Received: 11 September 2019 | Revised: 29 May 2020 | Accepted: 20 June 2020
DOI: 10.1002/aqc.3427

SPECIAL ISSUE ARTICLE

Optimization of off-bottom spat collectors for restoration and production of the European flat oyster (*Ostrea edulis*) in Dutch coastal waters

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Funding information
ARK Nature; EU FP7 Support 2008-2 for SME's project Oysterecover, Grant/Award Number: 243583; Ministry of Agriculture, Nature Management and Food Safety; WWF Netherlands

Abstract

1. Efficient spat collection is essential to both the successful restoration and cultivation of the European flat oyster (*Ostrea edulis*).
2. The results of four different experiments investigating larval abundance, and the use of seven different types of suspended spat collectors in seven locations in the SW Netherlands were compared. These different types included Chinese hats, Vexar mesh, PVC tubes, and four types of bivalve shells.
3. Larval abundance, and timing of peaks in abundance for both *O. edulis* and the non-indigenous Pacific oyster (*Crassostrea gigas*) varied between locations and years.
4. The most successful spat collectors were sacks of bivalve shells.
5. In general, most spat were observed on collectors deployed 1–2 weeks after the peak in larval abundance was detected.
6. Fouling was heavy and may have prevented larval settlement on collectors deployed too early. Suspended sacks of bivalve shells show great promise as *O. edulis* spat collectors intended for reef restoration purposes but may need further development for cultivation application.

KEYWORDS
aquaculture, coastal, estuary, invertebrates, new techniques, *Ostrea edulis*, reproduction, restoration

1 | INTRODUCTION

The European flat oyster (*Ostrea edulis*), was once widespread in the southern North Sea (Olsen, 1883). The flat oyster not only acted as a keystone species for a rich and productive ecosystem, but also served as an economically profitable fisheries product (Gercken & Schmidt, 2014). Over recent decades, *O. edulis* has declined considerably due to increased mortality as a result of overfishing around the end of the 19th and beginning of the 20th centuries (Gercken & Schmidt, 2014) and more recently due to the parasite *Bonamia ostreae* (Engelsma et al., 2010). However, the flat oyster population in the Dutch Delta area is showing signs of recovery, particularly in Lake Grevelingen (Smaal, Kamermans, Van der Have, Engelsma, & Sas, 2015). This may therefore be the ideal time to attempt to restore flat oyster reefs. Recently, several *O. edulis* restoration projects have been set up in the North Sea, off the coast of the Netherlands (e.g. Pogoda et al., 2019). Efforts to restore *O. edulis* in the North Sea and Dutch delta are motivated by: 1) restoring the local ecosystem and increasing the biodiversity in and around the reefs; 2) utilizing the possible ability of the reefs to reduce erosion around the base of planned wind turbines; and 3) the economic possibility of restoring the flat oyster market to its former glory.

Restoring *O. edulis* in the North Sea is not a simple task. The oyster experiences environmental pressures and consequent population bottlenecks at every life stage. The spawning success of adults can vary between locations and seasons (Korringa, 1940). In addition, flat oysters are a brooding species, i.e. the eggs are fertilized within the mantle cavity where they develop into larvae over 6–10 days (Korringa, 1940). Subsequently, *O. edulis* larvae are released into the water column and after a pelagic stage of about 10 days the larvae search for suitable hard substrate on which to settle and develop into oyster spat, this last stage is called spatfall. There is generally a considerable loss of individuals between the larval and spat phases, potentially due to unsuccessful metamorphosis, predation of larvae in the water column, and the lack of appropriate substrate on which to settle (Filgueira, Brown, Comeau, & Grant, 2014; Korringa, 1940). Settled spat is also under pressure from predation, and from competition for space with conspecifics or other settling organisms (Engelsma et al., 2010). Since its introduction in the Netherlands, the Pacific oyster (*Crassostrea gigas*) has become widely established in the same waterbodies that *O. edulis* occupies (Troost, 2010). Larvae of both oyster species are often found at similar times in the water column, and as spat, the two oyster species compete for space on appropriate substrate (Smaal & Lucas, 2003). However, there is evidence suggesting that adult *C. gigas* may facilitate settlement of larvae and survival of juvenile *O. edulis* by providing substrate and refuge from predation (Christianen et al., 2018).

The pressure on the survival of the oysters is further confounded by the presence of the parasite *B. ostreae*, which affects the health of the oyster and increases mortality at a later age (Engelsma et al., 2010). The cumulative effect of environmental pressures at each life stage results in a paucity of healthy, surviving adults. Providing more appropriate substrate for larvae to settle onto will relieve some of this pressure at a particularly vulnerable life stage and may enhance the amount of spat available to grow into adults. More surviving adults may increase the success of restoring oyster reefs and compensate for the lower survival rate of farmed oysters caused by *B. ostreae*.

Spat collectors are structures designed to provide artificial surfaces for spat to settle onto and grow to a size where they can be collected. The amount of *O. edulis* spat collected is influenced by the availability, type, and placement of the substrate provided. In general, oysters from the family Ostreidae typically prefer calcium carbonate rich substrate such as the shells of other bivalves (Smyth, Mahon, Roberts, & Kregting, 2018). Dutch oyster farmers generally use loose mussel shells scattered on the sea floor as spat collectors. These are later collected and transported to culture plots. This method of sowing loose shells minimizes fouling compared with off-bottom collectors (P. Kamermans, personal observation). However, the efficiency of this method is dependent on collecting as many of the scattered shells as possible after the spatfall. In addition, spat growing on sowed shells probably risk being smothered by bottom sediment, and are possibly more vulnerable to predation by crabs, fish, sea stars and oyster drills compared with those growing on off-bottom structures. Off-bottom spat collectors may therefore provide an alternative method of spat collection with lower mortality rates.

Various off-bottom spat collector types are currently in use in Europe to collect the larvae of *O. edulis* including, trestles (tables with bags of shells), and stacked PVC cones (‘Chinese hats’) and tubes sometimes with a calcium based coating (Freeman & Denny, 2003; Van den Brink, 2012). The objective of these spat collectors is to concentrate the number of oyster spat onto a relatively small surface area so that the spat can be efficiently gathered, removed from the collector, transplanted, and reared. The efficiency of a collector is influenced by several factors including geographic location, depth of the collectors in the water column, temperature, and competition for space with other fouling species such as the introduced Pacific oyster *C. gigas* (Bataller, Burke, Ouellette, & Maillet, 2006). For the commercial cultivation of oysters as well as for restoration efforts, spat collectors must be cost efficient. For commercial cultivation the product should also have a nice shape (Nalesso et al., 2008), but for the ecological restoration of flat oyster reefs the aesthetics of the oysters are not a priority. An efficient spat collector may therefore collect a large amount of spat over the spawning season without excess investment.

Various research projects (from 2003, 2011, 2017, and 2018) investigated the efficiency of different types of off-bottom spat collectors for *O. edulis*. Each year larval abundances in the water column were measured and attempts were made to deploy spat collectors throughout the period of larvae settlement to study optimal timing of collector deployment. The insights presented here may prove useful for the purposes of either improving the success of oyster reef restoration or increasing commercial production of *O. edulis*.

The selection of collectors tested in the different studies was based on existing methods of spat collection used in commercial production, suggestions from the literature, or recommendations by oyster farmers (Smyth et al., 2018; Van den Brink, 2012).

1.1 | Chinese hats

Chinese hats are an industry standard in oyster spat collection, particularly in France (Freeman & Denny, 2003). Chinese hats are shallow plastic cones with a diameter of about 15.5 cm with a surface area calculated as 377 cm^2 (Kamermans, Brummelhuis, Poelman, Van Gool, & Troost, 2004). The hats can be stacked together into towers and deployed vertically into the water column. Despite reports of extremely high fouling rates on Chinese hats (Van den Brink, 2012), the structures were developed specifically for the purpose of efficient spat collection and have proven successful in doing so (Anonyme, 2014). Furthermore, because these structures are widely used commercially, testing Chinese hats may also act as a type of control with which the other tested collectors can be compared; if another collector is shown to be more efficient than Chinese hats, it may be worth replacing them on a commercial scale. Communication with oyster farmers indicated that a calcium coating is necessary for the efficient removal of spat from the collectors. In addition, Smyth et al. (2018) indicate that *O. edulis* has a preference for calcium rich substrate.

1.2 | PVC tubes

PVC tubes are used on a large scale in oyster production in France and Canada (Poiriera et al., 2019). They are 1.2 m long with a 2.2 cm diameter. Like Chinese hats, the collected spat can be removed mechanically from the tubes. This is useful for oyster farmers as their final product is individual oysters. For restoration purposes removing the oysters means an extra step, because you do not want to leave the collectors at the location indefinitely.

1.3 | Vexar mesh

Vexar mesh is made of sheets of hard, black plastic strips in a cross-hatched formation. The mesh was reported to be not only successful in collecting a mid-range to high number of spat (of *Crassostrea virginica*), but it was cheap, reusable and user-friendly; easy to prepare, deploy, remove the spat and to store (Freeman & Denny, 2003). Furthermore, spat on the mesh also yielded the highest spat growth of all the collectors tested by Freeman and Denny (2003). Vexar mesh bags were also successful in collecting *O. edulis* spat on the Canadian Atlantic coast (Bataller et al., 2006).

1.4 | Bivalve shells

Bivalve shells are widely used in shellfish aquaculture and generally readily available and successful spat collectors (FAO, 2019; Lok & Acarli, 2006; Smyth et al., 2018). Using net stocking or bags of shells allow oyster spat to recruit onto a large surface area with minimal wasted volume.

2 | METHODS

2.1 | Larvae sampling

For each year a sample of water from the same area as the spat collectors was analysed once per week between weeks 24 and 35 for the presence of oyster larvae. A 100-μm plankton net was used to filter 100 L of water. The size of the net was based on the minimum size of around 160 μm that the flat oyster larvae have during the pelagic phase (Walne, 1974). The samples were fixed in 4% buffered formalin (diluted with filtered sea water) for later microscopic examination in the laboratory. The samples of larvae were filtered using a plankton-gauze (30 μm). The volume of the samples was reduced to 20–60 ml, depending on the amount of suspended matter. From the concentrated samples subsamples were taken for counting numbers of larvae. A Hensen plunger-sampling pipette was used to take subsamples. Bivalve larvae of *O. edulis* and *C. gigas* were identified and counted using a universal camera microscope (Reichert Me-F2, 52.6×). Three subsamples of each sample were analysed. Depending on the density of the samples, subsamples of 1–2.5 ml were counted. Larvae were identified according to Hendriks, van Duren, and Herman (2005) combined with data obtained from cultured larvae.

2.2 | Experiments with collectors

While there was some variation in the testing of the different spat collectors in the different experiments, all followed a similar protocol. Each experiment involved deploying spat collectors by attaching them onto a mounting structure that kept them constantly submerged in the water (Table 1). Collectors were deployed each week in June–August, corresponding with the period when high numbers of *O. edulis* larvae were measured in the water column (Figure 1a). Once all the collectors had been deployed, they were left until October/November to allow the spat to grow to an easily visible size at which time all collectors were retrieved. The retrieved spat collectors were transported to a laboratory where they were inspected for oyster spat and other fouling organisms.

TABLE 1 Years and locations of the various experiments presented and spat collector types tested in each

Collector type	2003 Lake Grevelingen	2011 Lake Grevelingen	2017 Voordelta	2018 Voordelta
PVC tubes	Stack of 5 replicates			
Chinese hats	Stack of 3 replicates	Stack of 3 replicates		
Vexar mesh		3 separate replicate pieces		
Mussel shells	1–3 separate 5-L sacks	3 separate replicate 5-L sacks	2 5-L sacks	2 5-L sacks
Pacific oyster shells			2 5-L sacks	2 5-L sacks
Flat oyster shells				1 5-L sack
Cockle shells				1 5-L sack

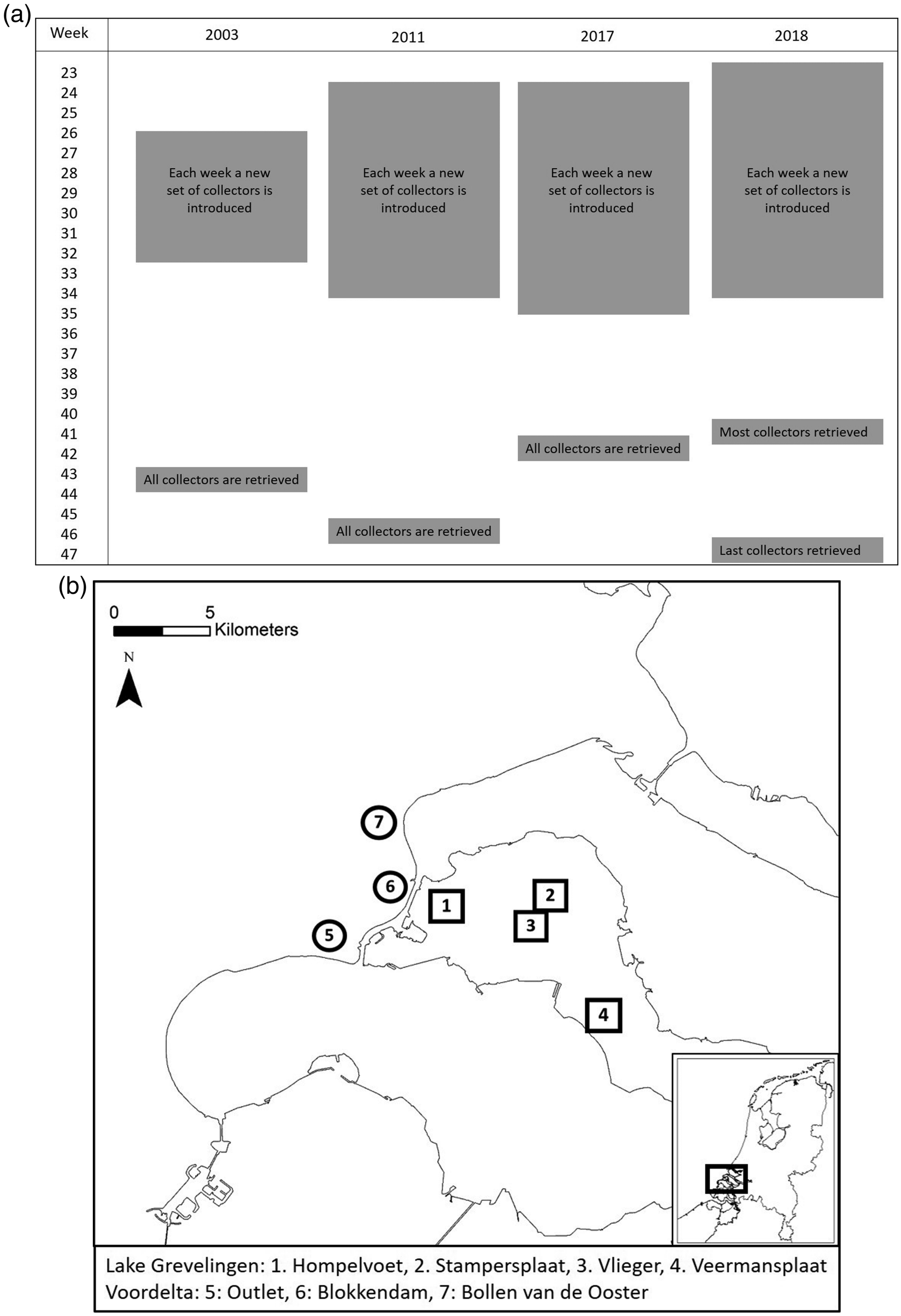


FIGURE 1 F Sample scheme (a) and locations (b) in Lake Grevelingen and the Voordelta (south-west Netherlands)

The mussel, oyster, and cockle shells used in the studies presented here were obtained from local farmers. The mussel shells were cooked and the cockle and oyster shells were weathered for a least 3 months. The shells were deployed inside 5-L mesh sacks.

2.2.1 | 2003

Spat collectors were deployed in three areas of Lake Grevelingen (south-west Dutch delta); Hompelvoet (1), Vlieger (3), and Veermansplaat (4; Figure 1b). All collectors were suspended in the water column attached to a buoy. A replicate set of five PVC tubes, three light brown Chinese hats stacked together and coated in calcium carbonate and 1–3 sacks of blue mussel (*Mytilis edulis*) shells were deployed. Each week a new set was put in from week 26 to week 32 (Figure 1a). The collectors were suspended below the water surface depending on the local situation at the culture plot (about 3 m below the surface at Vlieger, about 5.5 m below the surface at Hompelvoet, and about 2.5 m below the surface at Veermansplaat). All collectors were retrieved in October 2003 (week 43) for analysis.

2.2.2 | 2011

Spat collectors were deployed near Stampersplaat (2) in Lake Grevelingen (south-west Dutch delta; Figure 1b). A series of nine poles joined by cable in an open grid formation was constructed as a mounting structure in the water. From week 24 to week 34 a new set of three replicates of each collector type was deployed every week and suspended from the structure <1 m below the surface. The replicate spat collectors tested included a tower of three Chinese hats, three 30-cm^2 squares of 14-mm Vexar mesh (weighed down with a steel rod), and three 5-L sacks filled with blue mussel shells (average of 71, ranging from 56 to 96 shells per sack). All collectors were retrieved for analysis in November 2011 (week 46; Figure 1a).

The Chinese hats were identical to those used in 2003 but were coated in a calcium solution made of 50% calcium (calcium dihydroxide and magnesium dihydroxide) and 50% water. The collectors were left to dry until the coating was completely hardened prior to deployment.

2.2.3 | 2017

Spat collectors were deployed at Blokkendam (7; in the Voordelta, North Sea coastal area) and Outlet (6; where Lake Grevelingen feeds into the Voordelta; Figure 1b). The collectors tested were separate similar-sized sacks with 500 g mussel shells (average of 340 shells), or 1 kg Pacific oyster shells (average of 95 shells). One sack of each spat collector was attached to a float that was suspended in the water 1 m from the bottom, tethered to a heavy tile. Another sack of each spat collector was strapped directly onto the tile. A set of collectors was deployed each week from week 24 to week 35 and retrieved for analysis in October 2017 (week 42; Figure 1a).

2.2.4 | 2018

Spat collectors were deployed in two locations, the Outlet (6) and Bollen van de Ooster (5), both located in the Voordelta (Figure 1b). The spat collectors tested were separate similar sized sacks with 300 g mussel shells, 1,000 g cockle shells (*Cerastoderma edulis*), 500 g Pacific oyster shells, or 500 g flat oyster shells (*O. edulis*). The different weights of the shells were necessary to maintain similar volume in each sack (~5 L). Similar to 2017, each replicate spat collector was attached to a heavy tile connected to a buoy. A set of four different collectors types were attached to one buoy and were deployed weekly from week 23 until week 34 (Figure 1a). All four types of shell were attached to the float, and sacks with mussel and Pacific oyster shell were also strapped onto the tile. In the first 2 weeks, three replicate sets of spat collectors were deployed followed by one set of spat collectors per week after that. All collectors were retrieved for analysis in October (week 41), except for six at Bollen van de Ooster that had gone missing. These six were retrieved in November 2018 (week 47).

2.3 | Data analysis

Larval abundance per 100 L and spat per collector were plotted in graphs showing the development over time. Replicate collectors were either stacked together, resulting in pseudo-replicates, or not deployed consistently for each collector type and for each week in 2003, 2017 and 2018, or replicate collectors were lost in 2011. Therefore, no statistical analysis of the data could be carried out.

3 | RESULTS

3.1 | Larvae measurements

There was a high variation in *O. edulis* larval abundance over the years and locations of the different experiments. The highest abundance of larvae was observed in 2003, particularly at Vlieger on week 30 (2,610 larvae per 100 L water). The timing and height of the peaks in larval abundance varied over the years and locations (Figure 2).

During the sampling period, Pacific oyster larvae were always present in the water column. In 2003 Pacific oyster larvae greatly outnumbered flat oyster larvae (Figure 3). In 2011 at location Stampersplaat more flat oyster larvae were observed in the water column than Pacific oyster larvae. In 2017 Pacific oyster larvae maintained similar abundances as flat oyster larvae at the outlet but

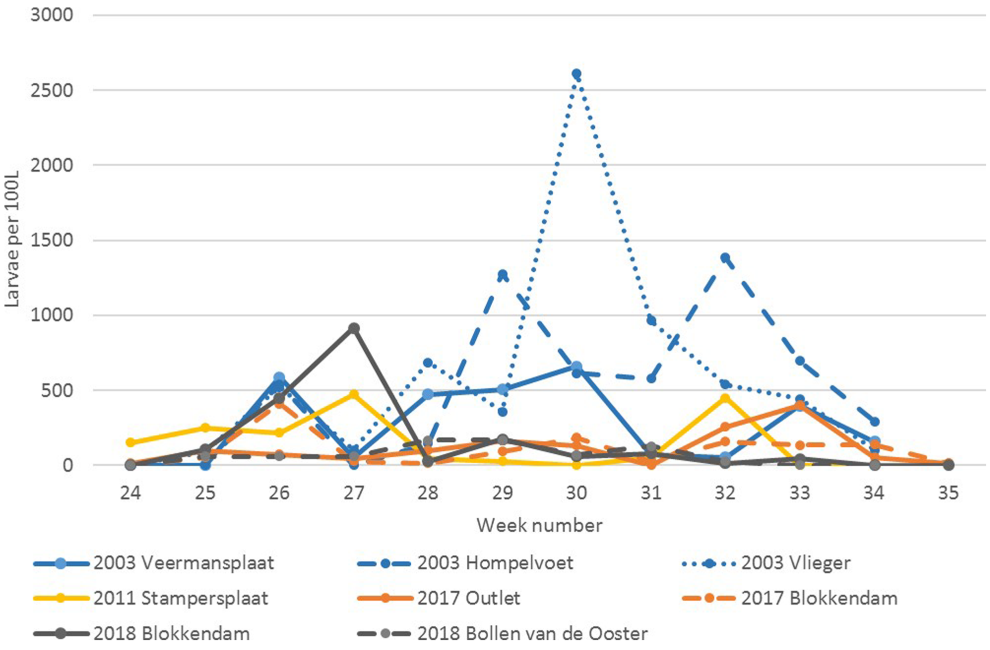


FIGURE 2 Variation in abundance of flat oyster (*Ostrea edulis*) larvae per 100 L seawater in the different studies over different years and locations

were largely outnumbered by flat oyster larvae at Blokkendam. In 2018 larval abundances for both species were relatively similar, excluding the peak in flat oyster larvae in week 27 at Blokkendam.

3.2 | Spat collection

3.2.1 | 2003

Of the tested collectors, the 5-L sacks of mussel shells collected the largest number of flat oyster spat per collector (Figure 4). At Veermansplaat a maximum of 102 spat were observed on the mussel shells deployed in week 27 of the year, one week after the first peak in larval abundance. At Hompelvoet a maximum of 333 spat were collected on the mussel shells in week 28, two weeks after the first, small peak in larval abundance. The sacks of mussel shells collected a maximum of 740 spat per collector in week 27 at Vlieger, one week after the first observed increase in larval abundance. However, no spat was collected following the largest peak in larval abundance in week 30.

Most of the spat collected on the PVC tubes were on those deployed early in the experiment. At Vlieger a maximum of 33 spat was collected on the tubes, at Veermansplaat and at Hompelvoet the tubes collected a maximum of 19 spat, all in week 27.

The towers of Chinese hats at Veermansplaat collected an average of 1 spat per replicate, and at Hompelvoet an average of 2.4 spat per replicate throughout the experimental period. The highest number of spat collected on Chinese hats were on those deployed in week 30 at Hompelvoet (total of 8.5 spat per collector). Unfortunately, all Chinese hats at Vlieger were lost.

3.2.2 | 2011

Peaks in flat oyster spat collection were observed on collectors deployed in week 27 and 29 of that year. The peak in week 27 coincided with a peak in larval abundance, and just followed the high larval abundance in week 25. The peak in spat collection in week 29 occurred two weeks after the second peak in larval abundance. Unfortunately, three towers of Chinese hats were lost in weeks 26, 27 and 28 of the experiment. And three Vexar mesh collectors were lost in week 26.

The mussel sacks were most successful at collecting spat. Mussel stockings collected more *O. edulis* spat per collector than either the Chinese hats or Vexar mesh in every week of the experiment that all three collector types were present. The most *O. edulis* spat on the mussel sack collectors was found on replicates deployed in week 29 with an average of 251 spat, followed by week 27 with an average of 211 spat. The number of spat found per Vexar mesh collector was negligible in comparison with the other collectors.

3.2.3 | 2017

The largest number of flat oyster spat collected at Outlet occurred in week 23, the first week of collector deployment, suggesting that the peak in larval abundance had occurred prior to the beginning of the experiment. There was a secondary peak in larval abundance in week 33, but no increase in spat collection was observed in the following week.

At Blokkendam, a total of 155 flat oyster spat was collected. The highest number of spat was found on collectors deployed in week 23.

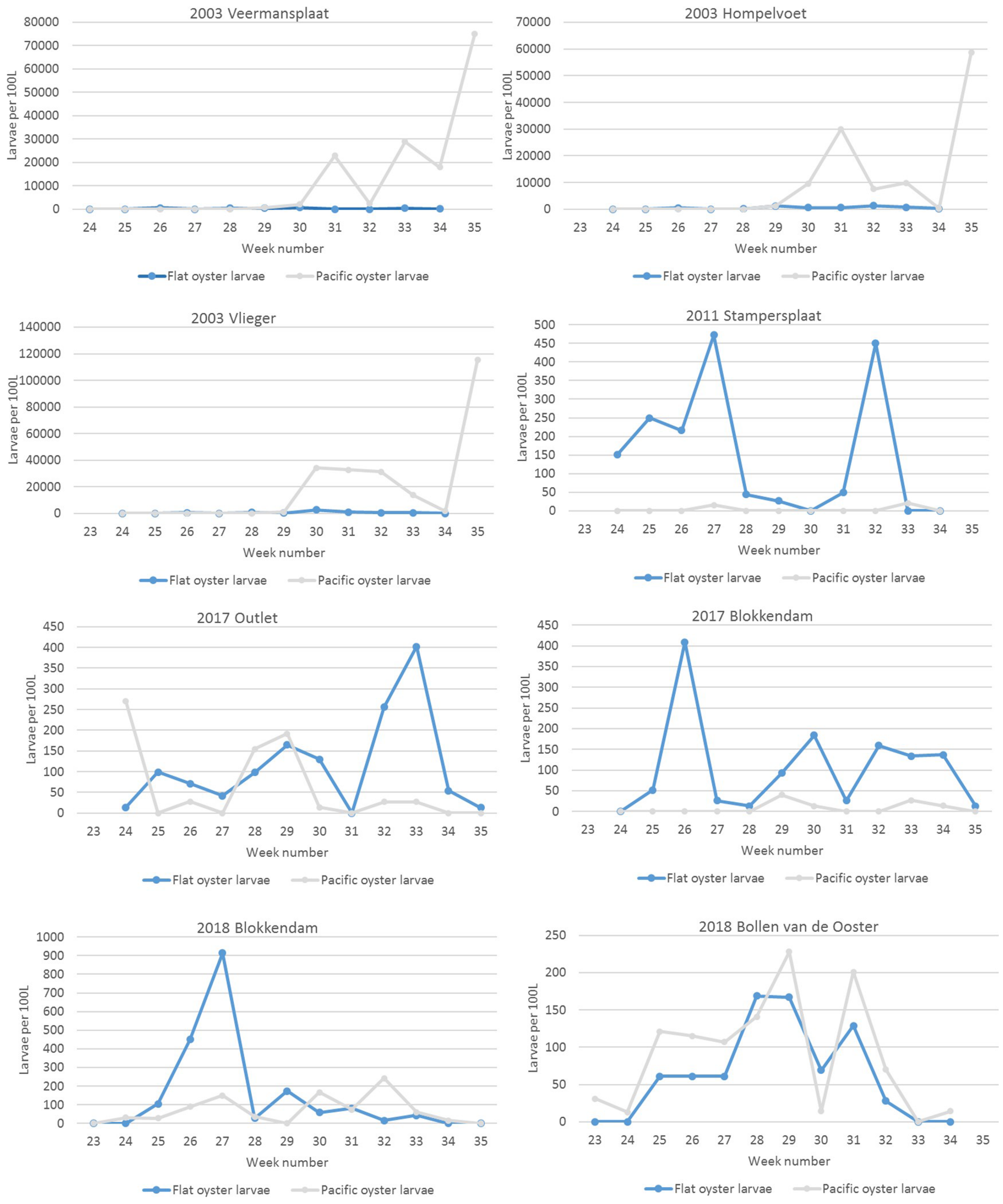


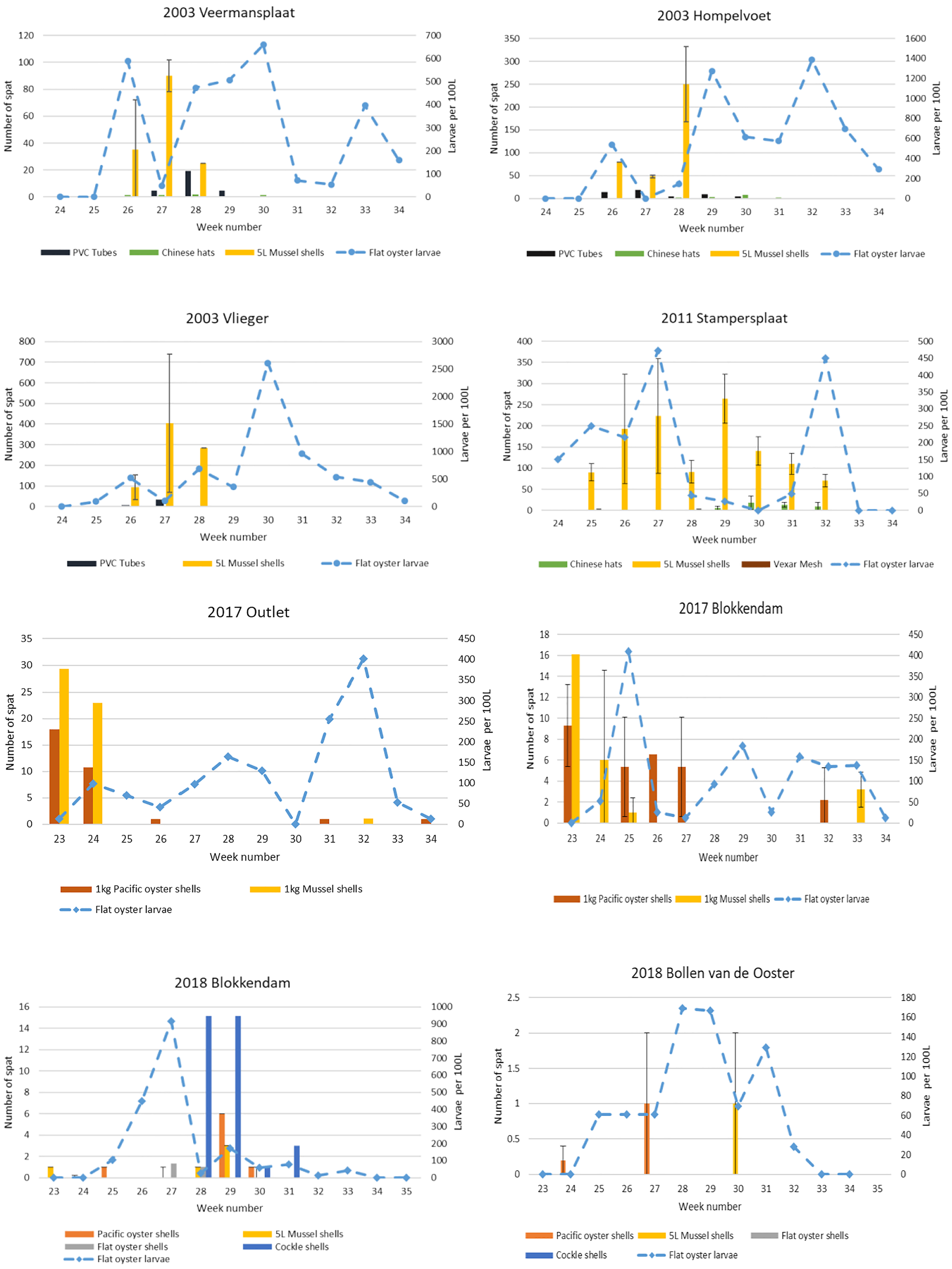
FIGURE 3 Abundance per 100 L seawater of flat oyster (*Ostrea edulis*) larvae and Pacific oyster (*Crassostrea gigas*) larvae in the water column in the different studies over different years and locations. Missing data points indicate where no measurements were taken

Larval abundance was relatively low with a maximum of 400 larvae per 100 L observed in week 25.

3.2.4 | 2018

At Blokkendam a peak in flat oyster spat collection was observed in weeks 28 and 29, one and two weeks after the peak in larval abundance in week 27. This peak was due to the high number of spat observed on cockle shell collectors. Throughout the experiment, cockle shells collected 34 flat oyster spat, compared to eight spat on Pacific oyster shells, five on mussel shells and two on flat oyster shells.

At Bollen van de Ooster only five flat oyster spat were found on the collectors. Of these, one was found on collectors deployed in week 24, and two were found on collectors deployed in weeks


2003 Veermansplaat

2003 Hompelvoet

2003 Vlieger

2011 Stampersplaat

2017 Outlet

2017 Blokkendam

2018 Blokkendam

2018 Bollen van de Ooster

FIGURE 4 Number of spat collected (bars with error margins indicate average) on different collector types and abundance per 100 L seawater of flat oyster (*Ostrea edulis*) larvae in the water column (line) in the different experiments over different years and locations. Xs at 2011 Stampersplaat indicate lost Chinese hats

27 and 30. Three of the spat were attached to Pacific oyster shells, while the other two spat were attached to mussel shells. Results regarding the type and timing of deployment and successful flat oyster spat settlement at Bollen van de Ooster cannot be reported separately due to the small number of larvae in the water column and spat collected.

3.3 | Fouling organisms

While fouling organisms were not specifically analysed in the experiments, observations were recorded. The amount and type of organisms fouling the collectors varied both between, and within experiments.

3.3.1 | 2003

Fouling was heavy on the PVC tubes, consisting mainly of Pacific oyster spat (Figure 5a), and on the mussel sacks, dominated predominantly by barnacles and tunicates (*Ciona intestinalis*; Figure 5b). Comparatively less fouling was observed on Chinese hats, consisting mainly of macroalgae (Figure 5c).

3.3.2 | 2011

While all three collector types were fouled on retrieval (Figure 6), the organisms could easily be removed from the Chinese hats due to the calcium coating, and from the mussel stockings when the mussel shells were removed from the mesh. Fouling was, however, particularly difficult to remove from the Vexar mesh, which resulted in the necessity to abandon removal attempts and manually search through the fouling for oyster spat.

There was a high degree of fouling on almost all collectors deployed during the experiment. However, the dominant species of fouling organism depended on which week the collector was deployed. Collectors from the first deployment week were heavily fouled with blue mussels, and mussel presence decreased with collectors deployed in later weeks. Other dominant fouling organisms included macroalgae, barnacles, bryozoans, tunicates (*C. intestinalis* and *Didemnum* sp.) and Pacific oyster spat.

3.3.3 | 2017

There was limited fouling on the shells at both experimental locations in the 2017 study. The fouling present was generally attached to the sack and consisted mostly of barnacles, bryozoans, tunicates (*C. intestinalis* and *Didemnum* sp.), macroalgae, and some Pacific oyster spat (Figure 7). There appeared to be little difference in fouling amount or species between the mussel shell and oyster shell collectors, but this was not explicitly analysed in this study.

3.3.4 | 2018

All collectors from both locations were heavily fouled, although there was some variation in type of fouling organisms depending on the substrate. In general, cockle shells trapped the most sediment, but were the least overgrown with other organisms, and these were almost exclusively barnacles. Flat oyster shells, Pacific oyster shells and mussel shells showed more variation in fouling coverage, with

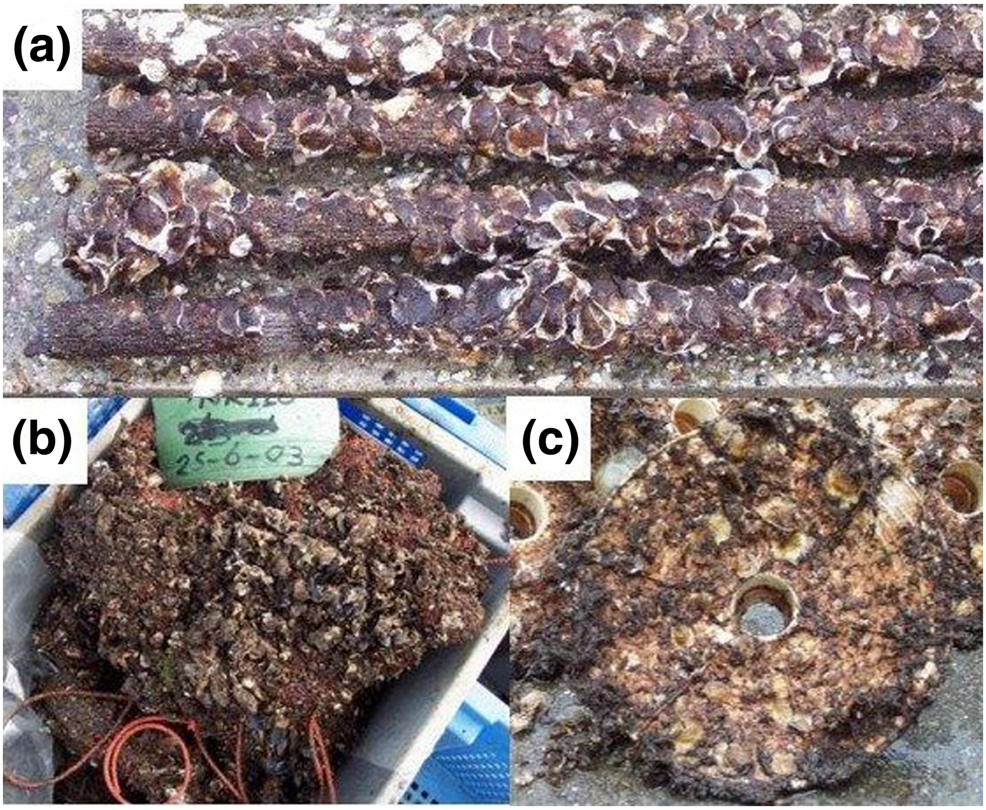


FIGURE 5 Example of epibiota fouling on the PVC tubes (a), mussel sacks (b), and Chinese hats (c) after retrieval from the water at the end of the experiment in Lake Grevelingen in 2003

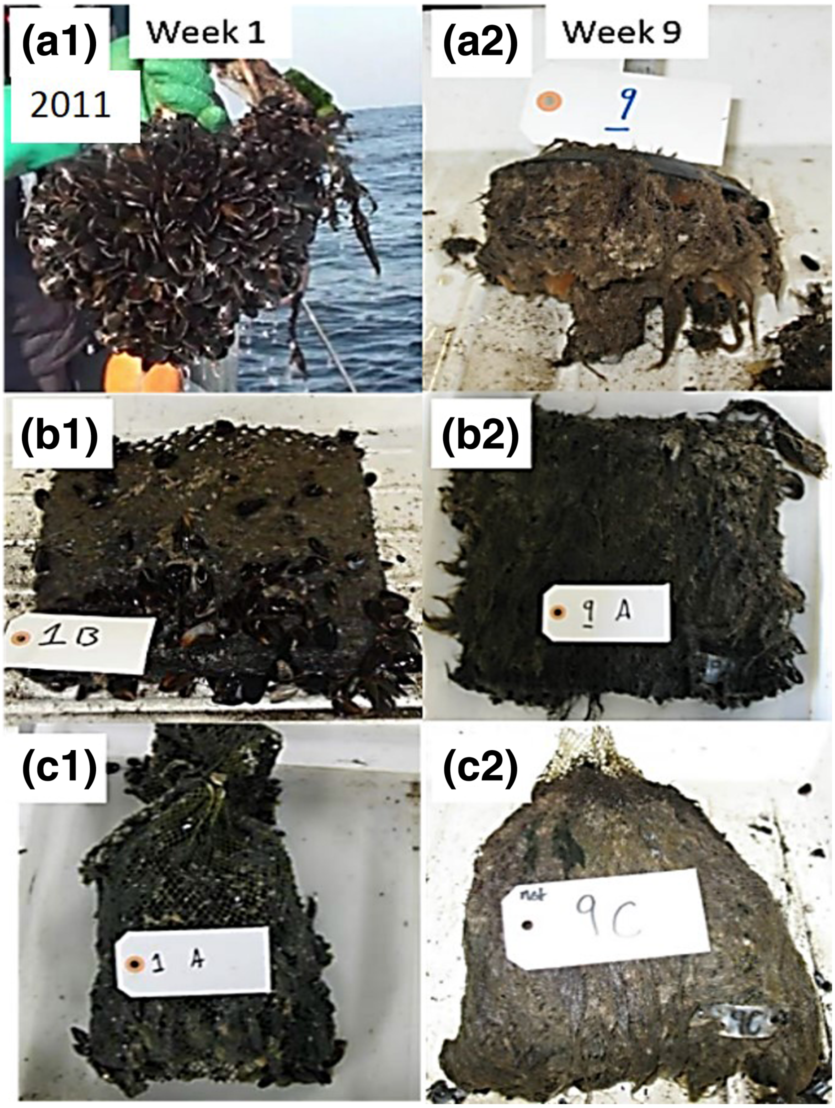


FIGURE 6 Example of epibiota fouling the spat collectors (Chinese hats, a; Vexar mesh, b; and mussel sacks, c) deployed in the first week (a1, b1, c1) and ninth week (a2, b2, c2) of the experiment in Lake Grevelingen in 2011

some shells being completely covered in fouling organisms, while others remained almost completely bare.

The most commonly observed fouling organisms on the collectors included bryozoans, barnacles, tunicates (*C. intestinalis* and *Didemnum* sp.), star ascidians (*Botryllus schlosseri*), sea lettuce (*Ulva lactuca*), blue mussels, crabs (*Cancer pagurus* and *Carcinus maenas*), polychaetes, asteroidea, and Pacific oyster spat (Figure 7).

4 | DISCUSSION

Enhancement of natural production of flat oysters, whether for ecological restoration or cultivation purposes, is a challenging objective. The environmental requirements of the oysters need to be met while other factors such as predation, competition, diseases, and parasites should be actively mitigated to give the oysters their best chance for survival. The early life stages show high mortality rates, and are a significant bottleneck in the population due to high levels of predation of both larvae and young spat and the requirement that the larvae have for available, appropriate substrate on which to settle (Korringa, 1940; Rodriguez-Perez et al., 2019; Smyth et al., 2018). By providing more appropriate substrate on which to settle, at the right time in a way that minimizes the competition pressure exerted by other species, more flat oyster spat will survive this population bottleneck. In this way, the potential for successful restoration and cultivation of the oyster will increase.

The different experiments presented here explored possible methods to actively promote flat oyster populations during the larval settlement stage by using off-bottom spat collectors in the Dutch delta and coastal waters. Lake Grevelingen is a relatively sheltered oyster production area, and the Voordelta is open to the influence of the North Sea. Despite the environmental differences between these two areas, flat oysters are known to grow and reproduce in both areas (Pogoda et al., 2019; Smaal et al., 2015).

The comparative abundance of flat oyster and Pacific oyster larvae varied among the experiments. Due to a high abundance of Pacific oyster larvae in 2003, they may have outcompeted the flat oyster spat for space on the collectors, resulting in the low numbers

FIGURE 7 Example of epibiota fouling on the mussel (left) and Pacific oyster (right) sacks in the experiment in 2017 (top); and cockle, mussel, flat oyster, and Pacific oyster (top to bottom) shell sacks in 2018 (bottom), after retrieval from the water at the end of the experiments in the Voordelta

of flat oyster spat observed following the peaks in larval abundance. In 2011 flat oyster larvae greatly outnumbered Pacific oyster larvae in the water column, and much higher numbers of spat were observed on the collectors compared with 2003. The reason for this is unclear; perhaps peak larval abundance for flat and Pacific oysters occurred at different times at Stampersplaat in 2011, and the experiment coincided with the peak in flat oyster larval abundance and not that of the Pacific oyster. In general, differences in flat oyster larval abundance between years has been observed in the past in the nearby Oosterschelde estuary (Korringa, 1947).

Pacific oysters were also one of the most common fouling organisms on the collectors. Pacific oysters are a non-indigenous species in the Netherlands, introduced in the 1960s to supplement the flat oyster stocks that were failing due to the severe winter of 1963/64 (Engelsma et al., 2010). The Pacific oyster is a successful species with a wide environmental tolerance range and high reproductive rate (Troost, 2010). The introduction of this species therefore may have added yet another pressure to the already stressed population of flat oysters in the Netherlands. However, Christianen et al. (2018) suggested that the presence of the Pacific oyster may facilitate the survival of the flat oysters by providing refuge against predation. In any case, developing a method of spat collection that distinguishes between these two oyster species is unlikely to be successful, but understanding the specific characteristics of the flat oyster related to spatfall and settlement may help increase the amount of surviving flat oyster spat.

4.1 | Timing of deployment of collector

To study the timing of deployment of collectors, testing it in different years and in different locations increases the strength of the conclusions. The results indicate that timing the collector deployment with the peak in larval abundance is important to the success of a spat collector. The pelagic phase of *O. edulis* larvae lasts only a few weeks in summertime (Korringa, 1947). In two of the four experiments (2011 and 2018), the peak in spat settlement occurred on collectors deployed 1–2 weeks after the peak in larval abundance. It would therefore be most efficient to concentrate the deployment of collectors shortly after the peak in larval abundance is detected.

If collectors are deployed too early, they may become fouled with other organisms that will prevent the spat from settling, or smother or overgrow spat that does manage to settle (Mackenzie, 1970). This competition for space was observed on the first collectors placed in 2011 when mussels settled before the flat oyster spatfall so that much of surface area on the collectors deployed was already occupied before the flat oyster spat settled. Other dominant fouling organisms observed on the collectors in all studies included algae, bryozoans, and colonial tunicates. These organisms can completely cover a surface, and essentially replace the hard, suitable substrate of the collector with a soft, gelatinous surface on which the spat will not settle.

If deployed too late, the collectors will miss the peak in spatfall, rendering them essentially obsolete for that season. In 2017, collectors were probably deployed after the peak in larval abundance, which was absent in our data but probably occurred earlier in the season. Consequently, collectors probably missed the peak spatfall as spat collection was highest on collectors deployed in the first week of the experiment. It would therefore be useful to regularly sample the larvae in the water and deploy the collectors only after a high number of larvae is detected.

Oyster breeding success varied from year to year and between locations. It is likely that high variation in timing of settlement exists amongst different geographical regions within the oysters' natural range. Therefore, it is difficult to predict when a peak in larval abundance occurs, or even what a 'high number' of larvae for that season will be. What causes this variation in reproductive success is not well understood but may be linked to water temperature. Temperature sum (accumulated temperature) is an important variable explaining larval abundance in modelled scenarios for bivalves (Broell, Mccain, & Taggart, 2017). Maathuis, Coolen, Van der Have, and Kamermans (2020) developed a model where the seawater temperature sum in early summer could be applied as a crude predictor of peak flat oyster larval abundance. In this study, larval counts of Korringa (1947) and the data presented here for 2003 and 2011 were included. Therefore, it is advisable to monitor the seawater temperature sum along with the larval abundance in the water column and deploy collectors 1–2 weeks after maximum larvae numbers are detected.

4.2 | Type of collector

Providing the appropriate type of substrate as a spat collector will greatly influence the success of spat collection. Seven different types of spat collector were tested, four of which involved the use of bivalve shells. In general, bivalve shells appeared to be the most successful spat collectors, but the species of shell most successful at collecting spat differed per study. Mussel shells were the only bivalve shell tested all years. In 2003 and 2011, it was the most successful collector type. In 2017, both mussel shells and Pacific oyster shells were used, both similarly successful. In 2018, cockle shells collected the most spat at Blokkendam, while the only spat found at Bollen van de Ooster were attached to mussel and Pacific oyster shells. The calcium carbonate rich substrate of bivalve shells provides a surface that larvae are well adapted to exploit (Smyth et al., 2018). Smyth et al. (2018) found a positive correlation between available shell material and spatfall for *O. edulis*, but similarly no preference for a particular species of shell. Naturally occurring flat oysters in the Dutch part of the Wadden Sea also settled on the substrate that was most available, in their case Pacific oysters and cockles (van der Have, Kamermans, & van der Zee, 2018). Furthermore, previous research on flat oysters in an oyster bed at Blokkendam, the same location as the experiment of 2018 presented here, showed that larvae predominantly settled on Pacific oyster shells, which was the most abundant substrate available (Christianen et al., 2018). However, a laboratory study showed that ‘sterile’ shells, cleaned of all biota, were less successful at collecting *O. edulis* spat compared with marine stones with habitat-associated biofilms (Rodriguez-Perez et al., 2019). Biofilms may provide a chemical cue for the larvae indicating the presence of appropriate substrate for settlement (Tamburri, Luckenbach, Breitburg, & Bonniwell, 2008). Our study shows that collectors placed 1–2 weeks after the larval peak yielded most spat. This probably allowed a biofilm to develop on the collectors during the pelagic phase of the larvae. The artificial spat collectors such as Chinese hats and PVC tubes are designed to simplify and mechanize the retrieval process by making individual spat easy to remove from the collector (FAO, 2019). However, the results suggest that the efficiency of these collectors was limited as they tended to collect fewer spat than the bivalve shells. The calcium coating on these artificial spat collectors is a practical solution to allow for easy detachment of spat during retrieval.

The potential and justification for investment into off-bottom bivalve shells as spat collectors depends on the intended purpose for the oyster spat. From a cultivation perspective, empty shells are most productive when a single oyster spat survives on each shell. As the oyster grows, the shell will eventually break away leaving an individual, unattached oyster. If more than one oyster grows on a shell, they may crowd each other and become attached to each other or warped in shape, which is not desirable from a producer's perspective as these oysters may only be suitable for the oyster meat market (Nalesso et al., 2008). From an ecological restoration perspective, crowded shells are not a problem as the aesthetics of the oyster are not a priority. In addition, potential empty bivalve shells can simply be translocated along with the oysters to the restoration site, thereby providing more substrate for future larval settlement.

For cultivation purposes, the costs of spat collection remain a regular, necessary investment. Collectors need to be prepared, deployed, retrieved and processed every season. Shells as spat collectors cannot be reused, because they degrade as the oysters grow. In addition, the availability of shells depends on shellfish production, which shows natural fluctuations. This has prompted the development of artificial, reusable spat collectors and mechanized harvesting techniques as seen with Chinese hats and PVC tubes in France (Anonyme, 2014). If the increased spat collection on off-bottom bivalve shell collectors could offset their drawbacks, and if the investment involved with their use could be made more economically viable with mechanization, bivalve shell collectors may be a good option as commercial spat collectors.

The intention of restoration efforts for the flat oyster are to create an eventually self-sustaining, natural oyster reef (Kamermans et al., 2018). The short-term investment intended for this type of restoration, therefore requires less financial and labour investment than for cultivation. Off-bottom bivalve shells are therefore a promising method of spat collection for restoration purposes.

5 | CONCLUSIONS

This paper describes results of spat collection in areas where adult oysters are present and larval abundance is not limiting. Under these circumstances the use of off-bottom spat collectors for restoration and production of flat oysters can be optimized when taking into account the following points:

- Monitor the abundance of larvae in the water column, along with the temperature of the water to determine larval peak.
- Deploy collectors 1–2 weeks after the peak in larval abundance to allow for maximum spat settlement, and to minimize the impact of other fouling species.
- The most successful spat collectors were suspended sacks of bivalve shells.
- For biosecurity reasons only cooked or weathered shells should be used.
- The use of off-bottom sacks of bivalve shells as spat collectors show great promise for oyster reef restoration and, if developed to be more economically viable, commercial cultivation purposes.

ACKNOWLEDGEMENTS

The Improve Oysterspat project of 2003 was funded by the Ministry of Agriculture, Nature management and Food safety. We thank the Dutch oyster producers organisation for cooperation in this project and Jaap Poelman and Emiel Brummelhuis of WMR (then called RIVO) for analysing the larvae samples and counting the spat. The 2011 study was funded by EU FP7 Support 2008–2 for SME's project Oysterecover (GA No. 243583). We thank the shellfish companies Roem van Yerseke and Prins en Dingemanse for cooperation in this project and Emiel Brummelhuis, Ad van Gool, Jack Perdon, Eva Hartog, Yoeri van Es, Noortje Ros of WMR (then IMARES) for fieldwork for and analysing the larvae samples and counting the spat. The 2017 and 2018 Voordelta experiments were part of a project of ARK Nature and WWF-Netherlands on shellfish reef restoration in the North Sea. We thank Joost Bergsma, Daniel Beuker, Martijn Dorenbosch, Udo van Dongen, Floor Driessen, Wouter Lengkeek, Michiel van de Vooren, Tom van der Have, and Hans Waardenburg of Bureau Waardenburg, and Peter van Rodijnen of Onderwaterbeelden for fieldwork and Pim van Dalen, Ad van Gool, Jesse van der Pool, Niels Wagenaar, Adryan Rademakers, Jetze van Zwol, and Yoeri van Es of WMR for fieldwork and laboratory analyses concerning larval abundance and spat collection.

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How to cite this article: van den Brink AM, Maathuis MAM, Kamermans P. Optimization of off-bottom spat collectors for restoration and production of the European flat oyster (*Ostrea edulis*) in Dutch coastal waters. *Aquatic Conserv: Mar Freshw Ecosyst*. 2020;30:2087–2100. https://doi.org/10.1002/aqc.3427