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Monitoring of the nature enhancement project in offshore wind farm Borssele III/IV, T3 2023

Scientific report

The Rich North Sea

Amsterdam, February 2024

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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 therefore explicitly included in the site decisions for developers. This enables them to contribute to the strengthening of nature, and the preservation and sustainable use of species and habitats that originally existed in the Netherlands (Blauwwind, 2019).

Blauwwind is currently operating the Borssele III and IV wind farm on the southern edge of the Dutch exclusive economic zone. Blauwwind developed its vision on how to design and construct the Borssele III and IV wind farm in such a manner that it matches the vision mentioned above, with the goal of contributing to a strong, healthy, and biodiverse North Sea (Blauwwind, 2019). In partnership with The Rich North Sea programme, a nature enhancement project is carried out and monitored.

In October 2020, biodiversity was monitored around eight wind turbines in the Borssele wind farm, site III and IV (T0). In addition, living flat oysters were installed at the scour protection of four wind turbines in site III. In July 2021, a second monitoring campaign (T1) was carried out and the scour protection of the eight wind turbines, consisting of different sizes of rock, was also partly covered with shell material to create settlement places for flat oysters. The eight wind turbine locations in sites III and IV were studied again in 2023 (T3) and are scheduled to be monitored and decommissioned in 2028. The scope of this monitoring entails measuring growth of the oysters, taking water samples for larvae and eDNA analysis, oyster samples from the baskets (for reproduction and Bonamia status), and a combined video and photo survey of the scour protection with an ROV.

Research questions which could (partly) be answered in the T3 are:

1) What is the survival and growth rate of flat oysters?
2) Do the flat oysters stay free of the parasite Bonamia?
3) Do the flat oysters produce larvae?
4) Do oyster larvae settle on different scour protection substrates (armour layer, filter layer and empty shells)?
    - Do they have a preference for any substrate type?
    - Can the oyster spat and/or larvae also be found in the control zone?
5) How is the general biodiversity developing on the different substrate variations?
6) Can patterns be found in oyster larvae settlement or species-specific responses in relation to the prevailing current?
7) Is the species data found by ROV (remotely operated vehicle) video and photo analyses correlated to the eDNA data? Which species are not found or only found by a certain method?
8) How fast does succession of a hard substrate community occur in the Borssele area?

The current report will describe the T3 monitoring in August 2023 in terms of a description of the observed site and a comparison with the T0 and T1 data (Schellekens et al., 2021; 2022). Methods are described in more detail in the next chapter, followed by the results and a discussion.

2 Material and methods

2.1 Location and project design

In Figure 1 an overview is given of the sampling sites in the Borssele wind farm. A total of eight monopiles and their surrounding scour protection area have been designated for the study. In October 2020, oyster tables were placed at the scour protection of each of the four selected monopiles in Borssele III. Each table contained 8 oyster baskets with 75 living adult oysters. Together with the installation, a first biodiversity analysis was performed using ROV photos, videos and eDNA techniques. Since there were no eDNA results in the T1, extra samples were taken at the monopiles where the oysters were installed in September 2022. Oyster larvae and eDNA were sampled earlier than the rest of the T3 in order to coincide with (the end of) the oyster larvae period. The two main campaigns (T1 and T3) were executed in July 2021 and August 2023, where the status of the oysters (survival, growth, reproduction status and Bonamia status), as well as the surrounding biodiversity was investigated. An overview of the different measurements performed per monopile is provided in Table 1.

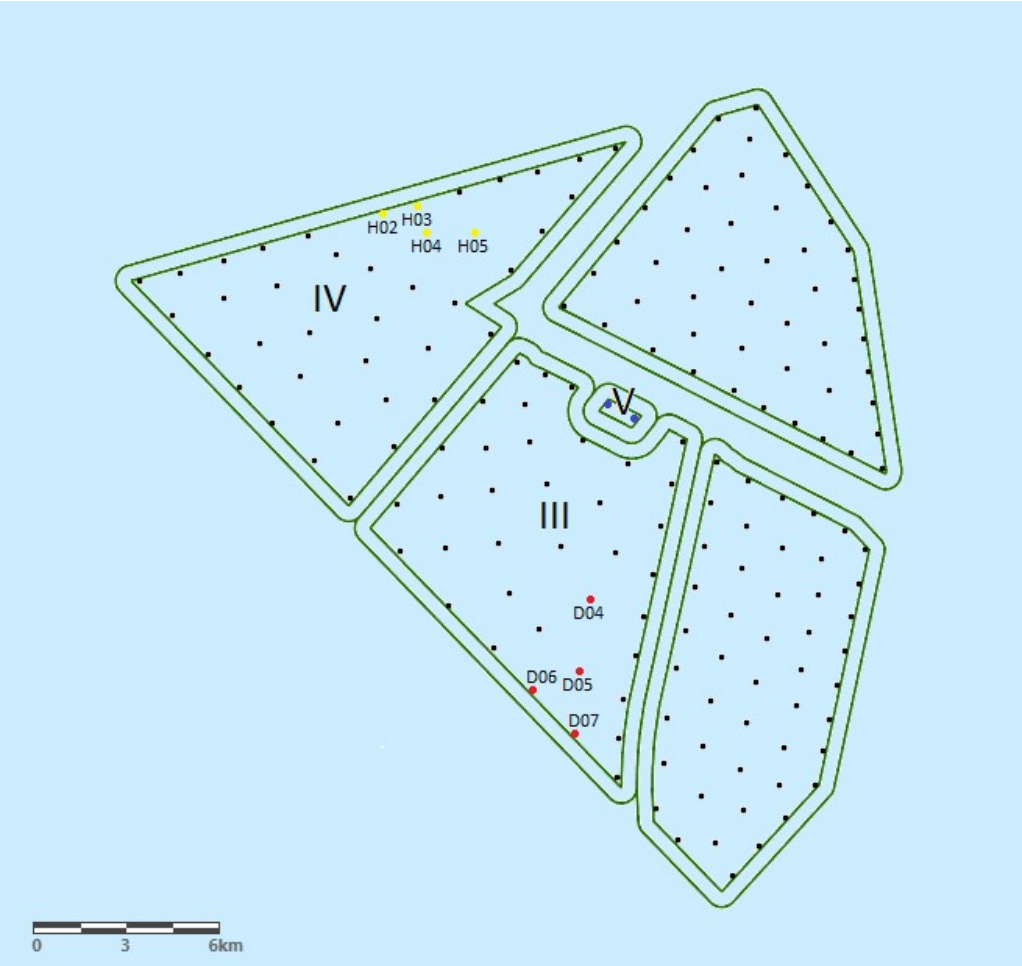

Figure 1. Overview of the Borssele offshore wind farm. Red dots indicate the locations of the oyster installation (north to south: B334-D04, B337-D05, B328-D06 and B327-D07), the yellow dots are the reference locations (west to east: B412-H02, B411-H03, B402-H04 and B401-H05). The blue dots indicate the locations of another oyster pilot in Borssele V.

Table 1. Overview of the activities performed since T1.

Period	Sample locations	ROV	Watersamples	Oyster measurements	Spat collectors
Sep 2022	4 at D-string		3x 1.5L for eDNA using Niskin		
July 2023	4 at D-string and 4 at H-string		2x 1.5L for eDNA using pump, 500L for larvae		
Aug 2023	4 at D-string	Video of oyster table and scour, 4*5 photos + 1 monopile	500 L for larvae at D07	Per basket: 25 oysters and 1 oyster sampled	All collectors removed (6 per table: 3 with oyster shells, 3 with mixed shells)
	4 at H-string	Video of scour, 4*5 photos + 1 monopile	500 L for larvae at H03 and H05		

2.2 ROV surveys

The scour protection around each monopile was divided into four sections. The first section is between leeward and windward side. The main current direction was always set to SW-NE or NE-SW, turning 180 degrees in between. The other category is 'perpendicular to the current'. The second division was made between coarse and filter grade scour. The coarse grade scour is located closest to the monopile (Figure 2). A finer grade is visually present at a further distance, up to 25 m from the monopile, but is also present below the coarser layer.

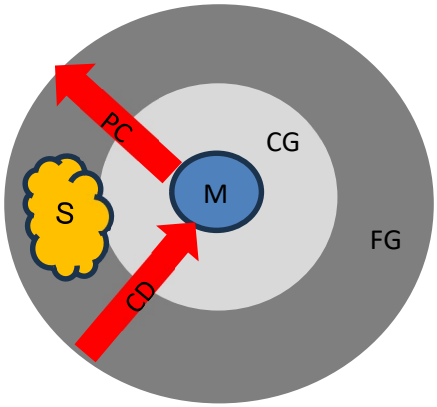

Figure 2. Positioning of coarse (CG) and filter (FG) gradings around the monopiles (M). The direction of the photo tracks in the current direction (CD) and perpendicular to the current (PC), as well as the shell (S) area are highlighted. These directions are different for all the foundations.

For the ROV survey, a Saab Seaeye Panther XT with a 4K video camera was operated by Bluestream (Figure 3). Two green subsea laser lines with a fixed distance of 42 cm were used during the video and photo survey as a scale, and was also used in the analysis to define a consistent surface area. The snail trail function was activated to see and track the live position of the ROV relative to the monopile, scour protection, vessel and tether management system. For each monopile, the two tracks (in the current direction and perpendicular) nearest to the shell area (this varied per monopile) were determined beforehand.

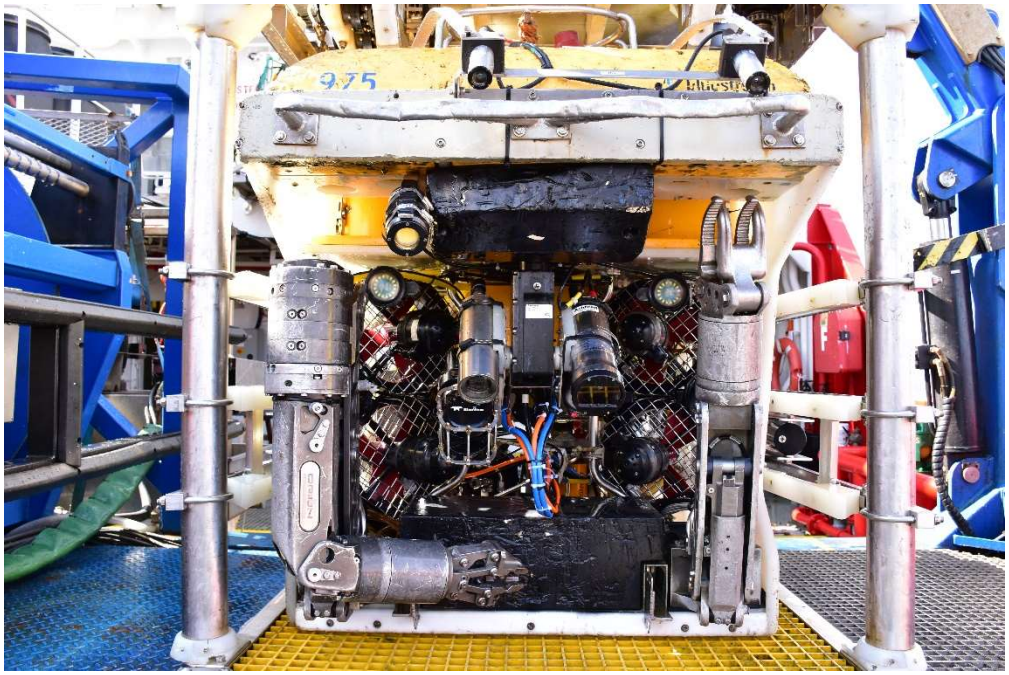

Figure 3. ROV setup with lights and lasers during T3.

The following steps were taken during the surveys:

1) After deployment of the ROV, the video was started. Using the navigational system and live knowledge about the current direction and strength, the ROV was moved to the outside of the scour protection, facing the monopile and current.
2) While getting closer to the monopile, the ROV landed on the scour protection aproximately every other meter to take photos (see next steps), as randomly as possible. The first five on the filter layer, the second five on the armour layer. The difference between these were theoretically visible on the navigational system and was visually confirmed with camera footage. After ten photos, the base of the monopile was photographed and the ROV turned 90 degrees alongside the monopile to face another direction while flying outwards and taking the other ten photos.
3) After landing and waiting until disturbed sediment had settled, the actual photo was taken with the 4K camera. It was possible to do this without having to stop the video. It was made sure that the photos did not have any overlap in the covered area. The tilt and focus of the camera were adjusted where needed.
4) Each photo was checked for clarity and focus; such that individual fauna can be identified from the photos.
5) After taking the photo, the following code was given to each photo: Monopile nr. – Current direction – Rock size – Photo number.
6) After the photo survey, a video survey took place up until the hour of net survey time was over or the currents became too strong to safely manoeuvre the ROV. During the video survey, it was the intention to cover the other sides of the monopile and scour protection that were not visible in the photo survey. Additionally, the shell area was further investigated with regards to the dispersal of shells /covered area, if this was not completely finished during the photo survey.

2.3 Oyster larvae and eDNA water sampling

Extra eDNA water samples were taken at the D-string monopiles in September 2022 (Table 2), as the samples from the previous (T1) campaign did not show results. There was an opportunity to combine this sampling with another project nearby, and so unfortunately there were

no H-string samples taken. This sampling was done using a 5L Niskin sampler, where 3 samples of 2.5L were taken per monopile.

In July 2023, water samples for oyster larvae count and eDNA analysis were taken at each of the eight monopiles in a separate CTV campaign (Table 2), as the peak in oyster larval production is in July and therefore sampling in this month will allow a more accurate snapshot of oyster larval production. The CTV was moored to the monopile with engines off during sampling, and the position of the CTV allowed all samples to be taken downstream of the monopile. Water was pumped from approximately 2m above the scour protection and within 25m of the monopile using a small weighted submersible pump, through a 100 µm (phytoplankton) net in a 100 L tub. This tub was emptied between sampling. In total, 500L of water was used for one larvae sample. The material that ended in a detachable part of the net was collected in a 200 ml jar using 96% ethanol. One larvae sample per monopile was taken. Two times 2.5L of water for eDNA purposes were directly sampled in two clean jars using the same pump. At sample D04 it appears that the sample was taken too high in the water column for comparable results to be obtained, possibly related to current strength or insufficient weighting on the pump.

During the ROV campaign in August 2023, extra water samples were taken where possible in order to record any larva that may be released later in the breeding season, as the exact length of time oyster larva are present in the water column is uncertain. This was done using a larger pump and an IBC-tote of 600 liters. The tote was filled and flushed with water from the location and at the same depth of the sample, before taking the actual larvae sample. Markings per 100L interval were made on this tote and samples of 500L were taken. These samples were not always sampled at a specific location around the monopile, but the vessel was on DP near the pile and placed the pump in the water using the crane. Samples were taken 2m above the sediment/scour, which was visually checked by the ROV. A spout was attached to the tap and the IBC-tote was placed on a raised surface, allowing easier tapping of the sample (Figure 4). A hard 125 µm sieve was used to filter the sample. Material collected in the sieve was completely emptied into a 0.5 L sterile bottle using a clean funnel and a rinsing bottle with 96% ethanol. The sample was stored in the freezer (-20°C).

Table 2. Overview of the water samples for eDNA and oyster larvae.

Monopile	Date	Larvae and/or eDNA samples	Method	Number of eDNA samples
D04	21-09-22	eDNA	Niskin	3
D05	21-09-22	eDNA	Niskin	3
D06	21-09-22	eDNA	Niskin	3
D07	21-09-22	eDNA	Niskin	3
H02	26-07-23	Larvae +eDNA	Pump (small)	2
H03	26-07-23	Larvae +eDNA	Pump (small)	2
H04	26-07-23	Larvae +eDNA	Pump (small)	2
H05	26-07-23	Larvae +eDNA	Pump (small)	2
D04	26-07-23	Larvae +eDNA	Pump (small)	2
D05	26-07-23	Larvae +eDNA	Pump (small)	2
D06	26-07-23	Larvae +eDNA	Pump (small)	2
D07	26-07-23	Larvae +eDNA	Pump (small)	2
D07	27-08-23	Larvae	Pump (large)	-
H05	28-08-23	Larvae	Pump (large)	-
H03	28-08-23	Larvae	Pump (large)	-


PUMPS
PUMPS

Figure 4. Photos of the (a) pump installation and IBC totes, (b) large pump, (c) sieve used in August '23, (d) phytoplankton net emptying using 96% ethanol.

2.3.1 eDNA sample processing

Bottles, tweezers, and other filtering equipment was cleaned beforehand with 2.5% bleach solution and fresh water. Unpowdered latex gloves were used throughout the sampling process. The eDNA-sample was collected on a sterile cup with a 0.2 µm filter by passing the water sample through a funnel, via the filter, into a sterile 2 L vacuum flask. A vacuum was created by an electric pump enabling a faster passage of the water through the filter. At each location, 1.5 L of water was filtered. After this step, the filter membrane containing environmental DNA was folded in half four times and placed in a 2 ml Eppendorf tube using tweezers. This tube was prefilled with 400 µL Zymo DNA/RNA Shield, which prevents DNA degradation. All vials were labelled with the name of their corresponding monopile location and date and stored in a freezer at -20°C.

2.4 eDNA analysis

Preserved samples were analysed by DNA metabarcoding in the lab at Marine Animal Ecology, Wageningen University. DNA was isolated and fish/vertebrate specific barcode fragments were amplified using PCR with the Mifish 12s primers. PCR was performed in triplicate to reduce stochastic effects in amplification. PCR produces were sequenced using the Oxford Nanopore MinION sequencer. Raw sequence reads were processed using the Decona analysis pipeline, which includes quality control, clustering and polishing to create consensus sequences, and subsequent taxonomic identification using a blast search against the Genbank genetic database, followed by post-processing to create an overview of detected species. This resulted in a species list, with numbers of 'reads' per species. More details on the method can be found in Doorenspleet *et al.* (2023).

All eDNA samples have been tested, in duplicate, for the presence of flat oysters. Specific *Ostrea edulis* primersets, developed by the Marine Animal Ecology lab, but not yet described elsewhere in literature, were used for this. In 2022, a 600 bp primer was used, while a newer, 250 bp, primer was used in the T3. These primers were recently developed especially for this type of nature enhancement projects and have extensively been validated.

2.5 Oyster measurements and sampling

Before recovering, the condition of the oyster table was checked with the ROV and a short video was recorded. The oyster tables were lifted onto the deck, after which photos were taken of the entire oyster table, spat collectors and all oysters from each basket. Before placing the oyster table back on the scour protection, photos were taken showing the placement of the oyster baskets and spat collectors. The total time on deck of the oyster tables was a minimum of 3 hours and 57 minutes and maximum of 6 hours and 20 minutes.

From each oyster table, the following samples were collected:

- Per oyster basket: one living oyster for research of reproductive status and Bonamia-infection, collected in a ziplock bag and stored in the freezer.
- Per oyster basket: dead specimens of oysters, collected in a ziplock bag and stored in the freezer. All spat collectors, collected in an onion bag and stored in the freezer.
- Scrape sample (not in the original scope): In order to assess the species composition of the new growth on the tables themselves, a qualitative scraping sample was collected with a putty knife from a variety of surfaces on the oyster tables. These included the top and undersides of the concrete table, the plastic oyster basket, the metal rods etc. during the processing of the oysters, additional samples were able to be taken from the inside of the basket and from between the oysters themselves. These samples were all preserved in 96% ethanol and frozen.

Due to time constraints, 25 live oysters out of 75 oysters per basket were measured using a digital calliper (maximum length and width) to assess their growth since T0, with length measured from the umbo. The number of dead and living oysters per basket were counted before placing the living ones back in the baskets.

2.6 Photo and video analysis

Photo and video analysis was conducted by specialists using the software TransectMeasure (from SeaGIS). The same specialists also worked on this project during the T0 and T1. Using the software, a 'window' was placed in each photo (Figure 5), with the laser lines comprising the vertical boundary, and the horizonal lines later added at a set distance from the edge of the photo. Within the window, all living fauna was identified and scored using the SACFOR scale.

Figure 5: View of an ROV photo in the software, with the area of interest being bordered by the red lines (added in the software) and green lasers. The photo was made at monopile H05 in the T3 with the filter grade and perpendicular to the current.

The SACFOR scale is an internationally recognised 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), which should allow for comparison with other (future). The anagram stands for (S)uperabundant, (A)bundant, (C)ommon, (F)requent, (O)ccasional, (R)are and supeRRare (RR). A specific adaptation of the Rijkswaterstaat protocol (RWSV) to the "standard" SACFOR scale is the omission of scoring individuals. Instead, the cover percentage of each taxon present was used to determine the SACFOR score. In Appendix 6.6 this adapted scale is shown, including relevant taxa in the different SACFOR categories. All present fauna in the photos were analysed by their cover and noted in the TransectMeasure software. The ROV videos were used to supplement photo analysis, as the quality of the video was much higher; more identifying details were visible, and the movement of the animals helped in the identification of the species. Eight photos were identified by both specialists for quality control.

Because the SACFOR score is ordinal categorical data, averages of the score cannot be determined. Therefore, the SACFOR scores were converted to a numerical scale for the purpose of data analysis (e.g. Leewis et al., 2000, Coolen et al., 2015, van der Stap et al., 2016). As, in the current study, only species cover was used and no counting of individuals, the average value of the range of each of the cover %- classes was used as its numerical counterpart (personal communication, Joop Coolen), see Appendix 6.6. These values were assigned to the letters of the SACFOR scale, depending on each "growth form", using the rationale in Strong & Johnson (2020).

Besides the photo analysis, the full length of the ROV videos (taken during the survey, before and after taking photos and lifting the tables) was inspected for additional benthic and pelagic species. Since the videos are not linked to a certain treatment, all these extra observations were reported per monopile. This species list was used in addition to the photo analysis to provide a qualitative overview of the total biodiversity per monopile.

2.7 Larvae, Bonamia, spat and reproduction analyses.

Larvae analysis

Larvae samples were inspected with a dissecting microscope to check for D-larvae. D-larvae are D-shaped larvae occurring as a developmental stage in a limited number of bivalve groups, which include oysters and mussels. D-larvae could not be identified to species level but were send for DNA-analysis to check whether they were flat or Pacific oysters.

Bonamia status

An infection with *Bonamia ostreae* manifests as dark lesions in the tissues of oysters. Oysters were opened with an oyster knife and the inside was inspected for the presence of dark lesions with a dissecting microscope.

Reproduction status

The oysters opened for inspection of Bonamia status were also used to assess the reproduction status. With a dissecting microscope the gonads were checked for specific colouring: clearly milky white (ready to reproduce), somewhat milky white (build-up to reproduction) or not milky white at all (already reproduced or no build-up).

Spat analysis

Spat collectors, consisting of empty oyster shells intended to attract oyster-settlement, were inspected for the presence of oyster-spat. When species identification could not be performed, spat was send for DNA-analysis to check whether they were of flat or Pacific oysters.

2.8 Data analyses

Data collection and preparation was done in Excel. Data-analyses were performed using R (R Core Team, 2023 and Primer 6).

3 Results

3.1 Stability, functioning and biodiversity of the oyster tables

All four oyster tables were recovered upright on the scour protection, therefore the tables were not covered with sediment and none were damaged. The surface of oyster table and baskets were covered with sessile organisms but the baskets themselves still had openings visible (see Figure 6). Species that were seen on the tables and baskets were for instance moss animals, barnacles, anemones, starfish, amphipods, crab, and slipper limpets. In several baskets, North Sea crabs were found.

Figure 6. Hoisting of the oyster table (left) and oyster table on deck, with both (yet unsampled) baskets covered in marine growth and cleaned and newly attached baskets (right).

Scrape samples

At each oyster table, a scrape sample was taken during the T1 and T3. These samples were analyzed in the lab using a binocular microscope. In the T1 and T3 respectively 45 and 57 species or taxa were found in the in total 8 scrape samples taken from the oyster tables (four per measurement year). Both years combined, 77 different species or taxa were found (see appendix 6.2 and Table 3). Crustaceans are most species rich with 18 species, followed by polychaete worms with 12 species. Cnidarians and gastropods are both represented with 9 species.

The oyster tables provide a habitat for several species less common or even rare on the Dutch coast, such as the queen scallop *Aequipecten opercularis*, the smallest saddle oyster *Heteranomia squamula*, the perforated barnacle *Perforatus perforatus* and two ribbed cowry species *Trivia arctica* and *T. monacha*. Several species on the oyster tables had only very rarely been recorded from Dutch coasts, such as the titan acorn barnacle *Megabalanus coccopoma* from the Pacific and the parchment worm *Chaetopterus variopedatus*. The occurrence of the parchment worm in the windfarm coincides with a remarkable increase in the number of parchment worm tubes, sometimes with a living worm, washed ashore on the Dutch coast during the winter 2023-2024. The occurrence of bobtail squid *Sepiola atlantica* eggs indicates that the sampled habitat not only provides a suitable habitat for bobtail squids, but a spawning ground as well. One species in the scrape samples has never been recorded from Dutch waters before; European big-claw snapping prawn *Alpheus macrocheles*. This species is common in the English Channel and seems to have been spreading northeast in recent years. There is only one other record from the North Sea, offshore the mouth of the river Thames in 2007 (DASSH Data Archive Centre – Statutary Surveys), and one record just west of the Strait of Dover in 2014 (Natural England monitoring surveys), while all other records in the English Channel are from the western and central part (GBIF, https://www.gbif.org/occurrence/search?taxon_key=2226097, 09/02/2024).

Table 3. Taxa found in scrape samples during the T3. Nomenclature according to TWN ("Taxa Waterbeheer Nederland").

Taxongroup	Taxon
Annelida/Platyhelminthes - Polychaeta	*Harmothoe*
Annelida/Platyhelminthes - Polychaeta	*Nereididae*
Annelida/Platyhelminthes - Polychaeta	*Phyllodoce groenlandica*
Annelida/Platyhelminthes - Polychaeta	*Sabellaria spinulosa*
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus lamarcki*
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus triqueter*
Annelida/Platyhelminthes - Polychaeta	*Syllis*
Bryozoa - Hydrozoa - Porifera	*Alcyonidium mytili*
Bryozoa - Hydrozoa - Porifera	*Amphiblestrum auritum*
Bryozoa - Hydrozoa - Porifera	*Callopora dumerilii*
Bryozoa - Hydrozoa - Porifera	*Cliona celata*
Bryozoa - Hydrozoa - Porifera	*Clytia hemisphaerica*
Bryozoa - Hydrozoa - Porifera	*Conopeum reticulum*
Bryozoa - Hydrozoa - Porifera	*Ectopleura larynx*
Bryozoa - Hydrozoa - Porifera	*Electra pilosa*
Bryozoa - Hydrozoa - Porifera	*Hydractinia echinata*
Bryozoa - Hydrozoa - Porifera	*Obelia bidentata*
Bryozoa - Hydrozoa - Porifera	*Porifera*
Bryozoa - Hydrozoa - Porifera	*Tubularia indivisa*
Crustacea - Amphipoda	*Jassa herdmani*
Crustacea - Amphipoda	*Monocorophium acherusicum*
Crustacea - Amphipoda	*Nototropis swammerdamei*
Crustacea - Amphipoda	*Phthisica marina*
Crustacea - Amphipoda	*Stenothoe marina*
Crustacea - Amphipoda	*Stenothoe monoculoides*
Crustacea - Amphipoda	*Stenothoe valida*
Crustacea - Decapoda	*Alpheus macrocheles*
Crustacea - Decapoda	*Athanas nitescens*
Crustacea - Decapoda	*Eualus cranchii*
Crustacea - Decapoda	*Hippolyte varians*
Crustacea - Decapoda	*Necora puber*
Crustacea - Decapoda	*Pilumnus hirtellus*
Crustacea - Decapoda	*Pisidia longicornis*
Crustacea - Remaining	*Megabalanus coccopoma*
Crustacea - Remaining	*Perforatus perforatus*
Crustacea - Remaining	*Verruca stroemia*
Echinodermata	*Asterias rubens*
Echinodermata	*Ophiothrix fragilis*
Echinodermata	*Psammechinus miliaris*
Fish	*Ciliata mustela*
Fish	*Pholis gunnellus*

Taxongroup	Taxon
Marine - Remaining	*Cylista troglodyta*
Marine - Remaining	*Diplosoma listerianum*
Marine - Remaining	*Lineus bilineatus*
Marine - Remaining	*Metridium senile*
Marine - Remaining	*Oerstedia dorsalis*
Marine - Remaining	*Sagartia undata*
Mollusca - Bivalvia	*Heteranomia squamula*
Mollusca - Bivalvia	*Mytilus edulis*
Mollusca - Bivalvia	*Ostreidae*
Mollusca - Gastropoda	*Aeolidia/Aeolidiella*
Mollusca - Gastropoda	*Crepidula fornicata*
Mollusca - Gastropoda	*Epitonium clathratulum*
Mollusca - Gastropoda	*Euspira nitida*
Mollusca - Gastropoda	*Nudibranchia*
Mollusca - Gastropoda	*Tritia*
Mollusca - Gastropoda	*Trivia monacha*

3.2 Installed shell areas and living oysters

All eight shell areas were found back at the location near the turbine foundation where they were installed during the T1. Most shell areas were still relatively extensive in size (up to about 10-15 m length), while it can also be seen that a substantial volume of the material must have been 'lost' in between the rocks, under the sand or away from the scour protection. This assumingly has no significant effect on the ecological result, as the depth of the shell material does not influence the habitat formed. Most shells themselves were not covered by many organisms, but there were numerous organisms such as anemones and hydroids which had begun to establish themselves on the harder shell substrate, and organisms living in between, such as gobies.

Figure 7. Screenshot of the D05 video survey in the T3. Several animal species, such as anemones, mussels and starfish are living in the area where the shell material was deployed.

Living flat oysters in the size category which could be expected from the young wind farm and installation of oysters in 2020 are difficult to find in between the placed shell material and on the scour protection rocks. Factors that make it more challenging are:

- camouflage of the oysters due to overgrowth by other species, which can be identical to the surroundings
- the fact that empty flat oysters shells were also installed during the T1
- found doublets of oysters could be either dead or alive, which is difficult to identify on ROV footage as the view is not always optimal to determine the presence of living organisms
- the ROV sometimes moves relatively fast over the area of interest

Despite these factors, flat oysters which we have assessed to be living were found on four monopile locations with enough certainty to conclude their presence (D06, D07, H04 and H05). Figure 8 shows an example of a living flat oyster on the scour protection. The shape, colour and shading of the area beneath makes this flat oyster relatively certain. The prevailing currents in this sample area mean that it is unlikely that an empty or single shell would stay in this position on a rock. Figure 9 shows an example of a living oyster in the shell material, although the original attachment is likely not visible here. These examples provide an indication of the difficulties in spotting these individuals.
Additional potentially living oysters were found at some of the same and two other locations (D04 and H02), see Figure 10 for an example.

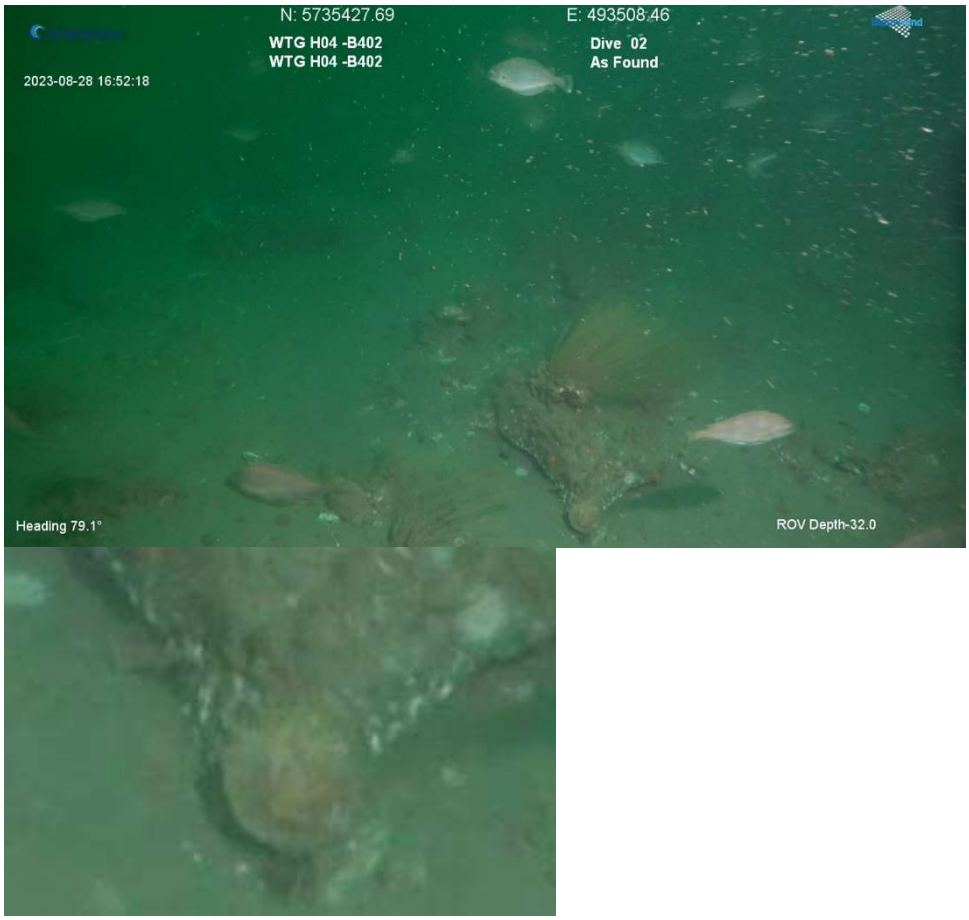

Figure 8. Screenshot from the video survey of the scour protection at turbine location H04, with a living flat oyster on a rock. The second photo is the same frame but with additional detail of a flat oyster

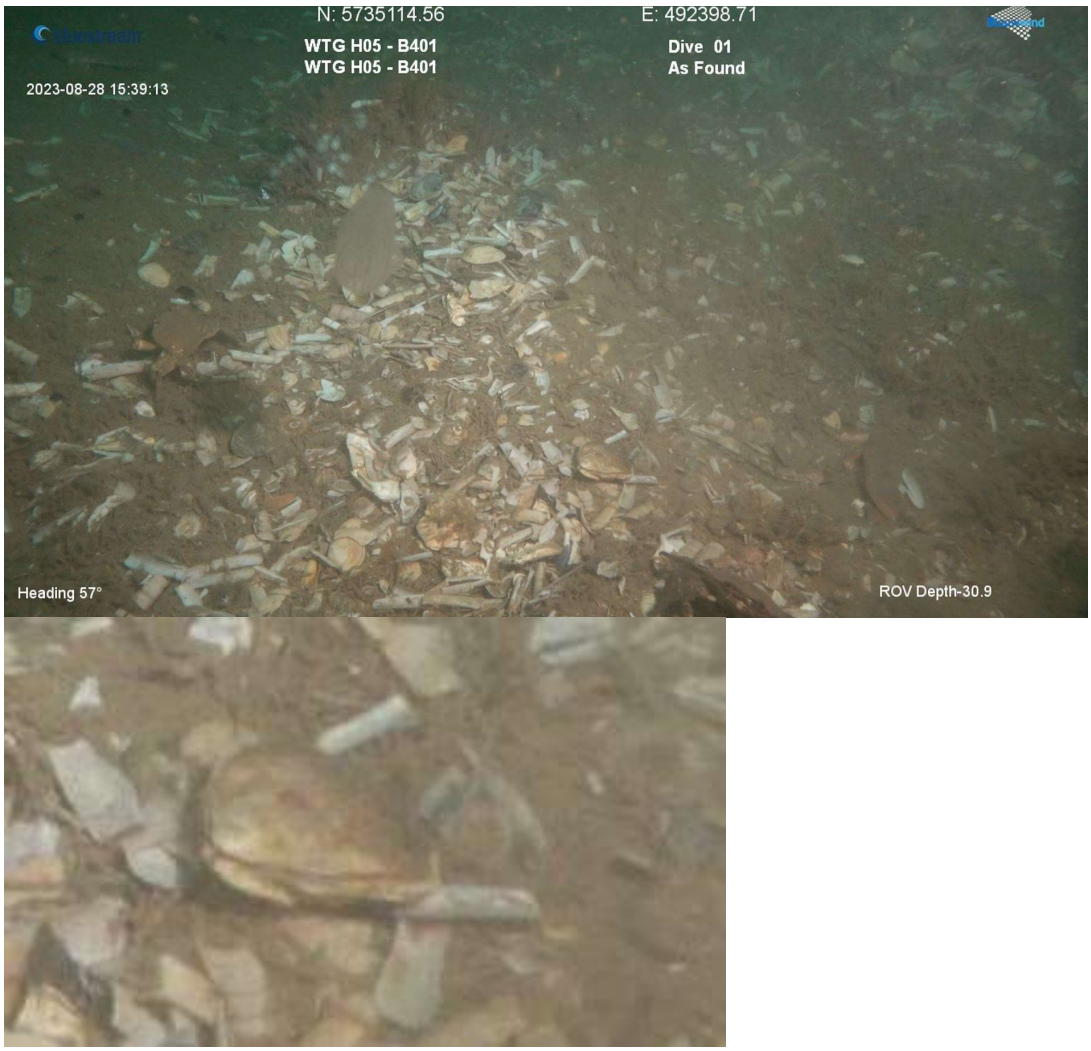

Figure 9. Screenshot from the video survey of the shell area on the scour protection at turbine location H05, with a living flat oyster. The second photo is the same frame but zoomed in on the flat oyster.

Figure 10. Screenshot from the video survey showing a potentially living flat oyster. This was taken on the filter grade of the scour protection at turbine location D04. The second photo is the same frame but zoomed in on the flat oyster. The white-coloured part is possibly an anemone.

3.3 Flat Oyster monitoring

3.3.1 Oyster survival

The number of living, dead, sampled and remaining oysters and the survival rate compared to the installation during the T0 are shown in Table 4.

In 19 of the 32 baskets there were oysters missing when compared to the remaining number of oysters in each basket after the T1. However, extra oysters are found in five of the baskets (indicated with a minus sign).

The average survival of oysters per basket when compared to the T0 (not including the 1 oyster that was harvested for Bonamia and gonad inspection) was 70%. Average of survival of oysters when compared to the T1 was 74%. Figure 11 shows that the variation in survival rate is high. There was no relation in the number of dead oysters between the T1 and the T3 within each of the baskets (R^2 = -0.004), meaning that if a particular basket had high mortality in the T1, it did not necessarily also have a high mortality in the T3.

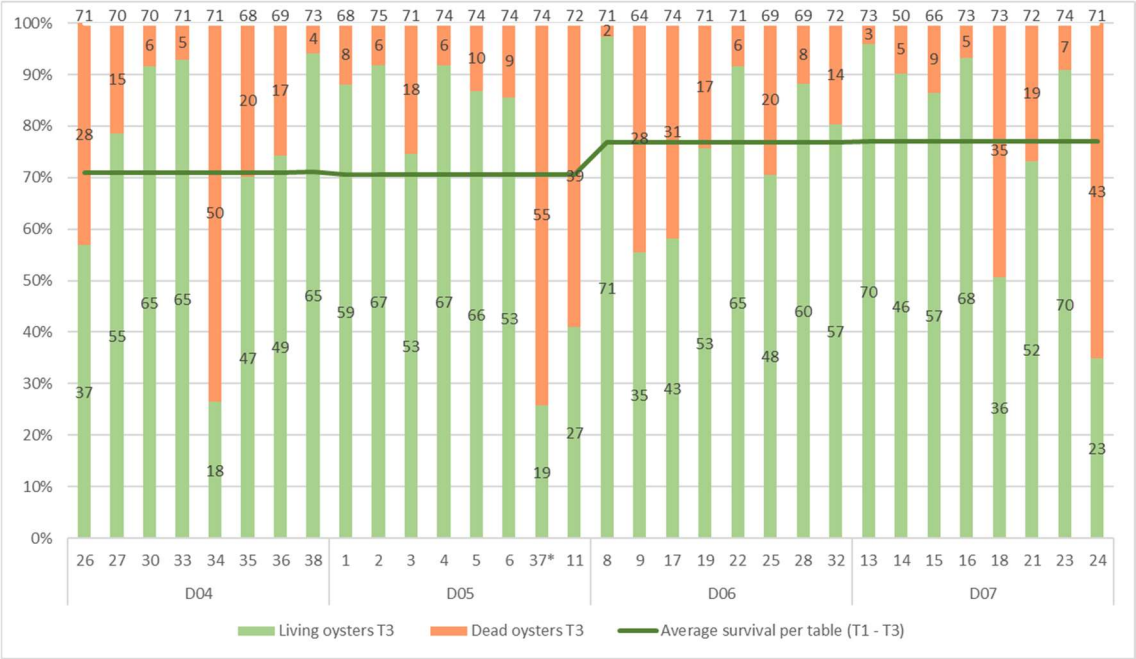

Figure 11. Percentage of living (green) and dead oysters (orange) found in each basket compared to the total number of oysters in each basket in the T3; numbers in stacked colums represent the number of living and dead oysters in the T3. Green line represents the average survival per oyster table compared to the T1; numbers above the columns represent the remaining number of oysters in the baskets at the end of the T1 (see also Table 4). Basket numbers and monopiles are on the x-axis.

*Table 4. Number of dead and living oysters per basket during T3, including survival rate compared to T0 and T1. * = basket number has been replaced during the T1, new number is 37.*

		T1	T3				survival	
Mono-pile	Basket number	Remaining living oysters T1	Total oysters in basket	Oysters missing	Living oysters	Dead oysters	survival T0 - T3	survival T1 - T3
D04	26	71	65	6	37	28	50%	52%
D04	27	70	70	0	55	15	74%	79%
D04	30	70	71	-1	65	6	88%	93%
D04	33	71	70	1	65	5	88%	92%
D04	34	71	68	3	18	50	24%	25%
D04	35	68	67	1	47	20	64%	69%
D04	36	69	66	3	49	17	66%	71%
D04	38	73	69	4	65	4	88%	89%
D05	1	68	67	1	59	8	80%	87%
D05	2	75	73	2	67	6	91%	89%
D05	3	71	71	0	53	18	72%	75%
D05	4	74	73	1	67	6	91%	91%
D05	5	74	76	-2	66	10	89%	89%
D05	6	74	62	12	53	9	72%	72%
D05	37*	74	74	0	19	55	26%	26%
D05	11	72	66	6	27	39	36%	38%
D06	8	71	73	-2	71	2	96%	100%
D06	9	64	63	1	35	28	47%	55%
D06	17	74	74	0	43	31	58%	58%
D06	19	71	70	1	53	17	72%	75%
D06	22	71	71	0	65	6	88%	92%
D06	25	69	68	1	48	20	65%	70%
D06	28	69	68	1	60	8	81%	87%
D06	32	72	71	1	57	14	77%	79%
D07	13	73	73	0	70	3	95%	96%
D07	14	50	51	-1	46	5	62%	92%
D07	15	66	66	0	57	9	77%	86%
D07	16	73	73	0	68	5	92%	93%
D07	18	73	71	2	36	35	49%	49%
D07	21	72	71	1	52	19	70%	72%
D07	23	74	77	-3	70	7	95%	95%
D07	24	71	66	5	23	43	31%	32%

During the T3, many brown crabs (*Cancer pagurus*) were found inside at least 18 baskets (Figure 12). Even an Atlantic spider crab (*Maja brachydactyla*) was found inside one of the baskets. It is suggested that these organisms entered the baskets while still juveniles, and subsequently grew too large to leave through the mesh. For this reason, and the fact that most baskets still had open holes, it was decided to try to replace the baskets with new and clean ones without the bigger holes that were created during the T1. Unfortunately, these baskets did not fit in the oyster table, so the old baskets were cleaned using water pressure and re-used.

Figure 12. Examples of brown crabs that were living inside the baskets. Some baskets also contained empty shells of the crabs. Basket 34 contained many empty/open oyster shells.

3.3.2 Oyster growth

Maximum width and length of oysters at T3 (25 individuals/basket in most cases; in baskets numbers 24, 34 and 37 not enough individuals were left to measure 25 individuals) were compared maximum width and length at T0 (75 individuals/basket) and T1 (25 individuals/basket). At T1 basket 34 was not measured correctly due to erroneous calliper calibration during the fieldwork. No comparison could be made for this basket in the T1.
The measurements per basket are displayed in the graphs in appendix 6.3. Note that, because the number of individuals measured is not the same between T0 and T1 and the T3 (75 vs 25 vs 25 individuals), the distribution around the average of measurements per basket do not compare one-on-one. Therefore, the most reliable comparison is to be found using the average and the differences between the measurement years.

For the oyster width there was an overall significant difference between the three measurement years ($p<0.01$). It was caused by the T3, that was different from the T0 ($p<0.01$) and the T1 ($p< 0.01$). This means there has been structural growth in width of the shells over the last two years (from T1 onward). Especially monopiles D07 experienced the largest growth in width, while D04 and D05 remained stable in oyster width over the measurement years see, Figure 13.

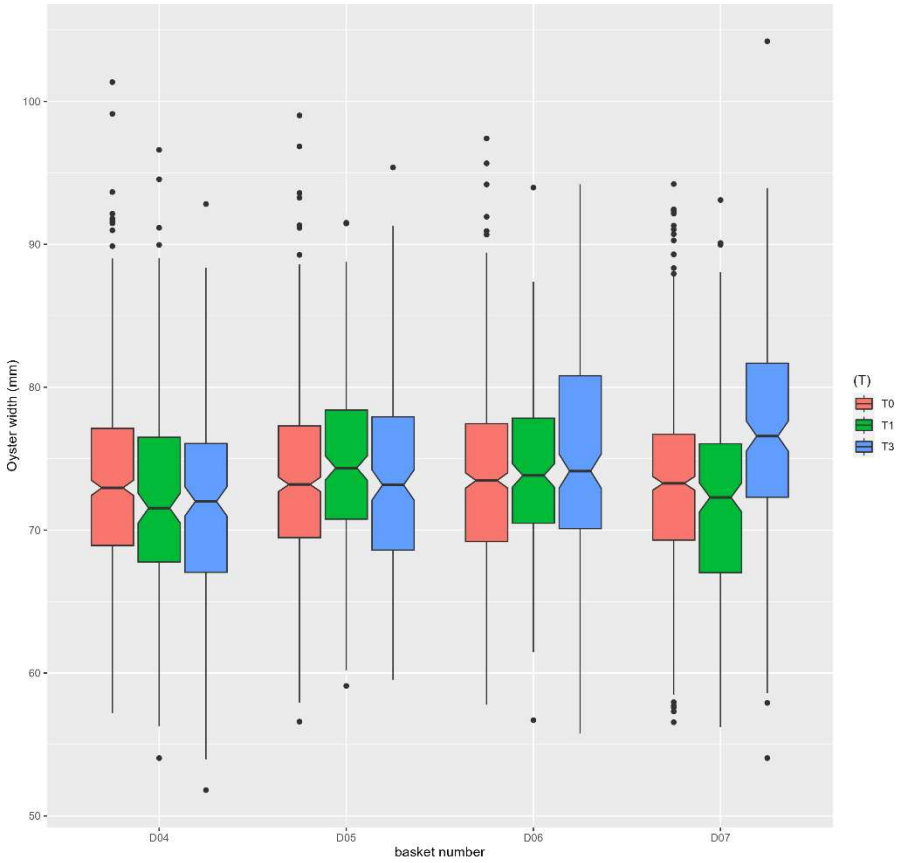

Figure 13. Notched boxplot of widest width of oysters measured in mm (75 individuals per basket on T0 (red), 25 individuals per basket on T1 (green) and 25 individuals on T3 (except baskets 24, 34 and 37), visualised per monopile over the measurement years. When notches overlap, no significant differences are present.

Oyster length showed a clear growth pattern, which was already noted in the measurements from T1. Overall, the measurement years were significantly different from each other ($p< 0.001$). Similar to the oyster width, D06 and D07 showed the largest growth in length, over all the years. Oysters on the tables near monopiles D04 and D05 experienced the most growth

from the T0 to the T1 and remained stable from the T1 onward (no significant differences between T1 and T3 for both monopiles, p> 0.95), see Figure 14. At T1, the oysters seemed to have grown mainly in length. However, at T3, also the width of oysters at D07 has increased.

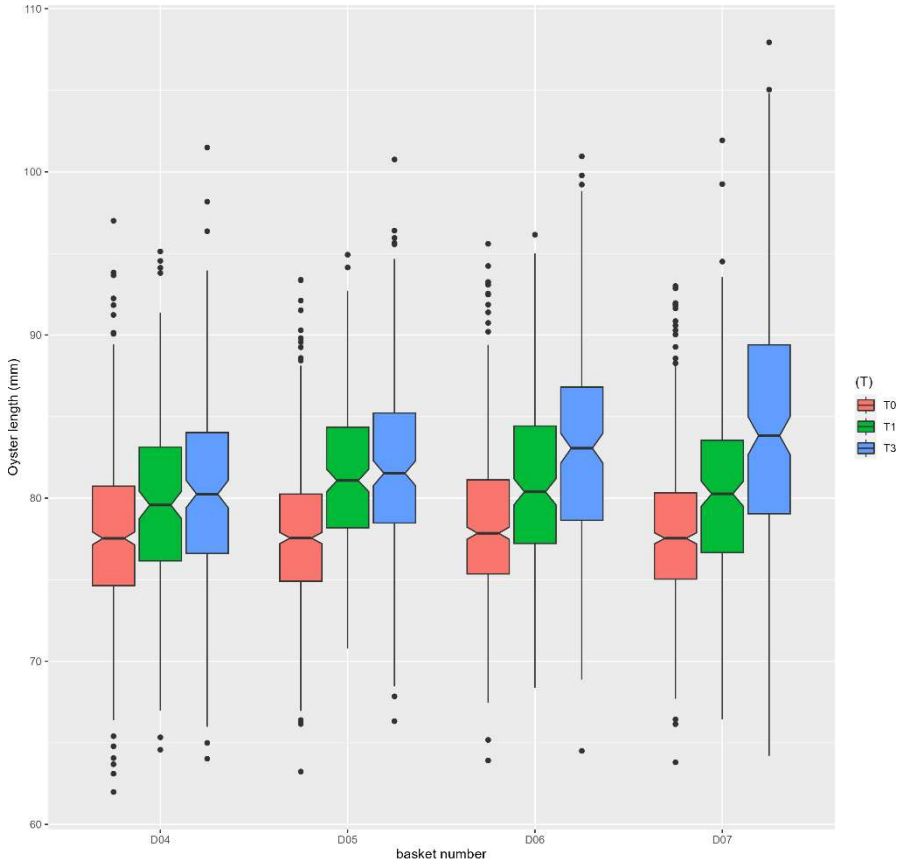

Figure 14. Notched boxplot of longest length of oysters measured in mm (75 individuals per basket on T0 (red), 25 individuals per basket on T1 (green) and 25 individuals on T3 (except baskets 24, 34 and 37), visualised per monopile over the measurement years. When notches overlap, no significant differences are present.

3.4 Larvae analysis

D-larvae were only found in two of the eleven larvae samples from the extra and T3 campaigns in 2023; at monopile D07 (1 larva) and H05 (3 larvae) (Figure 15). DNA analysis of the larvae samples established the presence of *Ostrea* sequences only in the sample of monopile H05.

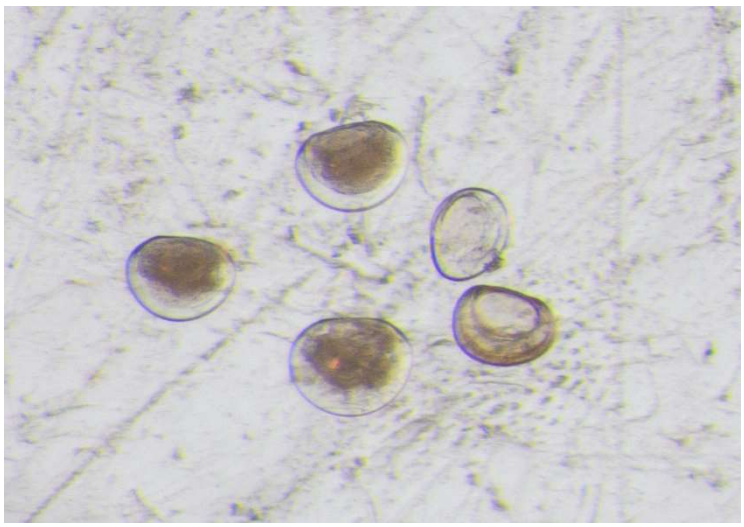

Figure 15. D-larvae found in H05 (of which 2 are empty)

3.5 Spat analysis

The presence of spat was assessed in two ways: first from the spat collectors that were attached to the oyster tables, and second from spat present inside the oyster baskets.

3.5.1 Spatcollectors

The spat collectors contained several benthos species. Noteworthy is the presence of the parchment worm *Chaetopterus variopedatus*, which is absent from the coast, the rare bryozoan *Biflustra tenuis* and the rare suborbicular Kellyclam *Kellia suborbicularis*, Dutch name holteschelpje. In accordance with its Dutch name this small bivalve prefers crevices etc. as a habitat, which in Dutch waters mainly are provided by oysters. Some saddle oysters (Anomiidae) were found, which superficially look like Ostreidae, but can be readily distinguished by their perforated lower shell. The taxa found in the spat collectors are shown in Appendix 6.4 as an addition to the scrape samples.

Six of the twenty-four spat collectors were not found ("NA" in Table 5). Another four contained no or very little collector shells, and no oyster spat (D04-IV and D07-IV, -V and -VI). Fourteen good collectors were left for analyses. On seven of these collectors a total of 11 live oysters were found (Table 5). The size of the collected oyster spat ranged from 12 to 51 mm in length (Table 6).

Table 5. Overview of spat collectors per oyster table

		D04		D05		D06		D07	
	shell type	collec-tor	spat	collec-tor	spat	collec-tor	spat	collec-tor	spat
Spat collectors	oysters	I	1	I	1	I	0	I	0
		II	0	II	1	II	0	II	1
		III	2	III	0	III	4	III	1
	mixed shells	IV	0	IV	0	IV	0	IV	0
		NA	-	NA	-	NA	-	V	0
		NA	-	NA	-	NA	-	VI	0

Table 6. Oyster length and width of the specimens harvested off the spat collectors

Table	Collector	Length (mm)	Width (mm)
D04	I	49	43
D04	III	27	17
D04	III	30	25
D05	I	41	30
D05	II	12	9
D06	III	51	40
D06	III	47	34
D06	III	42	25
D06	III	16	12
D07	II	15	15
D07	III	34	25

3.5.2 Spat in baskets

During the counting and measuring of the oysters from the baskets, the presence of oyster spat was also assessed. As can be seen in Table 7, many of the baskets contained several oyster spat. They were mostly attached to the adult oysters, both live animals and empty shells. Occasionally "loose" spat was found. Ten loose individuals that were measured ranged from 44 to 63 mm in length. In basket number 4, three spat specimens of the Pacific oyster (*Magallana gigas*) were present (not in Table 7).

Table 7. Number of oyster spat per monopile and basket.

	D04		D05		D06		D07	
	basket	spat	basket	spat	basket	spat	basket	spat
Oystertables	**26**	0	**1**	5	**8**	2	**13**	3
	27	3	**2**	14	**9**	1	**14**	5
	30	0	**3**	3	**17**	4	**15**	1
	33	4	**4**	0	**19**	7	**16**	3
	34	0	**5**	3	**22**	6	**18**	1
	35	6	**6**	4	**25**	8	**21**	1
	36	3	**37***	1	**28**	5	**23**	8
	38	3	**11**	0	**32**		**24**	5
total		**19**		**30**		**33**		**27**

3.6 Bonamia lesions and gonad development

In none of the oysters examined were *Bonamia* lesions found.

Twenty-six of the 32 oysters examined had well developed milky white gonads, indicative for an (almost) readiness to spawn. In two oysters the gonad was small or very small. In four oysters the gonad had a light brownish colour, which suggest a different stage of development, it is not clear whether this means recently spawned or an early stage of development. See Figure 16.

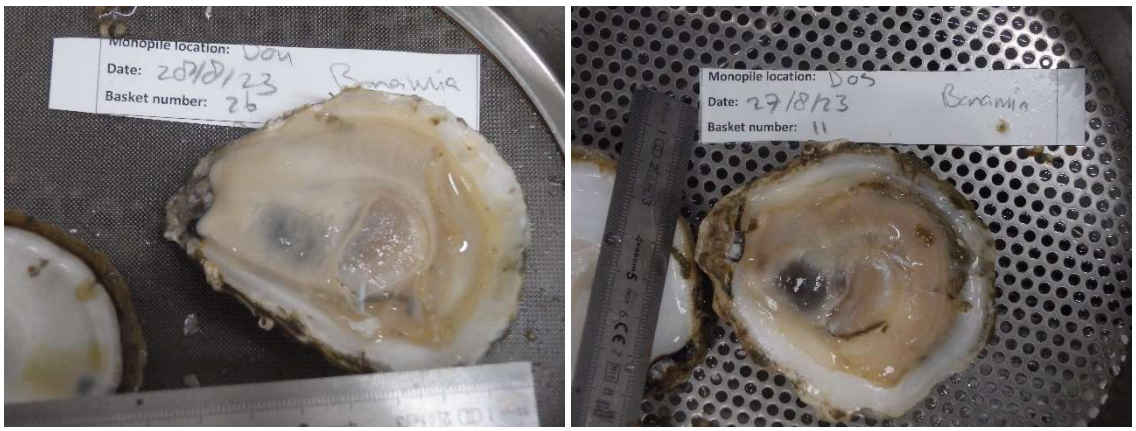

Figure 16. Inert of oyster showing gonad development (milky half-circle) on the left and brownish gonad colour on the right. Both have no Bonamia lesions.

3.7 ROV survey

In T3, a total of 35 taxa were identified from the photo analysis (20 photos around the monopile and 1 of the monopile itself). At one photo-location at H02 no taxa were seen; this location had 100% sand cover.
Additional to the photo analyses, ROV videos were analysed to find taxa that had not been seen on the photos. This was a non-quantitative analysis, in which an additional 39 taxa were found, resulting in a total of 74 taxa. In the table below (Table 8), the number of taxa per monopile per analysis type are listed. In appendix 6.4 the taxa found in the T3 are listed for their occurrence in the photo analysis and the video analysis.

Table 8. Number of taxa found in the T3 per monopile

monopile	photo	video
D04	20	30
D05	15	24
D06	11	35
D07	15	30
H02	13	31
H03	17	25
H04	16	23
H05	17	32

In the photo analyses of T0 and T1, respectively 41 and 36 taxa were identified, and an additional 24 and 30 taxa from the video analysis. This leads to a total of 65 and 66 taxa in the T0 and T1 respectively. In the T3 a total of 74 taxa were found in the combined photo and video analyses.

Combining the photo analyses of the three measurement years, a total of 65 taxa were identified, of which 9 higher taxa. These are mostly at family (e.g. Cottidae), class or even phylum level (e.g. Porifera). Appendix 6.5 shows the frequency with which species have been found per year per monopile (frequency of occurrence). That table gives an indication of how often species can be found and can be used to compare frequencies of occurrences of different species.

3.7.1 Multivariate analysis of the benthic species community

Because the SACFOR score is ordinal categorical data, its scores were converted to a numerical scale (see 3.7.2 and Appendix 6.6). The resulting numerical from the photo analysis was plotted in non-Metric multi-dimensional scaling plots (nMDS). The fourth root transformed cover values were used to calculate the Bray-Curtis similarity. A dummy variable (value 1) was added to deal with zero-inflated data, i.e. when samples have no taxa in common, for which Bray–Curtis would return zero similarity for those pairs of samples, leading to a "collapsed" plot (Clarke et al., 2006). This adjustment results in the 'zero-adjusted Bray-Curtis' similarity coefficient for each sample combination, which were visualised in nMDS plots.

The nMDS plots (Figure 17 to Figure 21) indicate the similarity in species-composition between multiple samples (the list of species found in each photo). The closer samples are grouped together, the higher the similarity between these samples. This means that the benthic community of samples that group together in a nMDS plot, have a high(er) similarity.

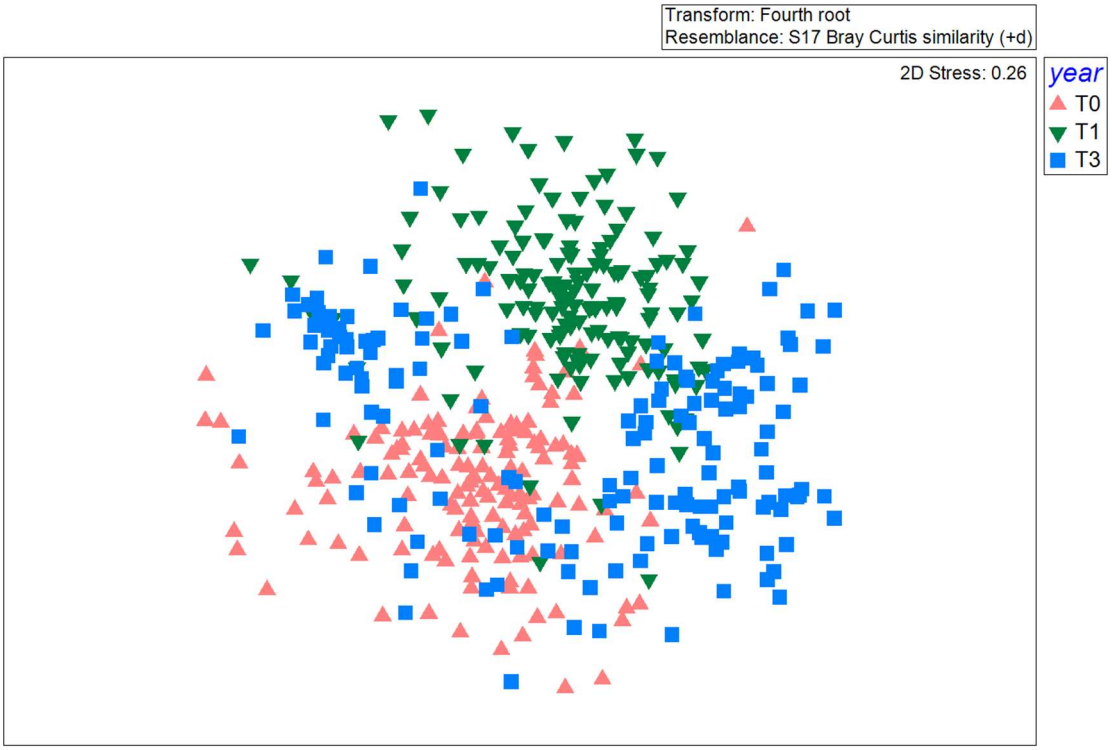

Figure 17. NMDS displaying the differences in species-composition between samples of T0, T1 and T3 (all samples except H3 in T0).

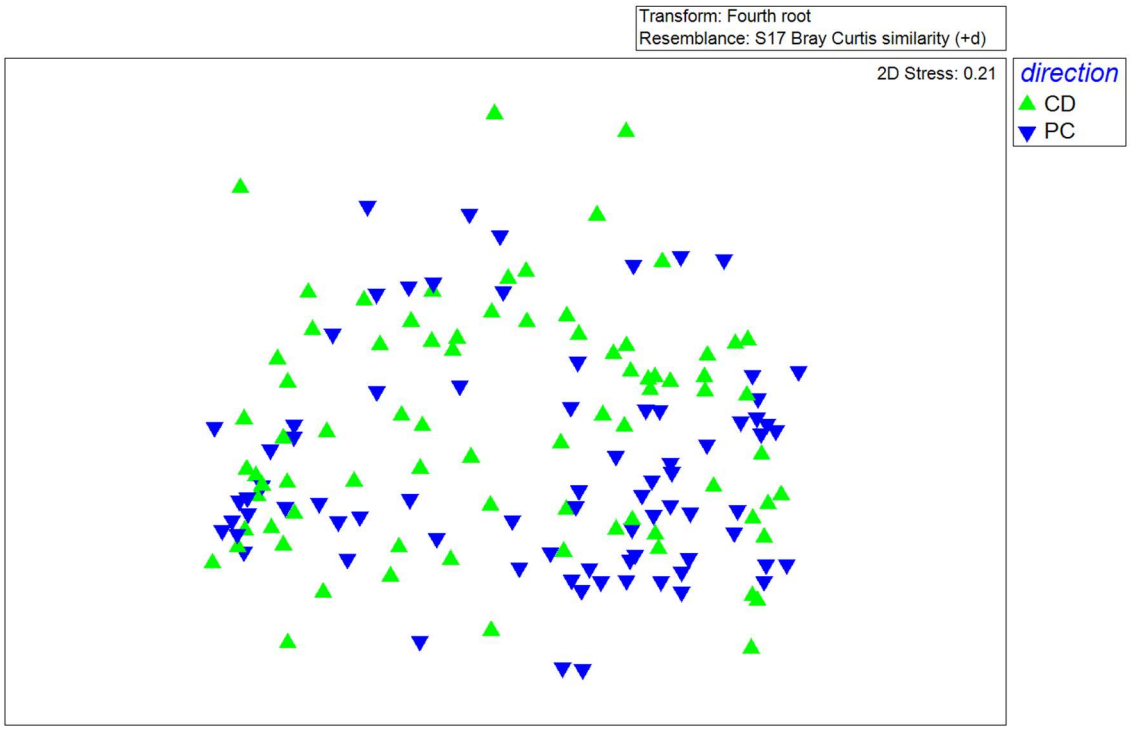

Figure 18. NMDS of T3 displaying the differences in species-composition between samples of current-direction (green) and perpendicular-current direction samples (blue).

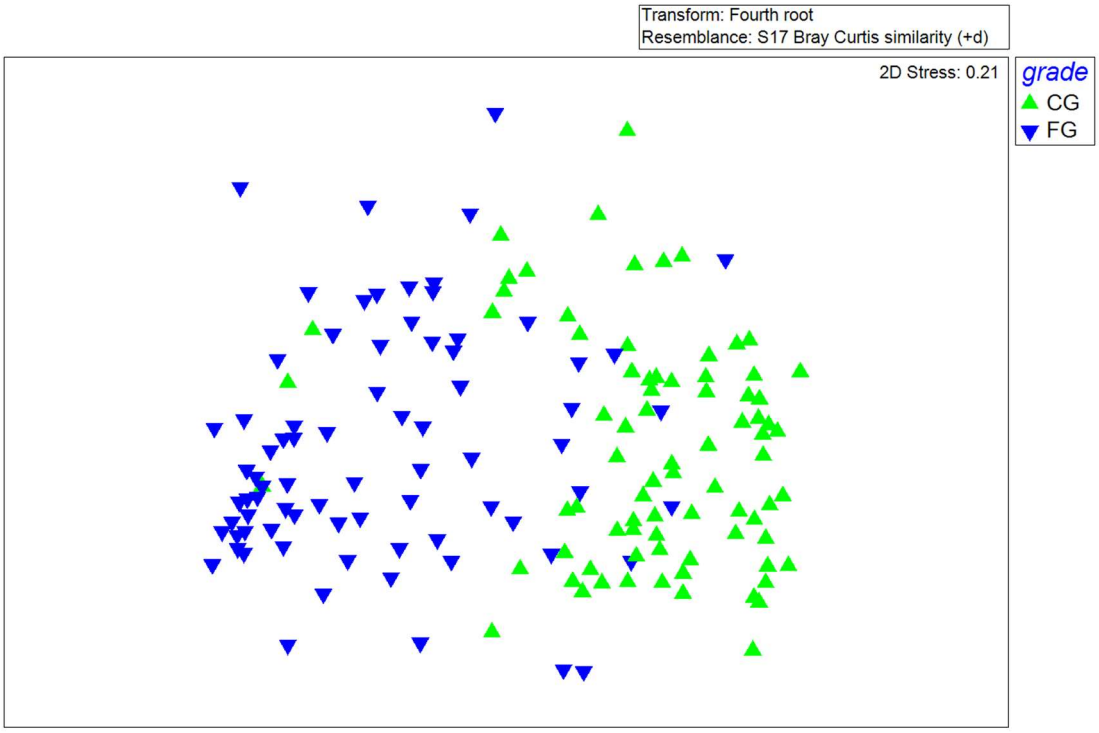

Figure 19. NMDS of T3 displaying the differences in species-composition between samples on coarse-grade (green) and filter-grade scour-protection (blue).

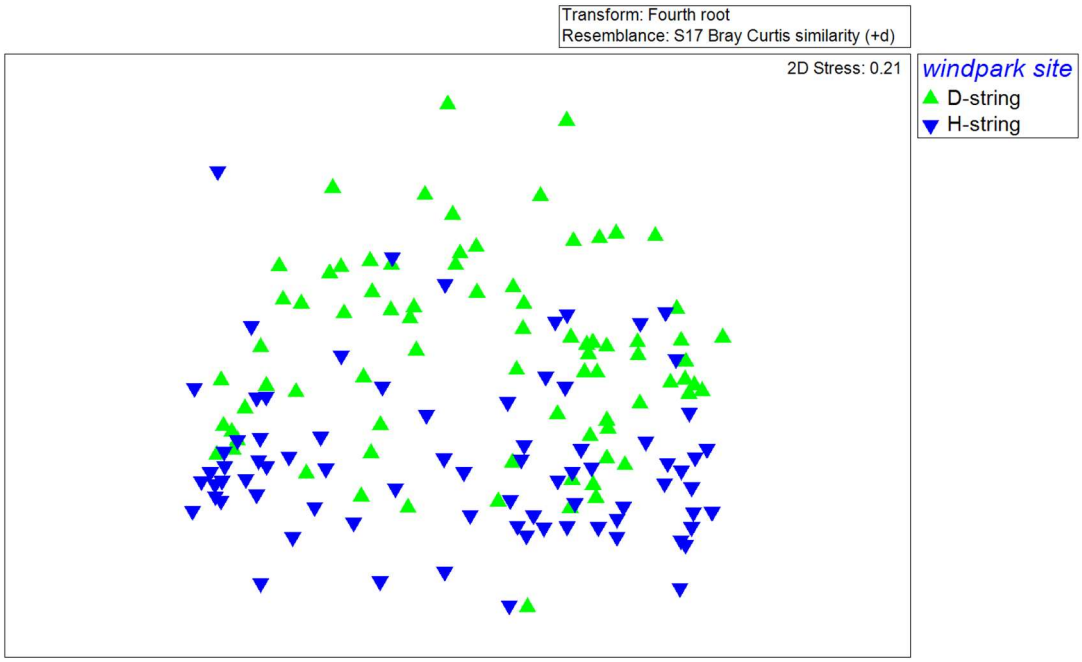

Figure 20. NMDS of T3 displaying the differences in species-composition between samples of Borssele III (D: green, with oyster tables) and Borssele IV (H: blue, without oyster tables).

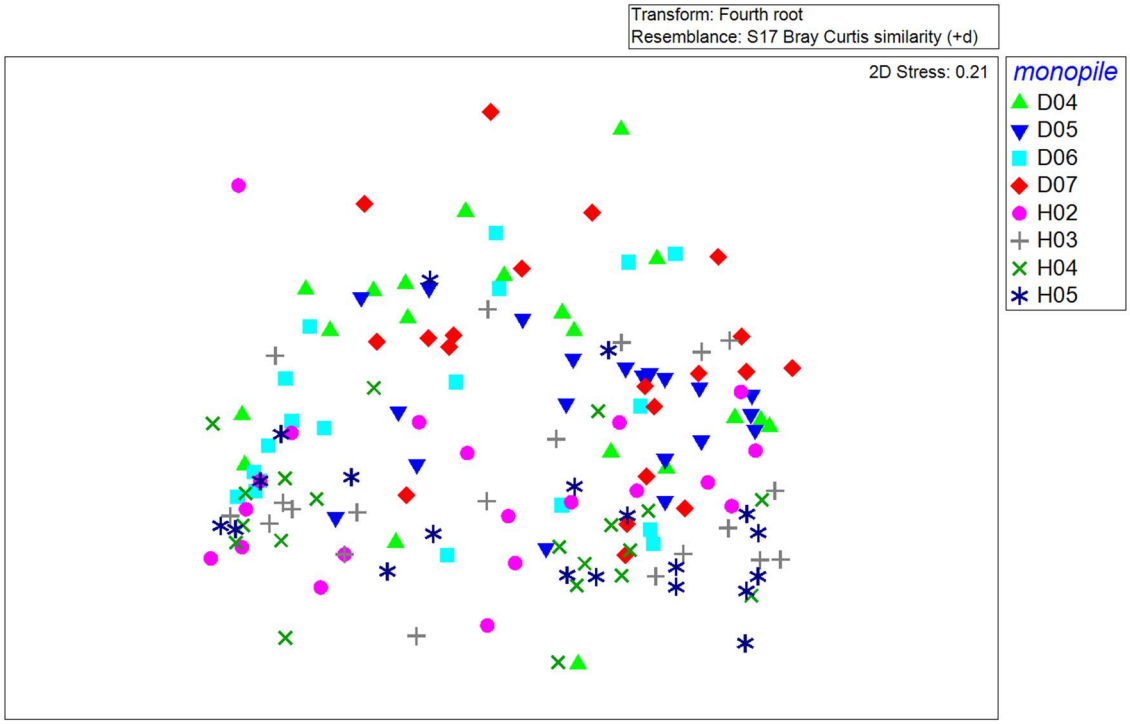

Figure 21. NMDS of T3 displaying the differences in species-composition between samples of different monopoles.

Because the nMDS-plots are a mere visual representation of differences between samples, the differences between groups of samples were also tested statistically using ANOSIM (Analysis of Similarities) and PERMANOVA on the same data as used for the nMDS.
In the ANOSIM the factor “Year” has been tested separately as an explanatory factor of the variation in species composition between samples. In ANOSIM, the R2 value indicates the amount of “separation” between the species composition in the groups under investigation.

R2=1 means the factor groups are completely different. R2=0 means they are completely similar.
In PERMANOVA the factors (type of scour protection, current direction, windpark i.e Borssele III (with oyster-table) or IV (without oyster-table)) and monopile) were tested as an explanatory factor in the variation between samples. Also, the amount of explanation for the variation each factor provides is represented by R2; its explanatory value. R2=1 means the factor explains 100% of the variation. R2=0 means it does not explain any variation.

In Figure 17 the nMDS is shown over all samples from all measurement years, it shows some grouping of the three measurement years. ANOSIM analysis indeed shows that there is a significant difference in the species composition between the years ($p<0.001$, GlobalR= 0.35). The pairwise tests are in Table 9 and are all significant ($p<0.001$). This means that there are differences in species composition between all three years, the difference between T0 and T1 is largest.

Table 9. Pairwise ANOSIM results on the factor year, Global R = 0.348

Year	R2
T0, T1	0.437
T0, T3	0.268
T1, T3	0.338

When all factors and measurement years were included in a PERMANOVA analysis, year explained most variance (0.18), followed by scour grade (0.06), monopile (0.04), wind farm site (0.01) and current direction (0.01). This still leaves 68% variance left that is not explained by these factors.

The PERMANOVA analysis on the factors for the T3, corresponding with Figure 18 to Figure 21 are shown in Table 10. It shows that the factor that explains the most variation in the species composition, is the grade of the scour protection (coarse or filter grade), since it has the highest R2. This means that species composition between the two scour types are relatively different from each other, this can also be seen in the nMDS plot. After this the monopiles explain the most variation, although this R2 value is relatively low. The corresponding figure indeed shows that there is no clear pattern in the samples of the different monopiles. This factor covariates partly with the factor wind farm site (D-string with oysters in Borssele III and H-string in Borssele IV), but some additional variation is still explained by the differences in species composition between the different monopiles. The small variance explained can also be seen in Figure 21, where there is no apparent grouping of the samples for the different monopiles. The factors wind farm site and current direction are significant, but with very low variance.

Table 10. PERMANOVA results on different factors in the T3, p<0.001 for all factors

factor	R2
scour grade	0.245
monopile	0.096
windpark site	0.052
current direction	0.028

From the table below (Table 11) it becomes clear that several species are common between the years. However, some species seem to be more specific in one year, such as the hydroid *Tubularia indivisa* in the T1 and the amphipod *Jassa herdmani/ Monocorophium acherusicum*

in the T3. It is striking that in T0 and T1 less species were responsible for a more or less similar average similarity between the samples. In contrast, in the T3 average similarity in species composition between samples was lower, and hence more species were "needed" to get to a cumulative contribution of ~90% to the total similarity. This is an indication that the species composition in 2023 was more variable and complex when compared to the earlier years.

Table 11. Characteristic species for each of the measurement years and their cumulative contribution (%) to the similarity in the species composition in the samples within each year (SIMPER analysis). Per year the average similarity in species composition between the samples is also shown.

T0		T1		T3	
Average similarity: 38.69	%	Average similarity: 45.65	%	Average similarity: 32.96	%
Cylista troglodytes	45.94	*Tubularia indivisa*	46.52	*Cylista troglodytes*	32.15
Asterias rubens	70.14	*Cylista troglodytes*	67.99	*Jassa herdmani / Monocorophium acherusicum*	46.33
Spirobranchus triqueter	86.39	*Asterias rubens*	79.68	*Spirobranchus triqueter*	59.09
Necora puber	90.00	*Spirobranchus triqueter*	87.83	*Lanice conchilega*	67.07
		Necora puber	93.19	*Necora puber*	74.38
				Asterias rubens	81.26
				Hydrozoa	85.26
				Cylista elegans	89.16
				Tubularia indivisa	92.14

3.7.2 Analysis of benthic species cover

In Figure 22 the average species cover (based on the converted SACFOR scores to a numerical scale) per photo is shown per monopile and type of scour protection combination.
From the graph can be seen that in general, species cover is higher in the coarse grade scour type. This is mainly due to the high cover of *Jassa/ Monocorophium*. Monopile D05 is exceptional in the high level of cover reported. Another notable finding from figure 18 is that in at the locations of the monopiles in the Borssele IV area (H-monopiles), the species cover is always higher than that at Borssele III area (D-monopiles). This is caused by *Lanice conchilega*, which was found most often and with high cover on the fine grade scour of the Borssele IV area. This species prefers fine to medium grained sand and seems to have established itself in the Borssele IV area, since it was absent at the T0 and present to a much lesser extent at the T1. The graph also shows that in all cases the filter grade (FG) scour has a higher sand coverage compared to the coarse grade (CG) scour. This was to be expected since any present sand is likely to enter the crevices in the coarse grade scour.

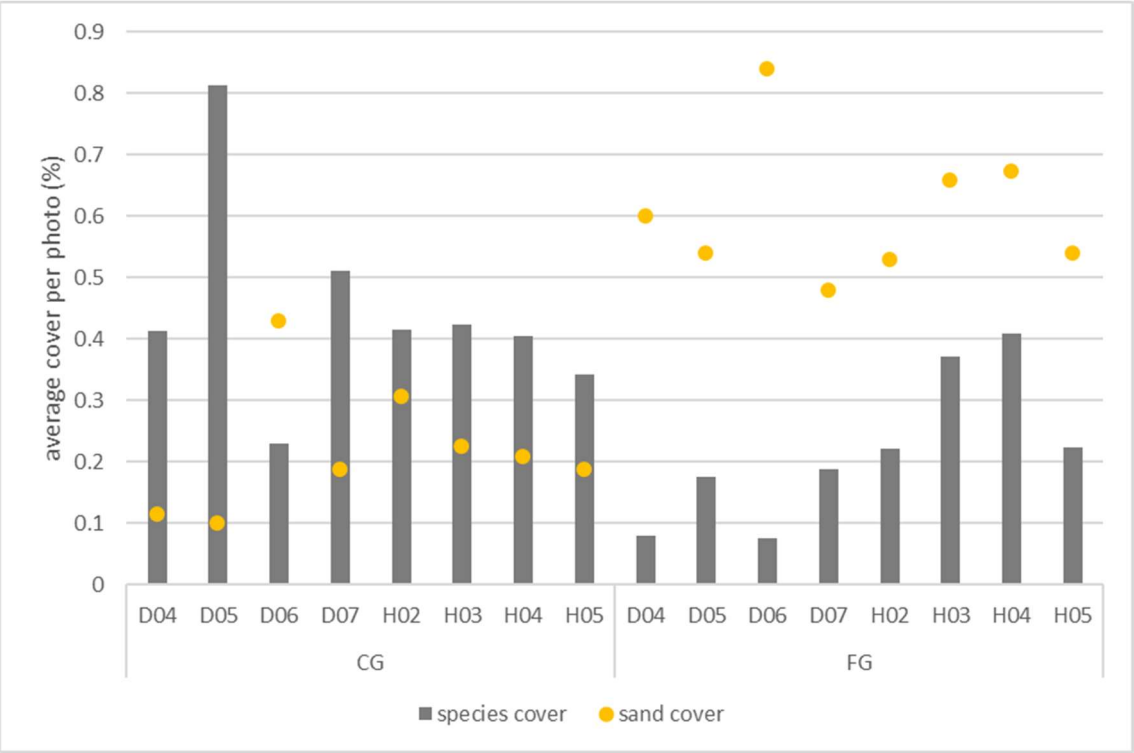

Figure 22. Average species cover per photo of the T3 per monopile and scour type (CG = coarse grade; FG = fine grade). Average sand cover is indicated with a yellow dot.

In Figure 23 a comparison is made between the three measurement years, with only the scour type visualised as factor. From the T0 to the T1 there is a clear increase in average species cover in both scour types. From the T1 to the T3 the increase is much less obvious, with a slight increase for the fine grade- and a small decrease for the coarse grade scour type. However overall, the cover of species on the scour protection seems to be increasing.

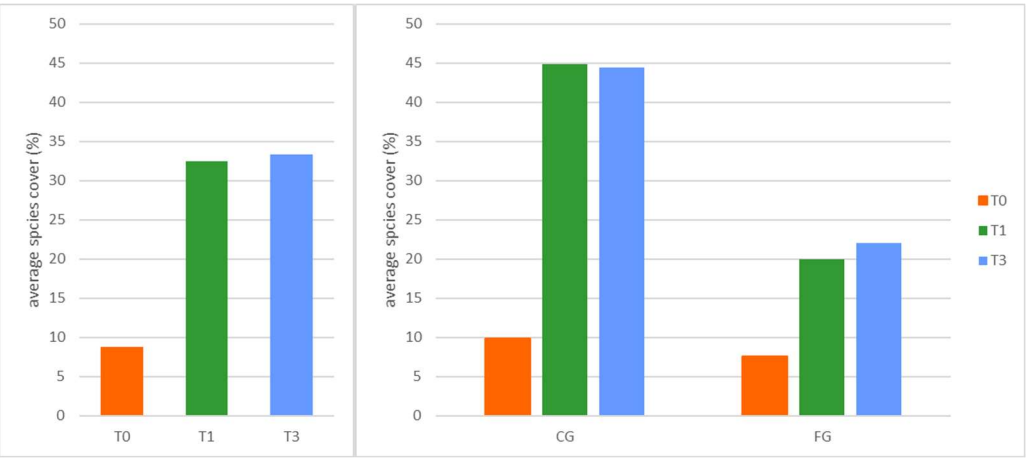

Figure 23. Average species cover in T0, T1 and T3 (left) and separate for coarse grade (CG) and fine grade (FG) scour protection (right).

3.8 (e)DNA

3.8.1 Fish

In 2022, eDNA samples were taken at the 4 D-string monopiles where oysters were installed and in 2023, both D-string and H-string were sampled at the 8 turbines with shell material. The eDNA results showed that the samples contained only fish species, which is the result of using

a fish primer (MiFish). In Appendix 6.7 is shown which species were demonstrated in the samples for 2022 and in the T3. In 2022 33 fish species were found, while in 2023 20 fish species were found. Both years had 12 species in common (Table 12).

Table 12. Species in common between 2022 and 2023 from eDNA analysis

Fish taxon
Ammodytes marinus
Dicentrarchus labrax
Engraulis encrasicolus
Gobius niger
Merlangius merlangus
Mullus surmuletus
Pegusa lascaris
Pleuronectes platessa
Pomatoschistus minutus
Sardina pilchardus
Scomber scombrus
Trachurus trachurus
Trisopterus luscus

The eDNA samples from the T3 were dominated by horse mackerel. After that, mainly sand-based species (flatfish, gobies, weevers etc.) and water column species (whiting, pouting, sardines, mackerel) were found. One fish species, sand sole (*Pegusa lascaris)*, was never seen before in eDNA samples by Wageningen University & Research, although the species is known from the southern North Sea.

Methods for fish detection

In the table below (Table 13), all fish taxa that were found in the different analyses are listed per method. It becomes apparent that in general, the different sampling methods are additive to each other. However, the video and eDNA analyses seem most suitable to detect fish, whereas the photo analysis only results in 2 unique taxa, of which 1 (*Actinopteri,* ray-finned fish) at a very high taxonomic level. The scrape samples contained 2 taxa of fish, of which 1 was unique (*Ciliata mustela*, fivebeard rockling).

Table 13. Fish taxa found in the different analyses in the T3.

Fish taxon	photo	video	eDNA	scrape
Callionymus lyra	x	x	x	
Trisopterus luscus	x	x	x	
Trisopterus minutus	x	x	x	
Conger conger	x	x		
Parablennius gattorugine	x	x		
Callionymus reticulatus	x		x	
Pomatoschistus minutus	x		x	
Actinopteri	x			
Pomatoschistus microps	x			
Trachinus draco		x	x	
Trachurus trachurus		x	x	
Pholis gunnellus		x		x

Ammodytidae	x		
Callionymus	x		
Cottidae	x		
Gadus morhua	x		
Microstomus kitt	x		
Mullus sermuletus	x		
Myoxocephalus scorpius	x		
Pomatoschistus	x		
Taurulus bubalis	x		
Ammodytes marinus		x	
Arnoglossus laterna		x	
Chelon auratus		x	
Echiichthys vipera		x	
Engraulis encrasicolus		x	
Gobius niger		x	
Merlangius merlangus		x	
Mullus surmuletus		x	
Pegusa lascaris		x	
Pleuronectes platessa		x	
Sardina pilchardus		x	
Scomber scombrus		x	
Ciliata mustela			x

3.8.2 Flat oysters

Next to the fish eDNA analyses, a specific primer for the flat oyster was also used. This resulted in the occurrence of oyster eDNA in only the 2023 samples, at the monopiles of D05 and D06, and at H03, H05 and H05 (Table 14).

Table 14. Results of eDNA analysis for Flat oysters

Sample location	Sample date	Flat oyster PCR 600 bp (2022) or 250 bp (2023) positive?	
		1/2	2/2
D4 A	21-9-2022	no	no
D4 B1	21-9-2022	no	no
D4 B2	21-9-2022	no	no
D5 A	21-9-2022	no	no
D5 B1	21-9-2022	no	no
D5 B2	21-9-2022	no	no
D6 A	21-9-2022	no	no
D6 B1	21-9-2022	no	no
D6 B2	21-9-2022	no	no
D7 A	21-9-2022	no	no
D7 B1	21-9-2022	no	no
D7 B2	21-9-2022	no	no
D4-1	26-7-2023	no	no
D4-2	26-7-2023	no	no

D5-1	26-7-2023	no	**yes**
D5-2	26-7-2023	no	**yes**
D6-1	26-7-2023	no	**yes**
D6-2	26-7-2023	**yes**	no
D7-1	26-7-2023	no	no
D7-2	26-7-2023	no	no
H2-1	26-7-2023	no	no
H2-2	26-7-2023	**yes**	**yes**
H3-1	26-7-2023	no	no
H3-2	26-7-2023	**yes**	**yes**
H4-1	26-7-2023	no	**yes**
H4-2	26-7-2023	no	no
H5-1	26-7-2023	no	no
H5-2	26-7-2023	no	no

4 Discussion and conclusions

The results will be further discussed in order of the research questions mentioned in the introduction. Some questions will be answered together.

What is the survival and growth rate of flat oysters?
The average survival at the T3 compared to the T0 was 70%, but with large variation between baskets (24% - 96%). Lowest survival was in D04, basket 34 (24%) as well as D05, basket 37 (26%) and D07, basket 24 (31%).
What is remarkable, is that some oysters seem to be appearing or missing. During the T1, oysters were missing in 14 out of 32 baskets. It was then assumed that some oysters might have been lost from their nets in the transport tank. In the T3, it was sometimes obvious that there were degraded or broken empty flat oyster shells in the baskets, which explains some missing ones, but it was impossible to estimate how many flat oysters these were originally. Since a number of baskets contained relatively large North Sea crabs, it is assumed that one major cause of oyster death was predation. However, this could have also happened due to other predators such as starfish. Moreover, crabs were also found in baskets with a relatively high survival rate, which indicates that their presence in a basket does not necessarily mean a high mortality in oysters.

The size and quality of the flat oysters indicate that the study set up at T0 was adequate. Measurements of the size of the oysters at the T3 have shown that growth is clearly present, especially in length, but also in width. There are small differences in growth between the tables at the different monopiles.

Do the flat oysters stay free of the parasite Bonamia?
There was no Bonamia detected in the oysters during the T3 during the inspection of oysters for lesions. Currently, no Bonamia has been seen in this offshore region.

Do the flat oysters produce larvae? Do the larvae become spat?
In only two out of eight locations where water samples were taken, were D-larvae present at the time of sampling. In only one sample (H05, July) was the presence of *Ostrea* indicated by DNA analysis. An explanation for this low number might be that most oysters had not spawned yet, which is further supported by the gonad development. It could also be that the oysters were in between spawning events, as this could happen multiple times a year.

Oyster spat was found on seven of the fourteen suitable collectors recovered from the oyster tables. The specimens that were found were approximately a few months - 2 years old, following the growth rates mentioned in Richardson et al. (1993), Smyth et al. (2023) and from the KOPON workgroup (Dubbeldam, 2023).

Inside the oyster baskets, spat was also found, mostly attached to larger oysters. The total number found ranged from 19-33 individuals per table. Ten loose specimens could be measured since they were not attached. They ranged from 44- 63 mm in length, indicating they are probably 1-2 years old. Although the abundances of spat in the baskets were not very high, this is evidence that the oysters seem to be reproducing and that the oysters are able to settle. Combined with the results of the spat collectors, it seems that the young oysters prefer other oysters to settle on. The spat collectors with the mixed shell material contained no spat in comparison to the spat collectors with oyster shells inside, although only 2 collectors with enough mixed shell material were left, compared to 12 with oyster material. Therefore, we surmise that spat collectors comprising mixed shells are not suitable.

Do oyster larvae settle on different scour protection substrates (coarse armour layer, filter layer and empty shells)? Do they have a preference for substrate type or is there a difference in relation to the current direction? Can the oyster spat and/or larvae also be found in the control zone?

The ROV video survey revealed that flat oysters are settling on scour protection, the monopile and empty shells. There were not enough observations to differentiate preferences between substrates. Young flat oysters were also seen in the videos of the reference area or control zone, site IV. It is possible that the oysters in this region originate from flat oysters living on other nearby hard substrates such as wrecks. Field observations from a wreck diver confirm the presence of flat oysters in this region of the southern North Sea (Olie, 2024). It is therefore recommended to further analyze future spat to determine the genetic origin of the oysters in both site III and IV of the Borssele wind farm.

How is biodiversity in general developing on the different substrate variations?
How fast does succession of a hard substrate community occur in the Borssele area?

It becomes clear from the results that there is a development in biodiversity and species composition over time (2020-2023). The total number of taxa found in the photo and video analyses increased from 65 and 66 in the T0 and T1 to 74 taxa in the T3. This development can also be seen from the multivariate analyses (nmds, SIMPER, ANOSIM and PERMANOVA), and in the species cover data (converted SACFOR). There are indications of differences between Borssele site III and IV, monopiles, years, scour types and current directions. However, most of these differences account for a very small part of the variation in species composition. From these differences scour type is most clear, since it explained the largest part in the species composition from the tested factor. However, a large part of variance in the species composition remains unexplained, possibly from (abiotic or biotic) variables that could not be measured. It did become clear from the results of the photo analysis that the sand cover was much larger on the filter grade scour type. This has resulted is a somewhat different species composition when compared to the coarse grade scour type, which has a more complex structure and hence more or different species. And this has also resulted in a clearly higher species cover in the coarse grade compared to the filter grade scour type. The analyses indicate that in the T3 the species composition might slowly become more complex, which could be expected at the current age of the structures. Analyses of the numerical SACFOR scores (indicating cover) have resulted in the impression that the sites have all become more populated in comparison with the T0 (cover is increasing towards T3), although the difference between the T1 and the T3 is less apparent. There are also differences in the most abundant taxa over the measurement years. This is most likely due to year-to-year variation, but it could also be explained in part by the month of monitoring. For example, *Tubularia indivisa* was abundant during the T1, which was in the beginning of July, however by late August (T3) and October (T0) this species has lost its polyps, which leads to a decrease in the measured surface area. In later months this species is also almost fully covered by *Jassa herdmani* and/or *Monocorophium acherusicum*.

Reflecting on the scrape samples that have been taken and analysed in the T1 and T3, the total number of species is relatively high when compared with species lists of windfarms. E.g. on both the scour protection and the monopiles of the Princess Amalia windpark, 88 species were found after a longer period of six years, in a larger number of 48 samples, while in other windfarms a lower number species were found (Vanagt & Faasse, 2013). Combined, 77 species were found during the T1 and T3 in 8 samples. These samples are qualitative in nature, taken

in order to gain a better overview of possible colonisation by organisms on the tables and inside the baskets, and therefore cannot be compared to other samples which have taken quantitative samples in this manner.

Is the species data found by ROV (remotely operated vehicle) video and photo analyses correlated to the eDNA data? Which species are not found or only found by a certain method?
The video analysis and scrape samples seem most suitable for detecting the presence and absence of species (Table 15). However, both these methods as used here are not suitable for indicating abundance or cover of species. The cover as analysed in this study is also not ideal, as the surface area analysed in each photo is not uniform, but it is the best available method, as conditions for monitoring are difficult with uneven surfaces and inside a wind farm. One other option would be to use a photo taken with a dropcam, but this would make the species recognition more difficult. The combination of both image methods with the scrape samples lead to the identification of 116 taxa. The eDNA analysis added another 12 fish taxa. On top of that, several additional taxa were found in the spatcollectors, which added another 9 taxa to the list. In Appendix 6.4 the full species list of the T3 can be found with the taxa found per monopile for each of the methods. So, while the comparison of results from photo-, video-, eDNA-analysis and scrape samples indicated some overlap, all types of analyses largely accounted for unique occurrences of taxa, showing the complementarity of all methods and the applicability of results in species composition analysis.

Table 15. Number of taxa found in the T3 by each method and the cumulative number of taxa added by each method.

	number of taxa	cum. nr. of taxa
photo analyses	35	35
video analyses	58	74
scrape samples	57	116
eDNA	19	128

The eDNA analysis was specifically used for detecting fish species, since these are relatively hard to detect from the other analysis due to their mobile nature. However, although the photo analysis hardly contributed to finding fish species, as well as the scrape samples, the video and eDNA analyses were largely complementary to each other. Of the total 31 fish taxa detected by video and eDNA, only 5 taxa were in common between the methods in the T3. The video and eDNA methods therefore resulted in respectively 12 and 14 unique taxa.

The eDNA species lists of 2022 and 2023 differ in the number of fish species found (33 vs. 20 species). Potentially, the main reason for this could be the poor quality of the 2023 samples. There were a lot of bacteria in these samples, so the PCR was less successful. The reason for this is unclear, as samples have been taken and treated according to the same protocol. A suggestion might be an (degrading) algae bloom in the water.
The flat oyster specific eDNA analyses showed the presence of flat oysters in both sites of the wind farm in the T3. The reason that these results are better than those from the extra samples taken in 2022, when no oyster presence was found, could be the newer primer. The first primer that was used was 600 base pairs long, while the new one is 250 base pairs. This results in a more sensitive primer that can better pick up the signal of flat oysters in environmental DNA. It could also be that there was less DNA in the water at the end of September (2022), compared to July (2023), because of less larvae in the water column. As no larvae samples were taken in 2022, this cannot be verified.

4.1 Conclusions

The oysters placed at Borssele III show good survival and growth after three years on the oyster tables. All live oysters were fit; no Bonamia-infection has been detected and most of the inspected oysters were getting ready to reproduce. At the time of the T3 survey, they did not seem to have recently spawned, because very little pelagic oyster larvae were found. However, in 120 specimens of Flat oyster spat were found, in the baskets (109) and spatcollectors (11) combined. They ranged in age from a few months to two years old.
Not only inside the oyster baskets, attached to the adults, and in the spatcollectors, but also on the monopile, on the scour protection rocks and on the sea bottom in the installed shell area, Flat oysters were found. They were seen at Borssele III (D-string), but also at Borssele IV (H-string), where no oyster tables were present. In the coming years the growth of yet established spat and coming spawning events will hopefully lead to larger and more adult flat oysters that will be noticeable in the T8 survey in 2028.

In total, 137 taxa (species and higher groups, such as family) were found during the T3, from all different methods combined: photo analysis, video analysis, eDNA analysis, scrape samples and . These methods were largely additive to each other in terms of the specific taxa that were found, where eDNA and video analysis were both best suitable to detect fish species.
From the T0 to the T3 there has been a development in species composition and also species cover seems to be increasing over the years. This development seems to be going hand in hand with scour type, which had a relatively large effect on species composition and -cover. In the T3, the species community is getting more complex when compared to the T0 and T1. This is consistent with the succession from a pioneer community with fewer dominant species towards the development of a more intermediate stage of the species community. The survey at the T8 in 2028 will have to show whether the species community will become even more mature.

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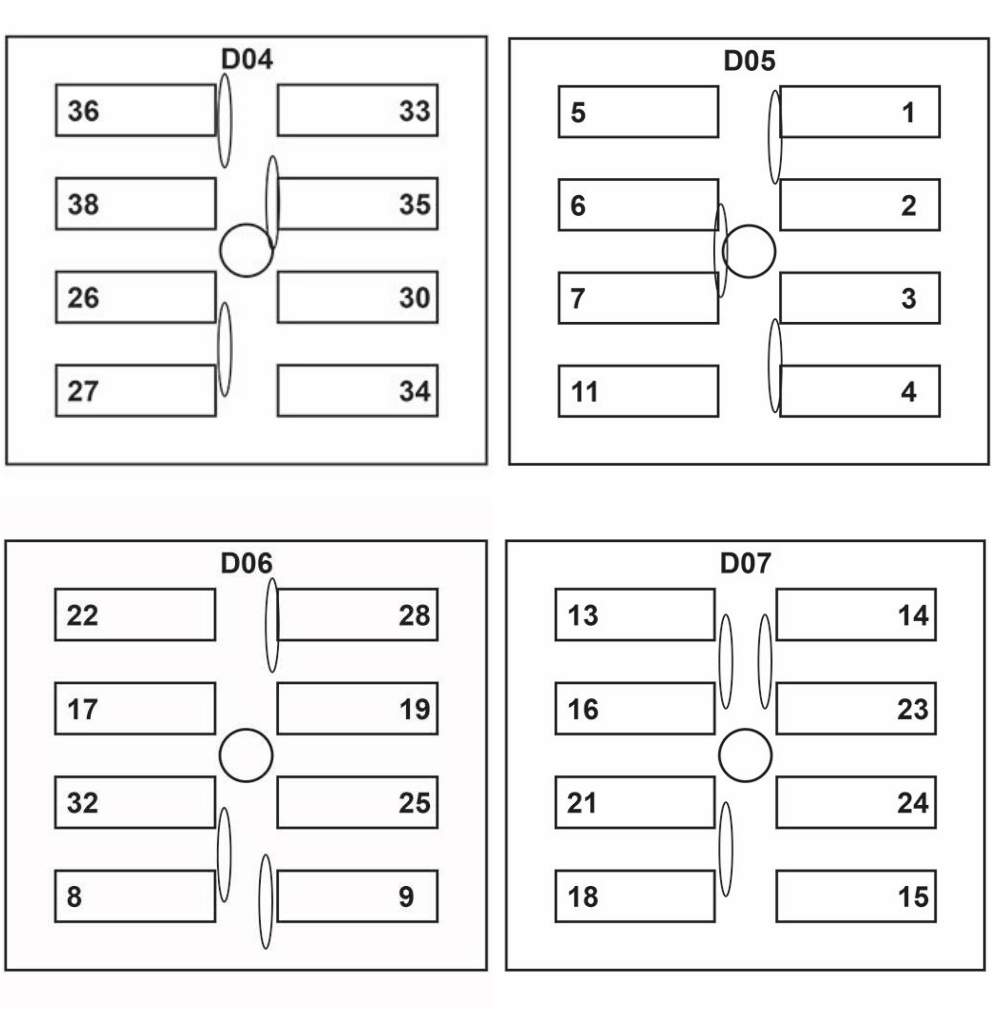
6 Appendices

6.1 Oyster table layout

Numbers corresponding to the baskets. Ellipses are the spat collectors. The number of basket 7 was replaced to 37 during the T1.



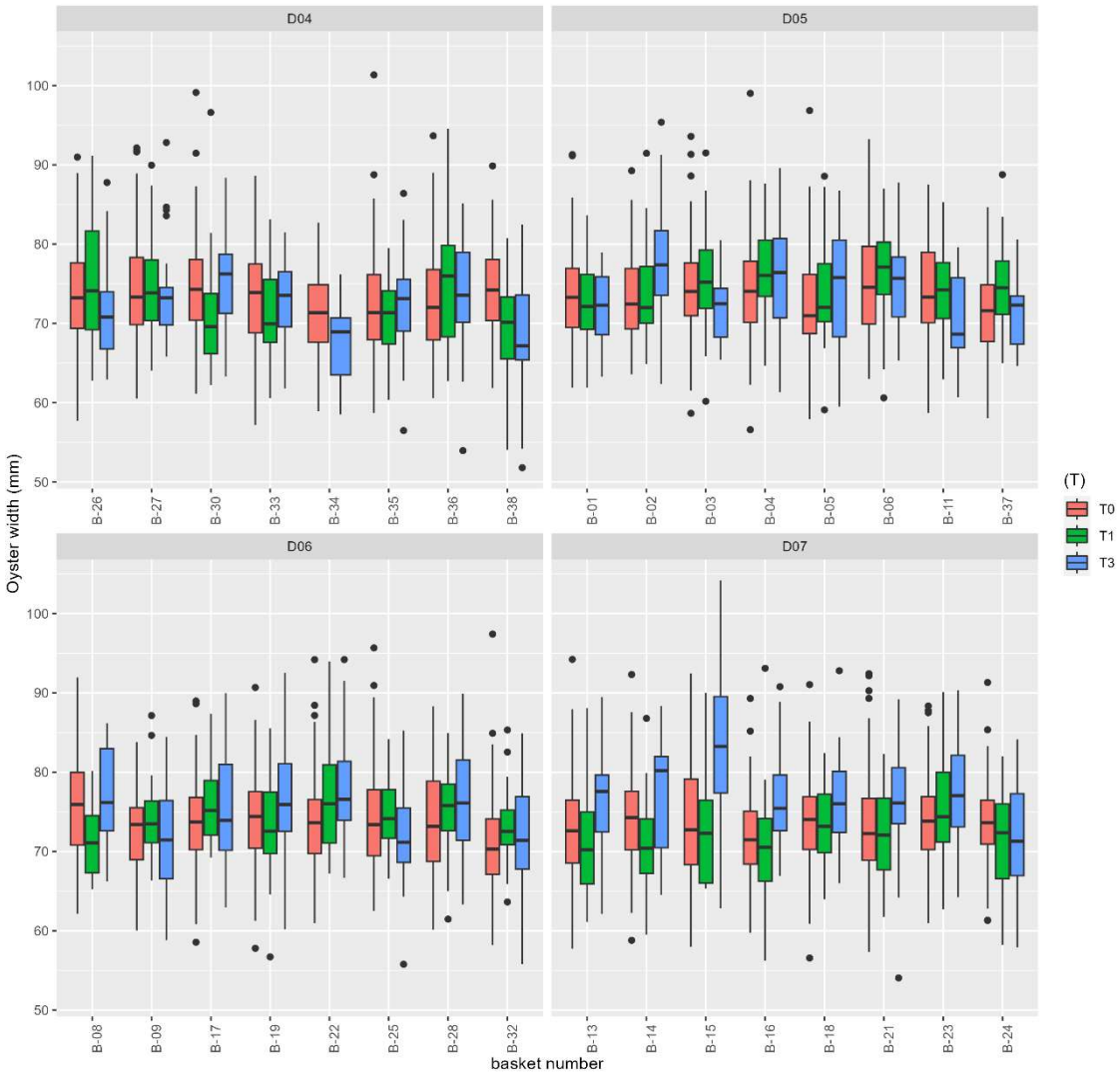
6.2 List of taxa found on scrape samples on oyster tables in the T1 and T3.

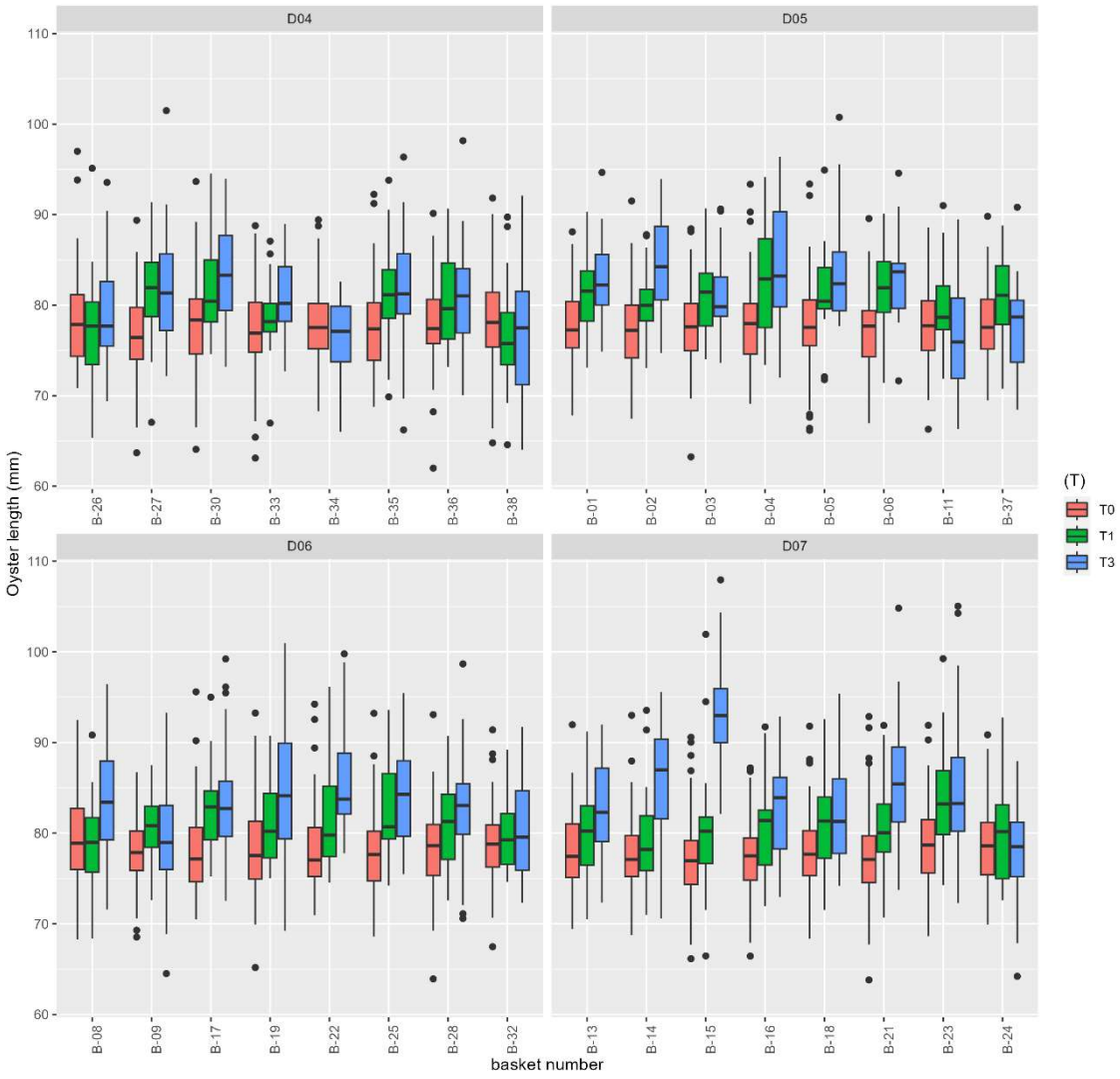
Numbers refer to the number of tables the species is found (max. 4). Nomenclature according to TWN ("Taxa Waterbeheer Nederland")

Group	Taxon	2021	2023
Annelida/Platyhelminthes - Polychaeta	*Chaetopterus variopedatus*	2	
Annelida/Platyhelminthes - Polychaeta	*Eumida*	3	
Annelida/Platyhelminthes - Polychaeta	*Eunereis longissima*	2	
Annelida/Platyhelminthes - Polychaeta	*Gattyana cirrhosa*	1	
Annelida/Platyhelminthes - Polychaeta	*Harmothoe extenuata*	3	
Annelida/Platyhelminthes - Polychaeta	*Harmothoe*		1
Annelida/Platyhelminthes - Polychaeta	*Lagis koreni*	2	
Annelida/Platyhelminthes - Polychaeta	*Lanice conchilega*	1	
Annelida/Platyhelminthes - Polychaeta	*Nereididae*	1	1
Annelida/Platyhelminthes - Polychaeta	*Phyllodoce groenlandica*	1	1
Annelida/Platyhelminthes - Polychaeta	*Sabellaria spinulosa*	3	4
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus lamarcki*		4
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus*	2	
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus triqueter*		1
Annelida/Platyhelminthes - Polychaeta	*Syllis*		1
Annelida/Platyhelminthes - Turbellaria	*Leptoplana tremellaris*	1	
Bryozoa - Hydrozoa - Porifera	*Alcyonidium mytili*		1
Bryozoa - Hydrozoa - Porifera	*Amphiblestrum auritum*		2
Bryozoa - Hydrozoa - Porifera	*Callopora dumerilii*		1
Bryozoa - Hydrozoa - Porifera	*Cliona celata*		1
Bryozoa - Hydrozoa - Porifera	*Clytia hemisphaerica*		3
Bryozoa - Hydrozoa - Porifera	*Conopeum reticulum*		1
Bryozoa - Hydrozoa - Porifera	*Ectopleura larynx*	2	2
Bryozoa - Hydrozoa - Porifera	*Electra pilosa*	4	3
Bryozoa - Hydrozoa - Porifera	*Hydractinia echinata*		1
Bryozoa - Hydrozoa - Porifera	*Obelia bidentata*		1
Bryozoa - Hydrozoa - Porifera	*Porifera*		2
Bryozoa - Hydrozoa - Porifera	*Tubularia indivisa*	4	4
Crustacea - Amphipoda	*Jassa herdmani*	4	4
Crustacea - Amphipoda	*Monocorophium acherusicum*	4	4
Crustacea - Amphipoda	*Nototropis swammerdamei*		1
Crustacea - Amphipoda	*Phthisica marina*	2	2
Crustacea - Amphipoda	*Stenothoe marina*	1	3
Crustacea - Amphipoda	*Stenothoe monoculoides*	1	4
Crustacea - Amphipoda	*Stenothoe valida*	4	2
Crustacea - Decapoda	*Alpheus macrocheles*		1
Crustacea - Decapoda	*Athanas nitescens*	1	3
Crustacea - Decapoda	*Eualus cranchii*		1
Crustacea - Decapoda	*Hippolyte varians*		1

Crustacea - Decapoda	*Necora puber*		1
Crustacea - Decapoda	*Pilumnus hirtellus*	1	3
Crustacea - Decapoda	*Pisidia longicornis*	4	4
Crustacea - Remaining	*Balanus crenatus*	3	
Crustacea - Remaining	*Megabalanus coccopoma*		1
Crustacea - Remaining	*Perforatus perforatus*		2
Crustacea - Remaining	*Verruca stroemia*		1
Echinodermata	*Asterias rubens*	4	4
Echinodermata	*Ophiothrix fragilis*	2	4
Echinodermata	*Psammechinus miliaris*	1	1
Fish	*Ciliata mustela*		1
Fish	*Pholis gunnellus*		1
Marine - Remaining	*Cylista troglodyta*		1
Marine - Remaining	*Metridium senile*		4
Marine - Remaining	*Sagartia troglodytes*	1	
Marine - Remaining	*Sagartia undata*		2
Marine - Remaining	*Alcyonium digitatum*	2	
Marine - Remaining	*Ascidiella aspersa*	1	
Marine - Remaining	*Ciona intestinalis*	1	
Marine - Remaining	*Diplosoma listerianum*	3	1
Marine - Remaining	*Styela clava*	1	
Marine - Remaining	*Lineus bilineatus*	1	1
Marine - Remaining	*Oerstedia dorsalis*	2	1
Mollusca - Bivalvia	*Abra alba*	2	
Mollusca - Bivalvia	*Aequipecten opercularis*	1	
Mollusca - Bivalvia	*Heteranomia squamula*	3	1
Mollusca - Bivalvia	*Mytilus edulis*	1	4
Mollusca - Bivalvia	*Ostreidae*		1
Mollusca - Gastropoda	*Crepidula fornicata*	2	4
Mollusca - Gastropoda	*Epitonium clathratulum*		1
Mollusca - Gastropoda	*Euspira nitida*		1
Mollusca - Gastropoda	*Nudibranchia*		1
Mollusca - Gastropoda	*Tritia*		1
Mollusca - Gastropoda	*Trivia arctica*	1	
Mollusca - Gastropoda	*Trivia monacha*	1	2
Mollusca - Gastropoda	*Aeolidia/Aeolidiella*		1
Mollusca - Gastropoda	*Dendronotus europaeus*	1	
Mollusca - Remaining	*Sepiola atlantica*	1	

6.3 Boxplots of all measured individuals per measurement year (T0, T1, T3)




D04
D05
D06
D07
Oyster length (mm)
basket number
(T)
T0
T1
T3
B-26
B-27
B-30
B-33
B-34
B-35
B-36
B-38
B-01
B-02
B-03
B-04
B-05
B-06
B-11
B-37
B-08
B-09
B-17
B-19
B-22
B-25
B-28
B-32
B-13
B-14
B-15
B-16
B-18
B-21
B-23
B-24

6.4 Comparison of taxa found in photo analysis (P), video analysis (V), scrape samples (S) and eDNA samples in the T3 per monopile.

x*: taxon found on/ in spatcollectors. Nomenclature according to TWN ("Taxa Waterbeheer Nederland")

		D04				D05				D06				D07				H02				H03				H04				H05			
Taxon group	**Taxon**	**P**	**V**	**S**	**E**	**P**	**V**	**S**	**E**	**P**	**V**	**S**	**E**	**P**	**V**	**S**	**E**	**P**	**V**	**E**	**P**	**V**	**E**	**P**	**V**	**E**	**P**	**V**	**E**	**P**	**V**	**E**
Annelida/Platyhelminthes - Hirudinea	*Nemertesia antennina*		x											x	x				x			x	x		x	x		x	x				
Annelida/Platyhelminthes - Polychaeta	*Chaetopterus variopedatus*			x*				x*							x	x*																	
Annelida/Platyhelminthes - Polychaeta	*Harmothoe*			x																													
Annelida/Platyhelminthes - Polychaeta	*Lanice conchilega*	x	x			x	x			x	x				x			x	x		x	x		x	x		x	x				
Annelida/Platyhelminthes - Polychaeta	*Lepidonotus squamatus*							x*																									
Annelida/Platyhelminthes - Polychaeta	*Nereididae*															x																	
Annelida/Platyhelminthes - Polychaeta	*Phyllodoce groenlandica*							x																									
Annelida/Platyhelminthes - Polychaeta	*Sabellaria spinulosa*	x		x		x		x				x				x																	
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus lamarcki*			x												x																	
Annelida/Platyhelminthes - Polychaeta	*Spirobranchus triqueter*		x				x				x				x	x			x			x			x			x				
Annelida/Platyhelminthes - Polychaeta	*Syllis*											x																					
Bryozoa - Hydrozoa - Porifera	*Alcyonidium mytili*			x																													
Bryozoa - Hydrozoa - Porifera	*Amphiblestrum auritum*											x				x																	
Bryozoa - Hydrozoa - Porifera	*Biflustra tenuis*															x*																	
Bryozoa - Hydrozoa - Porifera	*Bryozoa*	x	x			x	x															x	x					x	x				
Bryozoa - Hydrozoa - Porifera	*Callopora dumerilii*											x*				x																	
Bryozoa - Hydrozoa - Porifera	*Cliona celata*			x																													
Bryozoa - Hydrozoa - Porifera	*Clytia hemisphaerica*			x								x				x																	
Bryozoa - Hydrozoa - Porifera	*Conopeum reticulum*			x																													
Bryozoa - Hydrozoa - Porifera	*Ectopleura larynx*							x								x																	
Bryozoa - Hydrozoa - Porifera	*Electra pilosa*							x				x				x																	
Bryozoa - Hydrozoa - Porifera	*Hydractinia echinata*		x	x			x				x				x				x			x	x			x						
Bryozoa - Hydrozoa - Porifera	*Hydrozoa*	x				x				x				x				x			x			x			x	x				
Bryozoa - Hydrozoa - Porifera	*Leucosolenia variabilis*							x*																									
Bryozoa - Hydrozoa - Porifera	*Obelia*		x				x																										
Bryozoa - Hydrozoa - Porifera	*Obelia bidentata*	x				x						x		x				x						x									
Bryozoa - Hydrozoa - Porifera	*Obelia dichotoma*										x				x				x			x			x			x					
Bryozoa - Hydrozoa - Porifera	*Obelia longissima*										x																						
Bryozoa - Hydrozoa - Porifera	*Porifera*			x			x				x	x			x				x						x			x					

Taxon group	Taxon	D04				D05				D06				D07				H02				H03				H04				H05			
		P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	E	P	V	E	P	V	E	P	V	E	P	V	E
Bryozoa - Hydrozoa - Porifera	*Protosuberites denhartogi*		x																											x		
Bryozoa - Hydrozoa - Porifera	*Sertularia cupressina*	x				x																										
Bryozoa - Hydrozoa - Porifera	*Suberites*		x																													
Bryozoa - Hydrozoa - Porifera	*Suberitida*										x																					
Bryozoa - Hydrozoa - Porifera	*Tubularia indivisa*	x	x	x		x	x	x		x	x	x		x	x	x		x	x		x	x		x	x		x	x				
Crustacea - Amphipoda	*Caprellidae*		x																													
Crustacea - Amphipoda	*Jassa herdmani*	x		x		x		x		x		x		x		x		x			x			x			x					
Crustacea - Amphipoda	*Monocorophium acherusicum*			x				x				x				x																
Crustacea - Amphipoda	*Nototropis swammerdamei*							x																								
Crustacea - Amphipoda	*Stenothoe marina*			x								x				x																
Crustacea - Amphipoda	*Stenothoe monoculoides*			x				x				x				x																
Crustacea - Amphipoda	*Stenothoe valida*							x								x																
Crustacea - Decapoda	*Alpheus macrocheles*											x																				
Crustacea - Decapoda	*Atelecyclus rotundatus*										x																					
Crustacea - Decapoda	*Athanas nitescens*			x				x				x																				
Crustacea - Decapoda	*Cancer pagurus*	x	x			x	x			x	x			x	x			x	x		x	x		x	x		x	x				
Crustacea - Decapoda	*Eualus cranchii*							x																								
Crustacea - Decapoda	*Hippolyte varians*											x																				
Crustacea - Decapoda	*Homarus gammarus*						x				x				x				x													
Crustacea - Decapoda	*Liocarcinus*		x								x				x				x			x			x			x				
Crustacea - Decapoda	*Liocarcinus holsatus*													x																		
Crustacea - Decapoda	*Maja brachydactyla*																													x		
Crustacea - Decapoda	*Necora puber*	x	x			x	x			x	x			x	x	x		x	x		x	x		x	x		x	x				
Crustacea - Decapoda	*Pagurus bernhardus*		x							x	x																					
Crustacea - Decapoda	*Pilumnus hirtellus*			x				x				x																				
Crustacea - Decapoda	*Pisidia longicornis*			x				x				x				x											x					
Crustacea - Decapoda	*Porcellana platycheles*																													x		
Crustacea - Remaining	*Balanus crenatus*																													x		
Crustacea - Remaining	*Balanus perforatus*	x																												x		
Crustacea - Remaining	*Malacostraca*													x																		
Crustacea - Remaining	*Sessilia*																														x	
Crustacea - Remaining	*Verruca stroemia*			x																												
Crustacea- Remaining	*Megabalanus coccopoma*							x																								
Crustacea- Remaining	*Perforatus perforatus*											x				x																

Taxon group	Taxon	D04				D05				D06				D07				H02				H03				H04				H05			
		P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	E	P	V	E	P	V	E	P	V	E	P	V	E
Crustacea- Remaining	*Phthisica marina*											x				x																	
Echinodermata	*Amphipholis*											x*																					
Echinodermata	*Asterias rubens*	x	x	x		x	x	x		x	x	x		x	x	x		x	x		x	x		x	x		x	x				
Echinodermata	*Ophiothrix fragilis*			x				x				x				x																
Echinodermata	*Ophiotrix fragilis*										x																						
Echinodermata	*Psammechinus miliaris*			x							x																						
Fish	*Actinopteri*																				x											
Fish	*Ammodytes marinus*																x			x												x
Fish	*Ammodytidae*																		x													
Fish	*Arnoglossus laterna*								x																							
Fish	*Callionymus*										x											x			x				x			
Fish	*Callionymus lyra*	x																	x	x	x	x		x			x	x				
Fish	*Callionymus reticulatus*																							x					x			
Fish	*Chelon auratus*																								x							
Fish	*Ciliata mustela*			x																												
Fish	*Conger conger*	x									x				x																	
Fish	*Cottidae*										x				x				x						x							
Fish	*Echiichthys vipera*				x																					x						
Fish	*Engraulis encrasicolus*				x				x								x															
Fish	*Gadus morhua*						x																									
Fish	*Gobius niger*																			x			x			x						
Fish	*Merlangius merlangus*				x				x				x				x															
Fish	*Microstomus kitt*																		x													
Fish	*Mullus sermuletus*																		x												x	
Fish	*Mullus surmuletus*																									x						
Fish	*Myoxocephalus scorpius*														x				x												x	
Fish	*Parablennius gattorugine*	x	x			x	x				x				x				x		x	x			x		x	x				
Fish	*Pegusa lascaris*												x																			
Fish	*Pholis gunnellus*											x			x				x													
Fish	*Pleuronectes platessa*				x								x							x			x								
Fish	*Pomatoschistus*		x				x				x								x			x			x				x		
Fish	*Pomatoschistus microps*	x																						x								
Fish	*Pomatoschistus minutus*	x			x																			x			x					
Fish	*Sardina pilchardus*				x				x				x				x			x			x			x						x

Taxon group	Taxon	D04				D05				D06				D07				H02				H03				H04				H05			
		P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E
Fish	*Scomber scombrus*				x				x								x									x							
Fish	*Taurulus bubalis*										x				x								x										
Fish	*Trachinus draco*																			x											x		
Fish	*Trachurus trachurus*		x		x		x		x				x				x		x	x			x					x			x		x
Fish	*Trisopterus luscus*		x		x	x	x		x	x	x			x	x		x	x	x		x	x		x	x		x	x				
Fish	*Trisopterus minutus*		x		x						x			x	x							x						x				
Marien - Remaining	*Actinothoe sphyrodeta*	x	x			x	x			x	x			x	x			x	x		x	x		x	x		x	x				
Marien - Remaining	*Alcyonium digitatum*			x*																													
Marien - Remaining	*Cylista troglodyta*															x																	
Marien - Remaining	*Anthozoa*													x																			
Marien - Remaining	*Cylista elegans*		x				x				x				x				x				x				x				x		
Marien - Remaining	*Cylista troglodytes*		x				x				x				x				x				x				x				x		
Marien - Remaining	*Diadumene cincta*																	x	x								x						
Marien - Remaining	*Diplosoma listerianum*											x										x											
Marien - Remaining	*Folliculinidae*											x*																					
Marien - Remaining	*Lineus bilineatus*											x																					
Marien - Remaining	*Metridium dianthus*	x	x	x		x	x	x		x	x	x		x	x	x		x	x		x	x			x			x					
Marien - Remaining	*Mnemiopsis leidyi*		x				x				x				x				x			x						x					
Marien - Remaining	*Oerstedia dorsalis*															x																	
Marien - Remaining	*Sagartia undata*			x												x																	
Marien - Remaining	*Urticina felina*										x				x																		
Mollusca - Bivalvia	*Anomiidae*			x*				x*				x*				x*																	
Mollusca - Bivalvia	*Heteranomia squamula*							x																									
Mollusca - Bivalvia	*Hiatella arctica*							x*																									
Mollusca - Bivalvia	*Kellia suborbicularis*											x*																					
Mollusca - Bivalvia	*Mytilus edulis*	x	x	x			x	x			x	x			x	x		x	x														
Mollusca - Bivalvia	*Ostrea edulis*										x				x												x				x		
Mollusca - Bivalvia	*Ostreidae*			x																													
Mollusca - Bivalvia	*Spisula subtruncata*										x																						
Mollusca - Bivalvia	*Striarca lactea*											x*																					
Mollusca - Gastropoda	*Crepidula fornicata*		x	x			x	x			x	x			x	x			x				x				x				x		
Mollusca - Gastropoda	*Epitonium clathratulum*			x																													
Mollusca - Gastropoda	*Euspira nitida*											x																					
Mollusca - Gastropoda	*Nudibranchia*											x																					

Taxon group	Taxon	D04				D05				D06				D07				H02				H03				H04				H05			
		P	V	S	E	P	V	S	E	P	V	S	E	P	V	S	E	P	V	E	P	V	E	P	V	E	P	V	E	P	V	E	
Mollusca - Gastropoda	*Tritia*											x																					
Mollusca - Gastropoda	*Trivia monacha*			x				x																									
Mollusca - Gastropoda	*Aeolidia/Aeolidiella*											x																					
Mollusca - Remaining	*Alloteuthis media*		x																														
Mollusca - Remaining	*Sepia officinalis*		x				x																										
Mollusca - Remaining	*Sepiola atlantica*		x																														

6.5 Taxon list of the ROV surveys during the T0 (2020), T1 (2021) and T3 (2023)

Frequency of taxon occurrence per year per monopile in photo analyses. The frequency represents the presence of a taxon in a photo. Nomenclature according to TWN ("Taxa Waterbeheer Nederland")

Monopile	D04			D05			D06			D07			H02			H03			H04			H05		
Measurement year	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3
Max. frequency	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*20*	*21*	*21*	*20*	*21*	*21*	*20*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*
Actinopteri											1							1	1					
Actinothoe sphyrodeta			3			7	1		3	1		2			8			9			4			11
Alcyonium digitatum											1													
Anthozoa												1												
Ascidiacea																			5			2		
Asterias rubens	18	17	12	21	15	17	18	13	4	7	6	15	18	9	8	16	14	10	19	20	3	15	6	4
Balanidae							3												10					
Balanus crenatus																								1
Balanus perforatus			1																					1
Bryozoa			1	2		2	1						1	1		1		2				5		1
Callionymus lyra			1		2									2				1			3			1
Callionymus maculatus		1																						
Callionymus reticulatus										1											1			
Cancer pagurus		5	1	1	1	1	1	1	1	2		1		1	2		2	6			5	2		4
Cliona celata																				1				
Conger conger			1																					
Cottidae											1													
Cyanea lamarckii																								
Cylista elegans		6	4	3	3	6	6	3	5	2	4	6		3	7	4	2	10	1	5	10	1	2	9
Cylista troglodytes	20	16	15	21	19	16	18	17	18	21	20	13	17	14	16	15	15	16	19	21	18	19	21	15
Diadumene cincta															1									

Monopile	D04			D05			D06			D07			H02			H03			H04			H05		
Measurement year	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**	**T0**	**T1**	**T3**
Max. frequency	***21***	***21***	***21***	***21***	***21***	***21***	***21***	***21***	***21***	***20***	***21***	***21***	***20***	***21***	***21***	***20***	***21***	***21***	***21***	***21***	***21***	***21***	***21***	***21***
Diplosoma listerianum	1												6			5		1	10	6		6		
Echiichthys vipera																	1							
Echinocardium cordatum																								
Ectopleura larynx	2	1			3									9			10			3			6	
Gobius niger	1																							
Hydractinia echinata																	1	2						
Hydrozoa			5	2	1	9	11		7	1	1	10		1	8	1		7	7	2	2	4	3	8
Jassa herdmani / Monocorophium acherusicum	6	4	5		1	17		1	4	1	3	11	1		9			11		9	11	1	4	13
Lanice conchilega		3	3		9	2		6	9		5			3	11		4	8		4	10			7
Limanda limanda														1										
Liocarcinus holsatus												1												
Loligo vulgaris																			1					
Macropodia rostrata																1								
Maja brachydactyla													1									2		
Malacostraca												1												
Metridium dianthus	4	3	6	2	1	4	8	8	6	8	7	6	5	5	2	7	2	3	1	1		2	7	
Mullus surmuletus													1											
Myoxocephalus scorpius							2			2				1					2			1		
Mytilus edulis			1					2							2				1					
Necora puber	8	3	10	11	12	4	4	10	6	16	11	6	2	7	13	2	3	11	2	10	8	1	10	8
Nemertesia antennina											1	4	3			2	1	1			1	2	3	4
Obelia bidentata	2		3	6		1	8	1		3		1	6		2	5			5		4	6		
Ophiothrix fragilis																								
Ophiura albida																						1		
Ostrea edulis										1														

Monopile	D04			D05			D06			D07			H02			H03			H04			H05		
Measurement year	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3
Max. frequency	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*20*	*21*	*21*	*20*	*21*	*21*	*20*	*21*	*21*	*21*	*21*	*21*	*21*	*21*	*21*
Pagurus bernhardus									1								1							
Parablennius gattorugine			1			1					1							3		1			1	1
Pholis gunnellus								1																
Pisidia longicornis																					1			
Pomatoschistus microps			3													2					1			
Pomatoschistus minutus			2																		2			1
Porcellana platycheles																								1
Porifera																								
Psammechinus miliaris							7	1												1				
Sabellaria spinulosa			1			1																		
Sagartia undata		1																						
Sepia officinalis																								
Sertularia cupressina			6			2																		
Spirobranchus triqueter	16	7	16	11	9	16	14	7	9	18	14	18	13	14	11	16	13	10	10	15	13	18	21	15
Taurulus bubalis					1						1			2			1							
Trisopterus luscus	3			3		10	3		7	4		9	1		2	1		2	1		6	1		2
Trisopterus minutus												4												
Tubularia indivisa	6	19	3	1	17	5	5	19	3	4	19	7		18	4	1	21	6		19	10		18	9
Urticina felina		1		1			1	1		1	1		1											

6.6 SACFOR-scale – adapted from Rijkswaterstaat protocol (2020) and assigned numerical values

	Growth form classes					
cover (%)	**crust/ meadow**	**massive/ turf**	**1-3 cm**	**3-15 cm**	**> 15 cm**	**numerical value**
> 80	S					90
40-79	A	S	S			60
20-39	C	A	A	S		30
10-19	F	C	C	A	S	15
5-9	O	F	F	C	A	7
1-5	R	O	O	F	C	3
0,1-1	RR	R	R	O	F	0.5
0,01-0,1	RRR	RR	RR	R	O	0.05
< 0.01					R	0.005
Taxon group	**Example taxa**					
Annelida	*Sabellaria*	*Lanice*	*Spirobranchus*			
Anthozoa		*Alcyonium*	*Actinothoe*	*Metridium, Sagartia, Urticina*		
Ascidiacea	*Diplosoma, Ascidiacea*					
Bryozoa	Bryozoa	*Electra pilosa*				
Crustacea	*Jassa/ Monocorophium*		*Macropodia, Balanidae, Porcellanidae*	*Necora, Liocarcinus, Pagurus, Malacostraca*	*Homarus, Cancer, Maja*	
Echinodermata				*Psammechinus, Ophiura, Ophiothrix, Echinocardium*	*Asterias*	
Hydrozoa		*Tubulariidae*		*Nemertesia*	*Obelia bidentata*	
Mollusca			*Mytilus*	*Ostrea edulis*	*Loligo, Sepia*	
Pisces				*Gobiidae, Blennidae, Mullus*	*Pleuronectiformes, Callionymus, Dicentrarchus, Gadidae, Trachurus, Cottidae, Actinopteri*	
Porifera	*Halichondria*				*Haliclona oculata*	

6.7 Fish species found in eDNA in 2022 and 2023

Species	2022	2023
Ammodytes marinus	x	x
Ammodytes personatus	x	
Arnoglossus laterna		x
Belone belone	x	
Buglossidium luteum	x	
Callionymus lyra		x
Callionymus reticulatus		x
Chelidonichthys sp./Eutriglia gurnardus		x
Chelidonichthys spinosus	x	
Chelon auratus		x
Chelon labrosus	x	
Clupea harengus	x	
Dicentrarchus labrax	x	
Echiichthys vipera		x
Engraulis encrasicolus	x	x
Entelurus aequoreus	x	
Gadus morhua	x	
Gobius niger	x	x
Hippoglossoides platessoides	x	
Hippoglossus hippoglossus	x	
Limanda limanda	x	
Merlangius merlangus	x	x
Mullus surmuletus	x	x
Parablennius gattorugine	x	
Pegusa lascaris	x	x
Platichthys stellatus	x	
Pleuronectes platessa	x	x
Pomatoschistus	x	
Pomatoschistus minutus	x	x
Sander lucioperca	x	
Sarda chiliensis	x	
Sardina pilchardus	x	x
Scomber scombrus	x	x
Solea solea	x	
Spondyliosoma cantharus	x	
Syngnathus rostellatus	x	
Trachinus draco		x
Trachurus japonicus	x	
Trachurus trachurus	x	x
Trisopterus luscus	x	x
Trisopterus minutus		x