Autonomous eDNA sampling for:

Zero impact biodiversity monitoring

With marine life being under increasing pressure, the Flemish Institute for Agricultural, Fisheries and Food Research (ILVO) has started to look for a more sustainable, alternative method to study marine biodiversity.

As a research institute, ILVO has been monitoring the Belgian part of the North Sea, including wind farms, twice a year for the past 30 years to study which species of fish and invertebrates are found here and how biodiversity changes over time. The existing monitoring method is time-consuming, labour-intensive and involves removing the animals from their habitat and impacting the seabed.

Since 2019, ILVO coordinates the Interreg North Sea Regions project GEANS (Genetic tools for Ecosystem health Assessment in the North Sea region) which aims, among others, to develop and harmonize DNA-based time- and cost-saving techniques for genetic monitoring.

Sofie Derycke, senior researcher at ILVO: "But even with this method we still remove animals from their habitat. The only thing we bypass is the taxonomic expertise. In the challenge, we wanted to go a step further by studying the DNA released by fish and floating in the water column, the so-called environmental DNA (eDNA)."

eDNA

DNA molecules are released into the water when fish filter the water, but also through the production of eggs, sperm and faeces, she explains. The animals themselves are left alone. ILVO has determined around 130,000 sequences at twelve different locations in the Belgian North Sea by sampling the water, filtering it and analysing the part of the DNA found on the filter for biological patterns. This method has proven to be successful.

eDNA is not new. This method, for example, is already used in closed freshwater systems such as lakes. However, its application in the North Sea is still in its infancy, Derycke explains. That also makes it more challenging. The North Sea is shallow, up to 30 to 40 meters, with many water currents and wave action. A homogeneous mass can quickly develop. It is therefore important to know for certain where the DNA molecules have originated: from the place where the sample was taken or by currents that arrived there at the time of sampling. That was one of the questions they wanted to investigate for the challenge.

To be able to make that distinction, a link is made with available hydrodynamic models that accurately map out the water flows. Derycke: "At several locations where we know the communities are different, we sampled to see whether eDNA reveals the different communities or whether it has become one big soup due to wave and current action. We do see that there are geographic patterns reflected in the eDNA."

Manual sampling

Sampling of eDNA is currently still done manually. Special bottles are deployed from research vessels to certain water depths to collect water. The bottles still have to be brought down and up manually. ILVO cooperates with other institutes in Belgium; with the Institute for Nature and Forest Research (INBO) to further develop the eDNA method, and with the Flemish Institute for the Sea (VLIZ) and the Royal Belgian Institute of Natural Sciences (KBIN) to collect more samples in the North Sea. For example, through the Lifewatch campaigns of VLIZ, eDNA is now collected monthly at nine sites in the North Sea to also determine temporal patterns in eDNA. This could provide a more accurate picture of exactly when fish pawn.

The disadvantage of this practise is that it is dependent on the use of a research vessel and some locations are hard to reach. In the case of wind farms, you also need permission from the wind farm owner. ▶

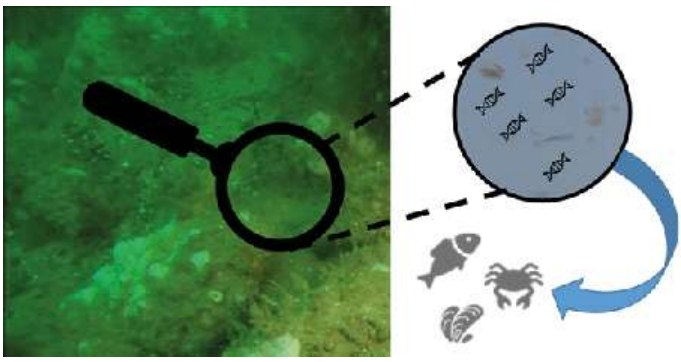

Illustration of eDNA

Autonomous sampling

"What if the sampling could take place autonomously," says Derycke; "without a research vessel and personnel. You can take samples using an autonomous underwater vehicle (AUV) which could also remain at sea longer."

Derycke already found a sampler that is commercially available. This can operate at depths up to 5 km and stay in the water autonomously for 14 months. Batteries ensure that everything keeps working and samples can be taken once in a while. The frequency of sampling can be set in advance.

The sampler can be lowered from a ship onto the seabed or attached to something, such as a buoy, but also, for example, to artificial reef structures. ILVO looked at the latter during the challenge with fellow participant Reefy. However, if the sampler can be converted and installed in on AUV, then sampling can also be done in different places.

"That also made participation in the challenge interesting, to actually take that step further, towards automation. So we can sample at multiple locations and thus study not only the temporal but also the spatial variation," says Derycke.

"First we need to test whether the sampler is entirely suitable for sampling eDNA. The sampler is currently being used to sample plankton. The principle is the same, but the concentration of eDNA is much lower compared to plankton," explains Derycke. The tubes that are used to take samples must be cleaned very well after use to prevent cross-contamination.

Link with taxonomic data

Derycke wants to emphasize that the (e)DNA method does not replace the taxonomic system. "With a DNA sequence in itself you are not going to know everything about the functioning of the marine ecosystem. You can see shifts in DNA frequencies but you also want to know what that means. Is it a particular species coming or going and what is the function of that species? It's important that you can link those sequences to species names.

So all the taxonomic work over the last few decades is very important to know what species are there. Creating a good reference database where species are linked to DNA sequences is a very important step to be able to build on with the DNA results, and this is happening within the GEANS project.

eDNA at wind farms

eDNA could be put to good use at offshore wind farms, as a monitoring strategy for offshore wind farms sites, as part of the pre-tender phase when the characteristics of the site are mapped. Based on the water samples, the biodiversity in the areas to be developed can be characterized. Even when the wind farm is in place, biodiversity can continue to be monitored in a simple manner during its lifetime.

Getting stakeholders and policy on board

ILVO is already fully convinced of the concept. To make the eDNA method the standard, however, there are still a few steps to take. The first step is to convince stakeholders that this method really has added value, not only for scientists and the environment but also for them. Using the autonomous method saves time and costs as monitoring is no longer dependent on the availability of a research vessel and personnel.

To convince that the eDNA method is a more sustainable way to monitor biodiversity, proof-of-concept is needed. In the meantime, ILVO obtained a ten months extension of the GEANS project. This was due to expire at the end of this year

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but has now been extended by ten months. "With the extension it was possible to add additional activities. We have now added the monitoring of eDNA in wind farms as a case study. The goal is to study the patterns inside and outside the wind farms C-power and Belwind and see if there are any differences between them. But also within the wind farm, for example close to the turbine, between the turbines or on the seabed where fauna lives on hard substratum."

A next step is to include the method in policy, at national but preferably also at European level. That's difficult and won't happen overnight, Derycke admits. "The most important message is that we are going for zero impact. That we do not disturb the animals or the habitat. Zero impact monitoring is the future, especially with automation."

Continuation

The challenge did not generate any new partners or projects yet but it did encourage ILVO to take real steps and gain confidence in the applicability of the concept. "Because we were in the final, we started contacting a number of companies ourselves here in Belgium, including our federal partner RBINS, to see if we could find funding for a sampler and we also looked at opportunities for grants."

That work has been rewarded because ILVO has now found funding through two projects to start the eDNA study in the wind farms and to purchase the sampler for this purpose. The first tests in wind farms will take place in October and November this year. This is not yet done autonomously but testing for automatic collection is expected to take place by summer next year.

Niskin-bottle, used for water sampling