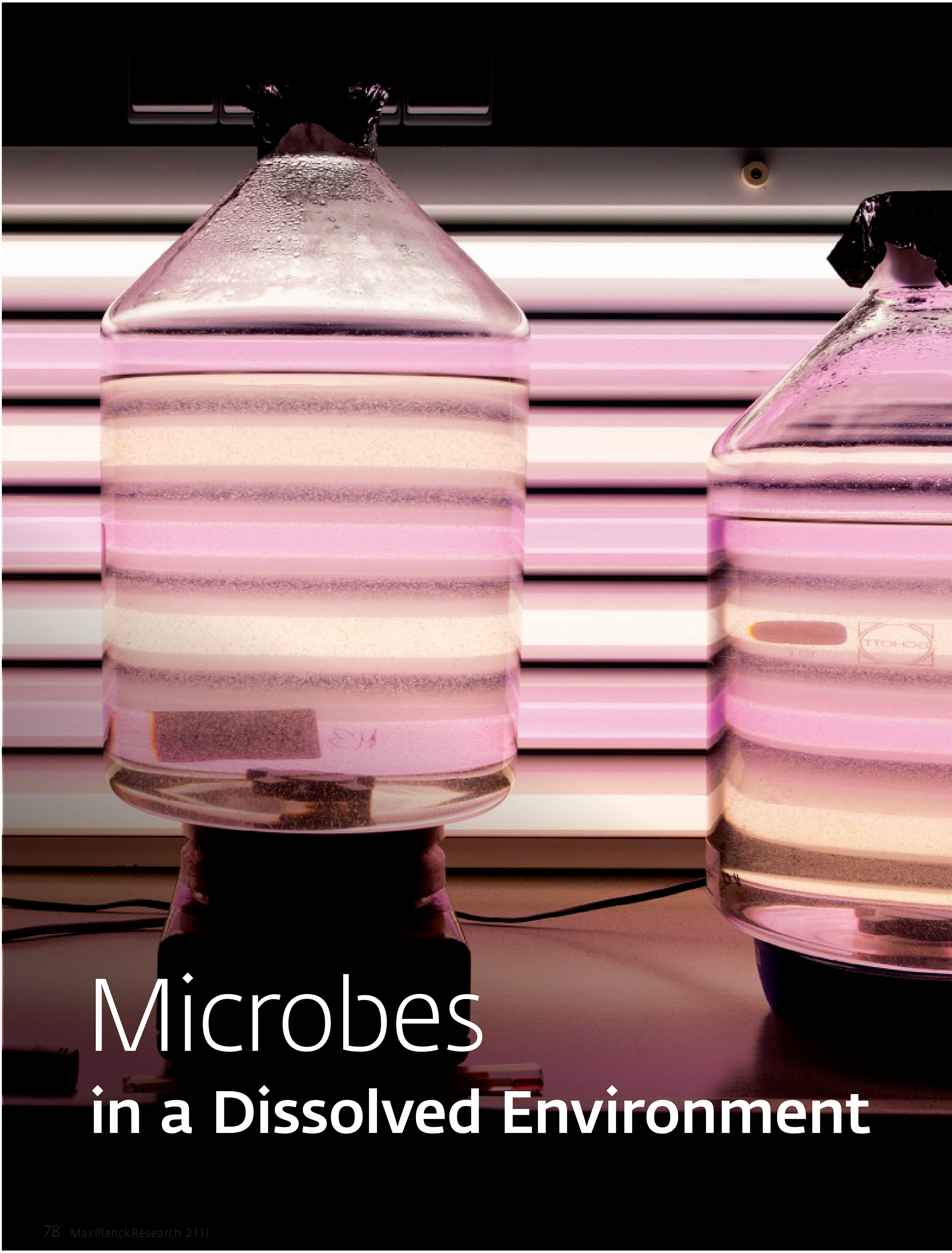


Microbes
in a Dissolved Environment

Algae bloom in the laboratory: Water from the North Sea is injected into artificial seawater. This produces an algae bloom followed by a bacteria bloom, both of which appear as particles in otherwise colorless water.

Photo: Bastian Ehl

Huge quantities of dissolved organic carbon are drifting around in the world's oceans, a ready-made meal for microorganisms. Yet strange as it may seem, they virtually ignore them. **Thorsten Dittmar** of the **Max Planck Institute for Marine Microbiology** in Bremen wants to close this and other knowledge gaps in marine research. After all, the eating behavior of microorganisms in the ocean plays a key role in the Earth's climate.

TEXT **NILS EHRENBERG**

"The subject of our research is in here," says Thorsten Dittmar, pointing to the table in front of him. On it stands a glass volumetric flask filled almost to the brim with water. Dittmar's office on the University of Oldenburg campus is flooded with light, yet the container appears to hold nothing but ordinary water. Slightly cloudy perhaps, but that's all. "Exactly one liter of North Sea water, full of single-celled organisms," he continues, "around a billion in total. A few algae, but mainly bacteria." In other words, there are as many single-celled organisms living in just seven liters of seawater as there are people on Earth.

But Thorsten Dittmar is less interested in all the microorganisms in his volumetric flask than in what they eat – or rather don't eat: dissolved organic material, all varieties of water-soluble carbon-containing molecules. A milligram of this material, no more than one grain of powdered coffee, is swimming around invisibly in the flask. That doesn't sound like much, but if we extrapolate the carbon bound in it to the total volume of all the world's oceans, it comes to an unimaginable 700 billion tons. This is the total amount of carbon contained in all the world's living organisms.

The main source of dissolved organic material is the photosynthesizing plankton in the sea – algae and bacteria that, as they grow, continuously deposit metabolic products into the water. These molecules are also released when plant plankton is eaten by animal plankton or destroyed by viruses. In turn, this dissolved material forms the diet of bacteria, which must get by without photosynthesis.

As the dissolved organic substances float in the sea, they are transformed in various ways by largely unknown processes, resulting in an almost infinite diversity. Dittmar estimates that there are between ten million and a hundred million different organic substances drifting in the ocean. "Only about 5 percent of this material can be assigned to known chemical structures, such as amino acids, sugars, fats and a few others. The remaining 95 percent are completely unknown," he explains.

BETWEEN BREMEN AND OLDENBURG

Accordingly, in September 2008, the scientist began setting up a new and unusual junior research group at the Max Planck Institute for Marine Microbiology in Bremen. Unusual not only because it would be working in an almost unknown area of research, but also because of its location: at the Carl von Ossietzky University in Oldenburg, an hour's drive from Bremen.

This is a unique model for the Max Planck Society, none of whose other working groups are based completely outside the parent institute. The advantages of the Oldenburg model work both ways: the microbiology-oriented Max Planck Institute in Bremen benefits from

» We're the Max Planck Institute's nutritionists so to speak.

the long geochemistry tradition of the Institute for Chemistry and Biology of the Marine Environment at Oldenburg University, which in turn can complement its own microbiology research with that of the institute in Bremen. Dittmar's working group itself, as a know-how interface, benefits doubly.

"I feel really at home in Oldenburg," says Thorsten Dittmar, who most recently conducted research in the US at Florida State University in Tallahassee. No wonder, since the Marine Geochemistry working group, of which he was originally the sole member, has now grown to 16 employees in less than three years. This is a clear indication of the scale of the need for research in this field.

The working group focuses on three main questions: What parts of the dissolved organic material do marine microorganisms eat? What do they not eat, and why not? How do the microorganisms' preferences affect the climate? "We are the Max Planck Institute's nutritionists, so to speak," says the scientist with a wink.

To answer these questions, we first have to know what is actually on the menu. The researchers are thus trying to pinpoint the identity of the many unknown substances – a monumental task. First they have to obtain the material for analysis, through investigations in every corner of the world – the Gulf of Mexico, the polar seas of the Arctic and Antarctic, deep-sea hydrothermal vents and right on their own doorstep in the North Sea off Helgoland.

The scientists make regular trips on the *Polarstern*, a research ship undertaking scientific expeditions commissioned by the Federal Republic of Germany and operated by the Alfred Wegener Institute in Bremerhaven. The water samples the researchers obtain from different depths are filtered on board to remove the microorganisms and other suspended matter, and then desalinated. Absorber cartridges are used to bind the organic components, which finally reach the laboratory as a yellowish, highly concentrated solution.

"Instead of 10 liters per water sample, we come home with only 10 milliliters. That saves a lot of room," says Dittmar. What follows in the laboratory in Oldenburg is one of the biggest challenges of all for an analytical chemist. For conventional molecular analysis, the individual components have to be separated from one another – an impossible task given the diversity of the dissolved organic compounds and their sheer number of over ten million different molecules.

ANALYSIS USING THE STRONGEST MAGNETS

Fortunately, Dittmar's laboratory houses a revolutionary instrument that cost more than two million euros and weighs four tons. Behind a transparent soundproof wall stands Germany's most powerful mass spectrometer, acquired in September 2010. "This piece of equipment is unique in marine research. We can use it to measure the mass of a molecule with a precision of less than the mass of an electron," Dittmar says with pride. There are only five of these systems in the world, and only the one in Oldenburg is used for marine research. The core of the system, which resembles a gas tank and is as tall as a human, is currently the world's strongest commercially available magnet used in mass spectrometry. And as luck would have it, the only company that sells these systems is essentially around the corner – in Bremen.

Ultrahigh resolution mass spectrometry has made what may well be the crucial breakthrough in research into dissolved organic material. This new technology measures so accurately that scientists can determine the precise chemical formula of each molecule. It also shows what elements make up a molecule and how many there are. Only with this new apparatus was the enormous variety of molecules in the oceans revealed. Some 5,000 to 10,000 different chemical formulas can be ascertained in a sample within half an hour. Using this method, the researchers have identified several tens of thousands in total.

But why don't bacteria simply eat their enormous pantry bare? Most dissolved organic material has been drifting around in bite-sized pieces in the deep sea for many thousands of years without a bacterium taking the bait. "Picture it as a non-stop Oktoberfest in the ocean, with a few grilled chickens being snapped up, but no one touching the beer," says Thorsten Dittmar.

But the scientists have solved at least one part of the puzzle. For the first time, they discovered a new and unexpected class of substances – functionalized polycyclic aromatic compounds. No previous knowledge existed of any organism that could produce these hydrocarbons, which consist of several carbon rings. They occur only when an organic substance burns or is heated by geothermal energy in deep sediment layers.

The research team found increased concentrations of these substances especially in very old deep water, which was last at the surface long before the beginning of industrialization. This rules them out as a source of manmade combustion processes, as most manmade substances that move from land to sea are broken down on the surface by sunlight.

The aromatic compounds that Dittmar and his colleagues have found evidence of everywhere in the deep sea may come from the sediments on the ocean floor. Dittmar suggests the following scenario: seawater that makes a sort of extra pass through the seabed

Penguins are constant companions on *Polarstern* expeditions (bottom), and stormy weather is also a routine event for the scientists (left). The scientists use a CTD rosette to collect water samples from varying depths (right).

» Picture it as a continuous Oktoberfest in the ocean, with a few grilled chickens being passed over the counter, but no one touching the beer.

Thorsten Dittmar opens the injection unit of the mass spectrometer. From the syringe at the right edge of the photo, the probe penetrates through the red sheathed capillary into the injection source.

appears again at, for example, hydrothermal sources with temperatures of more than 400 degrees Celsius. In this way, organic materials are presumably released from the sediments and washed, chemically altered, into the deep sea. Since destructive sunlight is not present here, they remain stable for a long time. “Up to 20 percent of dissolved organic material could be transformed in this way through the action of heat,” estimates Dittmar.

This would also explain why bacteria leave most of the dissolved organic substances in seawater alone – perhaps they simply don’t have suitable tools for breaking down the altered substances, forcing them to do without many originally tasty morsels.

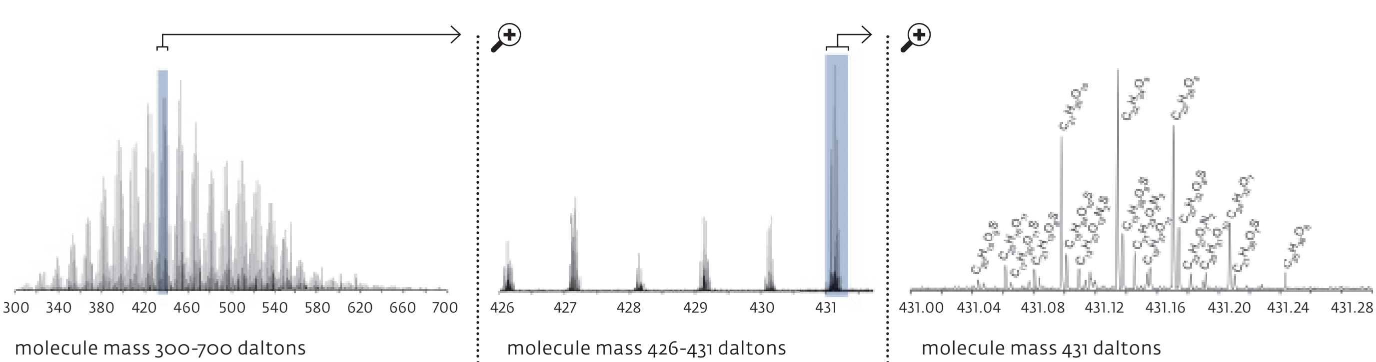
We can really only be thankful for this involuntary diet of the bacteria. If also the stable part of the dissolved organic material were eaten, releasing the carbon bound within, the atmosphere’s carbon dioxide content would easily double – with correspondingly disastrous consequences for the climate. However, the reverse scenario is also conceivable: if the bacteria were to suddenly reject part of their food, dissolved organic material in the ocean would build up at the expense of atmospheric carbon dioxide. An increase in dissolved organic material from the current 1 milligram to 1.5 milligrams per liter would halve the atmospheric carbon dioxide content and reduce it to the levels of the last ice age – producing a rather cool breeze in the process.

Bacteria may have triggered an increase in carbon dioxide in the atmosphere once before, 600 million years ago. At that time, the Earth was like a giant snowball: the global average temperature was well below the current figure, and the oceans were largely frozen. One theory (not without its detractors) suggests that more oxygen found its way into the oceans, enabling bacteria to break down dissolved organic material. The carbon dioxide released as a result escaped into the atmosphere and triggered a greenhouse effect.

Whatever the effect of global warming at that time, the eating habits of bacteria in the sea have the potential to influence the climate. No wonder, then, that scientists have recently begun talking about dissolved organic material in connection with geoengineering – manipulating natural cycles in order to counteract climate change (MaxPlanckResearch 3/2010, page 36 ff.).

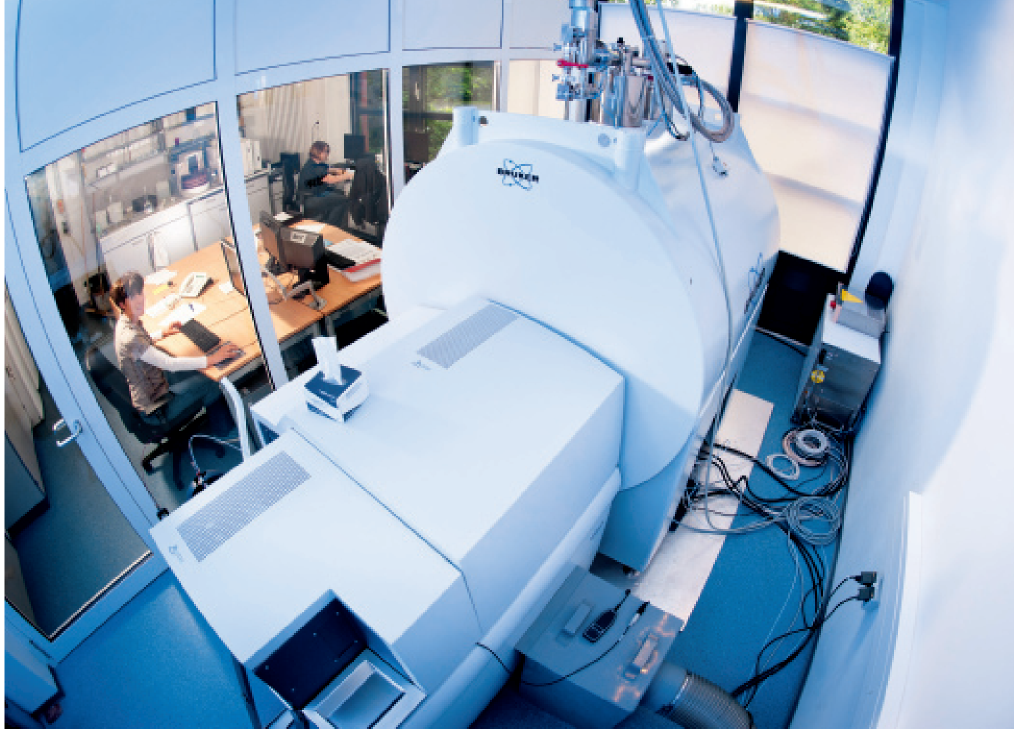
INTERFERING WITH NATURAL CYCLES CAN BE DANGEROUS

According to the theory, if the bacteria could be induced to ignore part of their food, the carbon dioxide levels in the atmosphere would fall and the greenhouse effect would be reduced. But Thorsten Dittmar is skeptical: “Before we start manipulating natural processes, we first need to understand every last detail. Without that knowledge, we won’t come anywhere close to estimating the impact of such interference.”



Mass spectrum of dissolved organic material in the deep sea. Modern ultra high resolution mass spectrometers make the huge diversity of substances in the survey spectrum visible (left). They even separate molecules with the same nominal mass, but different chemical formulas (center, right).

Photo: Bastian Ehl; Graphic: MPI for marine Microbiology



Oldenburg is home to Germany's most powerful mass spectrometer.

However, the reservoir of dissolved organic substances is already changing, even without geoengineering. Global warming is slowly melting the permafrost layers in the Siberian and Canadian tundra. This releases greater quantities of organic material, which is washed down the many rivers and into the North Atlantic. The intriguing question now is: what happens to this additional input of dissolved organic substances? Is it eaten, which would be bad for the climate, or does it remain stable and end up in the deep sea?

In order to discover what becomes of the additional dissolved organic material, the researchers must not only find out what bacteria leave on their plates, but also identify their favorite dishes. Thorsten Dittmar and his colleagues are currently analyzing water samples from the Helgoland long-term series: since 1962, researchers have been continuously measuring salt content, temperature and the species composition of plant, animal and bacterial plankton off the island. This is done once a week, always at the same location.

By comparing the Helgoland data on algae and bacteria density with the newly acquired information on the composition of dissolved organic material, the scientists have identified important key substances. When a certain single-cell diatom blooms, it produces, among other things, an organic substance that needs a bacterium in order to grow. "The organic substances are thus part of countless numbers of specific connection networks between the microorganisms in the sea," says Thorsten Dittmar.

Analysis is still in full swing, but the scientists have already discovered an exotic substance class in the waters off Helgoland: unusual sugar molecules that have never previously been found in the sea. Known in medical research for their enzyme-inhibiting effect, for instance, these substances are obtained from plants or synthetically produced for pharmaceutical use. "The exotic molecules appear in the water shortly after a particular group of algae blooms. This is the first proof of natural production of this substance class in the sea," says Dittmar. The working group's results may thus also have important medical implications.

Dittmar's working group has just been approved to take part in three further *Polarstern* expeditions to the Southern Ocean, all scheduled for next winter, which is summer in the southern hemisphere. The waters around Antarctica are the only place where marine researchers can analyze water from all of the Earth's regions in a single day. Water from Antarctica, the Arctic, the Atlantic and the Pacific all flows together there.

This allows dissolved organic material to be examined in waters where the living conditions for bacteria differ. These living conditions, such as water temperature, affect the composition and activity of bacterial communities, and thus the composition of dissolved organic material. In this way, the research can contribute to a better understanding of global materials cycles and possible alterations caused by climate change.

A brief glance at the most recent assessment report by the World Climate Council (IPCC) reveals the importance of the work being done by the Max Planck research group in Oldenburg. Published in 2007, its findings on climate change form the main basis for political decisions on climate protection. However, it is futile to search for any reference to dissolved organic material in the report, with runs to more than one hundred pages. Knowledge levels in 2007 were still far too patchy for meaningful integration of the 700 billion tons of carbon into global climate models. "Now it's time for an update," says Thorsten Dittmar. ◂

GLOSSARY

Organic molecules
Most carbon-containing compounds are known as organic compounds. After hydrogen, the element that can form the largest number of chemical compounds with other elements is carbon. For example, carbon can form chains and rings with itself and other elements through single or double bonds. That is why carbon compounds are considered to be the basis of all life on Earth.

Functionalized polycyclic aromatic compounds
Polycyclic aromatic compounds are organic molecules in which atoms are arranged in several rings. The binding electrons of the ring structures are not assigned to a single bond, which means that the molecules are highly inert and stable. Functionalized aromatic compounds further possess additional molecular residues, such as hydroxyl or amino groups, which increase the polarity of the compounds and thus their solubility in water.

Photo: Bastian Ehl