# DIVERSITY IN THE SAND

#### TEXT: NORA LESSING

Marine microorganisms play an instrumental role in Earth's elemental cycles: among other things, they help to maintain the ocean's role as a climate buffer. Katrin Knittel and her team at the Max Planck Institute for Marine Microbiology in Bremen study bacterial communities on the ocean floor in one of the planet's most inhospitable regions: the Arctic.

> An icy wind howls along the coast of Spitsbergen. Snow covers the shore, the valleys, and the mountains. In the distance, the ghostly silhouette of a polar bear moves along the coastline, barely visible under the ship's spotlight. It's Christmastime on Isfjorden near Longyearbyen, and the Arctic darkness reigns 24 hours a day. The researchers are aboard a small ship, 5 meters wide and 15 meters long, as

waves splash against the bow. Equipped with headlamps, they lower a custom-built sediment grab into the water. They call it the "Ellrott Grab," named after its inventor, Andreas Ellrott, an engineer at the Max Planck Institute in Bremen. With this device, the researchers can collect sediment samples without disturbing them or losing the porewater - that is, the water in the spaces between sediment grains. The grab is small and light, perfectly suited for the compact research vessel. With the help of a camera the researchers ensure that the device touches down gently on the ocean floor. In one smooth motion, the grab collects the sample: sand from the

ocean floor, covered by a thin layer of water. A lid seals the sample before the researchers pull it back aboard.

To the naked eye, the sediment sample does not look like anything out of the ordinary – algae, grains of sand in various sizes and colors, and graybrown silt. But under a microscope, it reveals astonishing biodiversity. "Between 100 million and several billion microorganisms belonging to thousands of different species live in a single gram of sediment," explains Katrin Knittel, Project Leader in the Department of Molecular Ecology at the Max Planck Institute for Marine Microbiology in Bremen. No matter

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# KNOWLEDGE FROM

**BIOLOGY & MEDICINE** 

Over 100 kilometers in length, Isfjorden on the west coast of Spitsbergen is one of the largest fjords on the island. Researchers at the Max Planck Institute for Marine Microbiology have chartered the 15-meter-long MS Farm on a regular basis in the past three decades to collect water and sediment samples for study in the lab.



During the polar night in Isfjorden, Dirk de Beer uses a small crane to lower the Ellrott grab into the water. The device is a sediment sampler designed by the researchers. It is equipped with a flashlight and a camera to illuminate and view the seabed and the surrounding area, and to ensure that the device touches down on the sediment gently. Weights stabilize the device during sampling.

PHOTO: KATRIN KNITTEL/MPI FOR MARINE MICROBIOLOGY

surprising discovery: unlike in other habitats, bacteria rarely form monospecific colonies on grains of sand. On the contrary, different species often thrive side by side in close proximity.

### Contrasting lifestyles

- Chyrene Moncada's doctoral thesis examines which microorganisms live where in the sediment and under what conditions. She counts the cells, identifies the different species, and analyzes their metabolisms. Moncada has developed a method to divide the bacteria into three groups based on their lifestyle in the sand: firmly attached bacteria, loosely attached bacteria, and porewater bacteria. The first are firmly attached to grains of sand and can only be separated by means of ultrasonication. They make up around 85 percent of all the microbes in the sediment, but are often less active than the other groups of bacteria, and usually reproduce slowly. The loosely attached bacteria also sit on grains of sand, but can be shaken off. These bacteria appear to be very active. They consume oxygen and process food more quickly than their firmly attached neighbors. The third group lives in the water between grains of sand, known as porewater. This group is among the most active in Moncada's experiments. "The bacteria in the porewater have first access to fresh nutrients from the environment," explains the microbiologist. "The loosely and firmly attached bacteria then process what the porewater bacteria leave behind."
- The porewater bacteria therefore have a locational advantage: food that sinks down from the water column above is immediately available to them. It would be reasonable then to expect these bacteria to be especially numerous. However, this is not the case. "There are far fewer bacteria in the porewater than on the grains of sand," says Moncada. "We speculate that they are eaten by grazing and filter-feeding animals or flushed out by currents." Any bacteria feasting on the plentiful food in the porewater clearly

where she and her team cast the grab; the sediment is always bursting with a diverse array of microorganisms, each playing a distinct role in a delicately balanced ecosystem.

Bacteria are tiny compared to a grain of sand; most are only 0.001 millimeters in size. To a bacterium, a grain of sand is like a little planet, with valleys, hills, chasms, and cracks. Many bacteria settle there gladly because the terrain offers protection from currents, abrasion, and predators. A single grain of sand can host up to 100,000 bacteria from 3000 to 6000 species. Sometimes bacteria settle side by side, while in other cases they leave vast, uninhabited regions. The reason behind this remains a mystery. Another do so at their own constant peril. The firmly attached bacteria that hunker down in the grain's cracks and fissures have opted for a safer neighborhood. However, less food arrives there. "They are safer and won't get washed away. But the competition with neighbors may be fiercer," adds Moncada.

In the bacterial communities that form on and around the grains of sand, cooperation plays a key role, perhaps even more so than competition. Sebastián Silva-Solar's doctoral thesis examines how these habitats are populated by different species and how the species deal with competition. His experiments involve mixing seawater with sterilized grains of sand and observing how different species of bacteria gradually colonize the surface or the cracks in a grain of sand. The first to arrive might possibly determine who settles there next. "I imagine this to be like the reseeding of an area covered in ashes after a volcanic eruption: at first there's nothing there. Over time, grass and bushes

take root, creating the preconditions for other species to move in."

One striking example of cooperation is how bacteria break down sugar. On the one hand, there is a "selfish" uptake method. The microorganisms use surface enzymes to grab sugar molecules, partially split them into smaller molecules, and transport them through their outer cell membrane. In the space between the outer and inner cell membrane, the sugar is further broken down and then taken into the cell. "Few or no degradation products are released into the environment, thus the food is protected from competitors," explains Katrin Knittel. On the other hand, some bacteria break down the sugar entirely outside the cell. The resulting degradation products can then also be used by other microorganisms. "The 'selfish' degradation pathway is rare in the sediment. The microorganisms here maintain a good neighborly relationship — they share," says Knittel. "The bacteria cohabiting in a confined space produce a cocktail of enzymes

that breaks down sugar molecules and makes them available to the community." However, the details on which species indulge in this "communist behavior" remain unclear. "The majority of the species that we find in seawater have not vet been cultivated in a lab," explains Knittel. Nevertheless, it is possible to study these bacteria and their role in the ecosystem. "We have developed a range of research methods that don't require us to keep the species in the lab. Genetic analysis of the sediment communities reveals, for example, the individual capabilities of organisms to break down sugars." Furthermore, it is often the case that the decisive factor is the community as a whole rather than the individual species.

#### Summers like winters

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Another research focus of Knittel and her team is how the complex bacterial community on the ocean floor reacts to changing environmental conditions. This ocean floor community has been shown to be surprisingly stable. The researchers went to sea and took samples during different seasons - in summer and winter, under the midnight sun and in the polar night. At first glance, the bacterial community composition hardly changed, despite the significant changes in the type and amount of sinking organic material (e.g., because algae, needing sunlight to grow, are hardly found during the dark season). While the bacterial community in the open water changes and adjusts with the changing seasons, the community composition in the sediments seems hardly affected by the change of spring, summer, fall, and winter. "The diversity of species and the number of individuals per species remains very stable throughout the year," explains Knittel. But are the sand bacteria really indifferent to the seasons, the midnight sun, and the polar night? A closer look revealed that some species were more active at certain times, even if the number of residents on the sand grains hardly changed. This raises the question: could seasonal changes in enzyme activity be linked

Microorganisms (blue) attached to grains of sand prefer to settle in cracks and hollows in the grains, where they are protected from environmental influences and predators. However, they have to cope with an oxygen shortage and increased competitive pressure from other species. (Grain size: 0.5 millimeters)

#### SUMMARY

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The coastal ocean floor harbors an incredibly diverse range of microorganisms. Up to 100,000 bacteria belonging to thousands of species can live on a single grain of sand.

The bacterial community in the seabed near the coast remains remarkably stable regardless of seasonal changes in food supply. The more diverse the community, the better its protection against disruptions. However, it remains unclear how these communities will cope with rising water temperatures and the acidification and pollution of the oceans in the long term.



After taking a sample in Isfjorden, Chyrene Moncada hauls the Ellrott grab back on board and checks whether the sample collection was successful.

to the varying types of food available on the ocean floor?

## Genes for breaking down sugar

To answer this question, the researchers from Bremen examined which genes the bacteria possess for breaking down algal sugars and to what extent they use these genes. Sure enough, they found clearer differences here compared to the composition of the bacterial community. Especially in spring and summer, when fresh material sinks down from the water, the sediment bacteria quickly take advantage of it. In addition, they possess the enzymes needed to consume the material that is present year-round or produced on the ocean floor, such as animal mucus and chitin. This, the

bacteria nibble on all year long and it is especially important in winter, when other food sources are lacking. The long-term availability of these substances on the ocean floor stabilizes the local bacterial community as a whole. "The enzymes reflect which algal sugars are available to the bacteria at different times of year," explains Knittel. "It's not so different from when we go to the farmers' market. In summer, there are lots of different local fresh fruits and vegetables for sale, but at some point in winter there's nothing left but potatoes from the cellar." The researchers suspect that the fluctuating availability of food affects mainly the porewater bacteria and loosely attached bacteria, but not so much their firmly attached neighbors.

It remains to be seen how this apparently robust ecosystem will respond to climate change. In polar regions such as Spitsbergen, this change is particularly dramatic. Researchers still know too little about how rising temperatures and changes in marine chemistry will affect life on the ocean floor. But the insights gained by Knittel and her team are a beacon of hope. The findings reveal a highly diverse bacterial community, and ecosystems with higher biodiversity are more resilient to environmental pressures. A healthy ecosystem can serve as a buffer for environmental fluctuations, even when several factors fall out of balance in the short term. Bacteria in the ocean could counteract climate change by, for example, decomposing environmentally damaging methane gas, which bubbles up from the seabed in various places. Nevertheless, Knittel stresses: "Like any ecosystem, this one has its limits. If its delicate balance is disrupted long enough, it will eventually reach its capacity."