Offshore flat oyster pilot Luchterduinen wind farm

Results campaign 2 (July 2019) and lessons learned

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Preface

Rijke Noordzee www.derijkenoordzee.nl is a program of Natuur & Milieu and Stichting De Noordzee. The aim of this program is to enhance nature development in offshore wind farms by a.o. artificial reefs and introduction of oyster reefs. The Luchterduinen oyster project is a first pilot.

Wind farm Eneco Luchterduinen (LUD) is located in the Dutch North Sea. Stichting De Noordzee and Natuur & Milieu, together with Van Oord, Eneco and ASN Bank, started a pilot to explore possibilities for nature enhancement with oysters in a wind farm in 2018. The project is realized in collaboration with the Flat Oyster Consortium consisting of Bureau Waardenburg, Wageningen Marine Research and Sascon. The project aims to get insight in the key success factors of active oyster restoration and aims to contribute to a blueprint for ecological enhancement options in offshore wind farms in the North Sea.

This report describes results of campaign 2 as part of the LUD oyster pilot.

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Summary

Shellfish reefs, mainly European flat oysters (*Ostrea edulis*), once occurred in 30% of the Dutch part of the North Sea. They have almost completely disappeared, due to overfishing, habitat destruction and diseases. Wind farms have been recognized as potential sites for the introduction of flat oysters, as they provide hard substrate and an undisturbed seabed due to the exclusion of trawling fishing boats.

In 2018 Stichting de Noordzee, Natuur en Milieu, Eneco, van Oord and ASN started a pilot on nature enhancement with flat oysters in offshore Windpark Eneco Luchterduinen (LUD).

The general objective of the LUD pilot is to elucidate how offshore nature enhancement, in particular with oyster reefs, can successfully be carried out in an offshore wind farm. The main objective is to identify key factors for success or failure in Windpark Eneco Luchterduinen. The second objective is to define what lessons learned from the pilot mean for the scalability of the used methods for the nature enhancement in offshore wind farms in the North Sea.

The results show that in the wind farm Luchterduinen survival, growth and reproduction (gonad development, larvae, swarming larvae in high concentrations) of introduced *Bonamia* free flat oysters from Norway was achieved in 2019 and that oysters remained *Bonamia* free. Furthermore, the installations attracted substantial biodiversity. These results indicate Luchterduinen wind farm can be a suitable site for flat oyster restoration and nature enhancement in general.

Considering the racks were found semi-submerged into the sea bed and the likelihood of these conditions reoccurring was deemed very high, the decision was made to interrupt and demobilise the pilot to prevent reoccurrence of this same phenomenon.

In conclusion:

- Use of adult *Bonamia* free Norwegian oysters to serve as broodstock in offshore wind farms show promising results on a biological level with respect to reproduction and swarming larvae as a result of this 8-month pilot.
- Sand accretion was observed around and in the oyster rack in the high dynamic conditions of Luchterduinen
- It is unclear whether the racks have sunk into the sand, or whether the sand has been accumulated into it. The design of the oyster racks should be revised for further testing at this location, or comparable sites.
- The sand accretion also indicates that the dynamic conditions in LUD need to be reviewed, especially in the context of the scalability of the pilot.

The next step in offshore oyster pilots should include redesigning equipment such as the oyster racks and monitor if the observed larvae in the water column have resulted in recruitment and survival of young oysters within the wind farm.

1 Introduction

1.1 Background

Shellfish reefs, mainly flat oysters (*Ostrea edulis*), once occurred in 30% of the Dutch part of the North Sea. They have almost completely disappeared, due to overfishing, habitat destruction and diseases, as was the case elsewhere in the marine world (Smaal et al., 2015; Airoldi and Beck, 2007). Wind farms have been recognized as potential sites for the introduction of flat oyster reefs (Lengkeek et al., 2017, Smaal et al., 2017; Kamermans et al. 2018), as they provide hard substrate and an undisturbed seabed due to the exclusion of trawling fishing boats.

More recently, scientist and practitioners throughout Europe have been focussing on the endangered status of *O. edulis* habitats and there is scope for restoration of, as well as nature enhancement with, oysters (Airoldi and Beck, 2007). Moreover, *O. edulis* reefs are now identified as a priority marine habitat for protection in European MPAs (OSPAR agreement 2008-6, OSPAR Commission, 2011) 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).
Over the past 3 years the first pilots with flat oysters started in the coastal zone of the Dutch North Sea (Voordelta, ARK/WWF, Sas et al., 2019). In 2018 three offshore flat oysterpilots were installed. The first at an offshore location near the Borkum Reef Ground (WWF), the second in wind farm Gemini (Gemini/WWF/ Edmelja) (Reuchlin-Hugenholtz, 2018) and the third in Wind farm Eneco Luchterduinen (LUD) (Stichting de Noordzee, Natuur en Milieu, Eneco, van Oord and ASN). Here we describe the first monitoring results of the LUD flat oyster pilot.

1.2 Research questions

The general objective of the LUD pilot is to elucidate how offshore nature enhancement with oyster reefs can successfully be carried out in an offshore wind farm. The main objective is to identify key factors for success or failure of nature enhancement with flat oysters in Windpark Eneco Luchterduinen. The second objective is to define what lessons learned from the pilot mean for the scalability of the used methods for nature enhancement with shellfish reefs in the North Sea.

In relation to the objectives the research questions in this pilot are:

1. How does the placed infrastructure perform?
2. Does the introduced population of oysters survive?
3. Do *Bonamia*-free oysters stay healthy?
4. What are risks for the introduced oyster population?
5. Does the introduced population reproduce?

6. Can larvae be detected in the water column?
7. Do the larvae settle on infrastructure?
8. What is the best location within a wind farm and best substrate for oyster settlement?
9. Which other species are stimulated by the oyster pilot?
10. What can we learn about success and failure factors with regard to nature enhancement with flat oysters in Windpark Eneco Luchterduinen?
11. What do the results and lessons learned mean for future pilots and the scalability of nature enhancement with shellfish reefs in the North Sea?

1.3 Reading this report

This report contains information on the results of the monitoring in July 2019. Chapter 2 describes the lay out of the pilot and monitoring methods, Chapter 3 the results and in Chapter 4 an evaluation of the results is presented. Chapter 5 contains a discussion of the results.

2 Materials and methods

2.1 Study area

Eneco Luchterduinen wind farm is located 23 km off the coast of IJmuiden. The wind farm is composed of 43 turbines and one offshore high voltage station (OHVS) in a 16 km^2 area and operational since 2015. The water depth at this location ranges between 19 m and 24 m relative to LAT.

The oyster pilot zone is placed within the concession boundaries of Luchterduinen (Fig. 1).

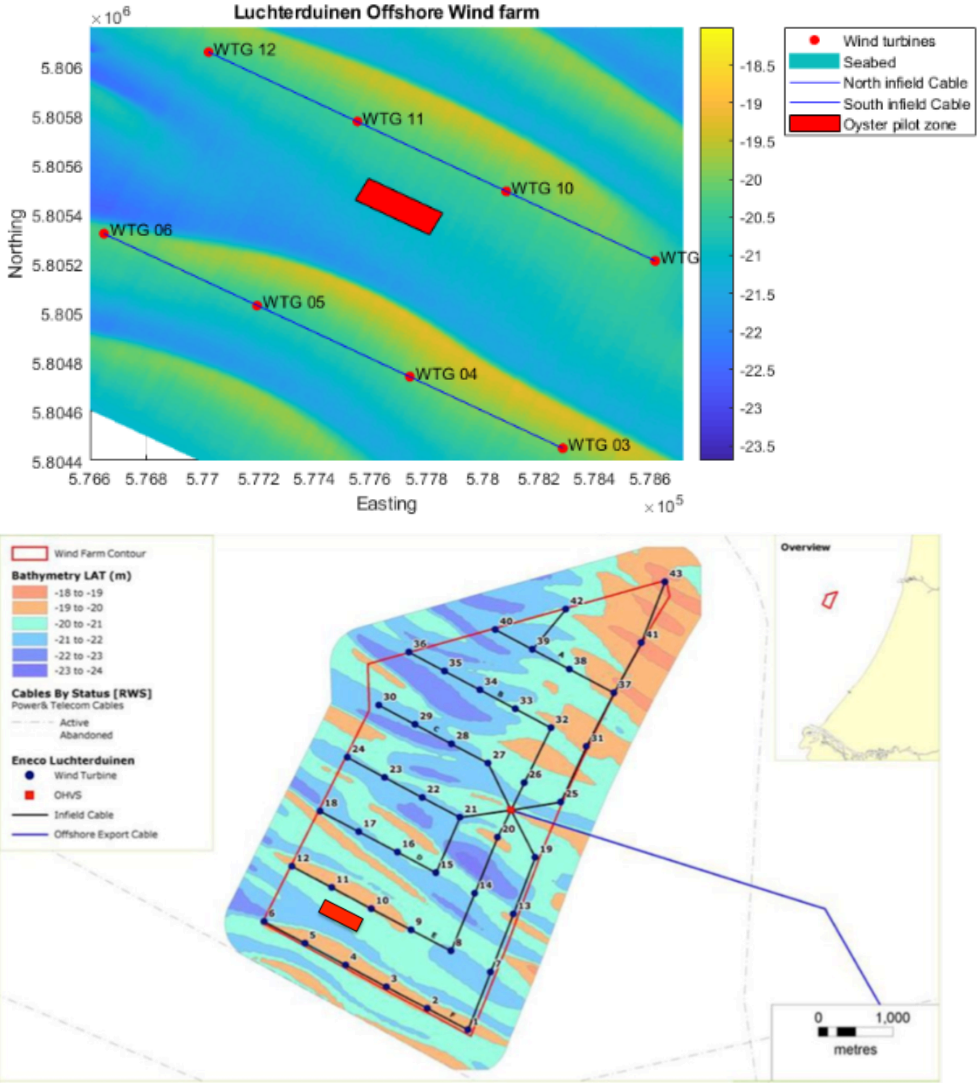

Figure 1. Overview of the Luchterduinen site. The oyster pilot is located on the seafloor in the area between turbines 11, 10, 5 and 4, indicated by the red quadrangle.

2.2 Pilot design

The design of the project includes racks designed for monitoring purposes: three oyster racks in which the oysters are placed, six substrate racks in which different substrate material is placed. Additionally two reef domes that facilitate locating the oyster racks and serve as additional protection were included in the design. The design characteristics and lay out of the Oyster Pilot Luchterduinen is provided in Fig. 2.

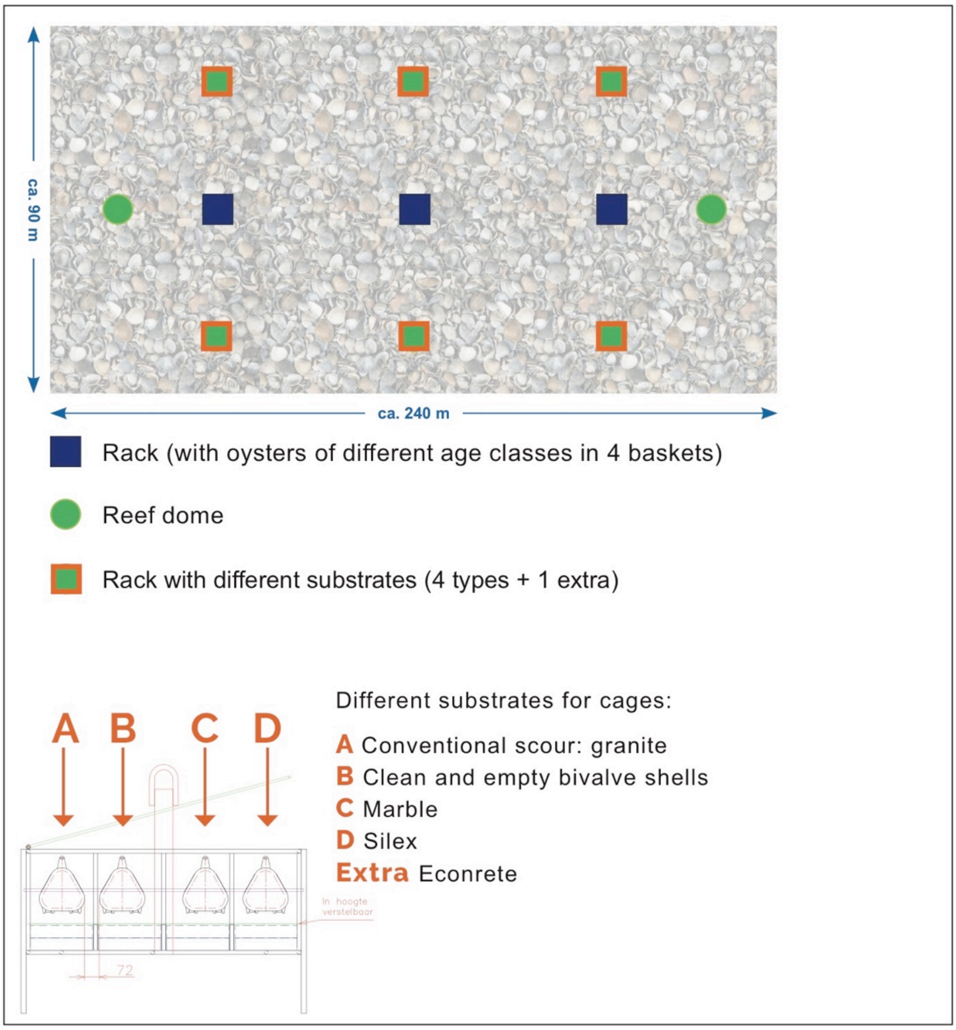

Figure 2. Design of Luchterduinen oyster pilot.

On the 5th of November 2018 3 oyster racks and 2 reef domes were installed on the seafloor using the side-stone-dumping vessel HAM 602. All infrastructure was marked with sub-sea buoys, that aid in retrieval for monitoring purposes and eventually decommissioning. A detailed as-built drawing after the first campaign was established (Appendix 2). A combination of multibeam survey with echoscope equipment was interfaced for the exact positions of installed infrastructure. Substrate racks were supposed to be installed in July 2019, but interruption of the pilot led to the demobilisation of all infrastructure on 9 July 2019.

2.3 Offshore equipment

For the operations an offshore supply vessel (VOS Star) with a heavy compensated deck-crane and ROV was used. Lifting operations were executed with the fixed off-shore crane on board of the ship. A ROV Eye Shank hook and HMPE 12-strand ropes were connecting the infrastructure to the off-shore deck-crane used for the recovery of the 3 oyster racks and 2 reef domes (van Oord 2019 a and b).

2.4 Monitoring parameters and activities

To answer the key questions, multiple parameters were measured during the monitoring campaigns (Table 1; Monitoring plan 2019). Monitoring was performed in line with Native Oyster Restoration Alliance (NORA) guidelines for restoration project metrics (Appendix 1). By using these guidelines, results obtained from this pilot experiment can be compared to flat oyster restoration efforts elsewhere in Europe. Table 2 shows monitoring activities and corresponding parameters.

Table 1. Research questions and corresponding parameters.

	Research parameters														
Question	Infrastructure	Position & persistance	Fouling organisms	Env. Developments	Pop. dynamics	Survival	Growth & condition	Gonad development	Seks ratio	Larvae	Spat	Risks	Predators & pests	Biodiv	# species groups
1 How does the placed infrastructure perform?		X	X	X											
2 Does the introduced population of oysters survive?						X									
3 Do infection-free oysters stay healthy?						X	X	X	X						
4 What are risks for the introduced oyster population?													X		
5 Does the introduced population reproduce?								X	X	X					
6 Can larvae be detected in the water column?										X					
7 Do the larvae settle on infrastructure?											X				
8 What is the best relative location and best substrate for oyster settlement?											X				
9 Which other species are stimulated by an oyster restoration pilot?															X
10 What can we learn about success and failure factors with regard to oyster restoration in OWFs?		X	X	X		X	X	X	X	X	X		X		X

Table 2. Activities and corresponding parameters.

Activities	**Infrastructure**	Position & persistence	Fouling organisms	Env. Developments	**Pop. dynamics**	Survival	Growth	Gonad development	Sex ratio	Larvae	Spat	**Risks**	Predators & pests	**Biodiv.**	# species groups
Check persistence infra.		X		X											
Underwater Video surv.		X	X	X									X		X
Oyster racks & measurements			X			X	X	X	X		X		X		X
Larvae counting										X					
Spat collection – spat collectors											X				
Spat collection - substr. racks			X								X		X		X
Temperature measurements								X		X					
Inspection infrastr. at decomm.		X	X	X							X		X		X

2.5 Methods

Position and persistence

The retrieval of the infrastructure gives information on the performance of the infrastructure. All structures were hoisted on board and visually inspected on deck. The structures were inspected on corrosion, wearing and biofouling.
Visual inspections were executed with a Remotely Operated Vehicle (ROV) on 9 July between 11:15 - 14:45 and 19:15 - 20:30. The infrastructure (reefdome, oyster rack) were inspected to check position and persistence of the oyster racks, and reef domes as well as obvious developments in the environment (i.e. presence of sand accretion or scour).

Fouling organisms

A brief scan of biodiversity was performed both during infrastructure retrieval and ROV inspections to identify whether fouling organisms were present on structures.

Environmental conditions

Temperature: Since spawning of the flat oysters is induced mainly by seawater temperature, this parameter provides useful insight on the relevant environmental conditions for projects on creating oyster reefs. Also, it determines the timing of varying aspects of the pilot project (e.g. placement of empty shells, larvae sampling) in relation to seawater temperature and is therefore important in determining an optimal methodology for oyster reef creation and restoration. Surface water temperature measurements were available via Eneco. Near-bottom temperatures were measured continuous with a temperature logger in each of the 3 oyster racks. A detailed multibeam survey was executed during installation in November by Van Oord.

Population dynamics

Three oyster racks, with 4 monitoring baskets each, were first inspected and monitored via underwater video recordings. Campaign 2 included hoisting the racks and performing detailed measurements of oyster survival length and weight (Photo 1) for all oysters.

Photo 1. Length measurements of oysters on board.

The following parameters were used to determine the fitness and reproductive status of the oysters.

- **Survival**
  - Survival rate (number of dead and alive oysters, N=480))
- **Growth & Condition:**
  - Width (mm, N=480))
  - Weight (mg) (wet (N=68) and Ash Free Dry Weight (AFDW (N=17)),
  - Condition Index = dry meat weight / dry shell weight (N=17)
- **Gonad development (N=17)**
  - Presence of gonads (0/1),
- **Sex ratio**
  - Sex ratio (male/female)
- **Larvae**

  Presence of larvae during the spawning season (July/ August, N=3)
  - Presence of larvae (0/1),
  - Concentration (#/100L)

After hoisting 3 research racks, all 12 baskets were emptied and oysters were assessed. Live and dead oysters were separated and counted. Of each live oyster, shell width (in mm; Photo 1a) and wet weight (in gram) was determined directly on board. A selection of

17 oysters, were retrieved and analysed in the lab providing data on condition, gonad development and sex ratio. Prior to installation the oysters were classified according to size (small and large) below or above a shell width of approximately 70 mm, respectively.

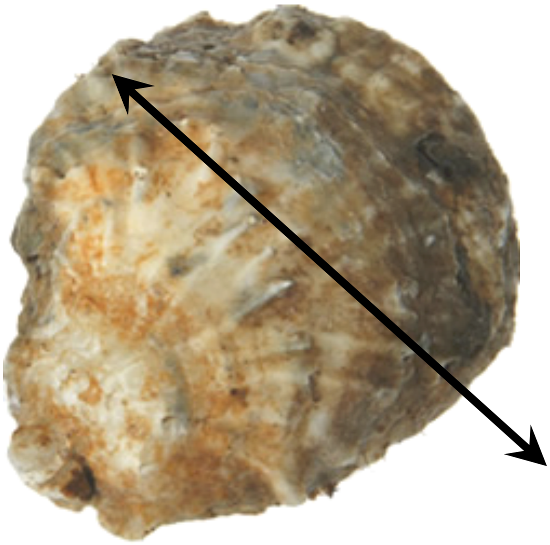

Photo 1a. Shell width: largest size perpendicular to hinge was measured (indicated by arrow).

17 oysters were collected and brought to the laboratory in Yerseke, where they were dried for 24 hours at 60°C. Afterwards the dry weight of the shell and soft tissue was measured. Condition Index was calculated according to Walne, & Mann (1975) as a ratio between dry weight of the oyster meat and dry weight of the oyster shell. This ratio was compared to the ratios observed by Pogoda et al. (2011). Condition Indices typically vary over the season due to investment in reproductive organs, decreasing the amount of energy spent on growth. Gonad development was inspected with a microscope in the laboratory in Yerseke. Gonads were described and presence of larvae was noted.

Larvae sampling was conducted at three locations neighbouring the oyster racks (Table 3) at 20 – 25 meters water depth. A set volume was inspected in the lab and counted to establish number of larvae per 100 litre of seawater.
Procedures in the field included:

- Place plankton net (100 µm) in 100-litre bucket.
- Use pump with 50 meter hose (end connected to the anchor, 2 meters above the sea floor) to collect 200 litres of seawater near the seafloor.
- Transfer plankton net material to labelled collection cup and add formol or ethanol as conservatives. Formol is the standard conservative, however 1 sample was conserved with ethanol in case further processing was needed (e.g. DNA analysis).

Table 3. Locations of flat oyster larvae sampling.

	Preservation	Northing	Easting	Sample size
Larvae sample 1	formol	52 23.4783498494	4 8.4330843168	200 litres
Larvae sample 2	ethanol	52 23.49010239186	4 8.4529041244	200 litres
Larvae sample 3	formol	52 23.49010239186	4 8.4529041244	200 litres

In the lab the samples of larvae were filtered using a 30 μm plankton gauze. The volume of the samples was reduced to 20 – 60 ml, depending on the amount of suspended matter. From the concentrated samples subsamples were taken for counting numbers of larvae. A Hensen plunger-sampling pipette was used to take subsamples. Bivalve larvae were identified and counted using a universal camera microscope (Reichert Me-F2, 52.6x). Three subsamples of each sample were analysed. Depending on the density of the samples, subsamples of 1 to 2.5 ml were counted. Larvae were identified according to Loosanoff et al (1966) and Hendriks et al (2005) combined with data obtained from cultured larvae.

Risks: Predation and pests

Oysters were brought to the laboratory and a small piece of tissue was clipped to assess infections using genetic markers. Of each oyster a small piece of gill was preserved in ethanol for analysis of *Bonamia* prevalence. The detection of genetic material of *Bonamia* by real time PCR was carried out based on the real time PCR described by Marty et al. (2006).

Biodiversity: number of species groups

To assess how the pilot structures influence biodiversity, a quick scan of the biodiversity on oyster racks, and reef domes was performed on deck. All racks, baskets and reef domes were photographed upon hoisting and a scan determining of number of main species(groups) was performed. The following parameters were noted

- presence (0/1)
- number of species(groups) (number per m^2 based on visual inspection only).

Racks were photographed on all four sides and from above. Baskets were photographed on the heads and on the side. Reef domes were photographed in their entirety, and in close up with a quadrant of 25x25 cm as a reference. An quantification of abundance and frequency of species was estimated using Tansley classes.

Statistical analysis

Differences in size and weight between November 2018 and July 2019 were compared using an Analysis of Variance (ANOVA). Linearity of the data was examined with residual plots. The homogeneity of variances was tested with a Levene test. Since the variances were not distributed homogeneously, the data were log transformed. A significance level of $P < 0.05$ was used. Significant effects were examined using posthoc Bonferroni tests. Statistical analyses were performed using IBM SPSS Statistics 23.

3 Results

The paragraphs in this chapter correspond tot the list of parameters in table 1 and table 2 (section 2.4).

Infrastructure

3.1 Position and persistence

Visual inspections with the ROV showed that the placed racks and reef domes were partially covered in sand. All 3 racks were not horizontal on the seabed, but diagonal and partly buried in sand (Photo 2). Domes were horizontal on the seabed but submerged in sand for 10 – 20 cm (Photo 2, Photo 4)

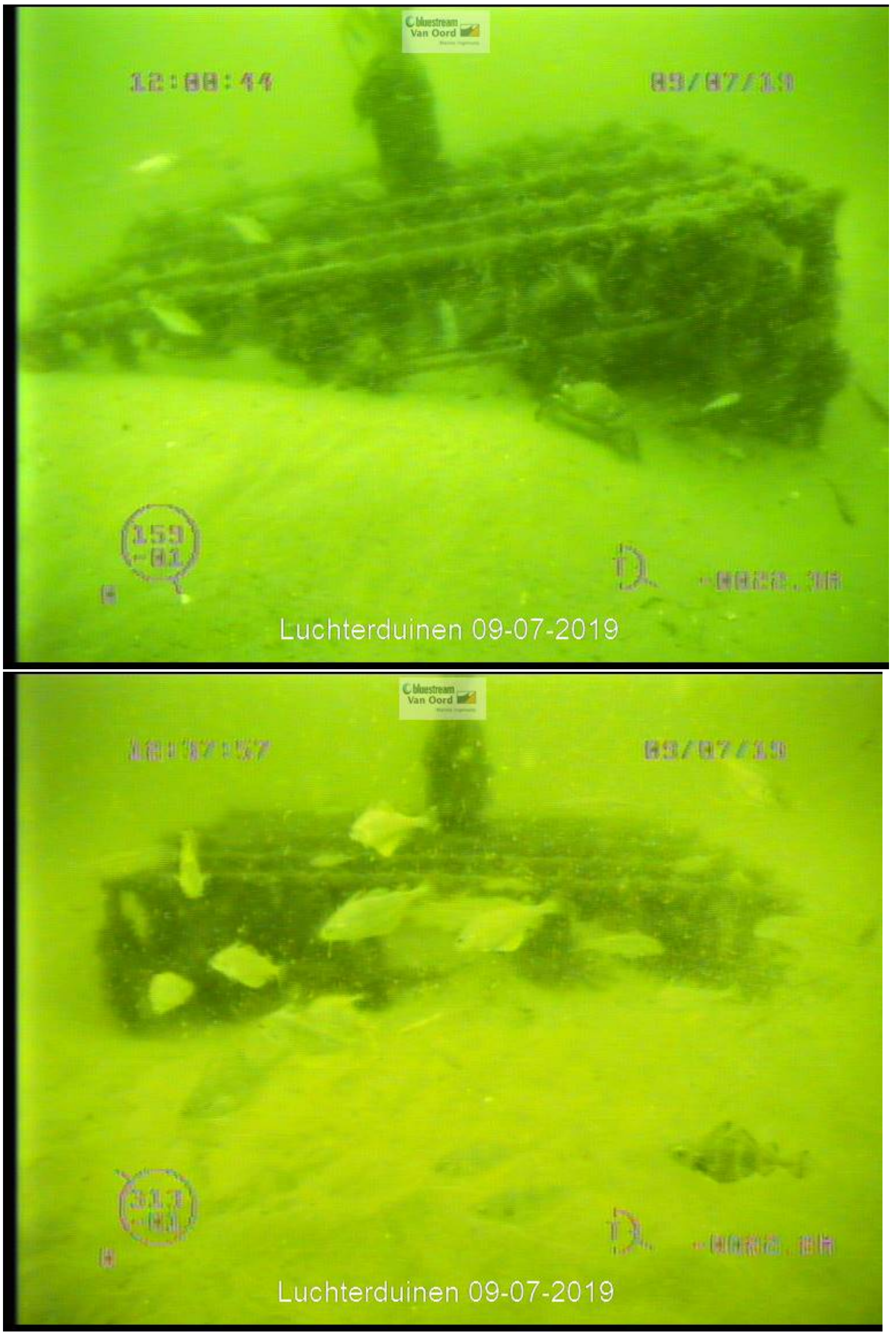

Photo 2. Oyster racks in Luchterduinen wind farm. Top – bottom, Rack 1 – rack 3. Sand aggregating in the structures, fish (pout) and crabs are visible in all 3 pictures.

Photo 2a. Reef dome in Luchterduinen wind farm. Sand aggregating around the bottom of the structure. Additionally fish (pout, school of juveniles), a crab and a plumose anemone are visible in this picture.

Inspection infrastructure on board

The racks showed very little corrosion. The zinc anode did show significant corrosion and performed its task (Photo 3).

Photo 3. Photo zinc anode in November 2018 (top) and July 2019.

Sand and fouling species were covering large parts of the infrastructure. Sand and signs of anoxia were visible as a strong gradient of parts that had been buried in the sand. Main fouling species include oaten pipes hydroid (*Tubularia indivisa*) as the most abundant fouling species and tube building amphipods (*Jassa sp*) as second most abundant.

Photo 4. Rack (bottom) and reef dome (top) at decommissioning.

3.2 Fouling organisms

See sections 3.1 and 3.11

3.3 Environmental conditions

Temperature

The temperature monitoring showed a gradual decline in temperature from 13 ^{0}C at installation of the oyster racks on November 4th 2018 and to 6.6 ^{0}C in February 2019 (Fig. 3). After this it increased gradually to 18 ^{0}C when the racks were hoisted on the 9th of July 2019. Variation between racks was minimal.

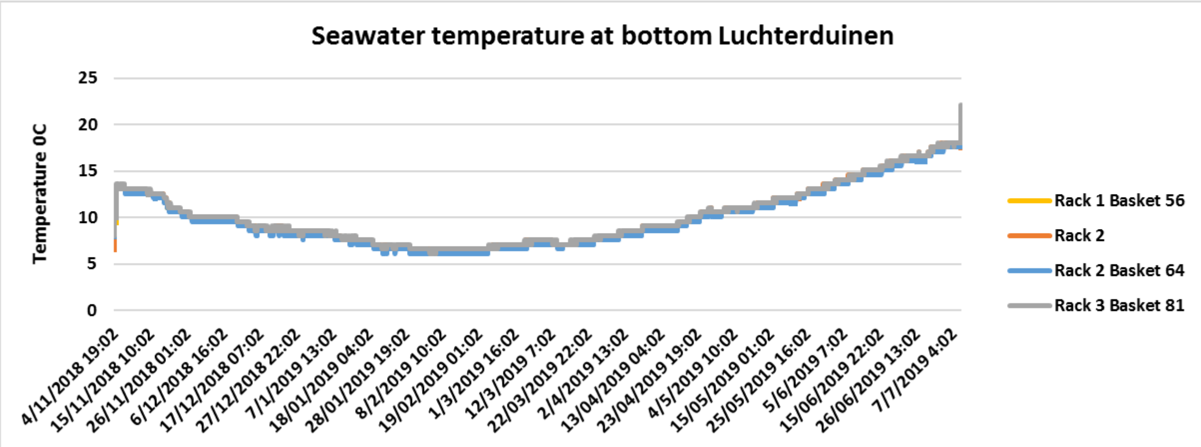

Figure 3. Temperature as measured near-bottom in the oyster racks (ibutton).

Temperature and prediction of larval release

The temperature development was used to predict the presence of larvae in the water column. Maathuis *et a*l (2018) recently developed a formula based on a large historical dataset describing *O. edulis* larvae counts. It was shown that the temperature sum is a major predictor of the timing of *O. edulis* larval release and the first major peak in larval concentrations is expected at 593-660 degreedays. In the case of Luchterduinen in 2019 the 593 threshold was reached on July 11th (Fig. 4).

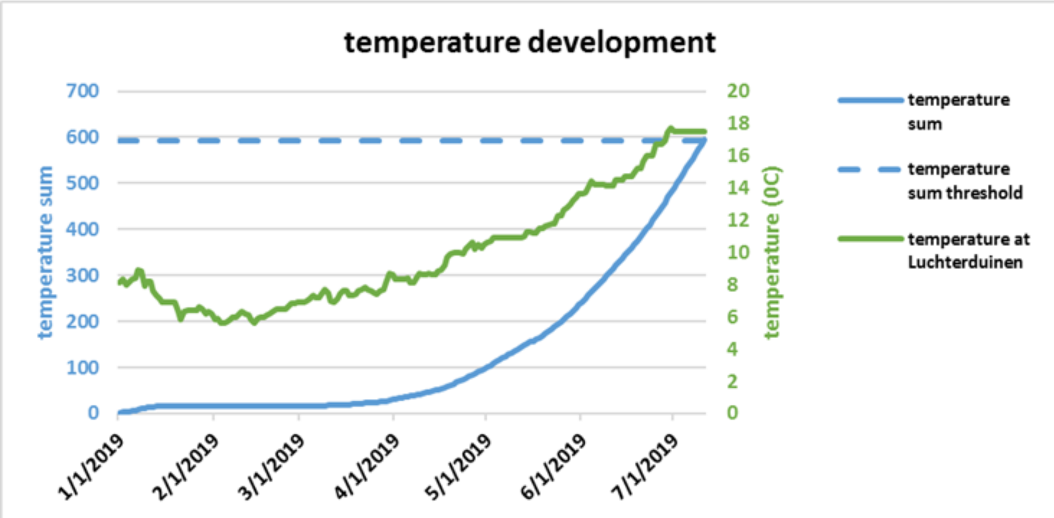

Figure 4. Surface water temperature and temperature sum development at Luchterduinen.

Bathymetry

The expected sediment movement was also taken into account when selecting the pilot area (Sas *et al.* 2018). Multibeam data collected in 2018 showed minimal change in depth compared with survey data for the construction of Luchterduinen (2014). A map was constructed showing the 2014 – 2018 difference (Fig. 5, Appendix 2). Maximum difference is 9 cm and there was no dune or trench visible.

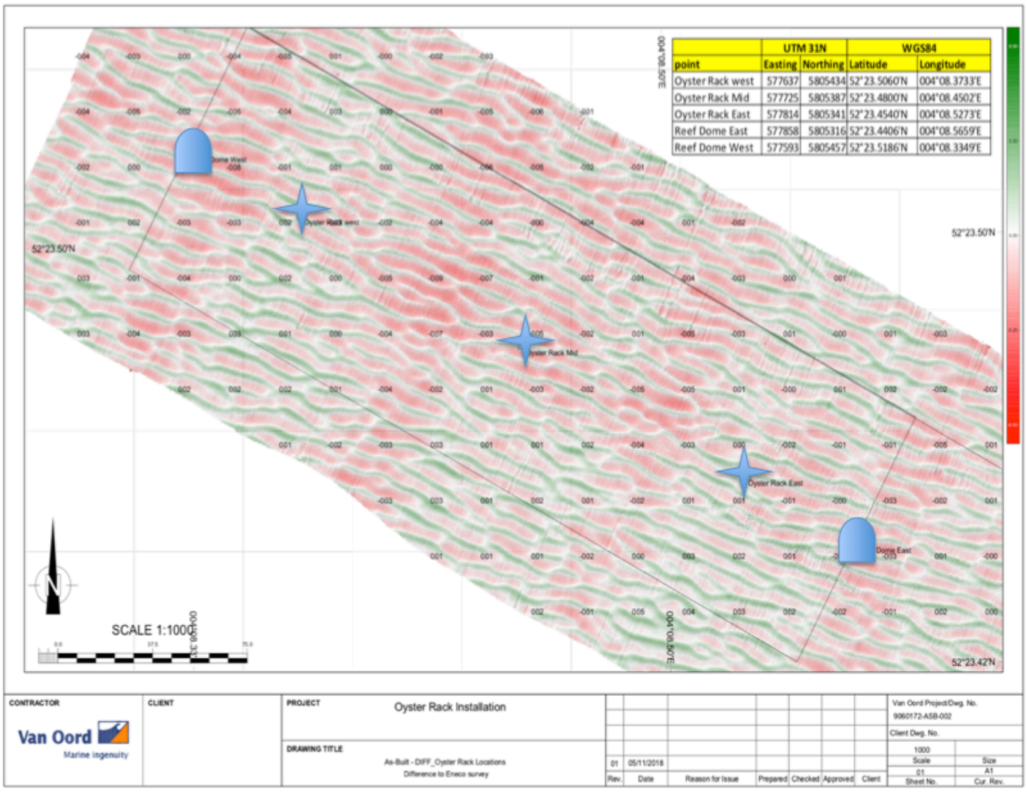

Figure 5. Difference in bottom depth between 2014 and 2018 (in m). In the top-right corner of the map, the coordinates of the research racks and domes can be found, which are also indicated in the map by star- and dome shapes respectively (Full size in Appendix 2).

Population dynamics

3.4 Oyster survival

Monitoring of survival of the oysters in the racks showed that 14.2 % of the oysters survived (Table 4) on average with minima of 0% in 7 out of 12 baskets, but up to 80 % in basket 72. Low survival rates were due to burial of the racks by sand, as was visible on ROV images (§3.1).

*Table 4. Survival of oysters in monitoring racks in July 2019. * Prior to installation the oysters were classified according to size (small and large) below or above a shell width of approximately 70 mm, respectively.*

Rack number	Basket number	Oyster size*	Number of oysters at start	Alive	Dead	% Survival	% Growth edge
1	56	large	40	3	37	7.5	10.0
	58	large	40	1	39	2.5	0.0
	59	small	40	0	40	0.0	20.0
	62	large	40	0	40	0.0	0.0
2	64	large	40	3	37	7.5	50.0
	67	large	40	0	40	0.0	50.0
	72	large	40	32	8	80.0	25.0
	79	small	40	29	11	72.5	20.0
3	81	large	40	0	40	0.0	15.0
	82	large	40	0	40	0.0	47.5
	85	large	40	0	40	0.0	12.5
	92	small	40	0	40	0.0	67.5
					average	**14.2**	**26.5**

3.5 Oyster growth

Growth edges were detected on oysters in 10 out of 12 baskets indicating oysters had been alive and increasing in size prior to burial with sand. Growth edges were found on 26.5 % of oyster shells (dead and alive) (Table 4). This was mainly on the part directly opposite the hinge (Photo 5). Shell width is measured perpendicular to this line and therefore, does not include all increase in shell size. A comparison of shell width in November 2018 and July 2019 showed no significant increase (ANOVA, P=0.516) (Fig. 6). Oyster wet weight was determined in the live animals. Only baskets 72 and 79 contained enough live oysters for statistical analysis. A significant increase in wet weight was detected (ANOVA, P=0.029, N=61) (Fig. 7).

Photo 5. Oysters showing distinct growth edges.

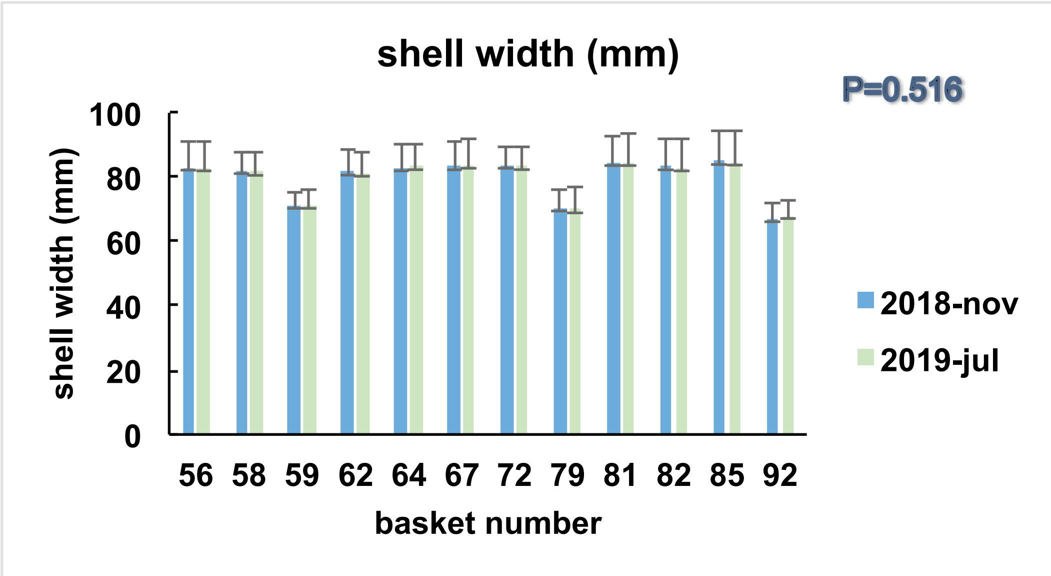

Figure 6. Shell width (in mm) of oysters in November and July.

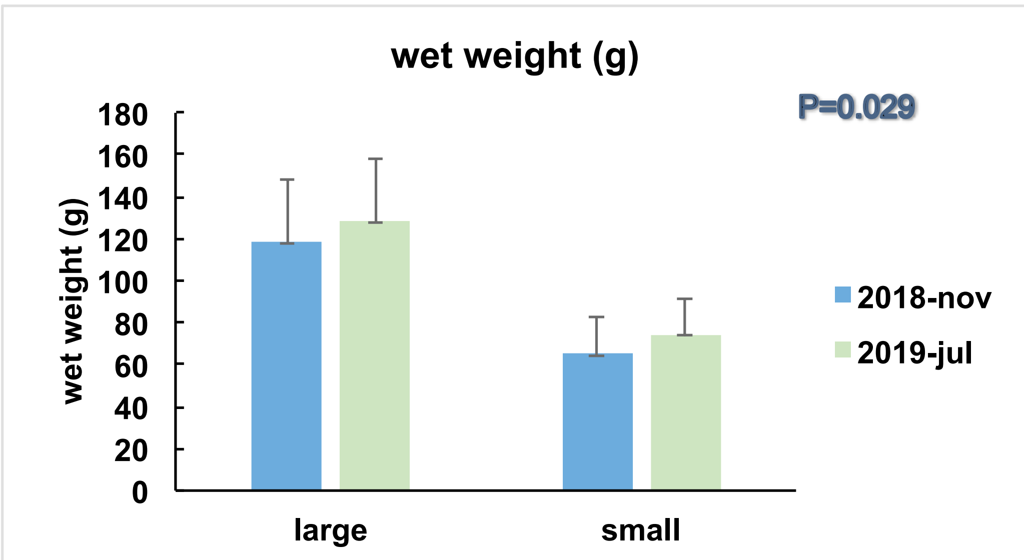

Figure 7. Wet weight (in g) of oysters in basket 72 (large) and 79 (small) in November and July.

3.6 Oyster gonad development

Microscopic analysis of gonad tissue smears showed that out of 17 oysters 7 were female, 1 male and 9 undetermined (Table 5). One female oyster contained larvae (Photo 6).

3.7 Sex ratio

Sex determination indicates a sex ratio of 7:1 females to males.

3.8 Oyster condition

The condition index of the live oysters was significantly lower in July 2019 than at the start in November 2018 (ANOVA, P=0.025) (Fig. 8, Table 5). This could be in line with the natural seasonal variation of the Condition Index. For example, the offshore aquaculture study of Pogoda et al. (2011) showed a higher condition index of flat oysters in fall compared to summer (Appendix 3). One explanation is that in summer energy is allocated to gonad development.

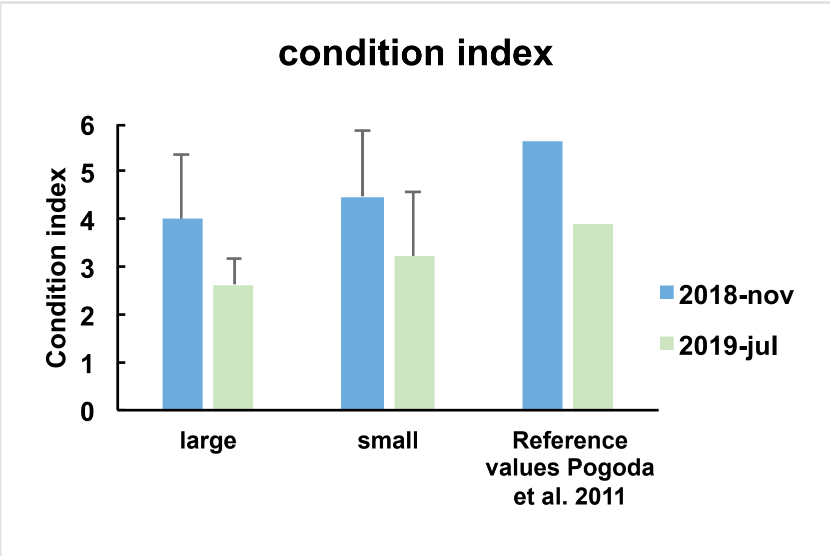

Figure 8. Condition of oysters in November and July 2019. Reference values are average condition index values of three German locations in fall (October : blue) and summer (June/August: green) (reference values taken from Pogoda et al. 2011; Appendix 3).

Photo 6. Larva found in mantle of oyster.

*Table 5. Condition index ([dry weight meat/ dry weight shell] * 100) and gonad development of oysters.*

Rack	Basket	Oyster	Growth edge	Shell width (mm)	Wet weight (g)	Dry flesh weight (g)	Dry shell weight (g)	Condition-index	Gonads
1	56	1		78.75	132.3	2.01	82.42	2.44	female
	56	2		70.16	95.6	2.17	60.58	3.58	female
	56	3	yes	86.81	149.4	3.77	99.36	3.80	female
1	58	1		81.22	109.6	1.70	74.17	2.30	undet
2	64	1		74.44	121.7	1.78	72.28	2.47	undet
	64	2		97.79	212.6	4.03	143.73	2.80	female
	64	3	yes	93.4	129.5	2.44	89.45	2.73	female
2	72	1		91.53	159.8	2.19	115.74	1.89	female
	72	2		89.56	135.1	2.38	92.75	2.57	undet
	72	3		80.29	134.3	1.92	93.67	2.05	undet
	72	4		70.52	92.8	1.47	63.72	2.31	undet
	72	5		81.53	79.8	1.63	59.64	2.74	undet
2	79	1		76.16	76.6	1.42	50.33	2.83	female
	79	2		69.29	85.4	2.72	50.28	5.41	undet
	79	3		66.53	47.1	0.93	29.96	3.09	male
	79	4		67.82	74.6	1.28	41.48	3.07	undet
	79	5		73.48	81.3	1.03	62.15	1.66	undet

3.9 Larvae

The water samples contained larvae with concentrations ranging from 90 -125 larvae per 100 litre (Table 6).

Table 6. Larval concentration in water samples

Sample number	Number of larvae per 100 litre
1	125
2	90
3	96

In comparison, larvae concentrations measured at Borkum Reef oyster pilot in 2018 were 5 to a maximum of 43 per 100 litre (Didderen et al. 2019).

Risks

3.10 *Bonamia* infection

17 individuals of adult flat oysters were tested negative for *Bonamia*. This is an indication that within Luchterduinen wind farm contamination with *Bonamia* and within 7 months after installation has not occurred.

Biodiversity

3.11 Number of species groups

ROV footage showed a rich life around and at the introduced structures, i.e. crabs, fish (Photo 1), mussels and squid eggs on all images. A few species could be identified to species of genus level, namely pout (*Trisopterus luscus),* edible crab *(Cancer pagurus),* plumose anemone *(Metrdidium sp)* and common starfish *(Asterias rubens)* (Table 7*).* The surrounding seabed was only sandy showing no epifauna species and few mobile fauna species: flatfish, hermit crab and brittle stars*.* Image quality, ROV time and ROV position did not allow for detailed species identification or quantitative comparison of infrastructure vs. sea floor without infrastructure.

Table 8. Species observed on the racks and reef domes during ROV dives.

	Dive 1	**Dive 2**	**Dive 3**	**Dive 4**	**Dive 5**	**Dive 6**
Infrastructure	**Search and retrieve rack 2**	**Search and retrieve rack 3**	**Search and retrieve rack 1**	**Search reefdome**	**Search and retrieve reefdome 1**	**Search and retrieve reefdome 2**
pout (*Trisopterus luscus*)	+	+	+	+	+	+
edible crab (*Cancer pagurus*)	+	+	+	+	+	+
plumose anemone (*Metrdidium sp*)	+	+	+	+	+	+
common starfish (*Asterias rubens*)	+	+	+	+	+	+

Visual inspection on board showed 19 taxa on the infrastructure, including mobile species like lobsters, starfish and crabs and sessile animals like mussels and anemones (Photo 7, 9). Species identified to species level included Rock gunnel and Fivebeard rockling, as well as nudibranch *Polycera quadrilineata.* Interesting observation is the occurrence of long specimen of sand mason worm *Lanice conchilega* at the bottom of each of the oyster racks. Inside of the reefdomes aggregations of this species were recorded (Photo 8).

The majority of other species were only observed sparsely with 1-6 individuals per structure (Tansley class 1-2). Highest cover/ abundance of species include oaten pipes hydroid (*Tubularia indivisa;* 50-75% cover, class 8) as the most abundant species and tube building amphipods (*Jassa sp*) as second most abundant (25-50% cover, class 7). Plumose anemone, common starfish and sand mason worm were present with 6-50 individuals per structure (class 3).

Photo 7. Species observed in and on reef dome on board: Top: starfish (Asterias rubens) and plumose anemone (Mitridium sp), bottom lobster (Homarus gammarus).

Photo 8. Lanice conchilega aggregation inside reefdome.

Photo 9. Plumose anemone (Mitridium sp) on oyster basket

*Table 8. Species observed on the racks and reef domes on board. + Fouling species *potential predator of flat oyster.*

Latin name	**English name**	**Dutch name**	**Rack**			**Reef dome**	
			1	**2**	**3**	**1**	**2**
Ascidiacea^{+}	Sea squirt	Zakpijp	+	+	+	+	+
*Asterias rubens**	Common starfish	Zeester	+	+	+	+	+
Tubularia indivisa^{+}	Oaten pipes hydroid	Pennenschaft	+	+	+	+	+
Hydrozoa	Hydroid polyp	Hydroid poliep	+	+	+	+	+
Metridium sp	Plumose anemone	Zeeanjelier	+	+	+		
Sessilia	Barnacle	Zeepok	+	+	+		
Mytilus edulis	Blue mussel	Mossel	+	+	+	+	+
*Jassa sp**	Amphipod	Jassa kreeftje	+	+	+	+	+
Caprellidae	Skeleton shrimp	Spookkreeftje		+			
Cancer pagurus	Edible crab	Noordzeekrab	+	+	+	+	+
Liocarcinus holsatus	Swimming crab	Zwemkrab				+	+
Homarus gammarus	European lobster	Noordzeekreeft	+	+	+		
Pholis gunnelus	Rock gunnel	Botervis	+				
Phyllodoce maculata		Gestippelde dieseltreinworm eieren	+	+		+	+
Teuthida	Squid eggs	Pijlinktvis eieren	+	+	+	+	+
Lanice conchilega	Tube worm	Kokerworm	+	+	+	+	+

Table 9. Species observed on and in the baskets.

Latin name	English name	Dutch name	Rack 1				Rack 2				Rack 3			
			56	58	59	62	64	67	72	79	81	82	85	92
Asterias rubens[+]	Common starfish	Zeester	+	+	+	+	+	+	+	+	+	+	+	+
Teuthida	Squid eggs	Pijlinktvis eieren		+										
Tubularia indivisa*	Oaten pipes hydroid	Pennenschaft	+	+	+	+	+	+	+	+	+	+	+	+
Hydrozoa	Hydroid polyp	Hydroid poliep	+	+	+	+	+	+	+	+	+	+	+	+
Metridium sp	Plumose anemone	Zeeanjelier	+	+	+	+	+	+	+	+	+	+	+	+
Sessilia	Barnacle	Zeepok												
Mytilus edulis	Blue mussel	Mossel	+	+	+						+	+	+	
Cancer pagurus	Edible crab	Noordzeekrab	+	+		+	+	+			+	+		
Liocarcinus holsatus	Swimming crab	Zwemkrab										+		
*Jassa sp**	Amphipod	Jassa kreeftje	+	+	+	+	+	+	+	+	+	+	+	+
Pholis gunnelus	Rock gunnel	Botervis	+				+	+	+		+	+	+	
Ciliata mustela	Fivebeard rockling	vijfdradige meun	+				+							+
Phyllodoce maculata		Gestippelde dieseltreinworm eieren		+										
Epitonuum clathrus	Common wentletrap	Wenteltrapje				+								
Alitta virens	Sandworm	Zager						+				+		
Polycera quadrilineata		Harlekijnslak										+		
Nudibranchia		Zeenaaktslak	+											+
Corophium			+	+	+	+	+	+	+	+	+	+	+	+
Caprellidae	Skeleton shrimp	Spookkreeftje	+	+	+	+	+	+	+	+	+	+	+	+

4 Lessons learned and conclusion

4.1 Lessons learned

Figure 9. Different stages of the oyster life cycle. Green circles indicate: observed during research in Luchterduinen oyster pilot (figure adapted from Fitzsimons et al. 2019).

The main lessons learned in this oyster pilot are:

Introduced oysters

- The oysters that have remained above the sand show signs of growth, have developed gonads and produced larvae (Figure 9): indications that oyster are healthy and surviving in their new environment.
- The larvae production of this small number of living oysters resulted in detectable oyster larvae with higher concentrations then ever detected before in the Dutch North Sea in all water samples (3 taken from depths of 20-25 meter).
- Unfortunately, general survival of the introduced oysters was low, because the racks in which they where placed sank in the sandy bottom / were covered with sand and only a small proportion of the oysters remained above the sand.
- 17 individuals of adult flat oysters were tested negative for *Bonamia*. This is an indication that within Luchterduinen wind farm contamination with *Bonamia* and within 7 months after installation has not occurred.

- The import of disease free Norwegian adult oysters resulted in healthy, surviving and reproducing oysters (for the part that was not covered in sand). However in order to minimise oyster transfers from natural populations, it is important to create more oysters through e.g. hatcheries or spatting ponds to increase availability and avoid large scale exploitation of wild oyster beds.
- Due to the interruption of the pilot shortly after spawning it will remain unknown if young oysters (spat) will recruit within the wind farm area. Since larvae have been shown to be in the water column in high concentrations (90 -125 larvae per 100 litre), it is likely some of the larvae have been settling on clean hard substrates in the vicinity of the pilot area.

Infrastructure and techniques:
- With the equipment used in this pilot, it is possible to introduce installations with flat oysters on the sea floor at a deep (20-25 meter) offshore location, find them back with an ROV and retrieve them at a later point in time for monitoring purposes. All can be achieved in conformity of the prevailing (safety) regulations in an offshore wind farm (e.g. no diving, no anchoring).
- Despite being tested on 3 other locations (1 offshore, 2 nearshore), the oyster racks used in this pilot sank in the sandy seabed, resulting in high mortality of oysters and therefore failed their purpose.
- A new design of a structure that can be used to contain flat oysters for monitoring purposes in offshore conditions should be developed.
- The principle of retrievable racks (although different racks are needed) worked well to answer the research questions, however ROV hoisting has some method specific limitations (Appendix IV).
- The technique of using a larvae pump resulted in sampling flat oyster larvae at 20 metres depth in the water column. This is an innovative monitoring technique with unknown effectiveness beforehand. All samples taken (3) contained larvae.
- The ROV provided footage that helped inspecting and retrieving the installations and provided valuable information (e.g. position of infrastructure on the seabed, some species occurring around the structures). Images were not suitable for quantitative analysis of species(groups).

Other biodiversity:
- The introduction of oyster racks and concrete reef structures resulted in increased biodiversity compared to the surrounding sandy bottom. 19 different taxa where observed on the installations, with many hard substrate related species and large schools of pout could be seen around the installations on the ROV footage.

4.2 Research questions

Infrastructure

- *How does the placed infrastructure perform?*

In November 2018 infrastructure including 3 oyster racks, with 160 oysters each and 2 reef domes were placed at the seafloor of Luchterduinen wind farm. After 7 months the placed infrastructure worked well in hoisting operations, could be retrieved and stayed at its place on the seabed. ROV images show oyster racks are heavily covered with sand and only a small percentage of the infrastructure visible, indicating they sank in the seabed or got buried, presumably through scouring effects. This was a major failing factor in this pilot resulting in high oyster mortality. The oyster racks should be redesigned on this aspect.

Flat oyster survival health and risks

- *Does the introduced population of oysters survive?*
- *Do infection-free oysters stay healthy?*
- *What are risks for the introduced oyster population?*

Survival of flat oysters was low, with 14.5% on average. Although survival is 0 in most baskets likely due to burial effects, maximum survival observed is 80% in least buried baskets. Apart from the negative aspects of the sand on infrastructure, from the oysters above the sand we can conclude: reintroduced flat oysters show signs of good health, growth and reproduction within Luchterduinen wind farm.
17 specimen were tested negative for *Bonamia*, an indication that within Luchterduinen wind farm contamination with *Bonamia* has not occurred and poses no risk.
The only risks that could be determined in this pilot is that of mortality through failing infrastructure.

Flat oyster reproduction

- *Does the introduced population reproduce?*
- *Can larvae be detected in the water column?*

Monitoring in July 2019 showed that reintroduced flat oysters show gonad development: in total 50% of flat oysters showed development of reproductive organs, with a female to male sex ratio of 7:1. One individual even showed signs of internal fertilization, with larvae present inside the oysters. Furthermore flat oyster larvae were present in al three seawater samples, showing swarming larvae were present throughout the pilot area with concentrations of around 100 larvae per 100 litre.

Flat oyster settlement

- *Do the larvae settle on infrastructure?*
- *What is the best relative location and best substrate for oyster settlement?*

Since the pilot was interrupted in July 2019 these questions could not be answered. For future monitoring of hard substrates in Luchterduinen wind farm (scour, monopiles) could include flat oyster monitoring, since larvae swarming in 2019 (§3.9) might have settled on these structures.

Biodiversity and species enhancement

- *Which other species are stimulated by the oyster pilot?*

Substantial biodiversity has been observed on the installations. A detailed description is provided in paragraph 3.10.

Factors of success and failure

- *What can we learn about success and failure factors with regard to creating oyster reefs in Windpark Eneco Luchterduinen?*

See 4.3 Conclusion.

4.3 Conclusion

1. Use of adult *Bonamia* free Norwegian oysters to serve as broodstock in offshore wind farms show promising results on a biological level with respect to reproduction and swarming larvae as a result of this 8-month pilot.
2. Sand accretion was observed around and in the oyster rack in the high dynamic conditions of Luchterduinen
3. It is unclear whether the racks have sunk into the sand, or whether the sand has been accumulated into it. The design of the oyster racks should be revised for further testing at this location, or comparable sites.
4. The sand accretion also indicates that the dynamic conditions in LUD need to be reviewed, especially in the context of the scalability of the pilot.

The next step in offshore oyster pilots should include redesigning monitoring equipment, evaluate the local conditions in LUD and evaluate if the observed larvae in the water column have resulted in recruitment and survival of young oysters within Luchterduinen wind farm.

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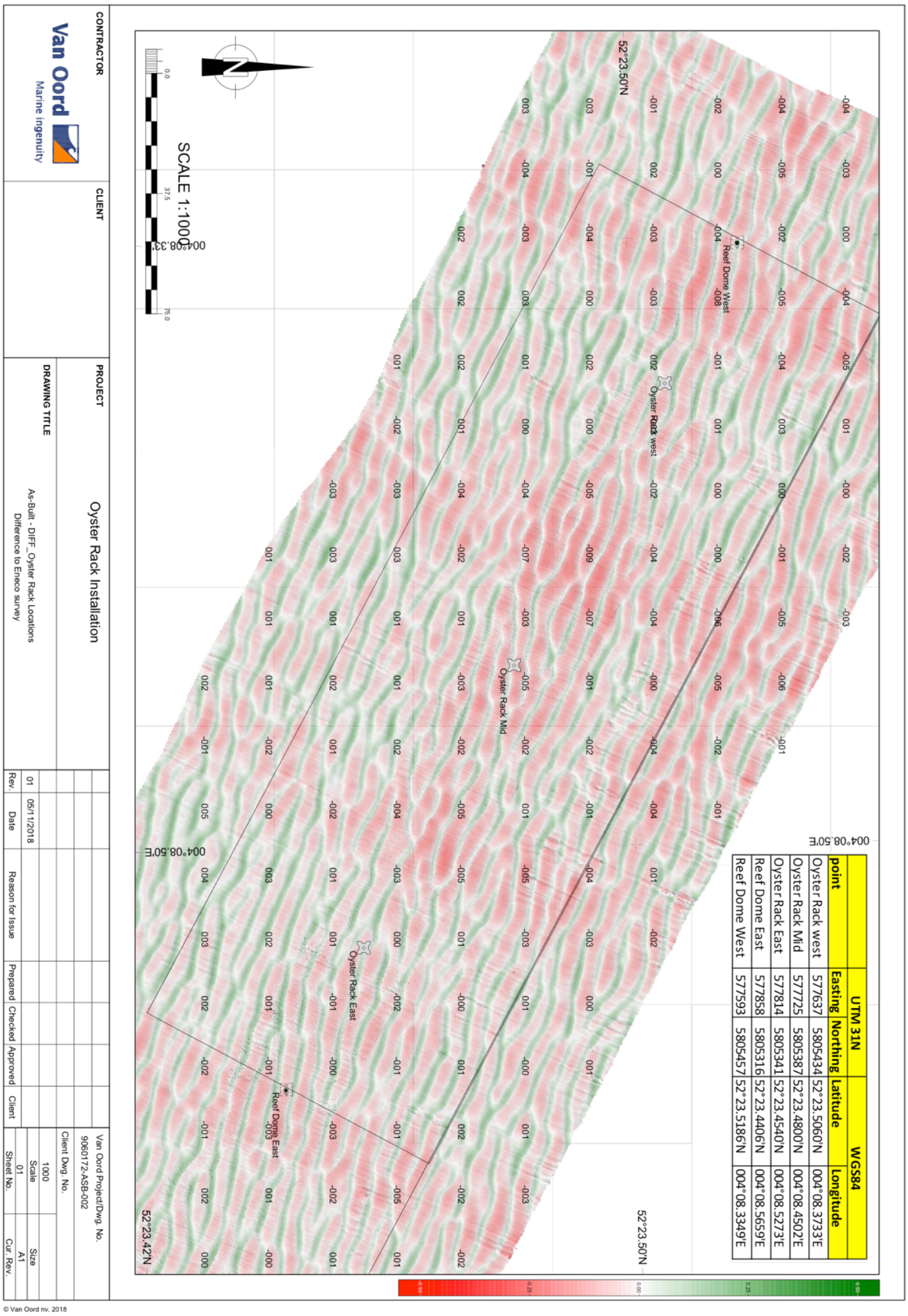
Appendix I NORA metrics

Box 1: NORA guidelines for oyster restoration project metrics
(X) indicate metrics that will be monitored in this pilot.

LUD pilot	1: Common Metrics:
	Metrics to be monitored across all European native oyster restoration projects:
	- Assessment of the before situation: assess suitability of site, reference T=0, water depth, ecological status and population dynamics of remnant oyster beds (if present) → baseline.
	- Population dynamics of restored oysters:
X	- Survival / density
X	- Growth (size, frequency distribution)
X	- Recruitment
	- Oyster bed areal extent and dimensions (height and area covered)
	- Persistence of the restored area and material added
	2: Diagnostic Metrics:
	Not all are needed, optional depending on site:
	- Key environmental variables:
	- Dissolved oxygen
	- Salinity
X	- Temperature
	- Chlorophyll (when considered limiting)
	- Reproduction
X	- Gonad development status
X	- Sex ratio
X	- Presence of larvae
	- Disease prevalence and intensity monitoring: *Bonamia, Marteilla*
X	- Presence of Predatory, Pest and/or Competitive Species
X	- Oyster Condition Index (Energetic measure)
	- Shell volume for determination of shell budget
	3: Ecosystem Services Metrics:
	Monitoring of benefit, optional, case-by-case:
X	- Biodiversity assessment
	- Quantification of ecosystem services
	- Water quality.

Appendix II pilot design (As built)



Appendix III Condition Index of flat oysters in the North sea

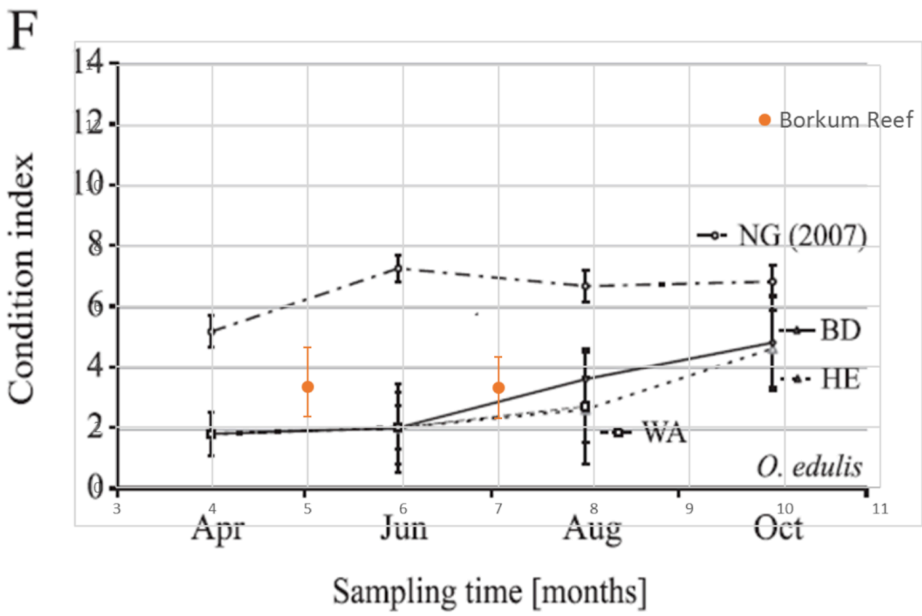


Figure II. Condition Indices of flat oysters in the German Bight according to Pogoda et al., (2011) (in black) and of reintroduced oysters at Borkum Reef Ground pilot in 2018 (in red) (Source: Didderen et al. 2019).

Appendix IV Oyster rack and ROV hoisting

Background

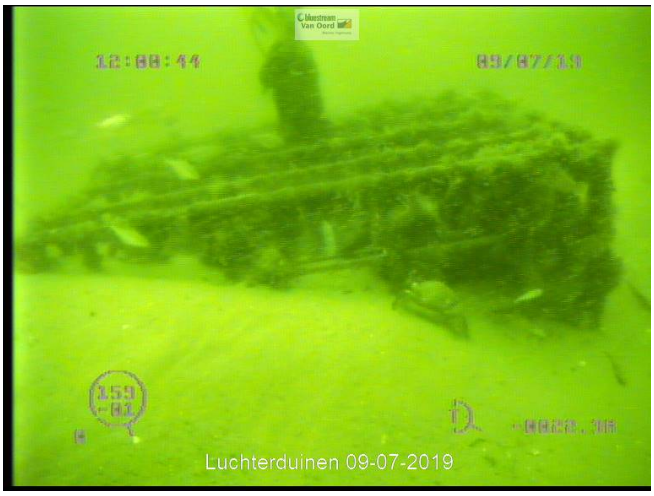
- In LUD flat oyster pilot 3 racks were deployed with the purpose of retrieval of a predefined and measured set of flat oysters.
- Oyster racks, with a comparable design used as in LUD have been used at 3 different offshore locations so far.
- At 2 sites (Voordelta, Borkum Reef ground) these racks are functioning fine, are retrievable, with racks above the sand and oysters alive after 1 year.
- However, other monitoring racks that have been trialled for flat oyster research (cubes, lanterns) have varying success rates and incidental losses are noted.
- The racks in LUD – based on stability requirements - were weighted with 3 layers of weights and composed of heavy steel wire. At sites Voordelta and Borkum Reef ground thinner wire and 0-1 layer of weights only were used. The extra weight of the former likely improved stability. At the same time probability of sinking into the sediment might be increased.

Observations

- Sand accretion in and onto the structures caused burying of the oyster baskets leading to high mortality rates (photo).
- It is unclear whether the racks have sunk into the sand, or whether the sand has been accumulated into it.

Observations ROV hoisting

- To make sure racks were detectable with ROV, a multibeam scan was made in June.
- Coupling the hoist eye of the buoy with ROV hook was difficult, the eye was overgrown with mussels and difficult to hook up due to movement.
- In the end ROV appeared to work well on the hoist eye of the rack itself, however this caused some bending of the rack and its hoist eye during hoist operations.
- Hoisting operations with ROV are challenging due to weather and current limits (availability of ROV time) and the necessity to use a large vessel.



Recommendations

- The design of the oyster racks should be revised for further testing at this location, or comparable sites.
- Fine sands and muds can promote sinking of structures. Apart from stability of a structure - focussing on a structure that stays in place and does not move along the sea floor - the sediment type at a pilot location should to be taken into account to prevent sinking of structures into the sediment.
- The sand accretion also indicates that the conditions in LUD need to be reviewed, especially in the context of the scalability of the pilot.
- Hoist configuration and method should be taken into account when redesigning future test facilities.

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