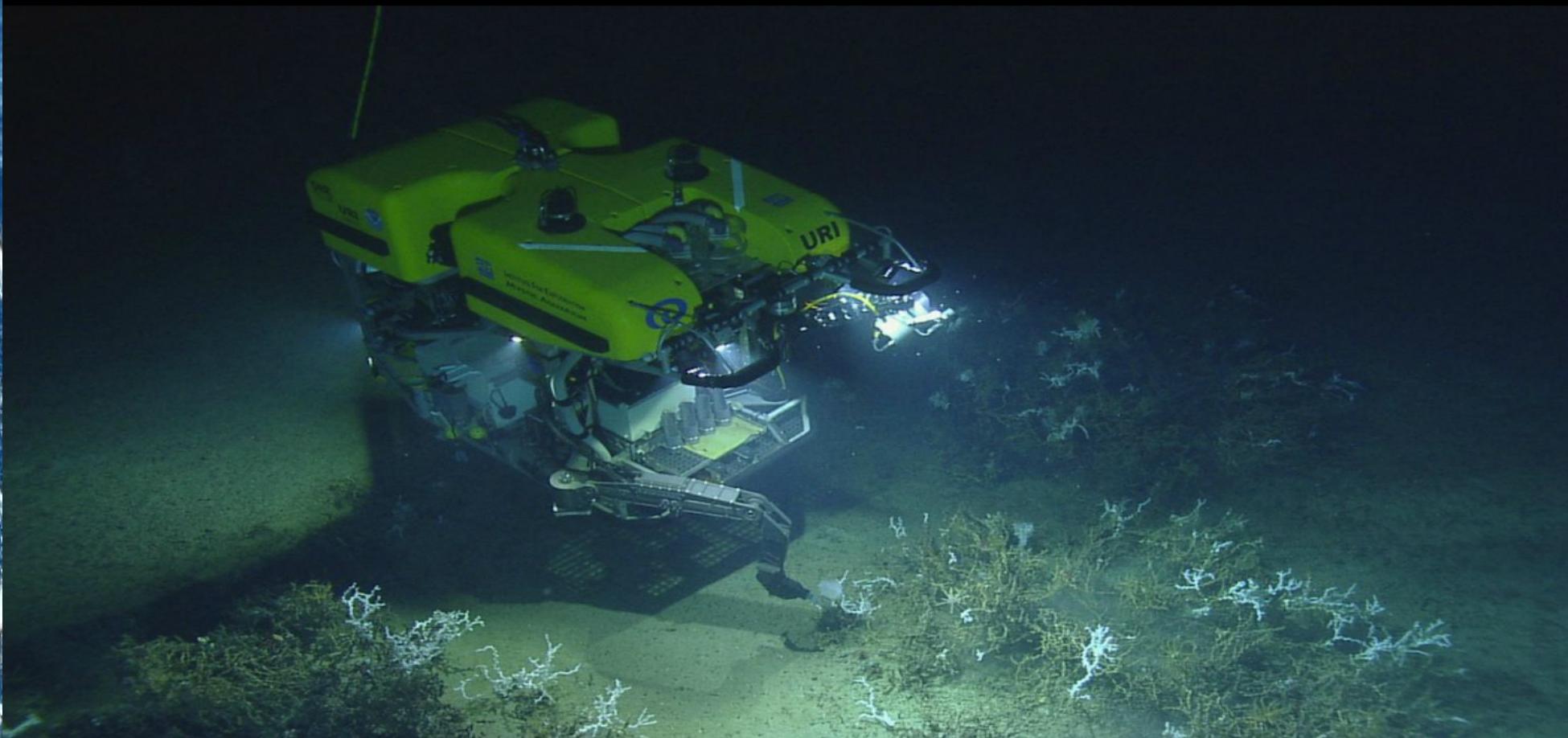
ATLANTIS
WOODS HOLE

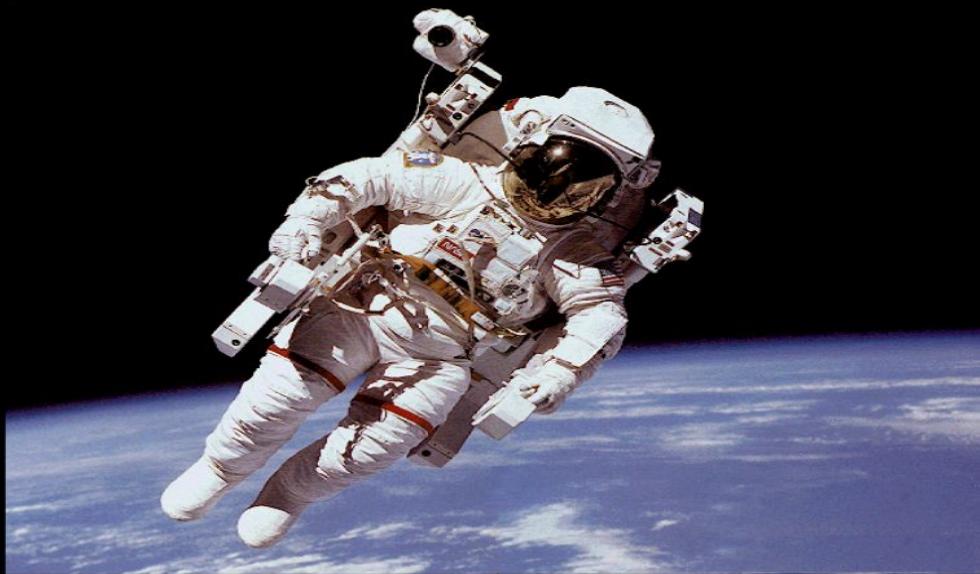
5000

ALVIN

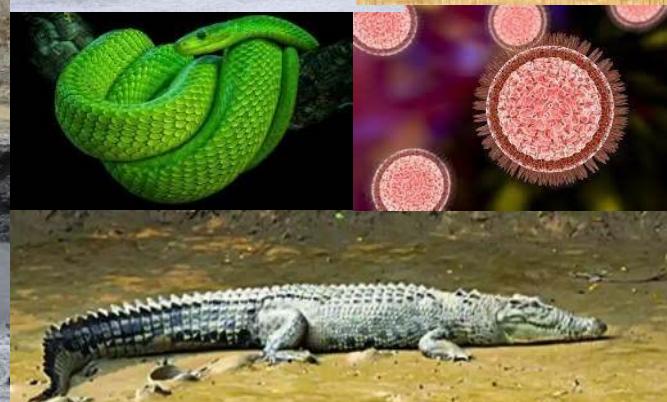
WOODS HOLE

ACOUSTIC







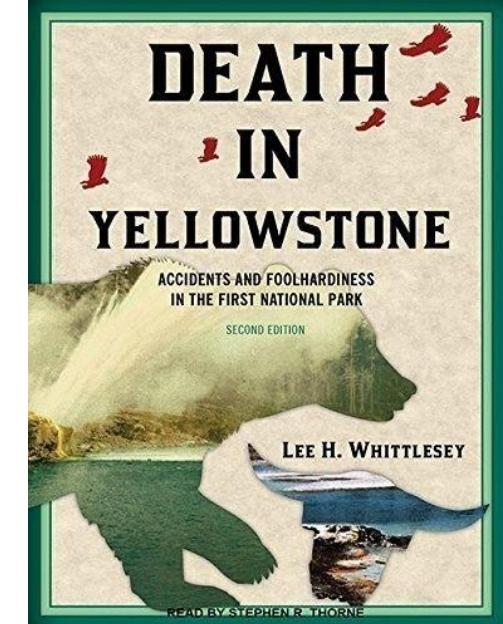


Sampling **hazards**

Remote locations and extreme environments pose a number of health and safety hazard on the research personnel involved in sample collection

Weather, environmental conditions, specific risks (e.g. volcanic eruptions), poisonous gases, wildlife, diseases, geopolitical risks all play a role and should be carefully evaluated and minimized

Accidents are not uncommon when working/sampling in remote areas





The Anthropology of Field work

The coordinate of the best location where to sample might be not available at the time of planning. This might require approximation in the sampling plan and unknown outcomes

Historical documents, archival research and interaction with local researchers and local populations is the key to a successful sampling

Most importantly, local scientist are valuable key collaborators on the science being done in the context of the sampling and involving them as collaborators adds tremendous value to the science being done, in addition to avoiding "scientific neo-colonialism" or "parachute science" type of behavior



A vertical strip on the left side of the slide shows a close-up view of a circular metal frame, possibly a scientific sampling device, set against a background of blue ocean water with visible ripples.

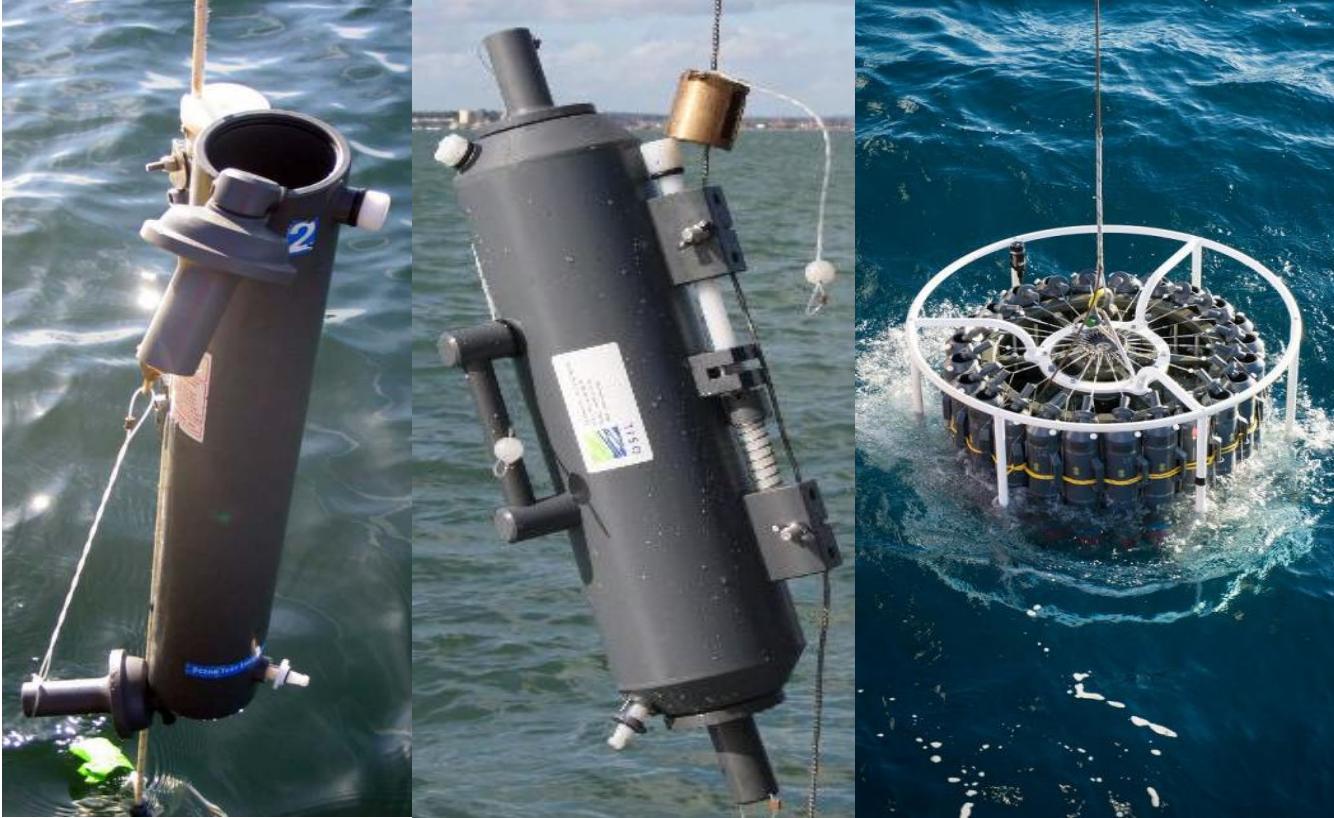
Sampling for Microbiology

sampling (**sea**)water

Seawater can be sampled in several different ways. The principal techniques are the use of hand-operated **sterile** or **acid-washed** bottles or containers, collection of known volumes using pumps and using the Niskin bottles, often mounted on a CTD-Rosette sampler



sampling (sea)water



sampling (sea)water

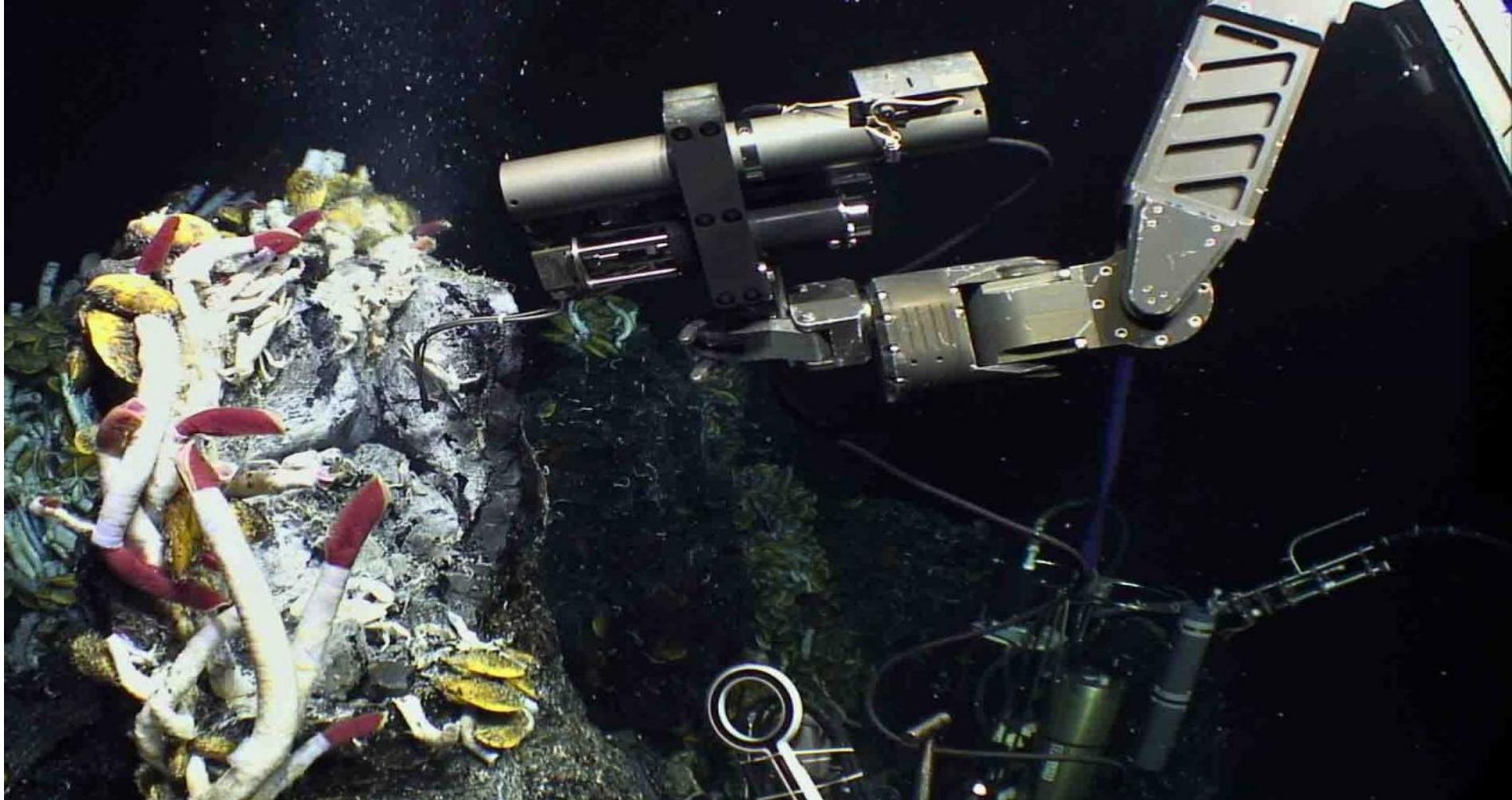


sampling Fluids

Fluids, like sediment pore fluids, or fluids seeping from a cold seeps or hydrothermal vent can be sampled using different approaches, that are specific to each case. Common approaches include centrifugation of sediments (pore fluids), core suction (pore fluids), pumps (fluids) and syringes (pore fluids and fluids)

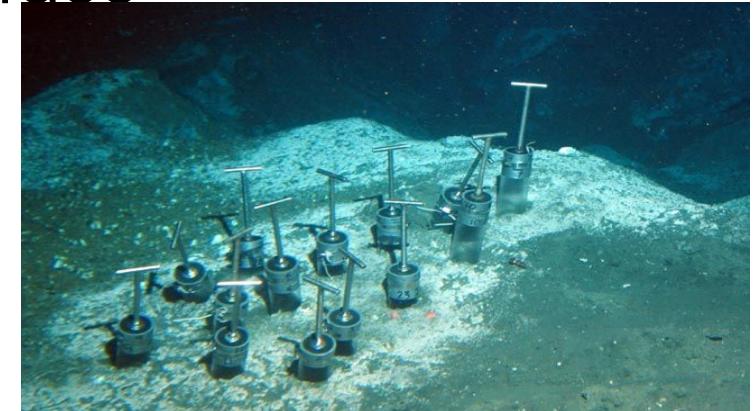


sampling Fluids



sampling Biofilms

Biofilms can be sampled through a syringe, a push core, or directly swabbing the surface

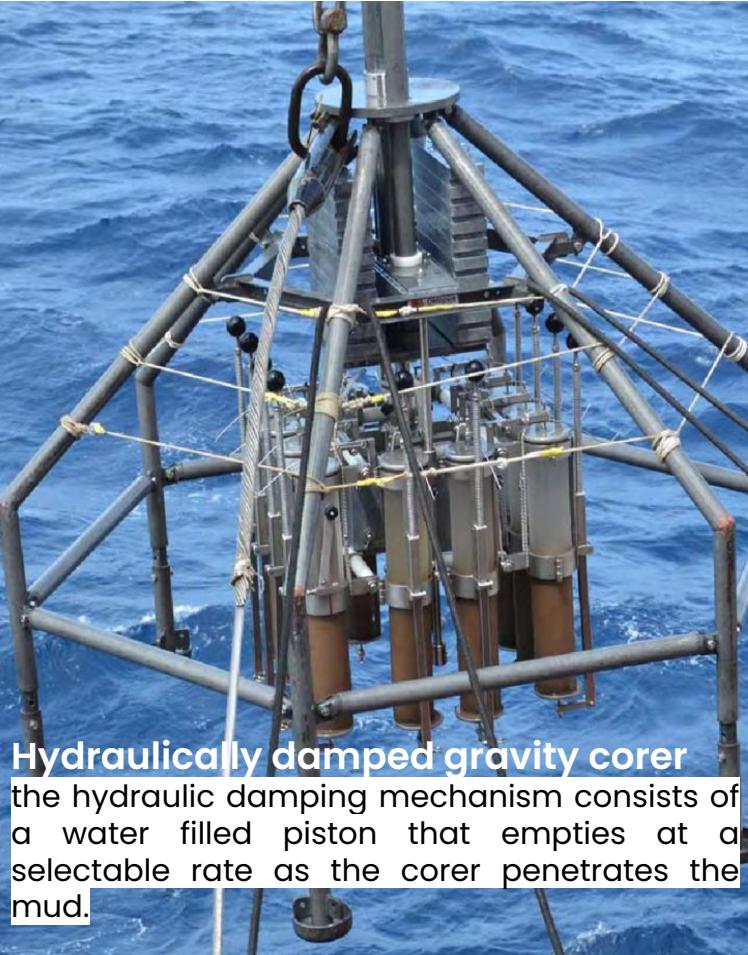


sampling **Sediments/Soils**

Sediments can be sampled in a variety of ways. The simples way is to use a sterile container (like a falcon tube), however the most used metods is a push core. Beyond that the use of multicorers, boxcorers, gravity corers and drilling is also used, depending on the purpose and location

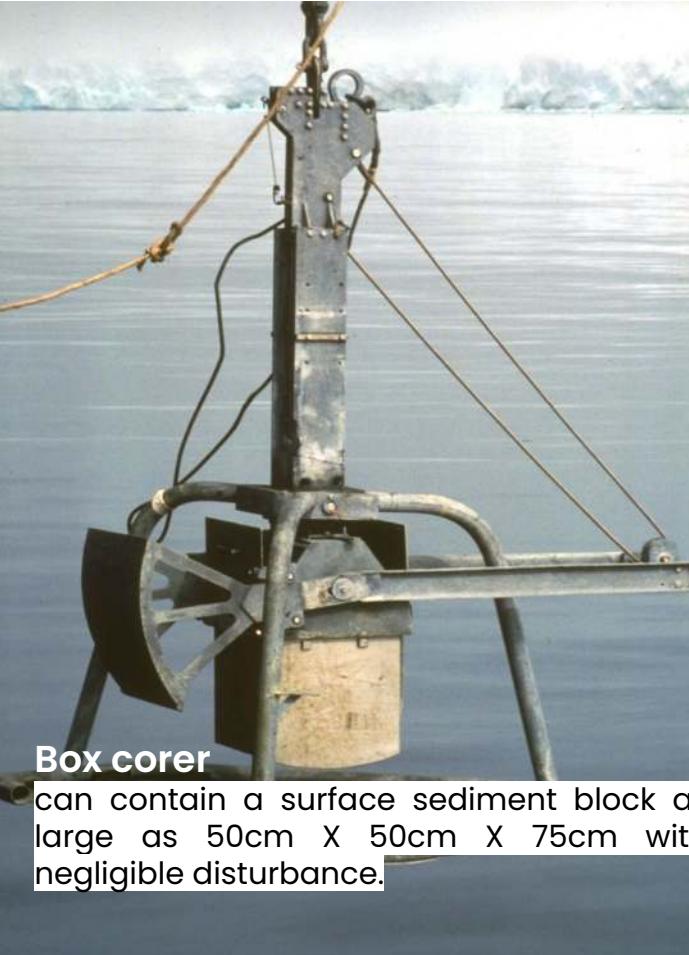


sampling Sediments



Hydraulically damped gravity corer

the hydraulic damping mechanism consists of a water filled piston that empties at a selectable rate as the corer penetrates the mud.



Box corer

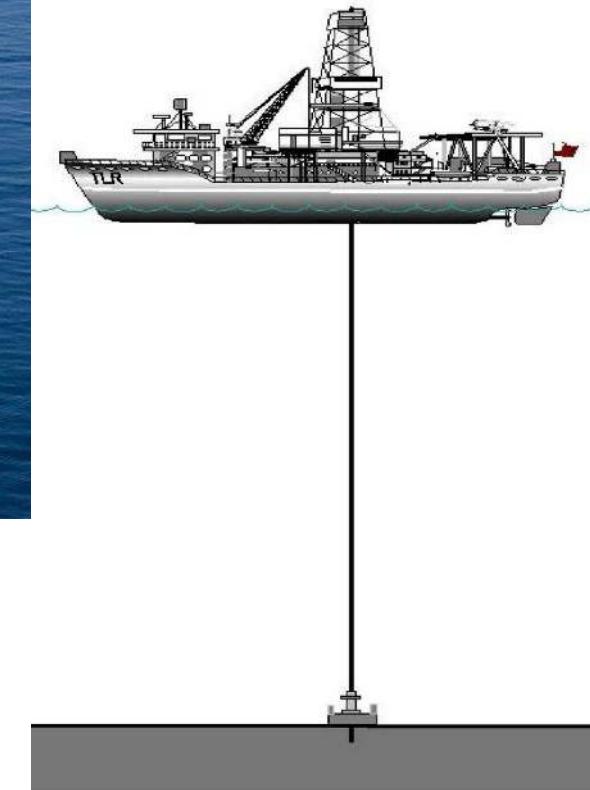
can contain a surface sediment block as large as 50cm X 50cm X 75cm with negligible disturbance.



Gravity corer

users need a boat with a winch powerful enough to lower and raise the corer and enough wire rope to reach the bottom of the water body.

sampling Sediments



sampling **Soils**



sample Preservation

Preservation is highly dependent upon the type of downstream analysis that needs to be carried out. There are some common preservation strategies that include the modulation of temperature or the addition of specific preservatives. These are often used in combinations

- Refrigeration at +4°C (culturing, enrichment, live specimens)
- Freezing at -20°C (viral counts, chemistry, DNA)
- Freezing at -80°C or liq-N₂ (DNA, RNA, proteins)
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CHOICE OF PRESERVATION TECHNIQUE NOT TRIVIAL WHEN DEALING WITH EXTREMOPHILES



