Haringvliet
Naar een dynamische delta

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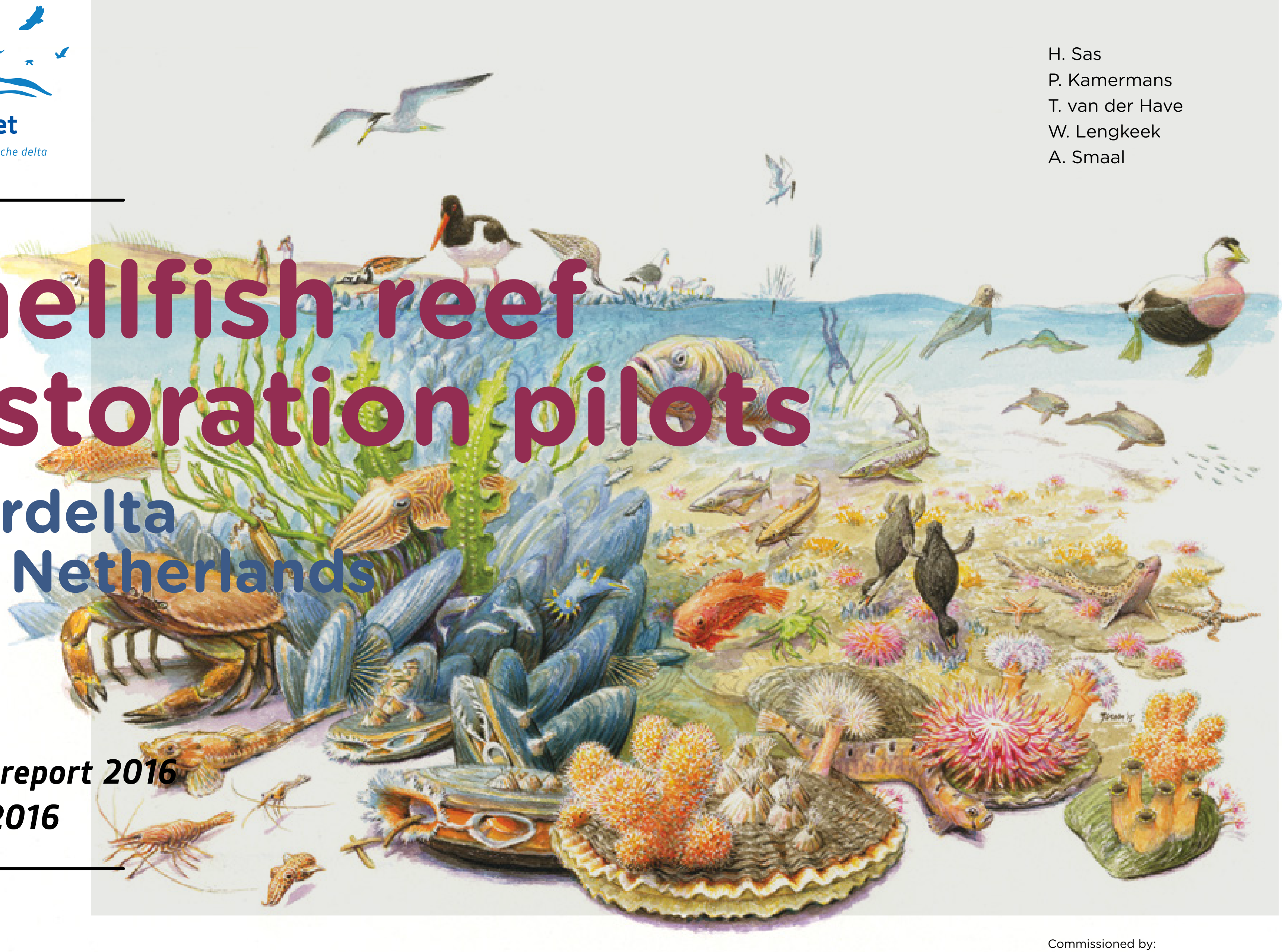

Shellfish reef restoration pilots

Voordelta
The Netherlands

Annual report 2016
20-12-2016

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Summary

The importance of shellfish reef restoration

Once, shellfish reefs - mainly flat oysters - covered about 20% of the North Sea floor, but diseases, pollution and overfishing have led to a significant decline. As part of the Haringvliet Dream Fund Project (www.haringvliet.nu), ARK Nature and World Wildlife Fund Netherlands are working on shellfish reef restoration. Shellfish, such as mussels and flat oysters, are keystone species for marine biodiversity, since they filter the water, provide shelter and nursery grounds for many marine animals, serve as attachment substrate for plants and sessile invertebrates and serve as food for a wide range of animals, including birds. They also play an important role in natural coastal protection.

Therefore, mussel and flat oyster reef restoration is attempted within the Haringvliet coastal zone (the so-called Voordelta).
This project is co-funded by the ministry for Economic Affairs, the ministry for Infrastructure and Environment, province of South Holland, Port of Rotterdam, National Postcode Lottery and LIFE. ARK Nature leads this project. The North Sea Flat Oyster Restoration Consortium (a cooperation of Wageningen Marine Research, Bureau Waardenburg and Sas Consultancy) is responsible for the execution of the current two pilots: maintenance, monitoring, analysis of monitoring results and reporting.

Primary objective of the project

Since shellfish reefs are considered extinct in the North Sea and Voordelta, too little is known about the critical success factors for development and sustainment of oyster and mussel reefs in this habitat. The current primary objective of the project is therefore to test and analyse these factors by means of pilot projects. At the beginning of 2016, two pilots were put into place, at locations with different environmental conditions (‘Blokkendam’ and ‘Hinderplaat’).

Main elements of the pilots are the following:

- The shellfish are placed into cages with different mesh size, in order to investigate the influence of predators of different size classes. These cages are placed into larger racks, for stability and protection.
- Empty mussel shells and settle plates are

Hinderplaat from the air.

- distributed around the racks, as settling substrate for spat which may originate from the pilot shellfish or elsewhere.
- Reef domes are put around the racks, for physical protection and as extra settling substrate.

Nine monitoring and maintenance visits were made to the pilots during 2016.

Discovery of shellfish reef

During one of the surveys at Blokkendam location, a mixed flat and Pacific oyster reef of about 10 hectares was discovered. Remarkably, the populations of Pacific and flat oysters are able to co-exist and maybe even to support each other. Larval monitoring as well as water flow modelling show that, most probably, the flat oyster population in the reef originates from Lake Grevelingen, which has its outlet near this location, but some local production of larvae may take place too. It appears that also mussels settle and grow well within the reef. The oyster reef appears to provide habitat to a large variety of organisms, attached as well as mobile.

Conclusions

One of the pilots was placed inside the reef, in order to study the factors which have led to its development. As could be expected, it was shown that conditions for mussels and oysters are good in the reef area. In this sense, the primary project objective, i.e. to find out whether environmental conditions ('critical success factors') for mussels and flat oysters are suitable to allow their growth, survival, reproduction and recruitment (SGRR) in the Voordelta, is already attained for one pilot location: at the Blokkendam. This is an unexpected early result of the project.

Blokkendam from the air.

At the Hinderplaat location, conditions appear to be much less favourable for flat oysters as well as mussels. This is probably due to regular occurrence of large freshwater inflow from the Haringvliet.

Recommendations

The conclusions of the first year of experiments lead to the overall recommendation to speed up the mussel and flat oyster reef restoration attempts in the Voordelta area. More in detail, our recommendations are:

1. Continue the current pilot in the oyster reef, at Blokkendam location, aimed at maximizing understanding of survival, growth and reproduction conditions of mussels and flat oysters and of enhancement methods for recruitment of these shellfish. Measure and analyse the critical success factors at this location.
2. Continue the experiment at Hinderplaat, but with much less intensity and mainly

Shellfish reef at Blokkendam location (flat oysters, Pacific oyster, mussels and epibionts).

aimed at the relation between fresh water concentration and shellfish mortality.

3. Attempt to extend the flat oyster reef at Blokkendam by stimulating spat fall in or around it. Motivation: it is an ideal location for experiments with recruitment enhancement and the abundance of life forms in and around it makes it a worthy habitat for extension. We propose the following methods:
   a. Distribution of empty mussel shells in or near the reef, around the time that larvae are expected to be in the water.
   b. As reserve, in case the amount of spat appears to be too low in or near the reef: distribution of mussel shells at another location where recruitment can be expected (e.g. in Lake Grevelingen), harvesting these after time allowed for growth and stronger settlement of the spat and distributing shells and spat at the desired location in or near the reef.
4. Continue monitoring of flat oyster larvae in the Voordelta, at least for several years to come (peak incidence, origin). Motivation: This constitutes an essential step towards better understanding of the mechanisms behind recruitment in the Voordelta. Besides, it yields key information for the timing of recruitment enhancement measures, such as under recommendation 3 and 5.
5. Attempt to stimulate oyster and mussel bed development at a new location in the Voordelta, accompanied by monitoring. Probably, the best locations will be those where shellfish beds (mostly mussels, as can be expected) can be found, or were found in recent years. In order to identify these locations, survey data should be analysed, to be verified by diving actions. Besides, of course, the locations should be free from bottom trawling fishery and strong fresh water fluxes and the legal regime should allow for maintenance and observation visits.
6. Investigate and monitor the existing oyster reef. Motivation for this is threefold:
   - Optimize protection and extension of this reef.
   - Derive guiding principles for stimulation of flat oyster reefs elsewhere in the Voordelta and even elsewhere in the North Sea at large.
   - Underpinning the importance of the oyster reef as key habitat species in the Voordelta and elsewhere in the North Sea.

1 Introduction

1.1 Background of shellfish reef restoration in the Voordelta

Within the Droomfonds ('Dream Fund') project 'Haringvliet - Towards a dynamic delta' (www.haringvliet.nu), partners work actively towards ecosystem restoration within and around the Dutch Haringvliet. This is a former estuary, through which most of the Rhine river water passes to the sea. In 1970, the Haringvliet was closed off unilaterally, by means of a system of lock doors, as part of the Dutch Delta works. Fresh water is flushed out via the doors, mostly at low tide (30 billion m3 per year). There are plans to leave the doors open more often, to allow migrating fish to pass inland (the so-called 'Kierbesluit'). This will also lead to a modest salt water gradient in the system.

Dutch nature conservation organizations have developed a plan to actively support the ecosystem restoration which is expected to result from the Kierbesluit. This is the 'Haringvliet - Towards a dynamic delta' plan. Within the Haringvliet coastal zone (the so-called Voordelta area), shellfish reef restoration is the main element of this plan, since these reefs are keystone organisms (bio-engineers) in this type of habitat.

Shellfish reefs, mainly flat oysters but also mussels, once occupied about 20% of the North Sea bottom. They have almost completely disappeared, due to overfishing, habitat destruction and diseases, as was the case elsewhere in the marine world (Beck et al., 2011; Smaal et al., 2015).

Shellfish reef restoration in the North Sea area is supported by current Dutch and EU government policy, among others through the Marine Framework Directive, for the Dutch North Sea area implemented by the Marine Strategy policy paper, part 3 (Mariene strategie, 2016).

In 2014, a feasibility study showed that the time is right to attempt to restore flat oyster reefs in the North Sea area, given the observation that the population in the Dutch Delta area is showing signs of recovery of the Bonamia disease (see Smaal et al., 2015). In particular, the population in the salt water Lake Grevelingen is thriving. This is close to the Voordelta area, which yields extra prospects for flat oyster restoration, as will be discussed later in this report. See fig. 4 in par. 2.2 for the geographical situation.

1.2 Project objectives

The overall project objective is to restore mussel and flat oyster reefs in the Voordelta area. However, since shellfish reefs were long since considered extinct in this habitat, too little is known about the conditions under which they can develop and sustain themselves there. The current primary objective of the project is therefore to test and analyse these conditions in practice. This is done by placing pilots with mussels as well as flat oysters at locations with different environmental conditions in the area.

The advantage of these pilots is that they yield a strong indication of the suitability of local environmental conditions. Shellfish reefs can develop and sustain themselves if they survive (S), grow (G), develop reproductive organs (R) and recruit within the neighbourhood of their parents (R). 'Recruitment' is defined as settling of young shellfish until they have grown to about 1 cm size. Hence, the direct objective of the pilots is to test whether SGRR occurs in the area. If that is so, the environmental conditions are apparently suitable for mussel and flat oyster reefs there.

However, in order to restore mussel and flat oyster reefs, we need to know the environmental conditions to the background of mussel- and flat oyster-SGRR. These are, therefore, the true critical success factors to be identified. Pilots do not measure these factors, but they can be analysed and measured once SGRR at a certain pilot location is proven. Therefore, attention should be given to analysing and measuring critical success factors at locations with high amounts of SGRR.

Previous to the pilot project, the Voordelta was investigated, whereby it was shown that the basic conditions, as derived from literature and experience elsewhere, are met for mussels and flat oysters. Only in the direct neighbourhood of the Haringvliet this may not be the case, due to strong fresh water outflow from this estuary. The role of such factors will be investigated by placing pilots at locations with different environmental conditions.

In summary: the direct objective of the current 2 pilots is to test if there is sufficient SGRR for mussel- and flat oyster reefs under different conditions in the Voordelta. With the current knowledge, the criterion for 'sufficient' cannot be determined yet. A certain amount of trial and error is needed in order to get to grips with this. The objective to the background of this is to identify the environmental conditions ('critical success factors'), which need to be satisfied for mussel and flat oyster reef development and sustainment.

Having said this, it should not be forgotten that reef restoration in the Voordelta is the overall objective of the current project. Therefore, the pilots are also designed in order to rapidly transform them into a true reef restoration attempt if they show sufficient SGRR. As will be discussed later, this opportunity indeed arises, due to the unexpected discovery of an existing oyster reef in the Voordelta (see Chapter 4).

A secondary project objective is to investigate the functionality and robustness of the pilot design as such. Robustness implies that the pilots should withstand the dynamic conditions in the Voordelta area. Functionality is that they fulfil the primary objective: reliable testing of SGRR in the Voordelta area.

1.3 Overall project planning

In order to investigate SGRR under various weather conditions and to allow sufficient time for experimenting with reef recovery, the pilots are designed for a duration of minimally 3 years and, in principle, for more than 2 locations. During the first year (2016) it will be decided how to continue, i.e. whether other pilot locations will be initiated, which investigations will be undertaken and whether more extensive attempts at reef restoration will be made. And if so: where and how. The discovery of the oyster reef at one of the locations will play an important part in the considerations about the future direction of the project.

The results of the Voordelta pilots are of interest for reef restoration in other parts of the North Sea area as well, including the Wadden Sea, but extension to these areas is no objective of the current project.

This annual report is the result of the first year of experiments, i.e. 2016.

1.4 Project organization

The reef restoration project in the Voordelta is part of The Haringvliet Dream Fund project (www.haringvliet.nu). This project is co-funded by the ministry for Economic Affairs, the ministry for Infrastructure and Environment, the province of South Holland, Port of Rotterdam, National Postcode Lottery and LIFE.

ARK Nature (Karel van den Wijngaard) leads the shellfish reef restoration project in the Voordelta, as one of the six partners in the Haringvliet Dream Fund project, and works closely together with WWF on this project. The North Sea Flat Oyster Restoration Consortium (a cooperation of Wageningen Marine Research – formerly IMARES -, Bureau Waardenburg and Sas Consultancy) is responsible for the execution of the current 2 pilots: maintenance, monitoring, analysis of monitoring results and reporting.

2 Methods

2.1 Pilot design and motivation

The pilots are schematically designed as presented in figure 1 below. The main elements are:

- Cages, with different mesh size, into which the shellfish are placed, in order to analyse the influence of predators of different size classes. Four different mesh sizes are employed: 4 mm, 2 cm, 9 cm and open. The racks are to keep the cages in place and to protect them against the dynamic Voordelta conditions. Per location, the pilots consist of 3 racks, with 12 cages each.
- Empty mussel shells, as settling substrate for spat, which may originate from the pilot shellfish or elsewhere.
- Reef domes, for physical protection of the pilots and as extra settling substrate. Per location, 8 reef domes are put into place.

Figure 2 shows an example of the racks and cages employed.

An example of the reef domes employed, while being placed at one of the locations, is shown in figure 3.

The cage experiments' main function is to test the SGRR variables at the chosen locations. If SGRR is shown to be successful, or if success is considered plausible on other grounds, shellfish reef extension can be considered. This can be done in two ways:

- If recruitment is expected at the desired reef restoration location: distribution of empty mussel shells there, around the time that larvae are expected to be in the water.
- If recruitment is not expected there: distribution of mussel shells at another location where recruitment can be expected (e.g. in lake Grevelingen), harvesting these after time allowed for growth and stronger settlement of the spat and distributing shells + spat at the desired location.

2.2 Pilot locations and motivation

Criteria for the choice of pilot locations in the Voordelta area were:

- No bottom trawling fishery occurs;
- Variation in physical circumstances (in order to investigate the influence of these circumstances);

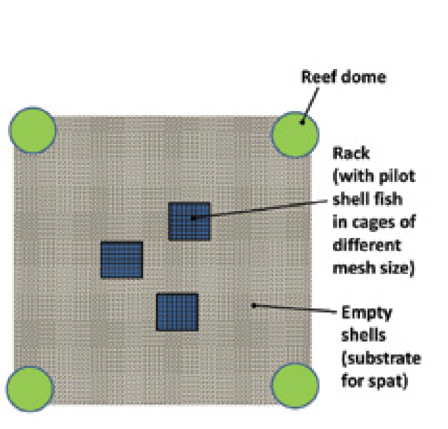


Figure 1: Schematic design of pilot locations.

Figure 2: Rack with cages employed in the Voordelta pilots. Shellfish are placed in the PVC cages (small, black), the larger steel racks are intended for physical protection and stability.

Figure 3: Placing of reef dome at Voordelta location.

- Relatively close to shore (in order to enable easy monitoring and maintenance).

The appropriate locations were identified by employing:

- A shellfish culture opportunities map for the Voordelta, developed by Wageningen Marine Research (WMR) and shellfish companies;
- Knowledge of Voordelta-experts from Bureau Waardenburg and MarinX;
- Data from standard monitoring, executed by WMR and others.

This lead to the choice of 2 pilot locations: 1b (‘Hinderplaat’) en 2b (‘Blokkendam’), indicated in figure 4 (next page). These locations indeed show a strong difference in physical conditions:

- Hinderplaat (location 1b): rather turbulent and with strong fresh water influence, geographically - and probably also hydrologically - isolated from the existing oyster reef.
- Blokkendam (location 2b): less turbulent and with low fresh water influence. Also: within the existing oyster reef.

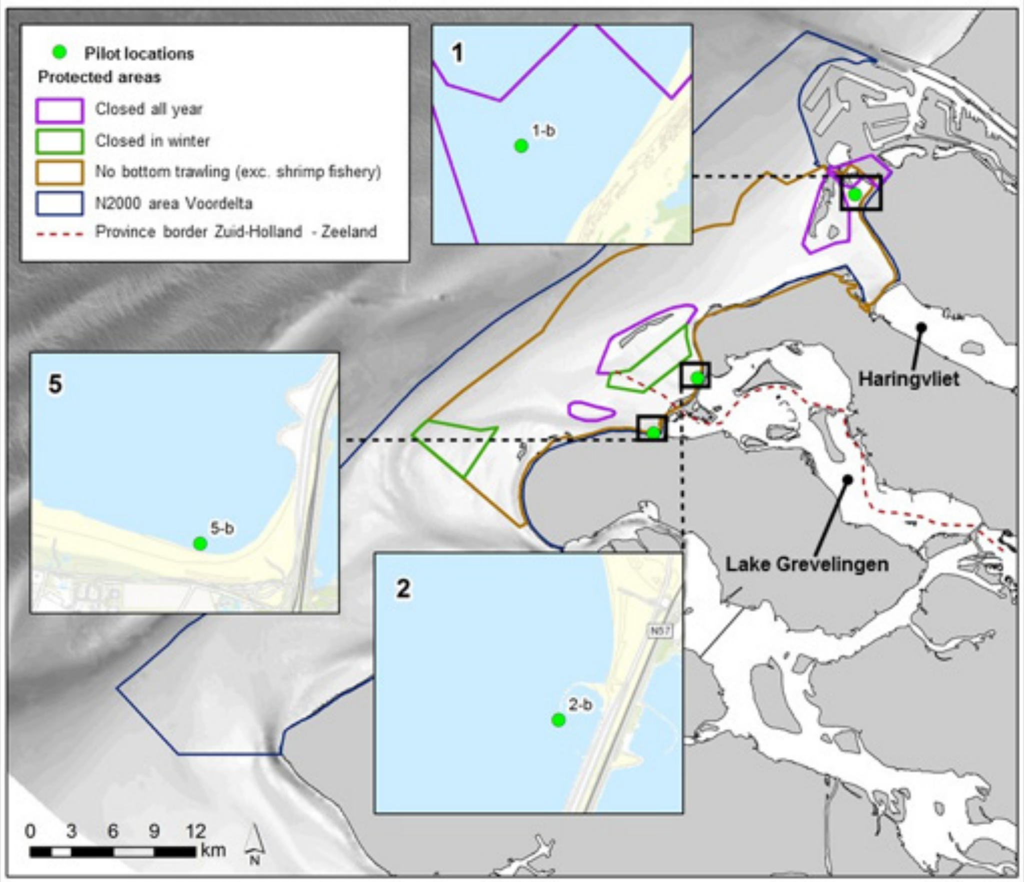
During the survey of location 2b, a mixed flat and Pacific oyster reef was discovered (also see Chapter 4). It was decided to place the pilot within this reef, in order to study the SGRR-variables and the influence of predation there. Reef domes were placed in order to protect the part of the reef with highest oyster densities at the North side. At the West and South side, this part of the reef is protected by large stones and at the East side a dam (the ’Blokkendam’) gives protection.

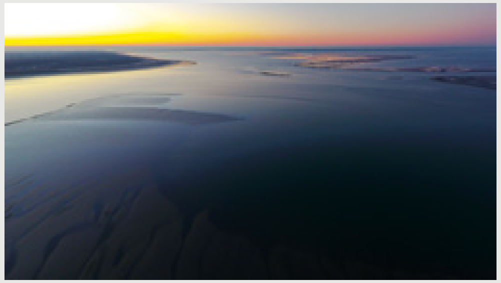
Racks and reefballs were put into place in January. Cages with shellfish were introduced in March.

2.3 Monitoring and maintenance

The pilots are regularly visited, for maintenance and monitoring purposes. In case of damage to racks or cages, they can be replaced by back-up material.

Attached organisms will grow on the cages, which may block the meshes, in particular those with smaller sizes. This will obviate nutrient flow to the shell fish, hence the cages must be cleaned regularly. During cleaning visits, also sediment





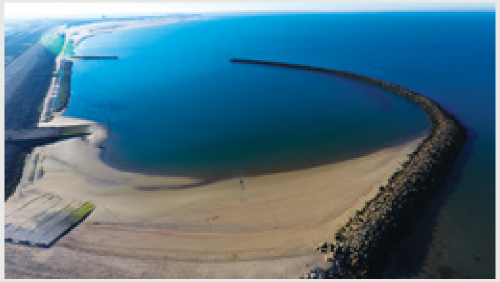


Figure 4: Pilot locations (1b and 2b were chosen) on map (upper frame) and impressions from the air (lower frames, left Hinderplaat and right Blokkendam).

material will be removed from the cages.

During 2016, 9 visits will be made to the pilots. In the period May to September, the average visit frequency is once per month, otherwise once per two months. The once per month visit frequency in the period May-September may be too low to sufficiently limit growth on the cages with small mesh size, but is chosen to limit project costs. If visit frequency appears to be too low in 2016, this aspect of the project should be redesigned (either increase visit frequency or abolish cages with small mesh size). The overall monitoring and maintenance scheme is shown in table 1 below. About each visit reports were provided to ARK Nature (references 2016-01 to 2016-14).

Per cage, pit tags were attached to a sample of mussels and oysters. This enables precise identification during analysis of survival and growth. See figure 5.

On June 6, settling plates were placed near the cages, in order to analyse possible spat fall and growth of other organisms. These were collected and analysed in the autumn.
During spring 2016, it was also decided to also perform monitoring on flat oyster larval incidence at several locations in the Voordelta. Together with modelling of water flow patterns in the Voordelta area, this should enable a first analysis of the origin of the discovered oyster reef (see chapter 4).

Activity	Execution	January	February	March	April	May	June	July	August	September	October	November	December
Placement of oysters and mussels in cages	BuWa/ WMR		X	X									
Inspection & maintenance of cages and reef domes	BuWa/ WMR				X	X	X	X	X	X	X		
Sampling for gonad development (April for mussels and June for oysters)	BuWa/ WMR				X		X						
Placement of settling plates	BuWa/ WMR						X						
Laboratory analysis of gonad development	WMR				X		X						
Sampling for survival, growth, meat content and presence of spat	BuWa/ WMR										X		
Laboratory analysis of survival, growth, meat content and presence of spat. Analysis of settling plates.	WMR										X		

Table 1: Monitoring and maintenance visits in 2016 (BuWa = Bureau Waardenburg).

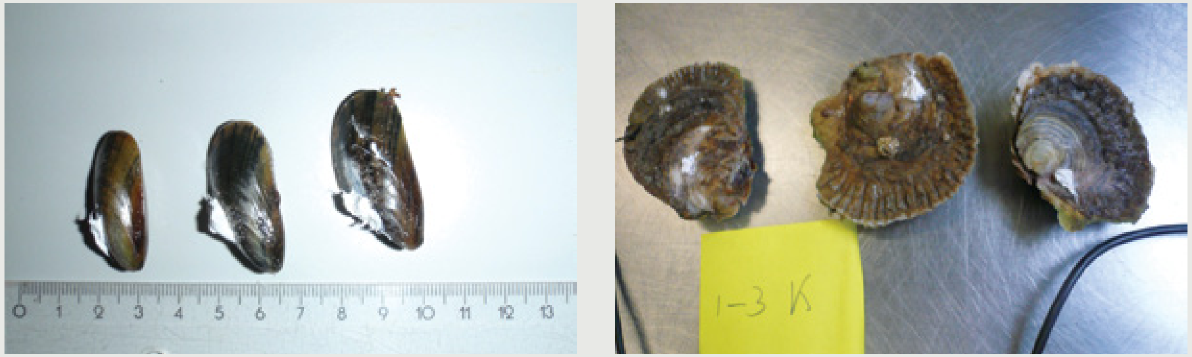


Figure 5: Left frame: small mussels with PIT tag and large mussels without PIT tag. Right frame: flat oysters with PIT tag.

3 Results

main findings and conclusions in relation to research questions

3.1 Growth

Growth was determined after the final sampling of October. See Annex 1 for methods.

Increase in shell length was determined with tagged shellfish. Survival of tagged oysters and mussels was best in 2 cm and 4 mm cages. Oysters at Blokkendam showed no significant increase in shell length (Fig. 6 ANOVA, P>0.05). Increase in weight was determined by comparing an initial sample with the final samples. No significant change in weight was observed in the flat oysters (ANOVA, P>0.05). This is possibly due to the relatively large size of the animals. Mussels at Blokkendam increased in weight and shell length (Fig. 6). This increase was not significant for shell length (ANOVA, P>0.05), but it was for weight (ANOVA, P=0.000).
At Hinderplaat, there was no survival of oysters and no mussels with tags survived (see 3.2), but comparison of an initial and a final sample showed a large and significant increase in mussel weight (Fig. 6 ANOVA, P=0.000).

Mussels at Hinderplaat showed a much higher weight than at Blokkendam (Fig. 6 and 7 ANOVA, P=0.000). This indicates that food conditions were good for those mussels that did survive. Cage mesh size did not have an effect on weight for both mussels and oysters (Fig. 7 ANOVA, P>0.05).

In October, condition of the mussels was also higher at Hinderplaat compared to Blokkendam (Fig. 7). However this was not significant (ANOVA, P>0.05). There was no significant effect of month, location or cage mesh size on mussel condition (ANOVA, P>0.05). Oyster condition was higher in February compared to June (ANOVA, P=0.009) and October (ANOVA, P=0.000). Condition in June and October did not differ significantly (ANOVA, P>0.05). Cage mesh size did not have an effect on oyster condition (Fig. 7 ANOVA, P>0.05).

3.2 Survival

Survival has been determined in cages with oysters as well as mussels. Mortality in cages with finer mesh sizes will generally be due to adverse conditions, since predators will not be able to enter the cages. Mortality and missing

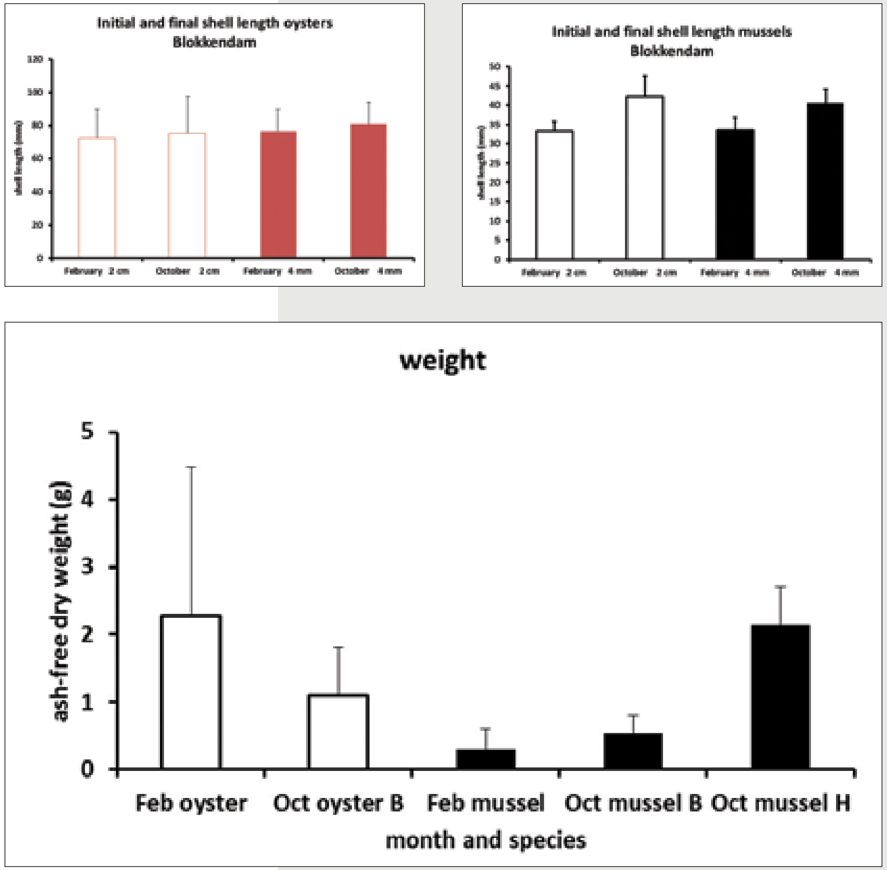


Figure 6: Initial (February) and final (October) shell length in 2 cm and 4 mm cages at Blokkendam (top) and weight (bottom) of oysters and mussels at Blokkendam (B) and Hinderplaat (H).

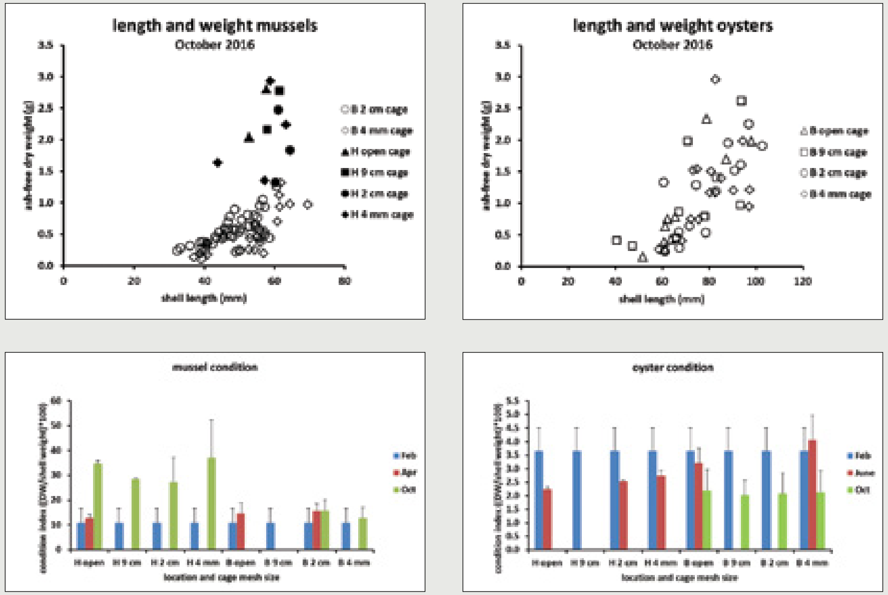


Figure 7: Relation between shell length and weight in October for mussels (top left) and oysters (top right) and condition index of mussels (bottom left) and oysters (bottom right) In February, April/June and October at Hinderplaat (H) and Blokkendam (B) in cages with different mesh size (open, 9 cm, 2 cm, 4 mm).

Figure 8: Brown crab in an open cage at pilot location Blokkendam.

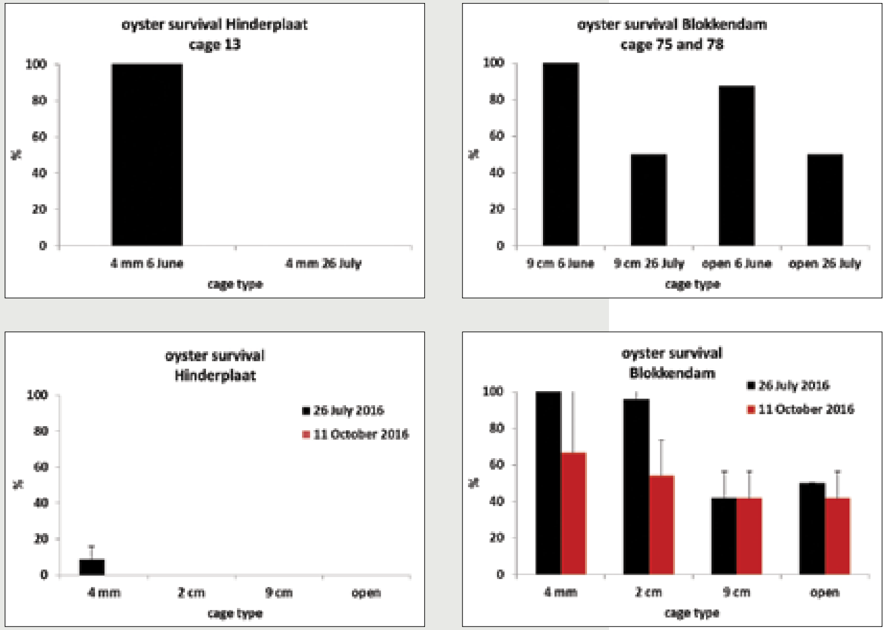


Figure 9: Flat oyster survival patterns at Hinderplaat and Blokkendam.

individuals from the cages with large mesh size, or open cages, may be a result of both predation and adverse conditions. Brown crabs were observed in the open cages (see Figure 8).

For all cages, survival was determined in October. For some cages also in June and July, which allows an impression of the survival pattern during the year, in relation with circumstances at the pilots.
Figure 9 shows flat oyster survival during 2016, at Hinderplaat as well as Blokkendam location. The upper part shows the results for a selection of cages in June and July, the lower part for all types of cages in July and October.

At Hinderplaat all oysters were dead in October while survival was 40-60% at Blokkendam (see Fig. 9 ANOVA, P=0.035). As can be derived from the fate of the oysters in the sample cage with 4 mm mesh size, death at Hinderplaat probably occurred between the beginning of June and the end of July. At Blokkendam, in cages with large mesh size or no mesh at all, there was mortality in the this period too, but much less. Survival at Blokkendam was slightly higher in cages with small mesh size (excluding predators) than with large mesh size, but this effect was not significant (ANOVA P>0.05).

Survival data for mussels are reported in Figure 10. These are all based on the October evaluation.

There was a significant effect of location (ANOVA, P=0.000) on and cage mesh size

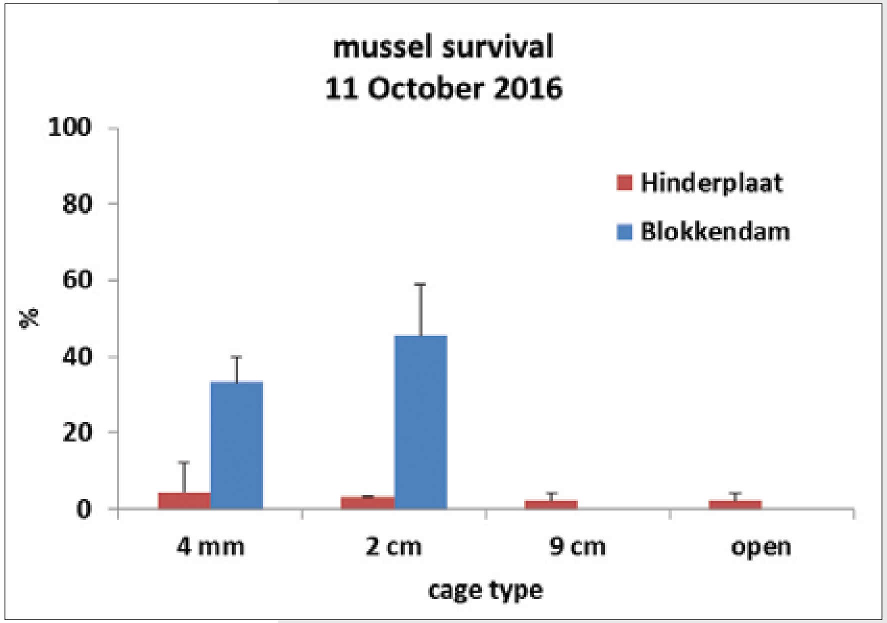
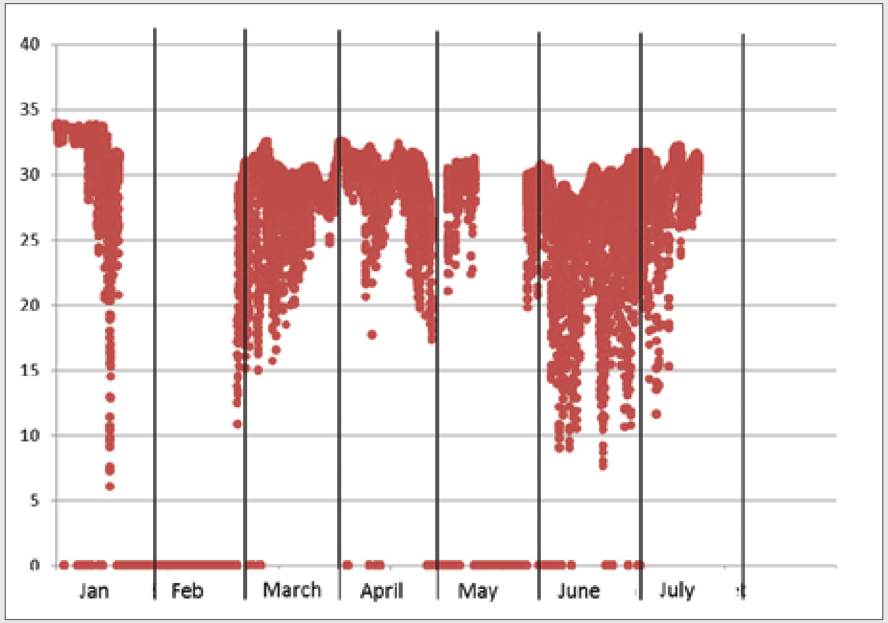


Figure 10: Mussel survival patterns at Hinderplaat and Blokkendam.



Salinity in surface water at Haringvliet mouth in 2016. Zero means no measurement. Source: Rijkswaterstaat.

(ANOVA, P=0.000) mussel survival (Fig. 10). At Blokkendam, there was no mussel survival in the open cages and in those with large meshes, which is most probably caused by predation (as illustrated by Figure 8). At Hinderplaat, some survival in the open and 9 cm cages suggests that predation pressure is lower at that location. The mortality in cages with small mesh size at Blokkendam may be caused by the extreme growth of attached organisms (see par. 3.5), blocking food supply to the inside.

These figures show that, in 2016, circumstances at Hinderplaat were adverse for both types of shellfish. Large-scale mortality probably occurred between the beginning of June and the end of July, and across all mesh sizes. This leads to the hypothesis that the main cause of death was fresh water outflow form the Haringvliet: heavy rains in the Rhine drainage basin caused extremely high outflow during June (Fig. 11).

3.3 Reproduction & recruitment

3.3.1 Reproduction

Mussels were sampled for gonad development on 12-04-2016 and oysters on 06-06-2016 (see Annex for method and results). Gonads were scored as either sperm cells, eggs or unclear or spawned. Results show that gonad development of both species takes place at both pilot locations. Spawning of mussels was early this year, as all mussels had spawned.

Figure 12: Flat oyster from Blokkendam location, opened for gonad inspection (scale is cm).

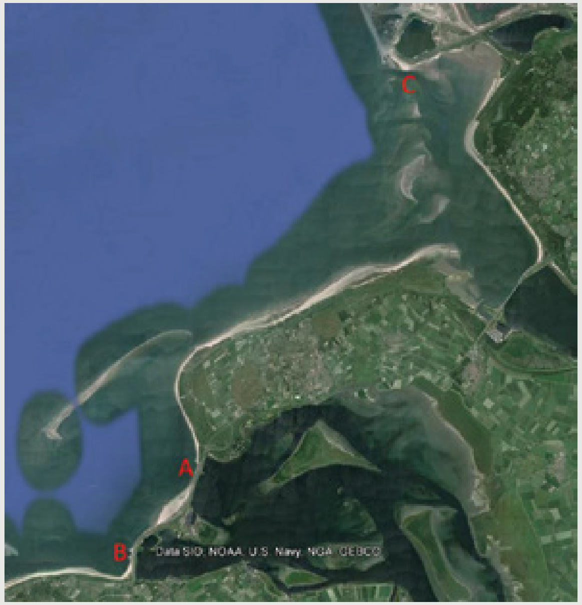


Figure 13: Relevant locations for flat oyster larvae distribution model and sampling.

Oysters showed ripe gonads. Figure 12 shows an oyster from Blokkendam location ready for gonad investigation; gonads are located in the flesh at the lower part of the animal. The oyster is in good physical condition, as can be derived from its flesh content.

Gonad development was more progressed at Blokkendam than at Hinderplaat, with 6 out of 9 individuals showing clear development compared to 2 out of 11 at Hinderplaat. This again indicates better shellfish conditions at Blokkendam.

3.3.2 Distribution of larvae in the Voordelta area

The distribution of flat oyster larvae in the Voordelta area was investigated in two ways:

- Employment of a water dilution model, by the Deltares institute (see Kleisen, 2016). Such a model can be employed to investigate the distribution potential of oyster larvae if they behave like inert particles. This is not (completely) the case hence the model is a first approximation of possible distribution patterns. It is assumed that larvae disperse into the Voordelta area from the Lake Grevelingen outlet, in order to investigate the hypothesis that the presence of oysters in the Voordelta is caused by dispersing larvae from the Grevelingen population.
- Oyster larvae sampling, in the period June-August, near de outlet of Lake Grevelingen to the Voordelta and at the two pilot locations.

Figure 13 shows the relevant locations: Lake Grevelingen outlet is at point B, Blokkendam location is point A and Hinderplaat location is point C. Samples at location B (near the outlet) show if larvae are flushed from Lake Grevelingen into the Voordelta. Comparison of the size of larvae at Location A (near the oyster bed) and Location B (near the outlet) can indicate if larvae were produced locally or supplied from Lake Grevelingen.

For larval transport, water dilution patterns as well as water transport time are relevant. The average model results for these two parameters are shown in Figure 14. Oyster larvae have a pelagic phase of about 2 weeks in which they grow up to metamorphosis after which they settle on the bottom. The general picture arising from the model, therefore, is that a certain amount of oyster larvae can indeed reach the Blokkendam in time to settle and that this is doubtful for the Hinderplaat location.

Flat oyster larvae sampling took place weekly in 2016, in the period that larvae are expected

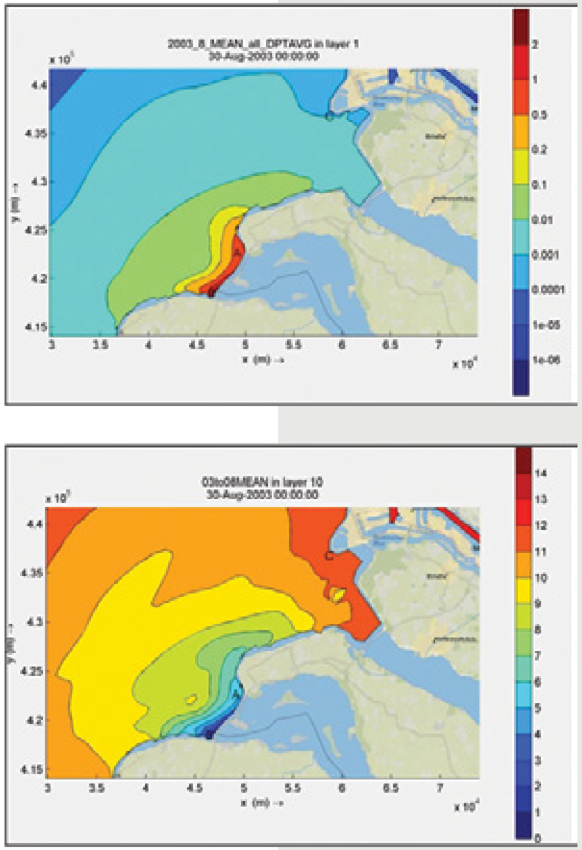


Figure 14: Model results for water dilution pattern (upper frame) and transport time (lower frame). From Kleissen 2016.

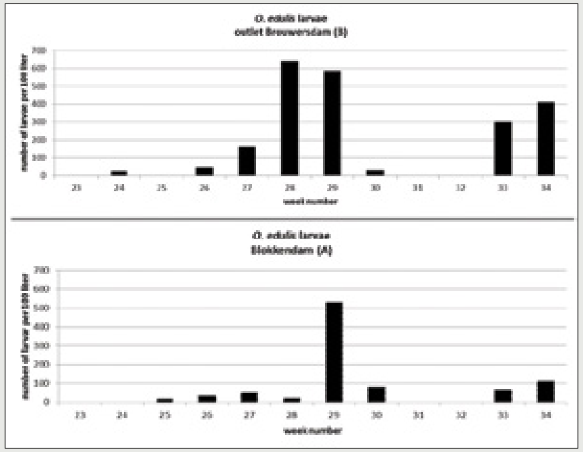


Figure 15: Concentration (in number per 100 litre) of flat oyster larvae at location B and A in 2016.

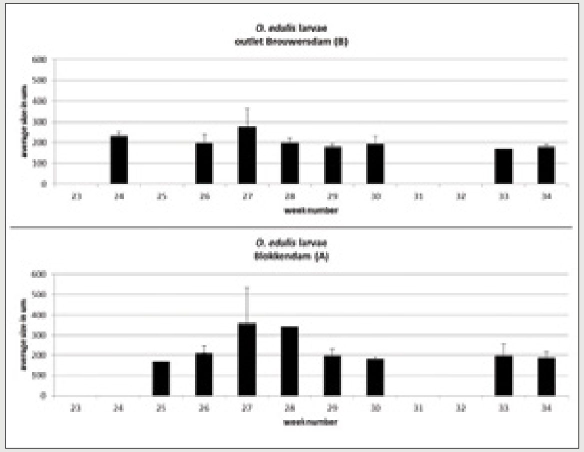


Figure 16: Average size (in µm with st. dev.) of flat oyster larvae at location B and A in 2016.

to be present, i.e. from week 23 to week 34. Larvae were found at Blokkendam (location A) and the outlet of Lake Grevelingen (location B), but not at Hinderplaat (location C). This confirms results of the model study. Apparently, Hinderplaat is too far from the outlet of Lake Grevelingen for flat oyster larvae to arrive there, in sufficient time and amount.
Highest larval concentrations were found in mid-July (week 28 and 29), see Figure 15. Concentrations were generally higher at the outlet than at Blokkendam. Sizes of larvae varied over time and were similar or even slightly larger at Blokkendam than at the outlet (Figure 16). The latter suggests that local production of larvae at Blokkendam location may take place.

It can be concluded that the oyster bed at Blokkendam may depend on local larval production. In addition, Hinderplaat is too far from the outlet to benefit from larval supply from the Grevelingen. This means that, for the Blokkendam location, supply of settling substrate such as empty shells can probably be used to increase the size of the bed. Empty mussel and oyster shells and settling plates were introduced in the water to conform this; see below for results.

3.3.3 Quantitative recruitment data

Recruitment was monitored in four different ways.
Firstly, empty mussel shells were distributed on the bottom around the pilot on 18-01-2016 and 26-07-2016. They were retrieved on 11-10-2016. See Figure 17.

Figure 17 shows that very few flat oyster spat settled, only on shells that were distributed in January. As can be observed in Figure 15, the summer peak in flat oyster larval concentration occurred just before the shells were distributed. Hence, this timing issue probably caused the poor settlement.

Pacific oyster spat was found at Blokkendam location on shells that were distributed in January, but also in July at both Blokkendam

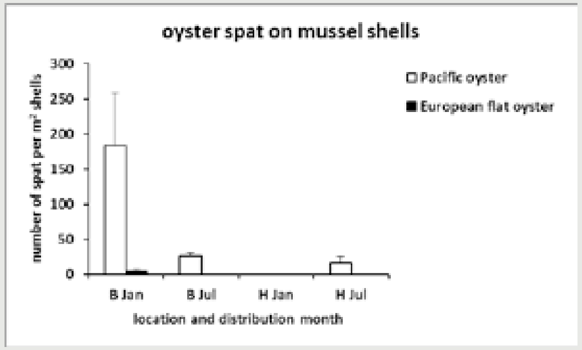


Figure 17: Oyster spat on mussel shells distributed on bottom in January and July, Blokkendam (B) and Hinderplaat (H) location.

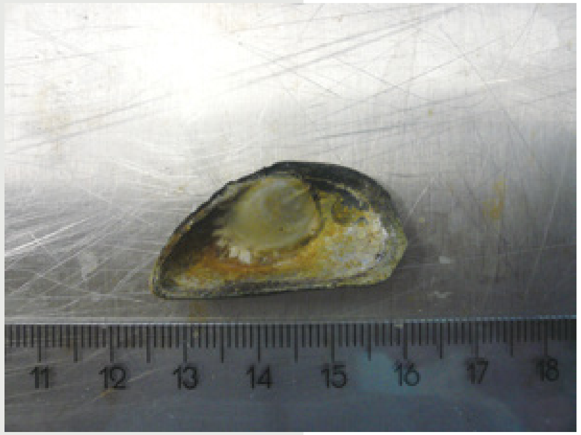


Figure 18: Pacific oyster spat (left) and Flat oyster spat (right) on empty mussel shells (top) and oyster shells (bottom) that were distributed at Blokkendam on 18-01-2016 (scale is cm).

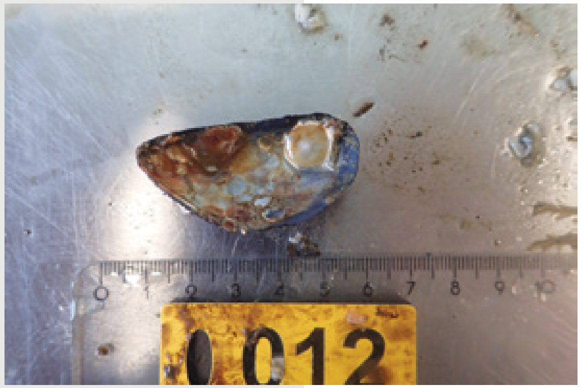


Figure 19: Small nets with oyster shells (left) and mussel shells (centre) introduced on 26-07-2016 at Hinderplaat and Blokkendam and flat oyster spat on mussel shell from net retrieved on 11-10-2016.

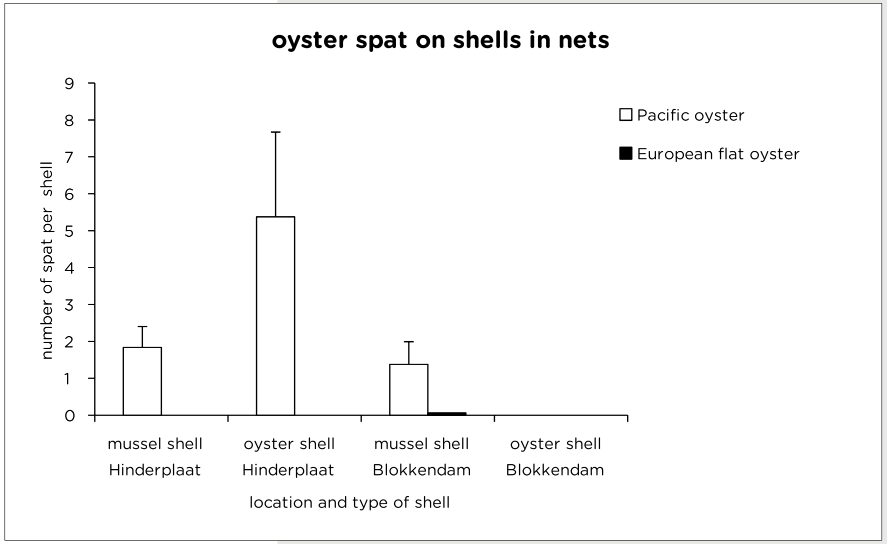


Figure 20: Oyster spat in small nets with oyster shells and mussel shells introduced on 26-07-2016 at Hinderplaat and Blokkendam. (results of oyster shell sampling at Blokkendam not yet obtained).

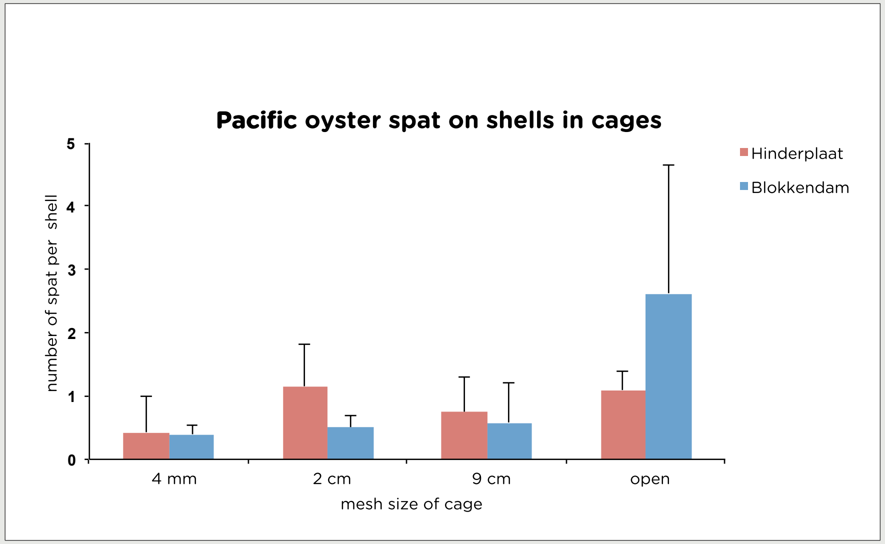


Figure 21: Pacific oyster spat at shells in cages, on both pilot locations.

and Hinderplaat location (Fig. 17). There was a significant effect of location on number of Pacific oyster spat (ANOVA, P=0.037), but not of month (ANOVA, P>0.05). Pacific oysters produce many more eggs (around 50 million per female) than flat oysters (1-3 million per female). This may explain difference in spat abundance. Remarkably, Pacific oyster spat was found at Hinderplaat on mussel shells distributed in July. Apparently, these larvae are able to reach this location. They probably originate from the Pacific oyster population at Blokkendam location. Figure 18 illustrates oyster spat fall on mussel shells.

Secondly, small nets with oyster and mussel shells were attached to the 6 racks on 26-07-2016, and were retrieved on 11-10-2016. See Figure 19 for illustration.

Results are shown in Figure 20.

Again, a large majority of the spat consisted of Pacific oyster. Flat oyster spat was rare, only on mussel shell at Blokkendam, see Fig 19). This may be due to the late distribution of the shell nets. Just as with the shells distributed on the bottom, shells in nets at Hinderplaat location shows considerable Pacific oyster spat fall (Fig. 20). There was no significant effect of location or type of shell on number of Pacific oyster spat (ANOVA, P>0.05).

Thirdly, empty mussel and oyster shells were placed in the cages at both locations on 26-02-2016, and retrieved on 11-10-2016. See Figure 21 and 22 for results: only Pacific oysters were found. There was a significant effect of location on number of spat (ANOVA, P=0.02), but no effect of cage mesh size (ANOVA, P>0.05).

Again, Hinderplaat location shows considerable Pacific oyster spat fall. There is no apparent effect of mesh size on number of spat (Fig. 21), so predation on spat seems not to be a major issue for Pacific oysters.

Fourthly, settling plates were placed at the pilots (3 plates per location) on 06-06-2016 and retrieved on 11-10-2016 (Fig. 23). See Table 2 for results.

Location	Table	Number plate holder	Total number Pacific oyster spat	Other organisms
Hinderplaat	1.1	90	2	barnacles
	1.2	91	0	barnacles
	1.3	92	0	barnacles
Blokkendam	2.1	83	3	Crepidula, sponges, sea squirts, mussel seed, Ulva
	2.2	85	0	Crepidula, sponges, sea squirts, mussel seed, Ulva, barnacles, crabs, star fish
	2.3	84	3	Crepidula, sponges, sea squirts, mussel seed, Ulva

Table 2: Species found on PVC settling plates at both pilot locations.

Deployment date	Substrate used	European flat oyster spat	Pacific oyster spat
18 January	Mussel shells on bottom	Yes	Yes
26 February	Mussel shells in cages	No	Yes
26 February	Oyster shells in cages	No	Yes
6 June	PVC plates	No	Yes
26 July	Mussel shells on bottom	No	Yes
26 July	Mussel shells in nets	Yes	Yes
26 July	Oyster shells in nets	Not known yet	Not known yet

Table 3: Summary of recruitment monitoring in 2016.

As Table 2 shows, hardly any oyster spat was found on the settling plates and these were only Pacific oysters. Since the settling plates were distributed earlier than the shells, timing is no explanation for the rare settlement. Possibly, the oysters - and particularly the flat oysters - do not favour the plates as settling substrate under the circumstances that are present in the Voordelta. The plates have been used successfully in Oosterschelde and Grevelingen.

Some European flat oyster spat was collected with empty shells deployed in January and in July (Table 3).

In conclusion, spat collection was most successful for the Pacific oyster, but some flat oyster spat was collected too.

3.3.4 Visual recruitment observations

3.3.4.1 Introduction

Observations are made on the cages as well as the reef domes placed to protect the cages (Hinderplaat) and oyster reef (Blokkendam) against sea floor disturbance. Both reef domes and cages provided extra artificial hard substrate in addition to the nearby rocks and breakwater, which were already present, and to the natural hard substrate of the oyster reef. During the inspections of the reef domes and cages anecdotal observations were made of the biodiversity associated with the artificial hard substrate of the cages and reef domes. Although these general biodiversity observations were not part of the project objectives, they provided a first impression of the biodiversity potential of natural hard substrate, which is now relatively rare in our coastal waters due to the disappearance of shellfish reefs in general and oyster reefs in particular.

On 16-08-2016 cameras were placed for several hours near two reef domes at the Blokkendam

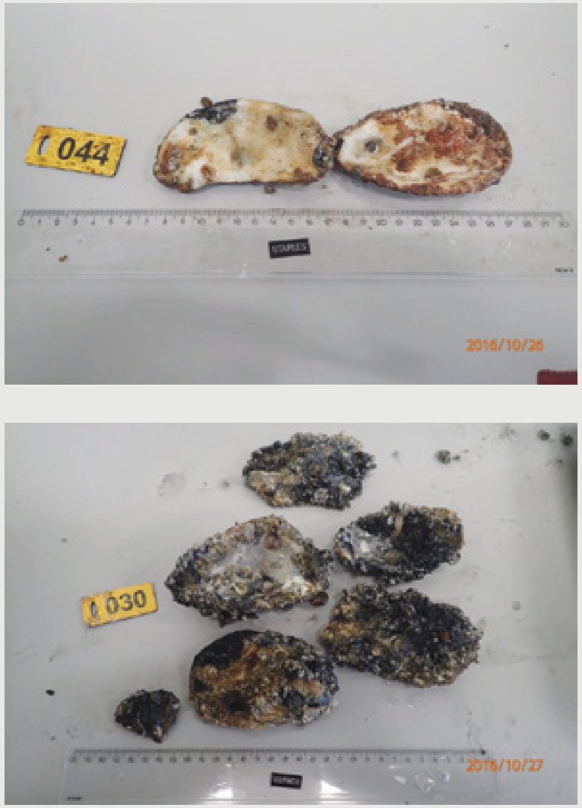


Figure 22: Pacific oyster spat on empty oyster shells that were placed in the cages at Blokkendam (upper) and Hinderplaat (lower) on 26-02-2016 (scale is cm).

Figure 23: Plate holders with PVC plates that were placed on the racks on 06-06-2016 at Blokkendam (upper) and Hinderplaat (lower).

pilot site to observe fish and large, mobile crustaceans. These films have not been analysed yet in detail. Several species of fish, crabs and prawn were observed in and around the reef domes.

On 22-09-2016 mussel seed on the reef domes, cages, oyster bed and the surrounding soft sediments were filmed and photographed.

These observations document the large mussel recruitment following the good spat fall of mussels in the Voordelta during spring 2016. Mussel seed that was attached to the cages was sampled on 11-10-2016 and had an average size of 20.89±4.89 mm at Blokkendam and 17.74±3.20 mm at Hinderplaat.

3.3.4.2 Mussel and oyster spat fall at Blokkendam location

During the field visit of 22-09-2016 a high density of mussel seed was observed on the reef domes, on the oysters and on the surrounding soft sediment of the Blokkendam location (Figure 24). This mussel spatfall was also noted in other regions of the Voordelta.

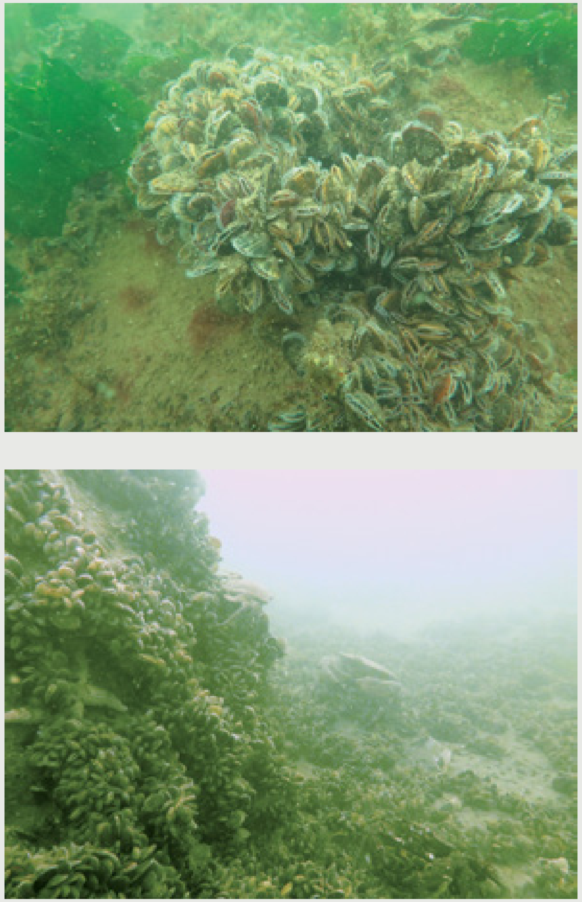


Figure 24: Mussel seed attached to reef dome, with macro algae (upper panel) and to soft sediment with Brown crabs, velvet crab and sea star (lower panel).

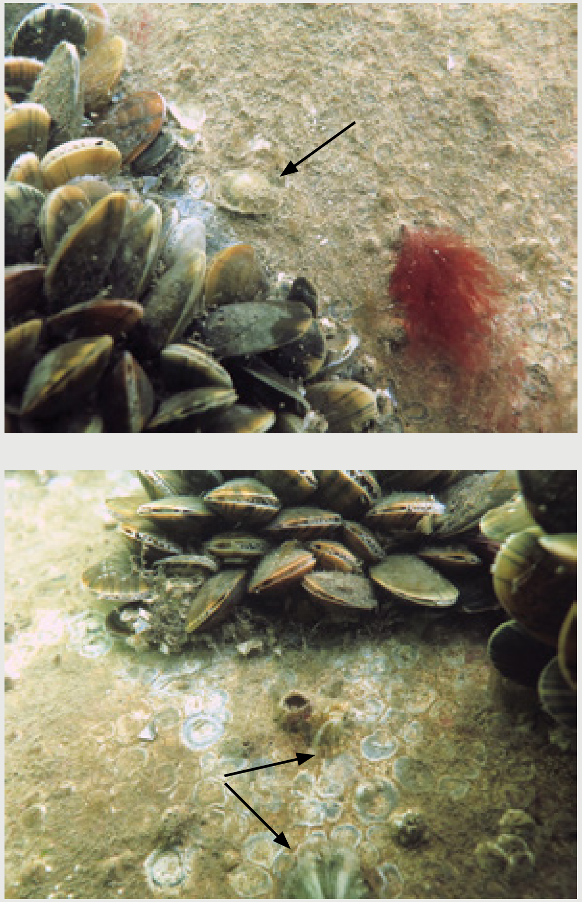


Figure 25: Mussel seed and possible flat oyster brood (upper panel, arrow) and pacific oyster brood (lower panel, arrows) attached to reef dome.

The reef domes close to the breakwater in more shallow water were covered with macroalgae, sea stars and crabs and very few mussels. The reef domes in deeper water further away from the breakwater were completely overgrown with mussels. Macroalgae were absent and only a few sea stars were present there. Several brown crabs, obviously enjoying this mussel rich environment, were observed on each reef dome and on the surrounding mussel beds (Figure 24).

Oyster brood was also observed on open spaces, i.e. without barnacles and mussels, on the reef domes (Figure 25). Apparently, the barnacles and mussels seem to be removed from the surface by an unknown cause (though crabs are among the usual suspects), resulting in a clear surface. Several Pacific oyster brood were found, which are clearly marked with violet-brown stripes One possible flat oyster brood was found, with a brownish, rounded shell without stripes.

3.3.4.3 Mussel and oyster spat fall at Hinderplaat location

The pilot site Hinderplaat is characterised by a high turbidity, which made it nearly impossible to obtain good observations of the biodiversity on the soft sediments, the reef domes and cages. The salinity is also highly variable due to the varying amounts of fresh water released through the Haringvliet sluices. Macroalgae are only found close to the surface and were hardly found on the reef domes. Bryozoa were dominant on the reef domes, in particular species, which are resistant to varying salinity and high turbidity, see Figure 26. As a result, the reef domes had a mainly greyish brown colour. Several fish species were observed (common goby, Fivebeard rockling) and several large, mobile crustaceans (common prawns, Palaemon serratus; European green crab, Carcinas maenas).

No flat oyster spat fall was observed at this location, but Pacific oyster spat was found on introduced substrate (see par. 3.3.3).

Figure 26: Feather bryozoan *Bugula plumosa* on a reef dome of the Hinderplaat (11 October 2016).

Figure 27: Mussels (left) and epiflora with sand smelt (right), attached to cages in rack at the Blokkendam location (the PVC plate holder is visible on top of the rack).

3.4 Bonamia prevalence

An important potential mortality cause of flat oysters is the parasite Bonamia. In December 2015 thirty oysters were sampled from the natural reef found near the Blokkendam (see Chapter 4). The Dutch Central Veterinary Institute (CVI) kindly agreed to analyse the oysters for Bonamia infection as part of their regular monitoring programme. No Bonamia was found. However, December is not the best period for sampling, since Bonamia development occurs mostly in spring. Hence, no conclusion can be drawn yet. Additional samplings are required to confirm the Bonamia free status of the oysters.

3.5 Robustness and suitability of pilot locations

Several storms occurred during winter and spring of 2016. This gave no noticeable damage. Hence, so, far the pilots have shown the required robustness.

In particular at the Blokkendam location, the cages show strongly attached growth, by several different types of epiflora and -fauna. See figure 27, which shows a photograph taken at the end of June 2016. Note the extensive spat fall and growth of mussels on the cage, as was the case elsewhere at this location (see par. 3.3.4.2).

The empty mussel shells, which were deposited close to the cage, remained in place during the whole study period despite several stormy periods. This suggests that this method to stimulate shellfish recruitment is feasible at both pilot sites.

The weather conditions in the first half of 2016 were often unsuitable for diving (high waves, poor visibility), so that some planned maintenance visits could not be executed or were delayed. The strongly attached mussels at Blokkendam, therefore, tended to block the smaller meshes of the shellfish cages, probably causing the lower shellfish survival recorded in these cages (see par. 3.2, Figure 10).

4 A mixed flat and Pacific oyster reef in the Voordelta

4.1 Discovery and location of oyster reef

During the first survey of the pilot locations in the Voordelta, on 30 October 2015, an oyster reef was found to the west side of the Blokkendam. Both flat and Pacific oysters were found in varying densities over an area of approximately 13 hectares. Mussels were found in the reef too.

During maintenance and monitoring visits to the pilots, there were incidental opportunities to collect information about the structure and associated organisms of this oyster reef, although this was not included in the monitoring programme. Most observations are, therefore, anecdotal and partly supported by photos and film (see photos in Figures 28 to 34, which were mainly taken on 28-06-2016 and photos in Figures 35 to 37, which were taken on 22-09-2016).

4.2 Impression of oyster reef: structure and associated organisms

Close to the Blokkendam, a subtidal zone of stones provides an artificial hard substrate and probably impedes bottom-trawling fishery. Many empty mussel and oyster shells are strewn among the stones too, providing a natural substrate for oyster and mussel recruitment (Figures 28 to 30). The distribution of oysters was patchy. Oyster densities varied from less than one per square meter up to five individuals per m2. Pacific oysters were mainly found on the stones, on other Pacific oysters or, more rarely, on flat oysters. Flat oysters were found in between the stones on soft sediment (sand or silt), where the bottom was covered with empty mussel and oyster shells. This distribution extended to several hundred meters from the Blokkendam. No oysters were found on the east side of the Blokkendam.

The stones and oysters were covered with numerous macroalgae and macrozoobenthos, including sponges (Porifera), sea squirts (Ascidiacea), bryozoids (Bryozoa) and other groups. Several species of large, mobile crustaceans were found in the oyster reef, including Brown crab, Velvet crab, Common shore crab and Long-legged spider crab

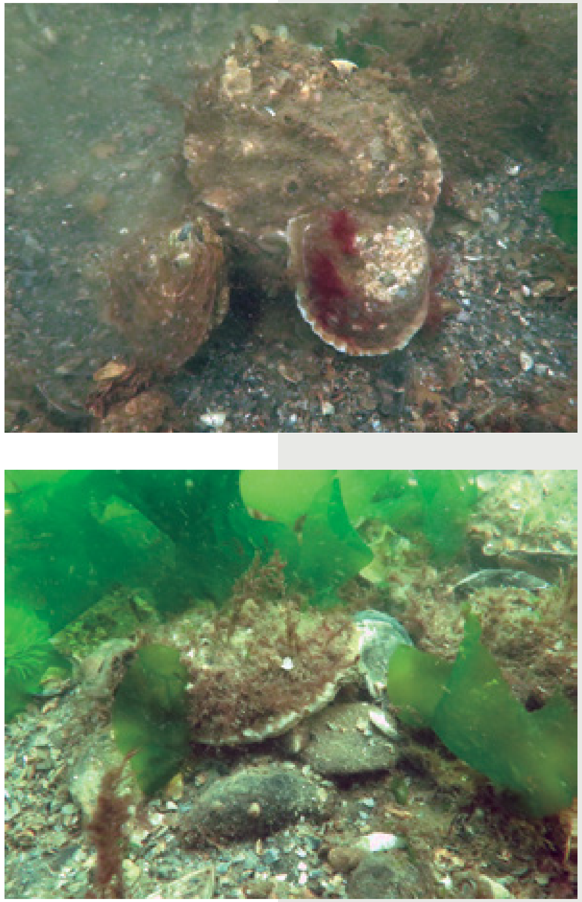


Figure 28: Upper: two Young flat oysters (right valve on top) attached to an adult flat oyster. Lower: adult flat oyster overgrown with macroalgae.

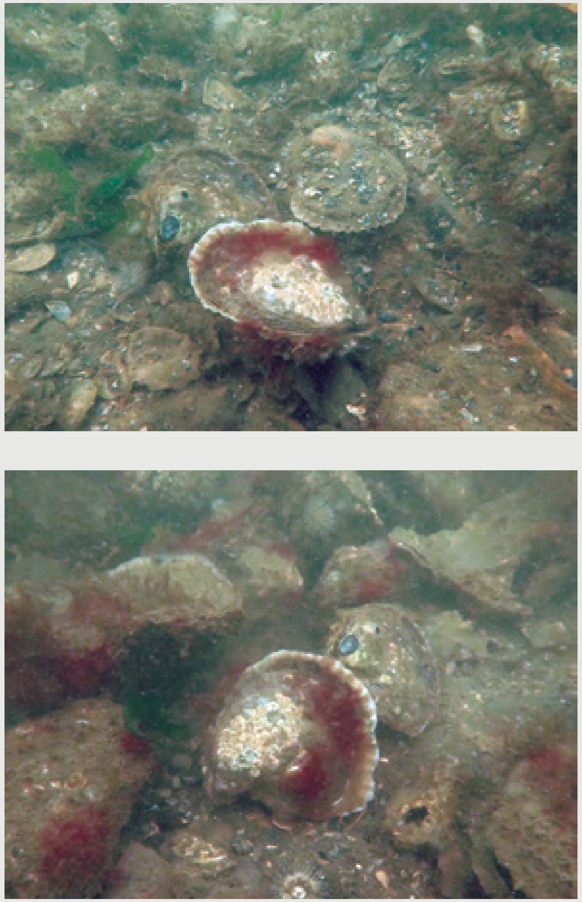


Figure 29: Upper and lower: characteristic view of a high density patch of mainly loose and scattered flat oysters. In most individuals the right (flat) valve is on top, which is overgrown with barnacles and macroalgae. Orange anemones are found between the oysters.

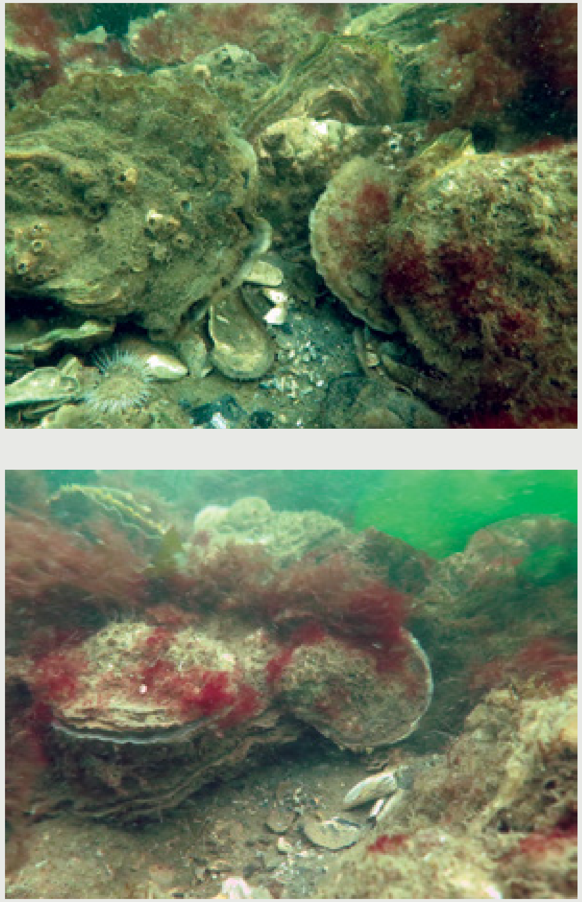


Figure 30: Upper: a large Pacific oyster (overgrown with barnacles) on the left and a smaller one above, a smaller flat oyster in the middle and a large flat oyster on the right, both overgrown with macroalgae. Lower: a large flat oyster with several smaller flat oyster on top, all with the flat right valve on top.

(Figures 31 and 32). Between the oysters, several species of anemones (Actiniaria) were found, including Dahlia anemone and Orange anemone (Figure 33).

On 28-06-2016 the entrance of the Blokkendam harbour was surveyed, but no oysters were found there. This area is more exposed to waves from the southwest and is also much more sandy than to the other side of the Blokkendam (where the oyster reef is). A large Sand mason worm reef was found here together with some mussels (Figure 34). Soft sediment samples were taken at some distance from the Blokkendam and many specimens of small shellfish (probably Abra alba) were found.

During the inspection and cleaning visit at the

Figure 31: Upper: Velvet crab (*Necora puber*) on stones with oysters, lower: European green crab (*Carcinas maenas*) in a macroalgae forest.

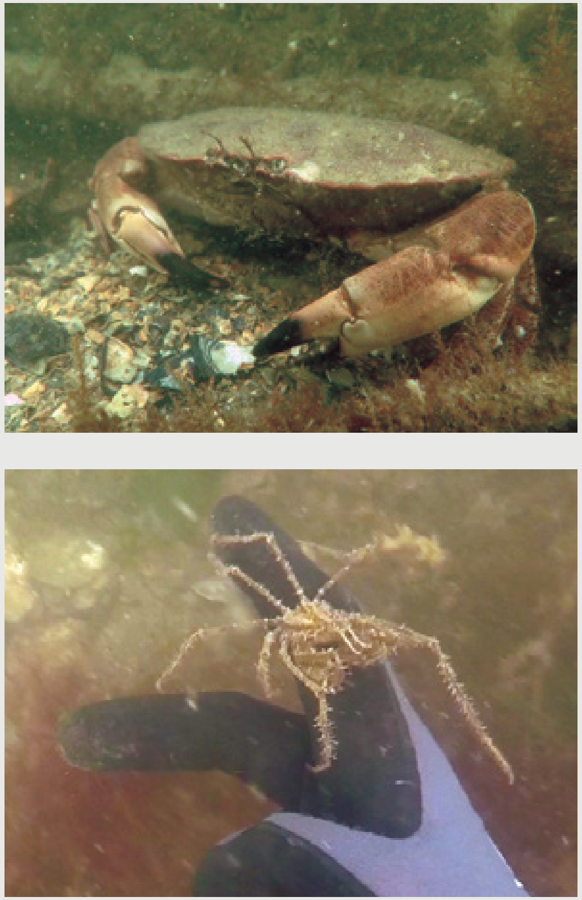


Figure 32: Upper: Brown crab (*Cancer pagurus*) close to one of the racks; Lower: Long-legged spider crab (*Macropodia rostrata*), which was hidden between the oysters.

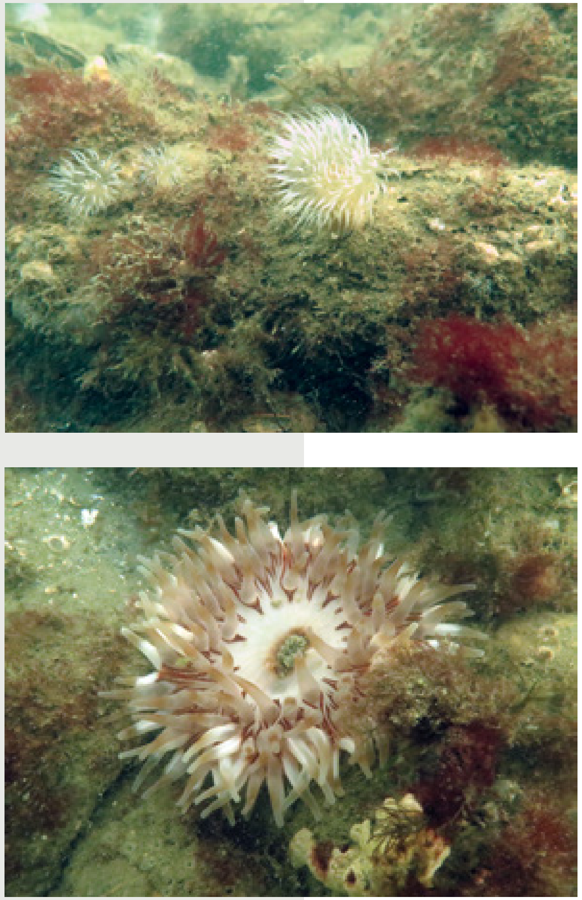


Figure 33: Upper: pale individuals of the Orange anemone (*Diadumene cincta*); lower: Dahlia anemone (*Urticina felina*) both on hard substrate.

Blokkendam pilot site of 22-09-2016 renewed observations on biota were made in and around the oyster reef. See Figure 35 to 37.
The mussels were found as clumps on the seafloor without any interconnections. This is in contrast with the network of mussels, which covered the reef domes. Several mussel clumps were attached to living flat oysters (Figure 35). Many mussel clumps were overgrown with macroalgae, bryozoa and ascidians (Figure 35). Several crabs, but very few sea stars were observed on the mussel clumps on the soft sediments surrounding the pilot (Figure 36).

A very large, (presumably) flat oyster (17 cm) was discovered living in the reef. It was measured, inspected and carefully returned to the seabed (Figure 37).
This single large oyster formed substrate for various other organisms, such as other (flat) oysters, young mussels and other epibionts.

Figure 34: Upper: A reef formed by the sand mason worm (*Lanice conchilega*) with a Small hermit crab (*Diogenes pugilator*); lower: Lanice-reef protruding from the sand covered by macroalgae. The Lanice reef is found at another location than the oyster reef (though close by).

Figure 35: Mussel seed attached to rocks with macroalgae, ascidians and other epibionts (upper) and among oysters (lower).

Figure 36: Dispersed clumps of mussel seed attached to oysters and soft sediment with sea star and velvet crab (upper) and brown crabs near mussel seed on soft sediment (lower).

When the oyster was inspected in the field, a total of 16 different epibiontic taxa where identified (see Table 4).

4.3 Implications for future pilots

The discovery of the flat oyster reef near the Blokkendam in the Voordelta raises many questions about its origin, population dynamics (reproduction, recruitment and survival) and ecology. Furthermore, it has several implications for the current and future pilots. The main implications for the pilot project are discussed in Chapter 5. However, some general observations can be made in advance.

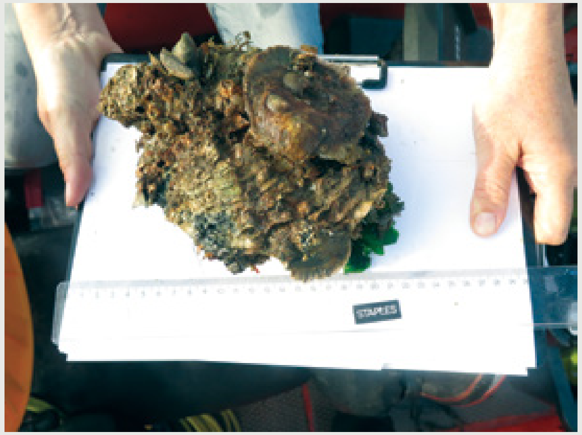


Figure 37: Large, presumably flat oyster and mussel seed attached to it (upper), with detail (lower).

Field inpspection biodiversity one large oyster		
Common name	**Scientific name**	**Number of individuals**
European flat oyster	*Ostrea edulis*	3
Pacific oyster	*Crassostrea gigas*	1
Edible blue mussel	*Mytilus edulis*	38
Yellow sea squirt	*Ciona intestinalis*	1
Star squirt	*Botryllus schlosseri*	
Long-clawed porcelain crab	*Pisidia longicornis*	1
Bread-crumb sponge	*Halichondria (Halichondria) panicea*	
Unknown bryozoan	*Bryozoa sp.*	
Barnacle sp.	*Sessilia sp.*	
Paddle worm	*Phyllodoce maculata*	1
Sea lettuce	*Ulva lactuca*	
Wireweed	*Sargassum muticum*	
Unknown other sea weed	-	
Encrusting bryozoan	*Conopeum reticulum*	
Yellow boring sponge	*Cliona celata*	
Amphipod sp.	*Gammarus sp.*	1

Table 4: Biodiversity description of organisms on the large oyster of Figure 37.

The site most likely has not been touched by bottom trawling fishery for many years, due to the presence of large stones. The Grevelingen outlet was probably the source of flat oyster larvae in the Voordelta, as the larvae sampling and water modelling reported in par. 3.3.3 also seem to indicate. This implies that possibly more flat oyster beds may be present at other unfished localities close to the Grevelingen outlet, or maybe even further away in the Voordelta. These could provide interesting starting points for reef restoration, as well as further development of restoration guidelines and the understanding of their ecosystem function.

The flat and Pacific oyster reef also provides a unique opportunity to study an ecosystem, which has long disappeared, in order to develop guidelines for oyster reef restoration and to understand the significance for biodiversity for the North Sea at large. In addition, it could directly provide the flat oysters for other pilots.

5 Conclusions and recommendations

5.1 Evaluation of pilots and oyster reef discovery in 2016

Based upon the observations made in 2016 on the Voordelta pilots, we can conclude the following on survival, growth, reproduction and recruitment (SGRR):

- Survival: Some mortality occurred at Blokkendam location, but this is probably caused by predation (on mussels mostly) and food supply blockage due to epibionts on cages with small mesh size (mussels and oysters). At Hinderplaat location, few mussels and no oysters survived, probably caused by strong freshwater flux in June.
- Growth: Oysters at Blokkendam showed no increase in shell length or change in weight and a decrease in condition possibly due to relatively large size of the animals. Mussels at Blokkendam increased in weight and shell length and surviving mussels at Hinderplaat showed a much higher weight and better condition than at Blokkendam. This indicates that food conditions were good as such.
- Reproduction: Gonad development, for both mussels and oysters, was more progressed at Blokkendam than at Hinderplaat. This indicates better reproduction conditions at Hinderplaat.
- Recruitment: Modelling of water flow and measurements of oyster larvae in the water of the Voordelta indicate that the flat oysters in the Blokkendam reef originate from the population in Lake Grevelingen. Possibly, some recruitment originates from the reef population itself. Flat oyster recruitment at Hinderplaat was poor: water dilution and distance being too high for larvae to arrive from Blokkendam or Lake Grevelingen
- Recruitment of flat oysters on mussel shells was poor, probably caused by a too late distribution of the shells. Pacific oysters recruited much more often on the mussel shells, probably because their recruitment was later in the season. Remarkably, Pacific oysters recruited at Hinderplaat location, which illustrates their superior spawning strategy over flat oysters. Mussel recruitment at Blokkendam location was massive and also occurred at Hinderplaat.

The discovery of the oyster reef near Blokkendam substantiates that SGRR

Mussel spat at Blokkendam.

conditions for Pacific and flat oysters at least in one area of the Voordelta are favourable, given:

- presence of larvae;
- absence of bottom trawling fishery;
- presence of suitable substrate (probably originally stones from Blokkendam, later on also the oysters and other shellfish).

SGRR conditions at Blokkendam location appear to be favourable for mussels too, given the observation of very substantial spat fall and growth on and around the pilots and the presence of older mussels in the oyster reef.

A remarkable extra observation is that the populations of Pacific and Flat oysters are able to co-exist and maybe even to support each other.

In this sense, the primary project objective, i.e. to find out whether environmental conditions (‘critical success factors’) for mussels and flat oysters are suitable to allow their growth, survival, reproduction and recruitment (SGRR), is already attained for one pilot location: at the Blokkendam location these conditions are proven to be very suitable indeed. At the Hinderplaat location, conditions appear to be much less favourable for flat oysters as well as mussels, probably due to high freshwater influx in June.

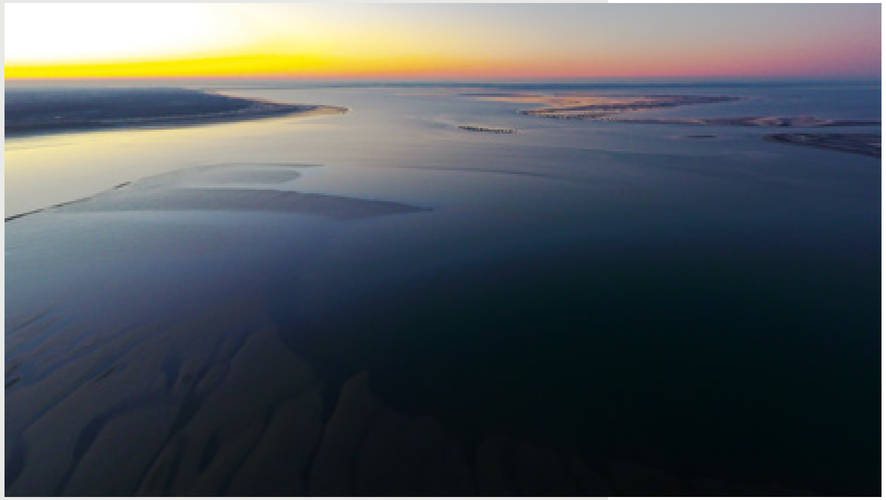
The oyster reef at Blokkendam shows a strong variety of organisms, attached as well as mobile. The pilot designs appear to be robust and suitable for the experiment, with the exception of cages with small mesh sizes (4 mm and 2 cm), since these tend to be blocked by epibionts. All in all, they have served to demonstrate the strong effect of predation on mussels and oysters. Most probably, this is caused by crabs, which, after they satisfied themselves on the contents of the cages seemed to proceed enthusiastically with the extensive mussel spat fall in the area.

5.2 Recommendations for pilots and research in 2017 and beyond

Given the fact that SGRR for both types of oysters and mussels could already be shown for at least one location, the project shows an unexpected early result. The question is: which are suitable next steps in this light? We recommend the following:

1. Continue the current pilot in the oyster reef, at Blokkendam location, aimed at maximizing understanding of survival, growth and reproduction conditions of mussels and flat oysters and of enhancement methods for recruitment of these shellfish. Measure and analyse the critical success factors at this location.
2. Continue the experiment at Hinderplaat, but with much less intensity and mainly aimed at the relation between fresh water concentration and shellfish mortality.
3. Attempt to extend the flat oyster reef at Blokkendam by stimulating spat fall

Blokkendam.



Hinderplaat from the air.

in or around it. Motivation: it is an ideal location for experiments with recruitment enhancement and the abundance of life forms in and around it makes it a worthy habitat for extension. We propose the following methods:

a. Distribution of empty mussel shells in or near the reef, around the time that larvae are expected to be in the water.
b. As reserve, in case the amount of spat appears to be too low in or near the reef: distribution of mussel shells at another location where recruitment can be expected (e.g. in Lake Grevelingen), harvesting these after time allowed for growth and stronger settlement of the spat and distributing shells and spat at the desired location in or near the reef.

4. Continue monitoring of flat oyster larvae in the Voordelta, at least for several years to come (peak incidence, origin). Motivation: This constitutes an essential step towards better understanding of the mechanisms behind recruitment in the Voordelta. Besides, it yields key information for the timing of recruitment enhancement measures, such as under recommendation 3 and 5.
5. Attempt to stimulate oyster and mussel bed development at a new location in the Voordelta, accompanied by monitoring. Motivation: Extension of shellfish beds is the primary objective of the project and many lessons will be learned by this new attempt. Probably, the best locations will be those where shellfish beds (mostly mussels, as can be expected) can be found, or were found in recent years. In order to identify these locations, survey data should be analysed, to be verified by diving actions. Besides, of course, the locations should be free from bottom trawling fishery and strong fresh water fluxes and the legal regime should allow for maintenance and observation visits.
6. Investigate and monitor the existing oyster reef. Motivation for this is threefold:
   - Optimize protection and extension of this reef.
   - Derive guiding principles for stimulation of flat oyster reefs elsewhere in the Voordelta and even elsewhere in the North Sea at large.
   - Underpinning the importance of the oyster reef as key habitat species in the Voordelta and elsewhere in the North Sea.

Literature

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Annex: Monitoring and analysis methods

1. Shellfish placement in cages

As explained in the main text, pilots are put into place at 2 locations in the Voordelta (Hinderplaat and Blokkendam). Each pilot consists of 8 reef domes and 3 racks. The racks contain 16 compartments (4x4) and can be opened and closed by a top lid.

Per rack, 12 shellfish cages are introduced, with have different mesh sizes: 3 x 4 mm, 3 x 2 cm, 3 x 9 cm, 3 x open.

All cages are given a unique number, indicated by a yellow label attached to it. Each cage contains 15 juvenile and 15 adult mussels, or 4 juvenile and 4 adult flat oysters. Within several cages, and also in empty cages, empty mussel and oyster shells were introduced, functioning as substrate for possible spat fall. See table A.1 for the complete administration. Mussels originate form Oosterschelde culture, flat oysters form the reef in the Voordelta.

Average shell lengths of shellfish placed into the cages were:

- Adult mussels: 51,83 ± 4,77 mm
- Juvenile mussels: 33,91 ± 3,58 mm
- Adult oysters: 86,21 ± 7,95 mm
- Juvenile oysters: 64,73 ± 7,81 mm

Per cage, passive integrated transponders (PIT tags) were attached to 10 juvenile mussels and to all oysters. This enables to identify and follow the development of individual shell fish. Of these, individual shell dimensions (length, width and height) and meat content (ash free dry weight) weight were determined before they were placed into the pilots. The cages are randomly distributed over the outer ring of the racks (see figure A.1).

Empty oyster shells are distributed around the racks too, in order to facilitate spat fall.

2 Analysis methods

Survival (in cages of different mesh size)

Mussel and oyster survival are priminarily determined in June or July, by counting the number of shellfish in the cages. In October, all cages are taken to the WMR lab for final annual

Location	Rack number	Mesh size cage	Lable number cage	Contents
1b Hinderplaat	1.1	4 mm	13	4* juvenile oysters + 4* adult oysters
			5	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			6	7 empty oyster shells
		2 cm	12	4* juvenile oysters + 4* adult oysters
			11	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			3	7 empty oyster shells
		9 cm	4	4* juvenile oysters + 4* adult oysters
			2	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			8	7 empty oyster shells
		open	1	15 juvenile oysters + 15 adult oysters
			10	4* juvenile oysters + 4* adult oysters
			7	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel1 shells
	1.2	4 mm	24	4* juvenile oysters + 4* adult oysters
			17	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			21	7 empty oyster shells
		2 cm	26	4* juvenile oysters + 4* adult oysters
			22	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			16	7 empty oyster shells
		9 cm	18	4* juvenile oysters + 4* adult oysters
			25	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			14	7 empty oyster shells
		open	15	4* juvenile oysters + 4* adult oysters
			23	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			19	7 empty oyster shells
	1.3	4 mm	40	4* juvenile oysters + 4* adult oysters
			34	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			39	7 empty oyster shells
		2 cm	32	4* juvenile oysters + 4* adult oysters
			35	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			41	7 empty oyster shells
		9 cm	33	4* juvenile oysters + 4* adult oysters
			34	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			28	7 empty oyster shells
		open	27	4* juvenile oysters + 4* adult oysters
			36	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			30	7 empty oyster shells

Table A.1: Distribution of flat oysters, mussels and empty shells over cages and racks, all with unique numbers. An * indicates PIT tag attachment.
Table continues on page 42.

Location	Rack number	Mesh size cage	Lable number cage	Contents
2b Blokkendam	2.1	4 mm	46	4* juvenile oysters + 4* adult oysters
			53	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			44	7 empty oyster shells
		2 cm	50	4* juvenile oysters + 4* adult oysters
			43	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			49	7 empty oyster shells
		9 cm	45	4* juvenile oysters + 4* adult oysters
			42	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			51	7 empty oyster shells
		open	48	4* juvenile oysters + 4* adult oysters
			52	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			47	7 empty oyster shells
	2.2	4 mm	54	4* juvenile oysters + 4* adult oysters
			66	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			56	7 empty oyster shells
		2 cm	64	4* juvenile oysters + 4* adult oysters
			55	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			59	7 empty oyster shells
		9 cm	63	4* juvenile oysters + 4* adult oysters
			58	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			67	7 empty oyster shells
		open	57	4* juvenile oysters + 4* adult oysters
			68	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			62	7 empty oyster shells
	2.3	4 mm	71	4* juvenile oysters + 4* adult oysters
			81	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			72	7 empty oyster shells
		2 cm	80	4* juvenile oysters + 4* adult oysters
			70	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			77	7 empty oyster shells
		9 cm	78	4* juvenile oysters + 4* adult oysters
			74	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			82	7 empty oyster shells
		open	75	4* juvenile oysters + 4* adult oysters
			79	15 juvenile mussels (10*) + 15 adult mussels + 7 empty mussel shells
			76	7 empty oyster shells

Rack 1.1 Hinder

1	2	3	4
13			5
12			6
11	10	8	7

Rack 2.1 Blokken

42	43	44	45
53			46
52			47
51	50	49	48

Rack 1.2 Hinder

14	15	16	17
26			18
25			19
24	23	22	21

Rack 2.2 Blokken

54	55	56	57
68			58
67			59
66	64	63	62

Rack 1.3 Hinder

27	28	29	30
41			32
40			33
39	36	35	34

Rack 2.3 Blokken

70	71	72	74
82			75
81			76
80	79	78	77

Figure A.1: Cage placement in racks.

analysis, among others in order to determine survival.

Survival depends on two factors:

- environmental circumstances;
- the incidence of predation;
- loss, out of the cages

It is assumed that at a certain location, survival due to environmental circumstances is equal for all cages. Hence, differences in survival will be due to predation or loss. Shells or shellfish cannot be lost out of cages with small mesh sizes, so that the relative influence of predation can be analysed by means of the difference in empty shells.

All analysis will be performed on two size classes, as it is assumed that the influence of predation on younger shellfish is relatively stronger.

Growth and condition

In October, annual growth (dimensions and shell weight), as well as ash free dry flesh weight are determined, for all pilot shell fish.

Growth is determined as the difference in dimensions and weight between February/ March and October.

Ash-free dry weight will be determined by first measuring the dry weight (DW) of the flesh after at least two days of drying at 70°C and cooling to room temperature in a desiccator. Ash-weight (AW) is analysed by ashing at 540°C and afterwards cooling down in a desiccator. The DW and AW were used to calculate the ADW by subtracted AW from DW (DW-AW).

Shellfish condition (CI) is determined by means of the relative meat contents, i.e.:
CI = [dry meat weight (g)/dry shell weight (g)] × 100

At the Blokkendam location, flat oysters from the reef outside the cages are collected and analysed for meat content and size, in order to analyse whether shellfish condition is pilot-dependent.

Reproduction

In June: random sampling of 5 adult oysters and 5 adult mussels per rack (totalling 15 per location). These shellfish are analysed for:

- gonad development (reproduction organs);
- whether spawning has taken place (also identified by analysing the gonads);
- presence of spat (only for oysters).

Spat fall, of both mussels and flat oysters, is also determined at other places:

- on empty shells in the cages (hypothesis: mainly mussels);
- on settling plates (hypothesis: mainly oysters);
- on empty mussel shells, dustibuted around the racks (these will be sampled by means of a frame with a standard surface area);
- on reef domes (hypothesis: these will become rapidly overgrown in Spring, which will hamper spat fall, occurring later in the season).

Bonamia incidence

The Dutch Veterinary Institute (CVI) kindly agreed to analyse the 30 oysters that were collected in December 2015 at the Blokkendam location for Bonamia infection as part of their regular monitoring programme.