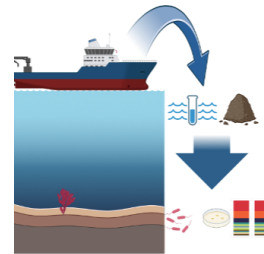


Aug 28, 2025

Water and sediment sampling in the sea to discover marine bacteria

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9rw9zv3e/v1>



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Protocol Citation: Karla Sofia Millan Lopez, Elva Escobar, Liliana Pardo-López 2025. Water and sediment sampling in the sea to discover marine bacteria. protocols.io <https://dx.doi.org/10.17504/protocols.io.x54v9rw9zv3e/v1>

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Protocol status: Working

We use this protocol and it's working

Created: January 18, 2025

Last Modified: August 28, 2025

Protocol Integer ID: 118646

Keywords: Sediment cores, Sea water, Ship sampling, marine bacteria this protocol, marine bacteria, sediment sampling in the sea, sediment sampling, sediment sample, sediment samples on board, oceanographic vessel at sea, oceanographic vessel, sea, obtaining water, sample

Funders Acknowledgements:

CONACYT PhD Scholarship

Grant ID: 957581

LANCO-CONAHCyT

Grant ID: APOYOSLNC-2023-80

DGAPA-PAPIIT

Grant ID: IG200223

Abstract

This protocol aims to show the strategy for obtaining water and sediment samples on board an oceanographic vessel at sea. The samples obtained can be used to identify marine bacteria associated with water and sediment samples.

Image Attribution

Marine Biotechnology Laboratory - IBT-UNAM

Guidelines

Samples must be processed immediately after collection.
Store samples cold after sampling.

Materials

Carousel equipped with Niskin bottles

Multinucleator

Spatulas

Glass containers

Container with liquid nitrogen



Protocol materials



Parafilm



Durapore® PVDF Membrane Filters: 0.22 μ m Pore Size, 0.22 μ m Pore Size; Plain; White; Dia.: 25mm **Thermo Fisher Catalog #GVWP02500**



Falcon Tube (50 mL) **Fischer Scientific**



Health and safety

1

Note

Make sure you have the necessary equipment to work on deck (overalls, suede gloves, helmet, safety boots, goggles or sunglasses). Maintaining adequate hydration is crucial, as deck operations are quite exhausting.

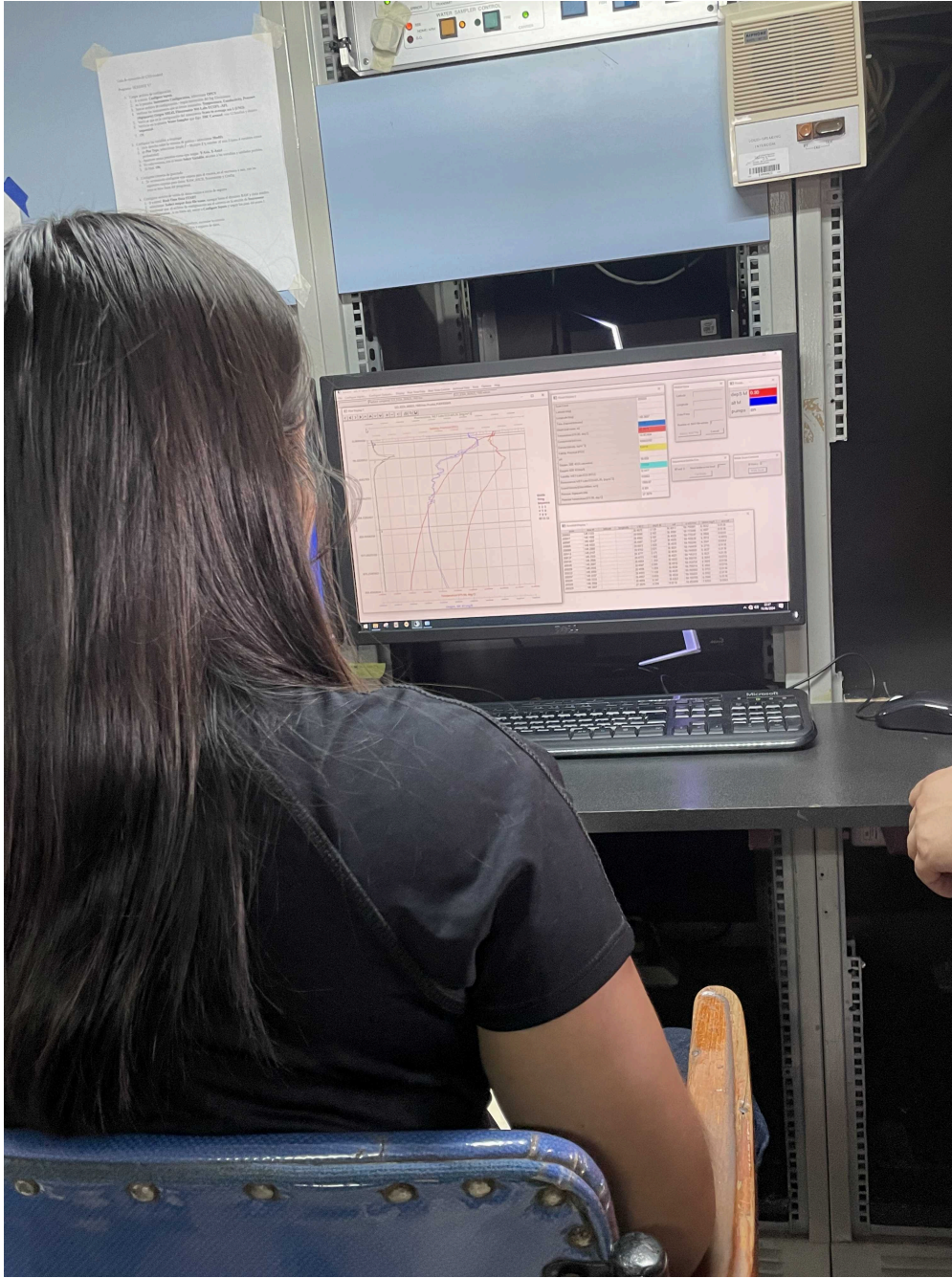
Arriving at the sampling point

- 2 When arriving at the sampling station equipments are deployed following the cruise plan. Each station is referred to by its geographical position and depth.

Note

The sampling operations were carried out aboard UNAM's Research Vessel *Justo Sierra*.

- 2.1 Upon reaching the designated sampling station, a SBE 9plus-Seabird Conductivity, Temperature, and Depth (CTD) profiler was deployed until reaching an approximate height of 50 meters above the seabed.



- 2.2 To record launch, bottom, and ascent information in an electronic and physical log, as well as to include any additional annotations.
- 2.3 The equipment is recovered on deck and the next equipment in the sequence is prepared to be deployed.



- 3 The PNF-300 fluorometer (Wet Labs) is deployed, and the graph is generated from the fluorescence profile of the water column. This is done to identify the maximum peak.
- 3.1 Be sure to remove the caps from the fluorometer sensors.
- 3.2 The fluorometer is recovered and the plugs are placed back on the sensors.
- 4 Seafloor is determined using a Kongsberg EM302 Multibeam Echosounder system.



Sampling in the marine environment: Sea water

- 5 The identification of specific zones of interest is to be based on depth measurements following site characterization

Note

The extent of this process is subject to considerable variation, due to the depth of the site. For instance, the process may take up to 2 hours at depths of 3,800 meters. The equipment is deployed and recovered at a rate of 1m/sec, however stabilization of the equipment for a few seconds is required before the acquisition of water samples

- 5.1 Water sampling is carried out using a SBE 32 – Seabird Carousel equipped with the capacity to activate 12 10-liter Niskin PVC bottles. The carousel is descended with the bottles open and at each of the previously defined sampling depths the bottles are closed remotely.



- 5.2 The first bottle to close is the deepest and is labeled bottom. That bottle is marked as background.
- 5.3 During the carousel's ascent through the water column, the bottles are sealed at the required depth, for example 1500 meters, 1000 meters, 500 meters and at the surface. The time, coordinates and bottle number are recorded in the logbook.



Water collection on the deck of the ship

6

Once all the bottles are closed, the carousel is transported to the deck of the ship and secured.



- 7 Taking into account the order of the bottles assigned to each sampling point, the sample is taken.
- 8 The hoses connected to the nozzles of each Niskin bottle are carefully placed in 20-litre bottles previously labeled for collection.



- 8.1  Sea Water from the surface (~5 meters) and bottom (~3600 meters) depth is transported to the laboratory on board the vessel.
- 8.2 Filter approximately  15 L of  Sea Water through 0.22 μ m filters placed in a filtration unit system, sucked in with a vacuum pump.

Equipment

Vacuum Pump

NAME

Millipore

BRAND

WP6211560

SKU

https://www.merckmillipore.com/MX/es/product/High-Output-Pump-115-V-60-Hz,MM_NF-WP6211560

LINK

115 V/60 Hz

SPECIFICATIONS



Durapore®; PVDF Membrane Filters: 0.22µ; Pore Size, 0.22 µ; Pore Size; Plain; White; Dia.: 25mm **Thermo Fisher Catalog #GVWP02500**

Equipment

PS reusable filtration unit

NAME

Wacol

BRAND

300-4000

SKU

<https://wacol.com.co/producto/unidad-de-filtracion-reusable-en-ps-de-250-ml-para-membranas-de-45-a-50-mm-diametro-cjx4-unidades/>

LINK





8.3 Saturated filters with biomass are stored in sterile aluminum foil and wrapped in



Parafilm .



8.4 A fraction of the filters are stored at  4 °C for later use and another fraction is stored in liquid Nitrogen. These filters are transported to the laboratory.



Note

Make sure to keep nitrogen containers on the boat tightly closed and secured.

Sampling in the marine environment: Sediment

9 In multiple sediment sampling events the research vessel is repositioned at the same geographical position if drifting occurred.





Before launching the multi-core, a full protocol is followed to guarantee safety during its operation and facilitated the successful sample collection.

Note

The time dedicated to collecting sediment samples is subject to significant variation, with factors including the depth of the station, prevailing climatic conditions at the moment of deployment, the performance during the descent, stabilization and ascent within the water column, the characteristics of the seafloor, and the retrieval of equipment.

9.1

The speed of the winch used for the descent of the multicorer depends on sea conditions. The multibeam system and from the penetration echosounder generate data that is then utilized to comprehend the nature of the seafloor where samples are being collected and to determine the probability of successfully extracting all cores.

9.2

The cable tension helps to identify the conditions during the descent of the equipment and the arrival of the multicorer on the seafloor.

9.3

Once the multicorer is retrieved and on deck, the equipment is secured before dettaching each core.

Note

If due to climatic conditions the sampling event was unsuccessful, additional sampling events are required in the same station an reposioning of the research vessel is needed.

10

Each core is documented and photographed on arrival before deattaching from the main frame. One by one each core is identified and deattached, core top and bottom are secured with rubber caps to avoid bottom water and/or sediment to be lost. Each core is placed and identified on a core rack for its later use. Depending on the use of each sample, cores can be processed either in a cold room, in the lab or on deck.



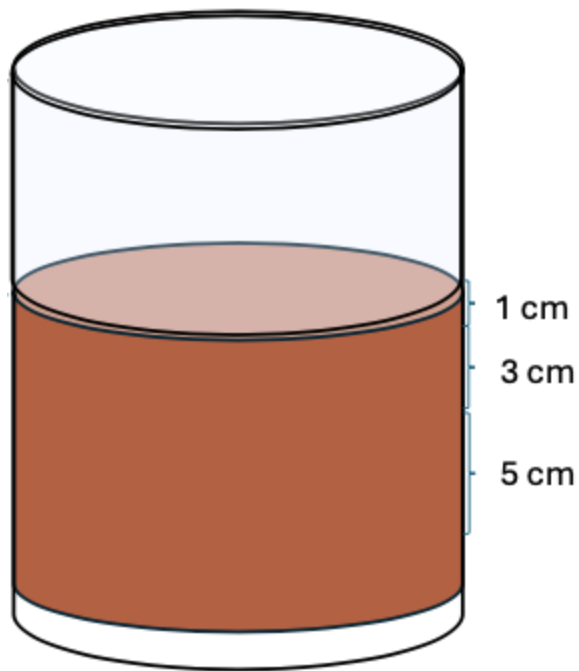


- 10.1 Placing the cores one by one in the core rack for their transport to the assigned processing area.
- 10.2 The processing of the cores can be performed by one or two persons depending on personal ability and experience. In cases where the core requires cutting in layers, the bottom can be retrieved and placed in an extruder. Otherwise no extruder is required. Bottom water can be of interest to some groups and preserving it properly should be considered in advance. Additional gadgets will be required depending on the nature of the study. In the event that the study is concerned with plastic, it is advisable to avoid the use of plastic tools, gloves, extruders, containers, hoses and rubber caps. Similarly, if metals are the focus of the study, it is recommended to avoid all metals tools for cutting the sediments slices. Avoid transcontaminating from diverse sources in case organic matter or microbiology are the topic of research. Use filtered water to clean, sieve to avoid incorporating water column biota into the samples.





- 10.3 Place the core with the sample and remove the caps firmly to prevent water or sediment leakage that can incline the sediment surface and affect the layers generating bubbles with the borrom water. Maintaining a quality control of the sediment sample is essential for achieving optimal results.
- 10.4 The depth of each sediment layer required for a study is determined by the specific objectives of the research. This procedure can be facilitated by the use of precut rings or with sediment dispensed, thereby ensuring uniform cuts. Each layer should be placed in a prelabelled glass or plastic container or bag. The selection is based on the nature of the study in question, with the objeotive of avoiding transcontamination and guarantee the quality of the sample. In the case of deep sea samples, it is usually advisable to expedite processing to minimize the risk of warming and poling exposure to sunlight.
- 10.5 Here we show an example of section sampling, the sediment is stored in glass jars.



Sampled sections representation.

- 11 Each sample is documented and fixed and stored in the cold room or the laboratory aboard the ship.
- 12 To carry out a subsampling of the obtained sediment, obtaining a sample for storage at 4°C in glass container with a lid and additional sample for storage in liquid nitrogen ($\approx 30\text{ g}$) in sterile containers Falcon Tube (50 mL) Fischer Scientific .
Subsampling can be done without perturbing the sediment layers by using hand held syringes without the tip.



- 13 The remaining sediment can be used by other team members, donated for art or returned to the sea.
- 14 Cleaning the corers and the multicorer is among the best ocean practices to avoid transcontaminating samples and habitats from one location to another.



Laboratory storage

15 Samples are carefully recovered from liquid nitrogen stores.



Note

Handle liquid nitrogen carefully, it reaches extremely low temperatures.

16 To store samples at 🌡️ -80 °C in an ultra-freezer in the laboratory at the institute

Equipment

GLACIER	NAME
-86°C ULTRALOW TEMPERATURE FREEZER	TYPE
NuAire	BRAND
NU-99728J	SKU
https://www.nuaire.com/products/ultralow-freezers/blizzard-nu-99728j-ultra-low-temperature-freezer	LINK
 GLACIER	