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## After 7000 years without light and oxygen in Baltic Sea mud: Researchers bring prehistoric algae back to life

A research team led by the Leibniz Institute for Baltic Sea Research Warnemünde (IOW) was able to revive dormant stages of algae that sank to the bottom of the Baltic Sea almost 7,000 years ago. Despite thousands of years of inactivity in the sediment without light and oxygen, the investigated diatom species regained full viability. The study, recently published in *The ISME Journal*, was carried out as part of the Leibniz Association-funded collaborative research project PHYTOARK, which aims at a better understanding of the Baltic Sea's future by means of palaeoecological investigations of the Baltic Sea's past.

Many organisms, from bacteria to mammals, can go into a kind of 'sleep mode', known as dormancy, in order to survive periods of unfavourable environmental conditions. They switch to a state of reduced metabolic activity and often form special dormancy stages with robust protective structures and internally stored energy reserves. This also applies to phytoplankton, microscopically small plants that live in the water and photosynthesise. Their dormant stages sink to the bottom of water bodies, where they are covered by sediment over time and preserved under anoxic conditions.

"Such deposits are like a time capsule containing valuable information about past ecosystems and the inhabiting biological communities, their population development and genetic changes," explains Sarah Bolius. The IOW phytoplankton expert is the first author of the study recently published in *The ISME Journal*, in which sediment cores from the Baltic Sea were analysed specifically for viable phytoplankton dormant cells from the past. "This approach bears the rather unusual name of 'resurrection ecology': Dormant stages that can be clearly assigned to specific periods of Baltic Sea history due to the clear stratification of the Baltic Sea sediment are to be brought back to life under favourable conditions, then they are genetically and physiologically characterised and compared with present-day phytoplankton populations," continues Bolius. By analysing other sediment components, so-called proxies, it will also be possible to draw conclusions about past salinity, oxygen and temperature conditions. "By combining all this information, we aim at better understanding how and why Baltic Sea phytoplankton has adapted genetically and functionally to environmental changes," the marine researcher explains the scientific approach of the study.

### Old genes, stable functions

The team led by Sarah Bolius, which included IOW experts as well as researchers from the Universities of Rostock and Constance, examined sediment cores taken from 240 metres water depth in the Eastern Gotland Deep during an expedition with the research vessel Elisabeth Mann Borgese in 2021. In favourable nutrient and light conditions, viable algae could be awakened from dormancy from nine sediment samples and individual strains were isolated. The samples were taken from different sediment layers that represent a time span of around 7000 years and thus the main climate phases of the Baltic Sea.

The diatom species *Skeletonema marinoi* was the only phytoplankton species that was revived from all samples. It is very common in the Baltic Sea and typically occurs during the spring bloom. The oldest sample with viable cells of this species was dated to an age of  $6871 \pm 140$  years. "It is remarkable that the resurrected algae have not only survived 'just so', but apparently have not lost any of their 'fitness', i. e. their biological performance ability: They grow, divide and photosynthesise like their modern descendants," emphasises Sarah Bolius. This even applies to the cells from the roughly 7000-year-old sediment layer, which proved to be stable during cultivation with an average growth rate of about 0.31 cell divisions per day – a value similar to the growth rates of modern-day *Skeletonema marinoi* strains, says Bolius. The measurement of photosynthetic performance also showed that even the oldest algae isolates can still actively produce oxygen – with average values of 184 micromoles of oxygen per milligram of chlorophyll per hour. "These are also values that are comparable to those of current representatives of this species," says the algae expert.

The researchers also analysed the genetic profiles of the resurrected algae using microsatellite analysis – a method in which certain short DNA segments are compared. The result: The samples from sediment layers of different ages formed distinctive genetic groups. Firstly, this ruled out the possibility that cross contamination could have occurred during the cultivation of the strains from sediment layers of different ages. Secondly, this proves that successive populations of *Skeletonema marinoi* in the Baltic Sea have changed genetically over the millennia.

### **Dormancy as a survival strategy – and as the basis for an exciting research tool**

The phenomenon that organisms survive in dormancy over very long periods of time and can therefore potentially recolonise habitats under suitable conditions is also known from other studies – for example for plant seeds or small crustaceans, some of which remain viable for several centuries, even millennia. However, the successful resurrection of a dormant stage after such a long time, as in the case of *Skeletonema marinoi*, has rarely been documented. At around 7000 years old, the tiny cells of this diatom are among the oldest organisms to have been successfully revived from an intact dormant stage. From aquatic sediments, no older such cases are known to date.

“The fact that we were actually able to successfully reactivate such old algae from dormancy is an important first step in the further development of the ‘Resurrection Ecology’ tool in the Baltic Sea. This means that it is now possible to conduct ‘time-jump experiments’ into various stages of Baltic Sea development in the lab,” says Sarah Bolius. The revived algae strains will therefore be further tested under different conditions in the future. “Our study also shows that we can directly trace genetic changes over many millennia – by analysing living cells instead of just fossils or DNA traces,” concludes the scientist. Further genetic analyses of the reactivated algae strains are expected to contribute to a better understanding of the causes of these genetic changes.

### **Understanding the future by travelling back in time**

The current study was carried out as part of the collaborative project PHYTOARK, which is funded by the Leibniz Association as part of the “Collaborative Excellence” funding line and coordinated at the IOW by Anke Kremp, head of the Phytoplankton Ecology working group. Nine other research institutions from Germany, Finland, Sweden and the USA are involved. The aim is to use the state-of-the-art methods of palaeoecology and biodiversity research to look back up to 8,000 years and reconstruct changes in Baltic Sea phytoplankton caused by natural climate fluctuations. This look into the past should help to better assess future climate change impacts in the Baltic Sea. More information: <https://phytoark.com>

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