Pilot to actively restore native oyster reefs in the North Sea

Comprehensive report to share lessons learned in 2018

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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 seafloor. 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 stands at the start of the restoration of 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.

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Content

CHAPTER 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). We can hypothesize that such a substantial natural hard substrate reef must have harboured extensive reef communities, largely consisting of other biodiversity than that is common on the present day soft bottom habitat. Furthermore, this reef of filter feeders would have had a major impact on visibility, water quality and carbon fluxes. The North Sea ecosystem would have differed substantially from that of today, being vastly more productive for hard substrate associated animals, however detailed knowledge on this reef ecosystem is not existent, since knowledge on this ecosystem disappeared with the last shellfish reefs.

Due to overfishing, habitat destruction and diseases, the North Sea epibenthic shellfish reefs have almost entirely disappeared, as is the case elsewhere in the world (Beck et al., 2011; Smaal et al., 2015). For a good hundred years, recovery was not to be expected, due to the absence of undisturbed areas. Epibenthic shellfish reefs take decades to develop, and a single human disturbance event is enough to destroy a shellfish reef entirely. In addition to that, high nutrient loads have in the past resulted in stratification and poor oxygen conditions in large area's of the North Sea.

More 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; 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). Furthermore, due to the designation of marine protected area's and the construction of offshore wind farms, area's with undisturbed seafloor are uprising. 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). This pilot stands at the start of the restoration 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.

The Borkum Reef Ground is known for its natural hard substrates, (stones and gravel) that provide habitat for a biodiverse community. The area was already documented in the 18th century by Guitet (1710) and historic maps of Olsen (1883) mention this area where it is mapped as 'Stones and Rocks'. The Borkum Reef Ground and other areas with reefs play an important role in the distribution across the North Sea of species associated with hard substrates. The remaining reefs form the last remnants of a once more extensive reef community habitat in the North Sea. Hence the fauna associated with these reefs is of importance for biodiversity conservation (Bos et al., 2014; Coolen et al., 2015). 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.

1.2 Objectives

In this 2018 pilot project, the possibilities for restoration of shellfish beds in the deeper parts of the North Sea are studied. The project entails 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 (Textbox).

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.

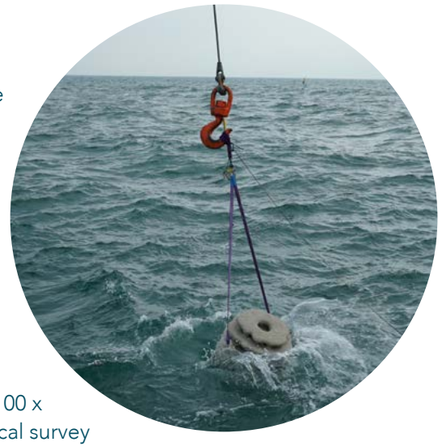
1.3
Reading this report

This report contains information on the installation of the pilot in May 2018 and first results of the monitoring in July 2018. 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 an outlook, with emphasis on monitoring plans for 2019. The Appendices contain detailed info on the pilot design and two field reports of two trips (installation and first monitoring).

CHAPTER 2

Methods

2.1
Study area



The Borkum Reef Grounds are situated in the North Sea north of the Wadden Sea island of Schiermonnikoog (Fig. 1). The area is characterised by water depths between 10 and 40 m, maximum currents between 0.4 and 1.0 m/s and water temperatures varying between 3 and 19 °C (Joschko et al., 2008, Coolen et al., 2015). The seafloor has previously been described as a 'rough ground', containing coarse sand, gravel and stone fields (Olsen, 1883; Tesch, 1910; Bos et al., 2014; Coolen et al. 2015). A pilot area of 100 x 100 meters was selected based on a geological survey (Deep, 2018, Appendix D) and situated within a 500 x 500 meters buffer zone (Appendix A, Figure 1).

The seabed is fairly unchanging in depth through this area areas, increasing by only 0.7m from northeast to southwest and water depths varying from 23-24 meters at low tide (data collected during this surveys) and 25-26 meters at high tide (Deep, 2018). ADCP data show the average current through the water column is 0.09m/s - 0.51m/s with an average of 0.32 m/s. The main current directions are around east to west and west to east. Before high and low tide the current is at its maximum. Sediments in the area consist of poorly sorted fine to very coarse sand and small amounts of very fine gravel to medium gravel and shells (Deep, 2018).

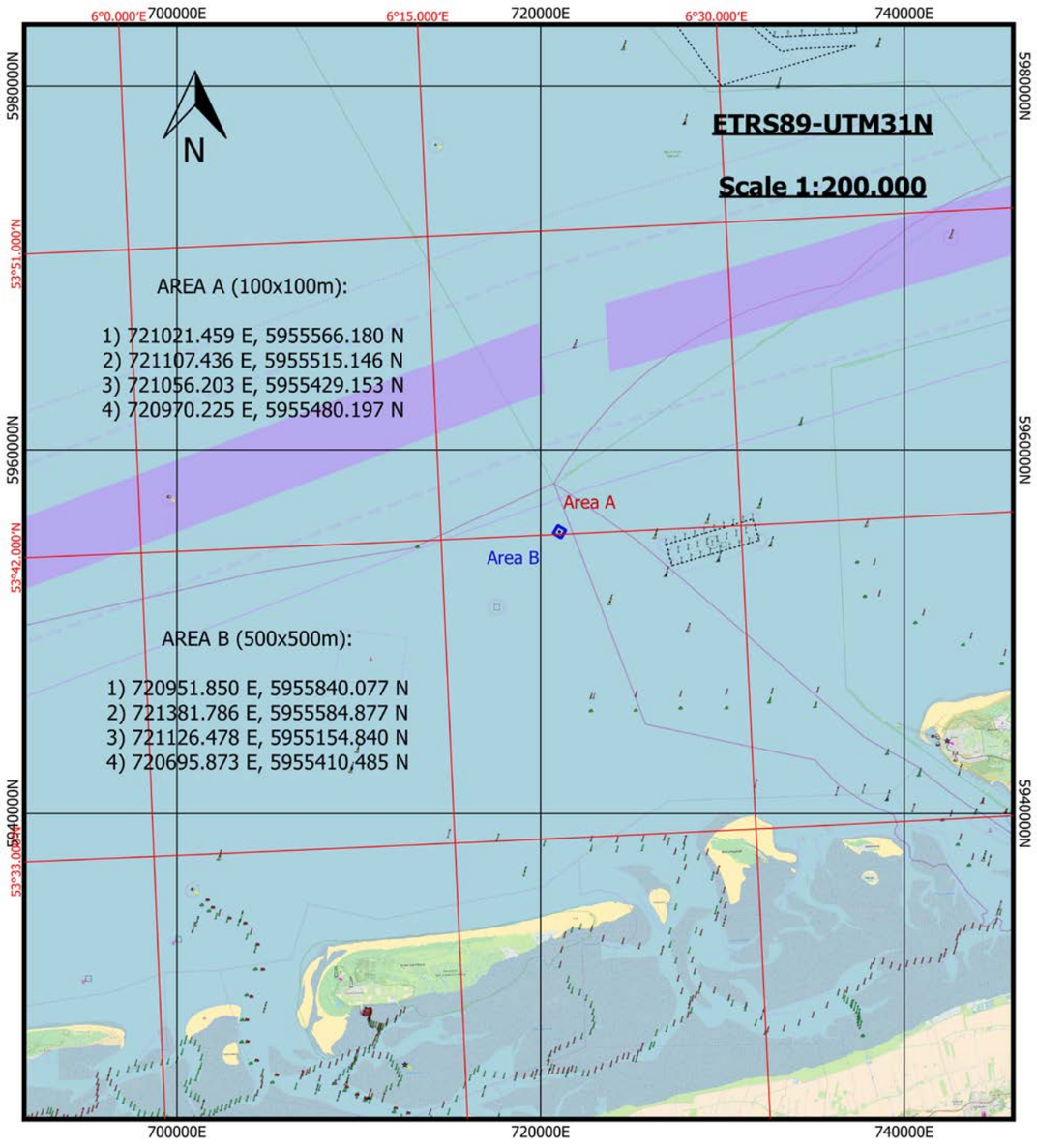


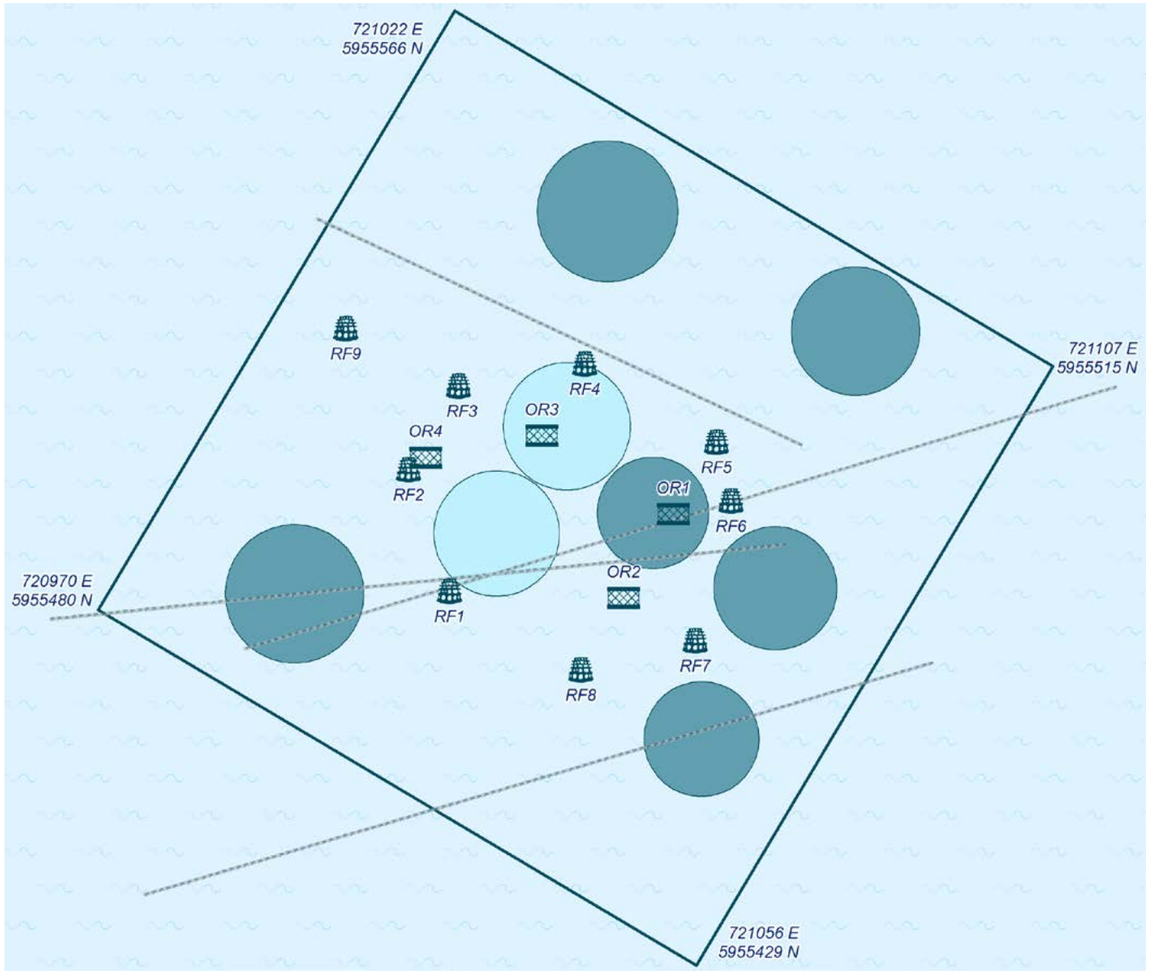
Figure 1. Project area indicated as area A, with a 500 x 500 m buffer zone (Area B).

2.2
Pilot design

The pilot design consists of the following components (Photo 1, Appendix A: Pilot and installation plan):

A. Live flat oysters
(approximately 5500 kg, estimated 80.000 individual oysters)
B. 4 research racks with flat oysters
C. 9 artificial 3D reef structures
D. Empty mussel shells (12 m3)

Flat oysters, research racks and 3D reef structures were installed on 24/25 May 2018 (A, B, C) According to the pilot layout in Figure 2. Empty mussel shells (D) were installed on 20 July 2018. Field reports are available upon request.



Borkum Reef Ground
Flat oyster pilot

- Oyster racks (OR)
- 3D reefs (RF)
- Empty shells
- Oyster location (n=7.500)
- Oyster location (n=10.000)

UTM 31N

Label	Easting	Northing
OR1	721053	5955494
OR2	721046	5955482
OR3	721034	5955505
OR4	721017	5955502
RF1	721021	5955483
RF2	721015	5955500
RF3	721022	5955512
RF4	721040	5955515
RF5	721059	5955504
RF6	721061	5955496
RF7	721056	5955476
RF8	721040	5955472
RF9	721006	5955521

Figure 2. Pilot lay out, 9 3D reefs, 4 monitoring racks and 8 patches of oysters were installed on 24 and 25 May 2018 (Appendix B).

Permits

Permits included, Water act permit, Nature conservation act permit (Appendix E), European Union Intra Trade Certificate to allow for export and import of livestock permits after inspection. WWF and ARK requested for an invasive species treatment protocol to be developed (van der Have & Schutter, 2018; van den Brink & Magnesen, 2018). Due to the vicinity to Natura 2000 areas Wadden Sea and Borkum Riffgrund, a Natura 2000 pre-assessment was required, which is available upon request (Van der Have, 2018).

Flat oysters

To ensure sustainable harvest and prevent overfishing of wild population, a sustainable fishing protocol was developed and WWF checked with Norwegian authorities and research institutes the status of the stock. Subsequently oysters were collected by divers in Norway at Hotate AS / Scalpro AS. Sizes ranged from 4 to 11 cm shell width. On 7 and 8 March 2018 oysters were inspected for epibionts (Van den Brink & Magnussen, 2018; der Have & Schutter, 2018). Treatment involved quarantine for at least one week (oysters in holding facility, filtering ozon treated water), then tumbling in freshwater in a cement mixer and submerging in freshwater with 5 ml per L of 15% sodium hypochlorite for 15 minutes.

Photo 2. Oysters on 24 May 2018

Oysters were put in stir foam boxes of 20 kg each and by cooling truck transported to Eemshaven, with a total of 6550 kg (94.000 approximately). 10 oysters were opened in Eemshaven and their condition was checked visually, meat content and smell were good (Photo 2). Oysters were stored at Eemshaven inside a cooling truck at 6 °C 1000kg (ca. 14.000) oysters were kept stored in the cooling truck for Gemini windfarm. One box with 350 oysters was further transported to IJmuiden uncooled, with condition checked at arrival and after 3 weeks. Part (120 of the 350) oysters from Norway were later placed in the Voordelta, to test and assess their resilience to *Bonamia*, to better understand and predict what will happen if *Bonamia* does at some point reach the oysters at Borkum reefground.

A selection of oysters (Table 1, Photo 3) was attached to the 3D printed sandstone reefs by means of Aquascape two-part epoxy putty that is developed for use in salt water and freshwater aquariums and underwater applications. This procedure took about 1.5-2 hours with 3 people. The outside air temperature was 18 °C on average in the sun and 14,5 °C in the shade. Oysters and structures were cooled with a seawater hose on deck.

NUMBER OF OYSTERS PER STRUCTURE

Table 1.

Reef	Reef numbers	Number of oysters on reef
small	2, 4, 5, 9	7
medium	1, 8	10
large	6, 7	20

Photo 3. Preparation of 3D reef structures with flat oysters.

2.4 Research questions

Based on the objectives of the pilot more detailed research questions were formulated (Table 2).

OVERALL RESEARCH QUESTION	PILOT RESEARCH QUESTION
Oyster bed development	
1. How many oysters are needed to create a viable and self-sustaining oyster reef?	Is it possible to create a viable oyster bed with active introduction of 100.000 oysters and approx. 10 oysters per /m2?
2. What is the optimal oyster density for creating an oyster reef?	Is there a difference between oyster beds of low and high density?
Reproduction & survival	
3. Do oysters reproduce in the deeper parts of the North Sea?	Is there gonad development visible in the introduced oysters?
4. Is offspring able to survive?	Are larvae and / or spat produced during the pilot?
5. What is the role of different types of substrate to enable spatfall?	What are spat densities on different types of shell material (broodcollector)?
6. Can 3-D printed reefstructures help in kick-starting oyster reefs?	What are spat densities on 3D reef structures 1) with and 2) without oysters cemented?
7. Will offspring recruit to the population?	*Question for year 2.*
Environmental	
8. How do environmental variables affect oyster population dynamics?	Is there a relation between sea water temperature and survival/ growth/ gonad development / larvae/ spat size of flat oysters? Are any other environmental variables stimulating or limiting to development?
Methodology	
9. What are the key lessons learned?	What do we learn by doing with regard to restoring oyster reefs in the North Sea?
10. How to best monitor population dynamics?	What do we learn by doing about how to best monitor population dynamics?
11. What do we learn by doing about interventions necessary to kick-start oyster reefs?	Does adding live oysters and 3D reef structures help to kick-start oyster reefs?
12. Biodiversity/ Community effects	How does oyster reef development change biodiversity, ecosystem function and ecosystem structure?

Table 2. Research question

2.5 Monitoring activities

Monitoring was carried out on 24 May (T0) and 20 July 2018, with activities as stated in the monitoring plan (Table 3).

Monitoring activities	Research questions	Common	Diagnostic	Ecosystem	Installation trip 2 May 2018	Monitoring trip 3 July 2018	Trip 3:2019
1. Dropcam survey	1-3, 6, 10	✗		✗		✗	✗
2. Temperature measurements	8		✗	✗			✗
3. Oyster measurements:	3-10	✗				✗	✗
Wet weight measurement		✗				✗	✗
Length measurement		✗				✗	✗
Condition assessment			✗			✗	✗
Gonad development			✗			✗	✗
DW determining			✗			✗	✗
Presence of Bonamia			✗				
4. Visual observation of survival	1, 4	✗				✗	✗
5. Visual observation of present life forms	1, 2, 5, 6	✗	✗	✗	✗	✗	✗
6. Visual observation of rack & 3D structure damage	9 - 11						✗
7. Visual observation of biofouling and predators	9 - 11		✗	✗			✗
8. Visual observation of settlement	3, 4, 5, 6, 7	✗					✗
9. Visual observation of oyster bed development	1, 2	✗					✗
10. Larvae sampling & counting	3, 8 - 10		✗			✗	✗
11. Spat collection	4, 5, 6, 7	✗					✗

Table 3.

2.5.1
Dropcam surveys

Drop cam surveys were used to:

1. Assess the pilot area before installation;
2. Asses the pilot area after installation;
3. Perform visual observations of oyster survival, present life forms, installation status and/ or damage (Table 2, 4-6).

Visual observations were performed on 20 July 2018. Drift 1 encompassed the whole length of the plot. Drift 2 and Drift 3 set within an oyster plot and near 3D structures (Table 4; Figure 3).

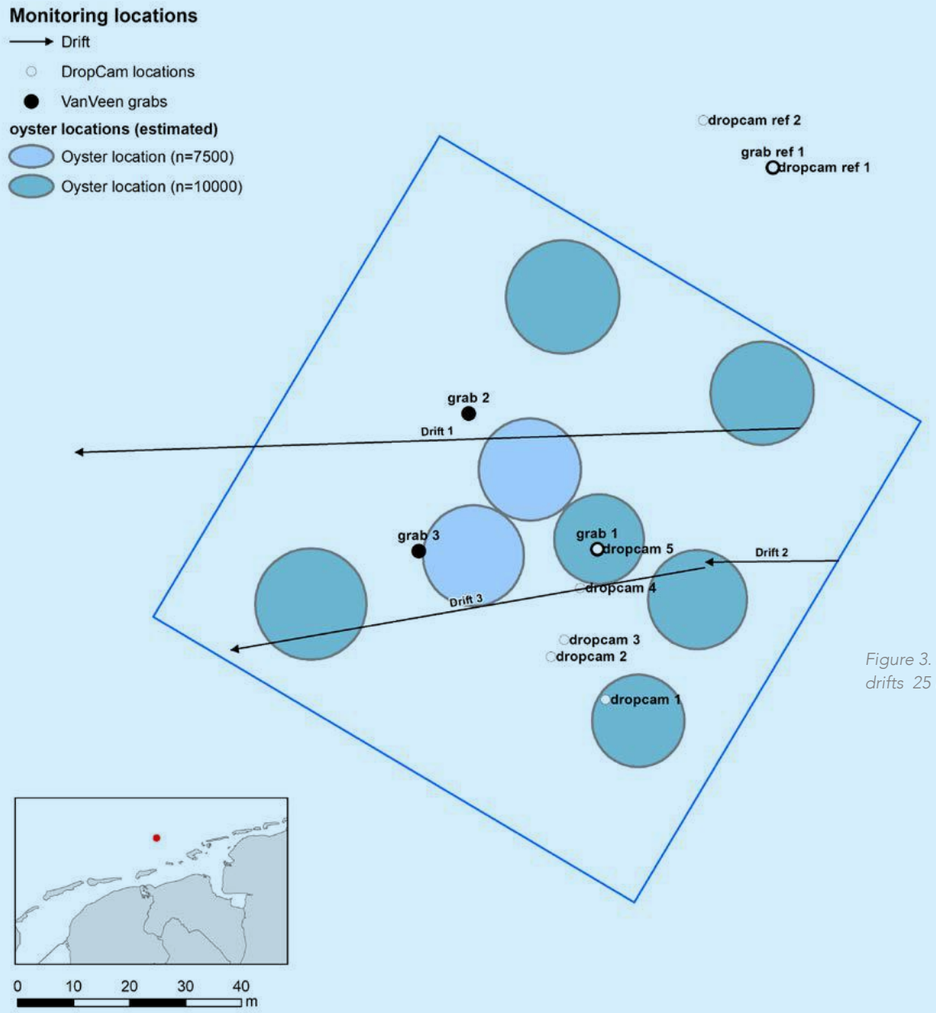


Figure 3. Drop cam drifts 25 May 2018

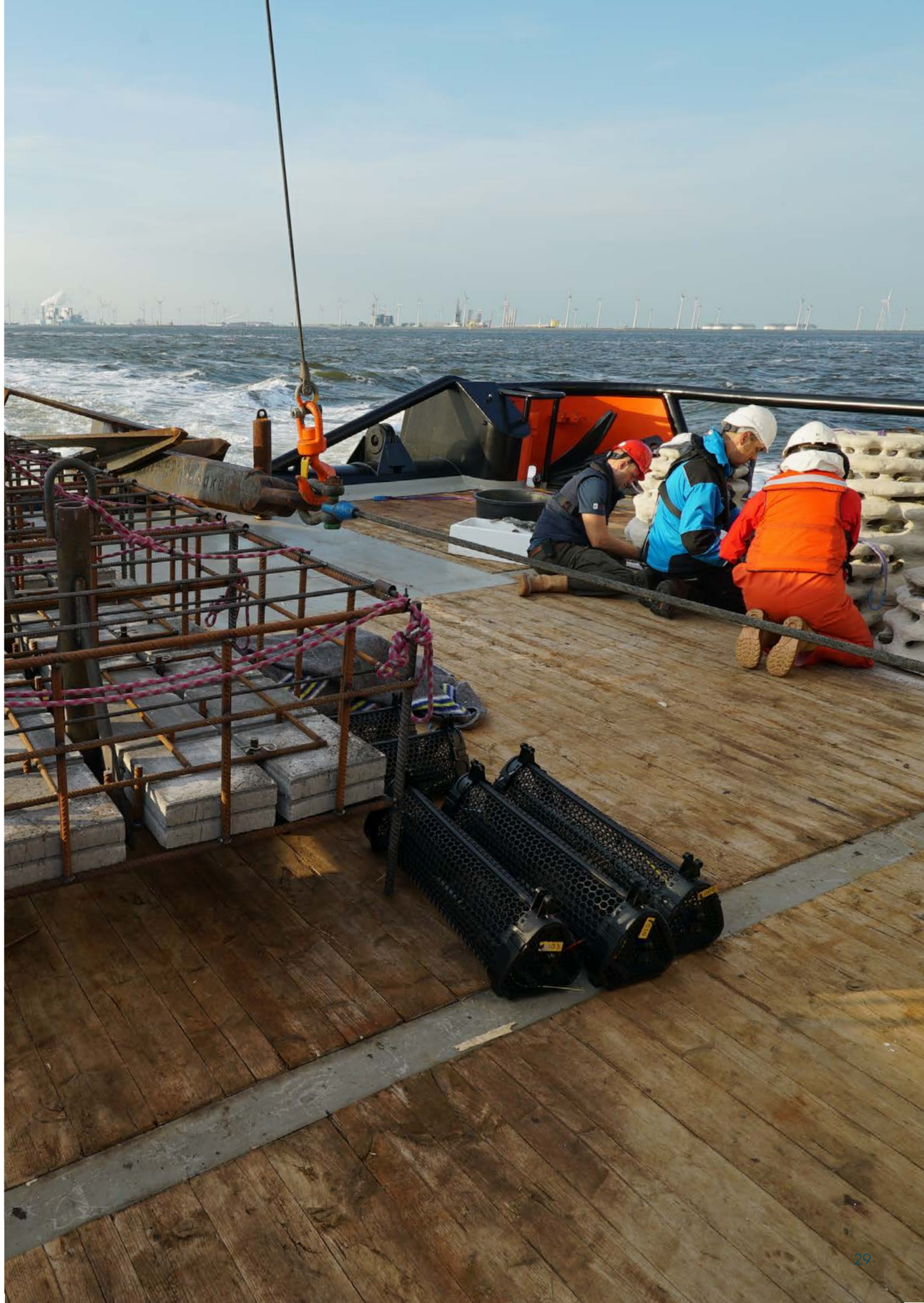
DROP CAM DRIFTS

Drift	Start	End
Drop cam Drift 1	N53,701216 E006,349184	N53,701115 E006,347890
Drop cam Drift 2	N53,701428 E006,349457	N53,701444 E006,347495
Drop cam Drift 3	N53,701212 E006,349546	N53,701216 E006,349184

Table 4. Drop cam drifts, 20 July 2018.

2.5.2 Environmental conditions: temperature

Temperature loggers 4 ibuttons, Fuchs Elektronik in Germany type DS1925L-F5 held in waterproof cases and attached to research cages and DS1921G-F5 for temperature logging during transport of oysters from Norway to Netherlands were placed in all racks. No ibutton data were retrieved due to software failure. However, later in July an ibutton of the neighbouring Gemini wind farm was collected. This ibutton was set to collecting temperature data every 30 minutes. Data of near-bottom and surface water temperature at Gemini were retrieved via Gemini and used in this report.



2.5.3
Oyster measurements

This report contains information on the installation of the pilot in May 2018 and first results of the monitoring in July 2018. 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 an outlook, with emphasis on monitoring plans for 2019. The Appendices contain detailed info on the pilot design and two field reports of two trips (installation and first monitoring).

Photo 4. On board measurements of flat oysters

Initial measurements and placement

At time of installation a total of 640 oysters were weighed (wet weight in gram) and measured (shell width in mm) and placed in baskets (Table 5) that were placed in research racks. Pictures were made of all oysters. Each research rack contains 4 baskets (Appendix A). The weighing and placement of 640 oysters spread over four cages took 7 hours with 2 to 3 persons. Oyster basket contained different subgroups includinged "holding tower", "small" and "large". Holding tower: In two baskets per rack the oysters are placed in holding towers, enabling monitoring of identified individuals (appendix A). Different size classes were ordered as source material in order to increase the change of introducing both male (smaller individuals) and female (larger individuals) oysters. Shell width of measured oysters ranged from 40.0 mm to 111.1 mm. A selection of smaller sized individuals were placed in baskets labelled "small", whereas larger individuals were placed in "large", with overlap in size classes (Table 5).

Survival and growth

On July 20th 2018 research rack 2 was hoisted and all 160 oysters were handled. Research rack 2 contained 4 baskets (Table 5). Per basket the live and dead oysters were separated and the live oysters were weighed (wet weight in gram) and measured (shell width in mm) and replaced in baskets. Pictures were made of all oysters.

Condition index

On July 20th 2018 research rack 2 was hoisted and all 160 oysters were handled. Research rack 2 contained 4 baskets (Table 5). Per basket the live and dead oysters were separated and the live oysters were weighed (wet weight in gram) and measured (shell width in mm) and replaced in baskets. Pictures were made of all oysters.

Gonad development & reproduction:

On July 21st 2018, 5 small and 15 large oysters, were transported to the laboratory in Yerseke in a cool box. Gonad development was inspected microscopically and all oysters were stored at -20°C for dry weight determination.

NUMBER OF OYSTERS PER RESEARCH RACK AND BASKET

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	88.5
	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
Position C1	5	40	holding tower	64.5	87.2
	24	40	small	45.6	75.0
	32	40	large	73.2	106.1

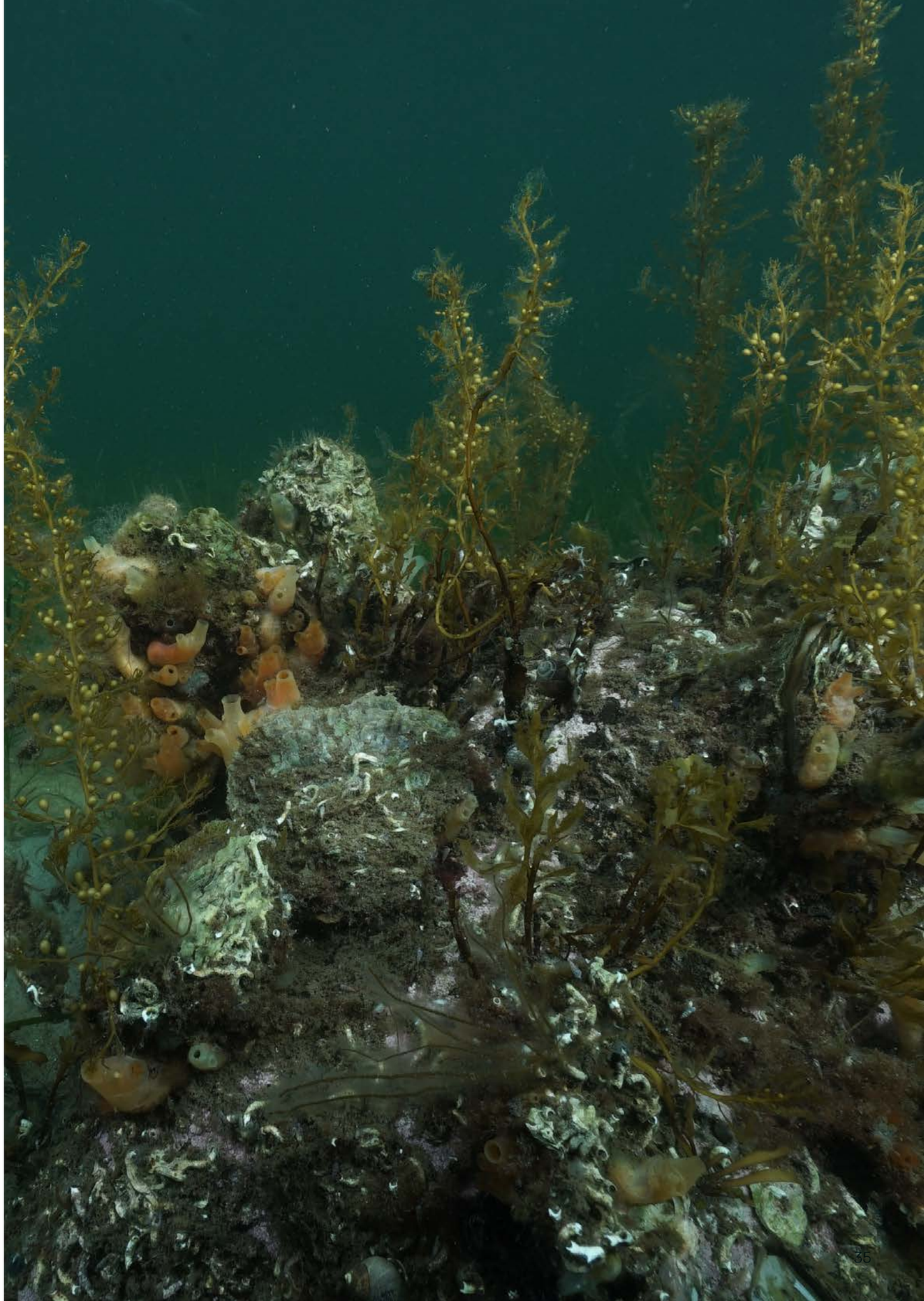
Table 5.

2.5.4
Visual observations survival

Results were retrieved by interpreting dropcam images (§2.5.1).

2.5.5
Visual observations present life forms

Results were retrieved by combining §2.5.1 (dropcam) and §2.5.3 (fouling on research rack).



2.5.6 Monitoring oyster larvae

Larvae sampling

Sampling included three samples of 3 times 200 litres (9 subsamples of 200 litres at three locations, 600 litre per location) (Table 6) from a water depth of 21 metres, 2 meters above the seafloor. Per location one sample was preserved for *O. edulis* DNA detection (qPCR analysis) and two for microscopic determination of flat oyster larvae. Procedures in the field included:

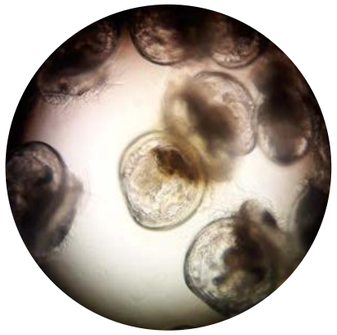
- Place plankton net (100 µm) in the 100-litre bucket.
- Use pump with 50 meter hose (end connected to the anchor, 2 meters above the sea floor) to collect 200 litres of seawater near the seafloor.
- Transfer plankton net material to labelled collection cup and add formol or ethanol (conservatives).

LOCATIONS OF OYSTER LARVAE SAMPLING

	Start	End	Sample size
Larvae sample transect 1	N53,700787 E6,352683	N53,700511 E6,359598	3x200 litres
Larvae sample transect 2	N53,700625 E6,345098	N53,700915 E6,349924	3x200 litres
Larvae sample transect 3	N53,702061 E6,346841	N53,702060 E6,350476	3x200 litres

Table 6.

Microscopic determination of larvae



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.

qPCR analysis

Besides microscopic determination of the larvae qPCR was performed on the DNA of one larvae sample per transect to compare between techniques. qPCR can potentially be used as an alternative method to detect and quantify *O. edulis* larvae as opposed to conventional counting of larvae. This analysis was part of a larger study towards the development of quantitative DNA detection methods of *O. edulis*. A total of 100L water sample was filtered and stored on ethanol. The organic material from the filter was lysed and the lysate was homogenized. Subsequently 200ml lysate was used for DNA extraction by means of the DNeasy Blood & Tissue kit (Qiagen). DNA detection was conducted by means of the newly developed detection-assay Oed2. This contains a short (<100bp) fragment of the ribosomal DNA that is multiplied using digital droplet PCR (ddPCR), a very recent alternative for qPCR analysis, which is able to perform quantitative DNA analysis with a higher accuracy (Hindson et al., 2011; Hindson et al., 2013). ddPCR is a method for performing digital PCR that is based on water-oil emulsion droplet technology. A sample is fractionated into ~20.000 droplets, and PCR amplification of the template molecules occurs in each individual droplet. In the droplets that contain *O. edulis* DNA this DNA is amplified, which results in a fluorescent signal. The fraction of positive droplets is then scored and fitted to a Poisson distribution to determine whether the complete interval is positive by means of a statistical analysis of the numbers of positive and negative droplets in a given sample. DNA extraction and qPCR analyses were performed by Wageningen Environmental Research (WENR).

2.6
Analysis

Condition Index

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 ratio's 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.

Statistical analysis

Differences in size and weight between May and July 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.

CHAPTER 3

Results

3.1
Visual observations

Pilot area

The seabed in both the pilot site and the reference area generally consisted of a mixture of sand and small patches of gravel and shell fragments (Appendix C; Appendix D). In grabs obtained during the geological survey in March 2018 grabs showed to contain sand mason worm *Lanice conchilega* lancelet *Branchiostoma lanceolatum*, anemones, unknown species of sea urchin, sand eel (*Ammodytus*) banded wedge shell (*Donax vittatus*) in areas bordering the pilot area (pers. obs. E. Reuchlin; Deep, 2018). In May during installation 2018 aggregations of Lanice (sand mason worm) were the main visible epifaunal organism and on some locations holes in the sediment indicated species of infauna. Apart from *Lanice* a few other species were observed: common sea stars (*Asterias rubens*), razor clam (*Ensis sp*), gobies (Gobiidae).

Installation inspection

Inspection in May 2018 found structures standing upright and oysters being alive and seeded on the seafloor (Photo 4). In July 2018 at least reef structure 3 and 4 and 9 were visible, standing upright and with the shape of epoxy glued oysters on the structure. It was not possible to see of these oysters were alive.

Photo 5. Seeded flat oysters on the sea floor (25 May 2018).

Oyster survival

On July 2018 images showed several live flat oysters on the seafloor (Photo 5; 6). No open (white) shells were observed. The inspected specimen showed a specific sign of being alive as valves where seen to close in front of the camera. New shell growth of flat oysters was also visible.

Fouling organisms

Both the rack itself and the baskets showed little fouling. The main fouling organisms were Bryozoa. Notably, a large amount of squid eggs was attached to the research rack. During inspection of the oysters in the research racks the tubeworm *Spirobranchus triqueter* has been observed to grow on the oysters. An anemone had attached itself to the tile used for weighing down the research cage. These species are all known to occur throughout Nortwestern European seas and also known from this area (Lengkeek et al., 2013).

Biodiversity

In July 2018 marine life, apart from flat oysters, included clusters of *Lanice* (>100), common starfish *Asterias rubens* (>50) and single observations of hermit crab, a species of flat fish, gobies, edible crab *Cancer pagurus* and squids (species unknown, 11), both near the substrate and in the pelagic (Photo 6; 7).
Drifts encompassed images of flat oysters (no open shells), with higher densities of oysters in drift 2 than drift 3.
3D reef structures contain crabs: in all four structures inspected in July several edible crabs were observed. Over ten individuals of squid were observed swimming in the pilot area, more specifically next to 3D reef structures in two cases (Photo 7), and eggs of this species group were attached to the research rack that was hoisted in July. Squid are known to depend on hard substrates for reproduction (attachment of eggs). These observations provide a first indication that adding hard substrate can improve habitat availability for this species group.

Photo 6. Seeded flat oysters on the sea floor (20 July 2018).

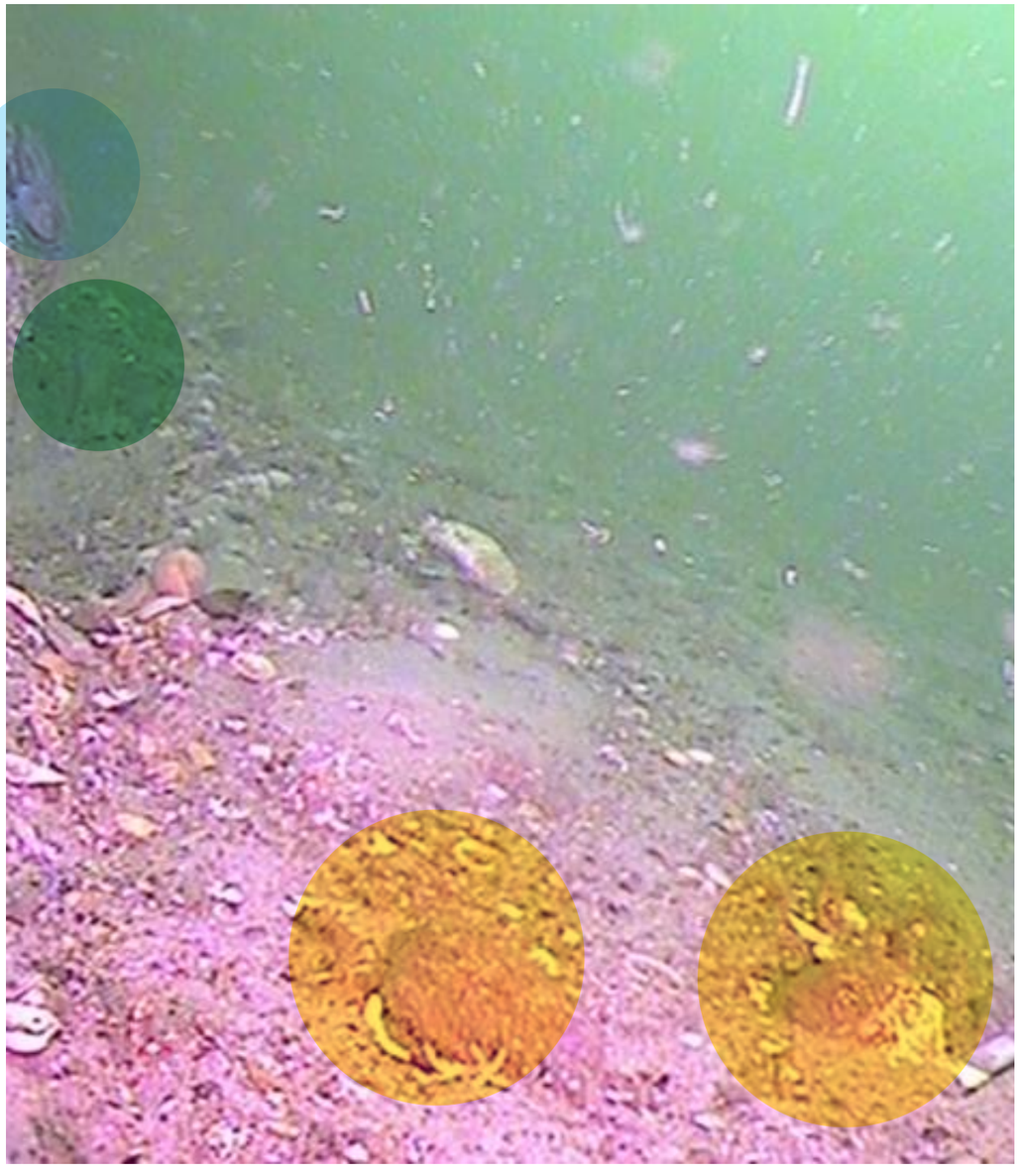


Photo 7. Imgages 20 July 2018. Top: Seeded flat oysters on the sea floor (yellow) squid (green) and edible crab (blue) near 3D reef structure. Bottom: 3D reefstructure with edible crab (blue) and oysters (yellow) and two squids (green) in the pelagic nearby.

3.2 Environmental conditions

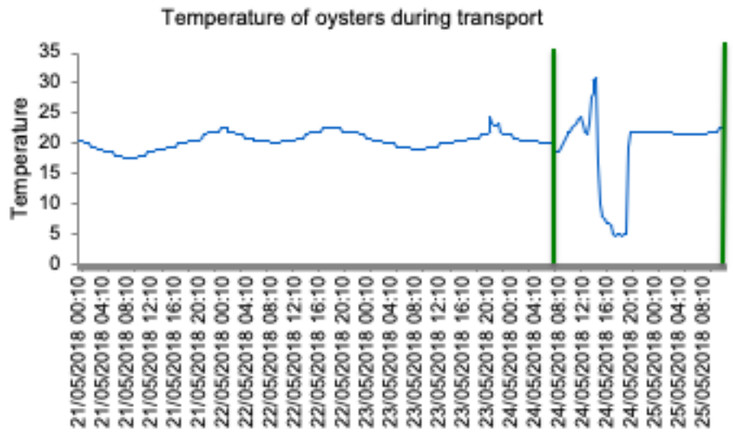


Figure 4. Temperature during transport of oysters. Green lines indicate period of installation.

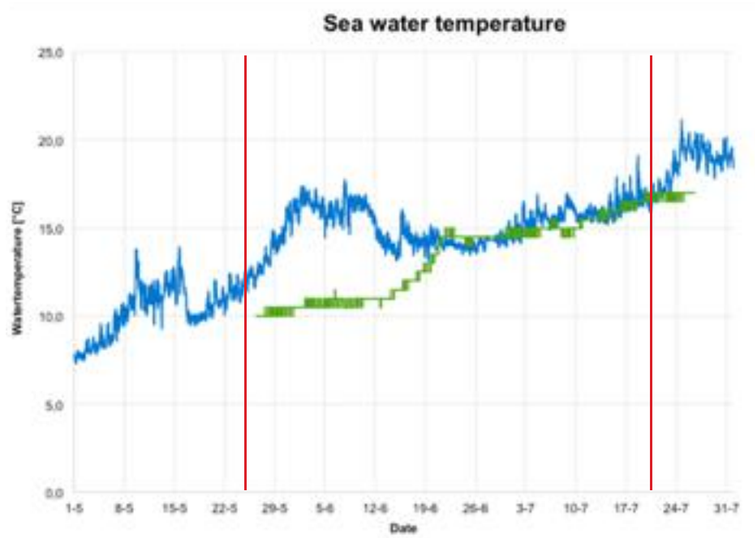


Figure 5. Water temperature at the research rack at Borkum Reef and surface water temperature at Gemini. Red lines indicate installation date (left) and monitoring date (right).

In March water temperature near the sea floor was 2.7 °C on average (Deep, 2018). Figure 5 shows the near-bottom water temperature, retrieved at a research rack at Gemini and surface water temperature at Gemini. At both locations a rapid increase in temperature is observed from May to July. The surface water temperature shows more fluctuations than the near-bottom temperature at this location. At time of installation surface water temperatures was 12 °C and just after installation near-bottom water temperature was 10 °C. Surface water temperature increased rapidly, while near bottom water temperatures increased slower. At 7June a maximum temperature difference of 7.3 °C was observed with near-bottom temperature at 10.5 °C and surface water temperature reaching 17.8 °C. At time of monitoring, 20 July there was no temperature difference between surface and near-bottom and temperature was 16.5 °C. The strong difference between rise in near- bottom and surface water temperature observed in spring could indicate that temperature stratification takes place.

3.3
Oyster survival

Flat oyster survival in rack 2 varied from 37.5 – 92.5% (Table 7). Minimum survival rate (37.5%) was observed for oysters that were placed in the holding towers (Table 7). The size of the oysters was probably too large for the holding towers, which presumably has hampered them in their feeding / breathing behaviour. Survival was best (92.5%) for the large oysters that were placed loose in a basket. When comparing survival for the loose oysters it was better oysters in basket 'large' (92.5%) than for (70%) oysters in basket 'small'.

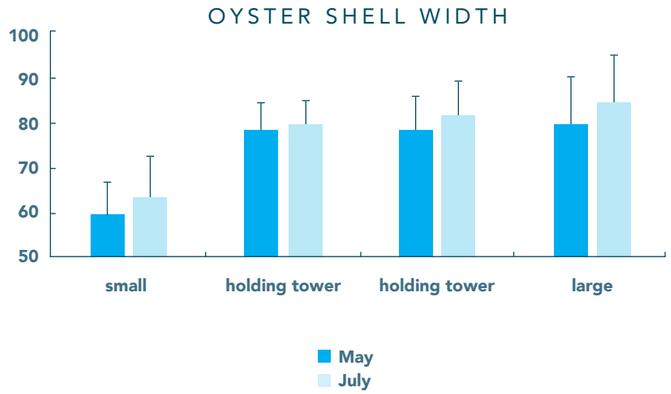
NUMBER OF LIVE OYSTERS PER OYSTER BASKET

Basket number	Content	Initial number of oysters in May 2018	Number of live oysters in July	Survival (%)	Min shell width (mm) in July	Max shell width (mm) in July
21	small	40	28	70	47.4	81.7
11	holding tower	40	15	37.5	72.0	88.8
6	holding tower	40	22	55	68.0	94.8
16	large	40	37	92.5	60.8	103.7

Table 7. research rack 2.

3.4
Oyster growth

The oysters sampled in rack 2 (basket number 21, 11, 6 and 16) showed an increase in shell width and wet weight in July (blue bars in Figure 6) compared to May (red bars in Figure 6). High variability in values is caused by the uncertainty of the weight determinations as a result of wind and waves affecting the balance. The increase in width and weight is not yet statistically significant (ANOVA P>0.05).



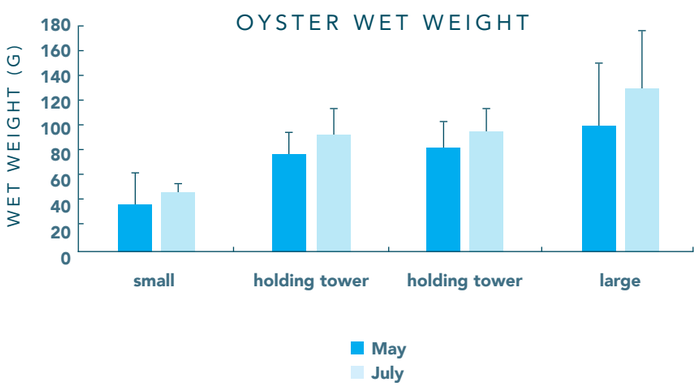


Figure 6. Average oyster shell width (in mm) above and wet weight (in g) below in the baskets in May (red bars) and July (blue bars). N = 40 in May and 15-37 in July with sd.

Condition Index

The oysters did not show a change in condition (relation between shell weight and soft tissue weight) between the start of the monitoring in May and the first sampling in July (Figure 7). stratification takes place.

When compared to Pogoda et al (2011) the Borkum Reef data fall within the range in condition index observed in the German Bight (Figure 8).

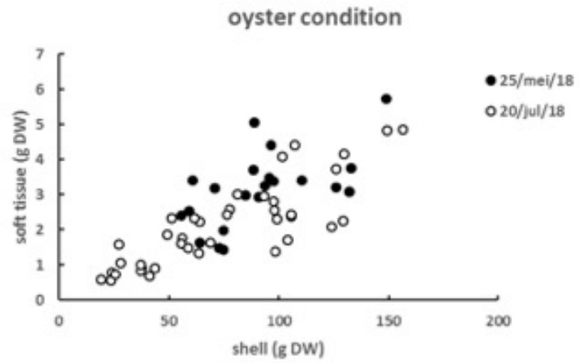


Figure 7. Relation between shell dry weight (DW) and soft tissue dry weight of oysters sampled in May and July.

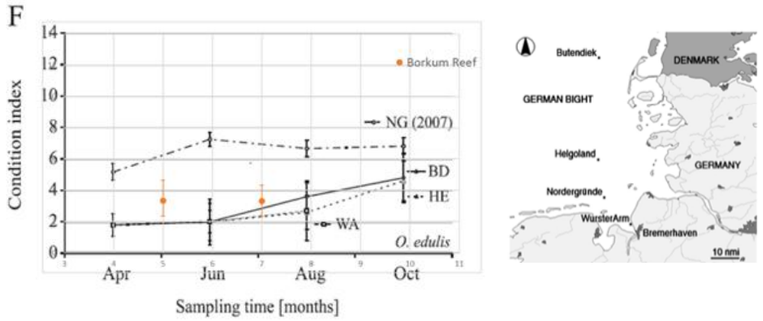


Figure 8. Condition Indices of flat oysters in the German Bight according to Pogoda et al., (2011) (in black) and of reintroduced oysters at Borkum Reef Ground pilot in 2018 (in red).

3.5
Oyster gonad development

Gonad development

Of 20 oysters, 10 (50%) showed development of reproductive organs (gonads). Of the 5 small oysters 2 contained sperm and 1 contained eggs (Table 8). Of the 15 large oysters two were breeding larvae (see photo 8 and 9) and five contained eggs.

Size	Gonad development
large	eggs
large	eggs
large	breeding
large	eggs
large	eggs
large	undetermined
large	undetermined
large	breeding
large	undetermined
large	undetermined
large	undetermined
large	eggs
large	undetermined
large	undetermined
large	undetermined
small	undetermined
small	eggs
small	undetermined
small	sperm
small	sperm

Table 8. Gonad development of collected oysters.

This means that male and female gonad development and even fertilization was observed. Breeding individuals are not often observed during sampling. Thus, finding two out of 15 large oysters is exceptional.

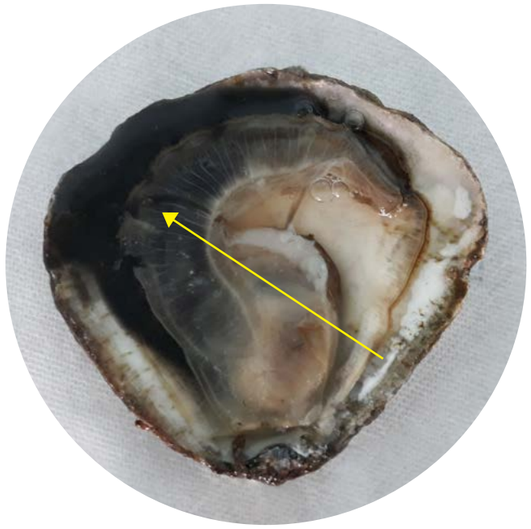


Photo 8. Oyster with larvae (yellow arrow) collected 20 July 2018 .

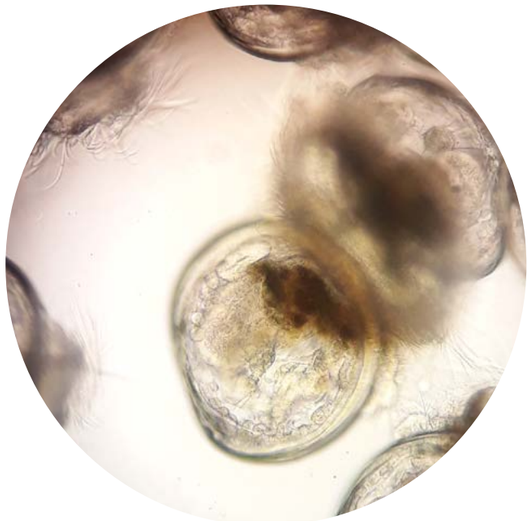


Photo 9. Detail of larvae collected 20 July 2018.

3.6
Larvae

Flat oyster larvae were detected in the water samples in low concentrations at all 3 locations, with numbers from 5 to 43 larvae per 100 liter (0.05 to 0.43 larvae per liter) (Table 9). Only one of 9 samples contained no flat oyster larvae. The qPCR results are comparable to the microscopic analysis and give positive results, yet the number of larvae detected is usually lower. These first results suggest that qPCR can also be used instead of microscopic analysis. However, more verification is needed.

LARVAE IN COLLECTED WATER SAMPLES

Location	Sample	# Flat oyster larvae per 100 liter
1	1	35
	2*	32
	3	0
2	1	16
	2*	11
	3	43
3	1	28
	2*	5
	3	8

*Table 9. *qPCR analysis.*

CHAPTER 4

Discussion

4.1
Research questions

Oyster bed development

Is it possible to create a viable oyster bed with active introduction of 100.000 oysters and approx. 10 oysters per /m2
Does adding live oysters and 3D reef structures help to kick-start oyster reefs?

In May 2018 approximately 80.000 adult flat oysters from Norway (due to poor weather conditions not the full amount could be picked by divers) were introduced at the pilot area in Borkum Reef Ground, the Netherlands. First monitoring shows a positive result so far: Reintroduced flat oysters show a high survival rate at this location and moreover showing signs of healthy growth, good condition and reproduction. Monitoring techniques did not allow for a quantitative comparison of survival rates of oysters outside of the cages.

Flat oyster survival and growth

Survival varied strongly between 32.5% for oysters in holding towers (which presumably suffocated the oysters) and 92.5% for large oysters that were kept loose within their basket. Drop cam footage showed that oysters on the sea floor do survive, and no white shells, as sign of dead individuals, were observed. Average size and wet weight increased by approximately 15-30 % from May to July within an research rack, however this was not significant. Additionally oysters on the sea floor showed signs of growth (visible grow edge).

Flat oyster reproduction

Is there gonad development visible in the introduced oysters?
Are larvae and / or spat produced during the pilot?

Monitoring in July 2018 showed that reintroduced flat oysters show gonad development: in total 50% of both male and female flat oysters showed development of reproductive organs. Two individuals even showed signs of internal fertilization, with larvae present inside the oysters. Furthermore flat oyster larvae were present in 5 of 6 seawater samples, showing swarming larvae were present throughout the pilot

Environmental factors

Is there a relationship between seawater temperature and survival/ growth/ gonad development / larvae/ spat size of flat oysters?

Temperature data of Gemini wind farm, at 20 km from the pilot site, show that the near-bottom water temperature at installation and monitoring was 10 and 16.5 degrees Celsius respectively.
In spring a difference between near-bottom and surface water temperature was observed, indicating temperature stratification. Since larval release is hypothesised to be strongly related to a temperature sum in spring (Maathuis, 2018), stratification with low water temperatures near the seafloor can increase the time to larval release. Temperature data of the Borkum Reef Ground pilot are yet to be retrieved. Since stratification might influence other environmental factors, like oxygen and chlorophyll concentrations, we recommend including more environmental factors in future monitoring.

Biodiversity

How does oyster reef development change the environment and biodiversity?

Although data on biodiversity were only retrieved by qualitative analysis of drop cam images and inspections of the research rack a few remarkable observations were made:

- Grabs and dropcam surveys allowed identification of several species groups in and around the pilot area including worms, bivalves, echinoderms, fish, anemones, squids, crabs and lancelets.
- Common species observed are sand mason worm *Lanice conchilega* razor clam *Ensis sp.* and common sea star *Asterias rubens.*
- 3D reef structures attract crabs: in all 4 structures inspected in July several edible crabs were observed.
- Over ten individuals of squid were observed swimming in the pilot area, more specifically next to 3D reef structures in two cases, and eggs of this species group were attached to the research rack that was hoisted in July. Squid are known to depend on hard substrates for reproduction (attachment of eggs). These observations provide a first indication that adding hard substrate can improve habitat availability fort his species group.
- Common starfish are present in the area and near the reintroduced oysters. Since starfish are known to be predators of oysters it is important to include a quantification of their presence and their possible influence on oyster restoration measures in future monitoring.
- The tubeworm *Spirobranchus triqueter* has been observed to grow on the oysters. This species is known to occur throughout Nortwestern European seas and also known from this area (Lengkeek et al., 2013)

4.2
Lessons learned

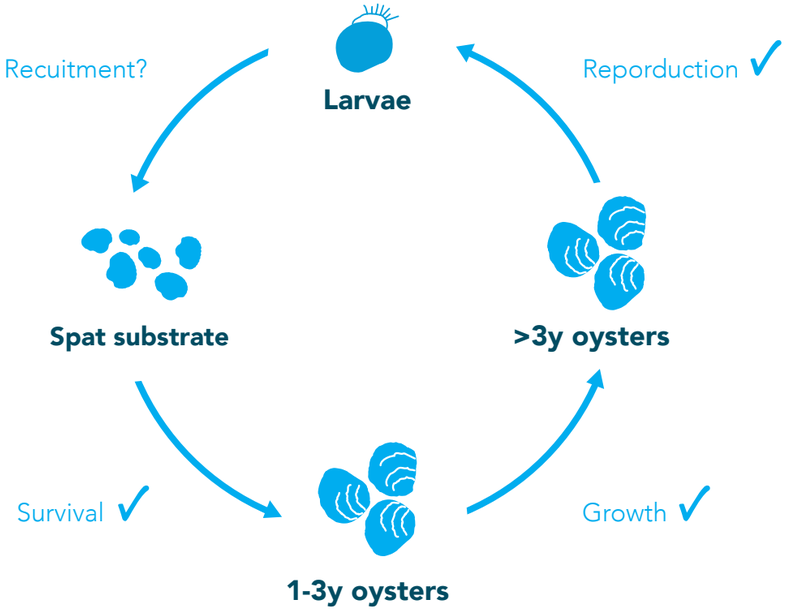


Figure 9. Different stages of the oyster life cycle. Green: observed Red: not yet observed in Borkum Reef Ground oyster pilot.

Oyster pilot

The main lessons learned in 2018 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 for monitoring purposes.

- The measure where 5500 kg, or 80.000 specimen, adult flat oysters are placed on the sea floor has so far led to the result of flat oyster specimen being alive, showing gonad development and signs of reproduction (oysters with larvae, larvae in the water column) (Figure 9). These results, two months after installation, are very promising.

- Due to the short period after installation, it is unknown yet:
  - if survival is high enough to maintain the reintroduced oyster bed
  - if young oysters (spat) will recruit to the population in the vicinity of the adults/source

Therefore, and although results are very promising so far, it is too early to evaluate if this measure is successful in restoring a long lost ecosystem.

Monitoring techniques

Most important lessons learned during monitoring were:

- Using in a rehoistable research rack is a good way to get quantitative results of oyster parameters like survival, growth and gonad development. At the same time spat collectors can be installed. Holding towers that were used inside the research racks were too tight, causing filtration problems for oysters and therefore generated additional mortality of oysters. These holding towers are preferably not used in future monitoring.

- Visual observations with drop cam images allow for qualitative analyses of the reference pilot area (sediment characteristics, present epifauna), survival of oysters and inspection of installations. However, it is not possible to quantify these results. Future monitoring should therefore include techniques that allow quantification.

- An innovative monitoring technique with unknown effectiveness beforehand, was sampling oyster larvae at large water depths. Since 5 out of 6 samples contained flat oyster larvae this technique is suitable for use in offshore parts of the North Sea. However, since it is well known that timing of the larvae peak largely correlates with the amount of larvae that can be detected it is unknown if this technique can be used in a period with fewer oyster larvae.

- To quantify *O. edulis* larvae qPCR can potentially be used as an alternative method to detect and quantify next to conventional counting of larvae.

- First monitoring shows the abundance of common starfish in the pilot site. This might be relevant for oyster survival at Borkum Reef Ground. Future monitoring should preferably include a technique targeting potential oyster pests and predators.

4.3
Conclusion and outlook

Results are all positive and very promising so far: Adult oysters survive, grow and oyster larvae have been observed. It is too early, however, to evaluate if this measure is successful in restoring the long lost ecosystem of an epibenthic shellfish reef with flat oysters. It is therefore important to take the next step in 2019 and evaluate if the observed larvae in the water column have resulted in recruitment and survival of young oysters. Furthermore, it is vital to keep track of the introduced adult population, to determine of the observed presence of common starfish (or other pests or predators) does not hamper the long-term survival of sufficient adult oysters.

Aspects and techniques that have already proven successful, such as the import of Norwegian oysters, the use of retrievable racks, the drop-cam monitoring, larvae pump and more, can be used in future oyster restoration pilots. However it is very important to create more oysters through e.g. hatcheries or spatting ponds to increase availability and avoid exploitation of wild beds.

5
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