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Scientific report

Installation of live flat oysters and monitoring benthic biodiversity in OWF Borssele III/IV; T-0 2020

de Rijke Noordzee

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1 Introduction

The Dutch government has a responsibility for the state of the North Sea environment following from the EU's Marine Strategy Framework Directive and Habitat and Birds Directive. In order to utilize the momentum of the large-scale development of offshore wind farms, a commitment was explicitly included in the site decisions for developers. This enables them to contribute to the strengthening of a healthy sea and the preservation and sustainable use of species and habitats that originally existed in the Netherlands. (Blauwwind, 2019).

Blauwwind is constructing Borssele III and IV wind parks on the edge of The Netherlands' exclusive economic zone. Blauwwind developed its vision on how to design and construct the Borssele III and IV wind parks in such a manner that it matches the vision mentioned above, trying to contribute to a strong, healthy and biodiverse North Sea. (Blauwwind, 2019).

With this in mind, the Borssele III & IV project aimed to install and monitor living flat oysters inside the Borssele Wind Farm Zone, Site III&IV as well as the monitoring of the surrounding epibenthic fauna, and investigating the success of oyster installations and different scour-protection. The main monitoring effort concerns status of flat oysters, as it is indicated as an umbrella species, assuming enhancement of the biodiversity in general.

The operational objective of this pilot was to increase the empirical evidence of the influence of habitat conditions on native biodiversity and flat oyster bed development by implementing various scour protection designs. This called for a scientific approach where the effects of various scour protection designs (treatments) can be investigated statistically to draw conclusions on what is the most effective approach (Blauwwind, 2019).

Based on this the monitoring objectives are:

1. What is the survival and growth rate of the live oysters introduced (as larvae source) in the wind farm?
2. Do oyster larvae settle on the various design substrates (scour protection and empty shells)? And if so, which scour protection variations are the best for oyster settlement?
3. How is biodiversity in general developing on the various design substrates (scour protection and empty shells)? Are there differences on the different scour and empty shell variations?

The main monitoring effort concerns status of flat oysters, as it is indicated as an umbrella species, assuming enhancement of the biodiversity in general.

The Blauwwind Pilot scientific scope (2019) consists of the monitoring of the Borssele 3/4 project in order to find answers to the monitoring objectives. This scope entails taking larvae samples, oyster samples from the racks, video surveys with an ROV including grab samples and writing reports. This type of monitoring is to be executed in the specified windows during Year 1, 3 and 8.

In this study however, that functions as the T-0, monitoring like mentioned above was yet conducted. Here, live oysters were placed in cages on installations and lowered to the sea floor at 4 different windmills. The methods are described in more detail in the next chapter.
The ROV surveying and e-DNA sampling that was conducted will serve as a baseline for biodiversity at the research sites to compare to the future campaigns.

2 Material and methods

2.1 Sampling sites

In Figure 2-1 an overview is given of the sampling sites in the Borssele windpark for the field work. A total of 8 monopiles and their surrounding area have been designated for the study in Borssele 3/4. In the vicinity each of monopoles in Borssele 3 there were Oyster tables placed with each table containing 8 oyster baskets with 75 living oysters (red locations). Next to this an ecological study was executed for the T-0 assessment using ROV images and eDNA sampling. In Table 2-1 an overview of the used methods per sample location is provided.

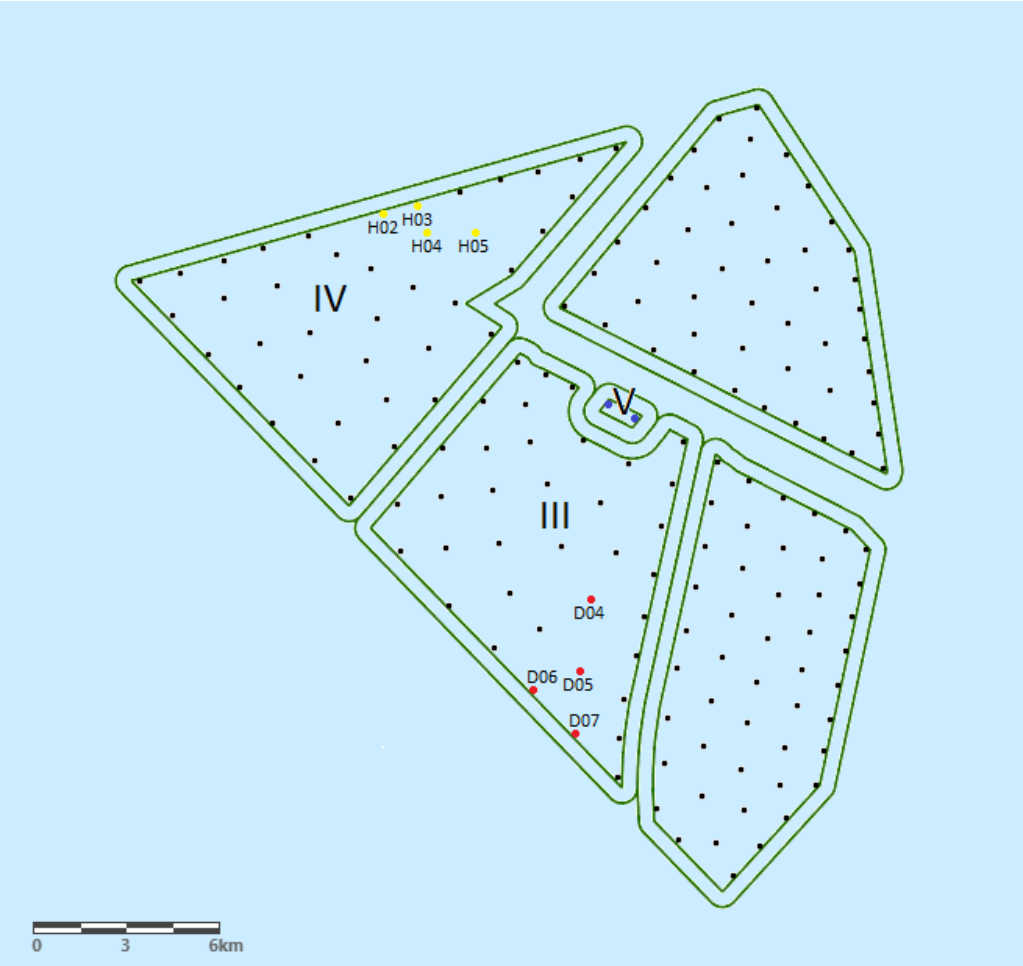

Figure 2-1: Overview of the Borssele offshore wind park. Red dots concern the locations for the oyster installation (from top to bottom B334-D04, B337-D05, B328-D06 and B327-D07), the yellow dots are the reference locations (from left to right: B412-H02, B411-H03, B402-H04 and B401-H05). The blue dots concern locations on Borssele 5.

Table 2-1: overview of methods used/actions performed per location

Sample location	ROV	eDNA	Oyster-installation
B334-D04	4*5 pictures	2 liter watersample	8 baskets
B337-D05	4*5 pictures	2 liter watersample	8 baskets
B328-D06	4*5 pictures	2 liter watersample	8 baskets
B327-D07	4*5 pictures	2 liter watersample	8 baskets
B412-H02	4*5 pictures	2 liter watersample	
B411-H03	4*5 pictures	2 liter watersample	
B402-H04	4*5 pictures	2 liter watersample	

B401-H05	4*5 pictures	2 liter watersample

2.2 Preparation of the flat oysters

2.2.1 Check and measurement of oysters

The measurement and monitoring of the flat oysters (*Ostrea edulis*) for the T-0 assessment took place in Yerseke on the 29th of September 2020. The oysters were removed from the Aqualife tank. For the oyster experiment, only live oysters without juvenile Oyster spat were selected in order to prevent young oysters from being to be falsly found during the experiment. The longest length and widest width of each Oyster was measured and saved in a database (Figure 2-2).

Figure 2-2:Measuring oysters size using digital calipers

Figure 2-3: The 75 oysters of cohort 1

After measuring the 2400 oysters were divided into 32 cohorts of 75 individuals. Each cohort was assigned a specific number (Figure 2-3). The oysters of one cohort were then deposited together with a label for the cohort number in a thin mesh bag (onion bag), so that the cohorts on board the ship could easily be transferred into the oyster baskets. Following, the oysters were placed back in the Aqualife tank. In Appendix 1, a picture of each cohort is displayed.

2.2.2 Flat oysters preservation

The water temperature and the oxygen supply of the flat oysters in the Aqualife tanks (Figure 2-4) have been monitored (by van Oord) between measuring the oysters and the placement of the Oysters in the baskets on the sample sites. The oysters have been kept in these tanks from September 16th 2020 until October 12th at a constant temperature to keep them healty and alive and keep their metabolism low.

Figure 2-4: Aqualife-tanks with oysters.

2.3 Installation of oyster tables

On board of the vessel the Oysters were transferred from the Aqualife tanks to the Oyster baskets using the cohort number as a number for each basket. The number tags were attached to the basket doors and the baskets were filled with the Oysters. For each Oyster table, 8 Oyster baskets were filled with 75 living Oysters per basket. In total 2400 Oysters were brought down on the seafloor in 32 cohorts on 4 Oyster tables. After positioning and securing the cages on the Oyster tables 3 additional bags containing empty Oyster shells were attached to each oyster table as a potential substrate for Oyster spat. The positions of the cohort numbers on the oyster tables are indicated on the following diagrams (Figure 2-6). In this figure the bags with empty Oyster shells is indicated with an ellipse.

The oyster structures were placed on the scour protection at 4 monopiles in Borssele 3 offshore wind park (locations B328-D06, B337-D05, B334-D04 and B327-D07, red dots in figure 2-1). Another 4 monopiles (locations B401-H05, B402-H04, B411-H03 and B412-H02, yellow dots in Figure 2-1) serve as reference locations (no installation of oysters).

On board of the research vessel, the cohorts were taken out of the Aqualife tanks and put in cages with their corresponding label tie-wrapped to the cage doors. One ‘oyster table’ consists of 8 cages (Figure 2-5).

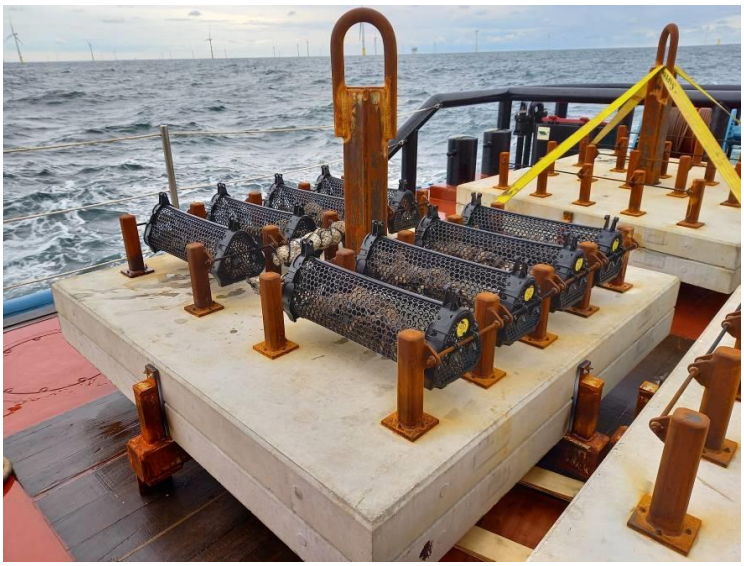

Figure 2-5: Oyster table with 8 oyster baskets with their corresponding labels and cohortnumbers attached.

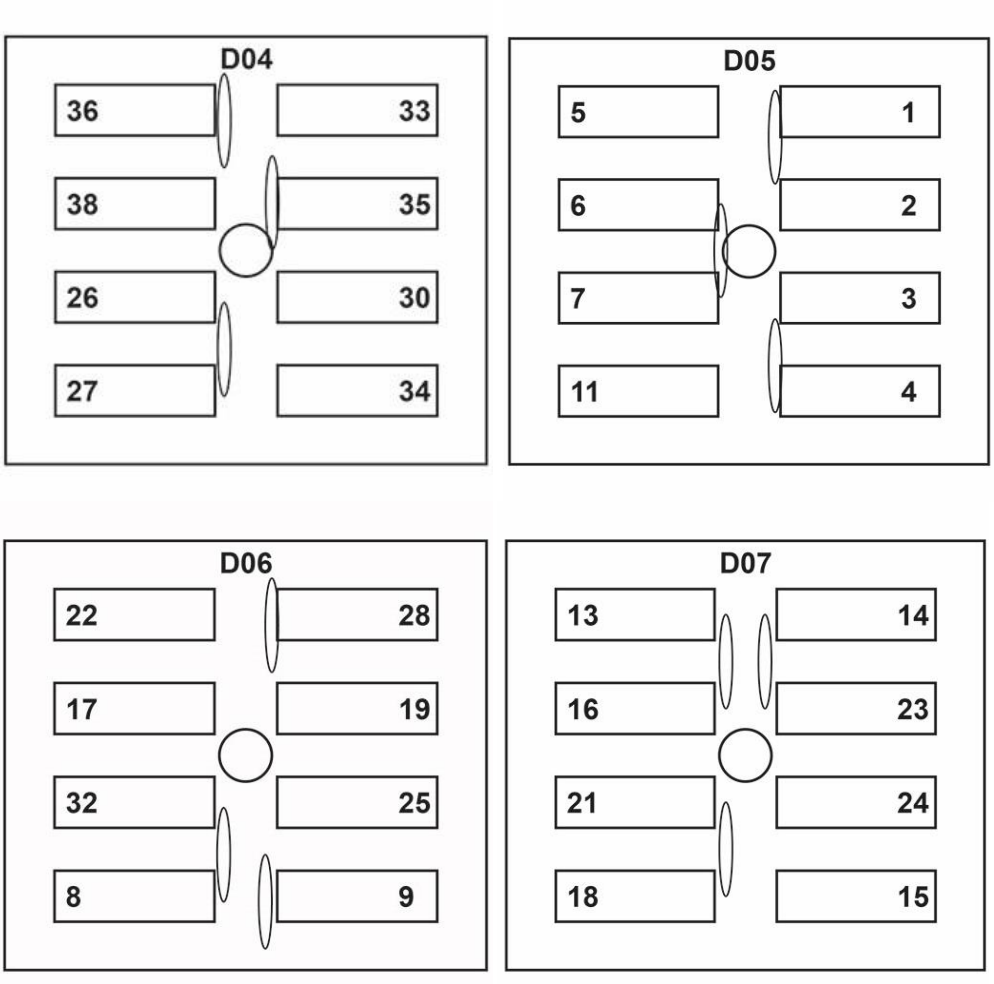

Figure 2-6: Schematic representation of the Oyster tables and the placement of the cohort numbers relative to the different monopiles at the locations D04, D05, D06 and D07. The ellipses indicate the placement of the bags with empty shells (noted at D07 on the basis of photos).

2.4 ROV surveys

At all 8 sample sites, an ROV survey was performed to take high resolution pictures of the benthic fauna living around the monopiles. For each turbine in the investigation four different types of scour-protection were used. The area around the base of a monopile is divided into 4 sections. The first division is between leeward and windward side. The second division is between coarse and filter grade scour. The coarse grade scour is closest to the base of the monopile (see Figure 2-7). A finer grade is present at a further distance.

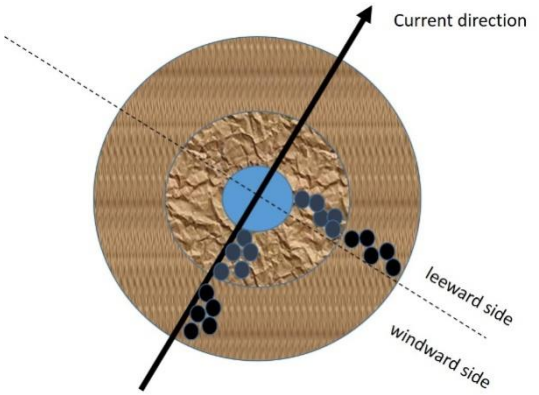

Figure 2-7: the positioning of coarse and filter gradings around the monopiles, and the approximate positioning pictures (black dots in filter grade, gray dots in coarse grade)

The fauna present in these images of the T-0 study is compared with that seen on images from future campaigns to assess the succession and development of the benthic fauna community on the different scour-types and to be able to assess the most successful type of scour protection for nature inclusive design.

For the ROV setup a Saab Seaeye Cougar with an 4K video camera was used. Two green subsea lasers with a fixed distance of 42.5cm were in the image as a scale for measuring fauna.

The following steps were followed during the individual surveys:

1. Using the cameras, a type of scour-protection was identified and visually confirmed.
2. Within the scour-protection type 5 photo's were randomly taken. The ROV was landed on the sea floor. After a 10 second stand still a picture was taken and the ROV was moved to the next location. It was made sure that the pictures did not have any overlap. If needed the tilt and focus of the camera was ajusted.
3. Each picture was checked for clarity and focus; such that individual fauna can be identified from the pictures. The stills/photos were inspected on a high resolution screen.
4. Each picture-frame contained the coordinates, date and time. This information is needed for the analysis of the pictures and the next campaigns to more easily identify the different scour types.
5. After a picture was taken, the type of scour and the file-number was written down on a sampling-form along with the code for the monopile.
6. If special flora or fauna (sharks, shark-eggs, special benthic fauna, et cetera) were seen during the 10 second ROV movement between pictures, extra pictures[1] were taken.

[1] These pictures are not used as one of the 5 random pictures for the community analysis.

Photos of the scour protection were taken according to the protocol in document J00002898_WMSv01 and as described in the steps above. The two ROV teams used a different approach to take photos. These are explained below. A detailed description of the ROV survey for each of the monopiles can be found in Appendix 3.

ROV team 1 started outside the scour protection and flew towards the monopile taking pictures. Successively the Filter Grade (photo # 1 to 5) and then the Coarse Grade (photo # 1 to # 5) was photographed. ROV team 1 then flew away from the monopile, against the current direction. Succesively the Coarse Grade (photo # 1 to 5) and then the Filter Grade (photo # 1 to # 5) was photographed. ROV team 1 aimed for the edge of the monopile at the start, so photos were taken next to the survey line (as in Figure 4 of the protocol). This approach is applied for monopiles DO4, DO7 and HO5.

ROV team 2 started outside the scour protection and flew towards the monopile taking pictures. Successively Filter Grade (photo # 1 to # 5) and then the Coarse Grade (# photo # 1 to # 5) was photographed. ROV team 2 then flew out of the scour protection to reposition the ROV and fly back towards the monopole against the current. Succesively the Filter Grade (photo # 1 to 5) and then the Coarse Grade (photo # 1 to 5) was photographed. This adjustment was made so the location of the monopile could be used to keep the correct heading for the ROV.

In 2020 there was no location beacon present on the ROV. Therefore the exact coordinates of the ROV were not recorded and printed in the photo frames. This means that not all requirements from step 4 could be met. Since it is expected to be more difficult in the future to identify the scour protection type, it is recommended to add a location beacon to the ROV, so that the precise position for each photo can be retrieved.

Photos were coded in the following way:
Current direction – scour grade - photo number - date and time - monopile code
For current direction the abbreviations PD (perpendicular to current) and CD (in direction of current) were used. For the scour grades the abbreviations CG (coarse grade) and FG (filter grade) were used.

The current direction was always SW-NE or NE-SW, turning 180 degrees in between. Current direction was noted for each ROV survey due to the direction of current and the order of the photos. Appendix 3 indicates the approach method and photo locations for each monopile.

2.4.1 Photo and video analysis

Photo and video analysis was executed by specialists using special analysis software for biological data. Therefore a species list was made and added to the software. From each photo all the living fauna was identified and written down.

Figure 2-8: Examples of some ROV photo's from the T-0, 2020 survey. Left image: H03-PC-CG-01 right image: H03-CD-FG-04

For species that can be counted a counting was done. For species with a coverage the SACFOR scale was used as an indication for the presence of the species in the image. Especially clustering or crust forming organisms can be quantified using this scale. The SACFOR scale is an internationally used unified system for recording abundance of marine fauna in biological surveys (JNCC). The SACFOR scales used in this project were derived from the video analysis protocol of Rijkswaterstaat (Rijkswaterstaat, 2020). This is done to be able to compare results with other projects if needed. All present fauna in the photos was analysed and noted in the software.

Next to the photo analysis all full ROV videos were also inspected for extra species. Since the videos are not linked to a certain treatment all these extra observations were written down on the level of each location. These observations can be used for the total biodiversity analysis of per monopile or per area.

2.5 eDNA-sampling

2.5.1 Water sampling

To identify as many species as possible water sampling was conducted at every sample site. With this water sample an environmental DNA (or e-DNA) sample was retrieved. For each sampling site approximately 2000 ml of seawater was collected with either a Niskin bottle or from water pumped from a water-pump placed in an IBC-tank on-board.

Due to the strong currents, it was not possible to manually lower and operate a Niskin bottle, due to high current and the low weight of the Niskin bottle. To mitigate this problem, two Niskin bottles were attached to the teather management system of the ROV (Figure 2-9). Before the start of each sampling, the Niskin bottles were rinsed with a 50% bleach solution in order to prevent DNA contamination.

After performing the ROV survey, the Niskin bottles were closed using the ROV manipulator. This happened at a height of 2 meters above the scour protection. After the ROV was retrieved on deck of the vessel, the eDNA-sample was collected. As the Niskin bottles remained attached to the cage, it was usually not possible to sample 2000 ml from 1 Niskin bottle. Therfore this was supplemented with water from a second Niskin bottle.

Figure 2-9: ROV in cage, with 2 Niskin bottles attached vertically.

2.5.2 *Filtration*

After collecting sampling water, the eDNA-sample was collected on a sterile cup with a filter (0.2 µm) by passing the water sample through a funnel via the filter into a sterile 2000 ml vacuum flask. Before every sample site, all bottles, tweezers and other equipment is cleaned with 50% bleach solution. Also latex unpowdered gloves were used thoughout the sampling process. A vacuum was created by a hand-pump enabling a faster passage of the water through the filter. Figure 2-10 provides an overview of the set-up. Table 2-2 summarizes the volumes of water that were filtered per location.

Figure 2-10: eDNA filtration set-up on the research vessel.

Table 2-2: Overview of eDNA-samples taken per sampling location

Location	Sampling device	Volume filtered	Remarks
334 D04	Niskin bottle	2000 ml	
337 D05	Niskin bottle	2000 ml	
328 D06	Niskin bottle	1650 ml	1 Niskin bottle did not close properly because handle was loose
327 D07	Niskin bottle	2000 ml	
412 H02	Niskin bottle	2000 ml	
411 H03	Niskin bottle	1500 ml	Smaller volume due to break down of 2 Niskin bottles
411 H03B	Pump	1850 ml	Sample from the water tank (filled with water via the water pump, at H03). There is more sedimentation, possibly this sediment has come from out of the tank. Tank has not been pre-bleached or rinsed.
411 H03C	Pump	2000 ml	Second sample from the tank (of H03), now let the water run out of the tank a little longer, there is less sediment but a few live animals (crimson shrimp and amphipode?)
402 H04	Niskin bottle	2000 ml	Due to uncertainty about the moment of impact of the Niskin bottles, a sample was also taken using the pump.
402 H04B	Pump	2000 ml	Sample taken using the pump at a depth of 30 m in a sanitized bucket (instead of a tank).
401 H05	Niskin bottle	2000 ml	

2.5.3 Preservation

After filtering, the top and bottom were disconnected to expose the filter membrane containing environmental DNA. With a forceps (decontaminated with 50% bleach solution), the filterpaper was folded filter paper in half, for four times. The filter was kept stable with a clean gloved finger to prevent it from unfolding. After folding the filter was placed in a 2 ml vial filled with 1,5ml molecular-grade ethanol (96%) for preservation. All vials were wrapped in aluminum foil, labeled with the name of their corresponding monopile location and stored in a freezer at -20°C.

2.5.4 DNA-Analysis

Preserved samples were sequenced using PCR in the lab by BaseClear BV using two primers: CO1 (for benthos) and 12s (for fish). Sequencing was repeated for each sample depending on the primer. CO1 was replicated 6 times, 12s was replicated 12 times for better and more reliable results. The resulting sequences were 'blasted' against the genetic databases BOLD and GenBank, and post-processed, using algorithms deciding what reads are valid and what reads are false/faulty developed by BIOMON (Leiden) which resulted in a species list with amounts of 'reads' per species.

3 Results

3.1 Flat Oyster monitoring

Before deployment a total of 2400 flat oysters have been measured. The average length was 77.9 (± 4.6) mm, the average width was 73.5 (± 6.3) mm. The measurements of the flat oysters show that each cohort displays a normal distribution in size (Figure 3-1). Also, the size of oysters is normally divided among all cohorts together, with the exception of one cohort (basket 8) as it includes more on average larger individuals than in other cohorts (see Figure 3-2 and Figure 3-3). The individuals in basket 8 in itself, however do exhibit a normal distribution in size. Overall the Oysters were quite similar sized.

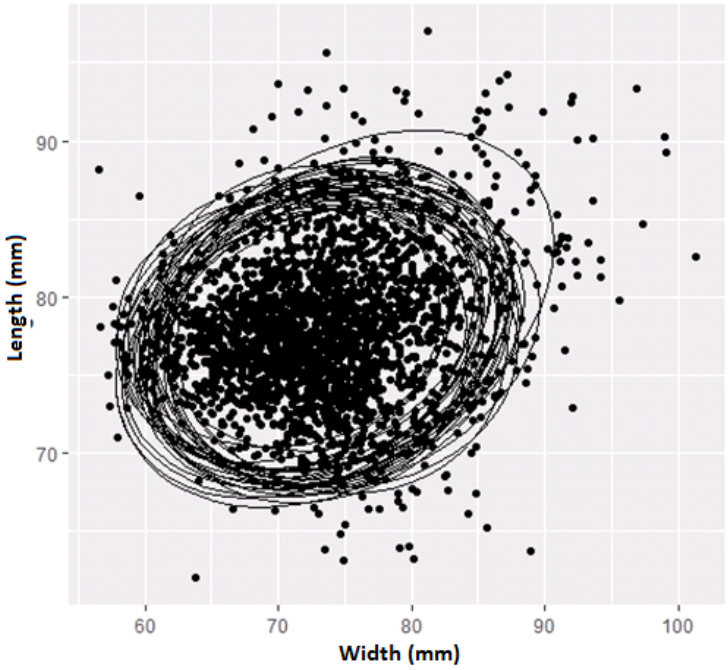

Figure 3-1: X-Y plot of all individual oysters with length and width in mm. Circles indicate the 95 percentile distribution of measurements by cohort. The centers of these circles are located on top of each other (with the exception of 1 circle (basket 8). This indicates that although the distribution contains slightly larger individuals (in the upper right corner), each cohort contains as many of the larger individuals (basket 8 slightly more large individuals than average).

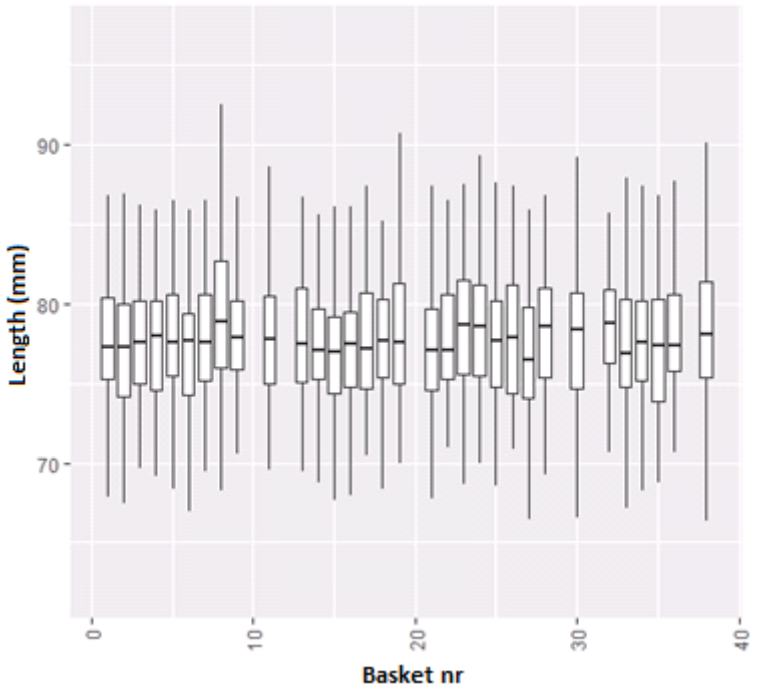

Figure 3-2: Distribution of measured shell lengths (longest length in mm) per cohort/basket in the 2020 baseline.

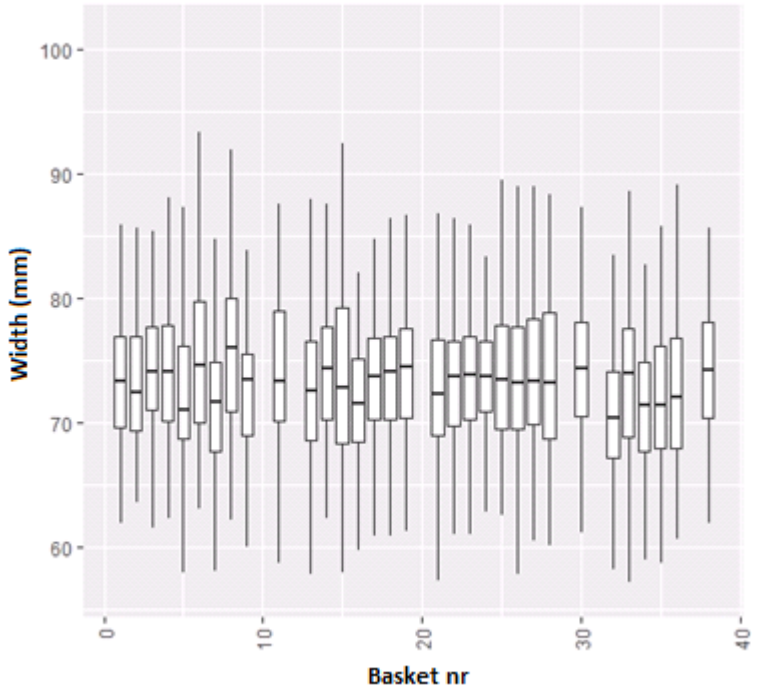

Figure 3-3: Distribution of measured shell widths (widest width mm) per cohort/basket in the t-0 2020 baseline.

3.2 ROV image analysis

In total 34 species and 7 higher taxa were identified in the ROV images. Using data from the extra photos and the data from the ROV videos another 17 species and 16 higher taxa were found in the video analysis in the T-0 of 2020. Among these species and taxa there are a lot of fish species, which are often not in the photo, but are more easily found in the video. The photos show about 70% of the identified species in the total photo and video survey. For the video analysis a lot more identifications were done on a higher taxon level, because the images are moving and therefore the identification often has a lower certainty. The lower image quality causes that the amount of identifications found with a higher species level to increase.

In Table 3-1 the taxa list per monopile for the ROV photo analysis for the T-0 in 2020 is given. On average about 16 species were found per monopile. The locations D04 and D05 have a slightly lower amount of species in the T-0 compared to the other locations.

Table 3-1: Presence/Absence table for taxa per monopile in T-0, 2020

Taxon name	Type	D04	D05	D06	D07	H02	H03	H04	H05
Actinopterygii	Fish				x			x	
Actinothoe sphyrodeta				x	x		x		x
Ascidiacea							x	x	x
Asterias rubens		x	x	x	x	x	x	x	x
Balanidae				x				x	
Bryozoa			x	x		x			x
Callionymus reticulatus	Fish				x				
Cancer pagurus		x	x	x	x	x	x	x	x
Diplosoma listerianum		x				x	x	x	x
Ectopleura larynx		x							
Enophrys bubalis				x		x	x		
Gobius niger	Fish	x						x	
Hydrozoa			x	x	x	x	x	x	x
Jassa falcata				x			x		
Jassa herdmani		x				x			x
Liocarcinus holsatus			x		x				
Macropodia rostrata							x		
Maja brachydactyla						x		x	x
Malacostraca				x					
Metridium senile		x	x	x	x	x	x	x	x
Mullus surmuletus	Fish					x			
Myoxocephalus scorpius	Fish			x	x	x		x	x
Mytilus edulis								x	
Necora puber		x	x	x	x	x	x	x	x
Nemertesia antennina						x	x		x
Obelia bidentata		x	x	x	x	x	x	x	x
Ophiura albida									x
Ostrea edulis					x				
Pagurus bernhardus				x			x		
Pomatoceros triqueter		x	x	x	x	x	x	x	x
Pomatoschistus minutus	Fish						x		
Porifera			x	x			x		
Psammechinus miliaris				x					x
Sagartia elegans			x	x	x	x	x	x	x
Sagartia troglodytes		x	x	x	x	x	x	x	x
Sagartiogeton undatus					x				
Sepia officinalis			x			x			

Trisopterus luscus	Fish	x	x	x	x	x	x	x	x
Trisopterus minutus	Fish			x			x		
Tubularia indivisa		x	x	x	x			x	
Urticina felina			x	x	x	x			
Higher taxa		***0***	***3***	***5***	***2***	***2***	***3***	***4***	***3***
Species		***13***	***13***	***18***	***17***	***18***	***18***	***15***	***17***

3.2.1 First analysis of the benthic community

For the research set-up there is an important variable the grade of the scour protection. The presence and absence data from the photo analysis was added into one non-Metric multi dimensional scaling plot, using a Bray-Curtis similarity. These plots indicates the similarity between multiple samples. The closer samples are grouped together, the higher the similarity between these samples. This means that the benthic community within grouped samples has a high(er) similarity.

In Figure 3-4 an n-MDS plot of all the samples from the research are added together. In this plot most of the locations and treatments are quite similar. However there are some photos that are placed quite outside of the clustering of the locations. These locations are mainly consisting of locations (H02, H03 and D07) within the filter layer which have a lot of sand in the photos. Therefore the benthic community is different compared to the more rocky and gravelly substrates in the fine and the coarse layer.

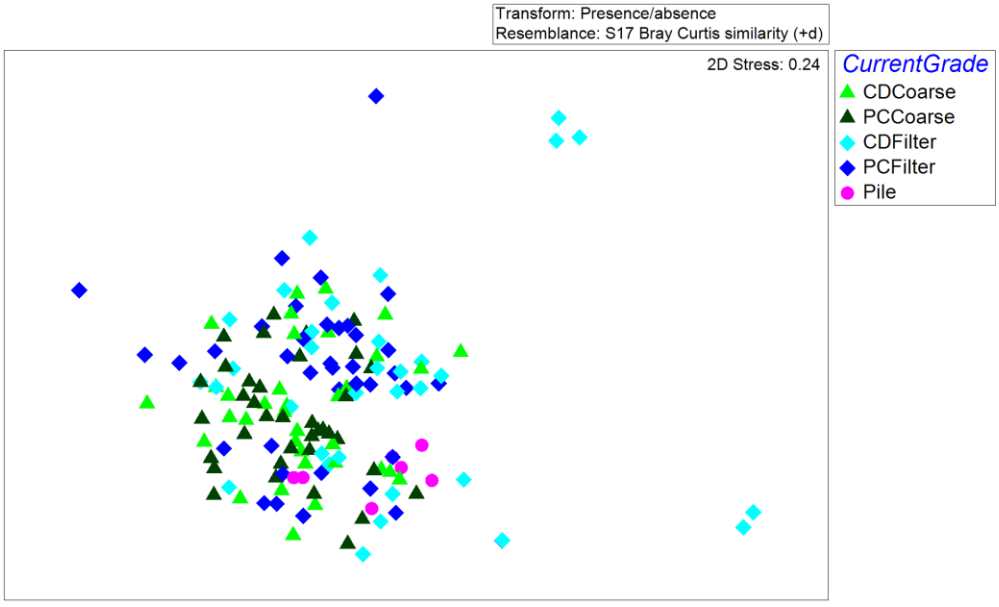

Figure 3-4: Non-metric multi dimensional scaling plot the for the current direction (CD = with the current, PD = perpendicular to the current), the grade of the sediment layer (filter and coarse grade) and the monopiles (pile). The presence/absence data with a bray-curtis similarity analysis was used.

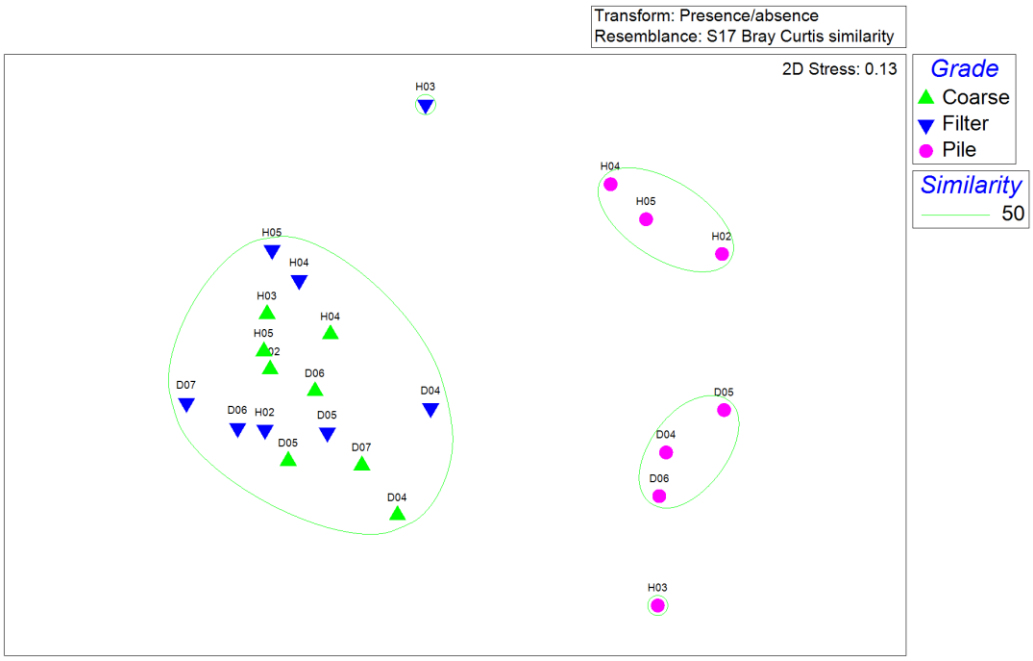

Figure 3-5: Non-metric multi dimensional scaling plot the for the grade of the sediment layer (filter and coarse grade) on each of the monopiles. The photo analysis of the monopiles are separately presented. The presence/absence data with a bray-curtis similarity analysis was used.

In Figure 3-5 the treatment of each of the locations is presented. It becomes clear that the analysis of the coarse and the filter layer have a high similarity and the locations are clustered together. The monopiles on the H02, H04 and H05 cluster together as a group and also the monopiles from D04, D05 and D06 cluster together. This means that the benthic community found on the monopiles is different from the community in the photos of the seabed, but also that the locations in Borssele area 3 (D series) are different from the community in Borssele area 4 (H series). In this plot treatment H03 Filter stands out. The reason for this deviation is that most of the photos for this treatment have an (almost) empty sandy seabed (see Figure 2-8, right photo) without any visible fauna. This causes a very distict deviation from the other treatments.

In Figure 3-6 a plot was given of the different areas in Borssele. In this plot the distinction between Borssele 3 (D locations) and 4 (H locations) can be derived. The locations from Borssele 3 seem to be clustered in the upper part of the plot and the locations from Borssele 4 are more clustered in the lower part of the plot. It is important to investigate the natural distinction between Borssele 3 and 4 area, because the H locations serve as a reference site for the treatment with the introduction of Oysters in the area. Any 'natural' differces in the two areas can be investigated using the T-0 data in the future. Awareness of the natural occurring differences is therefore important.

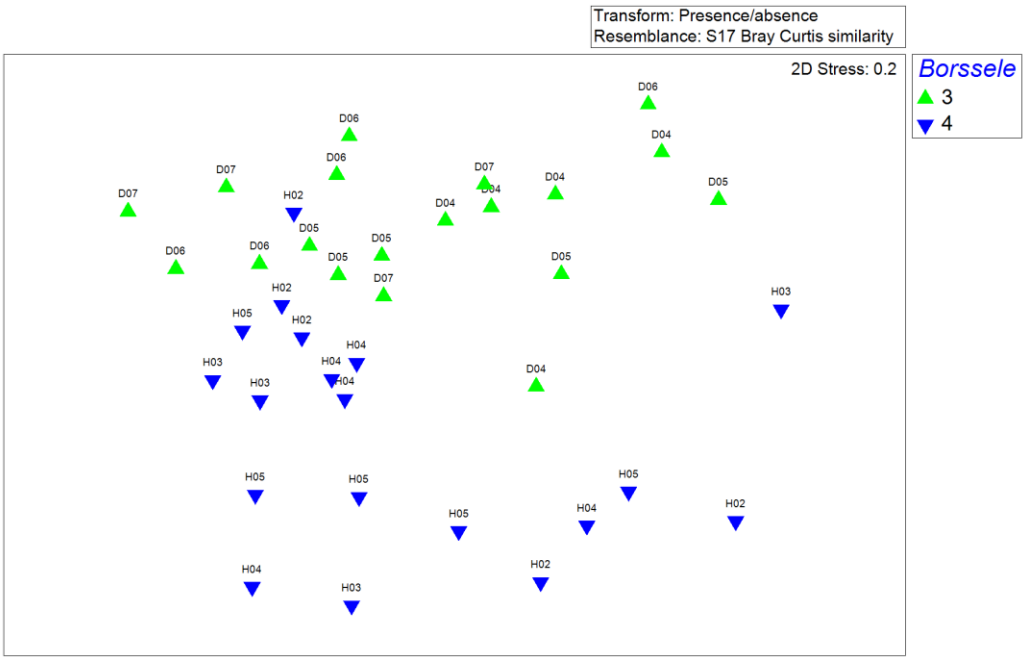

Figure 3-6: Non-metric multi dimensional scaling plot with the locations divided into Borssele 3 and 4 area. The presence/absence data with a bray-curtis similarity analysis was used. Treatment H03 CD FG was deleted from this analysis due to the deviation in this treatment (see text)

3.3 (e)DNA

The post-processing of the environmental DNA (eDNA) analysis resulted in a list of 62 taxa found in the 11 samples that were analysed. This list of taxa consisted of entries of both species and genera. From part of the list of taxa DNA was identified as a species, but from another part only the genus level could be identified. This resulted in instances were both the species and its genus were separately listed as taxa.

3.3.1 Comparison with species list from ROV photo/video

When comparing the species list of the video-analysis and DNA-analysis identification-levels become an issue. How can we compare species of a certain genus with its genus? Is the occurrence of the genus the same as the species or is it another species within that genus? Some species within a genus cannot be visually separated on video images, simply because they are too small or the identification depends on small characteristics not visible in the image. The cause of DNA-analysis not being able to identify to species level can be three-fold:

1. DNA (mostly eDNA) is too deteriorated to fully identify.
2. The genetic reference-databases (BOLD en GenBank) are incomplete for species (occurring in the North sea) of that genus, and the DNA-sequence differs from those of the species present in the databases.
3. The sequence for species in the reference-databases are taken only from a single or few organisms, or a single or few locations, probably not representing the full genetic diversity present, making it unable to attain a positive identification if a sequence originates from another genetiv pool of this species.

To tackle these identification-problems, we followed an assessment procedure. We first identified whether the occurrence of a genus was likely due to pelagic larvae (relatively high amount of reads in a water sample). If so, the genetic databases were checked for completeness (bullit 2) and representivity (bullit 3). If the databases were incomplete and/or irrepresentative the identication to genus level was counted as a taxum, since it could be another species within that genus that was not yet accounted for in the databases. If the amount of 'reads' were small

and the databases were complete and/or representative it was assumed the incomplete identification was due to deteriorated eDNA, resulting in the deletion of the count of the genus if a species in that genus was also identified. If species were identified using a small amount of 'reads' and the databases were not representative for these species, we critically assessed whether these species are likely occurring in the North Sea. The complete assessment procedure resulted in a list of 49 taxa identified using eDNA.

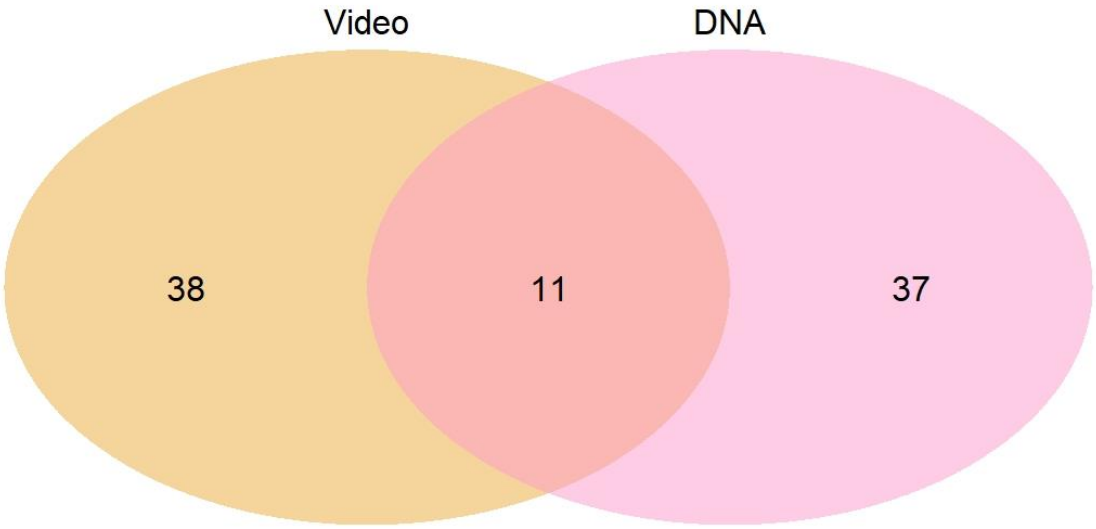

Figure 3-7: Venn-diagram showing the unique and overlapping taxa per analysis (video and DNA)

The eDNA-samples showed to be mostly adding to the complete species list; 11 taxa were correspondingly found in both the video and the DNA-analysis (see *Table 3-2*). 37 taxa were unique to the DNA-analysis, while 38 were unique to the video-analysis (see *Figure **3-7***).

Table 3-2: species identified in both DNA and video-analysis.

Asterias rubens
Echinocardium cordatum
Mnemiopsis leidyi
Metridium senile
Obelia bidentata
Ophiothrix fragilis
Mytilus edulis
Ophiura albida
*Ophiura ophiura**
Cylista elegans
Cylista undata

*by eDNA-analysis identified as O. albida/ophiura.

Of the 7 visible (potentially) pelagic species 4 taxa were not found with video (*Table 3-3*). These species might be visible in following videos, but their occurrence or positive identification (in case of Obelia dichotoma) in the video is unlikely.

Table 3-3: visible (potentially) pelagic species not seen in video.

Chrysaora hysoscella
Obelia (dichotoma)
Sardina pilchardus
Scomber scombrus

Of the 17 benthic species found using DNA, the following 2 species were not found in the video (*Table 3-4*). These species will likely become detectable in the video if these species survive and grow. Sabellaria spinulosa or Ross worm is a reef building species of particular ecological interest and is 'read' in high quantity at locations H05 and H04 both sampled using Niskin bottles. Both species *Echiichthys vipera* (the Lesser weaver) and *Sabellaria spinulosa* are associated with sandy sea beds, which are widely present outside the scour-protection of the offshore windfarm, but the video shows that sandy cover is also present on filter grade and in between coarse grade scour protection.

Table 3-4: Visible benthic species not found in video

Echiichthys vipera
Sabellaria spinulosa

Of the species found using DNA-analysis the following will not and will not ever be detectable using ROV photo or video (*Table 3-5*) because these species are simply too small to see or identify using images or video. The eDNA samples have uniquely added these species to the species list for the project.

Table 3-5: (pelagic) Species undetectable by video

Acartia (tonsa)
Amphinema dinema
Aphia
Calanus euxinus/helgolandicus
Centropages (typicus)
Eucheilota maculata
Eucheilota menoni
Eutima gracilis
Lovenellidae
Mitrocomella brownei
Oithona davisae
Paracalanus (parvus)
Pseudodiaptomus marinus
Sagitta (setosa)
Temora longicornis

3.3.2 Sampling techniques for eDNA sampling

In the T-0 campaign there were several sampling techniques used for eDNA sampling. There were Niskin bottles used, but also a pump was used to pump seawater towards the ship from near the seabed. With the present data no effect can be identified between the different sam-

pling techniques used for eDNA samples (Niskin bottle vs. waterpump; see table 2.1), simply because each eDNA sample differed from another irrespective of technique and not enough replicates were taken to test this effect properly. Nevertheless, both methods do seem to result in mostly the same species per location, but may have different amounts of reads per species. This indicates the pump may be more effective in picking up eDNA from certain species than the Niskin-bottle. From a qualitative view there is little difference between the two techniques, but there are some quantitative differences.

3.3.3 Comparison between impact and control sites

Even though the installation of scour protection was one year prior to the ecological campaign, differences in species composition between spatially distant locations was present both in results from video- and DNA-analysis. The additions to the species list from DNA-analysis do increase the difference between the impact (D-poles) and reference sites (H-poles) already found using the video-analysis (*Figure **3**-**8***). When considering the 37 taxa uniquely found using DNA-analysis, the impact area (D) holds 7 taxa not found in the reference area (H), while the reference area holds 18 taxa not found in the impact area. From the video this difference was less pronounced: of the 38 taxa uniquely found in the video 9 taxa were uniquely found in the reference area and 8 in the impact area (see *Figure **3**-**8***).

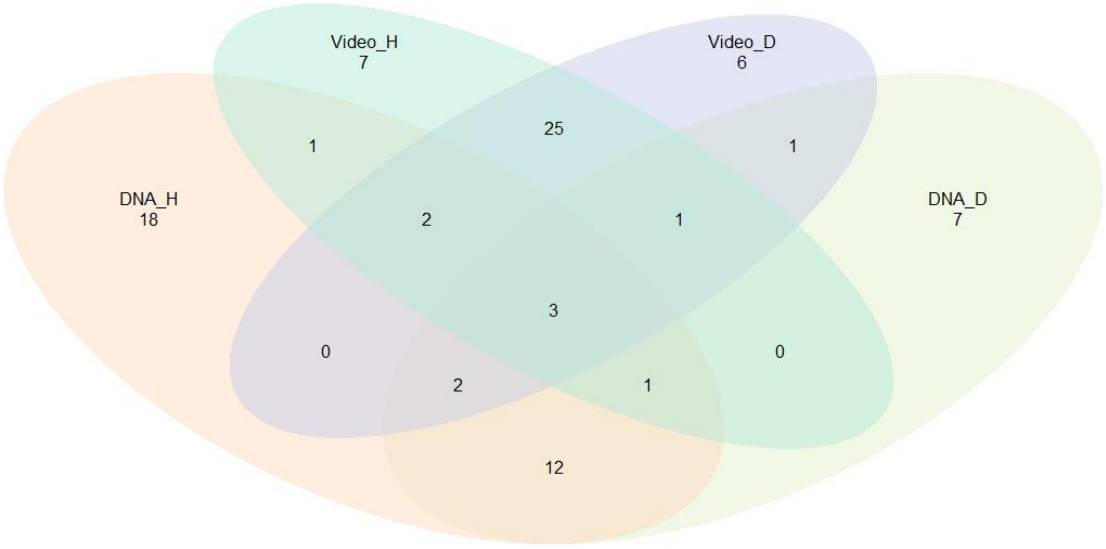

Figure 3-8: Venn diagram showing unique and overlapping taxa per analysis (video and DNA) and impact (D) and reference (H) sites.

4 Discussion and conclusions

In general we consider this T-0 campaign as a succesfull campaign. The Oysters were deployed successfully in the Oyster cages and the ecological monitoring was executed as planned. It will not be possible at this time to answer any of the research questions in this stage of the project, because more data is needed first to assess effects. In this chapter we will discuss and evaluate the used monitoring techniques.

4.1 Oysters

The size and quality of the Flat Oysters indicate that there is a proper set-up created for the pilot study. The size of the Oysters was very similar in the T-0 campaign. Measuring the size of the Oysters in the future field campaigns should result in a clear indication for growth and size. Also the quality of the Oysters seemed very high before deployment. Although the Oysters were kept in the Aqualife tanks much longer than anticipated, the mortality of the Oysters was very low.

4.2 Photo/Video analysis

The use of photos from the seabed as an indicator for the different treatments seem to be working as planned. It is possible to quantitatively analyse the photos and to compare different treatments and results. There were some interesting first results identified in the photo/video analysis.

An average of 16 species per location were found in the image analysis. However from the additional ROV video analysis about 30% extra species were identified. Also these are mostly fish species, which are less easily scored in the photos. The addition of the analysis of the video therefore can help to get a better indication of the total biodiversity in the area. It is therefore recommended that the ROV images are used for the quantitative analysis of the treatments and to perform the statistical analysis. The video can be used to obtain extra species in order to compare the separate areas and/or monopiles and perform a general biodiversity assessment.

There was a distinct difference between the results of the seabed photos and the photos from the monopiles. The benthic community on the monopiles have a different composition compared to the seabed.

There was not a distinct difference between the filter layer and the coarse layer. However the samples with sandy seabed and (almost) no fauna present in the photo were separated clearly in the analysis. The benthic fauna that can be observed with a camera is distinctly different on a sandy seabed compared to the scour protection.

All samples had a very high similarity. The reason for this high similarity can be that Borssele 3/4 is a very recent wind farm. So it is assumed that there is a development in the biodiversity going on. There is an indication that there is a difference between Borssele 3 (D locations) and 4 (H locations). The benthic community in Borssele 3 is slightly different form the Borssele 4 locations. The reason for this is not known, but it is likely that the conditions between the locations slightly differ. Since Borssele 4 serves as a reference for the Nature Inclusive Design plan, it is important to take this difference into account.

In the photo analysis we used a presence/absence analysis in order to compare all the different species from the analysis by the different image analysts that worked on analyzing the images. In the next years a comparison must be made using the different SACFOR analyses that

were done by the analysts. When using this methodology, it is very important that the analysis have a very high level of tuning the analysis, because this has the risk that it can be quite subjective. Therefore it is advised to have more tuning between analysts in the analysis of the T-1 images in 2021. Also it is very important that the same software package is used, so data from different analysts can be added together and analyzed as one dataset.

4.3 eDNA

The DNA-analysis proved to be a useful tool to indicate taxa difficult or impossible to see, and also point at the presence of species not yet spotted on video. While the comparison of results from video- and DNA-analysis indicated some overlap, both types of analysis largely accounted for unique occurrences of taxa, showing the potential of complementarity of both and the applicability of results in species composition analysis. Because of the complementarity of techniques, the combination of techniques increases the likelyhood that the species list is representative of what is actually present on site. Using a combined species list in data-analysis in the future will thereby increase the likelyhood natural variation and succession can be discerned from the effect of measures (installation of oysters).

Some issues remain unsolved, however. While the water-samples used for DNA-analysis are naturally suited to find pelagic species, some pelagic species were found in the video, but not in the eDNA-samples. Vice versa, most benthic species seen in video were not found in the eDNA-sample, while some cryptic (endo-)benthic species (of which it is unexpected to find eDNA) were found in DNA-analysis. Why some species are, and some species are not spotted using an eDNA-sample remains illusive, and may depend on species-specific characteristics (soft-bodied vs chitine-shelled), circumstance (reproduction for instance) or current direction and luck. Hopefully these issues will be resolved in due time, by using the technique more often.

5 Literature

Blauwwind, 2019, Wind Farm Borssele III & IV Nature Inclusive Design Plan, wind farm site decision regulatory basis 2.15, version 09

Blauwwind, 2020, BLAUWWIND – DRN – TENNET BORSSELE WIND FARM SITE III & SITE IV Nature Inclusive Design Scope of Work – Science Execution of Nature Inclusive Design Plan, rev. 0

Eurofins AquaSense, 2020, Work Method Statement Eurofins for scientific operations Eurofins in OWP Borssele 3/4, October 2020 for the Rich North Sea (De Rijke Noordzee)., J00002898_WMSv01

Rijkswaterstaat, 2020, Rijkswaterstaat Voorschrift, Analyse macrozoöbenthos, EUNIS habitat en antropogene materialen met behulp van een onderwatervideosysteem, code 913.00.B090, versie 1.

6 Appendices

Appendix 1: Collection of pictures of each oyster-cohort


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Appendix 2: Collection of pictures giving an overview of the oyster tables with baskets attached

D04

D05

D06

D07

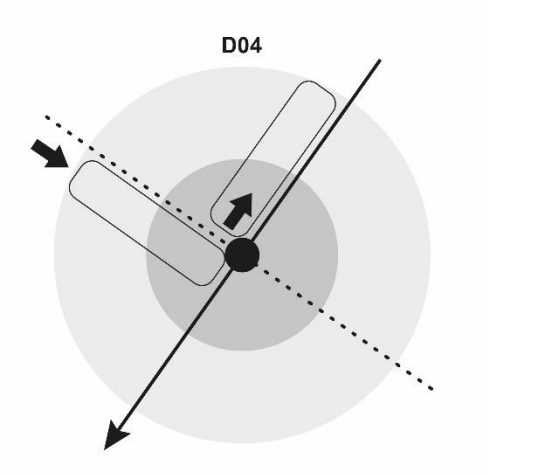
Appendix 3: Overview of ROV transects per monopile

ROV pilots:

- Team 1: pilots Stefano and Paul
- Team 2: pilots Sjoed and Nick

B334-D04:

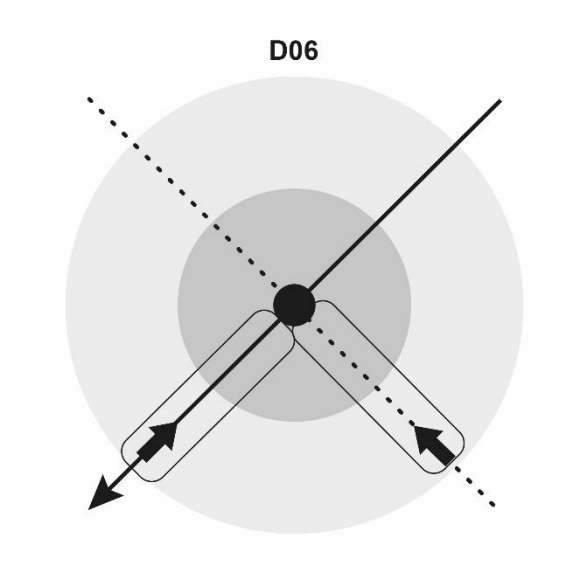
Date: 12/10/2020, 17:40 – 18:20
Notes: Hendrik Gheerardyn
ROV-team: Team 1
Current direction: 235°, NO to ZW
Route ROV: ROV starts from outside the scour protection, perpendicular to the current direction (series of photos PC-FG and CG); at the monopile, the ROV flies away from the monopile against the current direction, at the same time photos are taken (series of photos CD-CG and FG).



Chronologic order of photos (with distance to the monopile):
PC-FG-01-D04 (22m)
PC-FG-02-D04 (20m)
PC-FG-03-D04 (18m)
PC-FG-04-D04 (16m)
PC-FG-05-D04 (14m)
PC-CG-01-D04 (10m)
PC-CG-02-D04 (8m)
PC-CG-03-D04 (6m)
PC-CG-04-D04 (4m)
PC-CG-05-D04 (2m)
Monopile (Photo of the monopile-base)
CD-CG-01-D04 (just next to the monopile)
CD-CG-02-D04 (2m)
CD-CG-03-D04 (4m)
CD-CG-04-D04 (6m)
CD-CG-05-D04 (8m)
CD-FG-01-D04 (12m)
CD-FG-02-D04 (*14m*)
CD-FG-03-D04 (*16m*)
CD-FG-04-D04 (*18m*)
CD-FG-05-D04 (*20m*)

B328-D06:

Date: 12/10/2020, 21:30 - 22:00
Notes: Anke Engelberts
ROV-team: Team 2
Current direction: NO to ZW
Route ROV: ROV starts from outside the scour protection, perpendicular to the Current direction (series of photos PC-FG and CG); then the ROV flies away from the monopile to fly again from outside the scour protection to the monopile and take pictures against Current direction (series of pictures CD-FG and CG).

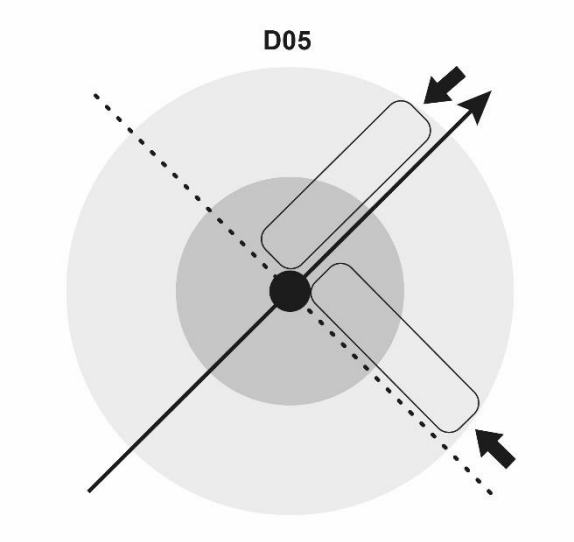


Chronologic order of photos (with distance to the monopile):
CD-FG-01-D06 (21m)
CD-FG-02-D06 (18m)
CD-FG-03-D06 (17m)
CD-FG-04-D06 (14m)
CD-FG-05-D06 (12m)
CD-CG-01-D06 (9m)
CD-CG-02-D06 (7m)
CD-CG-03-D06 (3m)
CD-CG-04-D06 (3,5m)
CD-CG-05-D06 (0m)
PC-FG-01-D06 (20m)
PC-FG-02-D06 (17,5m)
PC-FG-03-D06 (16m)
PC-FG-04-D06 (14,5m)
PC-FG-05-D06 (12m)
PC-CG-01-D06 (9m)
PC-CG-02-D06 (7,5m)
PC-CG-03-D06 (5m)
PC-CG-04-D06 (3m)
PC-CG-05-D06 (2m)

Extra observation: 22:02: Squid

B337-D05:

Date: 13/10/2020, 03:41 - 04:17
Notes: Anke Engelberts
ROV-team: Team 2
Current direction: ZW to NO
Depth: 30m
Route ROV: can be taken from the chronology of the photos

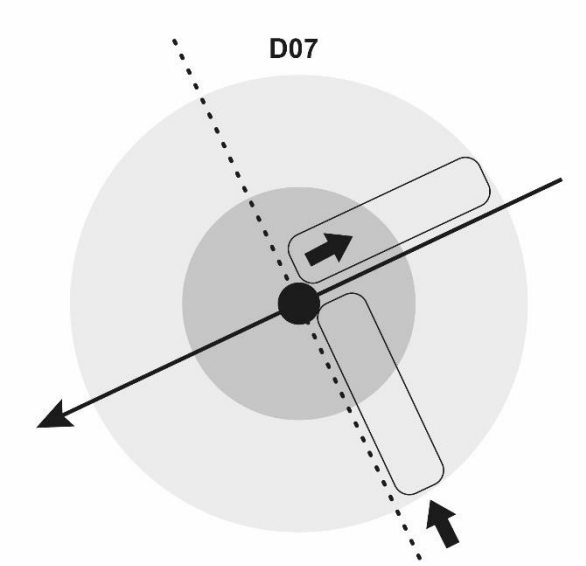


List of photos (with distance to the monopile) (for chronology check files)
PC-FG-01-D05 (17m)
PC-FG-02-D05 (14m)
PC-FG-03-D05 (13m)
PC-FG-04-D05 (12m)
PC-FG-05-D05 (9m)
PC-CG-01-D05 (7m)
PC-CG-02-D05 (4m)
PC-CG-03-D05 (4m)
PC-CG-04-D05 (4m)
PC-CG-05-D05 (1m)
CD-FG-01-D05 (22m)
CD-FG-02-D05 (20m)
CD-FG-03-D05 (19m)
CD-FG-04-D05 (15m)
CD-FG-05-D05 (11m)
CD-CG-01-D05 (10m)
CD-CG-02-D05 (7,5m)
CD-CG-03-D05 (5m)
CD-CG-04-D05 (3m)
CD-CG-05-D05 (1m)
Pile: Photo of the monopile-base

Extra observation: 03:55: sepia

B327-D07:

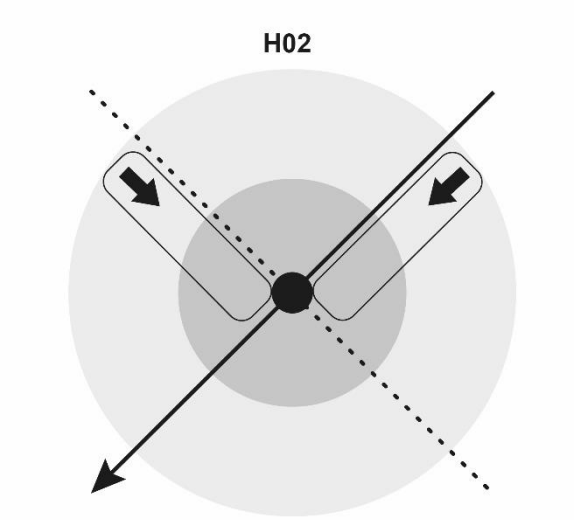
Date: 13/10/2020, start rond 14:45
Notes: Hendrik Gheerardyn
ROV-team: Team 1
Current direction: 205°, NO to ZW
Route ROV: ROV starts from outside the scour protection, perpendicular to the current direction (series of photos PC-FG and CG); at the monopile, the ROV flies away from the monopile against the current direction, at the same time photos are taken (series of photos CD-CG and FG).



Chronologic order of photos (with distance to the monopile):
CD-FG-01-D07 (20m)
CD-FG-02-D07 (18m)
CD-FG-03-D07 (16m)
CD-FG-04-D07 (14m)
CD-FG-05-D07 (12m)
CD-CG-01-D07 (10m)
CD-CG-02-D07 (8m)
CD-CG-03-D07 (6m)
CD-CG-04-D07 (4m)
CD-CG-05-D07 (2m)
PC-CG-01-D07 (2m)
PC-CG-01-1-D07 (extra)
PC-CG-02-D07 (4m)
PC-CG-02-2-D07 (detail)
PC-CG-03-D07 (6m)
PC-CG-04-D07 (8m)
PC-CG-04-1-D07 (8m) (extra)
PC-CG-05-D07 (10m)
PC-FG-01-D07 (12m)
PC-FG-02-D07 (14m)
PC-FG-03-D07 (16m)
PC-FG-04-D07 (18m) (already outside FG)
PC-FG-05-D07 (20m)
PC-FG-05-1-D07 (extra, close-up of Corophium?)

B411-H02:

Date: 14/10/2020, 22:20 – 22:45
Notes: Anke Engelberts
ROV-team: Team 2
Current direction: NO to ZW
Diepte: 30m
Route ROV: can be taken from the chronology of the photos

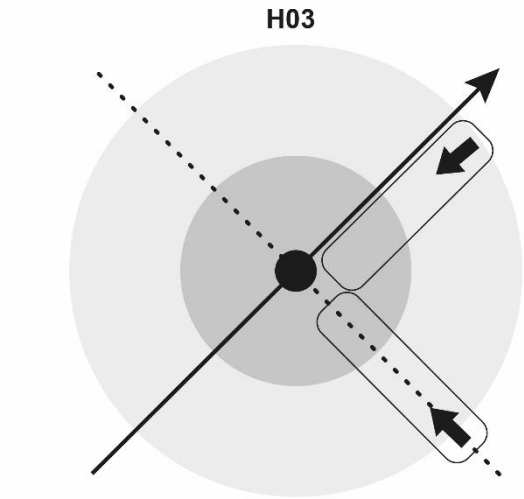


List of photos (with distance to the monopile) (for chronology check files)
CD-FG-01-H02 (18m)
CD-FG-02-H02 (16m)
CD-FG-03-H02 (14m)
CD-FG-04- H02 (10m)
CD-FG-05- H02 (10m)
CD-CG-01- H02 (8m)
CD-CG-02- H02 (6m)
CD-CG-03- H02 (4m)
CD-CG-04- H02 (2m)
CD-CG-05- H02 (2m)
PC-FG-01-H02 (18m)
PC-FG-02-H02 (16m)
PC-FG-03-H02 (15m)
PC-FG-04-H02 (14m)
PC-FG-05-H02 (11m)
PC-CG-01-H02 (9m)
PC-CG-02-H02 (7m) (observation: sea squirt)
PC-CG-03-H02 (5m)
PC-CG-04-H02 (3m)
PC-CG-05-H02 (9m) (9m is writingerror?)
Pile: photo of monopile-base

Additional observation: Mnemiopsis
Observation: Sand on filter layer
Note: photos against current were, according to the notes, taken with current. Maybe a writing error, but the Current direction still rotates 180 degrees at every turn.

B411-H03:

Date: 14/10/2020, 04:10 - 04:35
Notes: Anke Engelberts
ROV-team: Team 2
Current direction: ZW to NO
Diepte: 29m
Route ROV: can be taken from the chronology of the photos

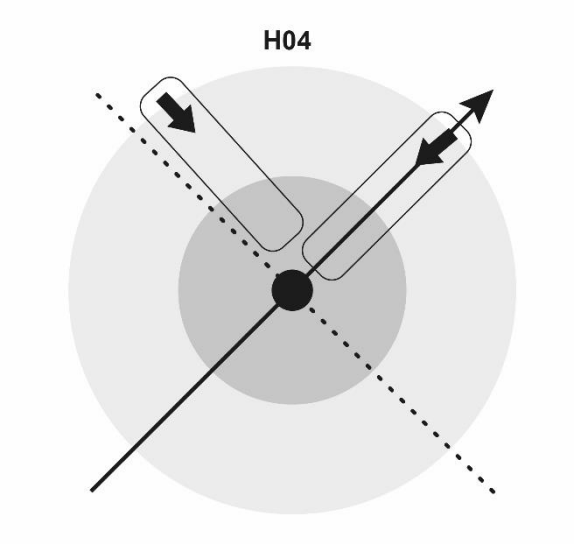


List of photos (with distance to the monopile) (for chronology check files)
CD-FG-01-H03 (20m)
CD-FG-02-H03 (17,5m)
CD-FG-03-H03 (15m)
CD-FG-04- H03 (13m)
CD-FG-05- H03 (11m)
CD-CG-01- H03 (8m)
CD-CG-02- H03 (7,5m)
CD-CG-03- H03 (5m)
CD-CG-04- H03 (3m)
CD-CG-05- H03 (2m)
PC-FG-01-H03 (19m)
PC-FG-02-H03 (17m)
PC-FG-03-H03 (15m)
PC-FG-04-H03 (13m)
PC-FG-05-H03 (12m)
PC-CG-01-H03 (9m)
PC-CG-02-H03 (6m)
PC-CG-03-H03 (5m)
PC-CG-04-H03 (4m)
PC-CG-05-H03 (1m)
Pile: photo of monopile-base

Additional observation: 4:24: Sepia
observation: Sand on filter layer

B402-H04:

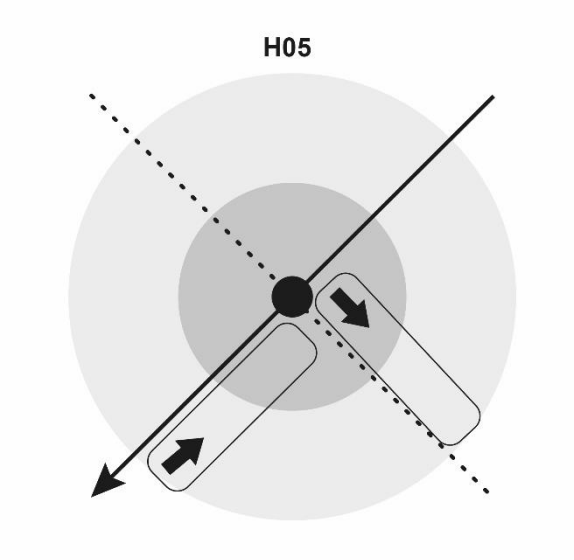
Date: 16/10/2020, 06:17 - 06:46
Notes: Anke Engelberts
ROV-team: Team 2
Current direction: ZW to NO
Diepte: 33m
Route ROV: can be taken from the chronology of the photos



List of photos (with distance to the monopile) (for chronology check files)
CD-FG-01-H04 (25m)
CD-FG-01b-H04 (20m)
CD-FG-02-H04 (18m)
CD-FG-03-H04 (16m)
CD-FG-04- H04 (15m)
CD-FG-05- H04 (14m)
CD-CG-01- H04 (10m)
CD-CG-02- H04 (7m)
CD-CG-03- H04 (5m)
CD-CG-04- H04 (4m)
CD-CG-05- H04 (2m)
PC-FG-01-H04 (18m)
PC-FG-02-H04 (16m)
PC-FG-03-H04 (15m)
PC-FG-04-H04 (14m)
PC-FG-05-H04 (12m)
PC-CG-01-H04 (9m)
PC-CG-02-H04 (8m)
PC-CG-03-H04 (6m)
PC-CG-04-H04 (5m)
PC-CG-05-H04 (2m)
Pile: photo of monopile-base
Extra observation: Spidercrab

B401-H05:

Date: 16/10/2020, 12:12 - 12:27
Notes: Anke Engelberts
ROV-team: Team 1
Current direction: NO to ZW
Depth: 33m
Route ROV: Start against Current direction (CD: photos of successively FG and CG), then perpendicular to Current direction (from pole to edge of scour protection; photos successively of CG and FG)



Chronologic order of photos (with distance to the monopile)
CD-FG-01-H05 (18m)
CD-FG-02-H05 (16m)
CD-FG-03-H05 (12m)
CD-FG-04- H05 (10m)
CD-FG-05- H05 (9m)
CD-CG-01- H05 (6m)
CD-CG-02- H05 (6m)
CD-CG-03- H05 (4m)
CD-CG-04- H05 (2m)
CD-CG-05- H05 (1m)
Pile: photo of monopile-base
PC-CG-01-H05 (1m)
PC-CG-02-H05 (2m)
PC-CG-03-H05 (4m)
PC-CG-04-H05 (5m)
PC-CG-05-H05 (6m)
PC-FG-01-H05 (8m)
PC-FG-02-H05 (10m)
PC-FG-03-H05 (12m)
PC-FG-04-H05 (15m)
PC-FG-05-H05 (18m)
Extra observation: 12:25: Spidercrab

Appendix 4: Species list for ROV Photo and video analysis

Scientific Name	Phylum	Class	Order	Remark
Actinopterygii	Chordata	Actinopteri		
Actinothoe sphyrodeta	Cnidaria	Anthozoa	Actiniaria	
Ascidiacea	Chordata	Ascidiacea		
Asterias rubens	Echinodermata	Asteroidea	Forcipulatida	
Balanidae	Arthropoda	Thecostraca	Balanomorpha	
Bryozoa	Bryozoa			
Callionymus	Chordata	Actinopterygii	Perciformes	
Callionymus lyra	Chordata	Actinopterygii	Perciformes	
Callionymus reticulatus	Chordata	Actinopterygii	Perciformes	
Cancer pagurus	Arthropoda	Malacostraca	Decapoda	
Cerianthus lloydii	Cnidaria	Anthozoa	Spirularia	
Cottidae	Chordata	Actinopterygii	Scorpaeniformes	
Dicentrarchus labrax	Chordata	Actinopterygii	Perciformes	
Diplosoma listerianum	Chordata	Ascidiacea	Aplousobranchia	
Echinocardium cordatum	Echinodermata	Echinoidea	Spatangoida	
Ectopleura larynx	Cnidaria	Hydrozoa	Anthoathecata	
Taurulus bubalis	Chordata	Actinopterygii	Scorpaeniformes	
Gadidae	Chordata	Actinopterygii	Gadiformes	
Gadus morhua	Chordata	Actinopterygii	Gadiformes	
Gobiidae	Chordata	Actinopterygii	Perciformes	
Gobius niger	Chordata	Actinopterygii	Perciformes	
Halichondria (Halichondria) panicea	Porifera	Demospongiae	Suberitida	
Homarus gammarus	Arthropoda	Malacostraca	Decapoda	
Hydrozoa	Cnidaria	Hydrozoa		
Inachidae	Arthropoda	Malacostraca	Decapoda	
Jassa falcata	Arthropoda	Malacostraca	Amphipoda	
Jassa herdmani	Arthropoda	Malacostraca	Amphipoda	
Lanice conchilega	Annelida	Polychaeta	Terebellida	
Liocarcinus	Arthropoda	Malacostraca	Decapoda	
Liocarcinus holsatus	Arthropoda	Malacostraca	Decapoda	
Loligidae				Not accepted by WORMS
Loligo	Mollusca	Cephalopoda	Myopsida	
Loligo vulgaris	Mollusca	Cephalopoda	Myopsida	
Macropodia rostrata	Arthropoda	Malacostraca	Decapoda	
Maja brachydactyla	Arthropoda	Malacostraca	Decapoda	
Malacostraca	Arthropoda	Malacostraca		
Metridium senile	Cnidaria	Anthozoa	Actiniaria	
Microstomus kitt	Chordata	Actinopterygii	Pleuronectiformes	
Mnemiopsis leidyi	Ctenophora	Tentaculata	Lobata	
Mullus surmuletus	Chordata	Actinopterygii	Perciformes	
Myoxocephalus scorpius	Chordata	Actinopterygii	Scorpaeniformes	
Mytilus edulis	Mollusca	Bivalvia	Mytilida	
Necora puber	Arthropoda	Malacostraca	Decapoda	
Nemertesia	Cnidaria	Hydrozoa	Leptothecata	
Nemertesia antennina	Cnidaria	Hydrozoa	Leptothecata	

Scientific Name	Phylum	Class	Order	Remark
Obelia bidentata	Cnidaria	Hydrozoa	Leptothecata	
Ophiura	Echinodermata	Ophiuroidea	Ophiurida	
Ophiura albida	Echinodermata	Ophiuroidea	Ophiurida	
Ophiura albida	Echinodermata	Ophiuroidea	Ophiurida	
Ostrea edulis	Mollusca	Bivalvia	Ostreida	
Pagurus bernhardus	Arthropoda	Malacostraca	Decapoda	
Parablennius gattorugine	Chordata	Actinopterygii	Perciformes	
Parablennius gattorugine gattorugine				Not accepted by WORMS
Pleuronectiformes	Chordata	Actinopterygii	Pleuronectiformes	
Spirobranchus triqueter	Annelida	Polychaeta	Sabellida	
Pomatoschistus	Chordata	Actinopterygii	Perciformes	
Pomatoschistus minutus	Chordata	Actinopterygii	Perciformes	
Porifera	Porifera			
Psammechinus miliaris	Echinodermata	Echinoidea	Camarodonta	
Cylista elegans	Cnidaria	Anthozoa	Actiniaria	
Cereus pedunculatus	Cnidaria	Anthozoa	Actiniaria	
Cylista undata	Cnidaria	Anthozoa	Actiniaria	
Sepia officinalis	Mollusca	Cephalopoda	Sepiida	
Sparus aurata	Chordata	Actinopterygii	Perciformes	
Taurulus bubalis	Chordata	Actinopterygii	Scorpaeniformes	
Taurulus/Myoxocephalus				Not accepted by WORMS
Taurulus/Myoxocephalus/Parablennius				Not accepted by WORMS
Trachurus trachurus	Chordata	Actinopterygii	Perciformes	
Trisopterus	Chordata	Actinopterygii	Gadiformes	
Trisopterus luscus	Chordata	Actinopterygii	Gadiformes	
Trisopterus minutus	Chordata	Actinopterygii	Gadiformes	
Tubularia indivisa	Cnidaria	Hydrozoa	Anthoathecata	
Urticina felina	Cnidaria	Anthozoa	Actiniaria	