Haringvliet
Naar een dynamische delta

H. Sas
P. Kamermans
T.M. van der Have
M.J.A. Christianen
J.W.P. Coolen
W. Lengkeek
K. Didderen
F. Driessen
J. Bergsma
P. van Dalen
A. van Gool
J. van der Pool
B.E. van der Weide

Shellfish bed restoration pilots

Voordelta
The Netherlands

Annual report 2017
30-03-2018

SASCON
Bureau Waardenburg Ecologie & Landschap
WAGENINGEN UNIVERSITY & RESEARCH

Commissioned by:
ARK NATUUR ONTWIKKELING
WWF

The Voordelta Shellfish reef restauration project is financially supported by:

Shellfish reef restoration pilots

Voordelta
The Netherlands

Annual report 2017
30-03-2018

Table of contents

Summary

Figure S.1 Map of the relevant parts of Voordelta and Lake Grevelingen.

Project background and context

As part of the Haringvliet Dream Fund Project (www.haringvliet.nu), ARK Nature and World Wildlife Fund Netherlands are working on shellfish bed restoration in the Haringvliet coastal zone (the so-called Voordelta). The project was started in 2016. Total project duration is at least 3 years. Results of 2017 monitoring and experiments are described in the current report. They are compared with 2016 results.

In 2016, a mixed Pacific and flat oyster bed was discovered at Blokkendam location (see map above). Most probably, the oyster larvae, which founded this bed, originate from Lake Grevelingen, which contains a large population.

Flat oyster spat settlement

We were able to demonstrate that, during the summer months, flat oyster larvae are present in the water near locations Outlet and Blokkendam. We collected spat with clean bivalve shells during the larval period. Deployment of clean bivalve shell substrate while the larvae are in the water is essential in stimulating flat oyster spat settlement. Possibly, the first flat oyster larval peak of the season has a much higher settlement capacity than those, which appear later.

Shellfish bed characterisation

It was estimated that the shellfish bed covers a surface area of at least 40 hectares, ranging several kilometres from the Blokkendam towards the north along the Brouwersdam. The bed is situated in 2-5 meters water depth. We found oysters of multiple size classes showing that environmental conditions for flat oysters were suitable to allow their survival, growth, reproduction and settlement. Sediment characteristics of the bed range from hard substrate, coarse, sandy sediment to fine mud and clay. The benthic community in and around the shellfish bed is 60% richer than in adjacent sandy sediment, including species of conservation interest. The oysters provide protection for blue mussels. Given the regular mussel spat fall in the area, oyster bed restoration also enhances the blue mussel population.

Bonamia presence

Bonamia tests show that this parasite is present in the flat oyster population of the Voordelta.

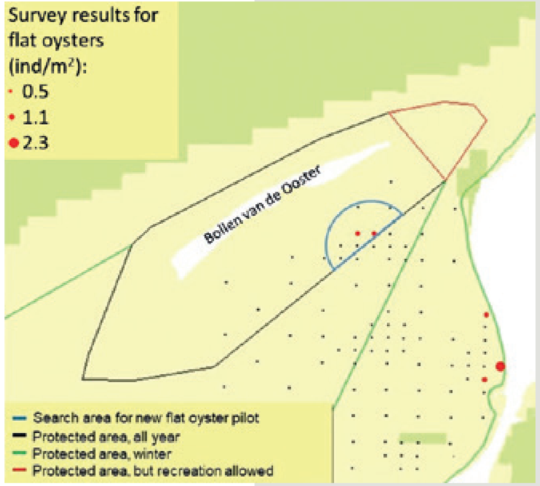


Figure S.2 Search area for new flat oyster pilot near Bollen van de Ooster.

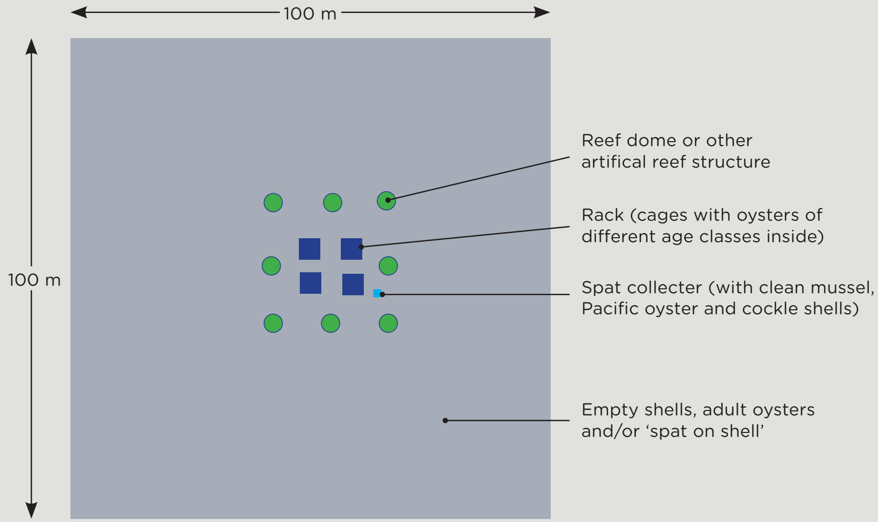


Figure S.3 Design of the recommended flat oyster pilot near Bollen van de Ooster.

Recommended new pilot location and design

We identified the south-east side of the tidal flat 'Bollen van de Ooster' as the best location for a new flat oyster bed restoration pilot in the Voordelta. Preferably within the N2000 protection zone, which surrounds this area, since bottom-dredging fishery is excluded. See figure S.2.

The recommended layout of the new pilot is as follows (see figure S.3):

- Adult oysters of different age classes are to be introduced. For monitoring purposes, these should be put into containers, attached to racks solidly placed on the sea floor.
- Empty and clean shell material, for monitoring purposes in spat collectors, should be distributed around the pilot, at the right time (onset of larvae swarming).
- Spat collected on empty shells elsewhere ('spat on shell') can be deployed, to speed up bed development.
- Reef domes or other artificial structures are to be added to enhance biodiversity around the pilot and for protection against disturbance.

Other recommendations

Other recommendations for the next project year are to continue spat collection (starting moment to be determined by continuous water temperature measurements) and to extend the number of collection locations, if possible even to Lake Grevelingen.

Given the importance of the shellfish bed at Blokkendam, Brouwersdam, as knowledge base for understanding flat oyster development and as species-rich habitat in the Voordelta, we also recommend continuing monitoring of the bed. Because of the biodiversity on the shellfish bed, it is recommended to provide more protection under the Dutch Nature Conservation Law.

We also recommend starting flat oyster bed restoration pilots elsewhere in the Voordelta and North Sea area at large. On the basis of the knowledge acquired in the Voordelta and other projects during 2016 and 2017, we formulated guidelines on how to design such restoration attempts.

1 Introduction

1.1 Background

Droomfonds Haringvliet
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 saltwater 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 bed restoration is the main element of this plan, since these beds are keystone organisms (bio-engineers) in this type of habitat.

Shellfish bed restoration in the Voordelta
The Voordelta is a nature conservation area and part of the EU Natura 2000 framework. A map of the designated Natura 2000 area is presented in figure 1.1.

Shellfish beds, mainly flat oysters (*Ostrea edulis*), once occupied about 20% of the Dutch part of the North Sea floor. 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 bed 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, 2015).

In 2015, a feasibility study showed that the time is right to attempt to restore flat oyster beds in

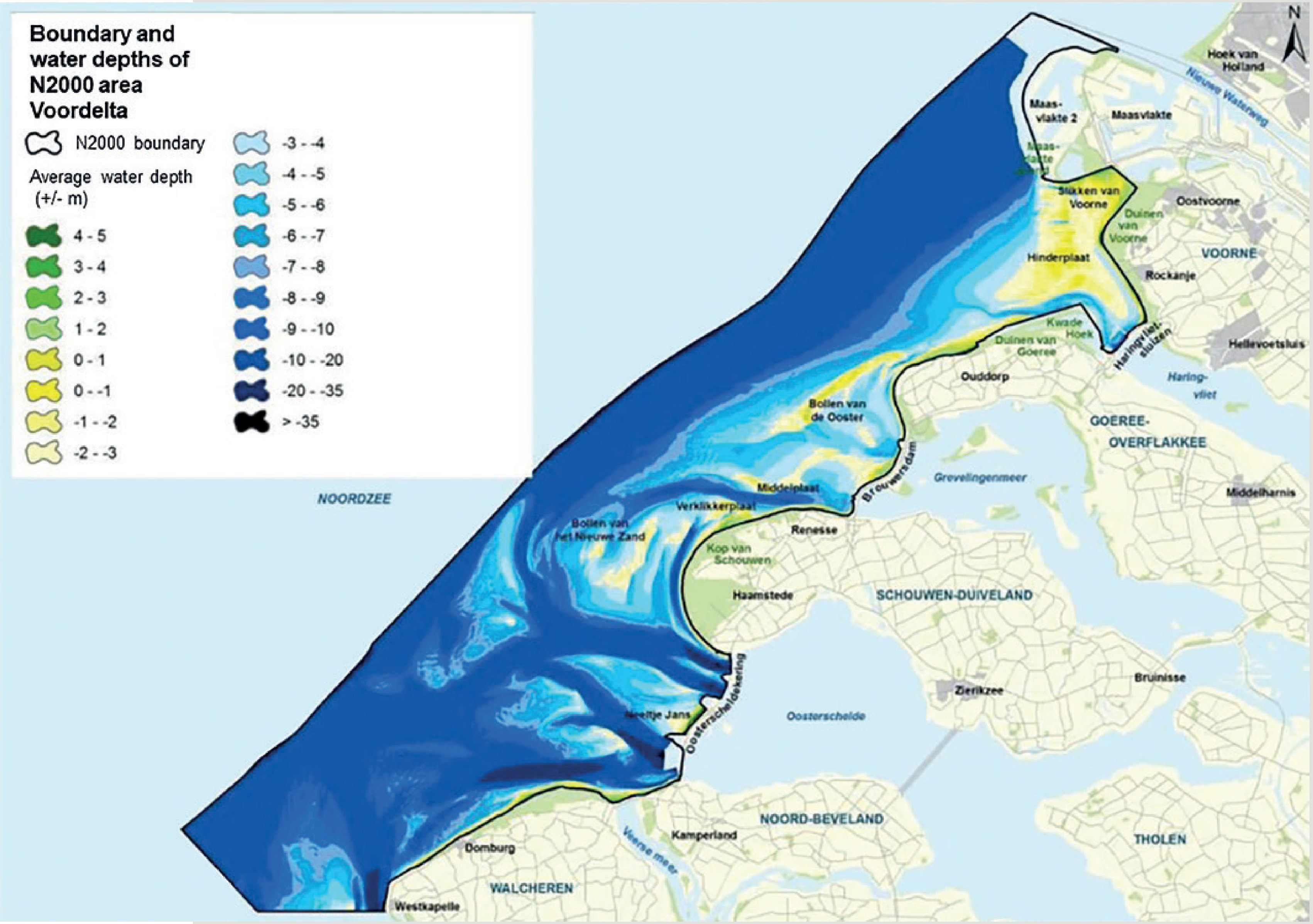


Figure 1.1 Map of the N2000 Voordelta area.

the North Sea area, among others since the population in the Dutch Delta area is showing signs of recovery of the Bonamia disease (Smaal et al., 2015).

In order to allow sufficient time for experimenting with shellfish bed restoration, the project is designed for the duration of minimally 3 years and, in principle, pilots are undertaken at 2 locations at least. This annual report is the result of the second year of experiments, that is 2017.

2016 discovery of mixed shellfish bed in the Voordelta

The current project was started in 2016 and reported in Sas et al. (2016). Main results of this first pilot year are:

- There is a mixed flat oyster and Pacific oyster (*Magellana gigas*, formerly called *Crassostrea gigas*) bed already present in the Voordelta area, near Blokkendam location.
- The flat oyster larvae most probably originate from Lake Grevelingen, a saline lake with limited tidal range and in open connection with the Voordelta since 1998. In this lake, flat oysters occur naturally and in aquaculture plots. As a result, in early summer, flat oyster larvae can be detected in the Voordelta area.
- Cage experiments showed that predation on flat oysters beyond the spat stage is minor.
- Conditions for flat oyster survival near Hinderplaat are unsuitable, probably due to large and sudden fresh water inflows from Haringvliet.
- Conditions for mussel spat settlement and growth are excellent, as was demonstrated by a massive spat fall in 2016 and consecutive growth measurements at pilot locations. Mussels appear to be protected by the oysters present in the shellfish bed.
- Predation causes the mussel population to decrease sharply after spat settlement in the Voordelta area, except in the Blokkendam bed. Cage experiments showed that predation on mussels can indeed completely decimate the population.

See figure 1.2 for a map of the relevant part of the Voordelta area and the locations mentioned above.

Figure 1.2 Map of relevant parts of the Voordelta and Lake Grevelingen; locations mentioned in the text are indicated.

1.2 Overall objectives of the project and specific objectives for 2017

The primary objective of the overall project (2016-2018) is to develop a method for creating shellfish beds in the Voordelta. In order to attain this objective, secondary objectives are:

- Understand the critical factors for formation and development of these beds.
- Identify suitable locations for shellfish beds in the Voordelta.

The 2017 project focused on flat oysters. Specific objectives and related activities for the experiments in 2017 are:

1. To unravel the dynamics of spat settlement as a basis for recruitment of flat oysters, since this probably constitutes the key threshold for the establishment of flat oyster beds. This is done by monitoring the occurrence of flat oyster larvae in the water column, deploying substrate when flat oyster larvae appear to be present and mapping the substrate used by flat oysters in the Blokkendam bed.
2. To map the existing shellfish bed at Blokkendam location and its associated biodiversity, in order to demonstrate the bed's value for marine nature development. Specifically, habitat conditions, spatial extent, species composition, epibenthic community, settlement preference and age structure of the existing bed are investigated.
3. To investigate the presence of the *Bonamia* parasite in the Blokkendam bed, by repeating the 2016 procedure for *Bonamia* detection, in the appropriate time interval.
4. To identify a new pilot location, where the results of the experiments with flat oyster spat settlement stimulation can be demonstrated to establish a new bed, by systematically investigating shellfish surveys conducted in the area.
5. To design a new pilot, on the basis of the knowledge acquired so far.

Given the large number of mussel recruits in 2016 and their protection within the Blokkendam shellfish bed, it was decided to merely focus on flat oysters in 2017. Given the unfavourable flat oyster survival conditions at Hinderplaat, it was decided not to monitor this location in 2017. The pilot infrastructure remains in place, for possible future use.

1.3 Project organization

As stated above, the shellfish bed restoration project in the Voordelta is part of The Haringvliet Dream Fund project (www.haringvliet.nu). The so-called Dream Fund is granted by the National Postcode Lottery. ARK Nature leads the shellfish bed restoration project and works closely together with WWF on this project. ARK and WWF are two of the six partners in the Haringvliet Dream Fund 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.

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 and LIFE. The Dutch WWF contributed additional funding, in order to investigate the Blokkendam bed structure and biodiversity (see Chapter 4).

1.4 Structure of this report

In line with the objectives and activities mentioned above, the report describes methods and results for the following subjects:

- Estimation of flat oyster larval abundance (Chapter 2).
- Test of shellfish bed expansion through stimulation of oyster spat settlement (Chapter 3).
- Shellfish bed structure: population composition, settlement substrate and biodiversity (Chapter 4).
- Estimation of *Bonamia* parasite prevalence in flat oysters of the shellfish bed at the Blokkendam location (Chapter 5).
- Evaluation of promising locations for shellfish bed restoration in the Voordelta (Chapter 6).

In Chapter 7, overall conclusions and recommendations are presented, whereas Chapter 8 summarizes and translates acquired knowledge into the design of new flat oyster pilots.

2 Monitoring of flat oyster larval abundance

2.1 Introduction

Larval abundance in the water is an important indicator of the productivity of a shellfish population (Korringa, 1940). We determined it in detail, throughout the growing season, since we suppose it is important to deploy settlement substrate when the maximum number of larvae is present in the water column (see Chapter 3).

2.2 Methods

Weekly larval concentrations were determined in the period June to August 2017. This was done at two locations: Blokkendam and Outlet (figure 1.1). A potential new location at Bollen van de Ooster (see chapter 6) was sampled once, on 21 July 2017. Samples at Outlet were taken around the tidal period when water started flowing from Lake Grevelingen into the Voordelta, since the majority of the larvae probably originate from the population in Lake Grevelingen. At each visit, 100 litres of surface water were filtered over a 100-µm plankton net (figure 2.1). The sample that remained in the net was preserved with formaldehyde. In the lab shellfish larvae were identified and counted.

An 'Ibutton' temperature data logger recorded the water temperature from June to October period, since it is known that a minimum temperature of 15°C is required for egg ripening and 19-20 °C for the swarming of larvae (Korringa, 1940, 1947).

Figure 2.1 Collecting plankton samples for estimation of oyster larvae abundance.

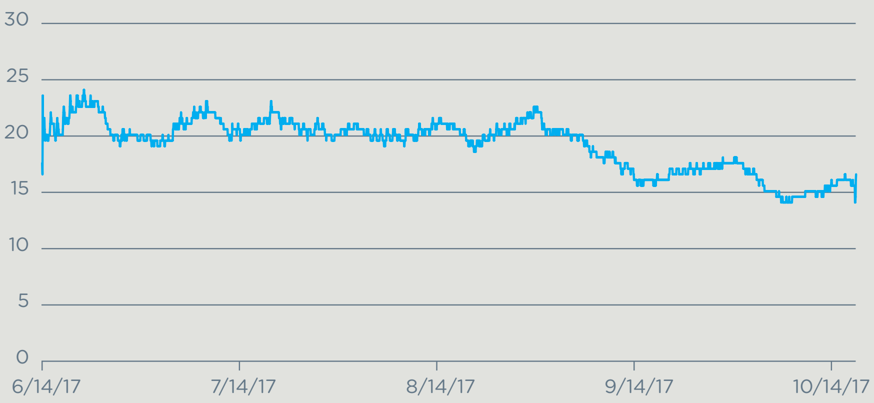


Figure 2.2 Water temperature (°C) at location Blokkendam, Voordelta.
June 14 = week 24, July 14 = week 28, August 14 = week 33 in 2017.

2.3 Results

In spring 2017, atmospheric temperatures were extremely high. Seawater temperatures followed this pattern, leading to unexpectedly high temperatures in June (up to 24 °C mid June, figure 2.2).

Apparently, water temperature in the Voordelta had attained 20 °C before we deployed the temperature logger. To analyse the situation, we retrieved seawater temperature data from 2016 and 2017, as monitored in the Voordelta by the government organisation Rijkswaterstaat. These are presented in figure 2.3.

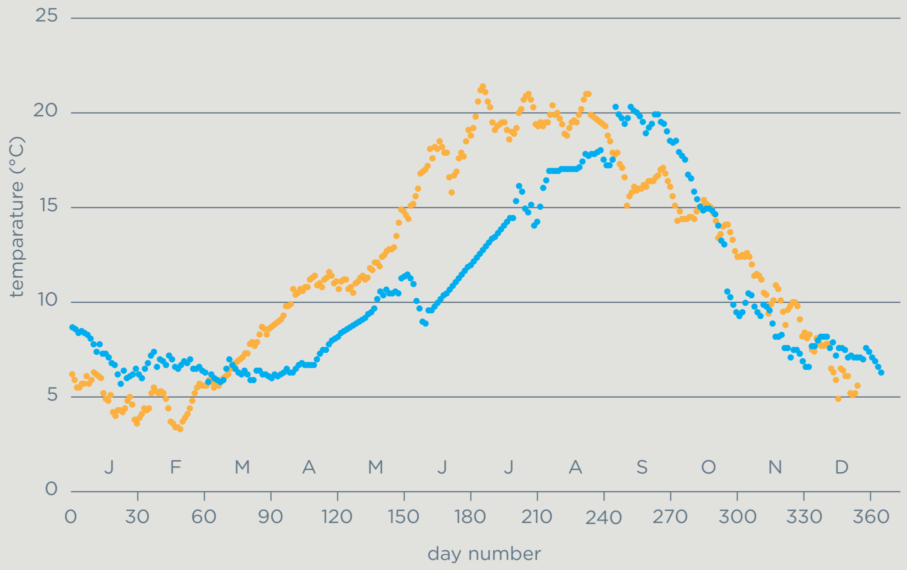


Figure 2.3 Water temperature (°C) at number of days since 1 January in 2016 and 2017 at location Outlet (Rijkswaterstaat data).

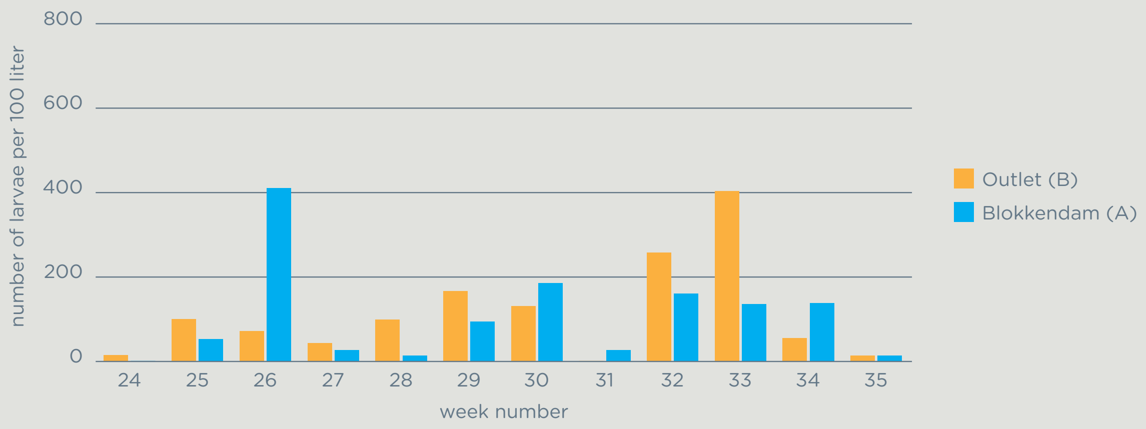


Figure 2.4 Abundance of flat oyster larvae at locations Outlet and Blokkendam, Voordelta, in 2017.
Week 24-26 = June, week 27-30 = July, week 31-35 = August.

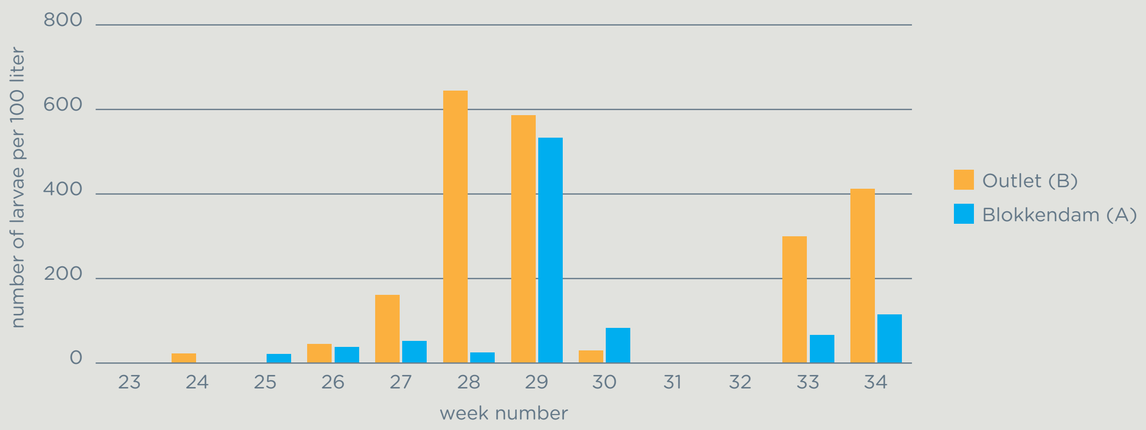


Figure 2.5 Abundance of flat oyster larvae at locations Outlet and Blokkendam, Voordelta, in 2016.
Week 23-26 = June, week 27-30 = July, week 31-34 = August.

It can indeed be observed that water temperature in June in 2017 was much higher than in 2016 (up to 5 °C by mid June).

The flat oyster larvae counts near Outlet and Blokkendam in 2017 are presented in figure 2.4. For comparison, also the data for 2016 are presented, in figure 2.5 (from Sas et al., 2016). As can be seen, the maximal numbers of flat oyster larvae recorded in 2017 were lower than during 2016. Because of the early high water temperature, the larval abundance peak has probably occurred earlier in June, so we have probably missed the maximum values in 2017 with our larval monitoring.

As figure 2.5 also shows, the larval abundance in June 2016 was low. This correlates well with the much lower water temperature in June 2016 (figure 2.3), once again demonstrating the effect of water temperature on flat oyster spawning and larval swarming.

We also sampled for larvae at location Bollen van de Ooster. Originally, it was intended to deploy spat collectors there (see Chapter 3), but we did not receive permission by the authorities. Therefore, we collected larvae instead. This was done at 21 July (week 28), yielding 34 larvae per 100 litre. This is a lower concentration than at Outlet (165 per 100 litre in week 29) and Blokkendam (93 per 100 litre in week 29), but is does show that larvae are present in the water column there.

2.4 Discussion and recommendations

Water temperatures in June 2017 were extremely high, probably resulting in a very early flat oyster spawning and larval swarming peak. Since we started our larval monitoring in the second week of June, we probably missed this early peak.

Hence, we recommend to record water temperature from the very start of the growing season onwards, and to be able to read the data in real time, i.e. not afterwards, as was done in 2017. This allows to deploy spat collectors as soon as the water temperature reaches 19-20 °C. This system should be used as of 2018 onwards.

3 Test of shellfish bed expansion through stimulation of oyster spat settlement

3.1 Introduction

Flat oyster culture farmers report that settlement of flat oysters is sensitive to substrate cleanness. This was confirmed by experiments of WMR in Lake Grevelingen (Kamermans et al 2004; van den Brink, 2012: van den Brink et al, 2013). Since flat oyster larvae are present in the water column for a limited amount of time (a maximum of ca. 10 days; cf. Walne, 1974), it is expected that clean substrate is most effective when a maximum concentration of larvae occurs. However, exactly when this maximum occurs is difficult to predict. Therefore new 'collector units', with clean substrate, were deployed every week of the June-August period. This was done at two locations: Blokkendam (near the pilot location) and Outlet. In October, the collector units were retrieved from the field by scuba divers.

3.2 Methods

A collector unit consists of two nets with clean mussel shells (0.5 kg, equalling an average of 340 shells each) and two nets with clean Pacific oyster shells (1 kg, equalling an average of 95 shells each). Settlement success was estimated by counting the number of spat per collector unit and also by recalculating the number of per kg of substrate.

One net with mussel shells and one net with oyster shells is placed on the bottom on a tile, while one net with mussel shells and one net with oyster shells is suspended 1 m above the bottom with a float (figure 3.1). In this way, both on-bottom and off-bottom collectors are tested.

All collector units that were retrieved from the field were temporarily stored in a basin with running seawater for a maximum of two weeks, until the shells could be checked for presence of flat and Pacific oyster spat in the laboratory.

In addition, mussel and oyster shells were placed on the seabed at location Blokkendam in the middle of the larval period (figure 3.1). Due to time constraints, sampling of the shells placed on the seabed is postponed to early 2018.

Figure 3.1 Collector unit for collection of oyster spat just before deployment (top left) and during deployment (top right) placed at Blokkendam (location A bottom left) and Outlet of Lake Grevelingen (location B bottom left). Introduction of mussel and oyster shells at location Blokkendam (bottom right).

Figure 3.2 Collector nets with mussel and Pacific oyster shells (left) and flat and Pacific oyster spat on shells (right).

3.3 Results

The collector units showed very limited fouling (figure 3.2). Both types of shells, as well as on-bottom and off-bottom collectors, had collected oyster spat (figure 3.3).

Totals of 155 flat oyster spat and 765 Pacific oyster spat were collected in 2017. The shells with spat are stored in running seawater, until they can be returned to the field. Location Outlet showed higher maximal numbers of spat settlement than location Blokkendam (figure 3.3), which is expected, considering the high amount of flat oysters in Lake Grevelingen.

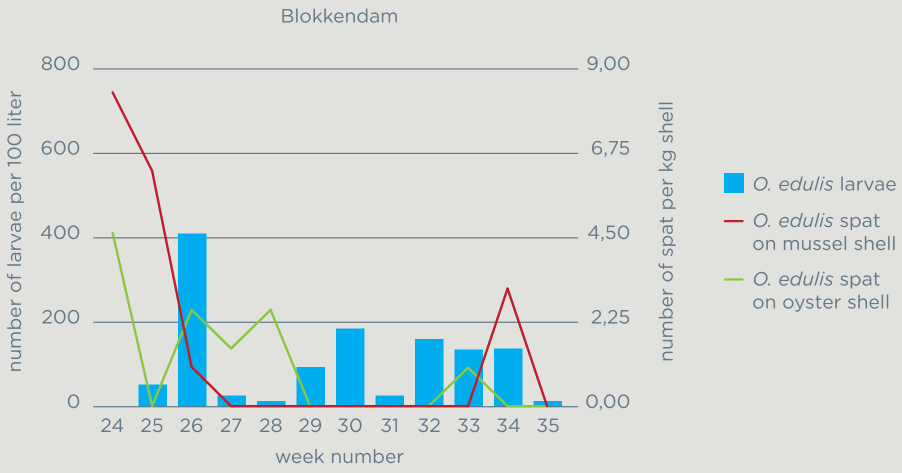
There is no clear spat preference for settlement at on-bottom ('bottom' in figure 3.3) or off-bottom ('top' in figure 3.3) collectors.

In order to investigate the relation between larval presence in the water and spat settlement in 2017, we plotted the observations in one graph (figure 3.4). It appears that the peak in spat collection occurs in week 24 (i.e. early in June), or even before. This coincides with the probable very early larval swarming peak before week 24, as discussed in Chapter 2, and the general observation that flat oyster larvae spend around 10 days in the water column before they settle onto hard substrate.

As can also be derived from figure 3.4, the first larval peak was the most productive for spat collection: the figure shows that the increase in the larval concentration at location Outlet in week 32 and 33 did hardly result in spat collection, whereas the early peak did. At Blokkendam this tendency appears to be a little less, but spat collection later in the season is relatively low also here. The settlement tendency of flat oyster larvae seems to be reduced later in the season, after the first peak. These observations concur with experiences by growers in Lake Grevelingen: also there water temperature was high early in June and spat fall on collectors was early too.

This phenomenon is not widely reported or analysed in the literature, but laboratory experiments of Robert et al. (1988) show less spatfall with lower quality of the larvae. Hence, larval

Figure 3.3 Abundance (number per bag) of flat oyster spat (*Ostrea edulis*) and Pacific oyster spat (*Magellana gigas*) on collectors with mussel shells (mussel) and oyster shells (oyster) placed on the bottom (bottom) and suspended 1 m above the seabed (top) at the locations Blokkendam and Outlet of Lake Grevelingen in 2017. The collector units deployed in week 29 at Blokkendam and week 28-31 at Outlet were lost. Week 24-26 = June, week 27-30 = July, week 31-35 = August.



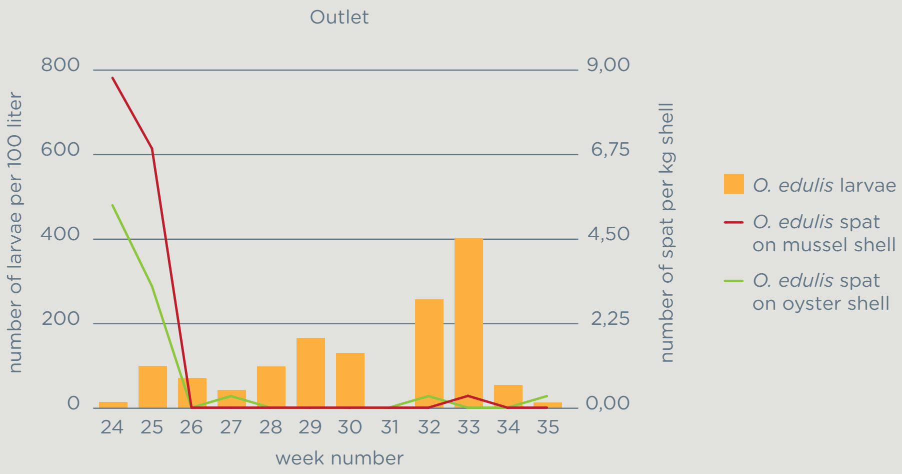


Figure 3.4 Comparison of larval and spat abundance at Blokkendam (top) and Outlet (bottom), Voordelta, in 2017. Week 24-26 = June, week 27-30 = July, week 31-35 = August.

quality may be lower later in the year. This could be a very important observation, although it is not clear yet whether this is a regular pattern. We need at least one year more of observations in order to conclude whether this is the case.

Pacific oysters show much more regular spat settlement throughout the summer period, while total as well as maximum settlement numbers were higher. The maximal number per kg shells was 29 for flat oyster spat and 55 for Pacific oyster spat. Pacific oyster spat preferred to settle on Pacific oyster shells suspended 1 meter above the bottom.

Flat oyster spat showed a slight preference for mussel shells above Pacific oyster shells in collector nets (see figure 3.3), but the difference is too small to suspect a systematic pattern.

Spat collection in 2016 was much less successful than in 2017 (see Table 3.1). Mussel and oyster shells placed on the bottom at location Blokkendam in January and February and in

Deployment date	Substrate used	Flat oyster spat result
18 January	Mussel shells on bottom	Yes (but very little)
26 February	Mussel shells in cages	No
26 February	Mussel shells in cages	No
6 June	PVC plates	No
26 July	Mussel shells on bottom	No
26 July	Mussel shells in nets	Yes (but very little)
26 July	Oyster shells in nets	No

Table 3.1 Summary of flat oyster spat collection at Blokkendam location in 2016 (Sas et al., 2016).

nets deployed in July yielded a total of just 4 flat oyster spat. In 2016 the timing of deployment was obviously not optimal. The placing of shell material in January and February was probably too early, resulting in fouling of the shells and limited flat oyster larvae settlement. In the last week of July the larval concentrations had already decreased at location Blokkendam (figure 2.5) and, possibly, their settlement capacity was reduced too. This underlines the importance of deploying clean settlement substrate when the larvae are in the water.

3.4 Discussion and recommendations

In summary, flat oyster spat collection with mussel and oyster shells was rather successful in 2017. Yet, we may have missed part of a very early spawning peak, occurring early in June with both larval monitoring and spat collection. Hence, water temperature measurement should start from the very start of the growing season onwards, and spat collectors should be deployed as soon as temperature is predicted to reach 19-20 °C within a few days, as already recommended in Chapter 2.

In 2017, the result of spat collection was also much better than in 2016. Clearly, deploying spat collectors during winter is too early, further supporting the hypothesis that flat oyster settlement is very sensitive to collector fouling.

Flat oyster spat settled on mussel as well as Pacific oyster shells in collector nets. During a flat oyster survey in the Dutch Wadden Sea in the summer 2017 (Van der Have et al., in prep.), in an area with mixed bivalve shell substrate present, it was observed that flat oysters use a wide array of shell types for settlement, with highest numbers of spat on empty cockle shells. For 2018, it is therefore advised to also deploy collectors containing clean mussel, Pacific oyster and cockle shells and/or to deposit clean shells of all three types on the bottom, before the occurrence of the first larval peak.

An intriguing larval settlement pattern appears from the 2017 experiment: the early spawning peak of flat oysters seems to have a much higher settlement capacity than the ones later in the season. It is unclear whether this is a regular pattern, and if so, what could be the mechanism behind it. Observations of this pattern, let alone explanations, could not be found in the scientific literature. Therefore, it is important to repeat the weekly deployment of collectors in 2018, and to start early enough in the season, in order to test whether it is a true pattern.

4 Shellfish bed structure: population composition, settlement habitat and biodiversity

4.1 Introduction

In this chapter, we report the investigations of the conditions of the mixed flay oyster-Pacific oyster bed at Blokkendam location, in order to get better knowledge on the requirements for large-scale restoration and the return of the flat oyster. With scuba divers an underwater survey was carried out based on visual observations and measurements of:

- The species composition of the bivalve community,
- The size-frequency distribution of flat oysters (to derive age structure),
- Epibenthic community structure on the shellfish bed,
- Substrate settlement preferences of the flat oyster,
- Habitat conditions inside quadrants that were distributed across the bed,
- Size-frequency distribution of blue mussels on the original pilot location within the bed.

Specifically, we also investigated the importance of Pacific oysters, as shell fragments or live individuals, as a settlement substrate for flat oysters.

4.2 Methods

Bed size and density

Fieldwork and measurements were carried out at the shellfish bed around the Voordelta pilot site located in the Voordelta in the Dutch part of the North Sea (figure 4.1). The site with the shellfish bed is described in the first report of the Voordelta project. The shellfish bed is situated at close proximity to the water outlet of Lake Grevelingen, which provides flat oyster larvae to disperse to the North Sea coastal zone (Sas et al., 2016).

Scuba divers discovered the shellfish bed with flat and Pacific oysters near the Blokkendam after pre-selecting a search area based on favourable conditions for flat oyster presence. These habitat conditions included the availability of suitable substrate for settlement of flat oyster larvae such as empty shells of bivalves (e.g., mussels and oysters), a nearby source of flat oyster larvae, and the absence of bottom disturbing activities.

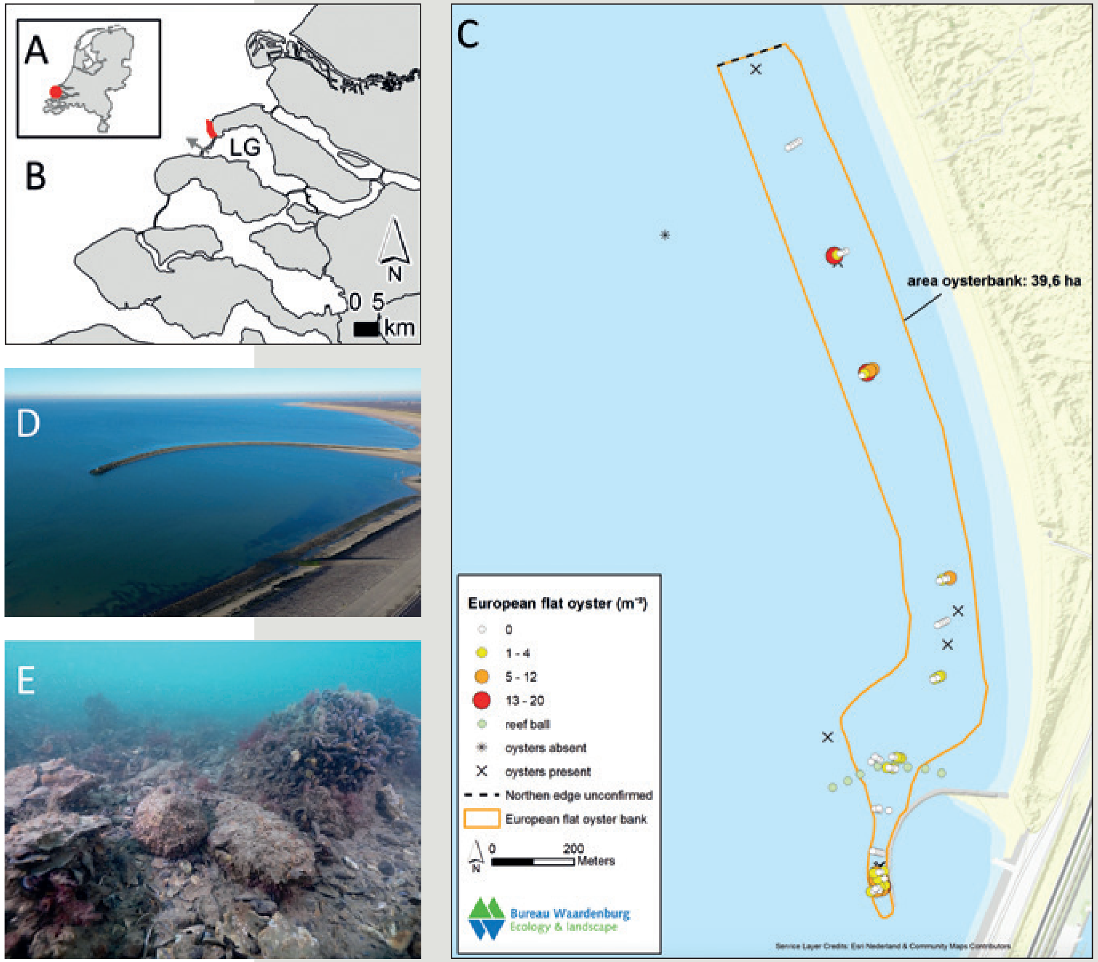


Figure 4.1
A) The Netherlands with in the Delta area
B) the location of the shellfish reef with European flat oysters (*O. edulis*), Pacific oysters (*M. gigas*) and mussels (*Mytilus edulis*) in the Voordelta, bed part of the North Sea. The tidal water outlet between Lake Grevelingen ("LG") and Voordelta is marked with a gray arrow.
C) A detailed map with contours of the shellfish bed (red line), and transect locations (squares), and
D) Blokkendam (© Peter van Rodijnen). E) Voordelta shellfish bed dominated by *Ostrea edulis* and includes also Pacific oysters (*M. gigas*), blue mussels (*M. edulis*) and epibionts (© by Peter van Rodijnen, onderwaterbeelden.nl).

The bed is located close to shore and is bordered by breakwaters on the south side. Large boulders are scattered over the sea floor in various, seemingly random artificial heaps, possibly as a result of spilling or dumping at the time when the Brouwersdam was constructed in the period 1962-1971. The presence of these stones probably prevents bottom disturbance by bottom trawling fishing activities. The rocks and boulders of the breakwaters are colonised by blue mussels and Pacific oysters, and the empty shells of these bivalves are deposited in the surrounding soft sediments. These empty shells can provide settlement substrate for both oyster species.

In August-October 2017 scuba divers surveyed the contours of the shellfish bed (figure 4.1C) by following the outer edges of the bed while deploying small buoys at regular intervals. These buoys where than plotted using a hand-held GPS by the boat crew. Subsequently, the presence of the shellfish bed formed by flat and Pacific oysters within the contours was validated by a sequence of haphazardly placed short dives at point locations and later also by the observations and measurement along census transects.

Visual census transects

Scuba divers conducted measurements on the composition of the bivalve community, size-frequency distributions of flat oysters, epibenthic community structure, substrate preference and habitat conditions in 75 quadrants that were spread across the shellfish bed along 15 transect lines. Along a transect line of 25 meters, quadrants (0.5 x 0.5 m^2) were spaced five meters apart, each transect line measuring 25 meters and five quadrants. For two transects the distance between quadrants was adjusted to 2.5 m to fit the transect line between border regions or contours of the bed. All transect lines were positioned perpendicular to the shore and are randomly setup across the shellfish bed in between edge of the bed. A handheld GPS marked the starting position and endpoint of the transect line.

All observations and measurements were conducted simultaneously in each quadrant by two observers using under water visual census and scuba. Quadrants were sampled between August and October 2017. Additional observations on fish and epibenthic species presence were collected between 2015 and 2017 using additional (video-)monitoring.

Bivalve age structure
To assess the population composition, we estimated the density and cover of flat and Pacific oysters, blue mussels, American razor clams and other bivalves. Additionally, we determined the size-frequency distribution from the 122 flat oyster individuals found in the quadrants. Flat oyster width was measured as the longest axis of the right valve (distance between the anterior and posterior tip) with a calliper to the nearest millimeter. A 'scratch' sample of the mussels on the pilot location was taken to determine the size-frequency distribution of mussels. Originally it was intended to follow the growth and survival of mussels in permanent quadrants, but the lack of visibility during the first dive in May made it impossible to start early enough to set a starting moment for the monitoring. We therefore investigated the mussel population development by sampling around the pilot location.

Settlement substrate and habitat conditions
The substrate preference of flat oyster settlement was investigated by careful visual inspection of all individuals found in the quadrants. We determined the (dead shell, complete or fragment) material attached to the umbonal area (or dorsal tip) of the left concave valve of each flat oyster and identified the shell or shell fragment to species level. After this inspection under water, scuba divers carefully placed the flat oysters back into their original position. For each quadrant we noted the sediment type: hard substrate (rock, stones), coarse sandy sediment and fine mud and clay sediment.

Biodiversity/epibenthic community
To assess the community structure, all epibenthic invertebrates (>5 mm) were identified and counted (solitary species) or estimated (percentage coverage of colonial species) within the quadrant in the field. The observed species were identified to species level or the most detailed taxonomic level possible. Several samples were taken to the laboratory, conserved on 70% ethanol and inspected with a binocular microscope to identify smaller species.
We estimated the size-distribution of mussels by taking several scratch samples around the pilot site and measuring shell length with a calliper to the nearest millimeter. Large numbers of mussels are still present on the rocks and soft sediments.

Prior to statistical model fitting, all observation and measurement data were checked for normality using Shapiro-Wilks tests ($p = 0.05$) and further confirmation by graphical validation of the final statistical model. No deviations from normality were observed and therefore no statistical transformations were needed. The differences in flat oyster *O. edulis* settling substrates and number of species per habitat were analysed with one-way ANOVA. Average values of the metrics are presented with standard errors (SE).

4.3 Results

Bed size and density
It was estimated that the shellfish bed covers a surface area of at least 39.6 hectares. The Northern boundary of the shellfish bed as presented in figure 4.1C is still not determined with complete certainty. The shellfish bed was situated in the shallow sublittoral, i.e. at water depths between 2 and 5 meters. Flat oyster density was 6.8 ± 0.6 oysters m^{-2} on shellfish patches on this bed.

Bivalve age structure
The size-frequency distribution of flat oysters suggests that the flat oysters on this bed consisted of individuals from different age classes (figure 4.2A). Flat oyster shell width ranged from 1.0 cm to 11.2 cm and averaged 6.6 ± 0.2 cm.

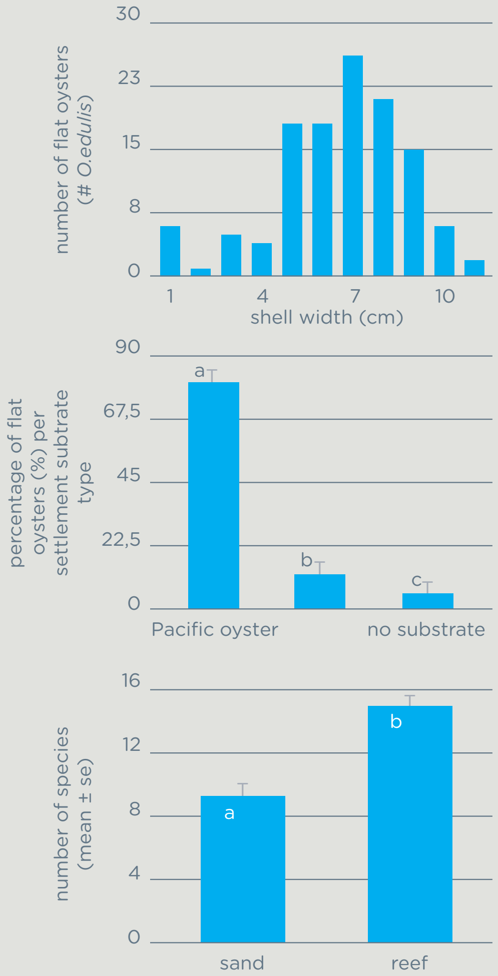


Figure 4.2 The shellfish bed structure consists of native *O. edulis* and invasive *M. gigas* oysters. A) Size-frequency diagram of *O. edulis* (n=122), B) settlement substrate (n=75) of O. edulis. Inset: native flat oyster (“NO”) settled on Pacific oyster (“PO”), (Pacific oyster preferred over other bivalves, p < 0.001. C) Comparison of the number of epibenthic species on the shellfish reef (>5 oysters m^{-2}) compared to adjacent soft sediment areas in the Voordelta. Total number of species was measured in quadrants of 0.25 m^2, ($\#species_{bed} > \#species_{sand}$, p<0.001).

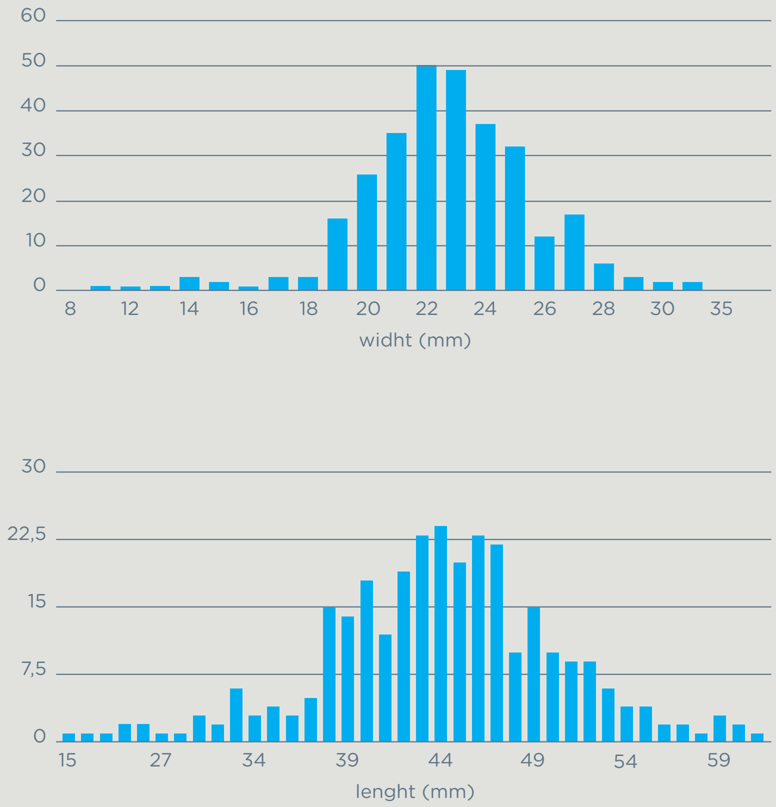


Figure 4.3 Frequency distribution of width and length of mussels at the Blokkendam pilot location (N=301).

We also investigated the size distribution of mussels found near the Blokkendam pilot location (figure 4.3). This investigation replaces the originally intended following of mussel growth and survival in permanent quadrants.

The peaks, at 14 and 22 mm (width) and 26 and 44 mm (length), suggests that at least two cohorts (2016 large peak, 2017 small peak) are present in the local mussel population. In December 2015, only a few mussels were present at the pilot location. These are possibly represented by the small peak at 59 mm (length). These results suggest that mussels of the 2016 spat fall show a high survival rate at the pilot location, in contrast to elsewhere in the Voordelta (see par. 6.3), confirming the protective function of the shellfish bed near the Blokkendam for mussels. Predation of mussels does occur in the Blokkendam bed, as was shown by the 2016 experiments (Sas at al., 2016). So the mechanism behind the apparent protective function is unclear.

Settlement substrate and habitat conditions
During our surveys at the Blokkendam bed, flat oysters were often found to settle and grow on hard substrate together with Pacific oysters and blue mussels. In the settlement substrate assessment, we found that flat oysters were predominantly attached to the Pacific oyster (81.3 ± 5.7%) and were less often attached to shell fragments of other bivalves (12.7 ± 4.7%) such as the blue mussels, common cockle and flat oyster (figure 4.2). This may not be a real preference, since there is a relatively high concentration of Pacific oysters in the sampled area. In any case, it shows that flat oysters are able to settle on Pacific oysters.

For a small number of flat oysters (5.8 ± 3.0%) we could not find a (shell) fragment attached. Sediment requirements were less clear. Flat oysters were located in areas with (but not attached to) hard substrate (rock, stones in 65.7 ± 3.4% of quadrants), but also on coarse, sandy sediment (14.5 ± 2.4%) and on fine mud and clay sediment (27.8 ± 3.9%). (Figure 4.2B, $p<0.001$).

Figure 4.4 illustrates a variety of flat oyster settling and growing behaviour.

Biodiversity/epibenthic community
The assessment of the benthic community in and around the shellfish bed with flat and Pacific oysters yielded at least 70 species (see Appendix). The species list includes some species of conservation interest that all used the shellfish bed as a habitat, such as the Dahlia anemone (Table 4.1). Mobile crustaceans (16 species) are most prominent in the shellfish bed assemblage, followed by molluscs (11 species, mainly species living on or attached to hard substrate), fish (10 species, predominantly smaller species associated with hard substrate), ascidians (9 species, all attached to hard substrate) and cnidarians (6 hard substrate species). Compared to soft sediments the number of species of annelid worms (4) and echinoderms (3) are relatively low.

We found a significant increase of 60% in the number of epibenthic species in quadrants with native flat oysters and Pacific oysters (14.9 ± 1.8 species m^{-2}) when compared to adjacent sandy sediment patches (9.3 ± 0.7 species m^{-2}) (figure 4.2C, $p<0.001$).

Phylum	English name	Scientific name	Conservation status
Annelida	Sand mason worm	*Lanice conchilega*	Natura 2000 - H1110A
Cnidaria	Dahlia sea anemone	*Urticina felina*	Red list Germany - score G
Arthropoda	Velvet swimming crab	*Necora puber*	Red list Germany - score R
Arthropoda	Hairy crab	*Pilumnus hirtellus*	Red list Germany - score 3
Arthropoda	European lobster	*Homarus gammarus*	Red list Germany - score 2
Mollusca	Grey chiton	*Lepidochitona cinerea*	Red list Germany - score 3
Mollusca	Steamer clam	*Mya arenaria*	Natura 2000 - H1110A
Mollusca	Blue mussel	*Mytilus edulis*	Natura 2000 - H1110A
Mollusca	European flat oyster	*Ostrea edulis*	OSPAR, Red list Germany - score 1
Mollusca	Sap-sucking slug	*Elysia viridis*	Red list Germany - score R
Chordata	Botrylloid	*Botrylloides leachii*	Red list Germany - score R
Chordata	Five-bearded rockling	*Ciliata mustela*	Natura 2000 - H1110A
Chordata	Rock gunnel	*Pholis gunnellus*	Natura 2000 - H1110A
Chordata	Short-spined sea scorpion	*Myoxocephalus scorpius*	Natura 2000 - H1110A

Table 4.1 List of species with special conservation status, which were found on the shellfish bed with flat oysters (*O. edulis*) and Pacific oysters in the period 2015–2017, in the Voordelta, Dutch part of the North Sea.

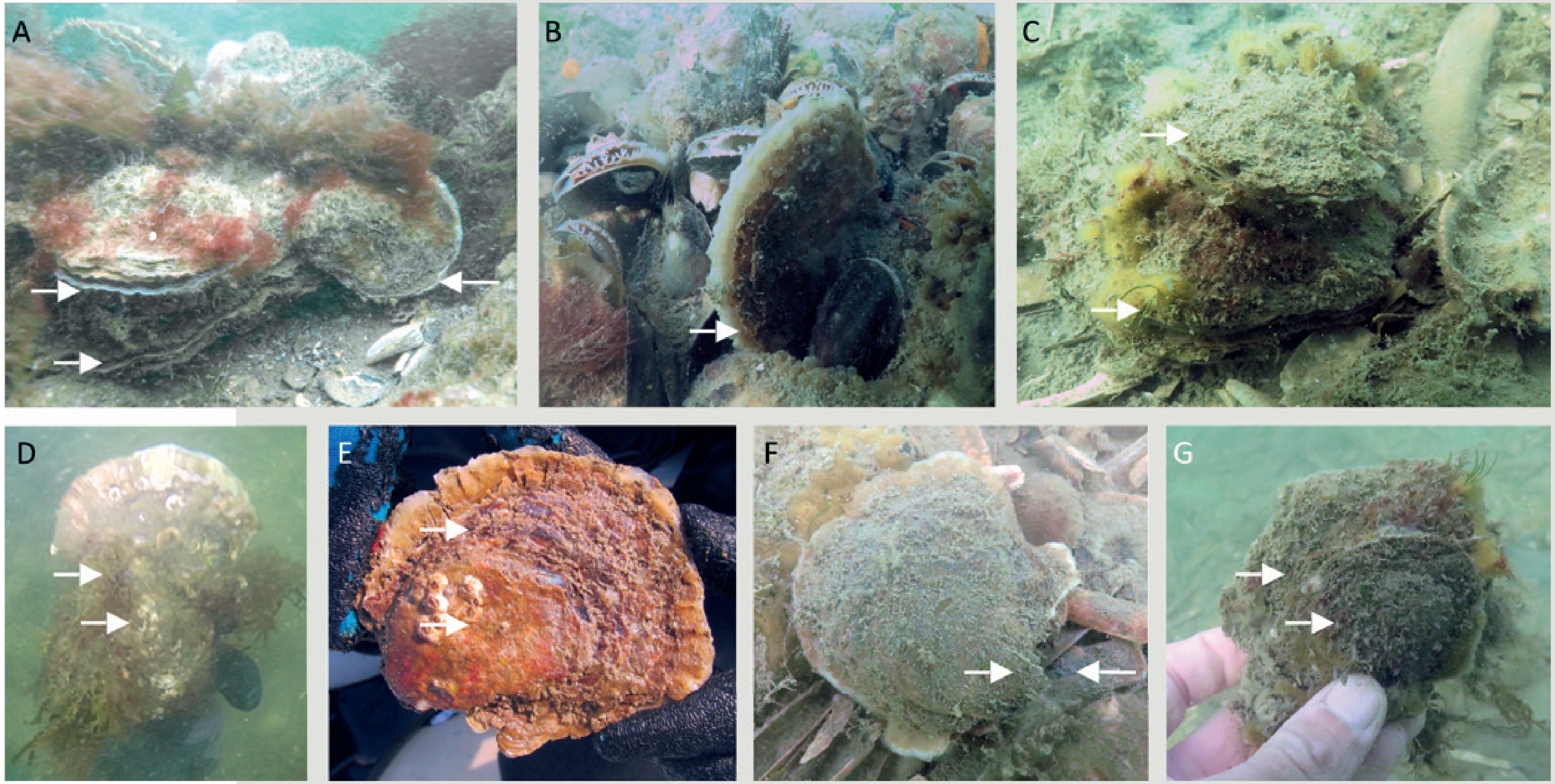


Figure 4.4 Native flat oysters (*O. edulis*) were found attached to several bivalve species that provided settling substrate for spat or recruits of this flat oyster (Voordelta, 2015-2017). A) Detail of a large flat oyster with several smaller flat oysters of different sizes attached on top, showing that the bed includes native flat oysters of different size classes (2015). All flat oysters have the flat, right valve facing upwards (© Wouter Lengkeek). (B) *O. edulis* in between and attached to blue mussels (2017). The oyster is positioned both standing straight up as well as C) positioned flat on the ground. Smaller flat oysters (spat or recruits) were found attached to larger flat oysters (C, E, G), to (D) Pacific oysters and to (F) American razor clams (2017). White arrows depict individual oysters.

4.4 Discussion and recommendations

At the Blokkendam location in the Voordelta, the first bed with flat oysters was discovered in the Dutch part of the North Sea. At this bed, we found oysters of multiple size classes (figure 4.2 and 4.4) showing that environmental conditions for flat oysters were suitable to allow their survival, growth, reproduction (yielding larvae, see Chapter 2) and settlement (see Chapter 3). Larval supply and availability of settlement substrate are the most important conditions for oyster bed development (Brumbaugh & Coen, 2009). This discovery shows that conditions for flat oyster recovery are suitable in the Voordelta and maybe also elsewhere in the North Sea area. Recent shellfish survey results suggest that the recovery is not limited to this single shellfish bed, as several flat oysters were also found in an area more than 6 km northwest of Blokkendam location (see Chapter 6). Besides, a flat oyster population was found in the Dutch Wadden Sea too, during a survey taking place in summer 2017 (Van der Have et al., 2018)

Our results may help to develop guiding principles for restoration and conservation of flat oyster beds in the Voordelta and elsewhere in Europe. We found that the native flat oyster used shells (live and dead) and shell fragments of bivalves, predominantly Pacific oyster, but also native blue mussels, invasive American razor clams, and the native common cockle as a settlement substrate. Our observations not only confirm the importance of substrate availability to restore oyster populations (Beck et al., 2011, Kennedy & Roberts, 2009), but also to the biocoenosis or assemblage of bivalves that were living in the same habitat (Smyth & Roberts, 2012; Zwerschke et al. 2016). Hence, to improve the selection process of suitable areas for flat oyster bed restoration, the availability of shell material ("shelliness") at the surface of soft sediments, should also be taken into account. Soft sediments areas with high availability of shell material are most suitable when selecting sites for flat oyster introduction to create or enhance the supply of larvae.

The Blokkendam bed shows that Pacific oysters and flat oysters share the shallow sublittoral (2 to 5 meter water depth) as habitat and that the former can facilitate the latter. Hence, in this habitat type, existing Pacific oyster beds should be important focal areas for flat oyster restoration projects.

Pacific oysters were found to live together and pave the way for native bivalves on other locations too. For example, co-existence between flat and Pacific oysters are reported in Ireland (Zwerschke et al., 2017) and the Wadden Sea (Van der Have et al., 2018). In the Wadden Sea, around 1990, mussel beds on the tidal flats were invaded by Pacific oysters, changing the bed from a mono-species (*M. edulis*) bed into a 2-species multi-layered mix bed of oysters and mussels (Reise et al., 2017). The relatively high survival rate of blue mussels in the Blokkendam shellfish bed underpins this point, although the mechanism remains unclear.

Our results show the importance of the shellfish beds with flat oysters as key habitat species in the Voordelta and elsewhere in the North Sea. Here we demonstrate that these beds not only enhance the available hard substrate in soft sediment ecosystems but also increase the species diversity and abundances of associated species, including several species of conservation interest (e.g. Dahlia anemone). These results support the value of restoring shellfish beds for biodiversity in general and for specific conservation goals in the North Sea. Although benthic assemblages associated with native and Pacific oysters can be similar (Zwerschke et al., 2016), the biodiversity is expected to increase when restoring flat oysters, due to the different spatial distribution of these two oyster species.

In contrast to flat oysters, Pacific oysters are restricted to a coastal (shallow) habitat, and therefore only the return of the flat oyster will allow for restoration of biogenic beds and enhancement of biodiversity in deeper offshore habitats. When flat oyster beds will return to offshore habitats in the North Sea, the net biodiversity and biomass of associated species is expected to be higher at a landscape scale, as epibenthic biogenic beds are currently absent there. One of the factors explaining the difference in distribution of the two oyster species are the different substrate requirements, as Pacific oysters attach more to rocks and native flat oysters attach more to shell material or living oysters.

Given the importance of the shellfish bed as knowledge base for understanding flat oyster development and as species-rich habitat in the Voordelta, we recommend proceeding with monitoring of the shellfish bed in 2018, along the same lines as in 2017. Special attention can be given to:

- Estimation of the abundance of large mobile fish and large mobile crustaceans.
- Exploration of the northward extension of the shellfish bed.
- Monitoring the availability of (dead) shells (‘shelliness’) in the soft sediments in and around the shellfish bed.
- Studying the settlement substrate of flat oysters in soft sediments without presence of Pacific oysters.

5 Analysis of the Bonamia parasite in the shellfish bed at the Blokkendam location

5.1 Introduction

The *Bonamia ostreae* parasite can cause a lethal disease in flat oysters. It is transferred from oyster to oyster. Hence, veterinary regulations impose strong restrictions to oyster transports from infected areas. However, if the disease is already present, these restrictions are superfluous.

Bonamia is present in Lake Grevelingen (Haenen & Engelsma, 2017). In order to know whether veterinary regulations allow flat oysters to be transported to the Voordelta area and to learn more about the spreading of *Bonamia* in a more general sense, it is important to know whether the parasite is present in the Voordelta population.

5.2 Method

In April 2017, a sample of 23 flat oysters larger than 7 cm shell width was collected at the pilot location Blokkendam. Previous sampling for *Bonamia* was done in December 2015. No *Bonamia* was found then (Sas et al. 2016). However, the parasite is more active in spring than in winter, so that the 2015 investigation may have resulted in underestimation of the prevalence of the parasite.

The sampled oysters were stored for 6 days at -20 °C until the samples were taken to Wageningen Bioveterinary Research for further analysis. The prevalence of the Bonamia parasite was determined with real time PCR.

5.3 Results

One of the 23 oysters sampled in 2017 tested positive for *Bonamia* spp. This amounts to a prevalence of 4% of the tested sample. The observation of *Bonamia* in 2017 confirms that it is indeed important to sample in spring instead of in winter.

5.4 Discussion and recommendations

A prevalence of 4% is low compared to the values found in Lake Grevelingen (23% in 2016; Haenen & Engelsma, 2017). However, it does show that *Bonamia* is present in the Voordelta population. The low prevalence may be related to the relatively low density of flat oysters in the Voordelta compared to the higher densities locally in Lake Grevelingen. The *Bonamia* presence in both populations confirms the close relationship between the Grevelingen and Voordelta populations.

Since *Bonamia* is confirmed to be present in the Voordelta area, there should be no veterinary restriction against employing flat oysters from Lake Grevelingen in the Voordelta area. It should be kept in mind that other types of transport restrictions may still apply. For instance, several types of oyster drills are present in lake Grevelingen, and these should obviously not be imported into the Voordelta by flat oyster transports.

6 Evaluation of promising locations for shellfish bed restoration in the Voordelta

6.1 Introduction

For 2018, an extension of the flat oyster population in another part of the Voordelta area is foreseen, among others to demonstrate that bed formation at a new location is possible on the basis of the knowledge created in the current project. Therefore, it was investigated whether there are other suitable locations present in the area.
The occurrence of any species of epibenthic bivalve shellfish at a certain location is considered to be an indication that this location is suitable for other epibenthic shellfish species as well. Shellfish survey data were collated in order to identify such locations.

6.2 Method

WMR regularly carries out benthic surveys in Dutch coastal waters. These include, among others:

- Shellfish surveys for the national government: since 1993, assigned by the ministry of Agriculture, Nature and Fishery (LNV).
- General benthic surveys: since 2009, assigned by the Port of Rotterdam (near Hinderplaat these surveys ended in 2015).

These survey data were checked for occurrence of flat oysters, Pacific oysters and adult mussels. Seed mussels were not considered, since many seed mussels do not remain after settlement, as they can easily be washed away during storms or predated by starfish and crabs.

The sampling equipment used is a towed bottom dredge with a knife. This dredge has a width of 10 cm and has a depth of 10 cm (figure 6.1). The dredge is towed for a known distance of 75-150 m.

6.3 Results

Figure 6.2 shows all locations sampled in the Voordelta since 1993. These include transects sampled in the context of a project studying benthic effects of shrimp fisheries, which show up in the maps as straight sampling point lines.
In January 2017 an extra survey was carried out, specifically aimed at locations with mussel seed from the large spat fall of 2016.

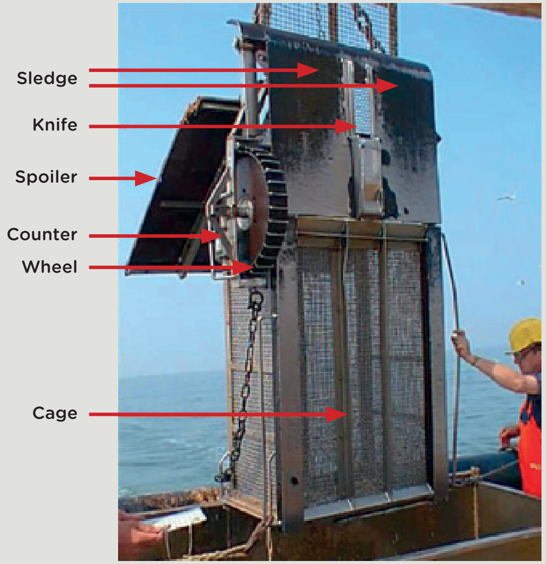


Figure 6.1 Towed bottom dredge with knife in front and box collecting sample in back.

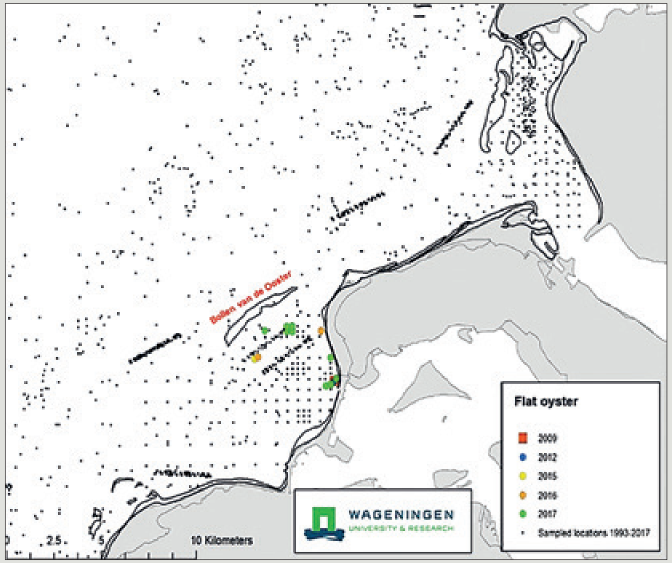


Figure 6.2 Survey points (black dots) and flat oyster observations in the Voordelta (coloured dots), found during WMR surveys in the period 2009-2017.

Figure 6.3 Flat oysters found in the Voordelta during the WMR mussel seed survey in January 2017.

Observations of flat oysters are also indicated in Figure 6.2. These are observed since 2009. All observations are restricted to the northern part of the mouth of Lake Grevelingen. Most observations occur near the Blokkendam location. In 2016 and 2017, several flat oysters were also found at the lee (southeast) side of the Bollen van de Ooster, the tidal flat to the northwest of the Blokkendam. The flat oysters found in 2017 are shown in figure 6.3. Their shell width ranged from 6 to 9 cm.

As oyster larvae are observed at Outlet and Blokkendam location, while water currents are dominantly to the north from there (Sas et al., 2016), this overall distribution pattern is in line with expectations.

The observed flat oyster densities varied between 0.2 and 3 per m^2 (Table 6.1). This is lower than reported at Blokkendam location (see par. 4.3), but survey methods are different. In particular, the survey samples reported here consist of 75-150 meters long dredges. Along this length, bare patches intersperse with oyster-rich patches.

The distribution of the Pacific oysters largely overlaps with the distribution of flat oysters (figure 6.4). Again, some individuals were found near the Bollen van de Ooster. Average water depth in the area where the Pacific oysters are found is similar to Blokkendam location (up to -5 meter; see figure 1.1). Pacific oysters are also found at a location further north, near the Hinderplaat, a tidal flat in the mouth of the Haringvliet.

Adult mussel distribution pattern (figure 6.5 and 6.6) overlaps with the Pacific oyster. They were observed both in the northern part of the mouth of Lake Grevelingen, near the Bollen van de Ooster and near the Hinderplaat in the mouth of the Haringvliet.

Although the distribution of mussels is widespread, the population immediately after spatfall is much larger. This was clearly shown in 2016, when most of the large spatfall population disappeared soon after settlement, being predated by starfish and crabs. This underpins the protective function of the shellfish bed near Blokkendam for mussels, which was also discussed in Chapter 5.

Year	Survey	Number per m^2
2009	PMR	0.19
2012	PMR	0.49
2015	WOT	3.00
2016	PMR	0.25
2016	PMR	0.32
2017	Mussel seed WOT	0.29
2017	Mussel seed WOT	0.23
2017	Mussel seed WOT	0.97
2017	Mussel seed WOT	0.54
2017	Mussel seed WOT	1.26
2017	Mussel seed WOT	1.02
2017	Mussel seed WOT	2.28
2017	PMR	0.25
2017	PMR	0.40
2017	WOT	0.26

Table 6.1 Density of flat oysters at locations shown in figure 6.2, as determined with bottom dredge during WMR surveys.

6.4 Discussion and recommendations

The survey data suggest that flat oysters are showing spreading capacity from the Lake Grevelingen outlet and the Blokkendam location. The direction is to the north and to the lee side of de Bollen van de Ooster. On most locations where flat oysters were found, Pacific oysters and mussels were also observed. This is in line with the observation of the co-occurrence of these three species in the Blokkendam bed.

We also observed flat oyster larvae in the water column near the Bollen van de Ooster (see par. 2.3).

Hence, on the basis of the surveys, as well as larval sampling, the area directly to the southeast of the Bollen van de Ooster is expected to be best suited for new flat oyster pilots. Probably, the whole area between Blokkendam and Bollen van de Ooster is also suitable, given the regular shellfish observations there. In other parts of the Voordelta much lower shellfish quantities were observed in the surveys, so these are probably much less favourable.

Obviously, bottom-dredging fishery should not occur at the pilot location. This is the case in the N2000 protection zone, which surrounds the Bollen van de Ooster (Beheerplan Voordelta, 2008). Therefore, it is recommended to plan this pilot within the protection zone, near the area where flat oysters, Pacific oysters and mussels were found.

The recommended search area for this new location is presented in figure 6.7.

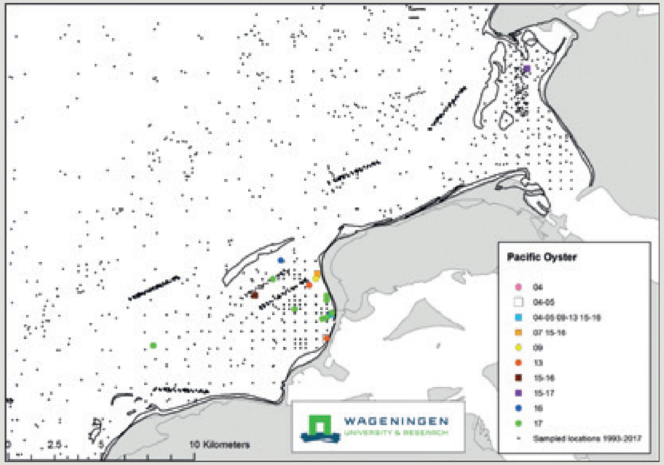


Figure 6.4 Pacific oyster observations in the Voordelta determined during WMR surveys in the period 2004-2017.

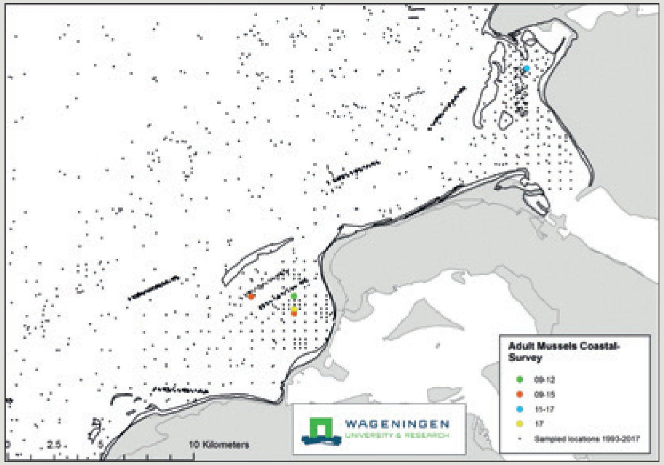


Figure 6.5 Adult mussel observations in the Voordelta determined during WMR surveys for LNV (WOT) in the period 2006-2015.

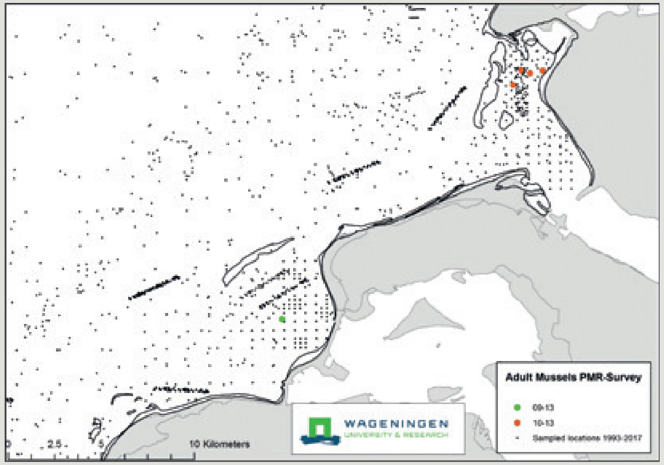


Figure 6.6 Adult mussel observations in the Voordelta determined during WMR surveys for PMR in the period 1993-2017. Surveys near the Hinderplaat ended in 2015.

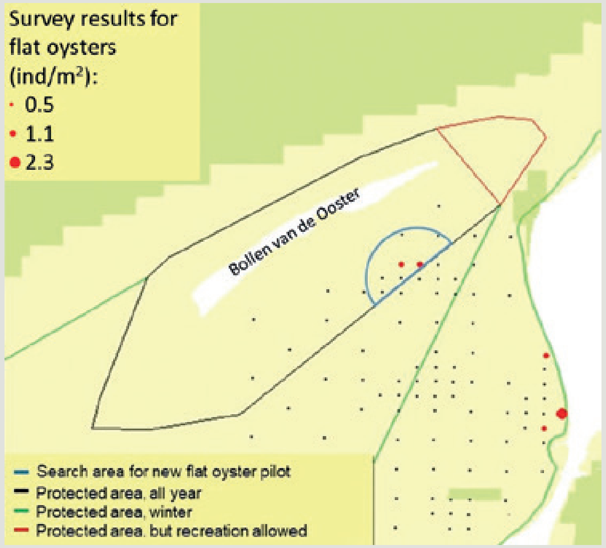


Figure 6.7 Search area for new flat oyster pilot near Bollen van de Ooster.

7 Overall conclusions and recommendations

The main conclusions and recommendations on the basis of flat oyster larval monitoring and spat collection are:

- In 2017, as in 2016, we observed flat oyster larvae in the water of the Voordelta. Yet, the maximal numbers of flat oyster larvae recorded in 2017 were lower than in 2016. Because of the high water temperature in spring 2017, the larval abundance peak may have been earlier than June, so that we have probably missed the maximum values in that year.
- The strategy adopted in 2017 for flat oyster spat collection, with weekly deployment of clean (mussel and Pacific oyster) shells was successful. Clearly, deploying spat collectors during winter, as was done in 2016, is too early, supporting the hypothesis that flat oyster settlement is very sensitive to collector fouling. Yet, we may have missed part of the very early spawning peak of 2017 with spat collection too.
- Our experiments with spat collection seem to indicate that the early spawning peak of flat oysters has a much higher settlement capacity than peaks later in the season. We do not yet understand the mechanism behind this, but this early peak may be very important for successful settling. We recommend measuring water temperature from the very start of the growing season onwards and use this as indicator for timely deployment of weekly spat collectors, as well as larvae monitoring, for experiments in 2018. This will enable investigation of settlement effectiveness during the full spawning period and thereby also to find out whether the higher settlement capacity of the early spawning peak, as observed in 2017, is reproducible.

Since spat collection is already proven to be successful, we recommend extending the number of locations to more than only Blokkendam location. If possible, even in Lake Grevelingen. Collected spat can be used to enhance the Voordelta flat oyster population and/or start another pilot there.

On the basis of observations made in the Wadden Sea, we also recommend to employ cockleshells additionally to mussel and oyster shells in spat collectors.

The main conclusions and recommendations on the basis of the 2017 structure and biodiversity monitoring of the Blokkendam shellfish bed are:

- Pacific oysters and flat oysters share the marine shallow sublittoral (2 to 5 meter water depth) as habitat. Probably, the former facilitate the latter. Mussels profit from the oyster presence too. Hence, in the shallow sublittoral, existing Pacific oyster beds are focal areas for shellfish bed restoration projects in general and flat oyster bed restoration in particular.
- Flat oysters use shells (live and dead) and shell fragments of bivalves, predominantly Pacific oyster, but also other shell types as settlement substrate. In the Wadden Sea, during a separate survey undertaken in 2017 and in an area with many different types of shell material, it was observed that flat oysters often settle on empty cockleshells. We therefore recommend employing clean cockleshells in spat collectors, beside mussel and Pacific oyster shells.
- A shellfish bed with flat oysters as key habitat species not only enhances the available hard substrate in soft sediment ecosystems but also enhances the species diversity and abundances of associated species, including several species of conservation interest (e.g. Dahlia anemone). These results support the value of restoring shellfish beds for biodiversity in general and also for specific conservation goals in the North Sea. Although benthic assemblages associated with native and Pacific oysters can be similar, the biodiversity is expected to increase when restoring flat oyster beds, due to the different spatial distribution of these two oyster species.

Given the biodiversity on the shellfish bed at Blokkendam, it is recommended that this bed be protected under the Dutch Nature Conservation Law.

Since the shellfish bed is also a rich knowledge base for understanding flat oyster development and the associated species in the Voordelta, we recommend continuing monitoring of the bed in 2018. Special attention should be given to:

- Estimation of the abundance of large mobile fish and large mobile crustaceans.
- Exploration of the northward extension of the shellfish bed.
- Monitoring the availability of (dead) shells (“shelliness”) in the soft sediments in and around the shellfish bed.
- Studying the settlement substrate of flat oysters in soft sediments without presence of Pacific oysters.

Testing for the *Bonamia* parasite shows that it is present in the flat oyster population of the Voordelta. This once again shows the relation between the Voordelta and Lake Grevelingen populations. Because of this, there should be no ‘*Bonamia*-based’ veterinary restrictions on flat oyster transports between the two systems. Other restrictions will still apply, in particular related to transports of invasive alien species, such as oyster drills.

8 Recommendations for the design of flat oyster bed restoration pilots

In Chapter 6, we identified the southeast side of the Bollen van de Ooster as the best location for a new flat oyster bed restoration pilot in the Voordelta. Preferably within the N2000 protection zone, which surrounds the Bollen van de Ooster, since bottom-dredging fishery is excluded there. But also elsewhere in the North Sea region, there is an increase in the number of areas where bottom disturbance by human activities is excluded, so that flat oyster bed restoration attempts could be successful.

To this end, it is important to know the primary conditions for a successful attempt for restoration and how such a pilot should be designed and monitored. Here we summarize detailed knowledge obtained in this and previous studies, on several crucial aspects that can influence the success of a restoration attempt:

Site selection

General conditions
Primary conditions for survival, growth, reproduction and recruitment of flat oysters are discussed in (Smaal et al., 2017). The report concentrates on the environmental conditions in wind parks (existing and planned) in the Dutch North Sea area, but it also gives an overview of suitable conditions in a wider area. It is shown that most primary requirements, such as food availability, oxygen concentration, water depth and temperature, salinity and current velocity are suitable in the Dutch North Sea at large. Bottom composition (sand and silt) is overall mostly suitable too for flat oysters, but should be investigated in more detail for a candidate location, as well as other physical conditions, such as sediment motility (sand waves) and bottom shear stress (by current and wave force).

Bottom shear stress
Our Voordelta experience indicates that the bottom shear stress threshold is possibly underestimated by (Smaal et al., 2017), since the shellfish bed at Blokkendam/Brouwersdam is thriving in an area with relatively high bottom shear stress. Hence, the suitable area is probably even larger than predicted by (Smaal et al., 2017).

Bottom disturbance
An oyster bed may take decades to develop. A suitable location is free of bottom disturbance for such a prolonged period of time.

Presence of other epibenthic shellfish beds
Presence of other epibenthic shellfish beds, such as mussels and Pacific oysters in relatively shallow water (< 5 meter depth) and horse mussels in deeper waters, is a good indicator of suitable conditions for flat oysters. But it is not a condition sine qua non. Presence of shellfish and other biota should be checked, to verify the T=0 situation. The T=0 survey can be done simultaneously with the investigation of the physical sea floor condition.

Presence of shell material
Presence of shell material (remnants of dead shellfish) is an essential condition, since it provides substrate for larval settlement. Our investigations, in Voordelta as well as Wadden Sea, has shown that flat oyster larvae can settle on a variety of shell types, such as Pacific oysters, blue mussels and cockles.

Field deployment

Providing adult oysters/creating a larvae source
An essential condition for bed formation and restoration is the presence of larvae in the water column. Hence, introduction of oysters on or near the sea floor at a location where a bed is planned is required, in order to locally enhance the number of larvae. Since it is known that oysters younger than 3 years old are all male and adult oysters can be female, both age classes should be deployed when trying to create a bed, in order to have a reproductive population in place.

Method for providing adult oysters
To allow for monitoring of the condition of the introduced oysters, their containment and fixation in containers, attached on or in racks, is required. To this end, the racks should be designed in such a way that divers can inspect them and remove the oysters or the total rack should be hoistable to the surface. The racks should be sufficiently sturdy and heavy, to survive the rough North Sea conditions. In order to retrace them, their position must be determined accurately. Reef domes (see below) can be deployed around the racks in order to assist traceability.

Adding shell material
If empty shells are not present in sufficient quantity, these should be provided in and around the planned location. We have shown that clean substrate material should be deployed when a high concentration of larvae is present in the water column.

Providing spat collectors
One of the most essential parameters for success is recruitment of oysters in a new location. Recruitment can be studied by providing spat collectors. Spat collectors (sacks with empty and clean shells, which can be collected after the growing season) can be deployed around the pilot when larvae are in the water.

Protection
It is strongly believed that no area in the North Sea is guaranteed free from bottom disturbance. Therefore, we recommend installing physical barriers around any oyster pilot. Heavy concrete reef domes have been used before for this purpose and provide extra substrate and habitat for bed-associated biodiversity in general (which often is a restoration goal that should follow with successful oyster bed restoration). Besides, they are instrumental in retracing a pilot in the often, turbid North Sea waters.

Monitoring

In order to determine success and gain better knowledge for future restoration attempts, at least the following parameters should be monitored:

- Survival of the introduced adults (inspection by divers, retrieving to the surface and/or valve position monitoring devices).
- Reproduction (gonad condition in the adults and/or larvae sampling).
- Recruitment (sampling of substrate or retrieving spat collectors).
- General condition of all deployed materials (cages, oysters, spat material, other substrates) (by divers, ROV/dropdown video and/or by retrieval to surface).

On this basis of this, the recommended layout of a new pilot is as follows (illustrated in figure 8.1):

- Adult oysters are introduced in containers attached to racks solidly placed on the sea floor.

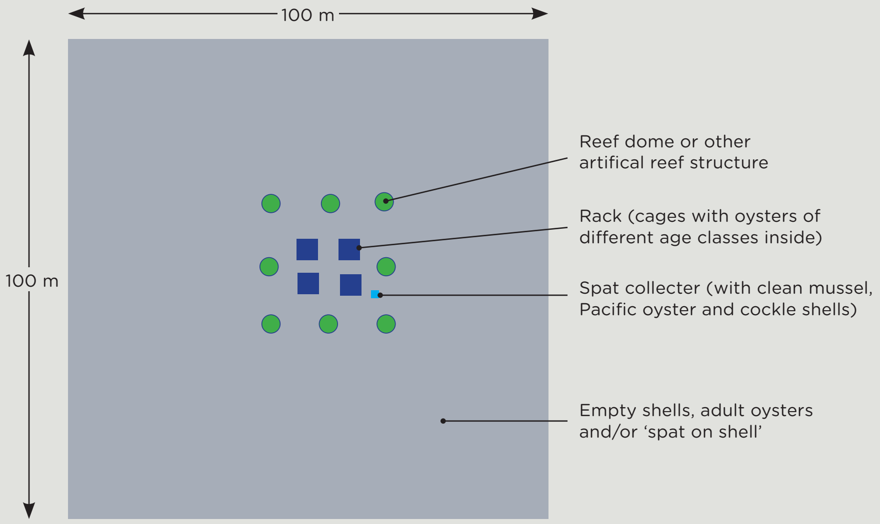


Figure 8.1 Basic layout of a flat oyster bed restoration pilot.

- Empty and clean shell material is to be distributed around the pilot, at the right time (onset of larvae swarming).
- Spat collectors can be deployed, in the manner recommended above.
- Spat collected on empty shells elsewhere ('spat on shell') can be distributed around the pilot, in order to speed up bed development.

Besides, artificial structures such as bed balls can be deployed in or around the pilot, for general biodiversity enhancement, protection against disturbance and traceability of the pilot.

As stated, the racks are instrumental to monitoring: by hoisting them up to the surface, survival, growth and reproduction capacity of the oysters can be monitored on a regular basis. Preferably, monitoring should be done at least twice a year: before and after the growing season. If spat collectors are deployed, they should be collected and inspected on the occurrence of spat settlement after the growing season. Since spat can be small, inspection should be done in the laboratory. In a later stage, samples can be taken of the substrate, which was distributed at the pilot location, to be inspected in the laboratory for the occurrence of spat settlement too.

Literature

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Defeo, O., Edgar, G. J., Hancock, B., Kay, M. C., Lenihan, H. S., Luckenbach, M. W., Toropova, C. L., Zhang, G. F. & Guo, X. M. 2011. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. Bioscience, 61(2), pp 107-116.

Beheerplan Voordelta. 2008. Spelregels voor natuurbescherming, Ministerie van Verkeer en Waterstaat, Provincie Zeeland en Provincie Zuid-Holland.

Brumbaugh, R. D. & Coen, L. D. 2009. Contemporary approaches for small-scale oyster reef restoration to address substrate versus recruitment limitation: a review and comments relevant for the olympia oyster, Ostrea lurida carpenter 1864. Journal of Shellfish Research, 28(1), pp 147-161.

Haenen, O.L.M., Engelsma, M.Y. 2017. Jaarverslag schelpdierziekten 2016; Resultaten van het onderzoek naar ziekten in schelpdierbestanden van het Grevelingenmeer en de Oosterschelde. Wageningen Bioveterinary Research, WBVR Report 1706707.

Kamermans, P., Brummelhuis, E., Poelman, J., van Gool, A. and Troost, K. 2004. Onderzoek naar verbetering broedvangst oesters. Nederlands Instituut voor Visserij Onderzoek (RIVO) BV report C003/04.

Kennedy, R. J. & Roberts, D. 2006. Commercial oyster stocks as a potential source of larvae in the regeneration of Ostrea edulis in Strangford Lough, Northern Ireland. Journal of the Marine Biological Association of the United Kingdom, 86(1), pp 153-159.

Korringa, P. 1940. Experiments and observations on swarming, pelagic life and setting in the European flat oyster, Ostrea edulis L. Archives Neerlandaises de Zoologie 5: 1–249.

Korringa, P. 1947. Relations between the moon and periodicity in the breeding of marine animals. Ecological Monographs 17: 347-381.

Ministry of Infrastructure and Environment. 2015. Mariene Strategie voor het Nederlandse deel van de Noordzee 2012-2020. Deel 3 KRM-programma van maatregelen, Bijlage 5 bij het Nationaal Waterplan 2016-2021. (Den Haag).

Reise, K., Buschbaum, C., Büttger, H. & Wegner, M. K. 2017. Invading oysters and native mussels: from hostile takeover to compatible bedfellows. Ecosphere, 8(9), pp.

Robert R., E. His, A. Dinet. 1988. Combined effects of temperature and salinity on fed and starved larvae of the European flat oyster Ostrea edulis. Marine Biology 97: 95-100.

Sas, H., Kamermans, P., van der Have, T., Lengkeek, W. & Smaal, A. 2016. Shellfish reef restoration pilots: Voordelta The Netherlands, annual report 2016.

Smaal, A. C., Kamermans, P., van der Have, T., Engelsma, M. Y. & Sas, H. 2015. Feasibility of Flat Oyster (Ostrea edulis L.) restoration in the Dutch part of the North Sea.

Smaal, A.C., Kamermans, P., Kleissen, F., van Duren, L., van der Have, T., Platte oesters in offshore windparken, Wageningen University & Research Report C035/17, 2017.

van den Brink A. 2012. OYSTERECOVER: The Efficiency of Different Types of Oyster Spat Collectors for Ostrea edulis. IMARES Report number C095/12.

van den Brink A., C. Fomsgaard, M. Nédélec, M. Hussenot, P. Kamermans. 2013. OYSTERECOVER: Testing the efficiency of different Spat Collectors. IMARES Report number C216/13.

Van der Have, T., P. Kamermans, E. van der Zee. 2018. Flat oysters in the Eijerlandse Gat,

Wadden Sea, Results of a survey in September 2017, Programme towards a Rich Wadden Sea.

Walne, P.R. 1974. Culture of bivalve molluscs; 50 years of experience at Conwy. Fishing News Books Ltd, Farnham-Surrey-England, 189 pp.

Zwerschke, N., Emmerson, M. C., Roberts, D. & O'Connor, N. E. 2016. Benthic assemblages associated with native and non-flat oysters are similar. Marine Pollution Bulletin, 111(1-2), pp 305-310.

Zwerschke, N., Kochmann, J., Ashton, E., Crowe, T., Roberts, D. & O'Connor, N. 2017. Co-occurrence of native Ostrea edulis and non-native Crassostrea gigas revealed by monitoring of intertidal oyster populations. Journal of the Marine Biological Association of the United Kingdom, 1-10.

Appendix: Species list on shellfish bed

List of species that were found on the shellfish bed consisting of flat oysters, Pacific oysters and mussels in the Voordelta, Dutch part of the North Sea, in the period 2015–2017 during scuba diving surveys and activities.

Phylum	Scientific name	English name	Dutch name	Conservation status	Method
Ochrophyta	*Phaeophyceae*	Brown algae	Bruinwier		T
	Sargassum muticum	Wireweed	Japans bessenwier		T
Rhodophyta	*Rhodophyta*	Red algae	Roodwier		T
Chlorophyta	*Ulva*	Green laver	Zeesla		T
Annelida	*Eulalia viridis*				
	Lanice conchilega	Sand mason worm	Schelpkokerworm	Natura 2000 - H1110A	T
	Lepidonotus squamatus				
	Mysta picta				
	Neoamphitrite figulus	Johnston's ornate worm	Slijmkokerworm		T
	Nereimyra punctata				
	Phyllodoce groenlandica				
	Polydora ciliata	Bristle worm	Slikkokerworm		T
	Sabella pavonina	Peacock worm	Waaierkokerworm		T
	Sabellaria spinulosa				
	Syllis gracilis				
Bryozoa	*Bryozoa*	Sea mat	Mosdiertjes		T
	Crisularia plumosa	Feather-bryozoan	Gepluimde hoorncelpoliep		T
	Conopeum reticulum				
Cnidaria	*Hydrozoa*	Hydrozoan	Hydroidpoliepen		T
	Urticina felina	Dahlia sea anemone	Zeedahlia	Red list Germany - score G	T
	Diadumene cincta	Orange anemone	Groene golfbrekeranemoon		T
	Sagartia elegans	Elegant anemone	Sierlijke slibanemoon		T
	Sagartia troglodytes	Mud sagartia	Slibanemoon		T
	Sagartiogeton undatus	Small snakelocks anemone	Weduweroos		T
	Metridium dianthus	Plumose anemone	Zeeanjelier		T
Arthropoda	*Austrominius modestus*				
	Balanus crenatus				
	Cancer pagurus	Edible crab	Noordzeekrab		T
	Carcinus maenas	Shore crab	Strandkrab		T
	Crangon crangon	Common shrimp	Gewone garnaal		T
	Decapoda	Decapod	Krabben juv.		T
	Hemigrapsus sanguineus	Pacific crab	Blaasjeskrab		T
	Hemigrapsus takanoi	Japanese crab	Penseelkrab		T
	Homarus gammarus	European lobster	Europese zeekreeft	Red list Germany - score 2	O
	Jassa herdmani				
	Liocarcinus navigator	Arch-fronted swimming crab	Gewimperde zwemkrab		T
Arthropoda	*Macropodia rostrata*	Long legged spider crab	Hooiwagenkrab		T
	Monocorophium acherusicum				
	Necora puber	Velvet swimming crab	Fluwelen zwemkrab	Red list Germany - score R	T

Phylum	Scientific name	English name	Dutch name	Conservation status	Method
Arthropoda	*Pagurus bernhardus*	Hermit crab	eremietkreeft		T
	Palaemon elegans	Grass prawn	Gewone steurgarnaal		T
	Palaemon macrodactylus	Oriental shrimp	Rugstreepsteurgarnaal		T
	Palaemon serratus	Common prawn	Gezaagde steurgarnaal		T
	Pilumnus hirtellus	Hairy crab	Ruigkrabje	Red list Germany - score 3 T	
	Porcellana platycheles	Great-clawed crab	Harig porceleinkrabbetje		T
	Praunus flexuosus	Chameleon shrimp	Geknikte aasgarnaal		T
Echinodermata	*Ophiothrix fragilis*	Common brittle star	Brokkelster		T
	Amphipholis squamata	Dwarf brittle star	Levendbarende slangster		T
	Asterias rubens	Common starfish	Zeester		T
Mollusca	*Lepidochitona cinerea*	Grey chiton	Asgrauwe keverslak	Red list Germany - score 3	O
	Crepidula fornicata	Slipper shell	Muiltje		T
	Venerupis corrugata	Pullet carpet shell	Gewone Tapijtschelp		T
	Mya arenaria Steamer clam	Strandgaper	Natura 2000 - H1110A		T
	Mytilus edulis	Blue mussel	Mossel	Natura 2000 - H1110A	T
	Ostrea edulis	European flat oyster	Platte oester	OSPAR, Red list Germany - score 1	T
	Magallana gigas	Pacific oyster	Japanse oester		T
	Nassarius reticulatus	Netted dogwhelk	Gevlochten fuikhoren		T
	Goniodoris castanea	-	Bruine plooislak		T
	Elysia viridis	Sap-sucking slug	Groene wierslak	Red list Germany - score R	T
	Aeolidia papillosa	Shag-rug aeolis	Grote vlokslak		T
Porifera	*Cliona celata*	Yellow boring sponge Boorspons			T
	Haliclona (Haliclona) oculata	Mermaid's glove horny sponge Geweispons			T
	Haliclona (Soestella) xena	-	Paarse buisjespons		T
	Porifera sp.	Sponges	Sponzen		T
	Hymeniacidon perlevis	-	Bleke piekjesspons		T
	Halichondria (Halichondria) panicea	Bread-crumb sponge	Gewone broodspons		T
Chordata	*Ciona intestinalis*	Yellow sea squirt	Doorschijnende zakpijp		T
	Didemnum vexillum	Compound sea squirt	Druipzakpijp		T
	Botrylloides violaceus	Colonial sea squirt	Eenkleurige botrylloides		T
	Botryllus schlosseri	Star sea squirt	Gesterde geleikorst		T
	Aplidium glabrum	-	Glanzende bolzakpijp		T
	Diplosoma listerianum	-	Grijze korstzakpijp		T
	Styela clava	Rough sea squirt	Japanse knotszakpijp		T
	Botrylloides leachii	Botrylloid sea squirt	Tweekleurige botrylloides	Red list Germany - score R	T
	Ascidiella aspersa	Dirty sea squirt	Ruwe zakpijp		T
	Ciliata mustela	Five-bearded rockling	Vijfdradige meun	Natura 2000 - H1110A	T
	Pholis gunnellus	Rock gunnel	Botervis	Natura 2000 - H1110A	T
	Gadus morhua	Cod	Kabeljauw		O
	Parablennius gattorugine	Tompot blenny	Gehoornde slijmvis		T
	Myoxocephalus scorpius	Short-spined sea scorpion	Gewone zeedonderpad	Natura 2000 - H1110A	T
	Taurulus bubalis	Sea scorpion	Groene zeedonderpad		T
	Gobius niger	Black goby	Zwarte grondel		T
	Gobiusculus flavescens	Two-spotted goby	Ruthensspar grondel		T
	Atherina boyeri	Big-scale sand-smelt	Kleine Koornaarvis		O

Acknowledgements

We want to thank M. Dorenbosch, D. Beuker, P. van Rodijnen and H. Waardenburg for their help in the field and Jack Perdon for preparing the distribution maps. R. Stijvers and M. Wildschut assisted in measuring the collected mussels.