WWF & ARK Borkum Reef Ground oyster pilot

Active restoration of native oysters in the North Sea - monitoring September 2019

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Preface

Shellfish reefs, consisting mainly of flat oysters (*Ostrea edulis*), once occupied about 30% of the Dutch part of the North Sea. However, due to overfishing, habitat destruction and diseases, they have almost entirely disappeared.

World Wide Fund for Nature (WWF) and ARK Nature, in collaboration with the Flat Oyster Consortium (POC), a consortium of Bureau Waardenburg, Wageningen Marine Research and Sas Consultancy, have been working in recent years on recovery opportunities for shellfish beds in the Dutch coastal zone (Voordelta).

Based on the experience gathered and knowledge developed via the Postcode Loterij Droomfonds Haringvliet pilots to restore native oysterbanks in the Voordelta, this project has been the first attempt to actively restore shellfish beds in deeper parts of the North Sea, in this case at the Borkum Reef Ground. Historically, flat oyster shellfish beds have been present in this area, however despite the presence of hard substrate as suitable habitat, flat oysters are currently absent.

This study was initiated and commissioned by WWF Netherlands. We would like to express our special thanks to Stichting Duik de Noordzee Schoon and her great crew of volunteers, the crew of the Cdt Fourcault and other crewmembers, Melchior Stiefelhagen, Peter van Rodijnen and Klaudie Bartelink.

Content

1 Introduction

1.1 Background

From historical documentation, we know that epibenthic shellfish reefs, consisting mainly of flat oysters (*Ostrea edulis*), once occupied about 30% of the Dutch part of the North Sea seafloor (e.g. Olsen 1883). Recently, scientist and practitioners throughout Europe have been focussing on the endangered status of *O. edulis* habitats and there is scope for restoration (Airoldi and Beck, 2007; Farinas-Franko et al. 2018; Gercken and Schmidt, 2014; Sawusdee et al., 2015; Smaal et al., 2015; Smyth et al., 2018). Moreover, *O. edulis* beds are now identified as a priority marine habitat for protection in European MPAs (OSPAR agreement 2008-6, OSPAR Commission, 2011).

In the Netherlands feasibility of the recovery of epibenthic shellfish reefs is estimated as feasible (Smaal et al., 2015). The time for restoration of epibenthic shellfish reefs is right and shellfish reef restoration in the North Sea area is now supported by current Dutch and EU government policy, among others through the Marine Framework Directive, for the Dutch North Sea area implemented by the Marine Strategy policy paper, part 3 (Marine Strategy, 2015).

Based on the first findings of natural flat oyster beds (Christianen et al., 2018; van der Have et al., 2016) and experiences with epibenthic shellfish reef restoration in the Voordelta, knowledge is being developed for near shore flat oyster reefs (Sas et al., 2017; 2018, Christianen et al., 2018). The Borkum Reef Ground pilot is the first example of shellfish beds in deeper parts of the North Sea. As a first pilot location for offshore flat oyster restoration efforts, the Borkum Reef Ground area was selected. Didderen et al (2019) describe the results of the first year of monitoring including detailed info on the pilot design and two field reports of two field trips - installation and first monitoring.

1.2 Objectives

In a pilot project, installed in May 2018, the possibilities for restoration of shellfish beds in the deeper parts of the North Sea are studied. The project entailed both kick starting shellfish beds in deeper parts of the North Sea and getting insight in the key factors for success and failure for active restoration of structure-forming shellfish beds in deeper parts of the North Sea (see Appendix A for overall research outline).

1.3 Reading this field report

This report contains results of the monitoring of the pilot 16 months after deployment. The monitoring campaign took place 9-11 September 2019.

2 Methods

Photo: Udo van Dongen

Table 1. Overview of all intended monitoring activities. This table is derived from Didderen et al., 2019 where all specific research questions are elaborated. The numbers in the first column correspond with the locations in Chapter 3 where these activities are addressed.

	Monitoring activities	**Trip 4: September 2019**	**Research questions**	**Common**	**Diagnostic**	**Ecosystem**
1	Dropcam survey		1-3, 6, 10	X		X
2	Temperature measurements	X	8		X	X
3	Oyster measurements:	X	3-10	X		
	Wet weight measurement	X		X		
	Length measurement	X		X		
	Condition assessment				X	
	Gonad development				X	
	DW determining	X			X	
	Presence of Bonamia	X			X	
4	Visual observation of survival	X	1, 4	X		
5	Visual observation of present life forms	X	1, 2, 5, 6	X	X	X
6	Visual observation of rack & 3D structure damage	X	9 - 11			
7	Visual observation of biofouling and predators	X	9 - 11		X	X
8	Visual observation of spat settlement	X	3, 4, 5, 6, 7	X		
9	Visual observation of oyster bed development	X	1, 2	X		
10	Larvae sampling & counting		3, 8-10		X	
11	Spat collector	X	4, 5, 6, 7	X		

2.1 Preparations

Mobilisation

The offshore research team and their equipment were mobilised to spend two weeks at sea to carry out different diving studies. The intended research period for the Borkum Reef oyster project was 10 -12 September. Additional specialist oyster restoration researchers were shipped in by means of crew tender.

As heavy weather conditions were predicted to occur at 11 September, the research period was advanced to 9 -11 September.

Briefing and safety

After arrival of the additional crew, a full briefing was held with the entire research team. In this briefing, everyone was updated on the project goals and instructed on their specific task during diving operations and on-board operations. A full safety briefing and Last-Minute Risk Analysis where also conducted. It was checked that all safety measures (e.g. decompression chamber, medical equipment, communications equipment, safety diver) was operational and had a designated operator.

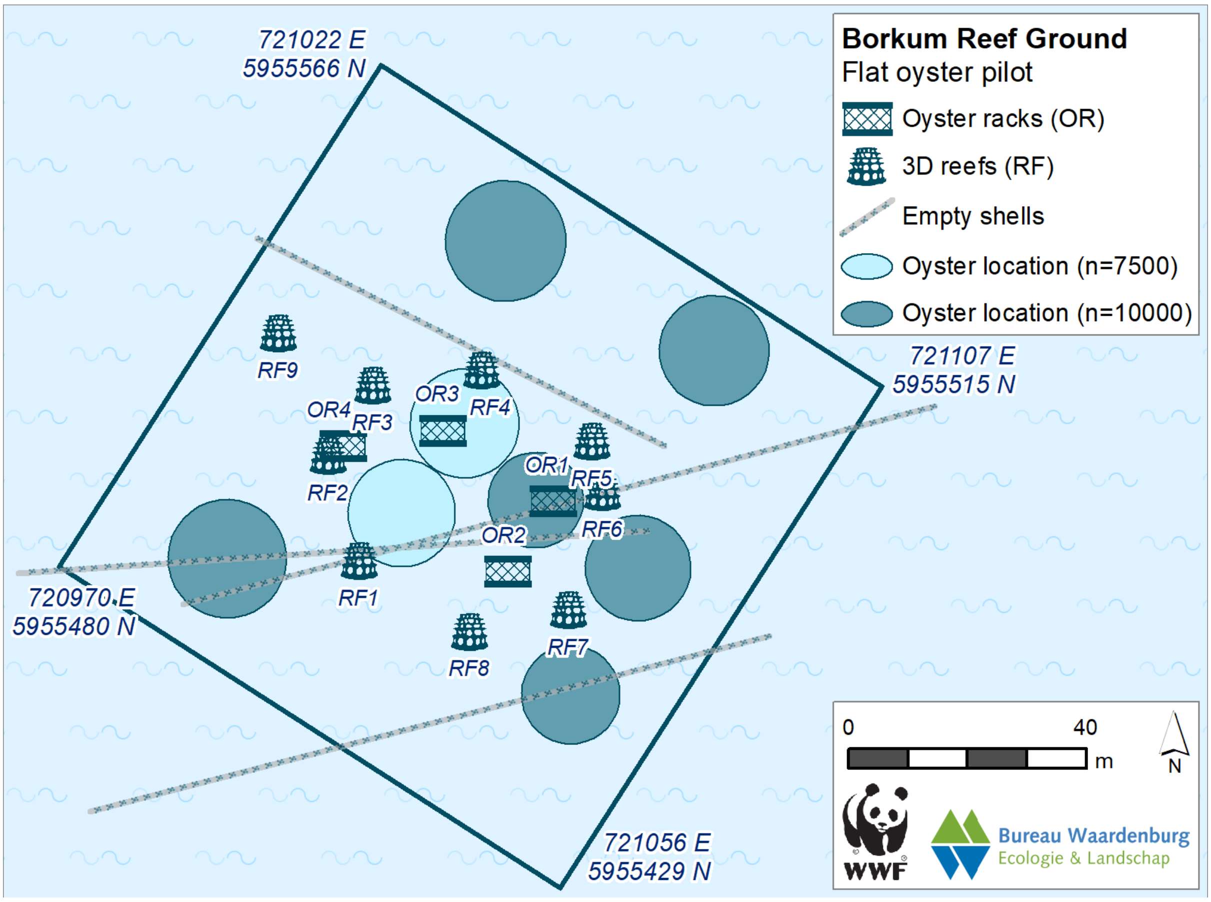

Figure 1. Pilot lay out, 9 3D reefs ,4 monitoring racks and 8 patches of oysters were installed on 24 and 25 May 2018 (Appendix B).

Diving
Weather conditions were particularly favourable at 9 and 10 September. 3 Research dives where made in optimal diving conditions, with 5 researchers each having 35 minutes bottom time. Diving depth was 26 meters. A Nitrox breathing gas was used with 35% oxygen. NOAA diving tables were used, and the 35 minutes dive time were well within the no-decompression limits.

2.2 Activities

The monitoring activities of this expedition included:
1. Research racks: retrieval of baskets, with oysters, spat collectors and temperature logger for temperature measurements (monitoring activity 2 in Table 1), wet weight and length measurements (3), spat settlement (8) and spat collector (11) and **laboratory analyses** for condition index and spat settlement.
2. Dive transects (UVS) for visual observations of oyster density and survival (4), present life forms / biodiversity (5), rack & 3D structure condition (6), biofouling and predators (7), recruitment / spat settlement (8), oyster bed development (9).

Top priority during diving was finding recruits on the seabed, as no other (remote sensing) technique is really suitable for finding recruits, and this was regarded as the most important result to obtain. Because no recruits were found in dive 1 and 2, most diving effort was focussed on searching for recruits, at the expense of obtaining quantitative data on oysters and biodiversity. Nevertheless, we ultimately succeeded in obtaining some quantitative data.

Photo 1. Divers retrieving oyster baskets from research rack (Udo van Dongen).

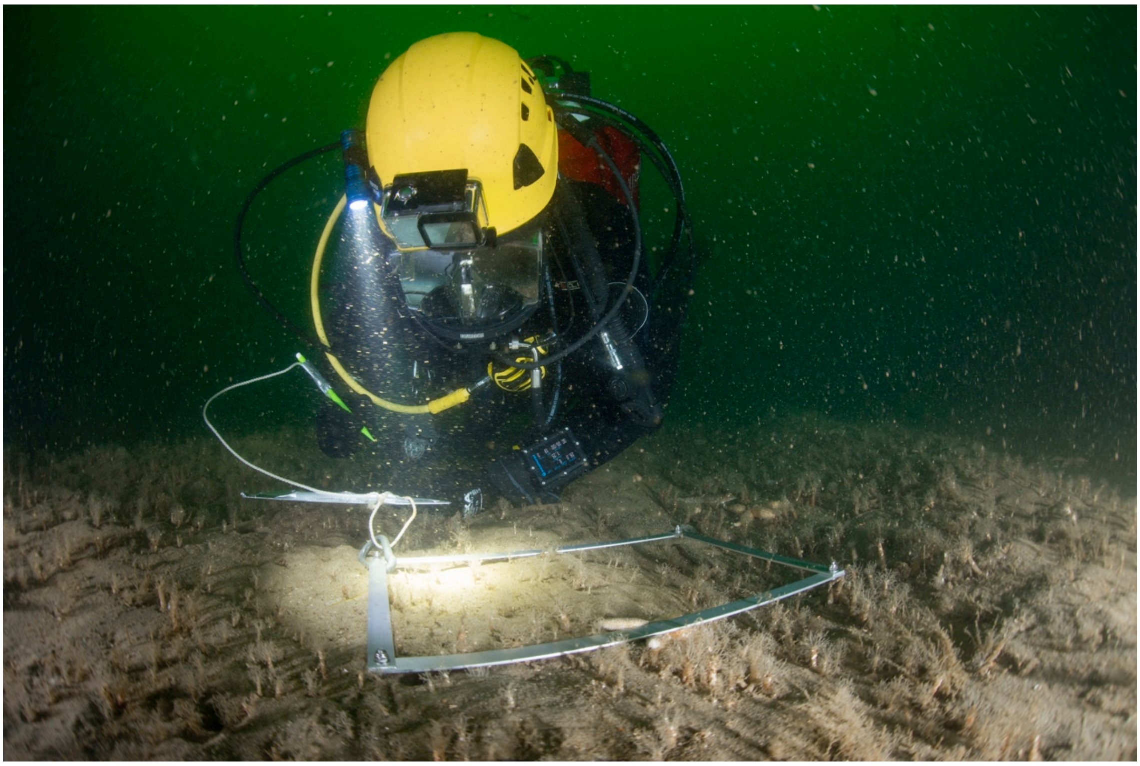

Photo 2. Divers retrieving biodiversity data within quadrant (Udo van Dongen).

2.3 Research racks

Acoustic release mechanism

In September 2019, two racks were equipped with acoustic releases (1 and 3). Both acoustic releases worked, and the location of research racks could easily be established. Acoustic release mechanisms were cleaned, and one was replaced at rack 1 (Frequency 101.1).

Retrieving oyster baskets

Each research rack contained 4 oyster baskets. The racks could be opened under water. The baskets where retrieved by a diver, packed in a large net, and sent to the surface by means of a lifting bag. The net with baskets was then picked up by a small RIB and transported to the ship. Baskets with oysters where placed in a holding tank on deck, with a continuous flow of fresh seawater.

Temperature data

Two Ibuttons were retrieved, one from each rack. New logger (CTD) was placed at rack 3.

Oyster survival, length, wet weight, condition index and *Bonamia* presence

Each research rack contains 4 baskets with 40 oysters (Table 2). Oyster basket contained different subgroups including “holding tower”, “small” and “large”. Holding tower: In two baskets per rack the oysters are placed in holding towers, enabling monitoring of identified individuals.

On September 9th, 2019, research racks 1 and 3 were hoisted and all 160 oysters per rack, were handled. Per basket the live and dead oysters were separated (Photo 3) and percentage survival was established. The live oysters were weighed (wet weight in gram) and measured (shell width in mm) and replaced in baskets. Pictures were made of all live oysters. Values for wet weight and shell width were compared with initial values obtained in May 2018 and values obtained in July 2018. An analysis of variance (ANOVA) was carried out on log-transformed data with a Bonferroni post-hoc test. Five oysters per basket were stored at -20 ^{0}C for determination of dry weight of meat and shell in the lab. Condition Index was calculated according to Walne, & Mann (1975) as the ratio between dry weight of the oyster meat and dry weight of the oyster shell. Condition Indices typically vary over the season due to investment in reproductive organs in spring and summer, decreasing the amount of energy available for growth. Per oyster a small piece of gill was sampled and send to Wageningen Bioveterinary Research for Bonamia analysis.

Recruitment

The spat collectors of both research racks were retrieved and brought to the lab for inspection. Furthermore, recruits that settled on live adult oysters were collected from the oyster baskets.

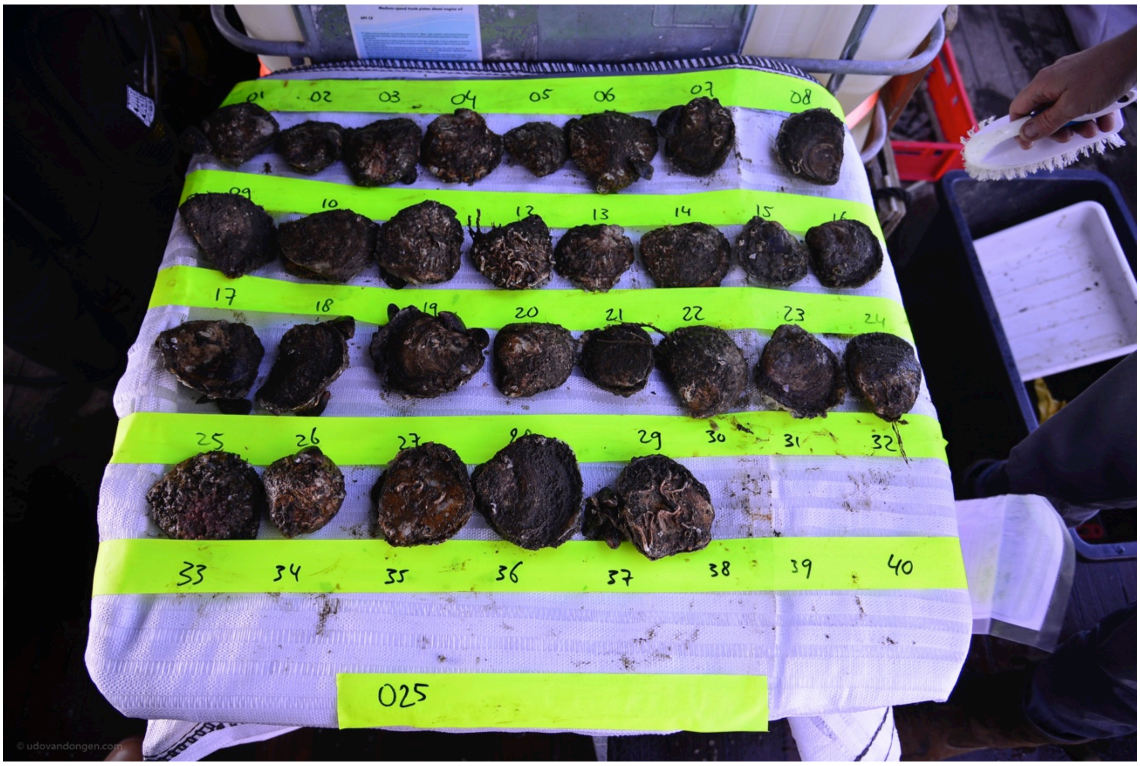

Photo 3. Live Flat oysters of basket 25. Dead oysters were removed and collected in a bag.

Table 2. Number of oysters per research rack and basket at time of installation.

Rack number	Basket number	Number of oysters	Comments	Min shell width (mm)	Max shell width (mm)
1	28	40	small	52.1	88.5
Position C3	8	40	holding tower	59.8	88.2
	10	40	holding tower	62.9	92.8
	17	40	large	68.6	111.1
2	21	40	small	40.0	76.3
Position C4	11	40	holding tower	64.2	95.4
	6	40	holding tower	64.5	93.0
	16	40	large	53.5	98.0
3	23	40	small	47.9	76.7
Position C2	3	40	holding tower	62.4	93.7
	12	40	holding tower	56.5	85.9
	25	40	large	71.1	106.1
4	1	40	holding tower	59.3	92.0
Postion C1	5	40	holding tower	64.5	87.2
	24	40	small	45.6	75.0
	32	40	large	73.2	106.1

2.4 Dive Transects UVS

Visual observations were performed on 9 and 10 September during 3 dives. Figure 2 depicts the different transects that where studied in the pilot area during the 3 dives, each dive with two teams doing separate transects.

During dives the following data were retrieved:

- **Pilot: Visual observation position and condition of infrastructure, including research racks, 3D-reefs and loose shell material.**
- **Oysters: Visual observations survival, density and growth of adult oysters, presence of recruits.**
- **Risks: Visual observations predators and other risks for oysters (e.g. pollution by the MSC Zoë).**
- **Biodiversity: Visual observations biodiversity on seabed, research racks and 3D reef structures.**

Oyster and predator density transects were carried out by following a transect line that is marked every meter and including a width of 2 meter for visual census. Each 5 meter (translating to 10 m^2) the number of oysters and predators was recorded. Oysters were classed as dead or alive based on visibly wide-open shells when oysters are dead and visible filtering activity from live oysters.

Biodiversity on the seabed was quantified by using a 20x30cm quadrant haphazardly placed on the seabed along the transect lines. In each quadrant, all visible species were recorded and named (to the highest taxonomic level of which our taxonomic specialist diver could be certain under water) and their density counted or estimated. This was repeated 26 times.

Biodiversity on the reefs and on research racks was observed and recorded, in part by photo and video analysis, densities were not quantified.

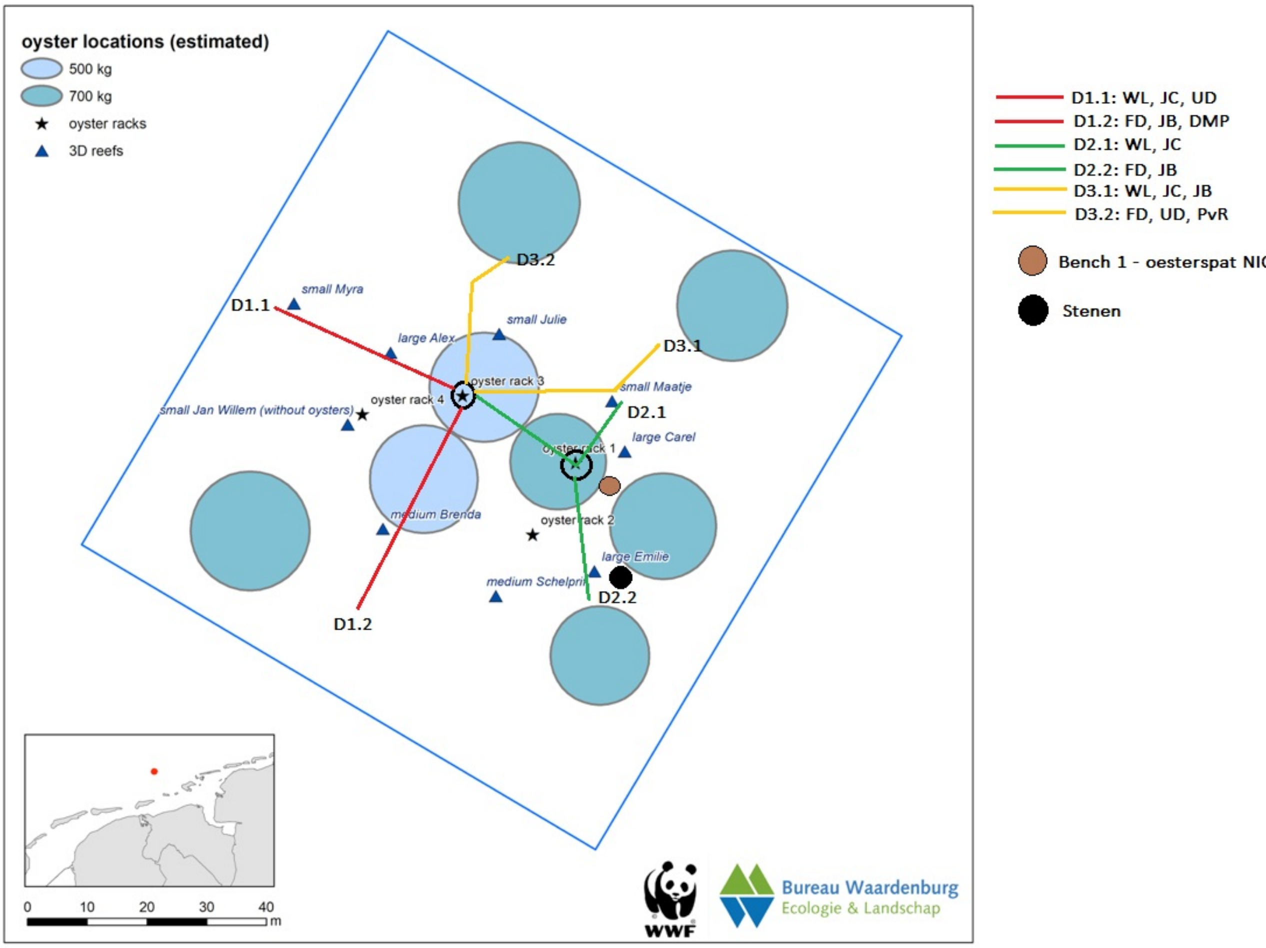

Figure 2. Dive transects in the pilot area. Indication of location of different dive transects (lines), live oysters (2018), oyster spat (brown circle, 2019), stones (black circle), 3D reef structures (triangles), oyster racks (asterix).

3 Results

Photo: Udo van Dongen

3.1 Temperature [2]

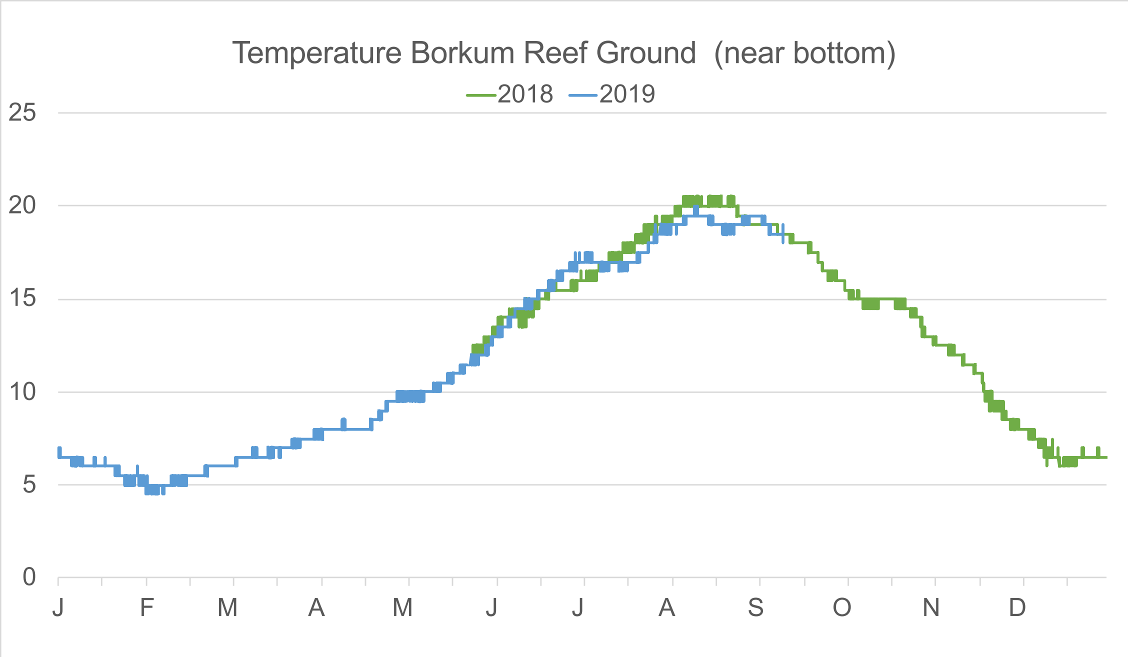

Figure 3. Near bottom temperature at Borkum Reef Ground 2018 (green line) and 2019 (blue line).

Temperature varied from 20.5 ^{0}C in the middle of August to 4.5 ^{0}C in the beginning of February (Fig. 3).
In 2018 the datalogger retrieved from wind farm Gemini showed lower temperatures than the one retrieved from Borkum Reef Ground (Didderen et al. 2019). Gemini is a deeper location than Borkum Reef Ground.
Recently a model was established indicating the importance of a threshold value for larval release of 576 degree-days (Maathuis et al. 2020). In 2018 this threshold value could not be established due to missing data at the start of the timeseries in that year. In 2019 the threshold was reached on 16 July 2019. This is a little earlier than the sampling date of 24 July 2019 when no larvae were detected (EcoFriend report in prep.), In 2018 larvae were detected on 20 July 2018, the only sampling date (Didderen et al, 2019).

3.2 Oyster width and wet weight [3]

Table 3. Flat oyster length and wet weight in research racks.

Rack	Bas ket	May 2018 Width (mm) MIN	May 2018 Width (mm) MAX	Sep 2019 Width (mm) MIN	Sep 2019 Width (mm) MAX	Sep 2019 Width (mm) AVG	Sep 2019 Wet weight (g) MIN	Sep 2019 Wet weight (g) MAX	Sep 2019 Wet weight (g) AVG	# oysters
1	28	52	89	65	109	85,8	79	147	112,9	26
1	8	60	88	62	92	79,6	61	143	100,0	18
1	10	63	93	74	90	80,2	70	127	98,8	21
1	17	69	111	80	117	97,1	89	360	205,6	29
3	23	48	77	48	95	80,7	34	152	101,3	25
3	3	62	94	67	89	77,2	55	124	94,1	23
3	12	56	86	68	92	80,6	64	145	96,6	17
3	25	71	106	82	114	93,8	117	355	201,7	27
			Total Sep 2019	48	117	84,6	34	360	132,0	186

Oyster width and wet weight
Flat oyster width in racks varied in September 2019 from 48 – 117 mm, with an average of 84,6 after, the range in May 2018 was 48-111 (Table 3), and wet weights of 34-360 g, with an average of 132,0 g.

Oysters of two racks were analysed and compared to initial values (May 2018) and values of the rack that was analysed in July 2018 (Fig. 4). Higher growth rates were observed in loose oysters than in oysters in holding towers. The highest growth rate was found in small oysters. They showed a significant increase in shell width and wet weight in September 2019 (Fig. 4 and Table 4). Large oysters showed a significant increase in wet weight, but not in shell width in September (Fig. 4 and Table 4).

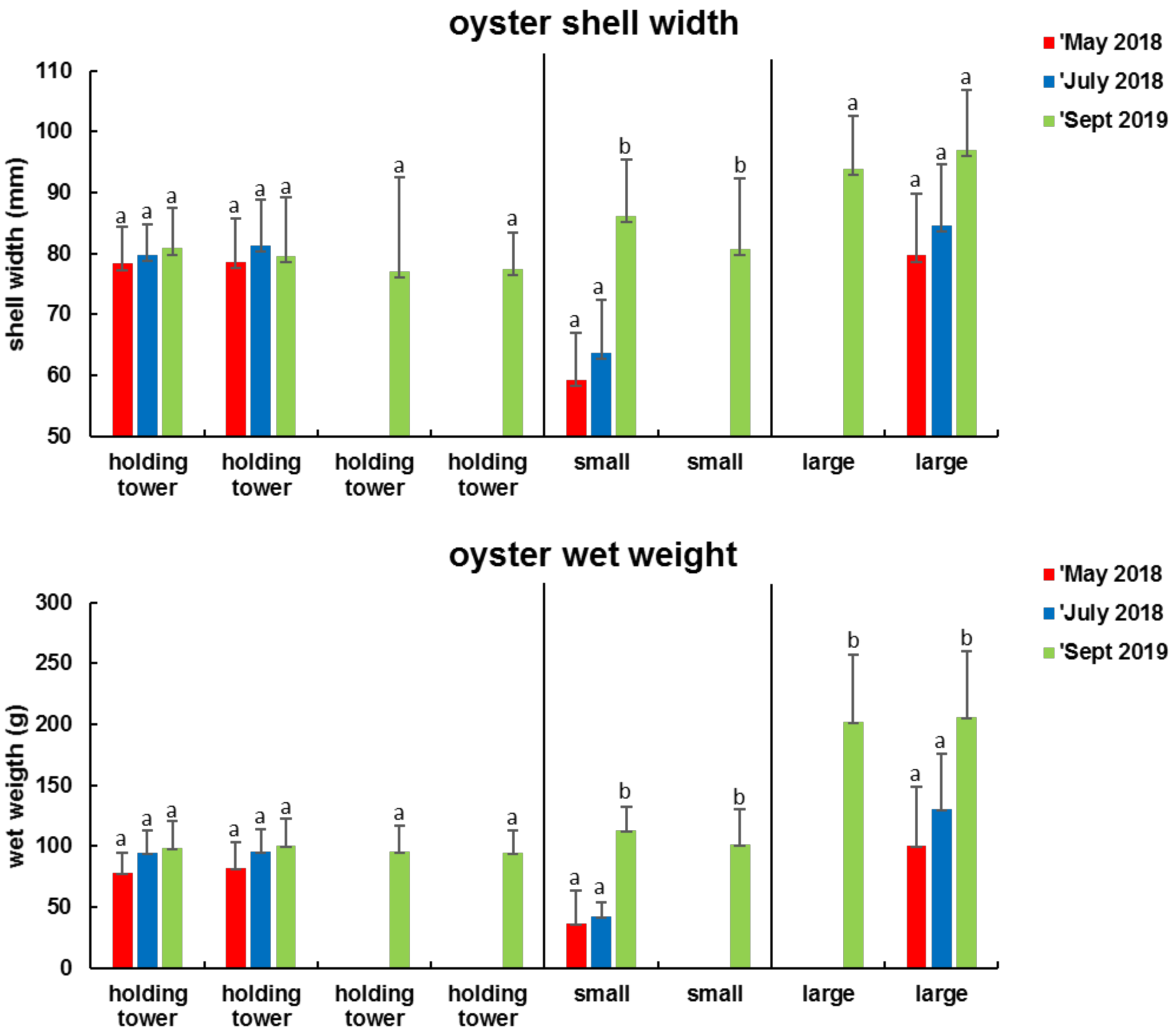

Figure 4. Shell width in mm (upper panel) and wet weight in g (lower panel) in initial subsample (May 2018), rack 2 (July 2018) and rack 1 and 3 (September 2019). Different letters indicate significant differences within groups (see also Table 4).

Table 4. Results of ANOVA on differences in wet weight and shell weight, ns = not significant.

Type	**Variable**	**Period**	**P value**
Holding tower .	Log width	May - Sep	ns
		July - Sep	ns
	Log weight	May - Sep	ns
		July - Sep	ns
Small	Log width	May - Sep	0.041
		July - Sep	0.003
	Log weight	May - Sep	0.000
		July - Sep	0.037
Large	Log width	May - Sep	ns
		July - Sep	ns
	Log weight	May - Sep	0.000
		July - Sep	0.002

Pogoda et al. (2011) observed increases in shell width from 30 mm to 50 mm in a period from April to October at an offshore location in the German Bight. This is a faster growth rate than observed in the Borkum Reef oysters. Pogoda's experiment was carried out in lantern nets suspended from a buoy. Therefore, it does not represent the conditions on the seabed. In addition, the initial size (age) of their oysters was much smaller (younger) and as younger oysters show higher growth rates (Fig. 4) this explains part of the difference in findings.

3.3 Condition index [3]

Condition index varied from 1 to 4.8 (Table 5). This is lower than values of 4.5-8 observed in October by Podoga et al. (2011) in the suspended offshore oysters in the German Bight. This difference might be explained by differences in food supply which may be better higher up in the water column. The lowest values were found for the oysters in three baskets with holding towers (basket 3, 8,10). This confirms the low growth in this set-up that confined oysters in space.

Table 5. Shell length, width, dried meat weight, dried shell weight and condition index of randomly selected oysters from 8 baskets.

Basket and oyster number	Length (cm)	Width (cm)	Dried meat (g)	Dried shell (g)	Condition-index
025-1	82.89	73.96	1.80	88.80	2.03
025-2	102.67	88.71	3.71	121.90	3.04
025-3	100.20	86.79	5.62	135.60	4.14
025-4	99.60	79.80	3.86	119.60	3.23
025-5	110.11	81.80	4.49	139.50	3.22
012-1	76.19	70.13	2.03	50.00	4.06
012-7	77.49	77.22	3.43	71.60	4.80
012-32	87.81	76.42	3.62	75.80	4.77
012-40	75.98	65.04	2.36	49.00	4.82
028-1	93.60	79.52	3.33	71.70	4.65
028-2	87.15	80.93	4.19	99.30	4.22
028-3	80.42	75.16	3.00	60.70	4.95
028-25	82.16	73.41	2.57	63.70	4.04
028-26	101.58	88.40	4.14	99.30	4.17
003-002	71.11	71.65	1.89	66.60	2.83
003-003	79.43	86.52	0.95	72.80	1.31
003-010	87.01	79.85	2.11	83.70	2.52
003-039	77.75	74.44	2.93	76.30	3.84
003-040	84.08	78.39	1.46	67.00	2.18
023-01	87.55	86.00	2.95	63.20	4.67
023-02	83.31	69.45	1.38	48.10	2.86
023-03	103.58	89.54	2.50	98.20	2.55
023-32	82.75	68.90	1.92	40.10	4.78
017-1	112.19	103.99	4.42	164.90	2.68
017-2	103.02	88.89	4.45	123.20	3.61
017-28	107.13	89.14	4.32	126.40	3.42
017-29	97.19	101.02	2.73	155.90	1.75
010-2	81.76	73.64	0.71	73.20	0.97
010-3	78.68	74.26	2.02	65.90	3.07
010-4	89.08	71.12	1.69	69.40	2.43
010-37	74.05	73.06	1.31	50.30	2.61
008-01	72.27	59.91	1.44	52.80	2.73
008-03	81.90	73.19	1.75	70.10	2.49
008-04	81.10	75.51	1.55	59.10	2.62
008-35	67.50	52.14	0.99	45.50	2.18
008-040	85.49	74.90	2.90	93.00	3.12

3.4 Bonamia presence [3]

36 oysters were tested by Wageningen Bioveterinary Research. All tested negative for Bonamia.

3.5 Oyster survival [4]

Table 6. Flat oyster survival rates in research racks.

Rack	Basket	Oyster size	Number at start (May 2018)	Alive	Dead	Survival % Sept ‘19	Number of recruits
1	28	small	40	26	14	65	0
1	8	holding tower	40	18	22	45	0
1	10	holding tower	40	20	20	50	0
1	17	large	40	29	11	73	3
3	23	small	40	24	16	60	0
3	3	holding tower	40	23	17	58	0
3	12	holding tower	40	16	24	40	1
3	25	large	40	27	13	68	0
						July ‘18	
2	21	small	40	28	12	70	0
2	11	holding tower	40	15	25	37.5	0
2	6	holding tower	40	22	18	55	0
2	16	large	40	37	3	92.5	0

Oyster survival in racks
Flat oyster survival in racks varied from 40 – 73 % after 16 months (Table 6). Minimum survival rate (40 and 45 %) was observed for oysters that were placed in the holding towers. The size of the oysters was too large for the holding towers, which highly likely confined them and therefore hampered them in their feeding / breathing behaviour. Highest survival rates per rack (73 % rack 1, 68% rack 3) were observed for baskets with ‘large’ oysters (Table 6). When analysing the oysters collected for condition index three oysters were not containing any meat. This suggests that the survival determined on board in September was possibly too high. As expected, survival after 3 months was higher than after 16 months, but in all cases large oysters showed highest survival.

Oyster density and survival on the seabed
The divers observed and recorded adult oysters on the seabed during every dive, although density seemed to vary substantially within the pilot area. Noteworthy, all live adult oysters had signs of recent growth (clean white shell edge). Furthermore, all oysters were observed to lie on their flat side (right). This is different from their initial position on the seabed as recorded by dropcam shortly after placement (majority on

concave side (left)). All oysters where partly covered by sediment, and many had other species growing on them, such as tubeworms or hydroids. Near gabions, several loose lying juvenile oysters where found on the seabed. According to their size of approximately 4 cm, these were most likely NIOZ-oyster spat, placed in 2 gabions on the seabed in April 2019.

Table 7 presents data from the quantitative oyster transect, obtained from transect D2.1 (Fig. 2). Table 8 presents data from 3 more oyster counts that where obtained whilst searching for recruits. In the transect D2.1 average oyster density was 0,37 oysters per m^2 (Table 7). Total recorded live oysters during counts and the quantitative transect was 108 and 10 dead oysters where recorded. This results in 92% survival, and 8% *recorded* mortality of oysters on the seabed.

Table 7. Quantitative transect data oyster census (obtained from transect D2.1; Figure 2).

Transect meters (2m wide)	**Substrate: shell %**	**Recruits present (y/n)**	**Oysters present (y/n)**	**# oysters alive**	**# oysters dead**	**Density (oyster / m^2)**	**Predator species**	**Density (starfish / m^2)**
1-5	15	n	n	0	0	0	*Asterias rubens*	0,9
5-10	5	n	y	3	0	0,3	*Asterias rubens*	0,1
10-15	5	n	y	4	0	0,4	*Asterias rubens*	0,3
15-20	5	n	y	4	0	0,4	*Asterias rubens*	0,6
20-25	5	n	y	5	0	0,5	*Asterias rubens*	0,6
25-30	5	n	y	3	0	0,3	*Asterias rubens*	2,5
30-35	2	n	y	4	0	0,4	*Asterias rubens*	0,7
35-40	2	n	y	4	0	0,4	*Asterias rubens*	0,4
40-45	2	n	y	4	0	0,4	*Asterias rubens*	0,7
45-50	5	n	y	5	0	0,5	*Asterias rubens*	0,4
50-55	3	n	y	5	1	0,5		

Table 8. Data from 3 additional oyster-counts to determine mortality on the sea floor %.

	# oysters alive	# oysters dead	mortality %
count 1	17	1	6
count 2	27	2	7
count 3	22	6	21

Photo 4. Flat oyster providing habitat for epifauna species: sand mason worm (Lanice conchilega) and queen scallop (Aequipecten opercularis) are attached to this adult oyster.

3.4 Biodiversity [5]

Seabed

The seabed consisted of sandy substrate, with patches of gravel, shell substrate, *Lanice* congregations and oysters (Photo 4). Table 9 presents data from 26 quadrants in which biodiversity is quantified. A total of 28 species was observed on the seabed (Table 9). Noteworthy were large quantities of juvenile queen scallop present on the seabed in the pilot area (Photo 4).

Reefs and racks

Substantial biodiversity was also observed on the 3D-reefs and research racks (Table 9,10). A total of 17 species were observed on these structures. Noteworthy were the substantial amounts of mobile species such as goldsinny wrasse and edible crab that were attracted by the reefs, and young recruits of the cold-water coral dead man's finger. A total of 42 species were observed by the divers in the pilot area.

Table 9. Biodiversity and density of species on the seabed in the pilot area presented as mean densities per square meter. Data retrieved from 26 quadrants of 20x30 cm.

Species %	**Common name**	**Mean coverage %**
Bryozoa	Moss animals	0,19
Hydrozoa	Hydrozoans	0,38
Spionidae	Spionid worms	0,15
Lanice conchilega	Sand mason worm	12,81
Diplosoma listerianum		0,77
Species n		**Mean number per m2**
Sagartiogeton undatus	Small snakelocks anemone	0,64
Sagartia troglodytes	Mud sagartia	0,64
Bivalvia	Bivalves	2,56
Ostrea edulis	European flat oyster	2,56
Aequipecten opercularis	Queen scallop	0,64
Aeolidia papillosa	Common grey sea slug	egg
Polycera quadrilineata	Four-striped polycera	3,21
Pagurus bernhardus	Common hermit crab	0,64
Pisidia longicornis	Long-clawed porcelain crab	0,64
Inachidae	Spider crabs	0,64
Macropodia		0,64
Liocarcinus		0,64
Asterias rubens	Common sea star	3,21
Gobiidae	Gobies	1,28
Ascidiacea	Sea squirts	11,54

Table 10. Total species list obtained during diving operations. Inspected 3D reefs where: Alex, Myra, Brenda, Maatje & Emilie (Fig. 2).

Total species list		**Seabed**	**Reefs**	**Research racks**
Scientific name	Common name			
Bryozoa	Moss animals	x	x	x
Hydrozoa	Hydrozoans	x		x
Chrysaora hysoscella	Compass jellyfish			
Metridium senile	Plumose anemone		x	x
Sagartiogeton undatus	Small snakelocks anemor	x		
Sagartia troglodytes	Mud sagartia	x		
Alcyonium digitatum	Dead mans finger		x	
Bivalvia	Bivalves	x		
Ostrea edulis	European flat oyster	x		
Aequipecten opercularis	Queen scallop	x		
Mytilus edulis	Blue mussel			x
Ensis	Razor shell	x		
Trivia	Cowrie		x	
Aeolidia papillosa	Common grey sea slug	x		
Polycera quadrilineata	Four-striped polycera	x		
Sessilia	Barnacles		x	x
Pagurus bernhardus	Common hermit crab			
Pisidia longicornis	Long-clawed porcelain cra	x		
Inachidae	Spider crabs	x		
Macropodia		x		x
Liocarcinus		x		
Liocarcinus holsatus	Flying crab	x		
Cancer pagurus	Edible crab	x	x	x
Spionidae	Spionid worms	x		
Lanice conchilega	Sand mason worm	x		
Sabellaria spinulosa	Ross worm			
Spirobranchus triqueter	Keelworm	x		
Asterias rubens	Common sea star	x	x	x
Astropecten irregularis	Sand sea star	x		
Psammechinus miliaris	Green sea urchin			x
Pholis gunnellus	Rock gunnel		x	x
Ciliata mustela	Fivebeard rockling		x	x
Parablennius gattorugine	Tompot blenny			x
Ctenolabrus rupestris	Gold sinny wrasse		x	x
Agonus cataphractus	Hooknose	x		
Raniceps raninus	Tadpole fish		x	x
Mullus surmuletus	Striped red mullet	x		
Callionymus lyra	Common dragonet	x		
Gobiidae	Gobies	x		
Limanda limanda	Common dab	x		
Ascidiacea	Sea squirts	x		
Diplosoma listerianium		x	x	x

Oysters

Although no quantitative data of oyster epifauna were collected, divers noted a large biodiversity on and underneath oysters. This included the sand mason worm (*Lanice conchilega*), the solitary individuals of ross worm (*Sabellaria spinulosa*) queen scallop (*Aequipecten opercularis*), nudibranchs, nudibranch eggs, different species of sea anemones and the tube worm (*Spirobranchus trique*ter). Furthermore, fish species such as the goldsinny wrasse and goby species hiding underneath oysters (Photo 5) were observed.

Photo 5. 3D Reef structure (Alex), schools of goldsinny wrasse, edible crab, anemones, hydroids and common starfish.

3.5 Performance of research rack and 3D-reef structure [6]

Inspection by divers in September 2019 showed structures, both racks and 3D reefs standing upright and intact (Photo 1,5,6). Only small reef Myra had sunk in the seabed almost entirely (Photo 7). The rest of the structures (also other small reefs) was undamaged, unaffected by scouring and was generally performing as intended. Only two epoxy glued oyster on a reef structure were observed and both were alive (reef Emilie, Photo 6). All other glued oysters were detached from the structures.

Photo 6. Reef Emilie, reef structure with 2 epoxy glued oyster, edible crab and goldsinny wrasse (Udo van Dongen).

Photo 7. Reef Myra, this reef structure has sunk into seabed (Udo van Dongen).

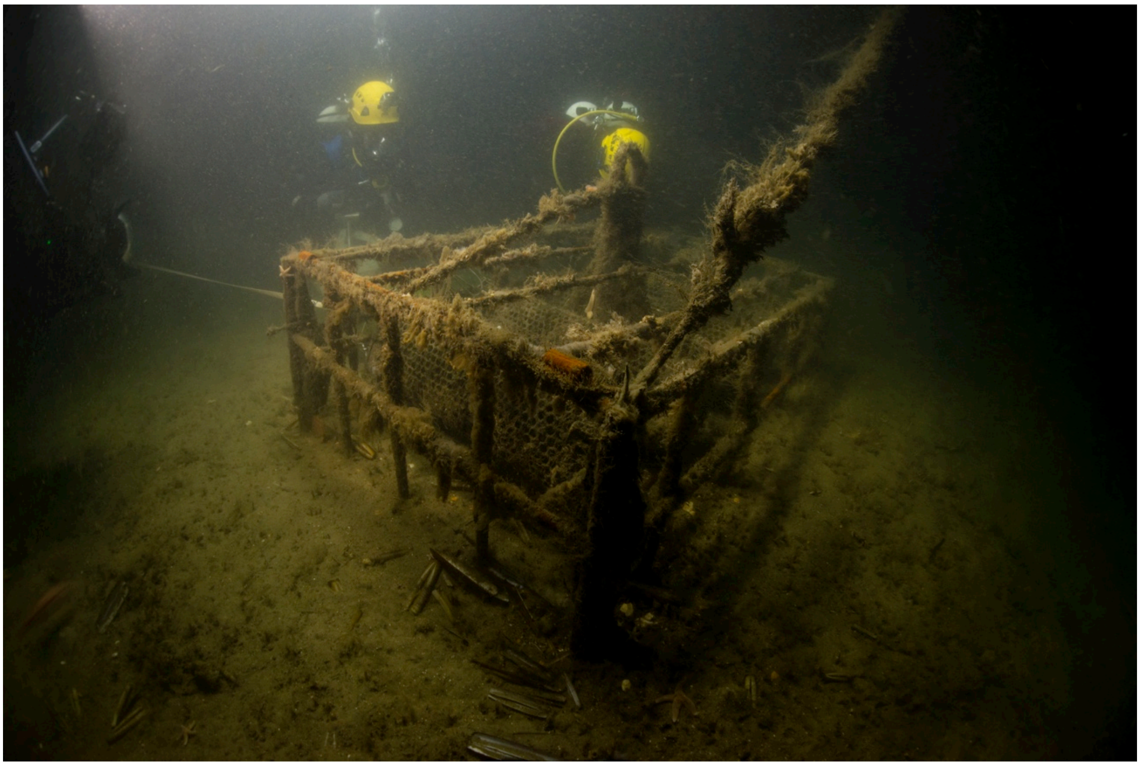

Photo 8. Research rack (Udo van Dongen).

3.6 Biofouling and predators [7]

Fouling organisms

The reefs where colonised by a diverse epifaunal community, which mostly consisted of mobile species. Both research racks and reefs where not greatly covered in encrusting sessile organisms. This has undoubtedly been beneficial to the condition of the oysters in the research racks, as the baskets where not overgrown to a state in which suffocating would be a risk. During inspection of the oysters in the research racks several species where observed to be growing on the oysters, such as blue mussel, hydroid species and the tubeworm *Spirobranchus triqueter*.

Predators

Common starfish (*Asterias rubens*) and edible crab are known predators, especially for young oysters, and were observed in densities up to 2,5 per m^2 on the seabed (Table 9), the reefs and the research racks. High survival percentage of adult oysters, however, suggests that this is no direct threat to the adult oysters.

Risk of pollution by MSC Zoë

Evidence of lost cargo by the MSC Zoë was found on and near the pilot area. Small strips of aluminium (possibly car-parts) and a jacket was found within the pilot area. Other cargo was found near the pilot area, mostly more car parts and jackets. The density of lost cargo items was small however, and so far we do not detect any direct effect from cargo items on the oyster pilot.

3.7 Oyster recruitment [8]

A total of 5 recruits were found: 4 inside or on the baskets in research racks, settled on adult oysters (Table 11; Photo 10); 1 on the seabed, settled on a live adult oyster (Photo 9). Sizes of recruits ranged from 57 to 71 mm. The size of the recruits indicates that they settled in 2018 and not in 2019.

Table 11. Data from oyster recruits.

Recruit	Basket/ Seabed	Width (mm)	Settle substrate
1	12	71,8	Holding tower + oyster
2	17	61,1	Adult oyster (alive)
3	17	66,2	Adult oyster (alive)
4	17	57,0	Adult oyster (dead)
5	Seabed	-	Adult oyster (alive)

Photo 9. Recruit on seabed (Wouter Lengkeek).

Photo 10. Three flat oyster recruits, likely to be recruited in 2018 (bottom right) and in comparison a juvenile, lab reared oyster that was outplaced in 2019 (top left) (Wouter Lengkeek).

In addition, smaller juvenile oysters (ca. 4 cm) were also found, lying lose on the seabed. However, considering their size, location and as no settlement substrate could be distinguished, it is most likely that these are the juvenile oysters produced in the NIOZ lab in 2019, that have been placed in April 2019.

Spat collectors

Eight spat collectors were examined. No *O. edulis* spat was observed, but eight individuals of *Heteranomia squamula* (UK: Prickly jingle, NL: Schilferige dekschelp) were found (Photo 11).

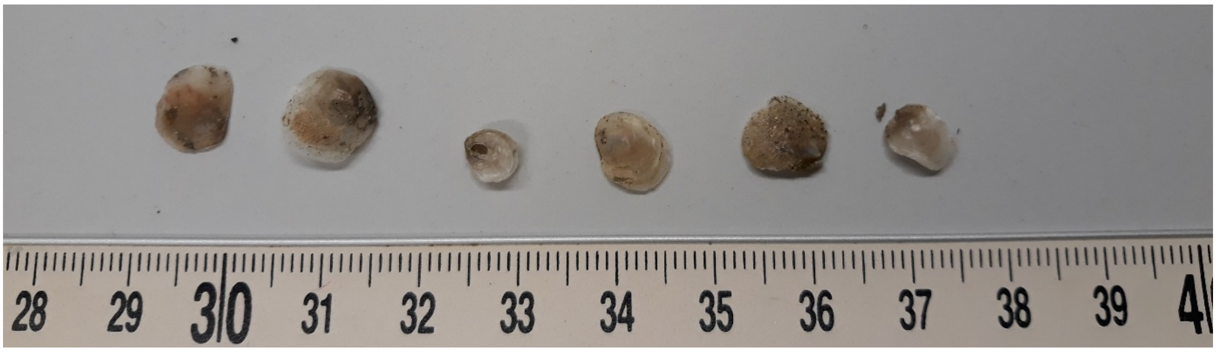

Photo 11. "Schilferige dekschelp" *Heteranomia squamula (Pauline Kamermans).*

3.8 Oyster bed development [9]

Large numbers of adult oysters are still present, growing and healthy. Density inside the pilot area seems lower than after deployment, but no signs of high mortality where observed. Recruitment was observed in the pilot area, but to date only in low numbers.

4 Discussion

Photo: Udo van Dongen

4.1 Conclusion

Results are positive and promising so far: Adult oysters survive and grow 16 months after installation. Moreover in 2018 and 2019 oyster larvae have been observed in the water column showing reproduction of flat oysters at and near this location (Didderen *et al.*, 2019; EcoFriend report in prep.). In September 2019 evidence is obtained that in 2018 settlement took place in this newly established offshore population in 2018, yielding 5,7-7,2 cm recruits that were observed during this expedition. In conclusion, the Borkum Reef Ground oyster reef, that was actively restored in 2018, shows survival, growth, reproduction and recruitment.

4.2 Lessons learned

Oyster pilot

The main lessons learned in 2019 are:

- It is possible to introduce installations and flat oysters on the sea floor at a deep (23 meters at low tide) offshore location and retrieve them at a later point in time (16 months) for monitoring purposes.
- The placement of 5500 kg, or 80.000 specimen, adult flat oysters on the sea floor has so far led to the result of flat oyster specimen being alive, showing growth and recruitment. These results, 16 months after installation, although largely based on qualitative data, are promising.
- On the seabed, a survival rate of adult oysters of 92% was observed. Although many starfish where present in the pilot area, they seem no direct threat to the adult oyster population. It is unknown if the starfish affected recruitment of new oysters.
- The observed density of adult oysters in the pilot area is lower than can be expected (expected is average 8 per m2, as 80.000 oysters have been placed on 10.000 m2). Mortality, however, is an unlikely cause because only few dead oysters could be found. Divers also searched in the seabed, as burial might be a cause, but could not find any buried oysters. One viable hypothesis is that the oysters are being moved during stormy conditions and are now distributed over a larger area than the initial pilot area.
- The 3D reefs and research racks performed well and as intended. Marine growth proved no threat to the oysters in the racks. Only small reef Myra suffered from scouring effects and had sunken into the seabed, other reefs including other small reefs where not affected. It is unknown why reef Myra sunk into the seabed, and other reefs not. A plausible hypothesis is that it is caused by local differences in characteristics of the seabed.

Monitoring techniques

Most important lessons learned during monitoring were:

- Divers can efficiently monitor several aspects of the oyster bed in two days' time.
- Using in a rehoistable research rack is a good way to get quantitative results of oyster parameters like survival, growth and presence of recruits.

4.3 Recommendations

These positive and promising results encourage further monitoring of the experiment. In subsequent monitoring expeditions, the following monitoring is recommended:

- Monitoring of recruits from more recent years than 2018
- Monitoring of larval presence
- Growth and survival of adult oysters
- Density development of adult oysters
- Biodiversity development on seabed, also compared to a reference area
- Biodiversity development on artificial reefs
- Searching for evidence of adult oysters being moved out of the initial pilot area
- Monitoring of 3D reefs and research racks durable functioning (e.g. scour effects).

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Appendix A

Textbox: Objectives of the Borkum Reef Ground oyster pilot (Source: Reuchlin-Hugenholtz, 2018)

The following overall objectives are formulated for this project:

1. Kick start shellfish beds in deeper parts of the North Sea;
2. Get insight in the key success and failure factors for active restoration of structure-forming shellfish beds in deeper parts of the North Sea;

More specifically, the 2018 pilot project at the Borkum Reef Ground aims at:

A. Developing a methodology for construction and restoration of structure-forming shellfish beds of mussels and flat oysters.

B. Construction of a pilot flat oyster bed at deeper water in the North Sea at the Borkum Reef Ground area, by placement of:
   - live flat oysters (originating from Norway) at the pilot area of 100x100m.
   - research racks with flat oysters to study survival, growth and reproduction.
   - 3D artificial reefs to study facilitation of oyster bed restoration (incl. elevation);
   - Shells (empty) in the surroundings of the live oysters to function as hard substrate for larvae settlement.

C. Learning from the pilot project by studying the following research questions in a field (and laboratory) monitoring programme:
   a. What is the mortality rate of introduced oysters, and what is the cause?
   b. Can the introduced oyster population survive and reproduce, and if (not), why (not) (long-term objective)?
   c. Can the introduced oyster population reproduce: i.e. produce gonads, resulting in larvae in the water column, resulting in recruitment on substrates?
   d. Is biodiversity enhanced in the vicinity of the pilot area, through the formation of a natural reef?
   e. Did oysters in the pilot die, and if so, why?
   f. What are the critical success factors for the pilot project?
   g. What are the critical fail factors for the pilot project?
   h. Is biodiversity enhanced in the vicinity of the pilot area?

All activities in the pilot project are closely monitored, to determine success and failure factors and based on these factors to determine and describe a successful methodology for restoration of flat oyster beds in the North Sea.

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