Assessing the potential to collect scientific samples using sailing vessels

Author(s): Author(s): Ninon Mavraki, Oliver Bittner, Linda Tonk, Reindert Nijland, Hans Verdaat, Enzo Kingma, Oscar Bos

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Client: TenneT
Attn.: Mrs Annemiek Hermans
Utrechtseweg 310
6812 AR, Arnhem

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Contents

Summary

The introduction of artificial hard substrates (e.g. offshore wind farms, oil and gas platforms, cable crossing berms) induces multiple changes on the marine environment. The most apparent effect is the creation of new habitat, which is exploited by marine growth. The presence of marine growth attracts larger organisms, i.e. crabs, lobsters and fish, increasing the local biodiversity and creating an artificial reef in the locations where artificial hard substrates are introduced. TenneT is the Dutch Transmission System Operator connecting offshore wind farms to the onshore electricity grid via subsea electricity cables. From a Corporate Social Responsibility driver, TenneT is currently designing different types of eco-friendly cable crossing berms to see whether they promote the local biodiversity. Together with DutchSail, a professional sailing company, they would like to investigate the possibilities of collecting high-quality scientific biodiversity data from the area of the TenneT cable crossings using sailing vessels that have a lower CO_2 footprint compared to the typical research vessels. They would like to investigate the options of collecting biodiversity samples using trained crew members, and thus, making the collection of samples more cost-efficient and reducing disturbance from the amount of vessels at sea. TenneT does not have experience in collecting such type of biological samples and has asked Wageningen Marine Research to teach and instruct the crew-members about the methods that need to be applied to obtain high-quality data, to perform the data analysis and obtain the results.
For this reason, two fieldwork campaigns were planned, where Wageningen Marine Research scientists joined, conducted and were assisted by crew members to collect biodiversity samples. Two cable crossing locations were visited and sampled during each sampling campaign. Two methods were applied during both samplings: 1) eDNA sample collection for vertebrate biodiversity identification and 2) visual censuses (where a remotely operated underwater vehicle -ROV – and a drop-camera were used) for invertebrate biodiversity exploration.
The eDNA analysis indicated that 30 vertebrate taxa were found in the general areas of the cable crossings. The visual censuses revealed the presence of 10 different invertebrate taxa. Overall, the collection and processing of eDNA samples was performed without significant difficulties on board of the sailing vessel and with the help of the trained sailing crew. Only when the weather conditions were not ideal, the water filtration could not be conducted on board. The quality of the eDNA samples indicated that the sample collection from the sailing vessel was successful, even though some contamination was observed. On the contrary, visual censuses were not as successful. This is both due to the incorrect coordinates, which may have resulted in the incorrect positioning of the vessel and thus, not being able to detect the cable crossings, and due to the difficulties when it comes to manoeuvring the ROV and the drop-camera. This project indicated that biodiversity data could partly be collected using sailing vessels and trained sailors but the quality (at least in terms of visual censuses) cannot be considered equal to similar scientific campaigns.

1 Introduction

With the awareness of climate change and the efforts of declining its effects on the environment, there is a continuous and increased demand for renewable energy (Soma et al., 2019). The offshore wind farm industry is, therefore, rapidly expanding the last decades in the Netherlands. Offshore wind turbines produce energy, which is transmitted to the onshore electricity grid via cables (Fig. 1). These underwater cables are mainly buried under the seafloor, although they emerge on the seafloor surface when they pass over an existing oil or gas pipeline. These exposed parts are called cable crossings, which are protected by a layer of rocks.

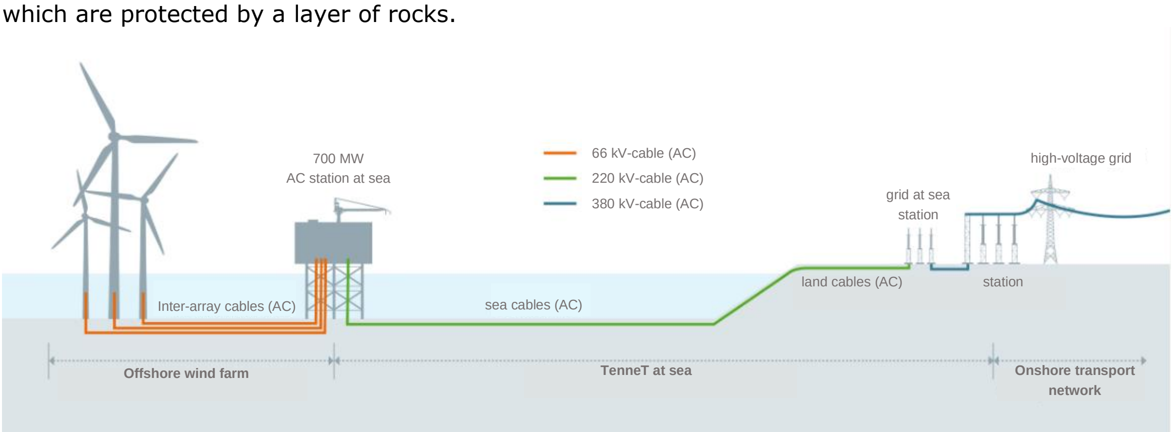

Figure 1: *Schematic overview of the TenneT grid network. Source: TenneT.*

TenneT, a transmission system operator, ensures that offshore wind farms in the Netherlands are connected via the offshore grid to the onshore electricity grid, so that the generated energy is transmitted to shore. Furthermore, TenneT 's aim is to develop infrastructures that cause no negative effects on nature by 2025 and thus, it is currently designing, developing and installing offshore grids that are, as much as possible, nature-friendly. By taking these additional measures, TenneT fulfils the agreements as stated in the climate accord (Klimaatakkord 2019).

In 2020, TenneT added berms on the cable crossings in a conventional construction design, which consist of a bottom layer of natural, crushed stones and a top, sprinkler layer of small, granite stones. TenneT has installed three eco-friendly cable crossing berms in the Hollandse Kust (zuid) area to promote biodiversity. These include berms, which have a top layer that consists of calcareous stones, such as marble or limestone, while the other three berms are of the previously mentioned, conventional design with granite stones. These were placed in the sea with the overall idea to examine whether calcareous stones would cause a higher increase in local biodiversity compared to the granite ones. This question is currently being examined. The location of the eco-friendly and the conventional berms of TenneT in the Hollandse Kust (zuid) area are shown in the red boxes in Figure 2. Every cable crossing location consists of two cable crossings, Alpha 1 and Alpha 2. The Alpha 2 cable crossings contain the eco-friendly designs, while the Alpha 1 cable crossing berms consist of the normal, granite stones.

The installation of the cable crossings and their subsequent berms add hard substrate to naturally soft-sediment areas, increasing habitat complexity. The introduction of hard habitats attracts multiple species. Initially invertebrate taxa, known as marine growth, attach on the newly introduced hard substrate (De Mesel et al., 2015). The increased amount of marine growth attracts larger organisms, such as large crabs, lobsters and fish, which can feed on the marine growth (Mavraki et al., 2020). Thus, the introduction of hard substrates is usually accompanied by an increased local biodiversity.

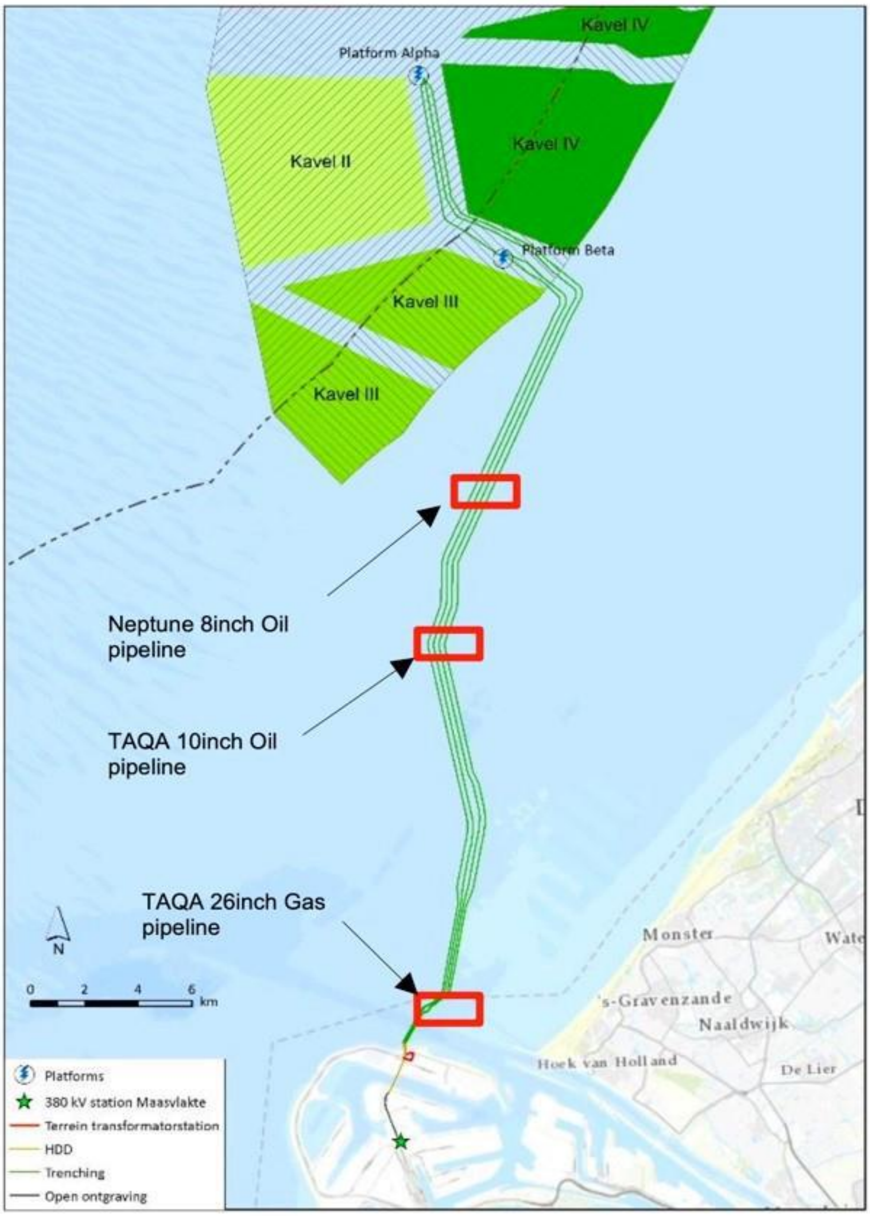

Figure 2: *Map showing the locations of the cable crossings in the Hollandse Kust (zuid) area. Source: TenneT.*

The biodiversity on the TenneT cable crossings is currently investigated using costly research vessels (often with a considerable CO_2 footprint). However, TenneT is interested in exploring different ways of collecting scientific biodiversity data. They were particularly interested in using sailing vessels and their crew to obtain data from the cable crossing berms.

In this project, a sailing vessel from the professional sailing company DutchSail (www.dutchsail.com) was used with the aim to conduct research whilst reducing the CO_2 footprint. This company aims at combining professional sailing (trainings) with durability and innovation. For some time, they have been attempting to combine sailing with research work, exploring their options as to which type of research they can perform using the sailing vessels. With this, they wish to prove that they can assist the scientific community, by acquiring valuable samples from locations that are costly and difficult to be approached by scientists and by vessels that emit less CO_2 compared to the vessels used by scientists. In addition, since the sailors need to train on sailing vessels, combining activities at sea could reduce the overall pressure of vessel traffic. However, they (similarly as TenneT) do not have the scientific knowledge regarding the way of collecting samples and assessing the quality of the data. For this reason, Wageningen Marine Research (WMR) scientists were involved in this project. The scientists would plan and execute the sampling campaigns, train the sailors to collect biodiversity samples and finally analyse and evaluate the samples taken.

2 Assignment

In this project, we investigated the possibility of measuring biodiversity data of scientific quality on the cable crossing berms of TenneT using sailing vessels and the crew of DutchSail. We further assessed if the crew would be able to collect high-quality scientific data independently in the future.

2.1 Research questions

The research questions were:

1. Can we collect eDNA samples using a sailing vessel?
2. Can we inspect biodiversity using visual censuses using a sailing vessel?
3. Are the sailors eventually capable of collecting samples on their own?

3 Materials and Methods

To investigate the biodiversity using sailing vessels and the help of the crew, we conducted two fieldwork campaigns. We applied two different methods (eDNA analysis and image recognition techniques) to estimate the local biodiversity that occurs on and around the cable crossing berms. Environmental DNA (eDNA), present in the seawater, allowed us to examine which vertebrate species are present in the general area of the cable crossings. Image recognition techniques were used to focus more on the epibenthos and the invertebrate species that occur on the seafloor in the area. These methods were chosen due to their general applicability when it comes to biodiversity data collection, we considered them easy to use on sailing vessels and are validated techniques. The best and most precise way to collect biodiversity data would be to conduct scientific diving, but this is costly and time-consuming and was not considered a possible method for this project.

3.1 Ship and crew

The fieldwork was conducted using the sailing vessel ‘van Uden’ by DutchSail, located in the harbour of Scheveningen, the Netherlands. The crew consisted of 9 professional sailors. For the purpose of this project, two WMR scientists and one employee of TenneT joined the cruises as well.

3.2 Sampling areas

In the trajectory of the cables, there are three locations were cable crossings can be found (see red boxes in Fig. 2). Two out of these three cable crossing locations, both located outside of the Dutch 12-mile nautical zone, were selected for sampling. At each location, two cable crossing berms can be found. During each fieldwork trip, both locations were visited and samples were collected as close to one of the cable crossing berms per location as possible (Table 1, Fig. 3).

Date	Cable crossing Location	Cable crossings	Lat (WGS84)	Y (WGS84)
6 July 2022	TAQA 10 inch oil pipeline	Alpha 1	52.8124	4.2754
6 July 2022	Neptune 8 inch oil pipeline	Alpha 1	52.12205	4.5346
30 September 2022	TAQA 10 inch oil pipeline	Alpha 2	52.2018	4.08853
30 September 2022	Neptune 8 inch oil pipeline	Alpha 2	52.1329	4.04994

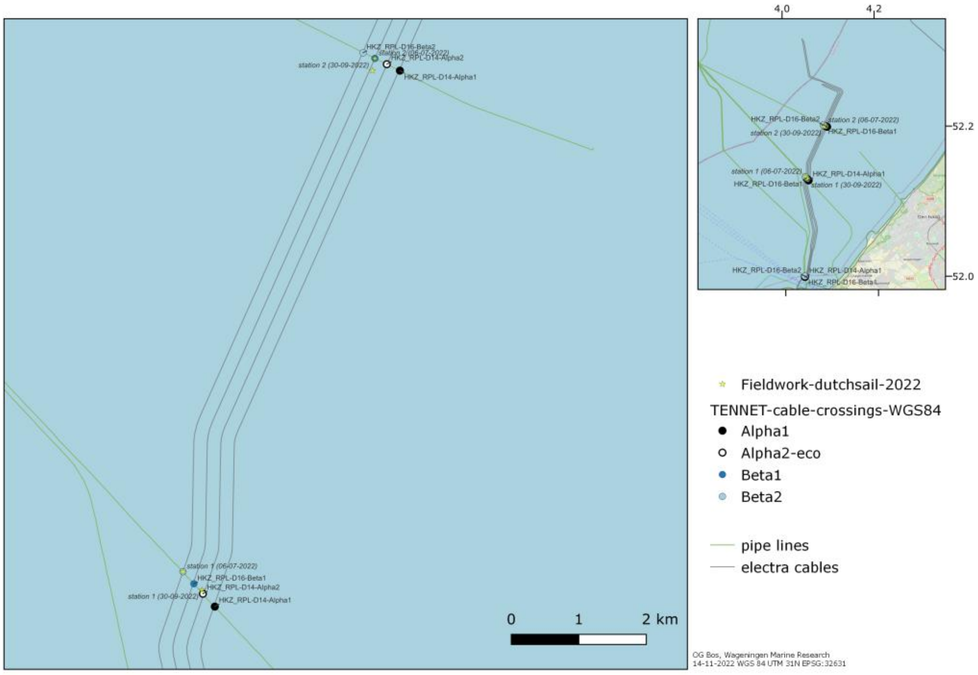

Figure 3: *Exact locations of the cable crossings that were visited and sampled.*

3.3 Collection of eDNA samples

Water samples were collected using a Niskin bottle of 2.5 L (Fig. 4a), of which 1 L was used. Gloves were used and the equipment was regularly rinsed with a bleach-water solution to avoid/ reduce the levels of contamination. The collected 1 L of water was filtered through a 0.45 µm pore-sized filter (diameter 47 mm) using a portable filtration system (Fig. 4b). Three water samples were collected from every cable crossing, thus, 12 samples in total were taken (2 cable crossing berms per sampling campaign * 3 eDNA samples * 2 sampling campaigns) as well as 2 control samples (filtered milliQ water). The filters were folded and placed in a small tube containing RNA stabilization solution to be transferred to the lab. The entire process was performed by WMR scientists with the assistance of the crew.
Immediately after arrival to the shore, the filters were transferred to the WMR lab and stored in the freezer (-20°C) until they were sent to Wageningen University for eDNA analysis to obtain information on the presence of vertebrate species that occur close to the cable crossings. Lab-work and bioinformation analysis followed all the internal standards and samples were processed following the rules of quality of Wageningen University and Research (see section 6 Quality Assurance).
The quality of the eDNA samples was examined by the eDNA expert of Wageningen University based on the identified species.

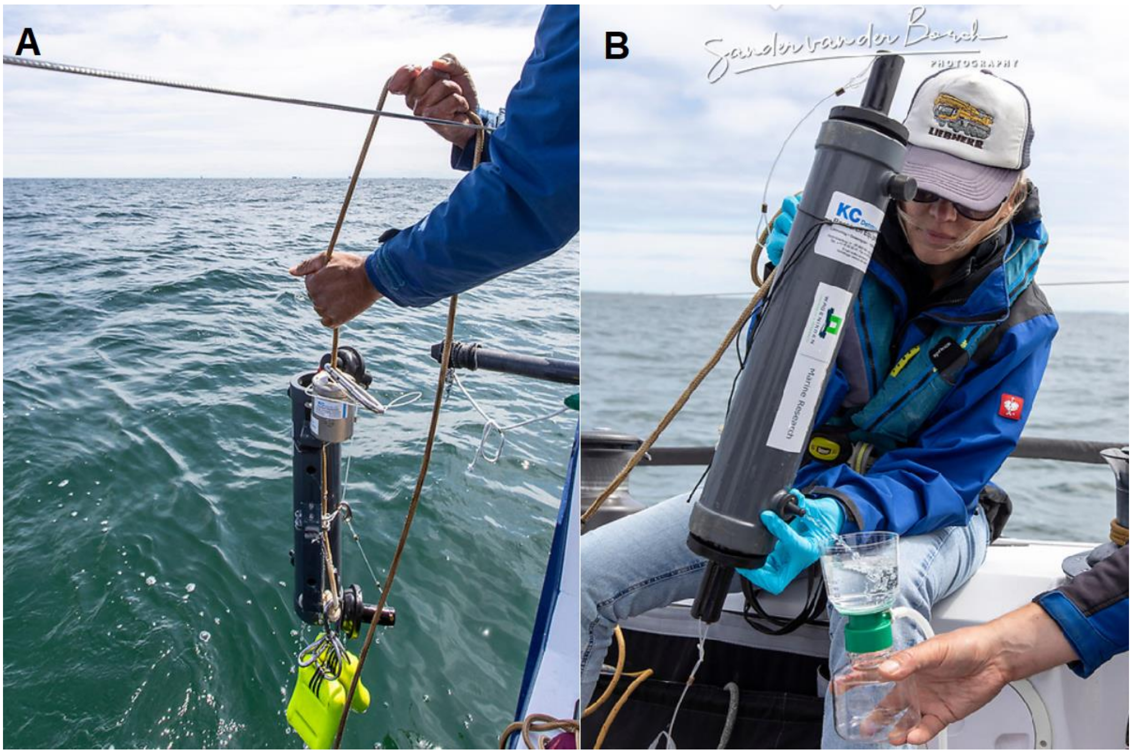

Figure 4: *A: Niskin bottle ready to be deployed at sea and collect a water sample. B: A scientist filtering the collected water through the portable filtration system with the help of a sailor. Photos: Sander van der Borch.*

3.4 Video techniques

3.4.1 Remotely operated underwater vehicle

The remotely operated underwater vehicle (ROV) of Wageningen Marine Research (Fig. 5) was used during the first sampling fieldwork to collect images from the area adjacent to and on the cable crossing berms. The ROV was manipulated by WMR scientists, while the crew inspected the process but did not assist in manoeuvring. The ROV is the BlueROV2 (https://bluerobotics.com/), a small 12 kg consumer type device, equipped with a GoPro (version Hero-7 black). The ROV is manoeuvred using a controller and it is connected to a laptop on board, which displays live video of the seafloor.

All the video material was analysed by benthos experts of WMR to identify the taxa present on and near the cable crossing berms using ImageJ and VCL media player. The videos were carefully inspected and all species present were noted and included in the final species list.

Figure 5: *The Wageningen Marine Research ROV (BlueROV2, Blue Robotics). Photo: Sander van der Borch.*

3.4.2 Drop-camera

During the second fieldwork campaign, a drop-camera (Seaviewer; https://www.seaviewer.com/) was used for collecting video footage from the seafloor by WMR scientists. This technique, which is considered easier to operate than ROV, was used as an alternative for the ROV method. The Seaviewer is designed to provide live video of the seafloor in currents. It was equipped with 10 kg of weight, a video light torch (Sealife seadragon) and a GoPro (version Hero-7 black). The camera was lowered at the initial positions of the transect as provided by TenneT. Unlike the ROV, the Seaviewer cannot be manoeuvred with a controller and does not provide additional information on its position, such as depth.

Again, all the videos collected from the drop-camera were processed by a benthos analyst, who noted the presence of species in the video images. All videos were analysed for their quality by the WMR benthos analyst, who assess the quality of images of the seafloor and the possibility of identifying most species present.

4 Results

4.1 eDNA

The eDNA sampling was proven to be generally successful, both as a method to conduct on board of a sailing vessel and from the quality of the samples. The water collection was easy and water filtration was successfully conducted on board (when weather conditions allowed it). The scientists, together with the assistance of the sailors, managed to collect 3 water samples per cable crossing berm and then they filtered the water mostly on board. However, on the second fieldwork day, this was not possible due to bad weather conditions. Furthermore, some difficulties were also encountered, especially with regards to space, which was limited on the sailing vessel. The lack of electricity for the filtration system was considered a minor problem, since we were equipped with portable batteries and managed to filter the collected water.
In total, 19 different taxa were found in all the eDNA samples (Table 2), from which 19 were identified in samples from the first campaign and 4 from the second campaign. The control samples indicated slight contamination. The same contamination was present in the field samples, where human DNA or human-related food DNA, e.g. chicken and pork, were identified. However, human DNA is commonly found in eDNA samples deriving from purely scientific campaigns. The human/food sources were not found in many DNA replicates, meaning that they did not affect the main results of this study. Therefore, we are confident that this contamination does not invalidate our results.

Table 2: *List of taxa identified by the eDNA samples collected at both locations during the two sampling campaigns. Common names in English and in Dutch are provided, as well as the location from which they were collected.*

Taxon	Common name (EN)	Common name (NL)	Location
Ammodytes marinus	Raitt's sand eel	Noorse zandspiering	Neptune
Ammodytes sp.	Sand lance	Straalvinnige vis	Neptune
Callionymus reticulatus	Reticulated dragonet	Rasterpitvis	TAQA
Clupea harengus	Atlantic herring	Haring	Neptune, TAQA
Cygnus olor	Swan	Knobbelzwaan	TAQA
Engraulis encrasicolus	European anchovy	Ansjovis	TAQA
Gobius niger	Black goby	De zwarte grondel	Neptune
Limanda limanda	Common dab	Schar	TAQA
Merlangius merlangus	Whiting	Wijting	TAQA
Mustelus asterias	Starry smooth-hound	Gevlekte gladde haai	TAQA
Phocoena phocoena	Harbour porpoise	Bruinvis	TAQA
Pholis gunnellus	Rock gunnel	Botervis	Neptune
Pleuronectes platessa	European plaice	Schol	Neptune, TAQA
Pomatoschistus minutus	Sand goby	Dikkopje	Neptune, TAQA
Raniceps raninus	Tadpole fish	Vorskwab	TAQA
Sardina pilchardus	European pilchard	Sardien	TAQA
Scomber scombrus	Atlantic mackerel	Makreel	TAQA
Scophthalmus maximus	Turbot	Tarbot	TAQA
Solea solea	Common sole	Tong	TAQA
Sprattus sprattus	European sprat	Sprot	Neptune, TAQA
Trachurus trachurus	Atlantic horse mackerel	Horsmakreel	TAQA

Some of the species identified in this study are highly associated with hard substrates, e.g. *Raniceps raninus*, *Merlangius merlangus* (can be found on gravel beds), *Pholis gunnellus*, etc., which could be an indication that we collected samples from the correct locations and thus, indicates that the method was successful. Furthermore, some interesting specimens, like sharks, harbour porpoises and swans, were found in the eDNA results. This further proves that the quality of the samples was good for biodiversity analysis.

4.2 Visual census

The cable crossing berms were not visible in any of the videos collected. The reason for this could be due to miscalculation of the coordinates between the parties involved, meaning that we did not reach and sampled the correct locations. Indeed, the coordinates of the two different sampling locations (Table 1) differ during the two sampling campaigns. Therefore, only the soft-sediment seafloor (probably very close to the berms) was monitored. The two methods used were considered generally difficult to handle from the sailing vessel, while they seem impossible to be performed by sailors alone. The ROV was very difficult to manoeuvre from non-trained people. The drop-camera is considered a good method when the vessel is equipped with a multibeam, a side scan sonar or a fish finder, but the sailing vessel was not equipped with any of these devices. Therefore, manipulating the drop-camera was challenging. In addition, the irregular speed of the sailing vessel made it difficult to tow the drop camera in a way that could provide useful images of the seafloor.
In total, 10 taxa were identified from both the ROV and the drop-camera videos (Table 3). Generally, the images collected with the visual censuses were of bad quality, the berms were not visible and, therefore, only a limited amount of taxa were identified. This is due to a combination of factors as mentioned above, i.e. wrong coordinates provided, the vessel was not completely equipped and the methods proposed were possibly too complicated to be executed from a not-easily-steerable sailing vessel. Apart from the identified taxa, a lot of marine snow was visible in all the analysed videos. In Figure 6, two individuals of the starfish species *Asterias rubens* and two of the genus *Ophiura* are visible, together with marine snow.

Table 3: *Taxa that were identified from the visual censuses in both locations and both sampling campaigns. Common names in English and in Dutch provided.*

Taxon	Common name (EN)	Common name (NL)
Actinopterygii	Ray-finned fish	Straalvinnige vis
Asterias rubens	Common starfish	Gewone zeester
Cylista troglodytes	Cave-dwelling anemone	Gewone slibanemoon
Electra pilosa	Thorny sea mat	Harig mosdiertje
Gastropoda	Gastropod	Slak
Lanice conchilega	Sand mason worm	Schelpkokerworm
Ophiura sp.	Serpent star	Gewone slangster
Ophiura albida	Brittle star	Kleine slangster
Pagurus bernhardus	Common hermit crab	Gewone heremietkreeft
Polychaeta	Polychaete	Borstelwormen

Figure 6: *Image of the ROV during the analysis of the videos. Four starfish individuals of two different taxa are visible in this image.*

5 Discussion, conclusions and recommendations

In this project, we applied well-known and previously frequently used methods that can provide valuable data on the local biodiversity. Even though the collaboration between sailors and scientists was perfect, the lack of electricity (mainly for the water filtration), a multi-beam or fish finder (to facilitate the drop-camera inspections) from the sailing vessel made the work (filtration and collection of videos from the seafloor) challenging.

5.1 eDNA sampling

Generally, the water sampling using a Niskin bottle to get eDNA information from the area worked perfectly during both sampling campaigns and could be handled by the crew members alone, without the assistance of WMR scientists.
The eDNA results indicated that we managed to collect good-quality eDNA samples, with which we could even indicate a first biodiversity difference between the two locations that are ca. 6 km apart from each other (TAQA and Neptune oil pipelines). However, human and human-related food contamination was noticeable in the samples. This did not affect the validity of our results, but it could be eliminated in the future by taking additional hygiene measures.
It can be concluded that water sampling for the purpose of eDNA analysis is possible using sailing vessels and by thoroughly instructed and trained crew members that have basic understanding of the contamination risks involved with collected eDNA samples. On a typical research vessel, which is larger and more stable, the range of weather conditions that allow for the filtration work to be carried out is larger. Improvements can be made in preventing contamination by cleaning more thoroughly all the equipment before and during use, assuring that everyone wears gloves and excluding animal food on board.

5.2 Video

The visual censuses used during the two sampling campaigns did not have the same success as the eDNA sampling. Both methods used during this project failed to find and monitor the cable crossing berms. This was most probably caused due to the wrong positioning of the vessel and maybe due to the methods applied in this project (e.g. drop-camera challenges). However, these two visual methods provided images from the soft-sediment seafloor. We observed that the use of ROV requires piloting experience, which the sailors do not have and it takes more time to acquire this knowledge. The ROV is generally difficult to manoeuvre and needs more practice and training. Because of these difficulties when it comes to ROV handling, we consider that ROV work cannot be performed by crew members alone. As an alternative, we decided to use an easier method for visual census during the second fieldwork day. We used, thus, a drop-camera, which also failed to provide us proper cable-crossing-berm images. Therefore, we believe that visual censuses are not easy to be done by sailing vessels (since they are not properly equipped for this type of techniques) and by crew members that are not highly trained to perform such methods. Maybe if a different type of drop-camera was used, such as one equipped with a frame (if it is not too large to handle from a sailing vessel) and is pointed vertically on the seafloor, positioned from place to place, then we could have obtained better images. To conclude, more skills are required in order to use visual census methods on a sailing vessel from sailors. It could potentially work if the sailing vessel is properly equipped and if the sailors learn how to pilot the cameras. However, at the moment, it does not seem favourable to use sailors to conduct biodiversity monitoring work using visual censuses.

5.3 Recommendations

- Drop-camera: we recommend to use a downward looking drop-camera with a frame, when the vessel is also equipped with drop-camera-specific devices.
- The crew could use one of the available apps (iObs or ObsMapp from Waarneming.nl) to record special wildlife that they encounter.
- Thorough instructions (including a step-by-step sampling protocol) should always be provided to the crew that needs to get a basic understanding of the sensitivity and contamination risks involved with acquiring eDNA samples.
- The collaboration with researchers remains necessary for the scientific approach and interpretation of the results.
- The sailing vessels should be better equipped with a multibeam or similar equipment to ensure that drop-cameras take images from the correct positions.
- In a potential future project, we could assess differences between eDNA samples collected from scientists vs sailors to see whether the results would significantly differ.

6 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

The Chemical and Benthos laboratory has an EN-ISO/IEC 17025:2017 accreditation for test laboratories with number L097. This accreditation has been granted by the Dutch Accreditation Council. As a result, the Chemical and Benthos laboratory has demonstrated its ability to provide valid results in a technically competent manner and to work in accordance with the ISO17025 standard. The scope (L097) of de accredited analytical methods can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to the results of those components which are incorporated in the scope, provided they comply with all quality requirements. The quality characteristic Q is stated in the tables with the original research results.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in proficiency tests.
In addition, a first-level control is performed for each series of measurements.

If desired, information regarding the performance characteristics of the analytical methods is available.

If the quality cannot be guaranteed, appropriate measures are taken.

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Justification

Report C078/22
Project Number: 4315100199

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Sander Glorius
Researcher

Signature:

Date: 25/11/2022

Approved: Drs. Jakob Asjes
MT member Integration

Signature:

Date: 25/11/2022

Wageningen Marine Research
T +31 (0)317 48 7000
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors' address

- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

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