Sight-Unseen: Characterising the Water Microbiome Using a “DNA Sequencing Lab in a Backpack”

DNA (deoxyribonucleic acid) is a molecule that carries the genetic information of living organisms[1]. DNA is made up of subunits called nucleotides, which each contain one of four bases: adenine, thymine, cytosine or guanine – represented by the letters A, T, C or G. The order, or sequence, of these letters can be read by devices in a process called DNA sequencing[2]. DNA sequencing can determine what an organism is, and what it is related to, and can identify organisms within mixed samples, such as seawater or soil.

Although marine mammals and fish might be the more well-known inhabitants of the water, the diverse communities of marine microbes are critical to the survival of these ecosystems[3]. The marine microbiome provides access to nutrients and recycles waste, allowing larger sea creatures to thrive by contributing to the ocean’s food web[4]. This hidden world is not only vital, but vast: with roughly 90% of the weight of all living organisms in sea being microbes[4]. One litre of seawater can contain between 10 and 100 billion microorganisms[5], and a single drop of water can contain 100 different species of bacteria[4].

Marine microbes absorb more carbon than all the forests in the world and produce as much oxygen – making them a crucial factor in regulating the Earth’s climate[3]. Human activity, pollution and the changing climate, however, pose a significant threat to marine microbiomes and the environments they support[6], with some species of microorganism identified as being indicators of environmental stress[7].

DNA sequencing allows the identification of communities of microbes inhabiting ecosystems. To characterise the microbiomes of the open sea, rivers, rock pools and geothermal springs during the NAIR, I took surface water samples to sequence microbial DNA. I also took temperature readings and noted the precise coordinate location of each sample site. I took the samples back to my “lab” on the boat, where I extracted microbial DNA from the samples and sequenced it.

Sequencing on board

My on-board “sequencing laboratory” consisted of the galley kettle, a BentoLab, a Nanopore MinION sequencer and a laptop. A BentoLab is a box that contains miniature versions of lab equipment like a centrifuge and a heating block. A MinION sequencer is a small DNA sequencing device, (about the size of a Mars bar), that plugs into a laptop. This device allows portable DNA sequencing in remote locations, dependent on the battery life of the laptop.

The Bentolab

My makeshift pipette tip bin!

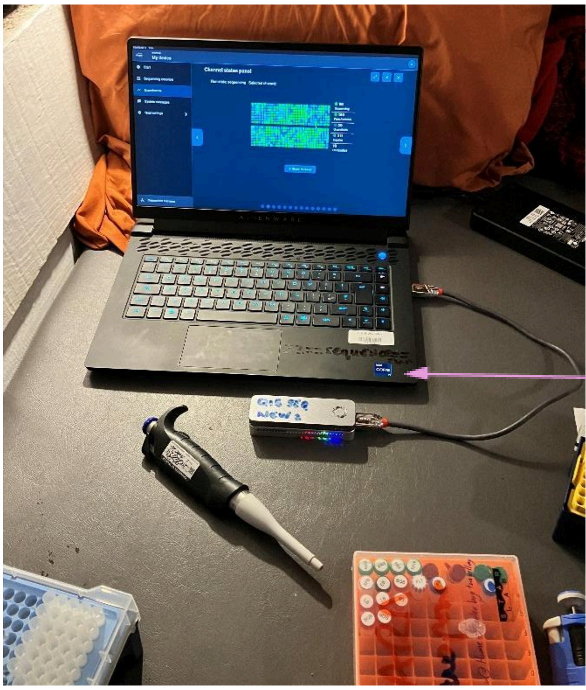
DNA extraction took place on the boat's galley table, in between meal preparation! To extract the DNA, I heated the samples in boiling water to burst open bacterial cells in the seawater. I used the centrifuge on the BentoLab to spin the heated samples at high speeds, to create a pellet of bacteria and the DNA released from the burst cells. The addition of cold ethanol precipitated the DNA out of the solution, and magnetic DNA-binding beads allowed the rest of the solution to be cleaned away, leaving behind extracted DNA resuspended in a buffer.

My "bunk-sequencing-lab" setup during the library preparation process

Once extracted from the water samples, the DNA had to be prepared so that the sequencer could read it, in a process called library preparation. The final product of library preparation is a DNA library, which is a single, combined collection of the DNA from all samples, prepared so that the sequencer can read them. The library preparation process took place in my (cleaned) bunk, as other crew members were preparing dinner for the boat at the time! Library preparation used the heat block in the BentoLab to attach adapters to the DNA to allow the sequencer to read it.

The outside view of "bunk sequencing"!

The finished DNA library was then loaded onto the Nanopore MinION sequencer, and the sequencing run was started. The sequencer ran for approximately 18 hours, before it was manually stopped. The sequencing data produced was taken back to the lab, where I could filter, analyse and visualise it.



Active sequencing of the water sample microbiomes

Nanopore MinION DNA sequencer

The data

The data are presented as interactive pie charts. Toggling through the list of sample names on the top left-hand corner of the charts allows you to compare the composition of each sample. As you can see, although there are some species common to most, or all, of the samples, the composition of each sample can vary hugely between samples, even if they are from similar-seeming habitats, or were taken from areas very close to each other. The map allows you to see where each sample was taken from, around the Faroe Islands.

Acknowledgements

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Bibliography:

1. Deoxyribonucleic Acid (DNA). https://www.genome.gov/genetics-glossary/Deoxyribonucleic-Acid-DNA.
2. DNA Sequencing Fact Sheet. https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Fact-Sheet.
3. Marine microbiome: the invisible majority of the Ocean | Microbiomes Mission. https://fondationtaraocean.org/en/mission-microbiomes/marine-microbiome-majority-invisible-ocean/.
4. Marine Microbes | Smithsonian Ocean. https://ocean.si.edu/ocean-life/microbes/marine-microbes.
5. Understanding the Invisible People of the Ocean | Mission Microbiomes. https://fondationtaraocean.org/expedition/mission-microbiomes/.
6. Walker, L. *et al. Applied Microbiology International Briefing - The Marine Microbiome*. https://sfam.org.uk/uploads/assets/uploaded/265c4291-2ffc-4518-b0d53bcdd4cd1fd0.pdf (2018).

7. Ramljak, A. *et al.* Microbial communities as indicators of marine ecosystem health: Insights from coastal sediments in the eastern Adriatic Sea. *Mar Pollut Bull* **205**, 116649 (2024).