Shellfish bed restoration pilots Voordelta, Netherlands

Annual report 2018

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Summary

Shellfish reefs, consisting mainly of flat oysters (*Ostrea edulis*), once occupied about 20.000 km^2 of the southern North Sea (Olsen 1883), but have almost entirely disappeared. The North Sea ecosystem would have differed substantially from that of today, being vastly more productive for hard substrate associated organisms. However, detailed knowledge of this reef ecosystem is not existent. Recently, scientists and conservationists throughout Europe have been focusing on the endangered status of *O. edulis* habitats and there is scope for restoration.

As part of the Haringvliet Dream Fund Project (www.haringvliet.nu), ARK Nature and World Wildlife Fund (ARK/WWF), in collaboration with the Native Oyster Consortium (POC, a consortium of Bureau Waardenburg, Wageningen Marine Research and Sas Consultancy), have been working on active restoration of shellfish reefs, with a focus on the European flat oyster in the Voordelta (Sas et al., 2016,2018; Christianen et al., 2018).

In 2016, a flat oyster reef was discovered at the Blokkendam (Figure 0.1) near the Brouwersdam, and the first experiments and measurements to gain knowledge of and experience with active shellfish reef restoration commenced. In 2017, these experiments and measurements continued, and the natural shellfish reef was studied intensively. This report is a continuation (year 3) of the shellfish reef restoration project in the Voordelta. The first objective for 2018 is to kick-start a new flat oyster pilot at the Bollen van de Ooster using methods derived from the pilots in 2016 and 2017 in the Voordelta. Objective 2 includes further research into the mechanisms behind the critical success factors for oyster reef restoration at the Blokkendam, specifically aimed at predicting peaks in larvae abundance to optimise timing of placement of settlement substrate. Also different techniques are tested for their applicability for enhancement of flat oyster spatfall (objective 3) and biodiversity (objective 4).

Figure 0. Rhine delta with Grevelingen (bottom) and Haringvliet (top) indicated location Blokkendam and Bollen van de Ooster.

Kick-starting a new oyster reef
Efforts to kick-start a new oyster reef included selecting a suitable location at Bollen van de Ooster (Figure 0) and installing a pilot with 15.000 live adult flat oysters, 4 research racks for monitoring purposes, empty shells of different bivalve species (cultch) and 8 artificial reef structures for protection (including reef domes, and 3D printed sandstone reef structures). Monitoring 4 months after deployment showed flat oysters had an average survival rate of 40-80% for oysters in research racks and 26% for oysters on the sea floor, with large oysters showing highest survival. Oysters increased in weight (on average 31% wet weight) and size (on average 12%, shell width) from May to October 2018. The mean condition index was between 4 and 6, which suggests a good condition. 50% of the sampled oysters showed development of gonads in the reproductive period. The concentration of oyster larvae was slightly lower than compared to the Blokkendam. Although only 5 flat oyster spat were observed at this location, there is evidence for recruitment at this location and spat is surviving in the first year. The first step in the installation of a new oyster reef can be considered successful with regard to survival and growth of the oysters deployed. The results suggest that the main factor influencing oyster survival is the size of the oysters used as source material. Large oysters, regardless of origin or treatment group survived better than small oysters, which showed a higher mortality, in particular in the first three months after deployment. This either suggests that larger oysters have reached a size, at which factors such as predation or food competition affecting survival are minimal or that storage of small oysters led to increased mortality. In order to learn if kick-starting the oyster reef was really successful, in future research rate of recruitment, i.e. presence of oyster spat, and long term survival of the source oysters should be studied.

Predicting larvae peaks
Research into critical success factor led to hypothesis that the temperature would influence the timing of reproductive processes in flat oysters most, and that temperature sum would be an appropriate parameter for predicting larval occurrence. The present study confirmed that temperature is an important explaining variable in using a model to predict larvae peaks. The temperature sum of 593-660 degree-days (°C*d) in spring and early summer can be used as a crude predictor of peak in oyster larval abundance in the Voordelta. Using this method the peak in larvae concentrations in the Voordelta could be predicted in 2 out of the 3 years that larvae were monitored (2016-2018). Spat settlement occurs 2 weeks after the first larvae peak. Monitoring the temperature sum provides a valuable tool for timing of deployment of substrates for spatfall enhancement in oyster reef restoration practices.

Oyster spatfall enhancement
Recruitment is defined as the inclusion of new individuals into the reproducing oyster population after successful settlement of oyster spat. This life cycle component is essential for the development of self-sustainable oyster reefs. The 2017 results showed that in the natural reef the majority of flat oysters settled on empty shells, predominantly Pacific oyster. To maximize settlement of flat oyster, clean hard substrate must be provided at the precise moment the larvae are ready to settle. We tested the relationship between spatfall and varying types and timing of substrate deployment. Additionally we investigated the possibility to enhance spatfall by adding large patches of shell material and 3D reef structures at different locations. Although the low number of flat oyster spat that settled during this study makes it challenging to draw reliable conclusions, some general observations could be made. Substrate type: Oyster spat settled on all four substrate types (4 different shell types)

suggesting that mussel, cockle, Pacific oyster and flat oyster shells can be classified as suitable substrate, with a slight preference for cockles and pacific oyster shells. Timing: The collectors collecting the majority of flat oyster spat (82 %) were two collectors deployed at 19th and 24th of July. This was approximately two to three weeks after the peak in larval abundance. Placement: Pacific oyster shells placed at one meter above the bottom were seven times more successful in flat oyster spat settlement than at the bottom. No flat oyster spat was observed in the samples of the seeded shells or on artificial structures. These substrates were deployed on the 7th and 19th of June, which was before the larval peak. However, at least a single flat oyster spat was observed by scientific divers on shells seeded in area C. Since the settlement rate is low and the window of spatfall restricted to narrow two-week period, a very precise timing of introducing substrate according to larvae abundance is necessary to enhance spatfall in oyster restoration practises. Resampling larger areas of cultch in future years, when spat has grown to a size detectable by visual inspection, will tell us if adding cultch in 2018 will yield new areas with flat oysters.

Biodiversity enhancement

In 2018 the oyster reef and 3D reef structures contained more species and more species of conservation interest compared to bare sediment. In accordance with the 2017 biodiversity study these results once more underpin the importance of the oyster reefs with native oysters to increase biodiversity in the Voordelta and elsewhere in the North Sea. Artificial reef structures can be a crucial component in kick-starting reef biodiversity and restoring heterogeneous habitats, with an additional potential to protect oyster reefs or pilots. Within the past three years we observed the transformation from mixed oyster reefs to mixed oyster mussel reefs with oyster dominance to co-dominance of oysters and mussels in 2018. Negative effects, where flat oysters are smothered by pseudo faeces, is locally observed. On patches with soft sediment mussels have less variation in size and less recruitment in 2018 compared to mussels collected from stones and the reef dome. The increase in mussel length and low recruitment of the soft sediment mussel population indicate that this mussel bed consisted mainly of a cohort from the massive spatfall in 2016, and chances are that if recruitment remains low the mussel bed will eventually disappear. Shellfish species interact, it is important to include population dynamics of other than target shellfish species as biotic parameters in restoration plans and outcomes.

Lessons learned

Future European flat oyster reef restoration projects are advised to incorporate the following lessons learned:

1) Preliminary results indicate at a recruitment and substrate limited site it is possible to kick-start an oyster reef at a new location by deploying adult oysters of mixed sizes and empty shells (cultch) on the sea floor.
2) Selecting oyster as source material for restoration includes checking disease status, IAS treatment and planning of collection and storage to secure mixed sizes, minimised storage and optimal condition.
3) When deploying structures and cultch, this is preferably performed at the same time due to efficiency.
4) Temporary storage of oysters used for active introduction should be minimised and after introduction, larger oysters might have a higher survival rate compared to small oysters.
5) To increase sustainability of shellfish restoration practises packaging material and other materials should be minimised, made from material that is and non-polluting and

biodegradable and cultch should be checked for pollution with man-made materials before deployment.

6) The temperature sum, of the sea water above 7 °C in spring - early summer can roughly predict the expected timing of the peak abundance of flat oyster larvae. A next step would be the validation of the models, with the goal of using it as a cost-efficient method for future restoration practices.
7) Relatively clean substrate for spatfall enhancement should be added exactly 2-3 weeks after the larvae peak, outside this period it is not useful.
8) Oyster reef restoration includes restoring the reef community and functions. Therefore kick-starting the reef community additional to the oyster reef itself is important in the context of oyster reef restoration.

General lessons learned from this pilot and other flat oyster restoration pilots for future shellfish restoration pilots are summarised in Sas et al., 2019.

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1 Introduction

1.1 Background

Shellfish reefs in the North Sea

Shellfish reefs, consisting mainly of flat oysters (*Ostrea edulis*), once occupied about 20.000 km^2 of the Dutch part of the North Sea floor (Olsen 1883). 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). We can hypothesize that such a substantial natural hard substrate reef must have harbored 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.

Flat oyster restoration

More recently, scientist and practitioners throughout Europe have been focusing 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 Commission, 2011) and part of the Marine Framework Directive, implemented for the Dutch North Sea area by the Marine Strategy policy paper, part 3 (Mariene strategie, 2015).

Best practices for flat oyster restoration

In the Netherlands the recovery of epibenthic shellfish reefs is estimated as feasible (Smaal et al., 2015). Based on the first findings of natural flat oyster reefs (North sea Christianen et al., 2018; Wadden sea van der Have et al. 2018) and experiences with epibenthic shellfish reef restoration in the Voordelta (Sas et al., 2016; 2018, Christianen et al., 2018) knowledge on flat oyster reef properties and key factors for flat oyster reef restoration is being developed. This includes research into functioning of the natural reef and development of restoration techniques based on best practices.

Oyster reef restoration

Definition of restoration (Source: Baggett et al., 2014. Oyster Habitat Restoration Monitoring and Assessment Handbook)
"The process of establishing or reestablishing a habitat that in time can come to closely resemble a natural condition in terms of structure and function." (modifed from Turner and Streever 2002). This definition includes activities aimed at returning degraded oyster habitat to its prior condition, and the construction of new oyster habitats of various forms and construction materials, either natural or man-made..

Restoration
Shellfish reef restoration is successful if high densities occur with substantial structural complexity. In the case of flat oysters such a reef should have a minimal density of 5 individuals per m^2 (OSPAR, 2000). This can be accomplished by (Beck et al., 2009):

- Providing or adding a mix of oysters of both sexes to produce larvae if the population is recruitment limited (which is the case in the North Sea)
- Providing settlement substrate for spat if the population is substrate limited (which may not be the case in some areas of the North Sea)
- Or both if oysters and suitable substrate are absent.

1.2 Voordelta shellfish bed project

As part of the Haringvliet Dream Fund Project (www.haringvliet.nu), ARK Nature and World Wildlife Fund (ARK/WWF), in collaboration with the Native Oyster Consortium (POC), a consortium of Bureau Waardenburg, Wageningen Marine Research and Sas Consultancy, have been working on experiments for the purpose of active restoration of shellfish beds in the Voordelta in recent years (Sas et al., 2016; 2018). The Voordelta is a nature conservation area and part of the EU Natura 2000 network. The project is designed for the duration of a minimum of three years at locations close to the Haringvliet.

The present work is a continuation of the existing flat oyster restoration project in the Voordelta that began in 2016. During the first phase of this project in 2016, a reproductive flat oyster reef was discovered at the Brouwersdam, located within the N2000 area the Voordelta in the Netherlands (Christianen *et al.* 2018; Sas *et al.*, 2016). The existence of this oyster reef indicates that this part of the North Sea provides adequate environmental conditions for oyster recovery and restoration (Smaal *et al.* 2015).

This 2018 report concerns the results of the third year of pilots and monitoring. Oysters were deliberately introduced to a second pilot location within the Voordelta in 2018 to kick-start a new oyster reef. Because restoration projects aim to cover more sediment surface with oyster reefs through creating self-sustaining beds, research and insight into best practices needed to identify how to successfully expand oyster reefs.

Shellfish beds and reefs
Shellfish reefs are defined as having significant vertical relief, >0.2 m above the surrounding substrate, while beds have lower relief, <0.2 m (Beck et al. 2009). In the interests of consistency and brevity, all oyster habitat restoration or construction projects, including those involving species that form beds rather than reefs, are referred to as “reefs” within the international oyster restoration community and literature (Beck et al. 2011; Baggett et al., 2014) and the term oyster reef will therefore be used in this report.

Overall objectives and research questions
The general objectives of the shellfish bed restoration pilots in the Voordelta are: to select suitable locations for shellfish bed restoration, identify critical factors for shellfish bed development, develop methods for shellfish bed restoration and explore the implications of this project for shellfish bed restoration in the wider North Sea area (Sas et al., 2016, 2018, www.haringvliet.nu).

General research questions:
A. How, when and under which circumstances does a shellfish bed develop?
B. When a beginning oyster reef is established, hoe does it develop further?
C. Which natural values (biodiversity) are associated with a shellfish bed?
D. How can spatfall of oysters and mussels be stimulated?
E. How can the development of a shellfish bed actively be initiated?

In summary the main objective is to gain knowledge on **where**, and based on what **method** and related **critical success factors** of flat oyster population dynamics, restoration efforts should best be implemented.

1.3 2018 objectives

The objectives for the activities in 2018 were testing methods for active oyster restoration and further research into the mechanisms behind the critical success factors for oyster reef restoration, more specifically:
1. Kick-starting a flat oyster reef at a new location (Bollen van de Ooster)
2. Further research into the mechanisms behind the critical success factors for oyster reef restoration at the Blokkendam, specifically aimed at predicting larvae peaks to optimally time placement of oyster settlement substrate.
3. Testing different restoration techniques for spatfall enhancement.
4. Testing different restoration techniques for biodiversity enhancement.

1.4 Research questions

The 2018 objectives were translated into research questions that address success factors to develop a flat oyster reef and ecosystem services provided by the bed.

KICK STARTING NEW OYSTER REEF

1. *What is the rate of oysters survival, growth, reproduction and recruitment at the Bollen van de Ooster?*
   This is a test to confirm the suitability of the selected new location and restoration method. Addresses general question A. For this 15.000 live adult oysters are placed on the bottom and 800 individuals in research racks.

FURTHER RESEARCH CRITICAL SUCCES FACTORS

2. *Can we predict larvae swarming (and subsequent spatfall) based on environmental data?*
   With this knowledge addition of settlement substrate can be timed in order to yield maximum results. This addresses general questions A, D and E.

RESTORATION TECHNIQUES FOR SPATFALL ENHANCEMENT

3. *What is the relationship between spatfall and substrate deployment a) type, b) timing (availability of larvae/ ambient temperature c) placement (on vs off bottom)?*

4. *Is it possible to collect spat near the existing oyster reef at the Blokkendam that can be used for oyster reef restoration in the future?*
   When successful, this method can be deployed in new areas where an oyster reef is being developed. Addresses general questions A, D and E.

5. *Is it possible to increase the surface area and improve the conditions / quality of the oyster reef of the oyster reef at the Blokkendam?*
   (a) by adding oyster and mussel shells in its vicinity?
   (b) by filling bare patches with shells?
   When successful, this method can be deployed in new areas where an oyster reef is developing. Preference for oyster or mussel shell is investigated. Addresses general questions D and E.

6. *Do 3D- reef structures (reef domes and 3D reefs) collect oyster spat?*
   (a) What position (deep vs shallow) of 3D-structures works best?
   (b) Do 3D-structures collect more spat than reef domes or loose shell material?
   When successful, this method can be deployed in new areas where an oyster reef is being developed. Addresses general questions A, D and E.

RESTORATION TECHNIQUES BIODIVERSITY ENHANCEMENT

7. *Do oyster reefs, 3D-structures and reef domes enhance biodiversity compared to bare sediment?*
   This is a test of the enhanced biodiversity of 3D reef structures compared to bare sediment. Addresses general question C.

8. *What additional insight into best practices for shellfish bed restoration can be generated from studying the reef ecosystem ?*
   Additional data of the ecosystem, biodiversity and population dynamics of blue mussel, were generated alongside other monitoring. This addresses general question C.

1.5 Outline

Chapter 2 includes a detailed description of the installation of oyster restoration pilots and techniques used. Specific research questions are addressed in the subsequent chapters (3-5) in which different components of the life cycle were tested. These include survival, growth, condition and gonad development of small and large oysters from different source populations (Chapter 3), concentration of larvae and spat settlement rates on different substrates (Chapter 4), timing of larval swarming (Chapter 5). These results will contribute to the efficiency of restoration practices as described in Conclusions and recommendations (Chapter 7).

Ostrea life cycle

Oysters in the genus *Ostrea* have a complex life cycle (Figure 1.1). After a pelagic period of 6-10 days flat oyster larvae settle permanently by cementing themselves to hard substrate (small shell fragments, complete shells or live oysters). In the first three years they function as males (protandrous life cycle) and in subsequent years they can function alternately as females or males. This sex change depends on environmental conditions (temperature, food; Joyce et al., 2013). The males produce sperm clumps (spermazeugmata), which after spawning are inhaled by the females (Ó Foigil, 1989). The females are larviparous, that is, the eggs are present in the cavity and after fertilization the larvae are brooded by the female for 8-10 days. After brooding the larvae swarm into the water column in June-August and are pelagic until settlement. During settlement larvae are attracted to conspecifics (spat, mature oysters) and the presence of a biofilm on the substrate (Rodrigez et al., 2018).

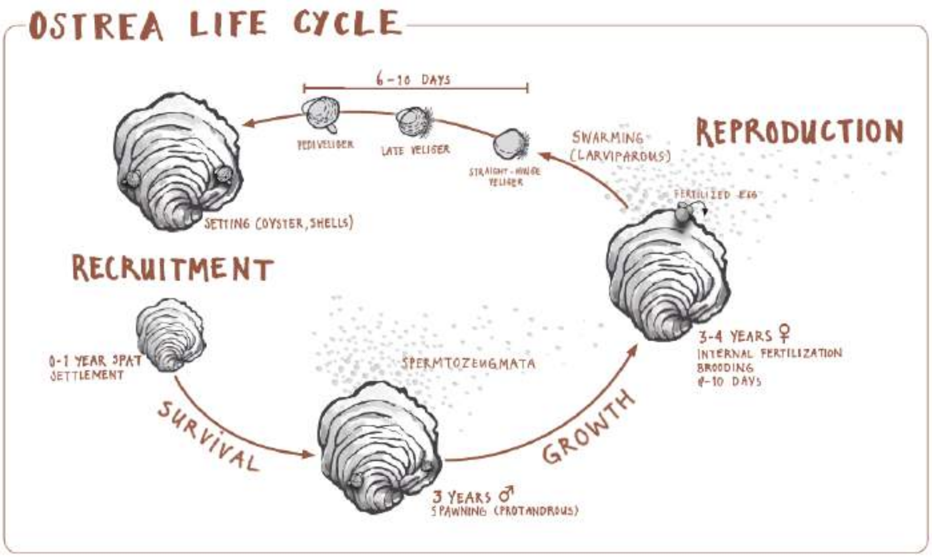


Figure 1.1 General life cycle of Ostrea oysters.

2 Oyster reef restoration pilots

2.1 Introduction

The primary objective of the overall project (2016-2018) is to develop a method for creating shellfish beds in the Voordelta. The ultimate goal is to realise self-sustaining shellfish beds and thereby restore a threatened ecosystem with its ecosystem functions (“work together towards the recovery of native oyster habitat and the associated services on an ecosystem scale”, NORA Conference agreement, Berlin 2017; Pogoda et al. 2017). This is accomplished by “learning by doing”, which implies that the success (or failure) of the implemented pilots are evaluated by a variety of monitoring techniques and metrics as recommended by Pogoda et al. 2017 (“NORA metrics”).

Restoration techniques
A good number of techniques have been developed for oyster reef restoration and the appropriate method generally depends on whether the local population is substrate limited, recruitment limited or both (Baggett et al., 2014; Brumbaugh et al., 2006, 2009). In addition, stress factors, such as fishing related mortality, need to be identified and mitigated (Baggett et al., 2014).

The development and sustainability of flat oyster reefs depends to a large extent on the successful completion of the flat oyster life cycle: from swarming of the larvae and settlement of spat to the survival, growth and reproduction of adult oysters (Sas et al., 2016, 2018). The potential steering factors of oyster reef restoration in the Voordelta are manipulating the number of adult oysters (to generate larvae) and the amount of shell material or cultch (to provide settlement substrate). Additionally, enhancing the reef community by providing hard substrates could potentially speed up the restoration process and at the same time protect the oyster reef.

This chapter
The first objective for 2018 is to kick start a new flat oyster pilot at the Bollen van de Ooster (BvO) using methods derived from the pilots in 2016 and 2017 in the Voordelta, including reef domes, 3D printed reef structures and empty shells of different bivalve species (cultch). Also different techniques are tested for their applicability for flat oyster spatfall (objective 3) and biodiversity (objective 4) enhancement. This chapter includes a detailed description of the restoration pilots, including techniques used.

2.2 Kick starting a new oyster reef (Pilot Bollen van de Ooster)

2.2.1 Location

The pilot is designed to kick start a new oyster reef in an area where a natural mixed oyster reef is known to occur (Figure 2.1; Objective 1, research question 1). Basic ingredients include deploying adult oysters on the sea floor and provide suitable substrate (clean, empty shells) before the period that the oyster larvae are predicted to settle.

Search area new oyster pilot Bollen van de Ooster
The search area for a new oyster pilot near the Bollen van de Ooster was motivated by the finds of flat oysters and blue mussels during shellfish monitoring surveys in 2016 and 2017 (WOT and PMR, ARK 2018, Sas et al., 2018). A massive spatfall of blue mussels occurred in spring 2016 and in January 2017 still live blue mussels were present near the BvO (Plan van Aanpak, ARK, 2018). This information suggests that this BvO location is suitable for shellfish bed development. Other positive factors for an oyster pilot include the protection of the BvO sandbank against waves from the North Sea and the closure for bottom trawling.

Location selection and T_0-survey
The locations of the pilot and reference site (Figure 2.1) were surveyed on 12 April 2018 with dropcam by scuba divers (Figures 2.2 - 2.3). At both sites the number of species was recorded by visual observation as T_0-survey. The site selected as reference had 60% cover of dead blue mussel shells (Figure 2.2) and an upper layer of soft clay (10-20 cm) on a more solid, sandy bottom. The location selected as pilot site had a 50% cover of dead shells dominated by the bivalve shellfish *Cerastoderma edule* and *Spisula solida*, together with *Mytilus edulis*, *Ensis directus* and *Limecola balthica* (Figure 2.3). The sea floor is relatively solid with soft patches and dispersed occurrence of the sand mason worm *Lanice conchilega*.

2.2.3 Pilot design

The basic design of the new BvO oyster pilot was adapted from the 2017 pilot at the Blokkendam (Sas et al., 2018). It includes (a) research racks (4) in the centre with live oysters for monitoring purposes, surrounded by (b) reef domes (4) and (c) 3D printed structures (4) for protection (Figure 2.4), (d) 15.000 live, adult flat oysters for additional production of larvae and (e) empty shells (Figure 2.5; blue mussel, Pacific oyster) as settlement substrate in addition to the natural supply of empty shells (“shelliness”).

Research racks
Oysters are placed in racks, which are positioned at the sea floor (Figure 2.7), to allow for optimal monitoring of the oyster condition. They consist of a steel frame (Frames made out of rebar with a length of 1.37 m, width of 1.02 m and height of 0.50 m, with 4 legs of 0.25 m) and a weight of 135 kg (excluding the oysters and inner cages). Flat oysters are individually placed in PVC holders, or BST oyster basket with a mesh size of 17 mm (Figure 2.7) in inner cages, in order to monitor their survival and growth individually. The cages enable easy removal and placement out of and into the racks. A holding rack within these baskets was used to interspace and track individual oysters in 6 of 16 baskets (Table 2.1).

This brings the total rack weight to ca. 500 kg. The racks are fitted with a cover, which can be opened and closed for oyster handling and an attachment point for a hoisting cable.

Reef domes

Reef domes are concrete, dome-shaped structures with holes and an attachment point for hoisting on the top (1 m diameter and 1 m height, Lengkeek et al., 2017). These are positioned around the pilot site to provide artificial hard substrate and protection against disturbance (van Duren et al., 2016). They weigh ca. 800 kg. Reef domes and other artificial structures put around the pilot must be removed once the pilot is finalised. Therefore, they should also be fitted with a system to enable rehoisting.

3D printed structure

The sandstone 3D printed structures (Figure 2.11) consist of Dolomite sand, Trassmehl™, water and Portland cement (Reuchlin-Hugenholtz, 2018) and are designed and printed by WWF Netherlands, Reef Design Lab and Boskalis. 30 live flat oysters were fixed to the 3D printed structures with Aquascape two-part epoxy putty that is developed for use in salt water and freshwater aquariums and underwater applications.

Empty shells (cultch)

Empty bivalve shells are suitable settlement substrate for flat oysters (Christianen *et al*., 2018; van der Have *et al*., 2017). The suitability of this substrate was confirmed by experiments of WMR in Lake Grevelingen (Kamermans *et al*., 2004; van den Brink, 2012: van den Brink et al, 2013). Clean cockle, mussel and oyster shells are all suitable. Roem van Yerseke provided clean shells of Pacific oyster (stored dry in open air for one month, 20 m^3) and Minnaard provided coocked clean shells of blue mussels (80 m^3, including accidentally a few shells of *Aequipecten opercularis*) (Figure 2.12). The Pacific oyster shells were checked for presence of epibionts before seeding. The mussel shells came from a shellfish boiling plant, which means that the shells had been boiled and all epibionts were dead.

European flat oysters

Live flat oysters from three different sources were used: Grevelingen and Oosterschelde (Netherlands) and Norway. International regulations and the Nature Conservation Law requires that flat oysters originate from a source area, which is free from (a) infectious diseases, such as *Bonamia* and *Marteilla* (proven by governmental surveillance); and (b) invasive alien species. *Bonamia* is endemic in the Grevelingen and Oosterschelde, and is present in the Blokkendam flat oysters, but no information is yet available from the North Sea. An exemption was granted for the use of oysters from the Grevelingen and Oosterschelde in the BvO pilot, because this location is very close to the BD shellfish bed, where the presence of *Bonamia* has been evinced (prevalence 4%, Sas et al, 2018).

Over several weeks in April and May ca 22.000 oysters (1.450 kg, average weight 65 g) oysters were gathered from different oyster culture bottom plots in Lake Grevelingen and Oosterschelde. These oysters were kept four weeks in storage (cooled and under flowing seawater) at the Roem van Yerseke. The oysters were inspected according the treatment protocol to avoid translocation of invasive alien species (van der Have & Schutter, 2018, van den Brink & Magnesen, 2018). No oyster drills, or other non-indigenous species not yet present in the Voordelta were observed during inspections of the oysters. 600 flat oysters, which were collected in the same period in the Grevelingen and Oosterschelde (*Bonamia* infected areas) as the above mentioned oysters were stored for 6 weeks before deployment of the research racks.

200 flat oysters were imported from Hafrsfjord, Norway, a *Bonamia*-free country, for comparison with the Grevelingen and Oosterschelde oysters from *Bonamia*-infected areas. The Norwegian oysters were treated according to the Flat Oyster Treatment Protocol, which is 100% effective (van der Have & Schutter, 2018, van den Brink & Magnesen, 2018) to prevent introduction of alien invasive species. Mature flat oysters were individually collected by scuba divers in Hafrsfjord, Norway and stored in nets without water by Hotate AS and transported to Scalmarin AS, where they were treated. Scalpro AS, an approved fish transporter, shipped the oysters to the Netherlands by cooling truck.

On 21 May the live flat oysters arrived in the Netherlands from Norway (Figure 2.8). After customs clearance they were subsequently stored in water tanks with flowing seawater of 12°C at WMR in IJmuiden. The oysters were transported to Yerseke on 28 May and stored in water tanks with flowing seawater at 15°C. A sample of the oysters were then opened and their condition assessed (figure 2.9). Three specimen were collected as a sample and frozen to determine the dry weight of meat and shell. The wet weight and shell width (mm) of all oysters (g) was measured per basket. For each research rack, 1 or 2 oyster baskets were equipped with dividers (holding towers) to allow identification of individual oysters (Table 2.1, Figure 2.7).

To maximize the chance that both sexes are represented, several age-classes are included in the seeding population: small oysters function as male, large oysters may function as female.

2.2.3 Installation

The BvO pilot was installed in several steps: (1) deployment of live flat oysters (15 May); (2) placement of racks with live flat oysters (29 May) (3) deployment of empty shells (7 June), (4) deployment of artificial structures (19 June).

Deployment flat oysters BvO (15 May 2018)
Of 22.000 oysters (c 1450 kg fresh weight) collected at lake Grevelingen and Oosterschelde, survival at deployment date showed to be 68,7%. This resulted in outplacement of ca 15.000 live oysters at the Bollen van de Oosters within a 45 x 45 m plot with a small boat (Figure 2.6). The resulting density was 7.5 oysters /m^2. Before placement, a sample of 50 oysters was taken for the estimation of condition and size at T_0.

Deployment research racks with flat oysters BvO (29 May 2018)
500 flat oysters, which were collected in the same period as the above-mentioned oysters in the Grevelingen and Oosterschelde (*Bonamia* infected areas) were stored for 6 weeks before deployment of the research racks. 200 flat oysters were imported from Hafrsfjord, Norway. Each research rack contained 4 hanging baskets with 40 (Grevelingen/ Oosterschelde) or 30 (Norway) oysters (loose or in a rack) of different sizes.

The oysters were divided into five different treatment groups, one per basket and four baskets per research rack (Grevelingen: small in rack or loose; Oosterschelde: large loose; Norway: large in rack or loose, see table 2.1). Separating oysters from different origins provided the opportunity to estimate growth rate of the different groups. Using holding racks in a basket made it possible to follow individual oysters over time.

***Table 2.1**. Number of oysters per basket for five different treatment groups. Small oysters from Lake Grevelingen, and large oysters from Oosterschelde. 'In rack' indicated oysters that were deployed in baskets attached in a holding rack (Figure 2.7), 'loose' indicated oysters that were loose in the basket.*

Research rack	Oyster basket #	Number of oysters	Treatment group
1	40	40	Small in rack
1	41	40	Small in rack
1	42	40	Small, loose
1	43	30	Large, loose
2	44	40	Small, in rack
2	45	40	Small, in rack
2	46	40	Small, loose
2	47	30	Large, loose
3	48	40	Small, in rack
3	49	40	Small, loose
3	50	40	Small, in rack
3	51	30	Large, loose
4	52	30	Large in rack, Norwegian
4	53	30	Large, loose Norwegian
4	54	30	Large, loose Norwegian
4	55	30	Large, loose Norwegian

Due to dangerous adverse weather conditions, they were first moved to a sheltered temporary location until they could safely be moved to the pilot location. The temporary location was inside of the Blokkendam area, outside the main shipping route, and within 5 m of each other. The tables were placed at 5.5 - 6 m depth at high tide and stood 40 cm above the sediment. A marker buoy was attached with a rope to each table. On 19 June the racks were then moved to the BvO pilot location for final installation.

Deployment of empty shells (cultch) at BvO and BD (7 June 2018)
At the BvO pilot location and BD test location B (see Figure 2.1 for the location of sites A, B and C) shells of Pacific oysters were placed first (10 m^3 per location) and subsequently shells of blue mussels (24 m^3). In test locations A and C of the shellfish bed only blue mussels were placed (16 m^3). The YE18 vessel placed the shells by opening the valves in the hold of the ship. During installation stir foam and plastics showed to be intermixed with cultch, this floating debris was removed manually.

Before the placement of shells the three test sites were inspected with a dropcam (location A, five samples; location B, 1 sample, location C, 2 samples) to estimate the presence of dead shells. At location A and B patches of live blue mussels were present (0 to 50% cover) on a relatively clean, sandy bottom (Figure 2.8). At location C the Pacific oyster cover was on average 50% (Figure 2.8).

Deployment of artificial structures (19 June 2018)
The YE42 vessel placed four reef domes and four 3D printed reef structures at the BvO and BD pilot sites (Figure 2.9). 30 live flat oysters from the Grevelingen were cleaned and glued to the 3D printed reef structures. Packaging material included several layers of plastics and a wooden box.

2.3 Oyster reef extension via spatfall enhancement (Pilot Blokkendam)

Location Blokkendam
The BD is an existing shellfish bed with an estimated flat oyster population of 6.8 ± 0.6 oysters/m² (Christianen et al., 2018; Sas et al., 2016, 2018). Furthermore, this oyster reef is located at close proximity to the Brouwersluis, the water outlet of the Grevelingen, and a habitat suitability survey concluded that the conditions at this location are favourable as oyster reef habitat (Kamermans et al., 2015). At this location the oysters are found up to a depth of 5 meters (Sas et al., 2017) and the tidal range is maximum 2.5 to 3 meters.

Substrate
The natural oyster bed at the Blokkendam is potentially a location where the natural reef can be enhanced and enlarged in the future serve as a source for nearby oyster reef developments. However, at present the population is still small, therefore it is crucial to increase the size and density of the existing reef. At the same time techniques to enhance spattfall (Objective 3, research question 4, 5, 6) and biodiversity (Objective 4, research question 7, 8) in a natural oyster reef can be tested.

In order to test whether the surface area of the bed can be increased, additional empty shells (blue mussel, Pacific oyster) were added to the BD oyster pilot in three areas (Figure 2.5). Two areas were in the vicinity of the shellfish bed (areas A and B), one area was a bare patch within the shellfish bed (area C). The timing of placement was chosen to optimize the chance of successful settlement of flat oyster larvae. If successful, this method would enhance the extention of the BD shellfish bed, fortifying the resilience of the natural reef and possibly creating spat on shell for other oyster restoration activities.

Additional artificial structures, 4 reef domes and 4 3D reef structures, were added to the BD oyster pilot in two areas: areas A at 5 meter water depth and at the boy at 2 meter water depth (Figure 2.5). These structures are protecting the pilot, whilst at the same time serving as a settlement substrate for flat oysters or other reef community members.

2.4 Lessons learned

Location

Site selection survey at BvO showed unexpected differences in abundance of empty shells. The prospecting scuba diving was very useful to determine this important parameter. Working conditions proved to be difficult at the new location, with strong currents and low visibility and workable conditions restricted to neap tide. These conditions were not taken into account when selecting the pilot area, and preferably would be taken into account in the future.

Flat oysters

Handling and time between collection and deployment of flat oysters should be minimized. The flat oysters, which were collected during spring in the Grevelingen, showed a relatively high mortality (30%) after harvest and during storage. This mortality shows that flat oysters are vulnerable during the reproductive period (spring, summer) and should preferably be relayed in autumn or winter. Since there is a high probability of introducing pest species like oyster drills or invasive species like Pacific oysters non-treated and inspected flat oysters should not be relayed or used in restoration pilots.

Deployment

When deploying structures and cultch, this is preferably performed at the same time due to efficiency. Small vessels showed to be sensitive for adverse weather conditions and non-sheltered location Bollen van de Ooster. Reef domes are robust and easy to deploy in near shore conditions. 3D printed reef structures are vulnerable for hoisting unevenness, a design that could be optimised in the future. To increase sustainability of restoration measures packaging material of artificial structures should be made circular and non-polluting and cultch should be check for pollution with packaging materials before deployment.

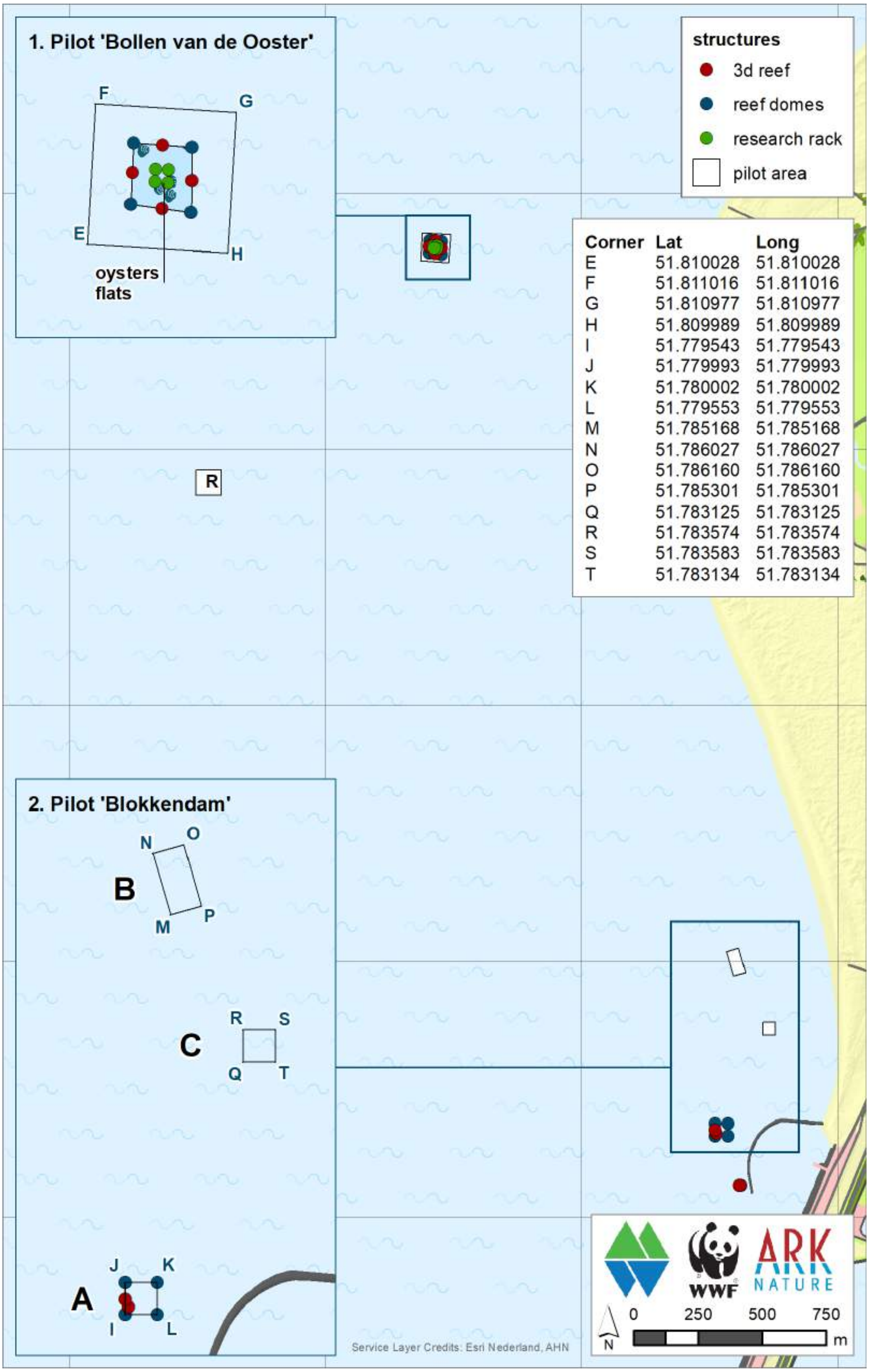


Figure 2.1 Map of the study area between BvO and BD, with reference (R) and pilot area (detail) of Pilot Bollen van de Ooster (top) and shell (cultch) deposition sites A B and C and reef structures at the pilot Blokkendam (bottom).

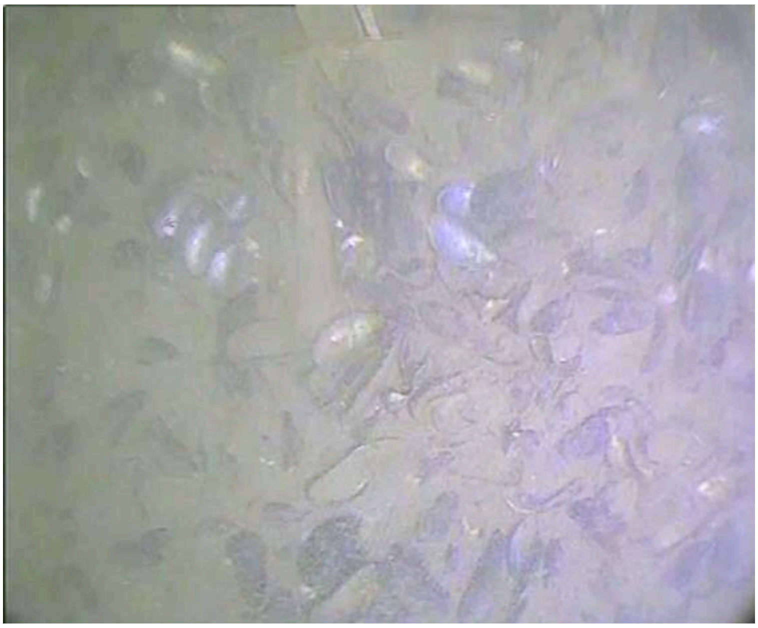


Figure 2.2 Empty blue mussel (Mytilus edulis) shells at the reference location BvO (12 April 2018, video still dropcam).

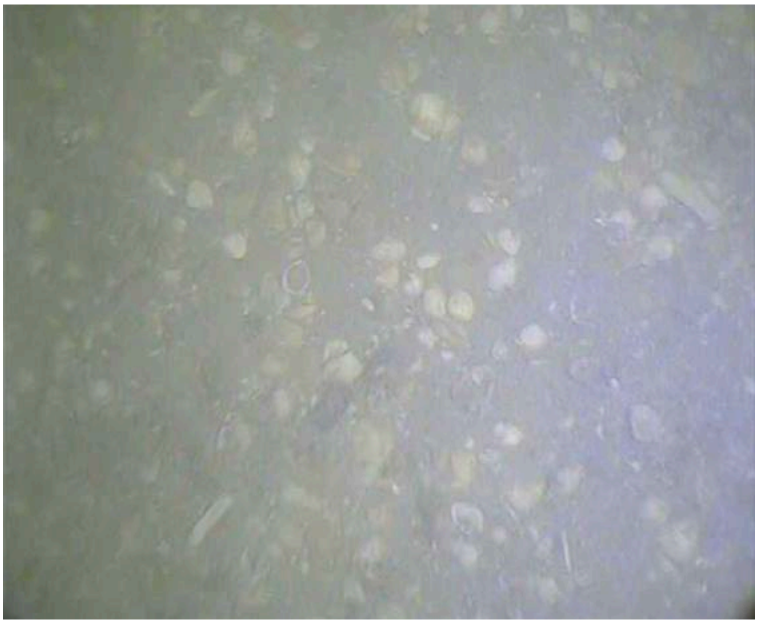
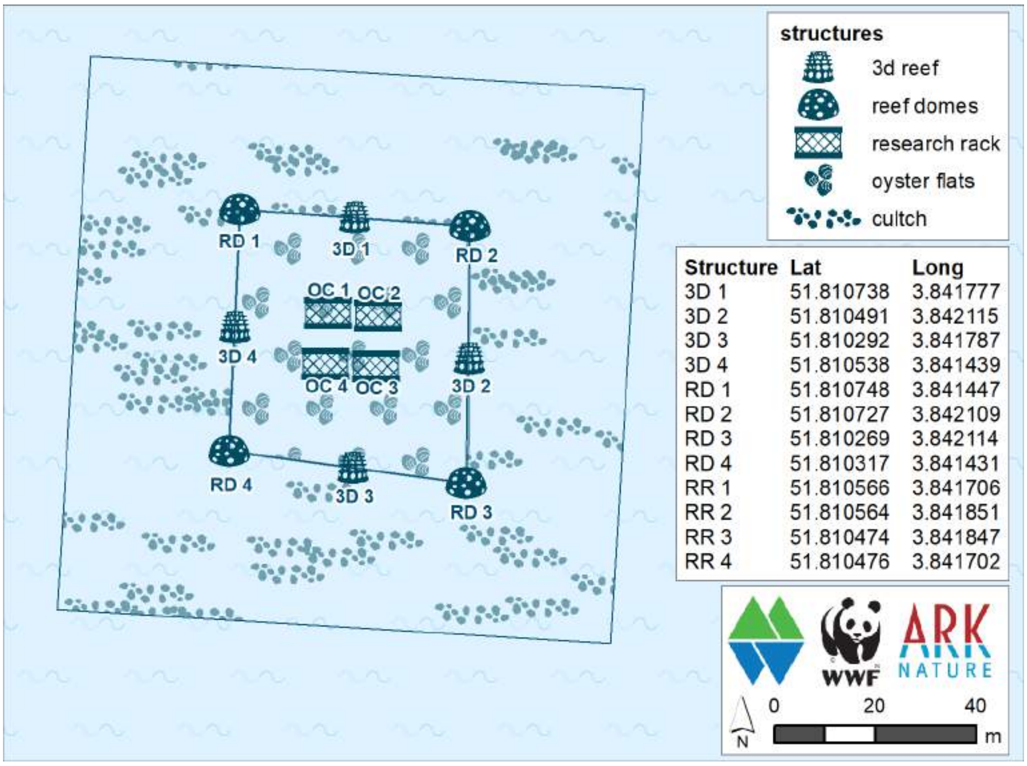
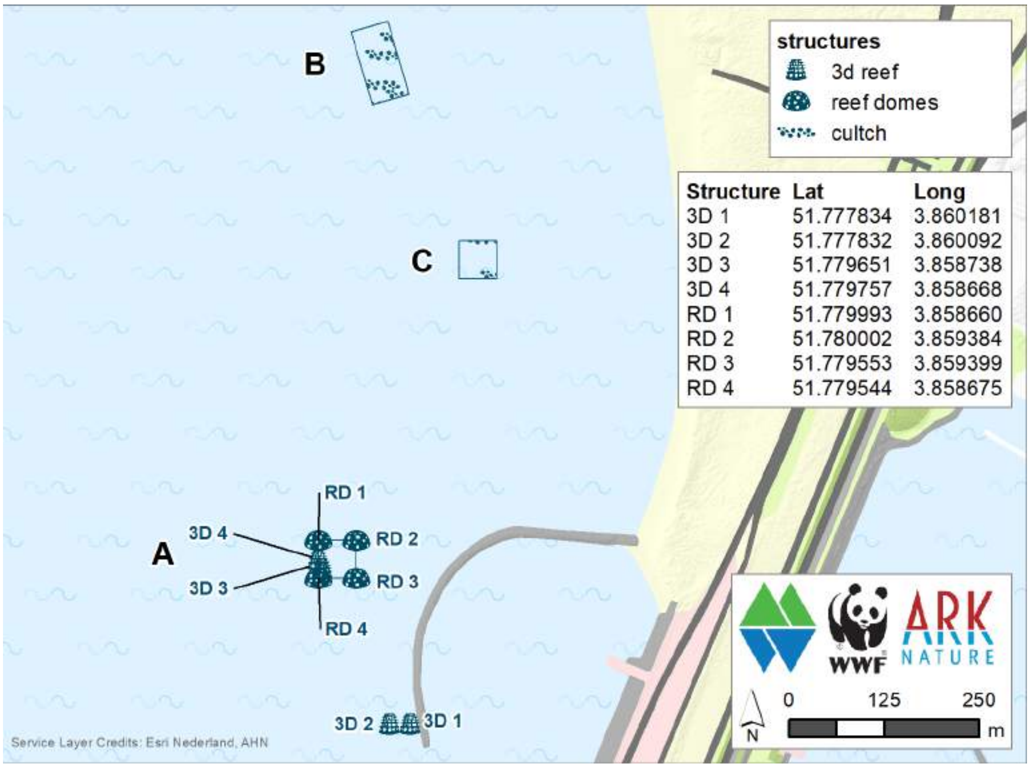


Figure 2.3 Empty shells (mainly Spisula solida, Cerastoderma edule and Ensis directus) at the pilot site BvO (12 April 2018, video still dropcam).



Structure	Lat	Long
3D 1	51.810738	3.841777
3D 2	51.810491	3.842115
3D 3	51.810292	3.841787
3D 4	51.810538	3.841439
RD 1	51.810748	3.841447
RD 2	51.810727	3.842109
RD 3	51.810269	3.842114
RD 4	51.810317	3.841431
RR 1	51.810566	3.841706
RR 2	51.810564	3.841851
RR 3	51.810474	3.841847
RR 4	51.810476	3.841702

Figure 2.4 Schematic representation of the BvO pilot location. The outer square (blue line) delimits the area with dead shells (cultch, c 110 x 110 m), the inner square (black line with dots) delimits the area with live oysters (c 45 x 45 m), reef domes, 3D structures and research racks.



Structure	Lat	Long
3D 1	51.777834	3.860181
3D 2	51.777832	3.860092
3D 3	51.779651	3.858738
3D 4	51.779757	3.858668
RD 1	51.779993	3.858660
RD 2	51.780002	3.859384
RD 3	51.779553	3.859399
RD 4	51.779544	3.858675

Figure 2.5 Locations of the three test sites for additional settlement substrate next to (areas A and B) or within (area C) the BD shellfish bed.

Figure 2.6 Deployment of flat oysters from the Grevelingen at the BvO pilot site (15 May 2018).

Figure 2.7 A small boat (lower left) was used to deploy the research racks (upper left) with baskets and holding towers (upper and lower right, 29 May 2018).

Figure 2.8 Norwegian oysters at arrival (Joost Bergsma)

Figure 2.9 Dutch (above) en Norwegian (below) flat oysters on May 28, 2018 (Pauline Kamermans)

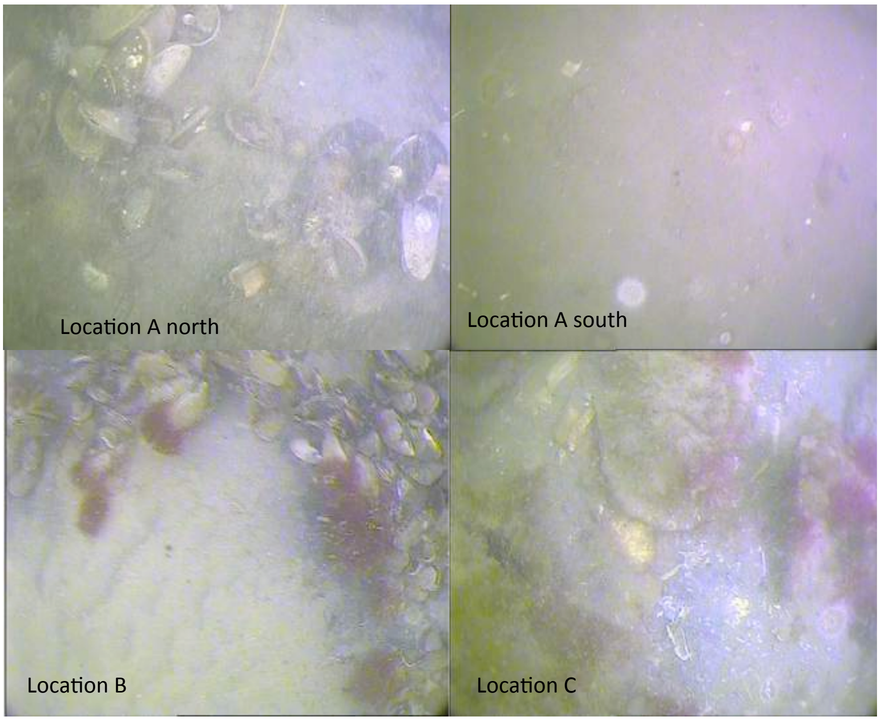


Figure 2.10 Dropcam images of the test locations at the BD shellfish bed before seeding shells showing patches of blue mussels (left), bare sand (upper right) and Pacific oysters with dead shells (lower right).

Figure 2.11 3D structures with live flat oysters (left) were placed at various locations (Figure 2.1) by the YE42 on 19 June 2018.

Figure 2.12 Loading dead shells on board of the YE18 for the BvO and BD pilots, left: blue mussel, right: Pacific oysters (7 June 2018).

3 Survival, growth and reproduction of European flat oysters in a new oyster reef

3.1 Introduction

The self-sustainability of an oyster reef depends on the life-cycle components survival, growth and reproduction of individual oysters. Once settled, oyster spat face various threats to their survival, including competition for space, the threat of smothering by other epibenthic organisms, and predation. Oysters can reduce the intensity of these threats by growing to a size at which these factors are of less importance. Growth requires a high input of energy and, therefore, oysters that can grow fast, whilst still enough energy available for investment in reproductive organs are likely to be those in the best condition. In terms of restoring a self-sustainable flat oyster reef, it is therefore essential that oysters have a high growth rate, a good condition and sufficient reproduction.

Growth in live oysters can be measured by the increase in shell width and wet weight with time. Alternatively, the condition of oysters can be determined using the condition index, the ratio of meat weight to shell weight. These measurements together can give a reliable indication of the condition of the oysters in the bed.

A good condition is also important for gonad development and reproductive output. Like growth, reproduction also requires a high energy input so that the oysters most successful in reproduction will be those with enough energy conserved to invest into the development of gonads, and (for females) the ability to brood eggs and larvae until the moment of swarming. Reproduction in flat oysters is essential for successful development and restoration of an oyster reef. The production of sufficient larvae will enhance the chance of settlement of larvae and recruitment of the resulting spat into the reproducing population and thereby the oyster reef to grow and become self-sustaining. It is therefore important that the oysters used in a restoration project are in good condition and are able to reproduce well under to the local conditions.

This chapter
This chapter concerns research question 1: What is the rate of oysters survival, growth and reproduction at the Bollen van de Ooster? During the 2018 pilot the survival, growth, condition and gonad development of flat oysters from various origins (Grevelingen, Oosterschelde, Norway) and kept in various experimental treatment groups were monitored to determine which treatment groups were most successful in the local conditions, and therefore recommended for use in oyster reef restoration. It is expected that successful restoration efforts lead to flat oysters that survive well, show growth, develop gonads and remain in a good condition.

3.2 Method

Figure 3.1 Monitoring rack with oyster baskets (Karel van den Wijngaard).

The methods includes hoisting or opening research racks (Figure 3.1) with a diversity of subsequent oyster related measurements:

- **Survival** was determined in June, August and in October by counting the number of life versus dead oysters in the individual baskets as a percentage per basket. All dead specimen were removed, so on each date survival is calculated as a percentage of live oysters.
- **Growth and condition:** On board of the YE42 the research racks were hoisted on 19 June to monitor growth. 13 flat oysters were sampled, five large (Oosterschelde) and five small oysters (Grevelingen) and three oysters from Norway. Most oysters had a pale edge to the shell, which shows that the oysters have grown. On the 9th of October after 134 days, all baskets were retrieved from the Bollen van de Ooster by divers. Per basket the live and dead oysters were separated and counted and the live oysters were weighed (wet weight in gram), measured (shell width in mm) and replaced in the baskets. Per basket 4 oysters (64 in total) were taken to the lab for determination of the condition. Pictures were made of all oysters to remember the order of measuring in case unlikely results were found. In addition 54 oysters were sampled from the bottom and treated as above.
- **Condition Index** was calculated according to Walne, & Mann (1975) as the ratio between dry weight of the oyster meat and dry weight of the oyster shell. Condition Indices typically vary over the season due to investment in reproductive organs in spring and summer, decreasing the amount of energy available for growth.
- **Gonad development:** On June 15th a total of 13 flat oysters were sampled from the bottom at Bollen van de Ooster. The oysters were transported in a cool box to the lab for gonad inspection and condition determination (see below). The quick gonad screening method entailed opening of the oysters and gently stroking the gonads with a pipette. The material that came loose was sucked up with the pipette and placed on a microscope

slide. This was then inspected microscopically at a magnification of 400x. Presence of sperm, eggs, or larvae was scored. If none was visible it was scored as no gonad development detected. This quick-scan method will not allow for all males to be detected, so they might be underestimated.

3.3 Results

3.3.1 Survival

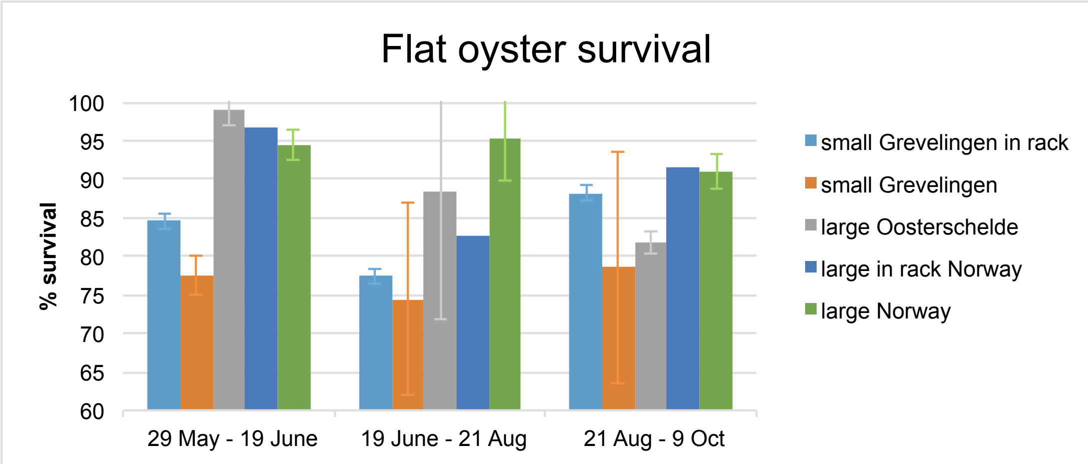
All treatment groups showed a decrease in number of oysters alive over the sampling period, with survival at the end of the experiment varying from 40% (small, loose Research Rack 1) to 84% (small in rack, on Research Rack 1) per basket. However, some treatment groups showed a larger decrease compared with others. The highest survival was in the large Norwegian oysters, which showed survival between 90 and 95% (Figure 3.2B) at each sampling date. On the contrary small, loose oysters showed a lower survival rate between 75 and 80% at each sampling date (Figure 3.2B) Overall survival showed a significant difference between the survival rate of small Grevelingen oysters compared to large Norwegian oysters (Tukey test; P=0.032; Figure 3.2A), all other groups showed no significant differences. Of small Grevelingen oysters only 52% was remaining at the end of the sampling period compared to 64% of small oysters in racks, 76% of large oysters and 82% of large oysters from Norway (Figure 3.2A).

Survival of the oysters that were placed directly on the sea floor was determined in October. Of the 54 collected oysters 14 were alive. This results in a percentage survival of 26%. The oysters placed directly on the sea floor were held in storage for a longer period than the oysters placed in the baskets. This difference in survival compared to the other placement methods is therefore likely a result of handling before and during placement.

<u>Large vs small</u>
In general, larger oysters from the Oosterschelde appeared to have higher survival compared to smaller oysters from Lake Grevelingen regardless of whether they were loose or in a rack. The Lake Grevelingen oysters were collected a few weeks earlier than the Oosterschelde oysters and stayed in storage longer.

<u>Holding tower (rack) vs loose oysters</u>
Small oysters in rack showed higher, but not significantly different, survival rates with 64% compared to 52% of small, loose oysters. The large, Norwegian oysters showed lower survival rates in the rack compared to loose Norwegian oysters.

<u>Overall survival from May to October</u>
Survival was lower in the period from June to August compared to May to June and August to October, but variability between replicated was high (Figure 3.2B).



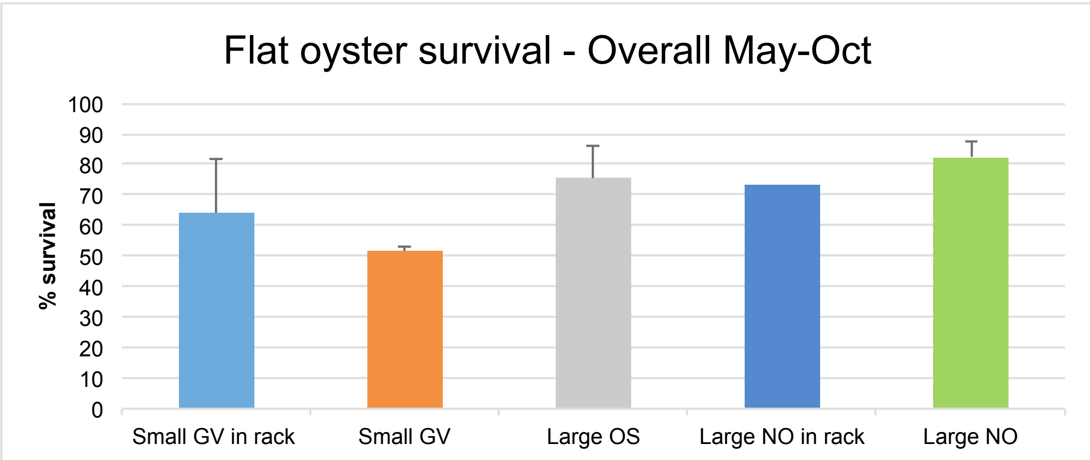


Figure 3.2 Flat oysters survival A. Overall survival May to October (average ± SD, N=3, except for Norwegian oysters in rack, N=1). B percentage of oyster specimen) alive (%) per sampling date. Dead oysters were removed at each sampling occasion. GV Grevelingen OS Oosterschelde NO Norway.

<u>Conclusion</u>
On average survival was 40-80% for oysters in research racks and 26% for oysters on the sea floor, 4 months after deployment. Large oysters showed highest survival.

3.3.2 Growth

All oysters which remained alive increased significantly in wet weight during the sampling period (Figure 3.3, Table 3.1). All differences were significant (Table 3.1), and wet weights in the beginning of the experiment in May were different for all treatment groups, but no treatment group showed significantly more growth than any other.

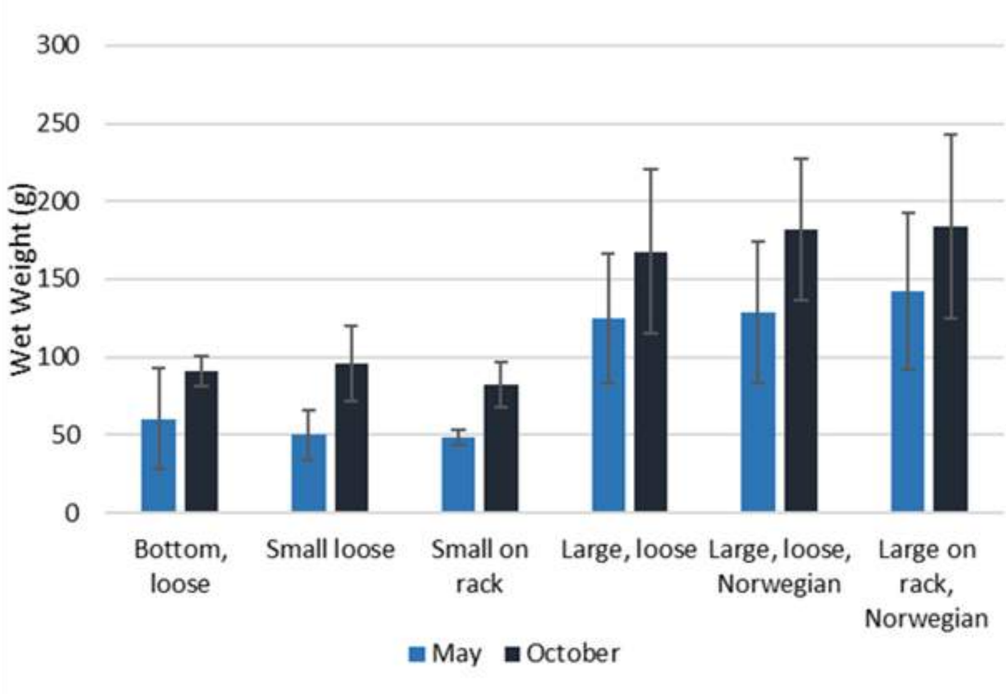


Figure 3.3 Wet weight (g) in flat oysters in the beginning (May) and end (October) of the sampling period of five different treatment groups.

Table 3.1 Results of t-test comparing the wet weight of oysters in different treatments in May and October 2018.

Treatment	***t***	***df***	**critical value**	***p* value**
Bottom, loose	3.5048	63	2	0.000424
Small, loose	-15.1643	185	1.976	< 0.00001
Small on rack	-32.3514	382	1.968	< 0.00001
Large, loose	5.6202	151	1.976	< 0.00001
Large on rack, Norwegian	-2.7555	50	2.009	0.004083
Large, loose, Norwegian	7.342	159	1.976	< 0.00001

All oysters appeared to increase significantly in shell width during the sampling period (Figure 3.4, Table 3.2). No shell width measurements were made for the oysters in the bottom, loose treatment in May, so no statistical test could be carried out. All differences were significant, and shell widths in the beginning of the experiment in May were different for all treatment groups, no treatment group showed significantly more growth than any other.

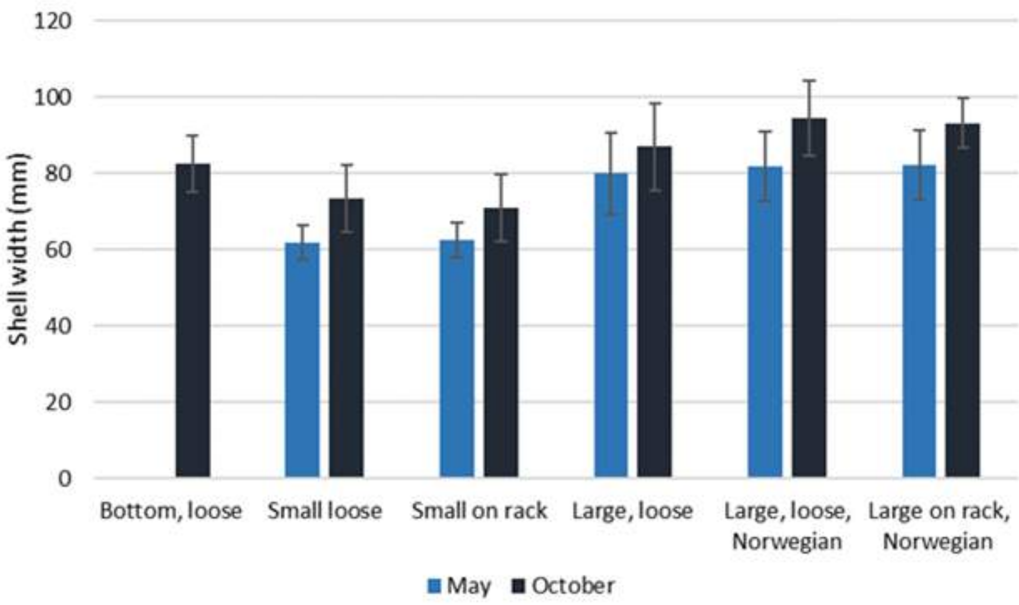


Figure 3.4 Shell width (in mm) of flat oysters in the beginning (May) and end (October) of the sampling period.

Table 3.2 Results of t-test of comparison of shell width of oysters in different treatment groups.

Treatment	t	df	critical value	*p* value
Bottom, loose	n.a	n.a	n.a	
Small, loose	12.3456	209	1.972	< 0.00001
Small on rack	-12.6139	411	1.968	< 0.00001
Large, loose	4.0067	161	1.976	0.000047
Large, loose, Norwegian	8.575	164	1.976	< 0.00001
Large on rack, Norwegian	-5.021	52	2.009	< 0.00001

Condition Index

Condition index ranged between 4 and 6 and showed no significant difference for oysters in any of the treatments between the beginning (May) and end (October) of the sampling period (Figure 3.5, Table 3.3). When compared to Pogoda et al (2011) the data fall within the range in condition index observed in the German Bight (Figure 3.6).

Table 3.3 Results of t-test of comparison of Condition Index (CI) of oysters in different treatment groups.

Treatment	***t***	***df***	**critical value**	***p* value**
Bottom, loose	0.1728	85	1.99	0.43161
Small, loose	1.4782	98	1.987	0.071281
Large, loose	0.2464	33	2.035	0.403449
Large, loose, Norwegian	-0.7776	15	2.131	0.218607
Small on rack	0.7219	39	2.023	0.237331
Large on rack, Norwegian	-0.9922	7	2.365	0.177121

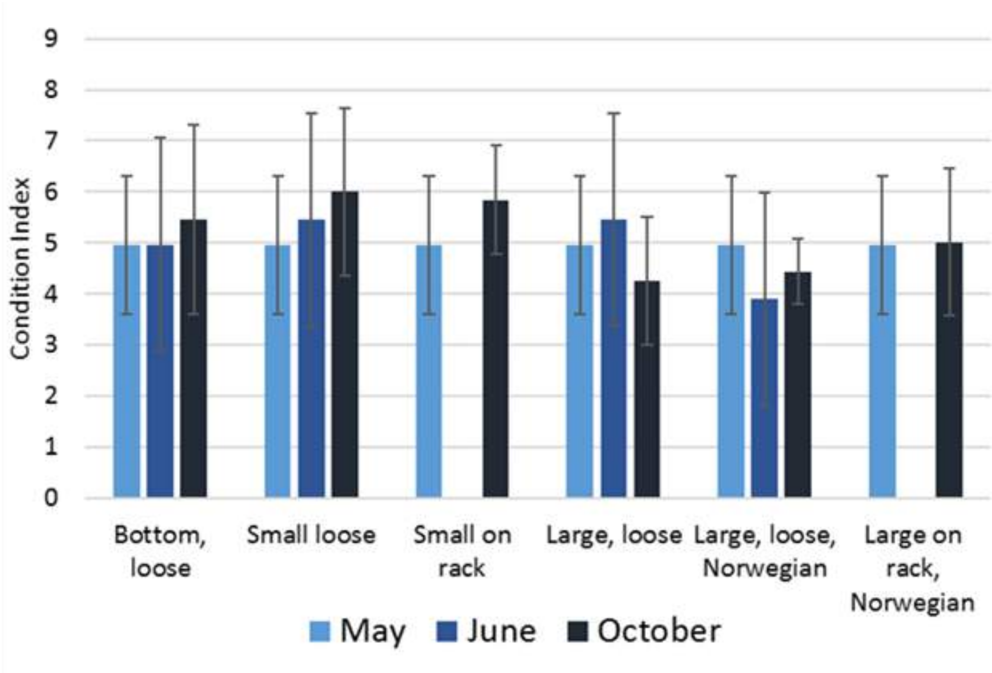


Figure 3.5 Condition index in Flat oysters in the beginning (May), mid (June) and end (October) of the sampling period.

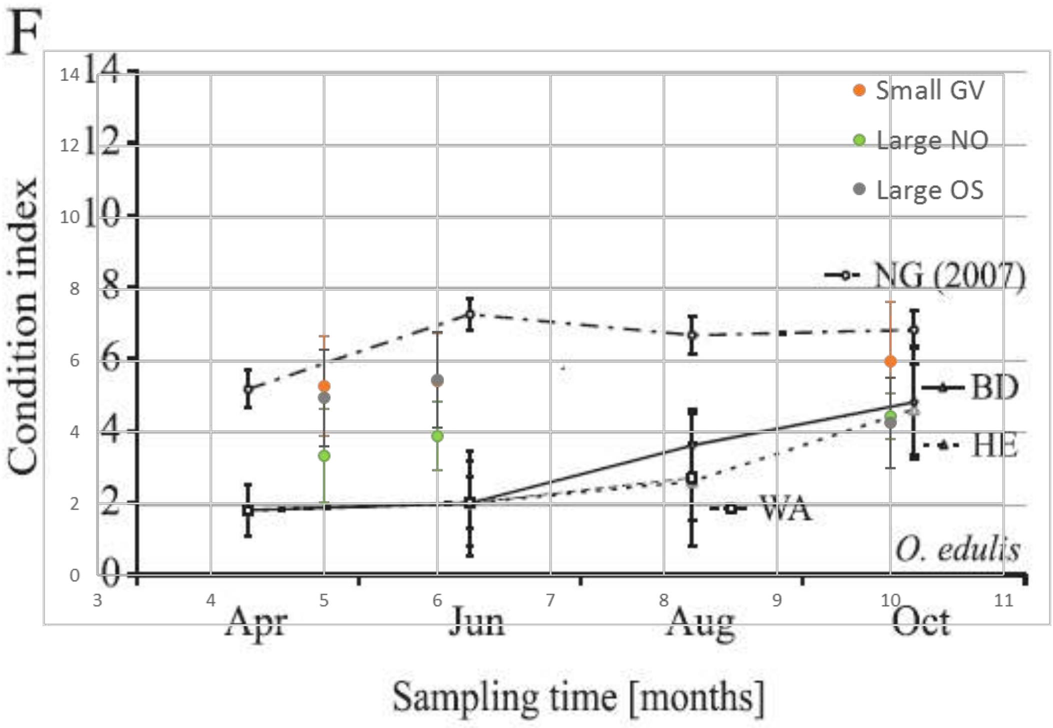


Figure 3.6 Comparison of condition index of oysters cultured in suspended lantern nets in the German Bight (Pagoda et al 2011) and 2018 data.

<u>Conclusion</u>
Growth of oysters showed to be significant for all treatment groups via either wet weight increase or increase in shell width. Oyster weight increased by 31% (wet weight) and length by 12% (shell width) on average from May to October 2018. Condition index showed values indicating a good condition that ranged between 4 and 6.

3.3.3 Gonad development

Of the inspected 26 flat oysters sampled on 15 and 19 June (Figure 2.9 and Table 3.4) 12 individuals contained eggs, 2 individuals sperm and in 12 individuals no gonad development could be detected. This indicates that 54% of oysters in the new oyster reef show gonad development. Condition index of males were 3.6 and 4.1 respectively and for females ranging from 2.9 to 7.6.

Table 3.5 Meat and shell dry weight (DW), Condition Index (CI) and gonad development of oysters in different treatment groups.

Date	Location	Number	Meat DW (g)	Shell DW (g)	Condition index	Gonads
15-6-2018	sea floor	1	1.89	56.18	3.39	eggs
		2	0.92	33.03	2.82	undet
		3	2.56	53.03	4.85	eggs
		4	1.73	61.00	2.85	undet
		5	1.94	56.43	3.47	undet
		6	2.25	74.92	3.01	eggs
		7	2.34	31.13	7.61	undet
		8	1.60	45.25	3.56	sperm
		9	3.88	48.15	8.13	undet
		10	3.39	48.07	7.11	undet
		11	1.99	55.76	3.60	undet
		12	2.70	36.17	7.55	eggs
		13	2.25	34.39	6.61	eggs
19-6-2018	Research Rack 1, small	1	1.69	28.22	6.08	eggs
		2	2.13	33.13	6.50	undet
		3	1.73	27.82	6.30	undet
		4	1.49	29.75	5.06	undet
		5	0.94	28.84	3.30	undet
19-6-2018	Research Rack 3, large	1	5.28	82.00	6.46	undet
		2	1.41	45.81	3.11	eggs
		3	3.58	62.28	5.79	eggs
		4	6.55	112.17	5.86	eggs
		5	2.38	39.70	6.05	eggs
19-6-2018	Research Rack 4, large Norwegian	1	5.21	128.19	4.07	sperm
		2	2.07	72.88	2.85	eggs
		3	1.56	33.19	4.76	eggs

3.4 Conclusion, discussion & recommendations

Flat oysters in the pilot showed an average survival rate of 40-80% for oysters in research racks and 26% for oysters on the sea floor, 4 months after deployment. Oyster weight increased by 31% (wet weight) and shell width by 12% on average from May to October 2018, while Condition Index ranged between 4 and 6 and 50% of the oysters showed development of gonads. With respect to the question about the possibility to kick-start a new oyster reef: The first step in the installation of a new oyster reef can be considered successful with regard to survival and growth of the oysters deployed.

Pogoda *et al.* (2011) found a much higher survival rate (>99% compared to 26% in this study) in *O. edulis* in the German Bight in 2007, but also reported mass mortality in 2004 at one sampling site. They suggested the die-off was caused by the high sediment load in the water, or pollutants entering the water from a nearby port. Survival of *O. edulis* is influenced by the energy expenditure required during spawning as well as the physiological pressure placed on the individual by multiple environmental stressors.

The results suggest that the main factor influencing oyster survival is duration of storage and the size of the oysters at the start of the pilot. Large oysters, regardless of origin or treatment group survived better than small oysters, which showed a higher mortality, in particular in the first three months after deployment. This suggests that larger oysters have reached a size, at which factors such as predation or food competition affecting survival are minimised. Smaller oysters are more vulnerable to these and other abiotic pressures and may therefore show lower survival rates. Furthermore larger oysters may be more adept to withstand the stress of being transported and deployed compared with smaller oysters. Similarly, during their experiment with flat oysters in suspended baskets in the German Bight, Pogoda et al. (2011) found that size class influenced the growth of the oysters, with larger oysters performing more successfully (based on growth) than smaller ones. They suggested that oyster size was an indication of robustness. Oyster handling, like collection, transportation and placementd in the field may have led to increased stress levels in the oysters, with smaller, weaker oysters less able to successfully endure. In addition, the smaller oysters were collected first and therefore remained in storage at Roem van Yerseke longer. This may have added to the stress.

Survival of oysters in baskets was better (up to 84%) than oysters that were relayed on the bottom (26%). The relayed oysters also had spent more time in storage than the oysters in the baskets. Condition and handling before placement may therefore be the main reason for the observed differences in survival.

All live oysters showed a significant increase in wet weight and shell width, but not in condition index during the study period. Despite the different origins of oysters and the treatment groups during the experiment, no group of still living oysters was more successful than another. All showed growth, and none changed significantly in condition. The condition index was comparable to what was observed by Pogoda et al. (2011). In general, condition decreases with the production and release of larvae and builds up again after that. This pattern is visible for the adult oysters, but not statistically significant. The experimental oysters came from the Oosterschelde, the Grevelingen and from Hafrsfjord, Norway, yet all showed similar success in the important life cycle components. This suggested that the origin of the oysters used in the restoration of the oyster reef has little impact on the success of the oysters. Provided that the oysters are in good condition prior to deployment, the results suggest that oysters from various origins, particularly larger individuals, have the potential to successfully survive and grow during the restoration of the oyster reef. From a study in the German North Sea, Pogoda et al. (2011) emphasized the importance of site selection for oyster production. Differences in growth rate were linked to different sites, suggesting that it is the location of deployment, rather than the origin of the oysters that has the most influence on the important life cycle component, such as survival, growth and reproduction. For restoration of flat oyster reefs and to ensure reproduction it is recommended to use a mix of large- and small sized oysters deployed in the most suitable location.

4 Patterns of European flat oyster larvae presence

4.1 Introduction

There is an increasing amount of scientific information contributing to the best practice for restoring oyster reefs (Beck *et al.*, 2011; Vera *et al.*, 2016; Smyth *et al.,* 2018). However, knowledge of how best to manage and promote the larval and spatfall phases, the most vulnerable stages of the oyster's lifecycle, is still limited (Korringa, 1940; Bromley *et al.*, 2016b). To optimise the success rate of restoring oyster reefs more insight into the steering factors of these processes is needed.

To increase settlement success of oyster larvae in restoration practices, it is important to deploy suitable substrate at the right time for maximal settlement success of oyster spat. This means deployment of suitable substrate when there is a peak in larval abundance has the best chance of successful settlement. Predicting the timing of peak larval numbers in the water column (swarming), and the deployment of substrate accordingly has the potential to greatly increase settlement success.

Maathuis (2018) investigated what conditions may determine the swarming of *O. edulis* in the Dutch Delta area, and if it is possible to make an adequate prediction of the timing of this process at the pilot sites. The factors that were examined as explanatory variables in the present study were temperature, tidal difference, lunar cycle, salinity and chlorophyll a. These factors were chosen based on suggestions from literature (Korringa, 1947; Ruiz *et al.*, 1992; Joyce *et al.*, 2013; Robert *et al.*, 2017) and the availability of the data. Generalized additive modelling was used to analyse the influence of these environmental factors on the larvae density in the water. Temperature was expected to have the most influence on the timing of reproductive processes. However, recent research suggests that temperature sum is a better parameter than daily temperature and more appropriate for studying bivalves (Broell *et al.*, 2017). The temperature sum (also known as growing degree days, heat units or thermal time) can be described as the accumulated temperature, if higher than the threshold, over a period of time (McMaster & Wilhem, 1997).

This chapter

In this chapter we describe research into the mechanisms behind the critical success factors for oyster reef restoration. More specifically we looked into swarming of *O. edulis* larvae in the Voordelta. In order to test the relationship between larvae production and ambient sea water temperature we a) used a model based on historical data to predict a larvae peak via a temperature based mode (Maathuis, 2018) and b) used larvae samples to establish knowledge on peaks of swarming larvae in the Voordelta at two locations. We hereby address the research question 2: *Can we predict larvae swarming based on environmental data?*

4.2 Method

Density of swarming oyster larvae in the Voordelta

Larval concentrations were determined weekly in the period June to August 2018 at Blokkendam and Bollen van de Ooster. At each visit, 100 litres of surface water were filtered with a plankton-net (100 µm mesh size). The sample that remained in the net was preserved with formaldehyde. In the lab the samples of larvae were filtered using a plankton-gauze (30 µm). 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.

Prediction of larvae concentrations based on sea water temperature

The study of Maathuis (2018) showed that most larvae were present around an accumulated sea water temperature of 593 °C and 660 °C (starting at 1st of January), for the Oosterschelde and the Grevelingenmeer respectively. The temperature sum is calculated by using daily temperature data measured at 8.00 h in the morning. The dataset starts when the temperature reaches a threshold of 7 °C. Each day the temperature above 7 ^{0}C is added to the sum. In that way the sum is reached at a certain day in June (Figure 4.1.). We tested if these temperature sums were able to explain the observed larvae counts in the Voordelta. Temperature data of Voordelta and Grevelingen for 2016, 2017 and 2018 were used. The test data on larval counts were collected in 2016 and 2017 at the Outlet of the Grevelingenmeer and at the Blokkendam. Moreover, the data collected during this study at the Blokkendam in 2018 was added to this test dataset.

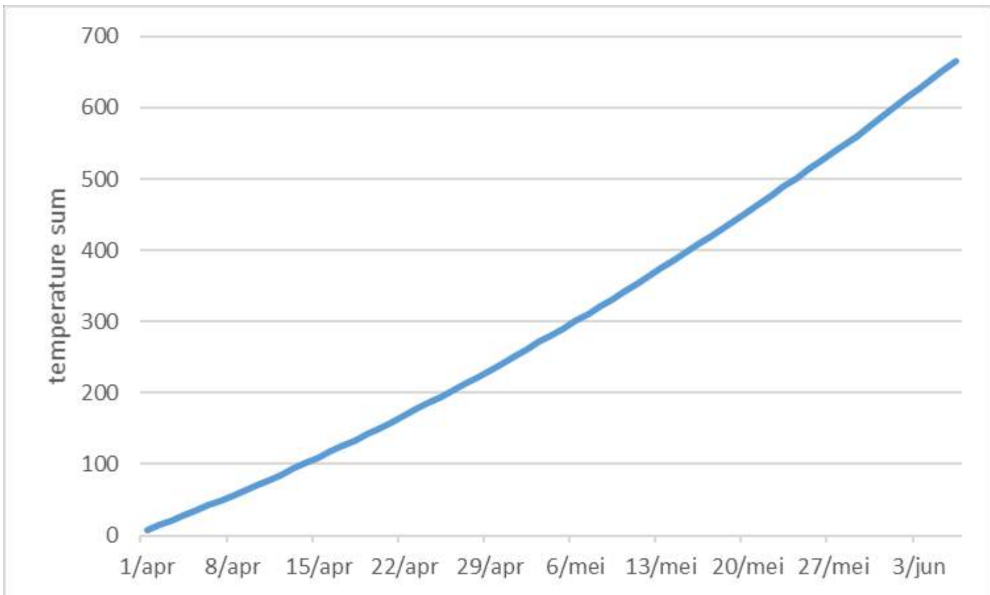


Figure 4.1 Fictive example of increase in temperature sum over time with 1 April as the first date with water temperature exceeding 7^{0}C at 8:00 h in the morning.

4.3 Results

4.3.1 Oyster larvae in the Voordelta

Concentration of larvae showed distinctive peaks in all three years that larvae abundance was measured (Figure 4.2). The highest concentration of larvae in 2018 was in week 27, with concentrations over 9 larvae per litre, while in the previous years larvae where at low densities in the same week. In 2016 highest concentrations were measured in week 28 (outlet) and 29 (outlet and Blokkedam). In 2017 the maximum concentration was measured in week 26 (Blokkendam) and week 33 (outlet).

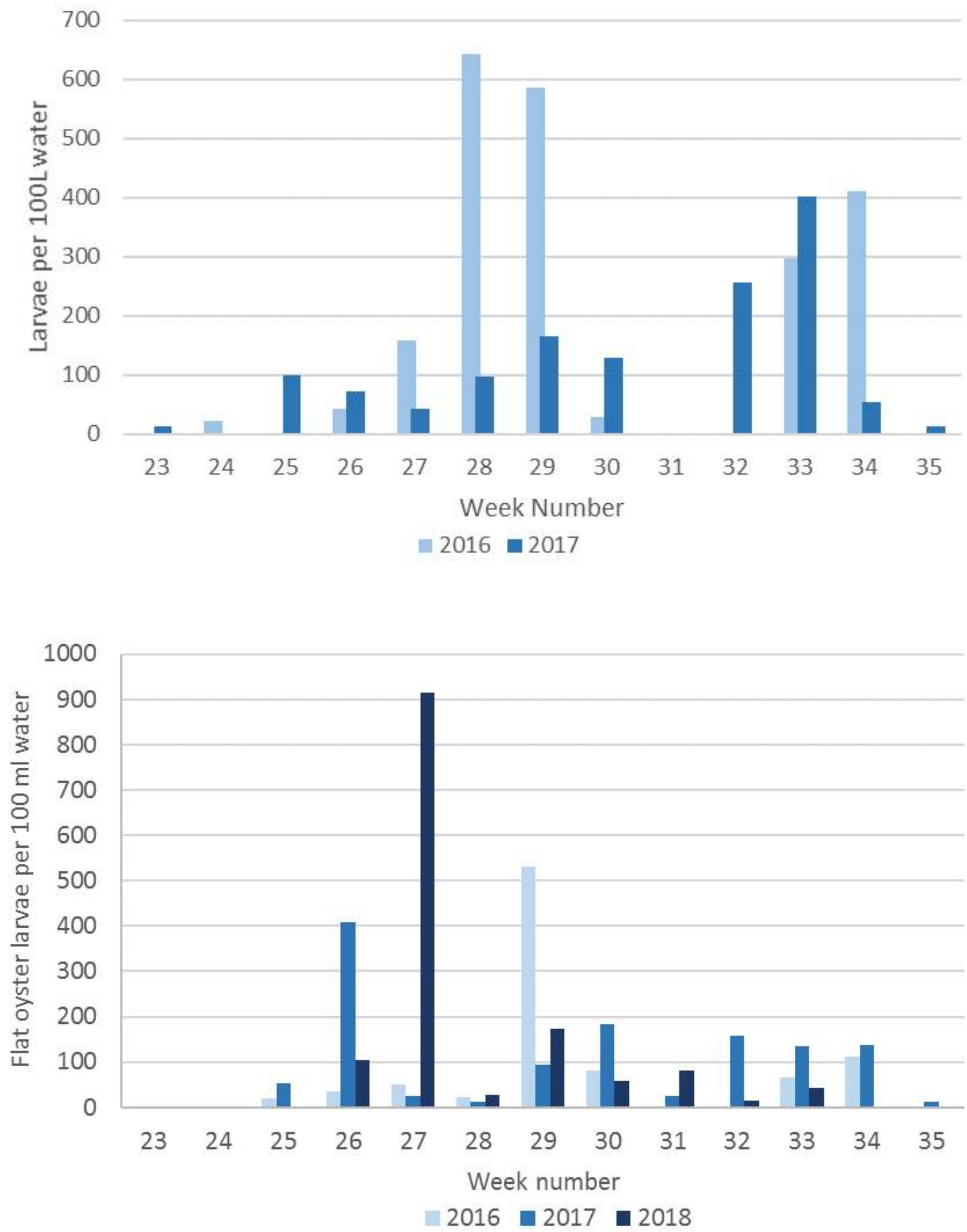


Figure 4.2 Larval concentration at Outlet (top) and Blokkendam (bottom) in week 23-35 2016, 2017 and 2018. No samples at Outlet in 2018.

4.3.2 Prediction of larvae concentrations based on sea water temperature

Using the temperature sum of 660 °C from the Voordelta to predict the day of the peak in larval abundance showed that in 2016 the larvae peak is observed 11 days later than predicted, but 2017 and 2018 show a deviation of 1 and 5 days respectively (see difference in daynumber on y-axis in Figure 4.3 for solid green and dotted orange line). The temperature sum of 660 °C from the Grevelingen predicts presence of the larval peak too early (see difference in daynumber on y-axis in Figure 4.3 for solid green and dotted red line). The temperature sum of 593 °C from the Grevelingenmeer is a good indicator of the first larvae in the water column, with 2-6 days difference between predicted and observed (see difference in daynumber on y-axis in Figure 4.3 for solid blue and dotted blue line). Furthermore, it can be concluded that the temperature sums of 593 °C of both waterbodies are too low to predict the exact day of the first peak. However, in all years it was the case that the day that the first larvae were present in the water column fell within the predicted number of days based on a temperature sum of 593 °C of Grevelingenmeer and Voordelta (see difference in daynumber on y-axis in Figure 4.3 for solid blue and dotted blue and green line).

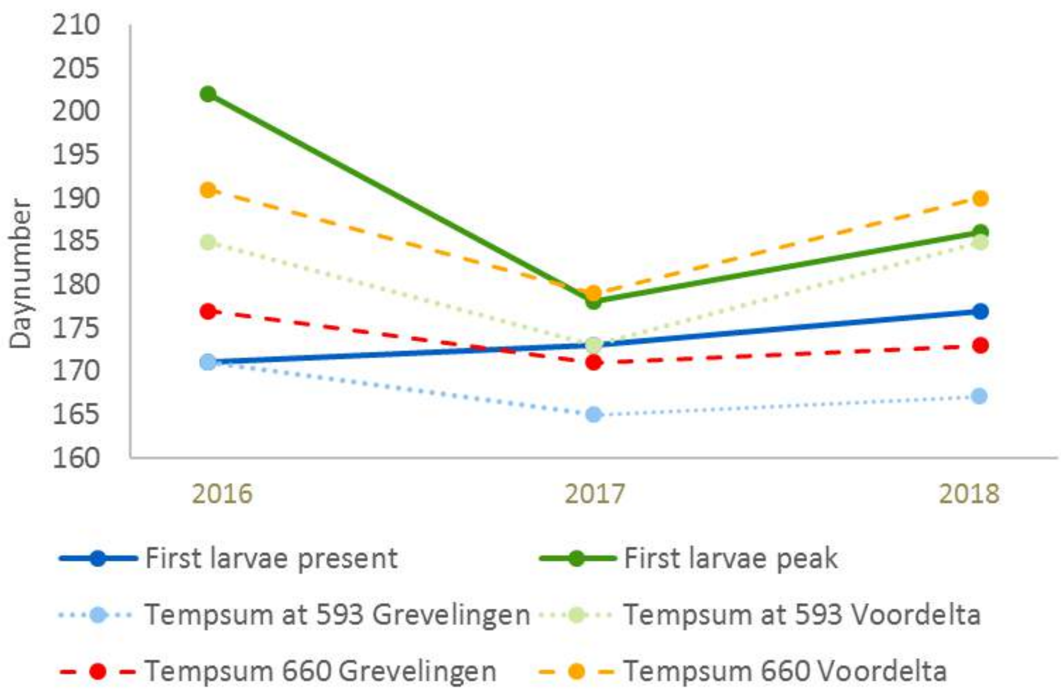


Figure 4 *Day numbers showing the timing of events over a three-year period, based on flat oyster larvae counts conducted at the Blokkendam, Voordelta. Blue shows the day that the first flat oyster larvae are present in the water column and orange presents the day of the first peak (>400 flat oyster larvae per 100L). The day number when the temperature sum of 593 °C is reached in the Grevelingenmeer (Bommenede) and at the Voordelta (Brouwershavensegat 8) are shown in grey and yellow respectively. The day numbers when the temperature sum of 660 °C*d is reached in the Grevelingenmeer and at the Voordelta are shown in light blue and green respectively.*
Day 180 = end of June.

4.4 Conclusion discussion & recommendations

Larval concentrations show obvious peaks coinciding with the simultaneous spawning of most of the oysters. However, the timing of these peaks can vary between years and also between locations. At Blokkendam the first peak in density varied over three years between week 26 and week 29. Accurate predictions of these peaks in density are important for the most effective timing for deploying substrate to enhance spatfall.

For the research question 2: *Can we predict larvae swarming based on environmental data?* Local conditions, such as water temperature, the occurrence of tidal movement and the concentration of chlorophyll-a, are probably drivers in the timing of gametogenesis processes in flat oysters. it was hypothesised that the temperature would influence the timing of reproductive processes in flat oysters most, and that temperature sum would be an appropriate parameter for predicting larval occurrence. The models of Maathuis (2018) were created based on the Grevelingen and Oosterschelde data and were used to predict the timing of larval occurrence in the Voordelta. When using the interval of the temperature sum of 593 and 660 °C of sea water temperature in the Voordelta for the 2016-2018 period, the peak of larvae concentrations are within this interval in two of three study years. Most flat oyster larvae could be found in the water column when the accumulated temperature is around 660°C, especially when this coincides with the end of the semi-lunar cycle. Thus, the accumulated temperature of 660 °C can be used as a first indicator of high concentrations of oyster larvae in the Voordelta. Using validated temperature-sum-models could be a future tool for shellfish restoration practices and a cost-effective method for predicting larvae peaks and timing of settlement substrate outplacement for a specific location. A next important step to achieve this would be the validation of the models.

5 Restoration techniques for spatfall enhancement

5.1 Introduction

One of the ways to expand an oyster reef is to provide settling substrate for oyster larvae. To maximize settlement success, hard substrate must be provided at the precise moment the larvae are ready to settle (Korringa 1940). Although bacterial biofilms are hypothesised to enhance settlement success (Tamburri *et al.*, 2008), extensive settlement by other organisms and sedimentation must be prevented as they obstruct oyster settlement (Korringa, 1940; Kamermans *et al.*, 2004; van den Brink *et al.*, 2013) and there for clean substrate must be provided.

Substrate type
The type of substrate available affects the density of oyster larvae that settle (Van den Brink *et al.*, 2013), Because there is a high mortality of larvae during the pelagic stage (Korringa, 1940; Filgueira *et al.*, 2014), and the larvae only settle if they encounter suitable substrate, more insight into suitable substrate could increase the success of oyster reef restoration. In general, oysters from the family *Ostreidae* typically like calcium carbonate rich substrate (Smyth *et al.*, 2018). Van den Brink (2012) and Van den Brink *et al.* (2013) concluded that mussel shells were the most appropriate collector type for oyster spat (settled larvae) in oyster culture, because it is efficient, affordable and user-friendly. Sas *et al.* (2018) also observed a slight preference of mussel substrate over Pacific oyster substrate in the Voordelta in 2017. However, a survey of naturally occurring flat oysters in the Dutch part of the Waddenzee, showed that most oysters settled on the substrate that was most available, which, in that case, were Pacific oysters and cockles (Van der Have *et al.*, 2017). Similarly, Smyth *et al.* (2018) found that the availability of a substrate is more important than the type of substrate. The shells of flat and Pacific oysters, mussels (*Mytilus edulis*) and cockles (*Cerastoderma edulis*) therefore make promising substrates. Furthermore, Korringa (1940) and Dijkema & Bol (1980) found most spat on the collectors close to the bottom, therefore it was expected that on-bottom substrate would collect more spat than off-bottom substrate.

Timing
Spawning in flat oysters occurs synchronously in 15 and 20% of the flat oyster population and lasts only a short period of time (Korringa 1940), indicating that there must be one or more environmental factor(s) controlling the timing of spawning. Being able to identify and measure the influential environmental factors to predict the timing of larval swarming would increase the success of oyster reef restoration (Chapter 4). Temperature is suggested most often as a factor that controls gametogenesis (Cano *et al.*, 1997; Yildiz *et al.*, 2011; Joyce *et al.*, 2013; Robert *et al.*, 2017). However, temperature alone is insufficient to predict the exact moment of spawning, swarming and spatfall (e.g. Korringa, 1940 & 1947). Other suggested stimuli include food conditions (Wilson, 1987; Robert *et al.*, 2017; Aranda *et al.*, 2014), chemical cues (Hadfield & Paul, 2001; Mesías-Gansbiller *et al.*, 2013; Smyth *et al.*, 2018) and the moon phase (Korringa, 1947).

Placement
Crabs and starfish are the main predators of recently settled oyster spat (Troost, 2010). Since these organisms live on the sea floor, predation may be more severe for spat collectors placed directly on the bottom compared to those that are suspended in the water column. Testing both heights can give insight in the importance of predation (Hein et al., 2017).

This chapter
Recruitment, defined here as the production of oyster spat, is essential when aiming for a self-sustainable oyster reef. Providing the right shell material at the right moment and right height in the water column will enhance the recruitment success. This chapter concerns objective 3 Testing different restoration techniques for spatfall enhancement. In order to test different restoration techniques for spatfall enhancement we tested the relationship between spatfall and varying types and times of substrate deployment. Additionally we investigated if it is possible to enhance spatfall by adding large patches of shell material and 3D reef structures at different locations.
Research questions 3-6 are addressed:
- What is the relationship between spatfall and substrate deployment a) type, b) timing (availability of larvae/ ambient termperature c) placement (on vs off bottom)?
- Is it possible to collect spat near the existing oyster reef at the Blokkendam that could possibly be used for oyster reef restoration in the future?
- Is it possible to increase the surface area and improve the conditions / quality of the oyster reef of the oyster reef at the Blokkendam?
  - by adding oyster and mussel shells in its vicinity?
  - by filling bare patches with shells?
- Do 3D- reef structures (reef domes and 3D reefs) collect oysters spat?

5.2 Method

Substrate type and placement
Four shell types were tested in spat collectors, on-bottom and off-bottom, which were deployed weekly at the location Blokkendam and Bollen van de Ooster in the Voordelta during the period of larval swarming. The collectors were retrieved in October and November 2018 and the number of spat (settled larvae) were counted and compared.
Water samples were taken weekly to estimate the density of oyster larvae in the water columnfor method see chapter 4. It is expected that settlement success is the highest during the period with peak densities of larvae. The collectors were deployed weekly so that there would be clean substrate available the entire larval swarming period.

A spat collector consisted of a heavy tile connected to a buoy. Nets with shells were attached to the buoy (Figure 5.1). Four types of shell material were attached to the buoy and include: mussel shells (*Mytilus edulis*), cockle shells (*Cerastoderma edulis*), Pacific oyster shells (*Crassostrea gigas*) and flat oyster shells (*Ostrea edulis*), placed in strong and coarse nets of equal volume. Since the average shell size of the four substrate types differs, the following weights were used: 500 g of oyster shell, 300 g of mussel shell and 1000 g of cockle shell per net with equal volume of 2.3 liters for each shell type. To test whether there is a difference in spat settlement off-bottom and on-bottom two extra nets with mussel and Pacific oyster shells were placed on the tile (near the bottom).

Figure 5.1 The oyster spat collector: a heavy tile connected to a buoy. Nets with shells of mussels, cockles, Pacific and flat oysters were attached to the buoy and shells of mussels and Pacific oysters were also attached to the tile.

Start of spat collection

Larvae were first observed at the Brouwersluis outlet of Lake Grevelingen into the Voordelta in week 22 (Table 5.1). Spat collectors were deployed weekly from week 23 until week 34. In the first two weeks three spat collectors were deployed followed by one spat collector per week after that. On June 7th empty shells were seeded at four different locations in the Voordelta (Figure 2.1, chapter 2). At Bollen van de Ooster and location B of Blokkendam both mussel (24 m^3 per location) and oyster shell (10 m^3 per location) was seeded. At location C en A only mussel shell was seeded (16 m^3 per location).

Table 5.1 Larval concentration at Brouwersluis outlet of Lake Grevelingen into the Voordelta.

Weeknumber	Date	Location	# Flat oyster larvae per 100 liter
21	5/23/2018	Brouwersluis	0
22	5/31/2018	Brouwersluis	111

Spat retrieval

Spat collectors: The spat collectors were retrieved by divers on the 8th of October and subsequently placed in a basin with running seawater at WMR. During the following three days all nets were opened and shells were checked for the presence of both flat and Pacific oyster spat, and the numbers of spat per substrate type was recorded. Six collectors at BvO could not be retrieved at the beginning of October due to strong currents that made lifting them into the boat impossible (deployment dates 7 June (2), 12, 19 and 31 July, and 7 August). Four were retrieved on the 22nd of November and analysed within three days. The two collector units that remain at BvO were deployed on the 7th of June. On this date three units were deployed at the same time and one of them was collected on the 8th of October. Thus, information on spat settlement is available for that deployment date. Some of the nets had been cut open, probably by abrasion of the sharp shells, resulting in the loss of some shells. This was corrected for by estimating the remaining volume as a fraction of 1 and dividing the spat counts by that fraction. At Blokkendam all collectors except one with cockle shells were still present at the time of retrieval at the location they were deployed. At Bollen van de Ooster one net with mussel shells was missing and five nets were not attached to the collector unit anymore. In addition, at both locations some nets had been cut open resulting in the loss of some shells. These results indicate that the collectors were able to withstand the currents and waves in the Voordelta, but that net strength and attachment to the buoy can be improved.

Cultch: On October 9th samples of shell material were collected at all four locations. At each location 3 squares of 0.25 m^2 were randomly placed on the bottom by divers and all shells present in the square were collected. The shells were transported to the laboratory where they were inspected for presence of oyster spat.

Artificial structures: Quadrats were also placed on artificial structures in three categories 1) 3D reef structures shallow water depth (Blokkendam) 2) 3D reef structures deep (Blokkendam) 3) reef domes (Blokkendam). For each category 3 quadrats of 0.25 m^2 were placed on reef structures and abundance of spat was noted.

5.3 Results

5.3.1 Flat oyster spat Blokkendam

In total, 49 flat oyster spat were found on the collectors of the Blokkendam (Figure 5.2). Of these 34 were alive and 13 dead. Furthermore, 1433 Pacific oyster larvae settled on the collectors and 97 unknown, dead oyster spat were found, with only the shell marks remaining. Nine collectors did not collect oyster spat on any of the substrates, both on top and bottom. This was probably due to the timing of deployment being at the start and end of the season.

Substrate type

Of all the nets placed at one meter above the bottom, cockle shells collected in total 24 settled flat oyster larvae, followed by 16 spat on Pacific oyster shells, six on mussel shells and three on flat oyster shells. In the first two weeks, three spat collectors were deployed to check for variation between the collectors. In this period, only one flat oyster spat was collected at a bottom net, consequently resulting in low variation. A total of three flat oyster spat settled on blue mussel shells, both on the top and the bottom nets (Figure 5.2 B).

Timing

The collectors that were most successful in collecting flat oyster spat were the two collectors deployed at 19th and 24th of July (**Fout! Verwijzingsbron niet gevonden.**A). This was a pproximately two to three weeks after the peak in larval abundance.

Placement: On vs off bottom

Pacific oyster shells placed at one meter above the bottom were seven times more successful in flat oyster spat settlement than at the bottom. Moreover, the number of dead spat settled on Pacific oyster shells at the top is higher than at the bottom. Due to the low numbers of flat oyster spat, Pacific oyster spat can provide more insight into the performance and survival rates of on- and off-bottom substrates for this species. In total 914 Pacific oyster spat settled on mussel and Pacific oyster shells, of which 773 on the nets placed in the water column and 141 at nets at the bottom. Spat survival rates were 35% and 28%, for nets placed in the water column and placed on the bottom respectively.

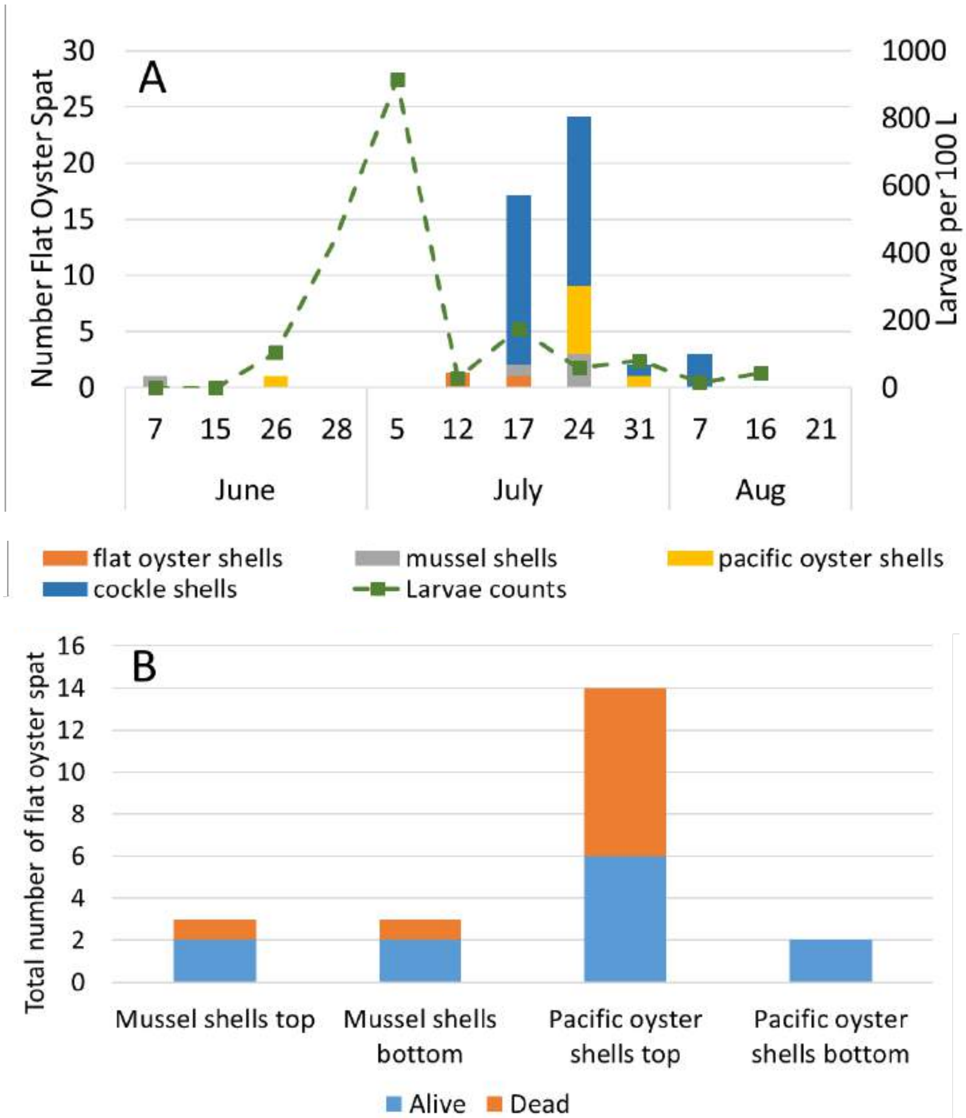


Figure 5.2 Oyster spat at Blokkendam. A. Total numbers of flat oyster spat on the nets placed off-bottom and the number of flat oyster larvae present in the water column at the same location. The results are shown per week and the columns indicate total sum. Note, because three collectors were deployed on 7 and 15 June, the average is shown. B. Total numbers of flat oyster spat on the mussel and Pacific oyster shells, subdivided by placement of the nets (bottom and top (1 m above the bottom)) and the state of the spat: alive (blue) and dead (orange).

5.3.2 Flat oyster spat Bollen van de Ooster

In total, only five flat oyster spat were found on the collectors of the Bollen van de Ooster, all of which were alive. Of these, one was found on collectors deployed on the 15th of June, and two were found on collectors deployed on the 5th and 24th of July (Figure 5.3). Three of the spat were attached to Pacific oyster shells, two on collectors on the bottom, and one on a collector one meter above the bottom. The other two spat were attached to mussel shells on the bottom. In contrast, 340 live, and 141 dead Pacific oyster spat were found on the collectors. All collectors included at least some Pacific oyster spat, however on some dates very few live Pacific oyster spat were found; on collectors deployed 7 June, only one, on 15

June, only three, and on 21 August, only four. Pacific oyster spat was found predominantly in collectors one meter above the bottom with 132 and 71 spat on mussels and Pacific oyster shells respectively, compared with those on the bottom, with 22 and one spat found on mussel and oyster shells respectively. On the mussel and Pacific oyster shell collectors placed 1 m from the bottom, 203 Pacific oyster spat were live, while 65 were dead, while on the bottom 23 were live, and six were dead.

Results regarding the type, timing and placement of deployment and successful flat oyster spat settlement at BvO cannot be reported separately due to the small number of spat.

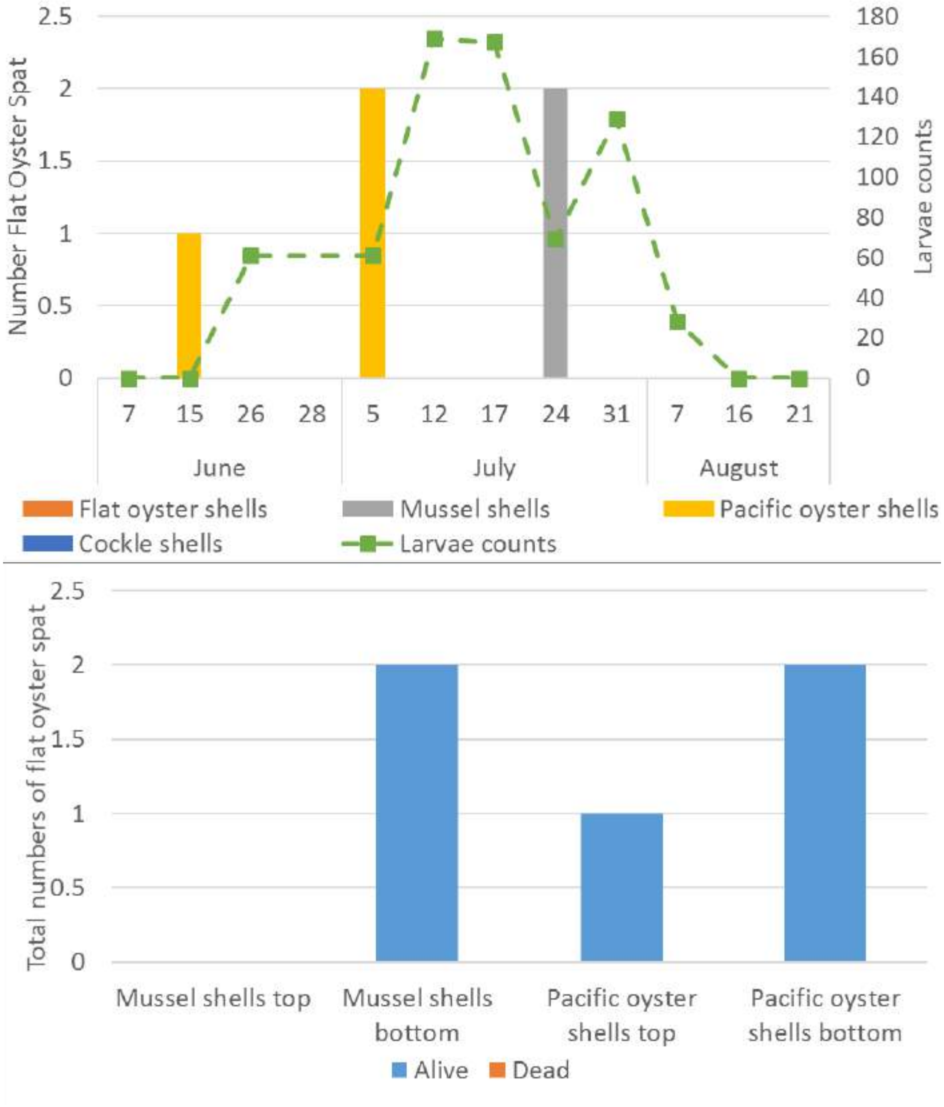


Figure 5.3 Oyster spat at Bollen van de Ooster. A. Total numbers of flat oyster spat on the nets placed off-bottom and the number of flat oyster larvae present in the water column at the same location. The results are shown per week and the columns indicate total sum. Note, because three collectors were deployed on 7 and 15 June, the average is shown. B. Total numbers of flat oyster spat on the mussel and Pacific oyster shells, subdivided by placement of the nets (bottom and top (1 m above the bottom)) and the state of the spat: alive (blue) and dead (orange).

5.3.3 Comparison larvae and spat of Flat oyster and Pacific oyster

In general, the amount concentration of oyster larvae in the water column was similar for both flat and Pacific oysters, with the exception of a definite clear peak in flat oyster larvae at BD on 5 July (Figure 5.4).

More Pacific oyster spat settled at both Blokkendam (BD) and Bollen van de Ooster (BvO) compared with flat oyster spat (Figure 5.4). During the sampling period at BD a peak of 405 Pacific oyster spat was collected on collectors deployed on 17 July, while, on the same date a maximum of 12 flat oyster spat settled on collectors deployed. At BvO a peak of 37 Pacific oysters settled on the collector that was deployed on 5 July, while only five flat oyster spat settled during the whole sampling period, one on 15 June, and two on 5 July.

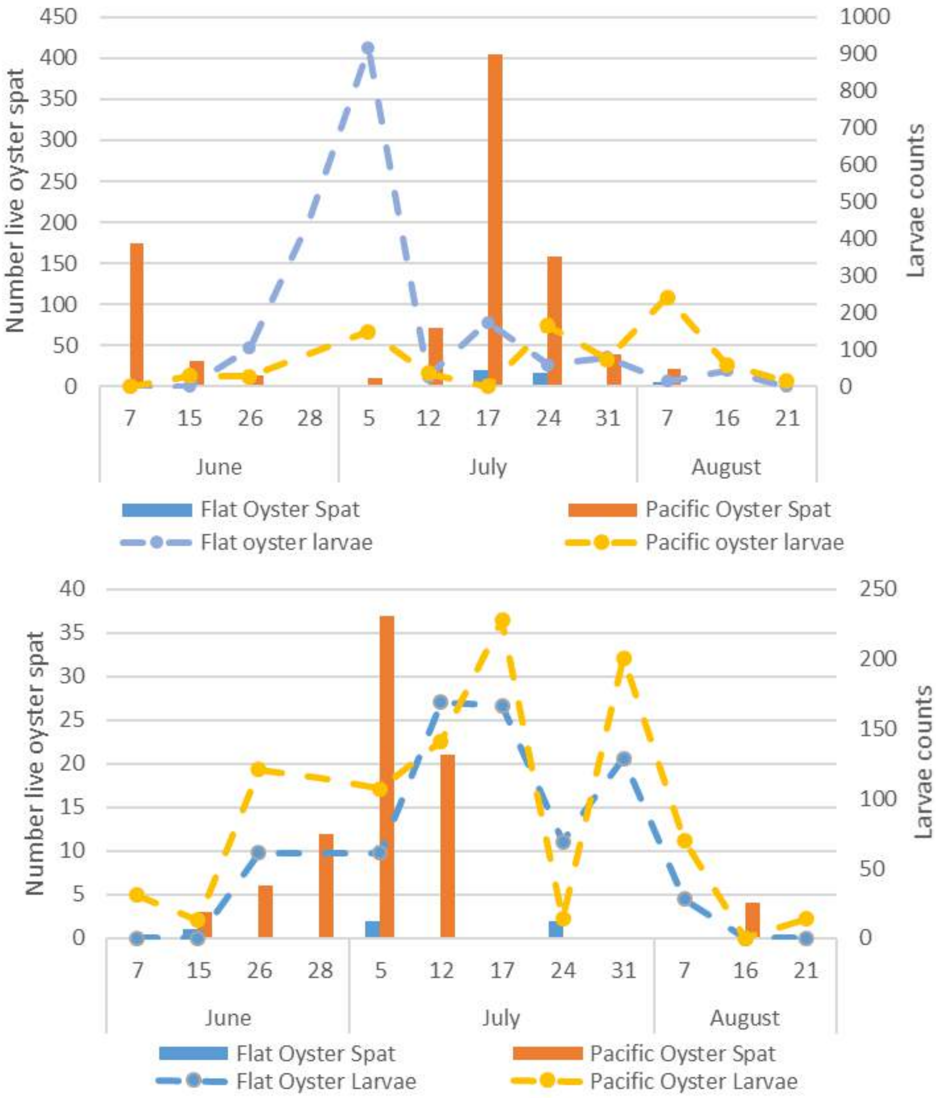


Figure 5.4 Concentration of flat oyster and Pacific oyster larvae measured in water column, and number of spat counted on collectors at Blokkendam (top) and Bollen van de Ooster (bottom). Dates indicate date of collector deployment. Oyster spat numbers are totals of all spat found on all available collectors. Data may indicate lower total spat where some collectors were missing.

5.3.4 Presence of epibionts

Epibionts can hamper settlement of oyster spat. There was a substantial cover of epibionts on the collectors from both locations, although there was some variation in type of epibionts depending on the substrate (Figure 5.5). In general, cockle-shells trapped in the most sediment, but were the least overgrown with other organisms, almost exclusively with barnacles. Flat oyster-, Pacific oysters- and mussel shells showed more variation in epibionts and sometimes the epibionts covered the shells completely.

Figure 5.5 Pictures of the shells and the fouling present on the shells at retrieval of the collectors. From left to right: examples of fouling on flat oyster shells, cockle shells, mussel shells, Pacific oyster shells and fouling totally sheathed substrate on some of the nets.

The epibiont community on the collectors also showed variation over the season. From week 30 onwards, the dominant type of epibiont were bryozoans, especially for the nets placed in the water column. Besides barnacles, some of the sessile species that were often encountered were: vase tunicates (*Ciona intestinalis*), star ascidians (*Botryllus schlosseri*), colonial tunicate species of the genus Didemnum, sea lettuce (*Ulva lactuca*) and blue mussels (*Mytilus edulis*). Furthermore, edible crabs (*Cancer pagurus*), shore crabs (*Carcinus maenas*), polychaeta spp. and different kinds of asteroidea, were non-sessile organisms often encountered in between the shells. No oyster drills (*Ocenebra inornata*) were found at the collectors.

5.3.5 Oyster spat on seeded shells and artificial reef structures

No flat oyster spat was observed in the samples of the seeded shells. Pacific oyster spat was observed at location A, B and C, but not at Bollen van de Ooster (Table 5.2). However, outside the quadrants, at least a single flat oyster spat was observed by scientific divers on shells seeded in area C.

Table 5.2. Number of Pacific oyster spat per m^2 of seeded shells.

Quadrat	Bollen van de Ooster	Location A	Location B	Location C
1	0	4	12	4
2	0	0	4	16
3	0	0	4	8

3D reef structures
No flat oyster spat was observed on 3D reef structures. In two quadrants a Pacific oyster spat was observed. Total sampled area was only 0,3 m^2 for each structure.

5.4 Conclusions, discussion & recommendations

Although the low number of flat oyster spat that settled during this study makes it challenging to draw reliable conclusions, some general observations could be made.

Substrate type
Oyster spat settled on all four substrate types, at least at Blokkendam, suggesting that all substrate types (4 different shell types) can be classified as suitable substrate. However, cockle shells and Pacific oyster shells appeared to be more successful than mussel shells and flat oyster shells.
The results do not support the expectation that spat settlement would be slightly higher on the mussel shells than the other substrate. Mussel shells are still commonly used as substrate by Dutch oyster farmers to collect flat oyster spat, as it is the most cost efficient method (Kamermans et al., 2004; van den Brink, 2012). Various studies have suggested other shell types as the most efficient flat oyster spat collectors. A study in Turkey found that total settlement was significantly higher on flat oyster shells than on mussel shells (Lok & Acarli, 2006). Moreover, in the Dutch part of the Waddenzee, live and dead Pacific oysters, and empty cockle-shells provided the main settlement substrate for flat oysters (van der Have et al., 2017). A study focussing on the shellfish bed at the Blokkendam showed that at this location the flat oyster spat mainly settled on Pacific oyster shells, which is also a dominant substrate type in this shellfish bed (Christianen et al., 2018). These different outcomes show that there is probably not one type of substrate superior for collecting flat oyster spat, but rather that the best substrate is dependent on the type of substrate available and the environmental conditions at the location of collection.

Timing
The results indicate that the timing of the deployment of the substrate is the most important factor for successful enhancement of spat collection (Korringa, 1940; Kamermans et al., 2004; van den Brink et al., 2013). At Blokkendam the spatfall was limited to a period of only a few weeks. The first collectors deployed were in the water before the peak in larval release, and did not attract oyster larvae to settle. The heavy cover of epibonts, which was present when larvae did occur, probably prevented spat from settling and attaching to the substrate. The peak in number of larvae was observed on the 5th of July, and most spat settled two to three weeks after this peak. This coincides with the study of Van den Brink

et al. (2013), where they found most spatfall one to three weeks after the peak in larval abundance in nearby Lake Grevelingen. The results from Bollen van de Ooster suggest that the first collectors were placed before the peak in spatfall occurred. Two flat oyster spat settled on the 24th of July. This suggests that the timing of deployment two weeks after the peak in larvae abundance in mid-July was yielding spat settlement, even with such a small surface area of collector material. The date when mussel and oyster shells were seeded (7th of June) was too early for flat oyster spat settlement. It is recommended that for optimal spat settlement, the water column should be regularly monitored for increases in larvae abundance before shells are seeded. With adequate scaling-up, or with more precise timing of seeding shells on the bottom according to larvae abundance, it could be possible to increase the collection of flat oyster spat.

Placement

Although flat oyster spat numbers were too low to draw conclusions it is indicated that flat oyster spat settlement on Pacific oyster shells was higher off-bottom than on the sediment. Conversely, in 2017 collectors at this location performed better at the bottom than the collectors in the water column (Sas et al., 2017). The 2018 results did not support the hypothesis that most spat would settle near the bottom due to the generally lower, and therefore more favourable current speeds (Korringa, 1940). Detailed research conducted in the Oosterschelde showed a uniform distribution of flat oyster larvae throughout the water column (Korringa, 1940). However, the general high variability in settlement success (Van den Brink et al., 2013) and the low numbers of spat that settled in this study make the current results less than reliable.

Seeded shells and artificial reef structures

Techniques that were used to enhance spat settlement including 3d Reef structures and large quantities of cultch in different areas did not perform well, since no flat oyster spat was observed in quadrants taken within these treatments. These substrates were placed on 7 and 19 June respectively. Spat collectors placed in this same week neither collected flat oyster spat (Figure 5.2). It is therefore hard to draw any conclusions on functioning of these substrates. Total sampled area was only 0,3 m^2 for each site and structure. With densities of natural recruitment of oyster reefs expected to be low, sampled area might be insufficient to detect oyster spat and future monitoring methods should included larger surface areas.

2016-2018

During the 2016 and in 2017 studies at the Blokkendam where flat oyster spat settled (Sas et al., 2016, 2017), only mussel and Pacific oyster shells were used as substrates. In 2016 the collectors were deployed in January, February and July, and resulted in a total of only four live flat oyster spat. In 2017, 42 live flat oyster larvae settled on the collectors, which were deployed from week 24 onwards. In 2018 a total of 34 live flat oyster spat were collected at Blokkendam. Deployment of collectors during this study began in week 23, just after the moment the first larvae were observed in the water column to minimise the possibility of missing spatfall. Even with the small surface area of collectors deployed, some flat oyster spat were collected.

Competition
In general, much fewer flat oyster spat were found at the Blokkendam and Bollen van der Ooster compared with Pacific oyster spat. Pacific oysters are highly fecund and can produce more than 50 million eggs per spawning (Troost, 2010), compared to 1 to 2.5 million larvae per brood for flat oysters (Helm et al., 2004). However, comparing the larvae counts between the two species suggest that both were present in the water column in similar densities during the sampling period, with the exception of a peak in flat oyster larvae on 5 July. It is therefore possible that competitive exclusion of the flat oyster larvae by the Pacific oyster larvae had occurred at the time of settlement. Furthermore, other fouling organisms were competing for space on the collectors. Mussels, ascidians, algae and various other organisms covered the collectors, which would have either prevented spat from settling, or potentially smothered those that had.

Survival
Alternatively the settling flat oysters may have had a lower survival rate than the Pacific oysters. Mortality during metamorphosis from larvae to spat is generally high (Helm et al., 2004). Juvenile stages of bivalves are also highly vulnerable to predators such as crabs (e.g. *Carcinus maenas*) and sea stars (e.g. *Asterias rubens*) (Troost, 2010). Both *C. maenas* and *A. rubens* were observed on and around the collectors. Successful flat oyster reef recovery may be improved if spat were able to settle and grow beyond a threshold of survival in an environment with minimal threat of competition and predation, such as a hatchery and nursery or a spatting pond before being deployed as single seeds or as spat on shell or on artificial structures into the field.

6 Biodiversity in shellfish beds

6.1 Introduction

Shellfish beds provide rare hard substrate in the North Sea, and therefore create a unique habitat for hard-substrate-associated species. Shellfish reefs have significantly higher species richness compared to surrounding soft sediments and harbour a range of endangered species (Christianen et al. 2018; Coolen et al. 2015). Due to overfishing, habitat degradation and diseases these shellfish reefs have become rare in the North Sea, worsening the plight of reef-associated endangered species. In recent years several projects have been executed to restore shellfish reefs in the Dutch North Sea, with emphasis on the native European flat oyster *Ostrea edulis*. These beds are now identified as a priority marine habitat for protection in European MPAs (OSPAR Commission, 2011) and part of the Marine Framework Directive, implemented for the Dutch North Sea area by the Marine Strategy policy paper, part 3 (Mariene strategie, 2015).
Although the main goal of these restoration efforts is to bring back *O. edulis* reefs, the ultimate goal is to restore the structure of these biogenic reefs and their associated species and ecosystem functions (Figure 6.1). Reefs of filter-feeding molluscs have a profound effect on visibility, water quality and carbon fluxes. They are a source of food and shelter for birds and fish, and provide a habitat for hard substrate-associated animals, as well as an attachment substrate for shark, ray and fish eggs.

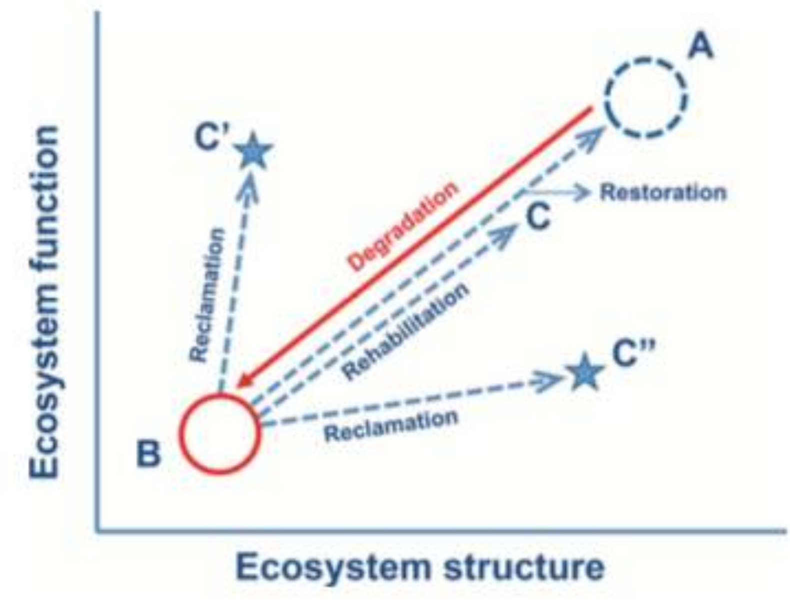


Figure 6.1 Schematic overview of restoration goals and efforts (source: Abelson et al. 2015).

Oyster reefs typically consist of several species of shellfish and form a biocoenosis of multiple bivalves interacting in the same habitat. In the Wadden Sea this co-existence for blue mussel and invading Pacific oyster is described in detail and show that interactions change over time from negative to positive, which result in “oyster reef” establishment (Reise et al. 2017). Pacific oyster induced habitat complexity and hereby positively effects the survival of small mussels by reducing predation by shore crabs (Waser et al. 2015). In the Voordelta native oysters use shell fragments of bivalves, predominantly the invasive Pacific oyster, but also blue mussels, American razor clams (Ensis leei), and common cockle (Cerastoderma edule) as a settlement substrate indicating a biocoenosis (Christianen et al. 2018).

This chapter
Since ecosystem restoration entails biodiversity enhancement this chapter describes options and techniques that could help achieve this goal. Specific research questions addressed are:

- *Do oyster reefs, 3D-structures and reef domes enhance biodiversity compared to bare sediment?*
- *What additional insight into best practices for shellfish bed restoration can be generated from haphazardly studying the reef ecosystem?*

Newly installed structures were sampled by visual census. Additionally during the monitoring surveys in the experimental region, scuba divers have monitored the biodiversity and sampled blue mussels to provide insights into other reef-forming species and the effect of these reef-formers on biodiversity. Here we focus on the native blue mussel *Mytilus edulis*, and present results of biodiversity surveys on shellfish reefs in the Voordelta.

Important note: In 2017 a full biodiversity survey was carried out on the natural oyster reef, which is described in Christianen et al. 2018. In 2018, this study was not repeated. Only limited capacity of the diving team (during other primary tasks focussing on oyster recruitment) was attributed to additional biodiversity observations and measurements. The results of these biodiversity survey and additional survey by volunteer divers, do contribute to the overall goal of this study however, and are therefore further described in this chapter.

6.2 Method

Biodiversity flat oyster reef-visual census
Visual census was performed using thee different methods: 1) quadrant analyses 2) noting rare and new species haphazardly encountered during operations, and 3) using a baited camera and determining the organisms observed in the video recordings. <u>Quadrants</u>: In October 2018 quadrats of 0.25 m^2 were placed across the experimental area and species were noted and filmed. The quadrats were placed randomly across the oyster reef at Blokkendam. Additional analyses included identification of species based on video images. The videos were analysed and observed species were identified to species level or finest taxonomic level possible. <u>Additional species during operations:</u> During monitoring surveys on the experimental sites in the Voordelta, scuba divers noted observations of particular species at the Blokkendam and Bollen van de Ooster.

Biodiversity on artificial reef structures
Quadrats were also placed on artificial structures in three categories 1) 3D reef structures shallow water depth (Blokkendam) 2) 3D reef structures deep (Blokkendam) 3) reef domes (Blokkendam).

Other shellfish species: population dynamics of blue mussel in the Voordelta
Mussels were collected in October 2018 by scuba divers near the Blokkedam in area A (oyster field with mussels, soft sediment), in area B (soft sediment near stone dams), in an area near the research racks (soft sediment), from the stones of the Blokkedam, from a reef dome (installation 2016) and from a location within the oyster reef One quadrant of 0.25 m^2 was randomly selected and all mussels in the quadrant collected. In the lab, shell length of at least 200 mussels was determined tot the nearest um with a digital calliper.

Schelpdierbankdag RAVON & Anemoon
On 13 October 2018 a shellfish reef day (Schelpdierbankdag) was organized by RAVON, Stichting ANEMOON and the Nederlandse onderwatersport Bond. In total 83 divers and one snorkeler contributed to the survey. The shellfish reef was divided into eight sections on the reef and near the reef. In each section ten divers recorded all species, made photographs for later determination and collected species that were later determined on shore. The group of voluntary divers included seven experts on algae, fish, sponges, sea squirts and nudibranches. All results of the survey were put together to create one list of observed species (Gmelig-Meyling & Ploegaert, 2019 in prep).

6.3 Results

Biodiversity flat oyster reef 2018
Organisms observed around the shellfish reefs include arthropods, bryozoans, tunicates, fish, anemones, molluscs, and sponges (Table 6.1, Figure 6.1). In total we observed 46 species, 7 species on reef domes, 9 on deep reef structures, 19 on shallow reef structures, 31 on the oyster reef compared to 12 on sandy sediment. After 4 months of installation artificial reef structures contained 23 species in total. On 22 August 2018 during at the Blokkendam area, a thornback ray (stekelrog, *Raja clavata*) was observed on the oyster reef. The thornback ray is classified as a near threatened species (IUCN, 2018). In October 2018, 4 months after installation, a European lobster *Homarus gammarus* (Red list Germany – score 2) was observed to live in a reef dome (Figure 6.1).

Table 6.1 Observed species in 2018 and specific habitat where species was detected. RSS: Reef structure shallow, RSD: Reef structure deep, OR: oyster reef, S: sediment, RD: Reef dome. R2: German Red list species category 2, RG: German Red List species G, RR: German Red List species R, O: OSPAR target species IU IUCN near threatened.

Name	Scientific name	Where observed				
Edible crab	*Cancer pagurus*				S	
Shore crab	*Carcinus maenas*	RSS	RSD	OR	S	
Asian shore crab	*Hemigrapsus takanoi*		RSD	OR	S	
European lobster	*Homarus gammarus*[R2]					RD
	Macropodia sp.	RSS				
Hermit crab	*Pagurus bernhardus*				S	
	Palaemon sp.	RSS	RSD			
	Sessilia sp.	RSS				RD
	Bugula plumosa	RSS		OR		
	Buguloidea sp.	RSS				
	Bryozoa	RSS		OR		
Green algae	Chlorophyta	RSS		OR		
Green laver	*Ulva*			OR	S	
	Ascidiella			OR		
	Botrylloides sp.	RSS	RSD	OR		RD
Star squirt	*Botryllus schlosseri*					RD
Yellow sea squirt	*Ciona intestinalis*	RSS		OR		
	Didemnum lahillei	RSS	RSD	OR		RD
	Diplosoma listerianum	RSS	RSD	OR		RD
Goby	*Gobiidae*			OR		
Black goby	*Gobius niger*	RSS		OR	S	
Two-spotted goby	*Gobiusculus flavescens*	RSS				
Tompot blenny	*Parablennius gattorugine*	RSS				
Rock gunnel	*Pholis gunnelus*	RSS		OR		
Thornback ray	*Raja clavata*[IU]			OR		
Viviparous eelpout	*Zoarces viviparus*				S	
Hydrozoans	*Hydrozoa sp.*			OR		
Mud sagartia	*Sagartia troglodytes*			OR		
Small snakelocks anemone	*Sagartiogeton undatus*			OR		
Dahlia sea anemone	*Urticina felina*[RG]				S	
Common starfish	*Asterias rubens*	RSS		OR	S	
Common brittlestar	*Ophiothrix fragilis*			OR		
	Sargassum muticum			OR		
Slipper shell	*Crepidula fornicata*			OR		
Sap-sucking slug	*Elysia viridis*[RR]			OR		
	Ensis sp.			OR		
Pacific oyster	*Magallana gigas*			OR	S	
Blue mussel	*Mytilus edulis*		RSD		S	
Flat oyster	*Ostrea edulis*[O]	RSS	RSD	OR		
Yellow boring sponge	*Cliona celata*			OR		
	Halichondria bowerbanki			OR		
	Halichondria panicea			OR		
Mermaid's glove horny sponge	*Haliclona oculata*				S	
Sponges	Porifera			OR		
Red algae	Rhodophyta	RSS	RSD	OR		RD

Figure 6.2 European lobster hiding in a reef dome (above). Sandstone 3D reef structure within a natural mussel bed. (above, 11 October 2018; Wouter Lengkeek).

Schelpdierbankdag RAVON & Anemoon

The volunteers recorded in total 150 taxa (Gmelig-Meyling & Ploegaert, 2019, in prep), ca. 50% of these species had not been recorded in the area before. The majority of new species included red algae, green algae, fish, and nudibranches,

Total biodiversity 2016-2018

The total number of observed species on the shellfish reef for the whole study period 2016-2018 and from all surveys is 160 (Appendix I). The list includes 20 mollusc species, 10 anemones, 11 sea squirts, 25 fish, 13 crabs, and 26 algae species. The list includes two OSPAR target species and four Red List species.

Other shellfish species: population dynamics of blue mussel in the Voordelta
Mussels collected from soft sediment and from the oyster reef had less variation in size compared to mussels collected from stones and the reef dome (Figure 6.3). There were only few small mussels on soft sediment, indicating that recruitment on soft sediment was low in 2018. Mussels collected from stones and the reef domes had higher variation in shell size, and two cohorts are distinguishable in the histogram (Figure 6.3). This indicates that recruitment has occurred in summer 2018 on stones and a reef dome. The larger-sized cohort (40-60 mm) is probably from 2016, when a massive spatfall of blue mussels occurred in the Voordelta (Sas et al., 2016, 2018).

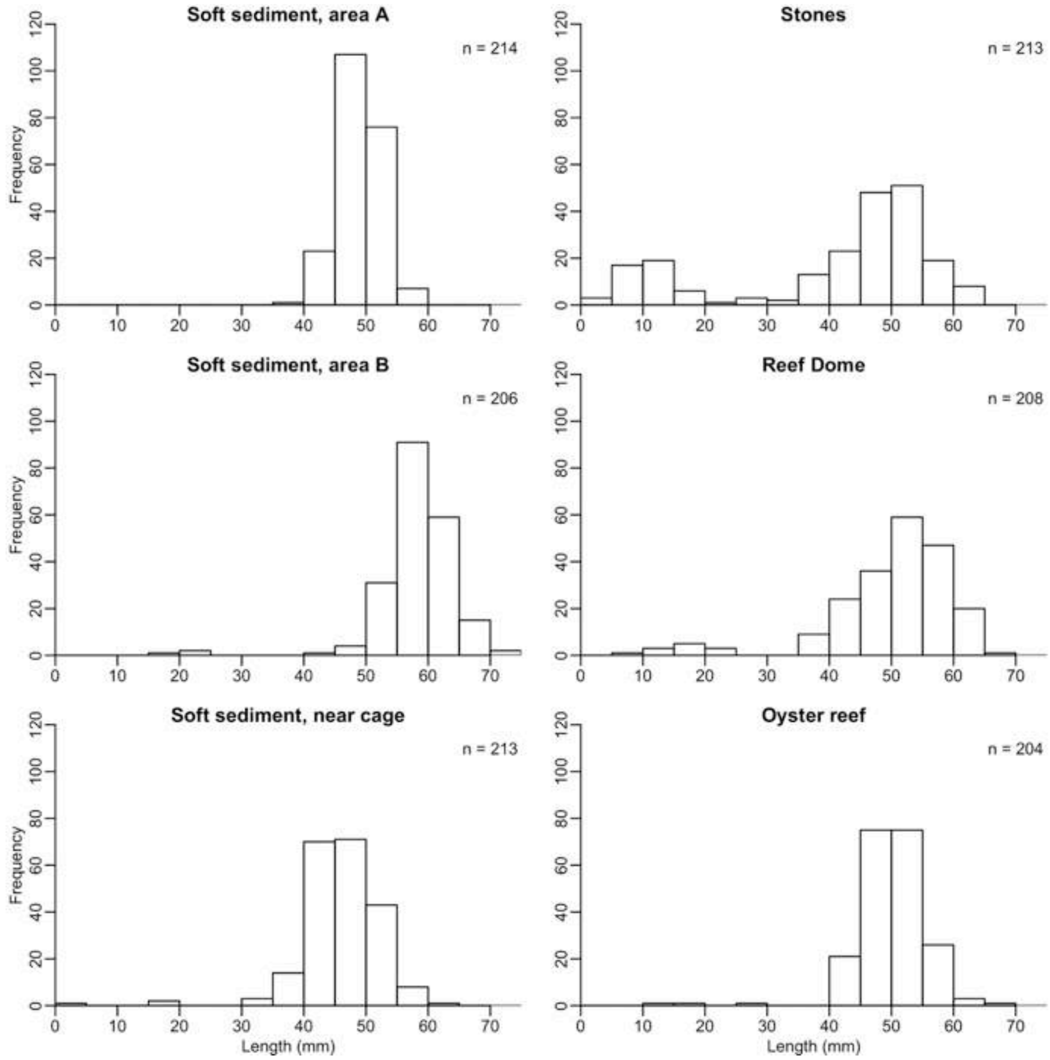


Figure 6.3 Size-frequency distributions (number per size class) of blue mussels collected in area A, B, near research racks, from stones, from reef domes and from an oyster reef.

6.4 Conclusions, discussion & recommendations

This chapter describes field-assessment results of biodiversity in the Blokkendam oyster reef and pilot area where artificial reef structures have been placed. Answering the question if oyster reefs and 3D structures enhance biodiversity: compared to sandy sediment the oyster reef and 3D reef structures contained more species and more species of conservation interest. Additional insight from the natural mussel bed shows that in patches on soft sediment mussels have less variation in size and less recruitment in 2018 compared to mussels collected from stones and the reef dome.

Our field assessment of biodiversity in 2017 showed the epibenthic community of the shellfish reef with *O. edulis* yielded 74 species and showing the number of epibenthic species was 60% higher compared to adjacent sand patches within the reef (Christianen et al. 2018). This years results confirm a higher biodiversity on the reef then adjacent sand patches. We observed many species in the reef, including some new species with conservation interest like thornback skate *Raja clavata*, The total number of species observed within the reef has increased to 167 (Appendix 1). These results underpin the importance of the oyster reefs with native oysters to increase biodiversity in the Voordelta and elsewhere in the North Sea, compared to bare sediment.

During surveys we observed a high biodiversity in the BD pilot. We identified 23 species on reef structures, compared to 12 different species on sandy patches. This indicates that introducing artificial structures mimicking reefs can potentially have a profound impact on the biodiversity. Although not essential for flat oyster recruitment artificial reef structures should be considered as a crucial component in kick-starting reef biodiversity and restoring heterogeneous habitats in coastal seas,. Additionally the structures might prevent man-made bottom disturbance, thereby protecting oyster reefs or pilots. Development of structures that are native to the area or dissolve over time will prevent costs related to obligated removal of artificial structures that is part of Dutch permits at sea.

Compared to 2017 (Sas et al. 2018), the mussel population around the BD reef slightly increased in length. In 2018 few small mussels were observed, indicating low recruitment for this year as in 2017. Recruitment on hard substrates (stones and reef dome) was higher, indicating that in order to sustain the mussel population in the reef, hard substrate is needed. The increase in mussel length of the 2016 cohort and low recruitment of the mussel population on the soft sediment indicate that this is an ageing mussel bed, and unless more hard substrate becomes available, chances are that the mussel bed will eventually disappear.

Within 3 years we observed the transformation from mixed oyster reefs to mixed oyster mussel reefs with oyster dominance to co-dominance of oysters and mussels in 2018. Negative effects, where flat oysters are smothered by pseudo-faeces were locally observed, yet no quantitative data were collected to substantiate this. Since most of the mussel samples contain merely one cohort of 2016, this is possibly just a temporal effect where the mussel population will decline in the near future when the cohort ages over time. Positive effects are expected where empty mussel shells will become available for oyster settlement. It is important to include population dynamics of other than target shellfish species in restoration plans and outcomes. Since effects of multiple species living together may vary in time and season and can be positive (accommodation, providing substrate Christianen et al. 2018) or negative (competitive displacement Helmer et al., 2019) or even changing over time (Reise et al. 2017) this factor is not easy to quantify, but should nevertheless not be overlooked.

7 Conclusions & discussion

7.1 Conclusions

Kick-starting a new oyster reef

The first step in the installation of a new oyster reef with the use of adult oysters, artificial reef structures and empty shell material can be considered successful with regard to survival, growth and reproduction of the oysters deployed. Additionally a few recruits have been observed on spat collectors. The results suggest that the main factor influencing oyster survival is duration of storage and the size of the oysters used as source material where large oysters, regardless of origin or treatment group survive better than small oysters.
In order to learn if kick-starting the oyster reef was really successful, rate of recruitment, i.e. presence of oyster spat, and long term survival of the source oysters should be studied in future research and continued monitoring is needed.

Predicting larvae peaks

The present study confirmed that temperature is an important factor for the timing of the peak occurrence of larvae peaks in the Voordelta. The temperature sum of 660 degree-days (°C*d), can be used as a first indicator of high oyster larval abundance in this area. Using this method the peak in larvae concentrations in the Voordelta could be predicted in 2 out of the 3 years that larvae are monitored. Spat settlement occurs 2-3 weeks after the first larvae peak. Monitoring the accumulated temperature provides a valuable tool for shellfish restoration practises timing of deployment of spat collecting substrates.

Flat Oyster spatfall enhancement

When testing the relationship between spatfall and varying types and times of substrate deployment we learned that all shell material can be classified as suitable substrate, with a slight preference for cockles and Pacific oyster. Timing is essential, since more then 80% of spatfall occurs within a two-week period (second half of July in 2018) and 2 to 3 weeks after the larvae peak. Since the settlement rate is low and the time window of spatfall restricted to a narrow two- week period, a precise timing of introducing clean substrate according to larvae abundance is necessary to enhance spatfall in flat oyster restoration practises.
When testing the use of cultch at different sites to collect spat for the purpose of oyster restoration or locally improve reef condition and extent, no flat oyster recruitment was observed, except for a single flat oyster spat observed by divers. Additionally no flat oyster spat was observed on 3D reef structures. Resampling larger areas of cultch in future years, when spat has grown to a size detectable by visual inspection, will tell us if added cultch in 2018 will yield new areas with flat oyster reefs.

Biodiversity enhancement

More species were found on the reef and on artificial structures compared to bare sediment, and included a very interesting observation of the near-threatened Thornback skate (*Raja clavata*). These results once more underpin the importance of oyster reefs (with native oysters) to increase biodiversity in the Voordelta and elsewhere in the North Sea. Artificial reef structures can be a crucial component in kick-starting reef biodiversity and restoring heterogeneous habitats, with an additional potential to protect oyster reefs or pilots.

Within 3 years we observed the transformation from mixed oyster reefs to mixed oyster mussel reefs with oyster dominance to co-dominance of oysters and mussels in 2018. Biotic factors can be crucial for flat oyster reef establishment and condition (Christianen et al. 2018; Helmer et al., 2019) and should not be overlooked.

7.2 Lessons learned

Future European flat oyster reef restoration projects are advised to incorporate the following lessons learned:

1) Preliminary results indicate at a recruitment and substrate limited site it is possible to kick-start an oyster reef at a new location by deploying adult oysters of mixed sizes and empty shells (cultch) on the sea floor.
2) Selecting oyster as source material for restoration includes checking disease status, IAS treatment and planning of collection and storage to secure mixed sizes, minimised storage and optimal condition.
3) When deploying structures and cultch, this is preferably performed at the same time due to efficiency.
4) Temporary storage of oysters used for active introduction should be minimised and after introduction, larger oysters might have a higher survival rate compared to small oysters.
5) To increase sustainability of shellfish restoration practises packaging material and other materials should be minimised, made from material that is and non-polluting and biodegradable and cultch should be checked for pollution with man-made materials before deployment.
6) The temperature sum, of the sea water above 7 °C in spring - early summer can roughly predict the expected timing of the peak abundance of flat oyster larvae. A next step would be the validation of the models, with the goal of using it as a cost-efficient method for future restoration practices.
7) Relatively clean substrate for spatfall enhancement should be added exactly 2-3 weeks after the larvae peak, outside this period it is not useful.
8) Oyster reef restoration includes restoring the reef community and functions. Therefore kick-starting the reef community additional to the oyster reef itself is important in the context of oyster reef restoration.

General lessons learned from this pilot and other flat oyster restoration pilots for future shellfish restoration pilots are summarised in Sas et al., 2019.

7.3 Recommendations

The flat oyster pilot at the Hinderplaat failed, probably due to high mortality after substantial fresh water outflow from the Haringvliet prolonged exposure to low salinity (Sas et al., 2016). The new pilot at Bollen van de Ooster is only in place for 6 months. In order to learn more about methods and success and failure factors for active restoration of European flat oyster it is important to collect long term data and evaluate success rate over longer time periods. For ecological processes such as reef establishment and restoration this implies at least a 10-15 year period. Since restoration efforts are now put into practise throughout North-western Europe (noraeurope.eu) the Voordelta, with a shallow and near-shore reef that can be easily studied, can serve as an important area for demonstration and knowledge development within the context of the North Sea or even North-western Europe.

During three seasons of field work, and the search for a suitable pilot location, by using local monitoring data, connecting with authorities and fishermen, we learned that low densities of flat oysters are occasionally observed throughout the Voordelta area. However, in current governmental policy flat oysters have been regarded as ‘absent’ and are therefore not included in the conservation status of the Voordelta area. Figures showing how unregulated harvesting can reduce a new flat oyster population quickly after establishment is available from recent literature (Smyth et al., 2018). It is important to use new insights of our three year project to enhance the conservation status of European flat oyster in the Voordelta and the Netherlands as whole by including it into governmental policy. In addition, a detailed survey of biogenic reefs and flat oysters in particular in the Voordelta and the wider Delta area will help to create a sound base for conservation of important natural values related to shellfish reefs.

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Appendix I **Biodiversity Blokkendam oyster reef**

Overview of observed species on the Voordelta shellfish reef in 2016-2018.

Group	**Scientific name**	**English name**	**Dutch name**
Annelida	*Arenicola marina*	Lug worm	Zeepier
Annelida	*Lanice conchilega*	Sand mason worm	Schelpkokerworm
Annelida	*Neoamphitrite figulus*		Slijmkokerworm
Annelida	*Phoronis hippocrepia*		Kleine hoefijzerworm
Annelida	*Pseudopolydora pulchra*		
Annelida	*Sabella pavonina*	Peacock worm	Waaierkokerworm
Arthropoda	*Praunus flexuosus*	Chameleon shrimp	Geknikte aasgarnaal
Arthropoda	*Cancer pagurus*	Edible crab	Noordzeekrab
Arthropoda	*Carcinus maenas*	Common shore crab	Strandkrab
Arthropoda	*Hemigrapsus sanguineus*	Pacific crab	Blaasjeskrab
Arthropoda	*Hemigrapsus takanoi*	Japanese crab	Penseelkrab
Arthropoda	*Hyas araneus*	Great spider crab	Gewone spinkrab
Arthropoda	*Inachus phalangium*	Leach's spider crab	Gladde sponspootkrab
Arthropoda	*Liocarcinus depurator*	Harbour crab	Blauwpootzwemkrab
Arthropoda	*Liocarcinus holsatus*	Flying crab	Gewone zwemkrab
Arthropoda	*Liocarcinus navigator*	Arch-fronted swimming crab	Gewimperde zwemkrab
Arthropoda	*Macropodia rostrata*	Long-legged spider crab	Gewone hooiwagenkrab
Arthropoda	*Necora puber*	Velvet swimming crab	Fluwelen zwemkrab
Arthropoda	*Pilumnus hirtellus*	Hairy crab	Ruig krabbetje
Arthropoda	*Pisidia longicornis*	Long-clawed porcelain crab	Glad porseleinkrabbetje
Arthropoda	*Sessilia*	Barnacles	Zeepokken
Arthropoda	*Mytilicola intestinalis*	Red worm disease	
Arthropoda	*Athanas nitescens*	Hooded shrimp	Kreeftgarnaal
Arthropoda	*Crangon crangon*	Common shrimp	Gewone garnaal
Arthropoda	*Hippolyte varians*		Veranderlijke steurgarnaal
Arthropoda	*Palaemon adspersus*	Baltic prawn	Roodsprietgarnaal
Arthropoda	*Palaemon elegans*	Grass prawn	Sierlijke steurgarnaal
Arthropoda	*Palaemon macrodactylus*	Oriental shrimp	Rugstreep-steurgarnaal
Arthropoda	*Palaemon serratus*	Common prawn	Gezaagde steurgarnaal
Arthropoda	*Pagurus bernhardus*	Common hermit crab	Grote heremietkreeft
Arthropoda	*Homarus gammarus*	European lobster	Europese zeekreeft
Arthropoda	*Porcellana platycheles*	Great-clawed crab	Harig porseleinkrabje
Bryozoa	*Anguinella palmata*		Slangmosdiertje
Bryozoa	*Conopeum reticulum*		Zeevitrage
Bryozoa	*Crisularia plumosa*		Spiraalmosdiertje
Bryozoa	*Schizomavella (Schizomavella) linearis*		Empingmosdiertje
Bryozoa	*Schizoporella cf unicornis*		
Bryozoa	*Tricellaria inopinata*		Onverwachts mosdiertje
Chlorophyta	*Bryopsis hypnoides*		Warrig vederwier
Chlorophyta	*Bryopsis plumosa*		Vederwier
Chlorophyta	*Derbesia marina*		
Chlorophyta	*Ulva australis*		Geperforeerde zeesla
Chordata	*Raja clavata*	Thornback ray	Stekelrog
Chordata	*Aphia minuta*	Transparent goby	Glasgrondel
Chordata	*Atherina boyeri*	Big-scale sand-smelt	Kleine koornaarvis
Chordata	*Atherina presbyter*	Sand smelt	Koornaarvis
Chordata	*Callionymus reticulatus*	Reticulated dragonet	Rasterpitvis
Chordata	*Dicentrarchus labrax*	Sea bass	Zeebaars
Chordata	*Entelurus aequoreus*	Snake pipefish	Adderzeenaald

Group	Scientific name	English name	Dutch name
Chordata	*Gadus morhua*	Cod	Kabeljauw
Chordata	*Gobius niger*	Black goby	Zwarte grondel
Chordata	*Gobius paganellus*	Rock goby	Paganel-grondel
Chordata	*Gobiusculus flavescens*	Two-spotted goby	Blonde grondel
Chordata	*Myoxocephalus scorpius*	Short-spined sea scorpion	Gewone zeedonderpad
Chordata	*Parablennius gattorugine*	Tompot blenny	Gehoornde slijmvis
Chordata	*Pholis gunnellus*	Rock gunnel	Botervis
Chordata	*Platichthys flesus*	European flounder	Bot
Chordata	*Pleuronectes platessa*	European plaice	Schol
Chordata	*Pomatoschistus microps*	Common goby	Brakwatergrondel
Chordata	*Pomatoschistus minutus*	Sand goby	Dikkopje
Chordata	*Pomatoschistus pictus*	Painted goby	Kleurige grondel
Chordata	*Solea solea*	Common sole	Tong
Chordata	*Symphodus melops*	Corkwing wrasse	Zwartooglipvis
Chordata	*Syngnathus acus*	Greater pipefish	Grote zeenaald
Chordata	*Syngnathus rostellatus*	Lesser pipefish	Kleine zeenaald
Chordata	*Taurulus bubalis*	Longspined bullhead	Groene zeedonderpad
Chordata	*Trisopterus luscus*	Pouting	Steenbolk
Chordata	*Zoarces viviparus*	Viviparous blenny	Puitaal
Chordata	*Aplidium glabrum*		Glanzende bolzakpijp
Chordata	*Ascidiella aspersa*		Ruwe zakpijp
Chordata	*Botrylloides violaceus*	Colonial sea squirt	
Chordata	*Botryllus schlosseri*	Star squirt	Gesterde geleikorst
Chordata	*Ciliata mustela*	Five-bearded rockling	Vijfdradige meun
Chordata	*Ciona intestinalis*	Yellow sea squirt	Doorschijnende zakpijp
Chordata	*Didemnum vexillum*	Carpet sea quirt	Japanse druipzakpijp
Chordata	*Diplosoma listerianum*		Grijze korstzakpijp
Chordata	*Molgula manhattensis*		Europese ronde zakpijp
Chordata	*Perophora japonica*		Japanse zakpijp
Chordata	*Styela clava*	Rough sea squirt	Knotszakpijp
Cnidaria	*Aequorea vitrina*		Lampenkapje
Cnidaria	*Halecium halecinum*	Herring-bone hydroid	Haringgraat
Cnidaria	*Hydractinia echinata*	Snail fur	Ruwe zeerasp
Cnidaria	*Kirchenpaueria sp.*		
Cnidaria	*Sarsia tubulosa*		Klepelklokje
Cnidaria	*Tubularia indivisa*	Oaten pipes hydroid	Penneschaft
Cnidaria	*Aurelia aurita*	Moon jellyfish	Oorkwal
Cnidaria	*Rhizostoma octopus*		Zeepaddestoel
Cnidaria	*Actinia equina*	Beadlet anemone	Rode paardenanemoon
Cnidaria	*Anemonia viridis*	Snackelocks anemone	Wasroos
Cnidaria	*Cerianthus lloydii*	Lesser cylinder anemone	Viltkokeranemoon
Cnidaria	*Diadumene cincta*	Orange anemone	Golfbrekeranemoon
Cnidaria	*Diadumene lineata*	Green anemone	Groene golfbrekeranemoon
Cnidaria	*Metridium senile*	Plumose anemone	Zeeanjelier
Cnidaria	*Sagartia elegans*	Elegant anemone	Sierlijke slibanemoon
Cnidaria	*Sagartia troglodytes*	Mud sagartia	Gewone slibanemoon
Cnidaria	*Sagartiogeton undatus*	Small snakelocks anemone	Weduweroos
Cnidaria	*Urticina felina*	Dahlia anemone	Zeedahlia
Ctenophora	*Beroe gracilis*		Meloenkwal
Ctenophora	*Mnemiopsis leidyi*	Sea walnut	Amerikaanse ribkwal
Ctenophora	*Pleurobrachia pileus*	Sea gooseberry	Zeedruif
Echinodermata	*Amphipholis squamata*	Dwarf brittle star	Levendbarende slangster
Echinodermata	*Asterias rubens*	Common starfish	Gewone zeester
Echinodermata	*Ophiothrix fragilis*	Common brittle star	Brokkelster
Echinodermata	*Psammechinus miliaris*	Shore sea urchin	Gewone zeeappel
Isopoda	*Idotea granulosa*		

Group	Scientific name	English name	Dutch name
Mollusca	*Buccinum undatum*	Common whelk	Wulk
Mollusca	*Crepidula fornicata*	Slipper limpet	Muiltje
Mollusca	*Elysia viridis*	Sap-sucking slug	Groene wierslak
Mollusca	*Tritia nitidus*		Grofgeribde fuikhoren
Mollusca	*Tritia reticulatus*		Gevlochten fuikhoren
Mollusca	*Aeolidia papillosa*	Common grey sea slug	Grote vlokslak
Mollusca	*Goniodoris castanea*		Bruine plooislak
Mollusca	*Hermaea bifida*		Slanke rolsprietslak
Mollusca	*Palio nothus*		Groene mosdierslak
Mollusca	*Thecacera pennigera*	Winged thecacera	Gestippelde mosdierslak
Mollusca	*Acanthocardia echinata*	Prickly cockle	Gedoornde hartschelp
Mollusca	*Acanthocardia paucicostata*		Tere hartschelp
Mollusca	*Cerastoderma edule*	Common cockle	Kokkel
Mollusca	*Magallana gigas*	Pacific oyster	Japanse oester
Mollusca	*Ensis directus*		Amerikaanse zwaardschede
Mollusca	*Mya arenaria*	Sand gaper	Strandgaper
Mollusca	*Mytilus edulis*	Blue mussel	Gewone mossel
Mollusca	*Ostrea edulis*	European flat oyster	Platte oester
Mollusca	*Venerupis corrugata*	Pullet carpet shell	Gewone tapijtschelp
Mollusca	*Lepidochitona cinerea*	Grey chiton	Asgrauwe keverslak
Nemerta	*Lineus longissimus*	Bootlace worm	Reuzensnoerworm
Ochrophyta	*Dictyota dichotoma*		Gaffelwier
Ochrophyta	*Sargassum muticum*	Wireweed	Japans bessenwier
Ochrophyta	*Undaria pinnatifida*	Wakame	Wakame
Phaeophyta	*Fucus spiralis*	Spiral wrack	Kleine zee-eik
Porifera	*Celtodoryx ciocalyptoides*		Gele wratspons
Porifera	*Cliona celata*	Yellow boring sponge	Boorspons
Porifera	*Halichondria (Halichondria) bowerbanki*	Bowerbank's horny sponge	Sliertige broodspons
Porifera	*Halichondria (Halichondria) panicea*	Breadcrumb sponge	Gewone broodspons
Porifera	*Haliclona (Haliclona) oculata*	Mermaid's glove horny sponge	Geweispons
Porifera	*Haliclona (Soestella) xena*		Paarse buisjesspons
Porifera	*Halisarca dujardinii*	Soft horny sponge	Weke balletjesspons
Porifera	*Hymeniacidon perlevis*	Crumb-of-bread sponge	Bleke piekjesspons
Porifera	*Leucosolenia variabilis*	Calcareous tube-sponge	Gewone buisjesspons
Porifera	*Mycale (Carmia) micracanthoxea*		Ruwe aderspons
Porifera	*Protosuberites denhartogi*		Oranje korstspons
Rhodophyta	*Aglaothamnion tenuissimum*		
Rhodophyta	*Antithamnionella spirographidis*		
Rhodophyta	*Caulacanthus okamurae*		Puntig korstmoswier
Rhodophyta	*Ceramium cimbricum*		Ceramium cimbricum
Rhodophyta	*Ceramium virgatum*		Rood hoorntjeswier
Rhodophyta	*Chondrus crispus*	Irish moss	Iers mos
Rhodophyta	*Cryptopleura sp.*		
Rhodophyta	*Dasysiphonia japonica*		Veelvertakt pluimwier
Rhodophyta	*Erythrotrichia carnea*		
Rhodophyta	*Gracilariopsis longissima*		
Rhodophyta	*Griffithsia devoniensis*		
Rhodophyta	*Hypoglossum hypoglossoides*		Tongwier
Rhodophyta	*Mastocarpus stellatus*		Kernwier
Rhodophyta	*Melanothamnus harveyi*		
Rhodophyta	*Nitophyllum stellato-corticum*		
Rhodophyta	*Vertebrata fucoides*		
Rhodophyta	*Polysiphonia senticulosa*		
Rhodophyta	*Pterothamnion plumula*		