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Monitoring of the nature enhancement project in offshore wind farm Borssele III/IV, T-1 2021

Scientific report

De Rijke Noordzee

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Author(s)	:	T. Schellekens, R. Olie, M. Faasse
Checked by	:	B. Dzon
Signature	:	
Approved by	:	A. de Beauvesère-Storm
Signature	:	
Contact	:	Eurofins Omegam B.V. Eurofins AquaSense H.J.E. Wenkebachweg 120 1114 AD Amsterdam-Duivendrecht Postbus 94685 1090 GR Amsterdam T +31 (0) 20 5976 680 www.aquasense.nl

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1 Introduction

The Dutch government has a responsibility for the state of the North Sea environment following from the EU's Marine Strategy Framework Directive and Habitat and Birds Directive. In order to utilize the momentum of the large-scale development of offshore wind farms, a commitment was explicitly included in the site decisions for developers. This enables them to contribute to the strengthening of 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, trying to contribute 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 (T-0). Also, living flat oysters were installed at the scour protection of four wind turbines in site III. The scour protection, consisting of different sizes of rock, will also partly be covered with shell material to create settlement places for flat oysters, during the T-1. This site, as well as site IV will be monitored for the development of the surrounding fauna at different scour-protections in 2021, 2023 and 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 that could (partly) be answered in the T-1 are:
1) What is the survival and growth rate of flat oysters?
2) Do the flat oysters produce larvae?
3) Do the flat oysters stay free of the parasite Bonamia?
4) How is biodiversity in general developing on the different substrate variations?
5) Are there species-specific responses in relation to current directions or rock size of the scour?
6) 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?
7) How fast does succession of a hard substrate community occur in the Borssele area?

The current report will describe the T-1 monitoring in 2021, its results in terms of description of the situation and a comparison with the T-0 baseline data (Schellekens et al., 2021). Methods are described in more detail in the next chapter, followed by the results and a discussion. This report does not, however, present conclusions on trends or effects of different treatments since this is only presentation of results of the T-1 monitoring, and hence too early to conclude on these results.

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 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. The next campaign (T-1) was executed in July 2021, where the status of the oysters (survival, growth, reproduction status and Bonamia status) as well as the biodiversity was investigated. An overview of the different measurements performed per monopile is provided in Table 1.

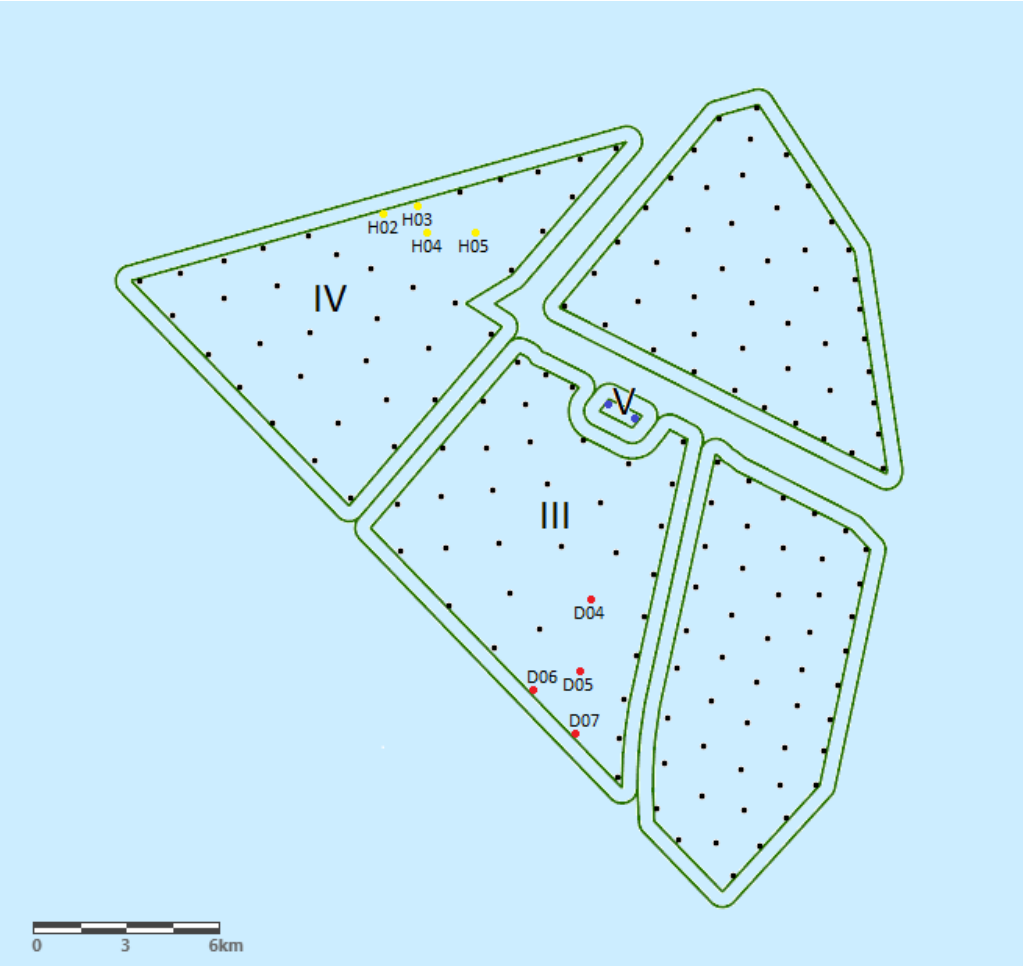

Figure 1. Overview of the Borssele offshore wind farm. Red dots concern the locations of the oyster installation (north to south: B334-D04, B337-D05, B328-D06 and B327-D07), the yellow dots are the reference locations (west to east: B412-H02, B411-H03, B402-H04 and B401-H05). The blue dots concern the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

*Table 1. overview of the activities performed per location. * At H02 and H03, originally 4*10 photos were taken.*

Sample locations	ROV	Watersamples	Oyster measurements	Spat collectors
D04 – D05 – D06 – D07	Video of oyster table and scour, 4*5 photos + 1 monopile	200 L for larvae, 2 L for eDNA	Per basket: 25 oysters and 1 oyster sampled	All 3 bags sampled and replaced with new ones
H02* – H03* – H04 – H05	Video of scour, 4*5 photos + 1 monopile	200 L for larvae, 2 L for eDNA	-	-

2.2 ROV surveys

The scour protection around each monopile was divided into four sections. The first division 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.

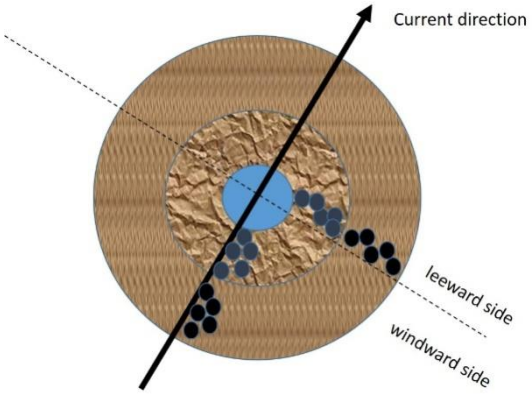

Figure 2. positioning of coarse and filter gradings around the monopiles, and the approximate positioning of photos (black dots in filter grade, gray dots in coarse grade).

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.

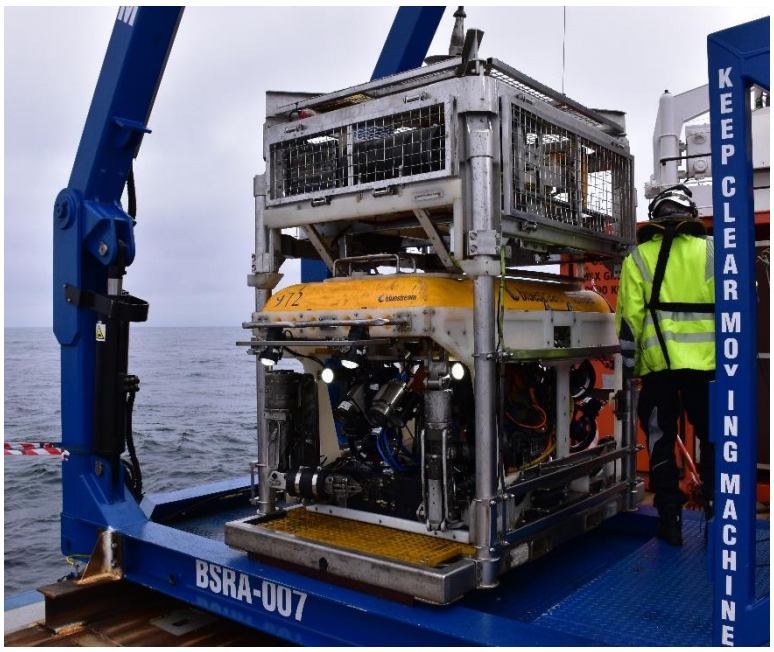

Figure 3. ROV setup with lights.

The following steps were taken during the surveys:

1) Using the ROV camera, a type of scour-protection was identified and visually confirmed.
2) Within the scour-protection type, five photos were randomly taken, but always viewing towards the monopile. To do this, the ROV landed on the sea floor. After a few seconds (when upwelled sediment had settled), the recording of the video was stopped for a moment to take a photo. After this, the recording continued. It was made sure that the photos did not have any overlap. The tilt and focus of the camera was adjusted, when needed.
3) Using sonar, the distance to the monopile was monitored while the ROV moved to the next location.
4) Each photo was checked for clarity and focus; such that individual fauna can be identified from the photos. The photos were inspected on a high resolution screen.
5) After taking the photo, the type of scour and the file-number was written down on a sampling-form along with the code for the monopile.

Photos were coded in the following way:
Monopile nr. – Current direction – Rock size – Photo number – Date – Time.

2.3 Oyster larvae and eDNA water sampling

Water samples for oyster larvae count and eDNA analysis were taken at each of the eight monopiles (Table 2). Water was pumped from about 2 m above the scour protection within 50m of the monopile using a submersible pump, into an IBC-tote of 600 liters. This was done with a long hose with weight attached near the end. The vessel moved downstream of the monopile and placed the crane with the pump in between the ROV and monopile. With this method, the ROV's sonar could see if the distance of the pump to the monopile was within the desired range. After that the ROV could safely approach to visually check if the height of the pump above the seabed was within 1-2 meters. The pump was placed about 10-15 meters downstream of the monopile, above the scour protection. The IBC-tote was marked at 100 liter intervals. The complete tote was filled and flushed to remove potential residue from a previous location. 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).

*Table 2. Overview of the 200L water samples for eDNA and oyster larvae. * = approximately, not noted*

Monopile	Date	Time (local)	Depth (m)
H02	02-07-21	01:18	27
H03	02-07-21	07:17	23
H04	02-07-21	12:20	29
H05	02-07-21	13:35	31.5
D04	03-07-21	06:40	28
D05	03-07-21	15:45	25*
D06	03-07-21	12:23	34
D07	04-07-21	03:10	30

Figure 4. Photos of the pump installation, pump and tank with markers and spout.

2.3.1 Larvae sample processing

A hard 200 µm sieve was used to filter 200 liters of seawater to collect oyster larvae. Material collected in the sieve was completely emptied into a 1 L sterile bottle using a clean funnel and a rinsing bottle with 96% ethanol. The sample was stored in the freezer (-20°C).

2.3.2 eDNA sample processing

During the oyster larvae sampling, 2 liters of seawater for eDNA analysis was collected into a sterile 2L jar, underneath the 200 µm sieve. Before every new location, all bottles, tweezers and other equipment was cleaned with 50% bleach solution. The eDNA-sample was collected on a sterile cup with a filter (0.2 µm) by passing the water sample through a funnel via the filter into a sterile 2 L vacuum flask. Also, latex unpowdered gloves were used throughout the sampling process. A vacuum was created by a hand-pump enabling a faster passage of the water through the filter. Figure 4 provides an overview of the set-up. At each location, 2 L of water was filtered.

After filtering, the top and bottom were disconnected to expose the filter membrane containing environmental DNA. Using tweezers, the filter paper was folded four times in half. After folding, the filter was placed in a 2 ml vial filled with 1,5 ml molecular-grade ethanol (96%) for preservation. All vials were wrapped in aluminium foil, labelled with the name of their corresponding monopile location and stored in a freezer at -20°C.

Figure 5. eDNA filtration set-up on the research vessel during the T-1.

2.4 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 on the deck, after which photos were taken of the entire oyster table, spat collectors, out- and inside of each of the baskets, 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 2 hours and 32 minutes and maximum of 7 hours and 15 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.
- Three spat collectors, collected in an onion bag and stored in the freezer.
- Scrape sample (not in the original scope): To get an idea of the species composition of the marine growth on the table and in the baskets, a qualitative scraping sample was collected from various surfaces of the oyster table (upper side and lower surfaces of concrete table, surface of plastic oyster basket, surface of central metal rod etc.) using a putty knife. During processing of the oysters various animal species present, inside the oyster basket and between the oysters, were added to the scraping sample for later qualitative analysis. The samples were preserved in 96% ethanol and stored in the freezer.

To limit the time spend on measuring, 25 of 75 oysters per basket were measured using a digital calliper (maximum length and width) to assess their growth since T-0. Furthermore, the number of dead and living oysters per basket were counted before placing them back in the baskets.

2.5 Photo and video analysis

Photo and video analysis was executed by specialists using the software TransectMeasure (from SeaGIS). These specialists also worked on this project in 2020. Using the software, a 'window' was placed in each photo (Figure 6). Part of the window were the laser lines already present. The horizontal lines were placed at set distances from the edges. Within the window, all living fauna was identified and scored using the SACFOR scale.

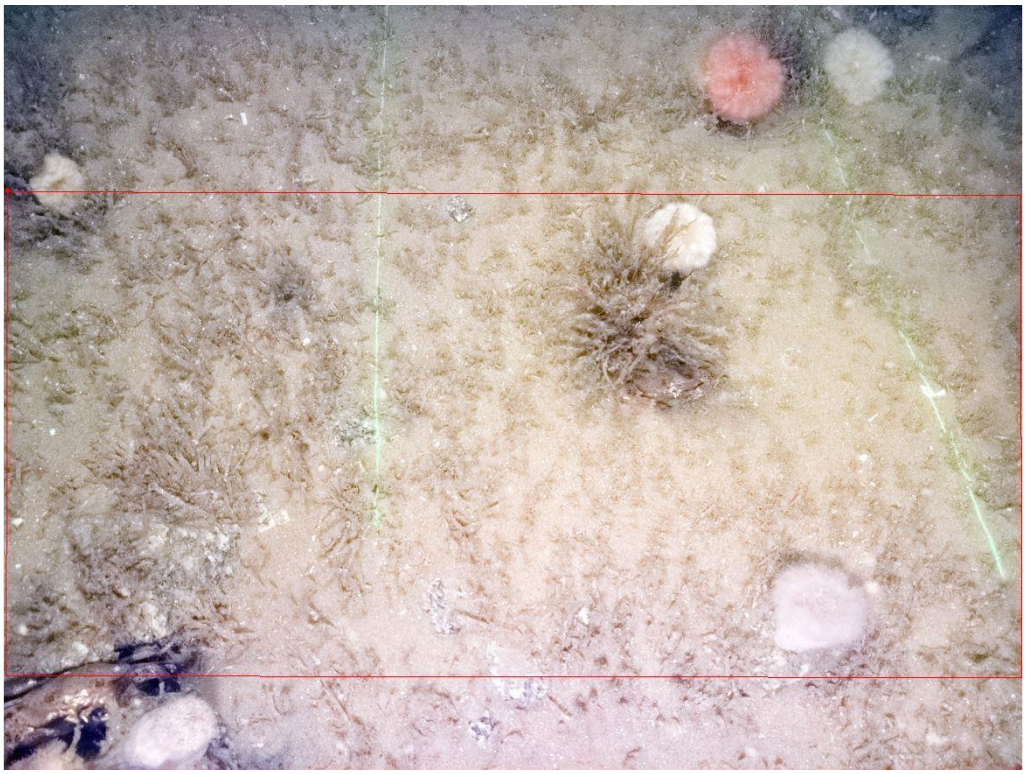

Figure 6: View of an ROV photo in the software, with the area of interest being bordered by the red lines (added in the software) and green lasers. The photo was made at monopile D06 in the T-1 with the filter grade and perpendicular to the current.

The SACFOR scale is an internationally used unified system for recording abundance of marine fauna in biological surveys (JNCC). The SACFOR scales used in this project were derived from the video analysis protocol of Rijkswaterstaat (Rijkswaterstaat, 2020). This was done to compare results with other (future) projects. The anagram stands for (S)uperabundant, (A)bundant, (C)ommon, (F)requent, (O)ccasional, (R)are and Superrare (RR). The scale is linked to abundances that differ in growth form and size of individuals (see Appendix 4). All present fauna in the photos were analysed and noted in the software. The ROV videos were used to better analyse the photos, as the quality of the video was much higher. More colour and details were visible. Also, the movement of the individuals helped in the identification of the species. One quarter of all photos were identified by both specialists for quality control. No significant difference in the identification of the species was found between the two specialists.

Besides the photo analysis, the full length of the ROV videos (taken during the survey, before, after and in between taking the photos) was inspected for additional benthic and pelagic species. Since the videos are not linked to a certain treatment, all these extra observations were written down per monopile. The species can be used for the total biodiversity analysis per monopile.

2.6 eDNA analysis

Preserved samples were sequenced using PCR in the lab with the Leray CO1 (for benthos) and Mifish 12s (for fish) primers. Sequencing was repeated for each sample depending on the primer. CO1 was replicated 6 times, 12s was replicated 12 times for better and more reliable results. The resulting sequences were 'blasted' against the genetic databases BOLD and GenBank thenpost-processed byusing algorithms (developed by BIOMON (Leiden)) to indicate which reads were valid and false/faulty. This resulted in a species list with numbers of 'reads' per species.

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 to the DNA-lab 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 ckecked 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. If identification could not be done, spat could be send to the DNA-lab to check whether they were of flat or Pacific oysters (all oyster spat was identified, so no DNA analysis was necessary).

2.8 Data analyses

Data collection and preparation was done in Access and Excel. Data-analyses were performed using R (R Core Team, 2021).

3 Results

3.1 Stability and functioning of the oyster tables

The oyster tables were still standing upright on the scour protection, therefore the tables were not covered with sediment and none of them were damaged. The entire surface of oyster table and baskets was covered with sessile organisms, but meshes of the baskets were still open. However, as the next campaign is in 2023, the choice was made to create a few larger holes in each new basket (Figure 7). There appeared to be more (black) sediment in all oyster baskets from location D06 than at other monopiles.

Figure 7. New basket, filled with remaining living oysters, and adjusted with four larger holes (one shown here).

Within and on the surface of the oyster baskets and tables, a variety of species were found and visually identified (in the field, using photos and the scrape samples). This is including, but not limited to the species shown in Table 3.

Table 3. Species list of fauna found on the oyster table and baskets, not exhaustive.

Group	Scientific name	Common name
Crustaceans	*Pilumnus hirtellus*	Hairy crab
	Pisidia longicornis	long-clawed porcelain crab
	Balanus crenatus	Crenate barnacle
	Stenothoe valida	No English name
	Monocorophium acherusicum	No English name
	Jassa herdmani	No English name
	Macropodia rostrata	long-legged spider crab
	Cancer pagurus	Edible crab
	Necora puber	velvet swimming crab
	Athanas nitescens	Hooded shrimp
Molluscs	*Aequipecten opercularis*	Queen scallop
	Venerupis sp.	Carpet shells
	Heteranomia squamula	Smallest saddle oyster
Group	**Scientific name**	**Common name**
	Crepidula fornicata	Slipper limpet
	Trivia arctica	Unspotted European cowrie
	Trivia monacha	European cowrie
	Abra alba	White furrow-shell
	Mytilus edulis	Blue mussel
	Dendronotus europaeus	No English name
Worms	*Gattyana cirrhosa*	No English name
	Sabellaria spinulosa	Ross worm
	Nereididae	Ragworms
	Lanice conchilega	Sand mason worm
	Lagis koreni	Trumpet worm
	Spirobranchus triqueter	Keelworm
Echinoderms	*Asterias rubens*	Common starfish
	Ophiothrix fragilis	Common brittle star
	Psammechinus miliaris	Green sea urchin
Anemones	*Sagartia troglodytes*	Mud sagartia
	Metridium senile	Plumose anemone
Hydrozoans	*Tubularia indivisa*	Oaten pipe
	Ectopleura larynx	Ringed tubularia
Ascidians	*Styela clava*	Stalked sea squirt
	Ciona intestinalis	Vase tunicate
Fish	*Pholis gunnellus*	Rock gunnel
	Myoxocephalus scorpius	Bull-rout
Nemerteans	*Oerstedia dorsalis*	No English name
Bryozoans	*Electra pilosa*	Hairy sea-mat

The sampled oysters were covered by a thick growth of sessile invertebrates, with several associated vagile species. Some species are noteworthy, such as the Ross worm, *Sabellaria spinulosa* (Figure 8), a reef building species of interest in the southern North Sea because of the quasi disappearance of such species. It was present on almost all oysters.

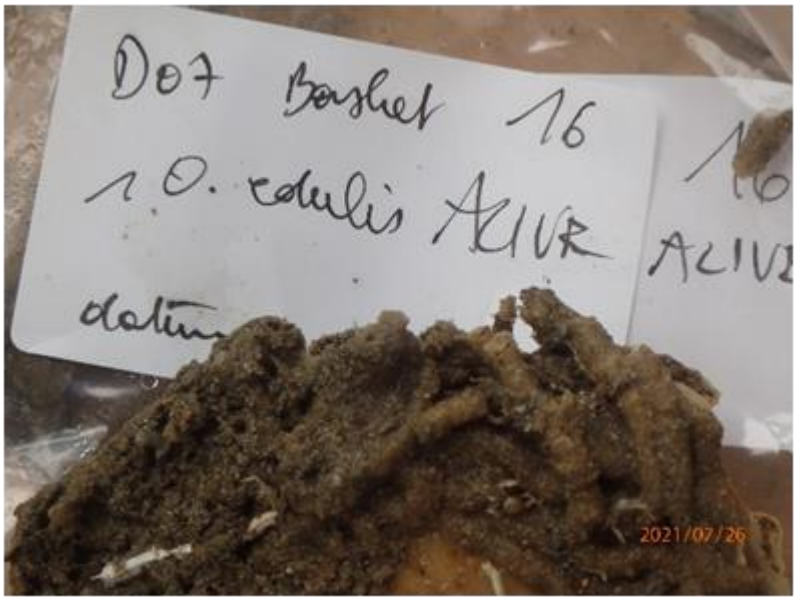

Figure 8. Sabellaria spinulosa found on oyster shells

Small colonies of dead man's fingers, *Alcyonium digitatum*, were often found (too small and contracted for a photo). Several specimens of the queen scallop *Aequipecten opercularis* (Figure 9) were found, a species less common along the Dutch coast.

Figure 9. Aequipecten opercularis found on an oyster shell.

The mudworm *Polydora hoplura* (Figure 10) was found several times. This worm bores into oyster shells, causing blisters which weaken the shell and require the oyster to spend energy on repair.

Figure 10. Polydora hoplura found on the inside of a (living) sampled oyster.

The slipper limpet *Crepidula fornicata* was found a few times. In theory this species is a food competitor of bivalves, but at low densities this will probably have little influence. An unknown flatworm species was found, *Prosthiostomum sp.*, which probably concerns a species introduced to Europe from another part of the world in the past (Gittenberger et al., in prep.).

These qualitative investigations of fauna from scrape-samples and oyster shells indicate that the oysters (or at least the oyster-table) enable the occurrence of a richer fauna. Although we have not put in the same effort to find these species in Borssele IV and are unable to spot most of these species on the photos or video (as of yet), we are confident most of these species are unlikely to occur in Borssele IV in such high numbers or even at all (like the *Polydora sp.*) because of their association with oysters.

3.2 Flat Oyster monitoring

Oyster survival

The number of living, dead, sampled and remaining oysters are shown in Table 4. Assuming that 75 oysters were installed in 2020, this also leads to an amount of missing oysters or shells of dead oysters in 14 out of 32 baskets. An explanation could be that during the installation of the oysters in 2020 something went wrong. Some of the oysters might have been lost from their nets in the transport tank. Therefore, not including the missing oysters, the average survival of oysters per basket was 96,3%. Only one basket (at D07) showed a relatively high number of dead oysters (24 of 75), but there was no significant difference in average survival between monopiles (T-test, $p>0.05$).

*Table 4. Number of dead and living oysters per basket during T-1 and additional information. * = basket number has been replaced, new number is 37.*

Location	Basket number	Living oysters	Dead oysters	Sampled (living) oysters	Oysters missing	Remaining living oysters
D04	26	72	3	1	0	71
D04	27	71	2	1	2	70
D04	30	71	3	1	1	70
D04	33	72	2	1	1	71
D04	34	72	3	1	0	71
D04	35	69	5	1	1	68
D04	36	70	4	1	1	69
D04	38	74	0	1	1	73
D05	1	69	0	1	6	68
D05	2	75	1	1	-1	75
D05	3	72	0	1	3	71
D05	4	75	0	1	0	74
D05	5	75	0	1	0	74
D05	6	75	0	1	0	74
D05	7*	75	0	1	0	74
D05	11	73	1(lost)	1	1	72
D06	8	72	2	1	1	71
D06	9	65	10	1	0	64
D06	17	75	0	1	0	74
D06	19	72	3	1	0	71
D06	22	72	3	1	0	71
D06	25	70	3	1	2	69
D06	28	70	5	1	0	69
D06	32	73	2	1	0	72
D07	13	74	1	1	0	73
D07	14	51	24	1	0	50
D07	15	67	6	1	2	66
D07	16	74	1	1	0	73
D07	18	74	0	1	1	73
D07	21	73	1	1	1	72
D07	23	75	0	1	0	74
D07	24	72	3	1	0	71

Oyster growth

Maximum width and length of oysters at T-1 (25 of 75) individuals/basket) were compared maximum width and length at T-0 (75 individuals/basket). At T-1 basket 34 was not measured correctly, probably due to erroneous calliper calibration during the fieldwork. No comparison could be made for this basket between T-0 and T-1.

All measurements are displayed in Figure 11 and Figure 12. Note that, because the number of individuals measured is not the same between T-0 and T-1, the distribution around the average of measurements per basket do not compare one-on one. Therefore, one must focus on the average and the differences between T-0 and T-1. For the width no significant structural growth of the shells has occurred. For the length there is an increase in the average length of the oyster shells. For only two baskets (26 and 38), the average length is lower in the T-1 measurement. This is a reminder for the potential sampling bias, as a smaller number of individuals were measured.

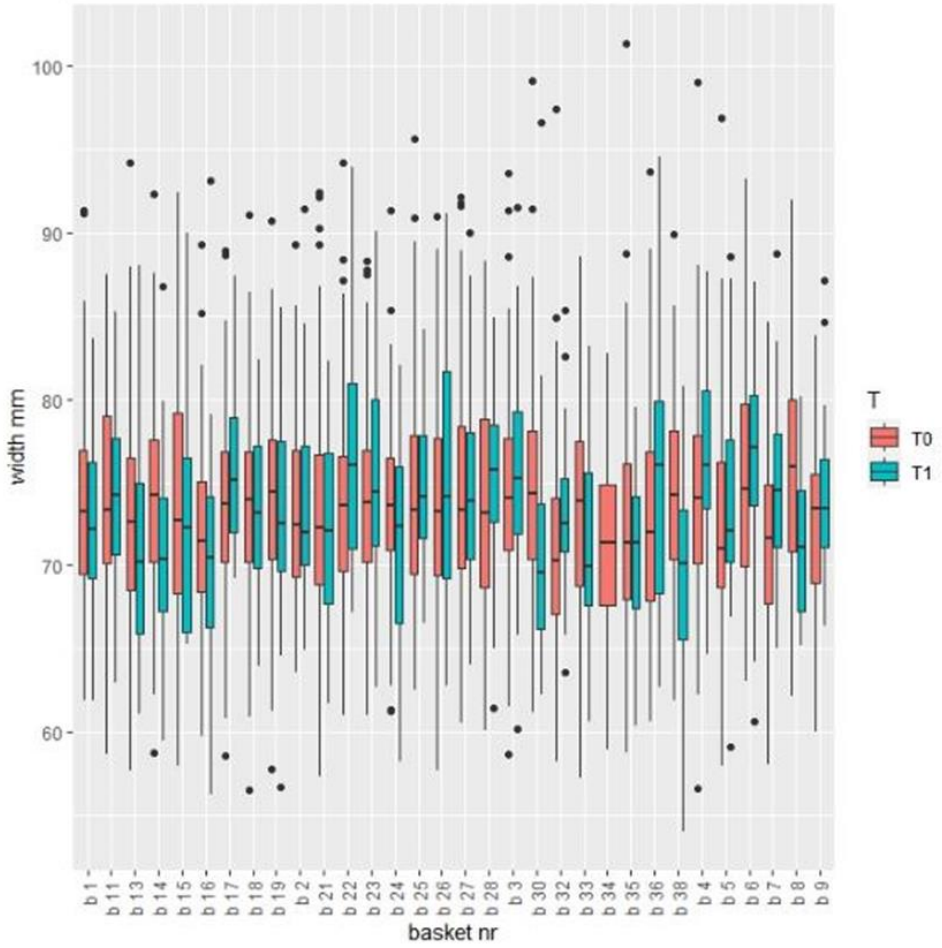

Figure 11. Widest width of oysters measured in mm (75 individuals per basket on T-0 (red), 25 individuals per basket on T-1 (blue)).

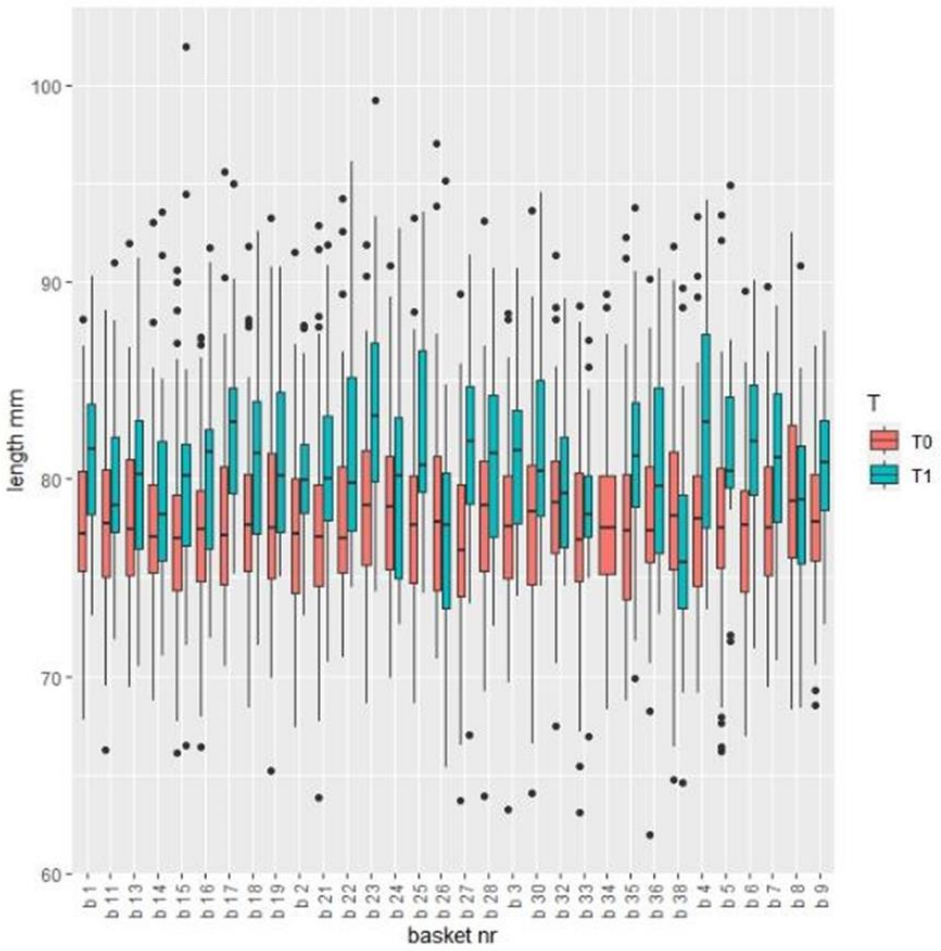

Figure 12. Longest length of oysters measured in mm (75 individuals per basket on T-0 (red), 25 individuals per basket on T-1 (blue)).

No prediction could be made in which direction oysters grow, in length or in width. To assess growth per location we calculated surface per oyster, assuming that surface is proportional to a circle with the average between maximum length width per oyster as the diameter (D) and the surface = $\pi*(D/2)^2$. The calculated surface of each measured oyster per basket per location is provided in Appendix 1. It seems that locations D05 and D06 showed a somewhat higher growth in oysters than locations D04 and D07 (see appendix 2).

3.3 Larvae analysis

Larvae samples

After initial inspection for the presence of suspended matter, four of the eight samples (D04, D05, D06, H02) were analysed to get an impression of the density of *Ostrea* larvae around the oyster baskets. Only certain groups of bivalves, such as oysters, mussels and soft clams (*Mya sp*) have D-larval stages. In all analysed samples, only one D-larva was found (D04) (Figure 13). In every sample either 2 or 3 unidentified non D-type bivalve larvae were found. All eight larvae-samples were also sent to the DNA-lab, where the samples were tested for the presence of oyster larvae (both flat and Pacific oyster). However, no oyster DNA was found.

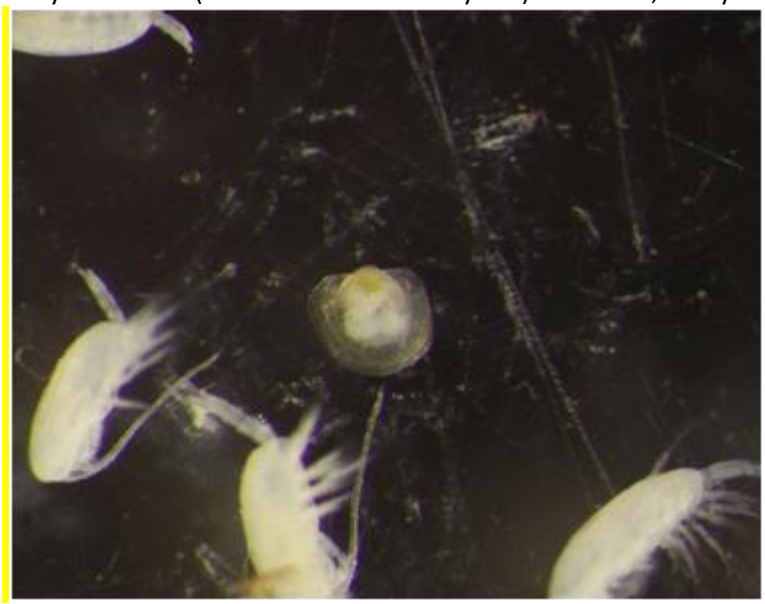

Figure 13. Only D-larva found in the samples

3.4 Spat analysis

Spat on the installed spat collectors (oyster shells in mesh bags) was identified. One juvenile *Ostrea edulis* was found, 17 mm in length. An oyster this size must have been present already at the moment of placement of oysters in the baskets. Five oyster-like bivalves smallerthan 1 cm were found. These were all identified as *Heteranomia squamula*, the smallest saddle oyster. So, no flat oyster spat of the installed oysters was found in the T-1.

3.5 Bonamia lesions and gonad development

The oysters collected for analysis of Bonamia lesions and gonad development were visually analysed. None of them yielded lesions related to the *Bonamia ostreae* parasite. Of 32 oysters, 26 clearly exhibited milky white gonads (ready to reproduce, see Figure 14), 5 additional oysters exhibited somewhat milky white gonads (build-up to reproduce). So, in conclusion: there was no Bonamia parasite found in the oysters that lived in the Borssele wind farm site III and most of the oysters were in the process of reproduction.

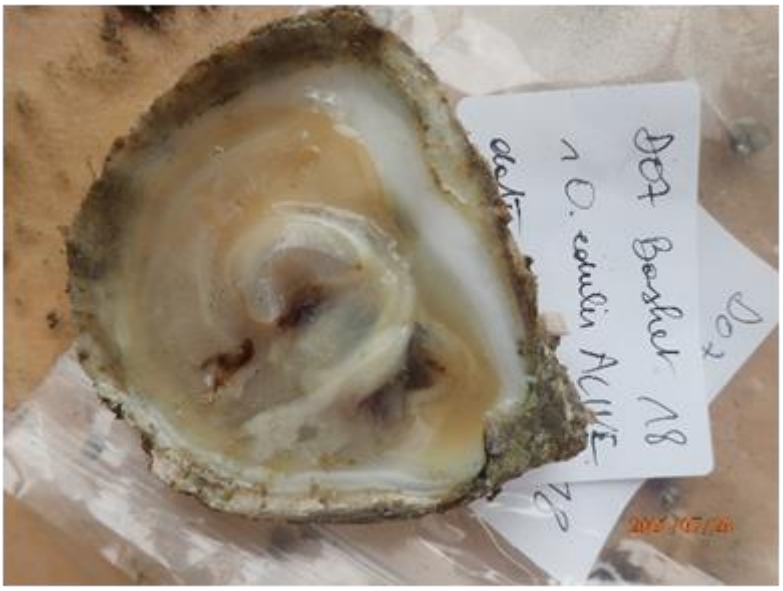

Figure 14. Inert of oyster showing gonad development (milky half-circle) and no Bonamia lesions.

3.6 ROV survey

In total during 2020 and 2021 89 taxa were identified of which 24 higher taxa (partly overlapping with species in the same genus). In 2020, 65 taxa were identified (photos plus video) of which 16 higher taxa, while in 2021, 66 taxa were identified (photos plus video) including 16 higher taxa (see Appendix 3). The table in Appendix 3 shows the frequency with which species have been found per year per monopole (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.

3.6.1 Analysis of the benthic community

The presence and absence data from the photo analysis was plotted in non-Metric multi-dimensional scaling plots (nMDS), using a Bray-Curtis similarity. These plots (Figure 15 - 19) 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 within grouped samples has a high(er) similarity.

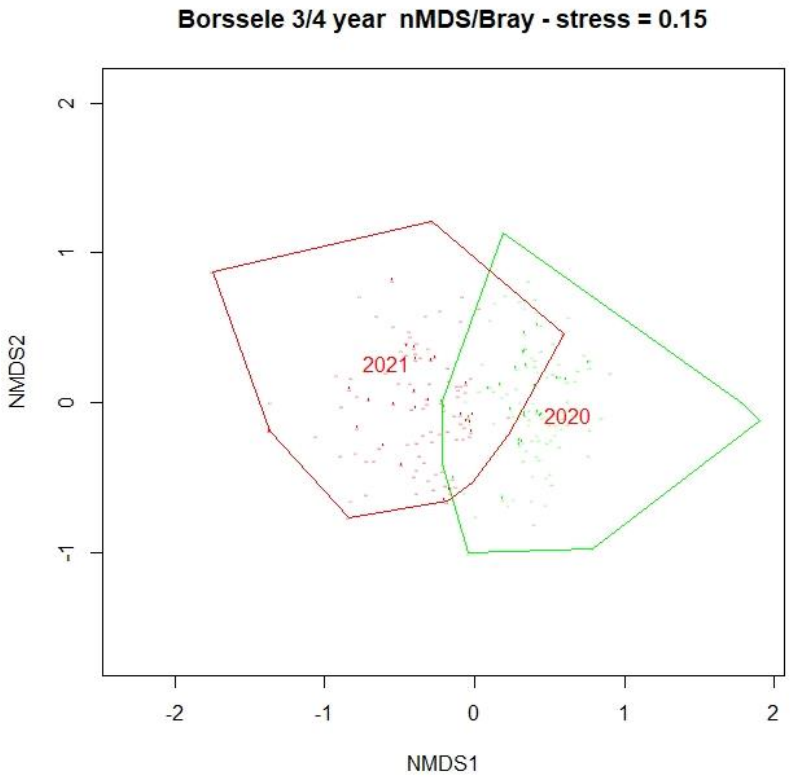

Figure 15. NMDS displaying the differences in species-composition between samples of 2020 and 2021 (all samples except H3 in 2020).

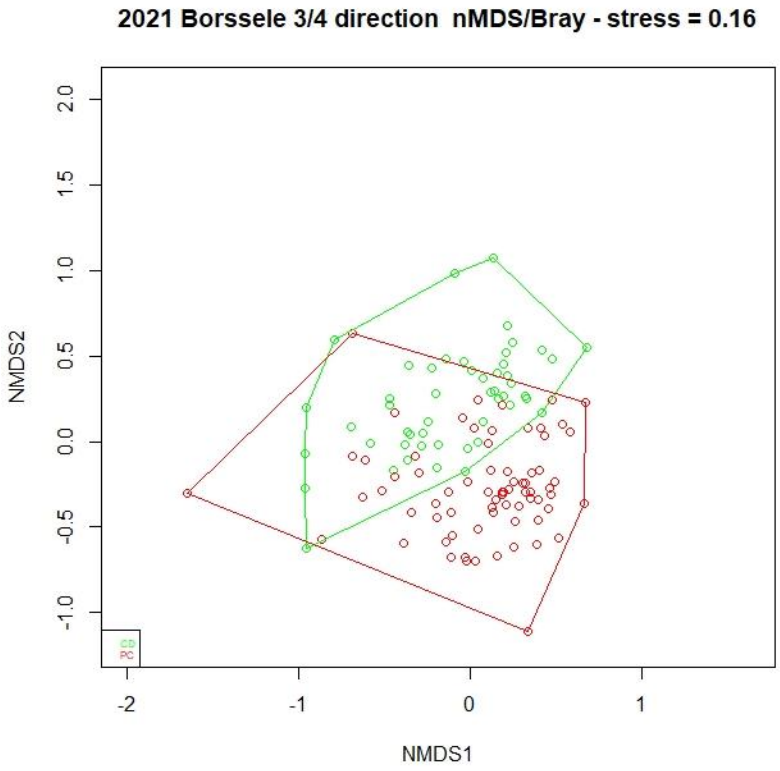

Figure 16. NMDS displaying the differences in species-composition between samples of current-direction (green) and perpendicular-current direction samples (red).

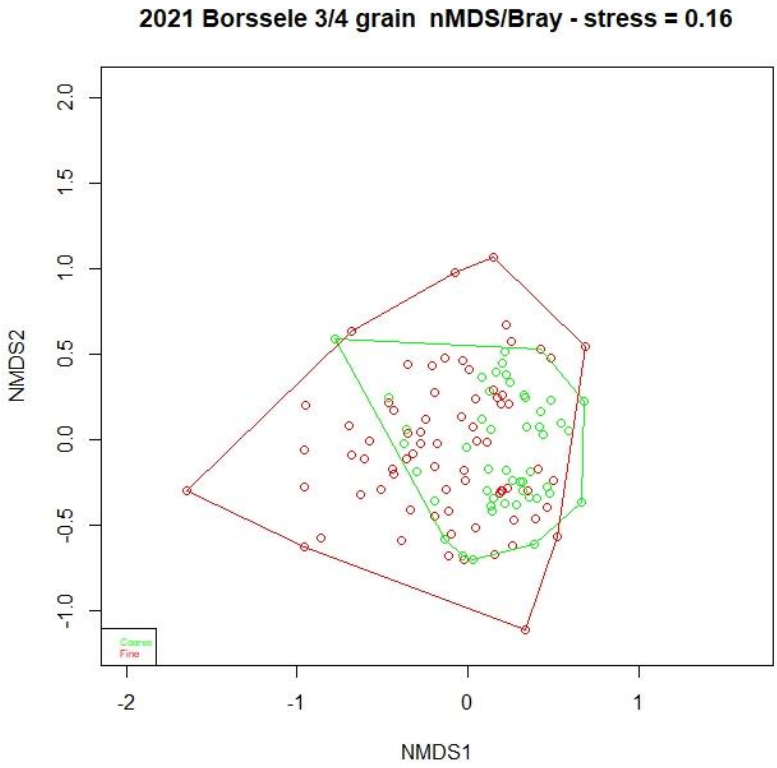

Figure 17. NMDS displaying the differences in species-composition between samples on coarse-grain (green) and fine-grain scour-protection (red).

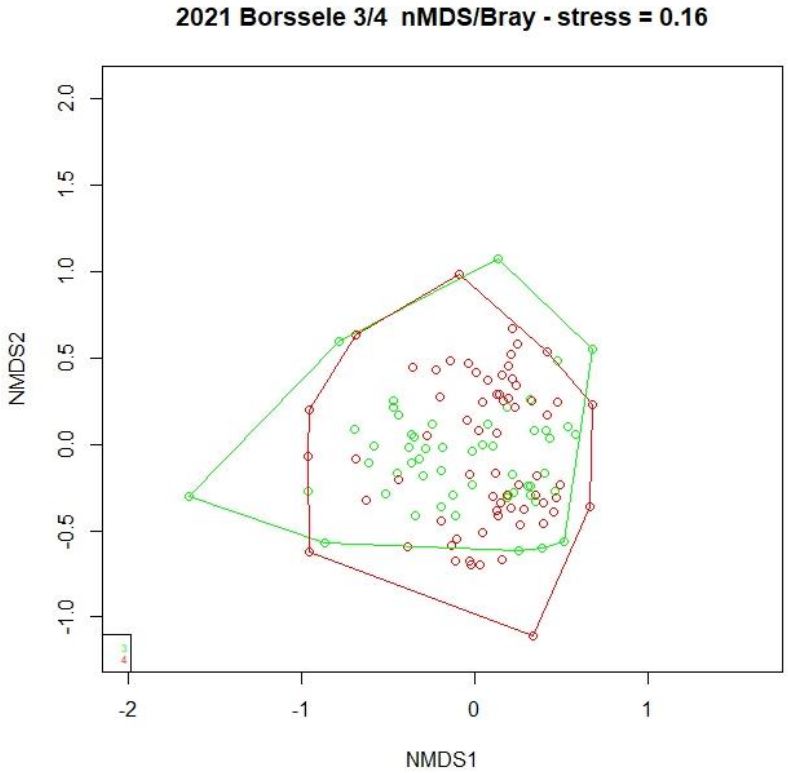

Figure 18. NMDS displaying the differences in species-composition between samples of Borssele III (green, with oyster tables) and Borssele IV (red, without oyster tables).

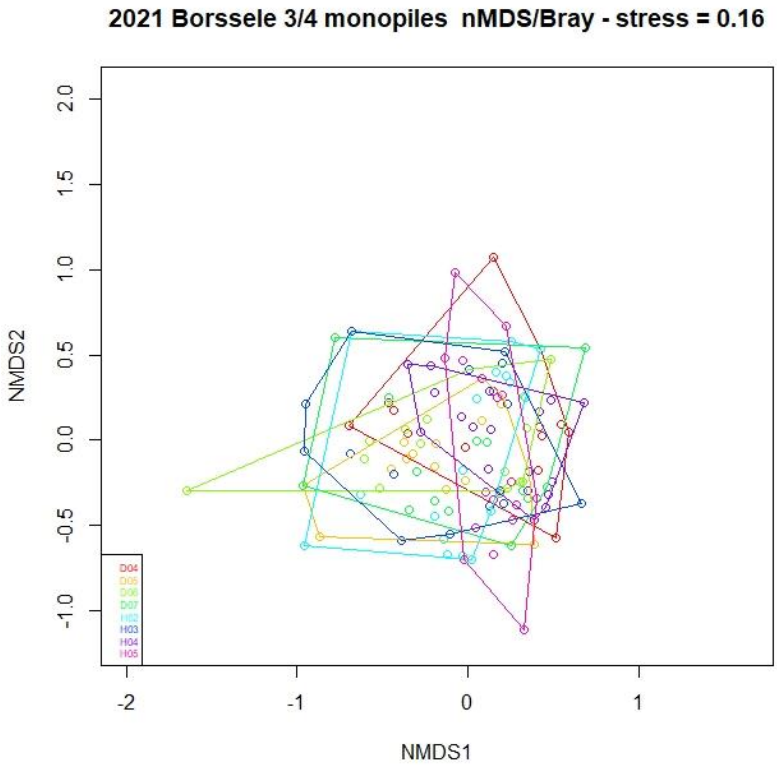

Figure 19. NMDS displaying the differences in species-composition between samples of different monopoles (D04=red, D05=yellow, D06=light green, D07=dark green, H02=light blue, H03=dark blue, H04=purple, H05=pink).

Because the nMDS-plots are a mere visual representation of differences between samples, the differences between groups of samples were also tested statistically using PERMANOVA. In PERMANOVA each factor (type of scour protection, current direction, Borssele III (with oyster-table) or IV (without oyster-table), monopile, year) is tested as an explanatory factor of 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 Table 5 below, all factors used with significant ($p<0.001$) explanatory value are displayed along with their explanatory value R2.

Table 5: PERMANOVA results (all displayed R2 have p<0.001) for 2020 or 2021 (or both in the year-to-year comparison)

Dataset:Variable	2020 R2	2021 R2
2020-2021:year	0.20	
monopile	0.17	0.13
current direction	0.01	0.12
scour-grain	0.16	0.05
Site III/IV	0.06	0.03

When analyzing the complete dataset of 2020 and 2021, the PERMANOVA-results remain qualitatively the same (Table 5). The factor “year”, however, adds 20% of explanatory value, indicating that successional change has played the largest role in shaping the species communities. Also, monitoring took place in different months of the year, which might explain part of this change as well. Furthermore, the PERMANOVA shows that each monopile is significantly different from another, explaining 13% of the variation in species composition in 2021. In 2020 the factor ‘monopile’ explained a larger part of the difference between samples, which might

indicate that the changes in species-composition between 2020 and 2021 are largely uniform over all samples and has reduced differences in species composition over time between monopiles.

Figure 17 shows that the species composition of coarse-grained photos are more alike than the species composition on fine-grained photos. Because the two groups overlap largely in species composition, however, PERMANOVA shows the explanatory value of the factor scour-type is only 5%. In Figure 18, the distinction between Borssele III (D-locations) and IV (H-locations) can be derived. Although the distinction is visually hard to interpret, the PERMANOVA shows it has a significant explanatory effect, but explains only 3%.

The species that most contribute to the difference in species composition between years can be assessed using a SIMPER-analyses. The SIMPER analysis points to *Tubularia indivisa, Necora puber* and *Spirobranchus triqueter* as important discriminants between years.

- *Tubularia indivisa,* a hydroid, is a prime pioneer on hard-substrate and was already present at most monopiles in 2020. In 2021, this species was seen in fewer photos than in 2020. This might have several ecological reasons: 1) being a pioneer it is likely to get overgrown. 2) this species reproduces by highly visible gonophores, which develop after summer and bud in April, making this species more visible after summer.
- *Spirobranchus triqueter* more than doubled in occurrence from 2020 to 2021. This annelid builds calcareous tubes which are highly visible and attaches these tubes on hard or solid substrates.
- *Necora puber*, or velvet swimming crab, was found in a larger amount of samples in 2021, but at fewer locations, concentrated mostly in Borssele III. This epibenthic species is a good swimmer and common in the open North Sea and associated with hard substrate as well.

3.6.2 SACFOR analysis

Because the SACFOR score is ordinal categorical data, averages/means of the score are not representative (a ½ S or A+ does not represent an actual situation). Instead, the mode (most abundant score) can be used to present the central tendency of categorical data. Also, the frequency/percentage of categorical data can be used to represent the data-spread. Table 6 presents the frequency of each scale of SACFOR found per monopile in each scour-type in 2021. The scour-type and the D/H-location is presented here because these factors have shown to influence the spread in SACFOR-scale as presented in table 6 and 7, while other factors (current direction) have shown not to have such an influence and are therefore not shown.

Table 6: frequency (relative to number of scores per row) of SACFOR scores per scour-type per monopile of 2021. Green numbers are the mode (highest frequency). CG=coarse grain, FG=fine grain.

	S	A	C	F	O	R	RR	sand-cover%
D04CG	0.17	0.13	0.06	0.19	0.28	0.09	0.09	15
D04FG	0.00	0.00	0.19	0.33	0.28	0.11	0.08	75
D05CG	0.15	0.07	0.07	0.28	0.24	0.07	0.13	15
D05FG	0.02	0.04	0.11	0.28	0.26	0.26	0.02	50
D06CG	0.14	0.09	0.16	0.19	0.23	0.14	0.05	25
D06FG	0.05	0.02	0.09	0.27	0.41	0.09	0.07	50
D07CG	0.18	0.00	0.11	0.18	0.25	0.20	0.07	20
D07FG	0.02	0.04	0.10	0.27	0.39	0.12	0.06	45
H02CG	0.00	0.07	0.17	0.28	0.37	0.09	0.02	40
H02FG	0.00	0.03	0.10	0.24	0.38	0.21	0.03	70
H03CG	0.02	0.15	0.17	0.28	0.24	0.06	0.09	25
H03FG	0.00	0.03	0.24	0.12	0.24	0.30	0.06	60
H04CG	0.13	0.04	0.09	0.35	0.18	0.16	0.05	0
H04FG	0.02	0.07	0.05	0.34	0.18	0.16	0.18	30
H05CG	0.06	0.00	0.06	0.34	0.50	0.04	0.00	50
H05FG	0.00	0.02	0.13	0.28	0.34	0.23	0.00	15

We notice a slight difference in abundances found between different scour-types of monopile D04, where the fine-grain section has no 'S' and 'A' abundances (while the coarse grained section does) and the mode of frequencies is 'F' instead of 'O' in the coarse grain section. There is also a difference in mode of frequencies in these sections (fine and coarse grained) in monopile H03. Some of the differences in SACFOR-score between coarse and fine-grain can be explained by the cover of sand, where there are less S-scores when the sandcover is higher. Another logical explanation is that coarse-grain simply presents more surface-area in the picture-frame than fine-grain because of its 3D-structure, therewith enabling a higher possibility for higher abundances and cover.

Between H (without) and D (with oyster tables) monopiles there seems to be a difference in the frequencies of 'S', because 'S' more often has no frequency in H-monopiles. This does not seem to correlate with the sandcover.

When we compare the SACFOR-scores of 2021 (table 6) with that of 2020 (table 7), we do not see great differences in the modes of frequencies, but do see a marked difference in frequencies of 'S', 'A' and 'C', indicating abundances have increased over time. These abundances can be attributed to the increase in abundance of *Spirobranchus triqueter* on coarse-grain scour-types, as well as *Necora puber.* Since in 2021 the H-monopiles still often have low frequency of 'S' (although *Spirobranchus triqueter* and *Necora puber* do occur more often in coarse-grained areas), the impression is that these monopiles have less or a slower development of abundances than the D-monopiles.

Table 7: frequency (relative to number of scores per row) of SACFOR scores per scour-type per monopile of 2020. Green numbers are the mode (highest frequency). CG=coarse grain, FG=fine grain.

	S	A	C	F	O	R	RR	RRR	sand-cover%
D04CG	0.00	0.02	0.07	0.34	0.23	0.09	0.25	0.00	5
D04FG	0.06	0.00	0.06	0.26	0.37	0.17	0.14	0.00	35
D05CG	0.00	0.05	0.09	0.28	0.37	0.05	0.16	0.00	15
D05FG	0.02	0.02	0.00	0.30	0.34	0.25	0.07	0.02	45
D06CG	0.00	0.02	0.03	0.38	0.41	0.07	0.07	0.03	0
D06FG	0.00	0.00	0.08	0.26	0.32	0.24	0.10	0.00	35
D07CG	0.00	0.04	0.07	0.37	0.24	0.04	0.24	0.00	5
D07FG	0.00	0.00	0.10	0.23	0.43	0.10	0.15	0.00	65
H02CG	0.02	0.00	0.07	0.38	0.24	0.20	0.09	0.02	35
H02FG	0.00	0.00	0.13	0.37	0.17	0.20	0.13	0.00	35
H03CG	0.00	0.04	0.02	0.33	0.38	0.17	0.06	0.00	0
H03FG	0.00	0.00	0.04	0.26	0.30	0.26	0.15	0.00	20
H04CG	0.00	0.02	0.04	0.33	0.40	0.07	0.07	0.07	5
H04FG	0.00	0.00	0.03	0.34	0.21	0.16	0.24	0.03	35
H05CG	0.02	0.02	0.09	0.29	0.33	0.22	0.04	0.00	5
H05FG	0.00	0.08	0.05	0.18	0.38	0.23	0.10	0.00	50

3.6.3 SACFOR trends

Besides giving a general indication of the abundance of fauna in a certain situation or spot, the SACFOR-scale has other possibilities. Even though the SACFOR-scale does not provide the opportunity to do classic statistical analysis with averages and variances because it is ordinal categorical data, it can be used to indicate trends over time of specific species in different treatments. These trends can be displayed in a x-y graph, where the y-axis represents the mode of SACFOR-scores for a species and the x-axis time. Using these graphs, the trend of a species in a specific treatment (for instance fine-grain) can be compared with the trend in another treatment (for instance coarse-grain) to indicate whether the treatment has an effect on the abundance of that species.

As indicated in the introduction, however, the fabrication and assessment of trends is not possible with two measurements in time, and needs at least three measurements in time. This analyses therefore will be saved for the report on T-3.

3.7 (e)DNA

Unfortunately, analysis of the eDNA-samples taken in July 2021 was not successful. It is unclear what went wrong, whether it was an error in sampling or processing, but analysis did not yield any results (blanco). Fortunately, during another field trip in Borssele in September 2022, three more water samples were taken at all four monopiles with oyster cages. These samples will be analysed by Wageningen University & Research. Results will be reported in the T3 scientific report.

4 Discussion and conclusions

The ecological monitoring of Borssele III/IV set out to answer the 7 questions stated in the introduction, and from this T-1 report we can state the following:

What is the survival and growth rate of flat oysters?
The average mortality at the T-1 was 3,7%, with just two baskets in D06 and D07 exceeding a mortality of 8%. Especially the baskets at D06 included some sediment, possibly explaining the increased mortality. One basket at D07, however, showed a mortality of 32%. It is unclear what happened there.
The size and quality of the flat oysters indicate that there was a proper set-up created for the pilot study at T-0. Measurements of the size of the oysters in T-1 has resulted in a clear indication for growth in size, especially in length.

Do the flat oysters produce larvae?
The water samples showed no flat oyster larvae around the eight monopiles at the time of sampling (beginning of July 2021). Also, no oyster-spat was found on the spat collectors. This coincided with the finding that most sampled oysters were (about to get) ready for spawning. Hence, at the time of the survey, oysters had not yet spawned.

Do the flat oysters stay free of the parasite Bonamia?
The quality of the oysters seemed very high before reinstallation. There was no Bonamia detected, either in inspection of oysters for lesions, nor RNA-analysis of both oyster-tissue and water-samples.

How is biodiversity in general developing on the different substrate variations?
Are there species-specific responses in relation to current directions or rock size of the scour?
How fast does succession of a hard substrate community occur in the Borssele area?
All samples had a very high similarity in species composition. The reason for this high similarity can be that Borssele III/IV is a recently constructed wind farm. It is assumed that there is a development in biodiversity and species composition over time (2020-2021). There are indications of differences between Borssele site III and IV, monopiles, years, scour-types and current directions. However, these differences account for a small part of the variation in species composition. For now, the species most influential in the differences between 2020 and 2021 are *Tubularia indivisa, Necora puber* and *Spirobranchus triqueter* all indicating the development/succession of a hard-substrate community. Analyses of the SACFOR scores (indicating abundances) have resulted in the impression that the sites have all become more populated in comparison with 2020 (abundances are higher in 2021) and Borssele IV (the H-monopiles) is lagging behind Borssele III in the development of abundances.

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?
In 2020 the environmental DNA-analysis proved to be a useful tool to indicate taxa difficult or impossible to see, and also point at the presence of species not (yet) spotted on video. While the comparison of results from video- and DNA-analysis indicated some overlap, both types of analyses largely accounted for unique occurrences of taxa, showing the potential of complementarity of both and the applicability of results in species composition analysis. Unfortunately, the T-1 analysis of eDNA somehow failed. Hopefully, the analysis of a new set of samples from Borssele (in 2022) will enable a comparison with the 2020 data. This comparison can hopefully be presented in the next report on T-3.

4.1 Conclusions

As stated beforehand, this report cannot draw conclusions on the success or trends in development of the ecology in Borssele Windfarm, because there is only the comparison with the T-0. From our analysis, however, it is clear that the development of species composition is still ongoing; there is a strong successive development in the whole area (Borssele III and IV) indicated by the difference in species composition between years. This development is largely overruling any effects that different treatments (oysters, current and scour type) may have. The photo/video analysis shows that differences between factors such as wind farm site are smaller in 2021 than in 2020. Possibly, the original differences found on substrate and bathymetry between these sites (in 2020 Borssele III seemed sandier than in 2021), has been erased or faded by the placement of the wind farm, moving sand waves and succession taken place. Even though this overruling effect of succession may indicate that the different treatments (scour types and oyster-tables) have little effect, this is not the end of the story since at least two more monitoring campaigns (T-3 and T-8) are planned.

The oysters placed at Borssele III show good survival and growth. 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 T-1 survey, however, they did not seem to have spawned yet, because no oyster-spat or pelagic oyster-larvae were found. Given the state the adult oysters were in, there is no reason to doubt that the oysters will have reproduced in the autumn of 2021. When this reproduction of 2021 has led to spat, that spat/ongrowth of young oysters will be big enough to be noticed at the T-3 survey in 2023.

5 Literature

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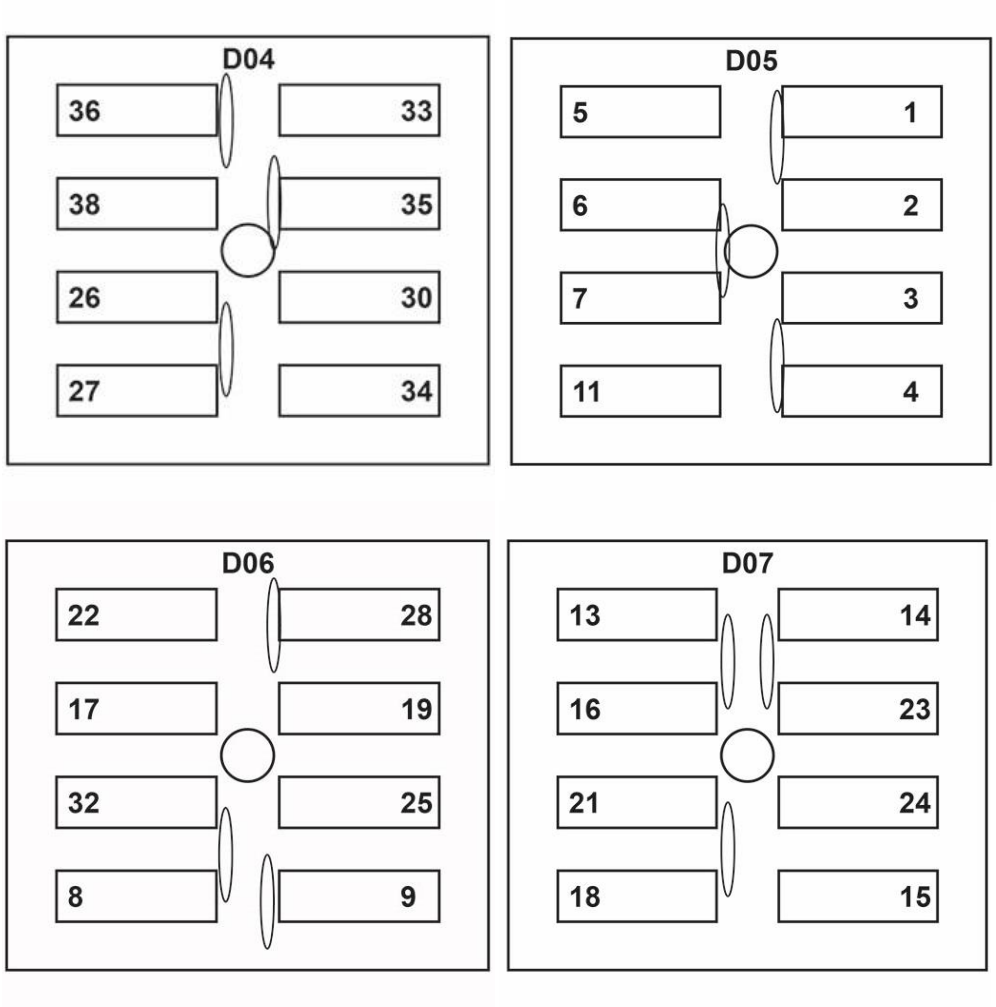
6 Appendices

6.1 Appendix 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 T-1.



6.2 Appendix 2. Surface growth of the oysters, per basket

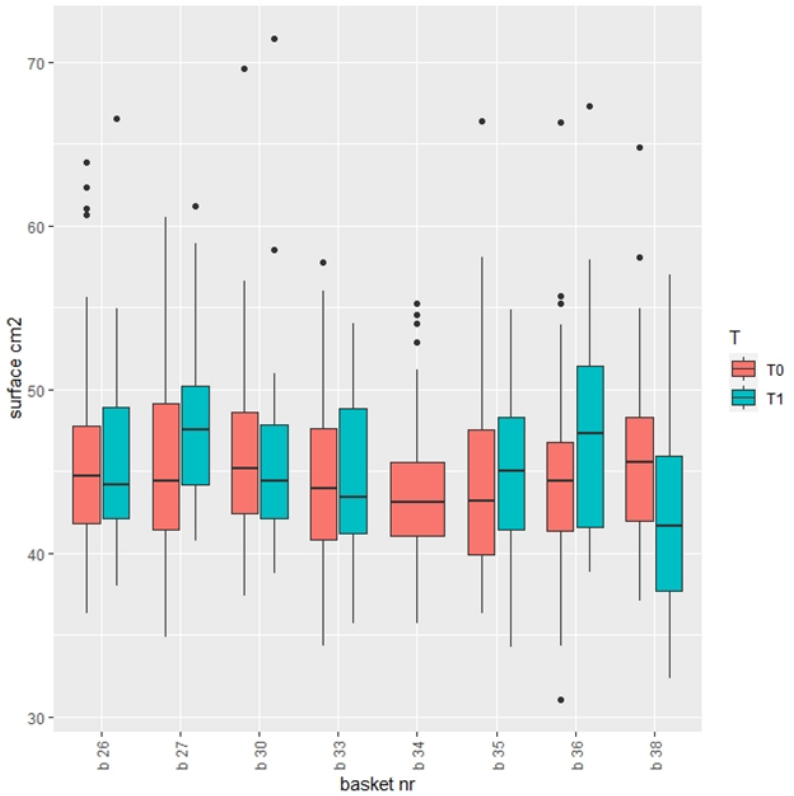


Figure 17. Calculation of surface of oysters measured at D04 (75 individuals/basket on T-0 (red), 25 individuals/basket on T-1 (blue)) in cm²

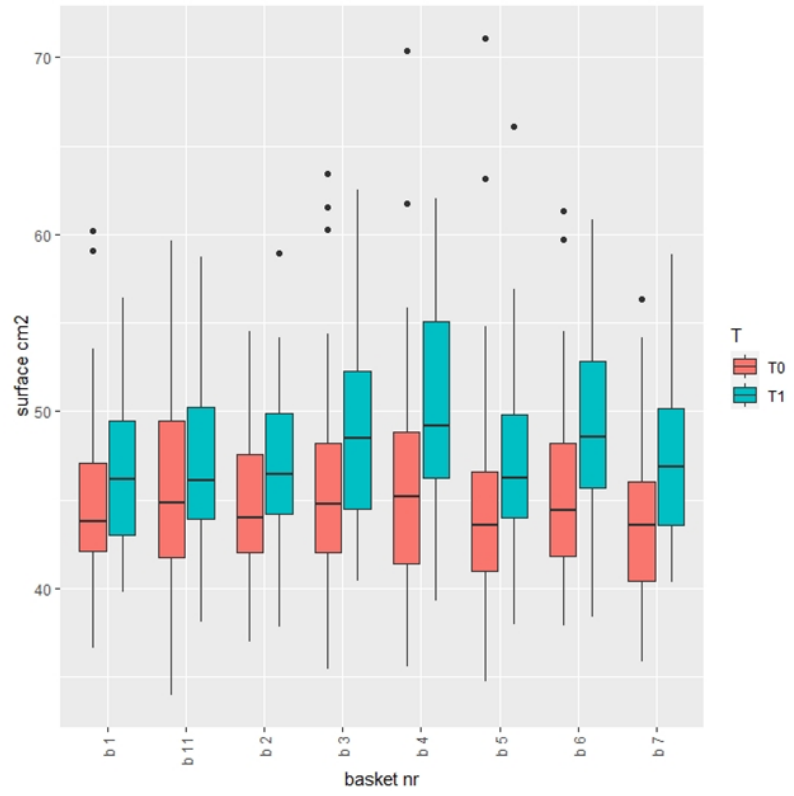


Figure 18. Calculation of surface of oysters measured at D05 (75 individuals/basket on T-0 (red), 25 individuals/basket on T-1 (blue)) in cm²

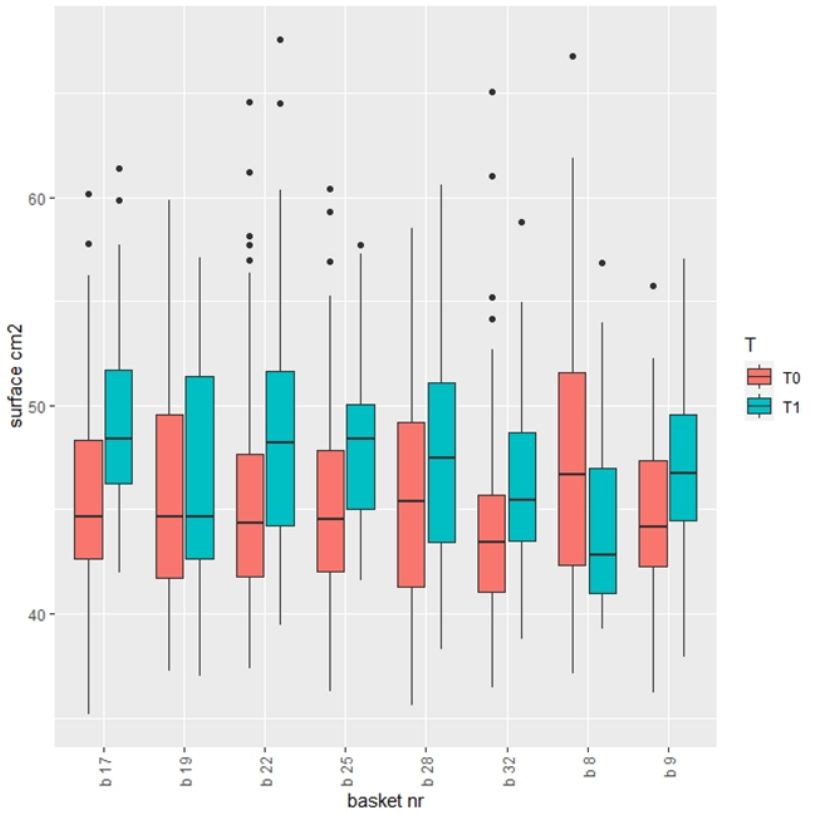


Figure 19. Calculation of surface of oysters measured at D06 (75 individuals/basket on T-0 (red), 25 individuals/basket on T-1 (blue)) in cm²

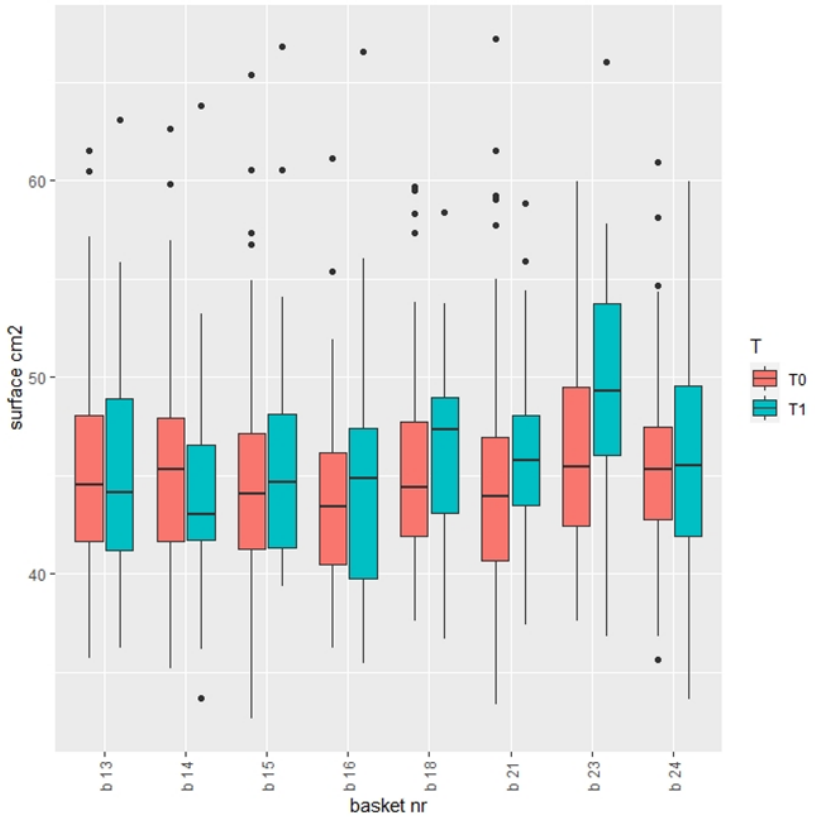


Figure 20. Calculation of surface of oysters measured at D07 (75 individuals/basket on T-0 (red), 25 individuals/basket on T-1 (blue)) in cm²

6.3 Appendix 3. Species list of the ROV surveys during the T-0 (2020) and T-1 (2021)

Frequency of occurrence per year per monopile. The frequency represents times a species has been spotted in pictures (21 per monopile per year, but 20 in 2020 for D07, H02 and H03) and video (1 per monopile per year).

Monopile	D04		D05		D06		D07		H02		H03		H04		H05	
Year	20	21	20	21	20	21	20	21	20	21	20	21	20	21	20	21
Maximum frequency	*22*	*22*	*22*	*22*	*22*	*22*	*21*	*22*	*21*	*22*	*21*	*22*	*22*	*22*	*22*	*22*
Actinopteri							1	1					1			
Actinothoe sphyrodeta					2	1	1	3		1	3	2			1	
Agonus cataphractus								1								
Alcyonium digitatum								1								
Ammodytidae								3								
Ascidiacea													5		2	
Asterias rubens	20	17	21	15	19	13	7	6	17	9	15	14	19	20	18	6
Balanidae					3								13			
Bryozoa			2		1				1	1	1	1			5	
Callionymus	1						1			1						
Callionymus lyra				3		1	1	1		3						
Callionymus maculatus		1														
Callionymus reticulatus							1									
Cancer pagurus	1	5	1	1	2	1	2	1	4	1	2	2	3		5	
Cerianthus lloydii	1															
Cliona celata														1		
Clupeidae		1								2						
Conger conger		1														
Cottidae		1	1			1	1	1	1	1				1	1	1
Cyanea lamarckii		3						1								
Cyanobacteria									1							
Cylista elegans		5	4	3	7	3	4	4	3	3	7	2	3	5	2	2
Cylista troglodytes	20	16	21	19	19	17	19	19	17	14	14	15	19	21	19	21
Dicentrarchus labrax			1						1			1				
Didemnidae														1		
Diplosoma listerianum	1		1						5		5		11	6	8	
Doris pseudoargus								1								
Echiichthys vipera				1								2				
Echinocardium cordatum					1								1			
Ectopleura larynx	3	1		3						9		10		3		6
Gadus morhua	1	1										1	2	1	2	
Gobius niger	1												1			
Halichondria panicea													1		1	
Homarus gammarus									1				1		1	
Hydractinia echinata		1								1		3		2		
Hydrozoa			3	1	10		1	1	1	1	1		7	2	5	3
Inachidae													1			
Jassa herdmani / Monocorophium acherusicum	6	4		1		1	1	3	1					9	1	4

Lanice conchilega		3	1	10		6		6		3		5		4		
Limanda limanda										3		1		3		
Liocarcinus			1		1			2								
Liocarcinus holsatus			1		1		2	1					1			
Liocarcinus vernalis								1								
Loligo vulgaris			6		2								2			
Macropodia rostrata			1				1				1					
Maja brachydactyla								1	5				5		10	
Metridium senile	4	3	3	1	10	8	11	7	6	5	9	2	1	1	7	7
Microstomus kitt						1						1	2			
Mnemiopsis leidyi									1							
Mullus surmuletus			1			1			3	1		1	4			
Myoxocephalus scorpius					2	1	2		2	1			4		2	1
Mytilus edulis				1		3		1					2			
Necora puber	9	3	18	12	9	10	18	11	7	7	3	3	5	10	1	10
Nemertesia	1															
Nemertesia antennina		2	1					4	10	1	11	2		1	8	4
Obelia bidentata	3		6		9	1	4		8		5		8		6	
Ophiothrix fragilis					1											
Ophiura albida			1												1	
Ophiura ophiura						1					1					
Ostrea edulis							1									
Pagurus bernhardus					1	1		1				2	2	1	2	
Parablennius gattorugine	2		2	1		1		2		2				1	1	2
Pholis gunnellus						2		1				1				
Pisces										1						1
Pleuronecta platessa										1						
Pleuronectidae				1		1	2		1	2		2				1
Pomatoschistus		1		1							1			1		
Pomatoschistus microps											2					
Porcellanidae														1		
Porifera			1											1		
Protosuberites denhartogi										1						
Psammechinus miliaris					9	2								1	1	
Sagartiogeton undatus		1			1		1									
Sepia officinalis			4						1		1					
Sepiola atlantica								1								
Sessilia												1				
Soleidae						1										
Spirobranchus triqueter	16	7	11	9	15	7	18	14	15	14	16	13	11	15	18	21
Spondyliosoma cantharus					1											
Taurulus bubalis		1		2		1		3	2	3		2	1			
Taurulus/Myoxocephalus							1		2		1		4		1	
Taurulus/Myoxocephalus/Parablennius							2								1	
Trachinus draco												2				

Trachurus trachurus		2	2		2	2		6	1	2	1		2	2		
Trisopterus										2						1
Trisopterus luscus	4		9		6		4		6		2		4		4	
Trisopterus minutus	1		1			1	1		1				1	1	1	
Tubularia indivisa	6	19	1	17	6	19	4	19		18	1	21	1	19		18
Urticina felina		1	3		3	2	3	1	3			1	1	1		1

6.4 Appendix 4. SACFOR-scale

	Growth form		Size of individuals/colonies				
% cover	Crust/meadow	Massive/Turf	<1cm	1-3 cm	3-15 cm	>15 cm	Density
>80%	S		S				>1/0.001 m^2 >10,000 / m^2 (1x1 cm)
40-79%	A	S	A	S			1-9/0.001 m^2 1000-9999 / m^2
20-39%	C	A	C	A	S		1-9 / 0.01 m^2 100-999 / m^2 (10 x 10 cm)
10-19%	F	C	F	C	A	S	1-9 / 0.1 m^2 10-99 / m^2
5-9%	O	F	O	F	C	A	1-9 / m^2
1-5% or density	R	O	R	O	F	C	1-9 / 10m^2 (3.16 x 3.16 m)
<1% or density		R		R	O	F	1-9 / 100 m^2 (10 x 10 m)
					R	O	1-9 / 1000 m^2 (31.6 x 31.6 m)
						R	<1/1000 m^2