Eco-friendly reef restoration pilots in offshore wind farms

Report Project ECOFRIEND 2019-2023

Author(s): Oscar G. Bos (1), Pauline Kamermans (1), Linda Tonk (1), Mirjam Schutter (2), Margot Maathuis (2), Ad van Gool (1), Tom van der Have (2), Joost Bergsma (2), Tim Raaijmakers (3), Luca van Duren (3), Antonios Emmanouil (3), Isabel Gerritsma (3), Frank Kleissen (3) en Hein Sas (4)

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(1) Wageningen Marine Research, (2) Waardenburg Ecology, (3) Deltares, (4) Sas Consultancy

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ECOFRIEND (2019-2023) is een samenwerking tussen Wageningen Marine Research, Bureau Waardenburg, Deltares en Sas Consultancy. De industrie partner is Gemini Offshore Windfarm.

Het project is uitgevoerd met subsidie (referentienummer TEWZ118017) van het Ministerie van Economische Zaken en Klimaat, subsidieregeling Top Sector Energie uitgevoerd door Rijksdienst voor Ondernemend Nederland.

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Contents

Summary

ECOFRIEND context and objectives

The ECOFRIEND project (2019-2023) is funded by the TKI Wind op Zee, a Dutch governmental funding program that aims to "stimulate, connect and supports Dutch organisations and knowledge institutes with the development and deployment of innovations to help speed up the transition to sustainable, reliable and affordable energy system, focussing on offshore wind." (https://www.topsectorenergie.nl/en/about-tki-wind-op-zee). The project partners are Wageningen Marine Research, Waardenburg Ecology, Deltares and Sas Consultancy. The industry partner is the Gemini Offshore Windfarm.

Offshore wind is increasingly important in the Dutch energy transition. Its development is not only influenced by environmental factors (e.g. distance to coast, water depth, seabed structure and dynamics) and technical developments, but also by ecological considerations. The Dutch Ministry of Agriculture, Nature and Food Quality intends to steer offshore wind farm design towards strengthening the North Sea ecosystem, by enhancing policy-relevant marine species. This is part of the North Sea 2050 Spatial Planning Agenda (Min I&M and Min EZ, 2014), the North Sea Agreement (OFL, 2020) and and is being operationalized trough permit-obligations. Offshore wind licence holders are required to make demonstrable efforts into this direction (e.g. wind energy tenders IJVER and HKZ).

European flat oyster (*Ostrea edulis*) reefs constitute a potential keystone habitat for the North Sea ecosystem, enhancing biodiversity. But a combination of diseases, pollution, cold winters and overfishing have caused their disappearance circa a century ago, so it is widely attempted to restore these reefs. The ECOFRIEND project aimed to develop and study new methods to re-introduce and monitor flat oyster reefs and related biodiversity in offshore wind farms, in cooperation with the wind industry.

The expected outcomes of the ECOFRIEND project (2019-2023) were a proof-of-concept for active re-introduction of offshore flat oyster beds, to show whether there would be a viable population of flat oysters in an offshore wind farm and to develop novel methods, including (predictive) models, to help monitoring the effectiveness of restoration initiatives. Thereby assisting restoration attempts in Dutch marine waters, but also providing competitive advantage for Dutch research organisations and industry and to increase the knowledge base for research organisations and industry that can be applied to initiatives in other North Sea countries. This report provides a summary of the project's main findings. Detailed findings are described in scientific articles and/or in technical reports which are being published separately and also listed in this report.

Proof of concept of active reintroduction and demonstration of a viable population

To set a first step in creating a flat oyster reef, an initial population of mostly adult oysters has to be introduced at the location where the reef is intended. In Gemini Offshore Windpark a flat oyster population was introduced in 2018 by Gemini, before the ECOFRIEND project started in 2019. The population appeared to be viable as shown by monitoring: the oysters showed a high level of survival, growth and also larval production. It is recommended to develop adequate settlement detection techniques. Further, it is recommended to develop a standardized monitoring protocol (for sampling larvae, growth, and condition), since this will enable comparison of monitoring results with other oyster restoration projects.

Development and testing of innovative monitoring methods

The ECOFRIEND project's objective was to develop and test a variety of innovative relevant monitoring techniques which can be applied in the - usually harsh - offshore conditions to follow several aspects of reef development. As test sites, flat oyster pilots in the Gemini wind park and Borkum Reef Ground (a WWF Netherlands pilot) were used.

Innovative oyster cage: WERC-dock

Usually, flat oyster restoration projects start with deploying an initial oyster population to the sea floor in a contained form, which is regularly hoisted up to monitor whether they remain alive and active. Traditionally, large cages are deployed for this, requiring heavy (hence costly) deployment and retrieval equipment, and environmental risks. Therefore, we developed a much lighter alternative, in the form of the so-called WERC-dock. This is a compact, but stable and robust device that holds oyster baskets which can be deployed to and lifted relatively easily from the seafloor. Hence, WERC-dock is a cost-effective alternative for the original large cages.

Measuring oyster activity: valve gape monitor

As stated above, cages can be used to inspect survival and condition of an oyster population. However, there is scientific and operational benefit if their condition can be monitored continuously and *in situ*. Therefore, the valve gape monitor was developed. This is essentially a frame which stands stably on the sea floor and to which oysters are attached, connected with electrodes for monitoring and recording their opening and closing. Combined with a Chlorophyll A sensor, turbidity and temperature, the valve gape monitor provides continuous insight in the oyster's survival and daily activity, e.g. in relation to food availability and other environmental parameters.

Simplifying sampling and larvae analyses

It is also essential to be able to monitor oyster reproduction, i.e. to detect whether there are flat oyster larvae in the water column. However, traditional sampling techniques are difficult to use under the often rough North Sea conditions. We therefore developed an easy-to-use larval sampling technique, by which larvae were sampled by filtering 200 L (2 x 100L) of seawater per sample from 0,5 – 1 m above the seafloor with the help of a 30 m long hose and a pump. This appeared to function well.

We also showed that larvae can be detected by DNA-analysis (qPRC), which is easier to use than the traditional visual analysis by microscope. We compared the results of DNA-analysis with the traditional (and reliable) visual inspection by microscope, and found high variability, hence it is recommended to further improve the DNA-technique.

Flat oyster produce larvae during relatively short peak moments (several weeks), hence it is important to be able to predict when these are present in the water to perform the sampling in the right period. Again, this is important for the work in offshore conditions (window of opportunity for sampling), since a fruitless sampling expedition bears heavily on a project's budget. The common prediction method employed is measuring the local seawater temperature, but this is usually too cumbersome in offshore situations. Therefore, we developed a method in which the 3D Dutch Continental Shelf Model in Flexible Mesh (3D DCSM-FM) can be used to predict the temperature development in a given year. This model is very versatile and can for example predict currents, temperatures, and other abiotic variables at any giving location in the North Sea. In this project we used and improved the model, among others with temperature data measured by logging devices attached to harbour seals, to produce the temperature predictions.

What causes oyster motion on the sea floor?

An important other application of the 3D DCSM-FM model in the ECOFRIEND project was the determination of flat oyster mobility on the sea floor. To create an oyster reef, it is important that the initial population (a number of young and/or adult oysters introduced from elsewhere) deployed at the intended restoration site remains in place there. However, of a relatively large population (80,000 oysters) loosely deployed oysters at the Borkum Reef Grounds (in a pilot undertaken by WWF Netherlands) only a few could be retraced in later monitoring. We therefore conducted an experiment with 'dummy oysters' at Deltares. Hundreds of vividly coloured oyster shells doublets, of different weights and sizes, were subjected to various wave regimes representative of the Borkum Reef Ground seafloor conditions, in a Deltares test basin. Then they were taken to the Borkum Reef Grounds and dropped at the seafloor. One year later a scuba-dive and ROV operation was organised to see whether they could be found back, but that was not the case. On the basis of the 3D DCSM-FM model and the results from the basin experiments, it could be shown that the turbulence caused by regular strong storm events indeed causes dispersal of flat oysters in the seafloor in water depths less than ca. 40

meters, as is the case in Borkum Reef Ground. For a variety of practical reasons, all other flat oyster pilots in the North Sea occur at less than 40 meter water depth. Hence, deploying loosely strewn flat oysters at the North Sea floor will not be conducive to reef formation in such projects. It is recommended to investigate and develop methods by which the initial population can be kept together on the sea floor, or measures to stabilise the seabed.

Where do oyster cages go?
We attempted to make 3D DCSM-FM model predictions of the movement of oyster cages if these, for one reason or the other (inadequate design, unforeseen turbulence etc.), happen to move over the seafloor. It is important to be able to trace these, since a moving cage can cause damage to the wind farm infrastructure and one does not want to lose the monitor population either. The model predicts at what level of hydrodynamic forcing the oyster cages start to move. The rough movement paths of such cages could indeed be predicted, but probably not precise enough to be able to find them back. However, the model can be used to design future deployment cages that will stay stable under the prevailing metocean conditions.

Oyster larvae detected far away from restoration projects
During ECOFRIEND fieldwork, larvae were sampled at 2 locations where oysters restoration pilots were located (Gemini wind farm and the Borkum Reef Ground), for which Norwegian oysters were used, as well as at a control location roughly halfway between these sites (the ‘Halfweg’ location). Surprisingly, in two consecutive years, the control location yielded higher larval concentrations than at the restoration sites. Modelling showed that the larvae at the Halfweg location were unlikely originating from either of these two sites. This would indicate to the presence of a relic population, capable of reproduction, of which the scale and position is currently not identified. Genetic analyses of the larvae should yield more insight in their potential origin.

Biodiversity monitoring
Since flat oyster reef restoration is intended to enhance the North Sea biodiversity, it is important to be able to monitor whether local biodiversity around an initial reef is indeed increasing. To get an impression of the (fish) biodiversity of the environment, we used environmental DNA (eDNA) sampling using Niskin bottles. This proved to work really well and is now commonly used in many other nature restoration pilot projects in the Dutch North Sea. We also conducted trials with bait cams and a consumer type underwater drone (ROV). Both methods proved to work (after some adaptations in the bait cam set-up) to get an impression of the fish and benthic community present. For example, fish species recorded on the bait cam also appeared in the eDNA results.

These results and recommendations have already been disseminated widely, via various communication methods and channels. This enables the flat oyster restoration community and other interested parties, such as the offshore industry, to use them for design, execution and monitoring of flat oyster restoration pilots in the offshore marine environment.

1 Introduction

Offshore wind is increasingly important in the Dutch energy transition. Its development is not only influenced by environmental factors (e.g. distance to coast, water depth, seabed structure and dynamics) and technical developments, but also by ecological considerations. The Dutch Ministry of Agriculture, Nature and Food Quality intends to steer offshore wind farm design towards strengthening the North Sea ecosystem, by enhancing policy-relevant marine species. This started as part of the North Sea 2050 Spatial Planning Agenda (Min I&M and Min EZ, 2014), is subject of the North Sea Agrement (OFL, 2020) and is currently being operationalized trough permit-obligations. Offshore wind licence holders are currently required to make demonstrable efforts into this direction (e.g. wind energy tenders IJVER and HKZ in 2022-2023).

European flat oyster (*Ostrea edulis*) reefs constitute a potential keystone habitat for the North Sea ecosystem, enhancing biodiversity. But a combination of diseases, pollution, cold winters and overfishing have caused their disappearance circa a century ago, so it is widely attempted to restore these reefs. The European oyster is internationally recognised as 'threatened and declining' in the NE Atlantic by OSPAR (OSPAR, 2008). At present European oyster beds are rare or absent in most of their natural range (OSPAR BDC, 2020) and several European countries have consequently adopted strategies for its conservation and restoration. Under the Marine Strategy Framework Directive, in the Netherlands one of the environmental targets is the "return and recovery of biogenic reefs including flat oyster beds (min I&W and min LNV, 2018). The species is also one of the focal species for nature inclusive building and restoration projects in offshore wind parks. The ECOFRIEND project aimed to develop and study new methods to re-introduce and monitor flat oyster reefs and related biodiversity in offshore wind farms, in cooperation with the wind industry.

This report provides an overview of the main findings made during the ECOFRIEND project (2019-2023). This is a Joint Industry Project, subsidised by the Dutch Ministry of Economic Affairs under the TKI Wind op Zee (Top consortium for Knowledge and Innovation Offshore Wind), under reference number TEWZ118017.

TKI Wind op Zee "stimulates, connects and supports Dutch organisations and knowledge institutes with the development and deployment of innovations to help speed up the transition to sustainable, reliable and affordable energy system, focussing on offshore wind" (https://www.topsectorenergie.nl/en/about-tki-wind-op-zee).

The project partners are Wageningen Marine Research, Bureau Waardenburg, Deltares and Sas Consultancy. The industry partner is Gemini Offshore Windfarm.

1.1 Project aims

The project aims of the ECOFRIEND project were:

1) Monitor pilots with restoration of European flat oyster beds in offshore wind farms;
2) Develop new monitoring methods and to assess the effectiveness for these pilots, reducing cost and increasing scientific output;
3) Understand the environmental background of the pilots by intensive monitoring and modelling of the surrounding parameters (e.g. temperature, salinity, food availability, turbulence);
4) Analyse, discuss and publish the findings in scientific and industrial literature;
5) Advise on eco-friendly design of future wind farms.

1.2 Expected results

The expected results of the ECOFRIEND project were:

1) Proof-of-concept for active reintroduction of offshore flat oyster beds;
2) Demonstrate whether there is a viable population of flat oysters in the Gemini offshore wind farm;
3) Novel methods to monitor the effectiveness of restoration initiatives providing competitive advantage for Dutch research organisations and industry;
4) Increased knowledge base for research organisations and industry that can be applied to initiatives in other North Sea countries (e.g., NORA).

1.3 Expected impacts

The expected impacts from the ECOFRIEND project were:

1) Economical - The gained experience with eco-friendly design in offshore wind farms will increase competitiveness of the involved industry partners in future wind farm applications;
2) Economical - Cost-efficient methods developed to monitor the effectiveness of biodiversity stimulation through flat oyster reintroduction will provide competitive advantage for Dutch research organisations and industry;
3) Ecological - Establishment of a viable population of flat oysters in offshore wind farms, kick starting offshore oyster bed restoration in the Netherlands and related biodiversity, increasing the feasibility of large scale offshore flat oyster restoration, one of the aims of the OSPAR convention;
4) Scientific - The increased knowledge base on flat oyster restoration will provide Dutch research organisations and industry the ability to export and facilitate similar initiatives in other North Sea countries (e.g., through the Native Oyster Restoration Alliance - NORA).

1.4 Project structure

The overall project manager is Wageningen Marine Research (WMR). The project consisted of the following work packages, which are detailed below:

- WP1: Monitoring (lead: Wageningen Marine Research)
- WP2: Innovation (lead: Bureau Waardenburg)
- WP3: Abiotics (lead: Deltares)
- WP4: Analyses (lead: Wageningen Marine Research)
- WP5: Advice (lead: SAS Consultancy)

WP1 Monitoring

Aims: Monitor pilots with restoration of European flat oyster beds in offshore wind farms

Tasks:

a. Monitoring the flat oyster pilot in Gemini wind farm;
b. Monitor the effectiveness of oyster reintroduction by measuring:
    - Survival and growth of the adult flat oysters;
    - Production of larvae by measuring larval concentrations in the surrounding sea water;
    - Larval settlement success on substrate provided in the reintroduction system;
    - Larval settlement success on other substrates in the environment,
c. Sample surrounding substrates to quantify the settlement of larvae on nearby e.g.:
    - Ship wrecks by scientific diver operated airlift sampler;
    - Natural rocky reefs by scientific diver operated airlift sampler.
d. Laboratory based analysis of water samples and samples taken on surrounding substrates to survey larval production by the oysters

WP2: Innovation

Aims: Develop new monitoring methods and to assess the effectiveness of these pilots, reducing cost and increasing scientific output.

Tasks:

a. Develop cost-efficient offshore methods to monitor the success of the flat oyster pilots and, where necessary, adapt the pilot design to these new monitoring methods;
b. Develop easy-to-monitor adult oyster cage system, combined with ROV retrieval system to be installed in future flat oyster pilots;
c. Develop novel cost-efficient offshore methods to monitor the presence of larvae in the immediate surroundings of the introduced adult oysters as well as settlement of these larvae on surrounding substrates;
d. Develop eDNA methods to quantify the number of larvae in the surrounding water after spawning of the adult flat oysters and apply in;
    - Near shore environment (also to try out the technique in a more accessible location than offshore);
    - Offshore environment, inside the Gemini wind farm.

WP3: Abiotics

Aims: Understand the environmental background of the pilots by intensive monitoring and modelling of the surrounding parameters (e.g. temperature, salinity, food availability, turbulence);

Tasks:

a. Collect complete time series of relevant environmental parameters (e.g. hydrodynamic climate, sediment concentration, temperature, turbidity etc.) for a full monitoring period by means of combining offshore measurements and numerical model hindcasts;
b. Setting up a numerical modelling framework to obtain time series of relevant environmental parameters, either to cross-check the measured parameters and to fill in any gaps in the time series, or to model parameters that cannot easily be measured; data will be supplemented by satellite data whenever necessary.

WP4 Analysis

Aims: Analyse, discuss and publish the findings in scientific and industrial literature;

Tasks:

a. Data-analysis;
b. To evaluate the results obtained in WP 1, 2 and 3;
c. To place the results in a wider context, by including data from other research, e.g. monitoring programmes on benthos and fish in the wider North Sea; the numerical modelling results will be used to explain observed differences between different locations/pilots;

WP5 Advice

Aim: Advise on eco-friendly design of future wind farms.

Tasks:

a. Advise on flat oyster pilots in other wind farms.
b. Advise on standardised, but location-specific changes to scour protection in future wind farms, making eco-friendly design easy and cost-effective.
c. To prepare the evaluated and contextualised results for dissemination to industry, government and nature conservation organisations;

2 Monitoring an oyster restoration pilot

2.1 Introduction

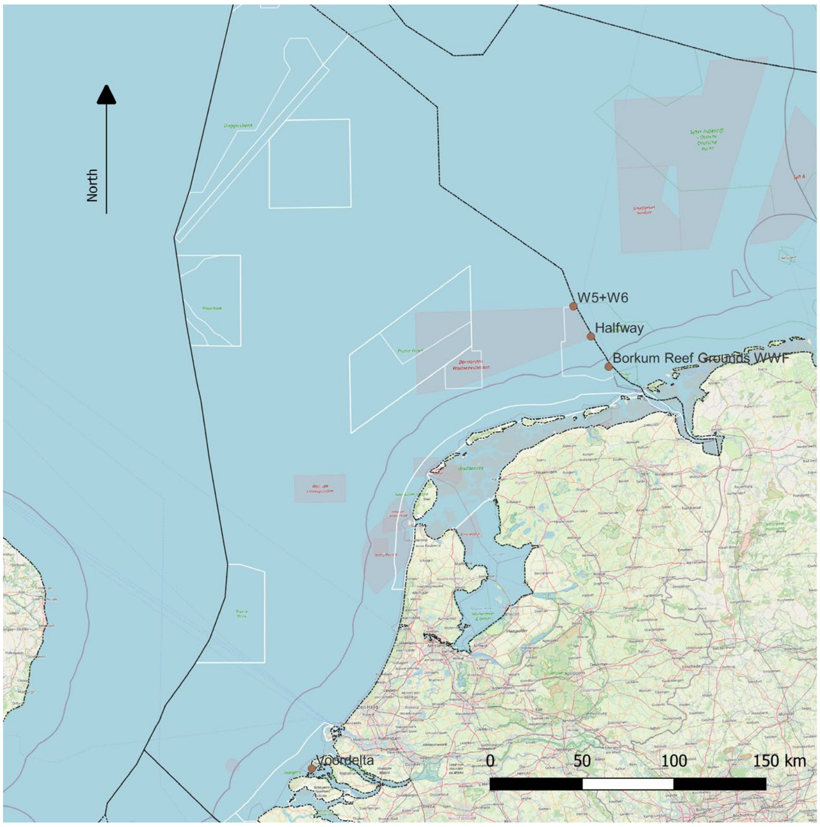
In the ECOFRIEND project we have monitored a pilot oyster restoration project in Gemini Offshore Windfarm. In short, the aim (see 1.4) was to see if oyster restoration was possible, if the oysters would grow and survive and produce larvae and if lighter and cheaper methods could be used for monitoring. In the windfarm, oysters imported from Norway were put on the scour protection and seafloor near two offshore wind piles. Lightweight experimental cages were installed with oysters in plastic baskets to be able to measure oyster growth. As added value, the oysters were individually numbered to be able to assess individual growth over a period of half a year. In the analysis, we compared our results to those of 5 other oyster restoration pilots that occurred within the period 2018-2021. Between 2019 and 2022 also oyster larvae were monitored and eDNA samples were taken to assess local fish biodiversity.

2.2 Study areas

The oyster restoration sites studied in the ECOFRIEND project were located offshore, north of the Dutch Wadden Sea, close to the German border (Figure 1): Gemini Offshore Windfarm (Gemini OWF) and Borkum Reef Ground. In both locations pilot oyster reefs were established in 2018 in collaboration with the Dutch World Wildlife Fund (WWF Netherlands) and a private partner, Edmelja B.V. Both sites are located in the historical distribution area of the European flat oyster (Olsen 1883) and are therefore considered to be suitable for oyster restoration (Smaal et al. 2015; Kamermans et al., 2018).

The Gemini OWF study site focusses on the area near two turbines: W5 and W6. Oysters were put on top and next to the scour protection of these turbines. These specific turbines were chosen because they serve as reference turbines for yearly inspections by a large ROV as part of the maintenance programme of the park. The other turbines and scour protections are inspected according to a multi-annul scheme.

The Borkum Reef Ground is a natural hard substrate area consisting of a mixed sandy and hard substrate seafloor that qualifies as reef habitat (1170) under the Habitat Directive (Bos et al. 2014, Coolen et al. 2015). In the hard substrate area, WWF has established an oyster reef consisting of 80.000 oysters, originating from Norway. Monitoring of the reef on the seafloor was led by Waardenburg Ecology (Didderen et al. 2019), while some additional monitoring in the water column was done as part of the ECOFRIEND project. This pilot location is marked by buoys.



ECOFRIEND sampling locations

Figure 1. Sampling locations in the North at Borkum Reef Grounds, halfway and in OWF Gemini (wind turbines W5 and W6) and Voordelta in the south of the Dutch North Sea (see 'valve gape monitor, section 3.4).

Figure 2. Turbine W5 in Gemini offshore windfarm.

2.3 Performance of flat oysters

The results on oyster growth performance are described in Bos et al. (submitted). We analysed the ECOFRIEND growth (Figure 4), survival (Figure 6) condition (Figure 7) and larvae (Figure 25) data of

Gemini offshore windfarm in comparison to those of 4 other oyster restoration pilots in the Netherlands (Borkum Reef Grounds, Luchterduinen, Bollen van de Ooster, Borssele V). Growth was expressed as increase in shell width (mm/month) (Figure 3). Survival is expressed as '% per month'. Condition Index is the ratio between dry weight (DW) of the oyster meat and the dry weight of the oyster shell: (DW meat/DW shell)*100.

For the Gemini site, our study showed that the Norwegian oysters grow and survive in the Dutch North Sea. It also found that the oysters had a healthy condition and that larvae were present in summer, pointing to a viable population. When comparing the five pilot oyster restoration pilots, we found that growth rate was explained by origin and average water temperature, to a lesser extent by the duration of the pilot (number of months), location and salinity and not to other environmental factors such as pH and O_2 (Bos et al., in prep.). Variations in growth rate were probably also related to the oyster size at the start of the pilot: detecting growth is more difficult in large oysters as increase in size reduces with age, compared to small oysters. Also the timing of sampling is important, since oysters do not grow all year round, but only grow during a part of the year. When comparing the different pilot oyster restoration monitoring studies, it became clear that there is an urgent need for a standardized monitoring protocol, because data were not always comparable.

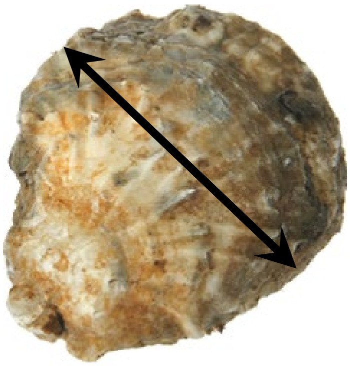


Figure 3. Shell width: largest size perpendicular to hinge (illustration: Pauline Kamermans, WMR).

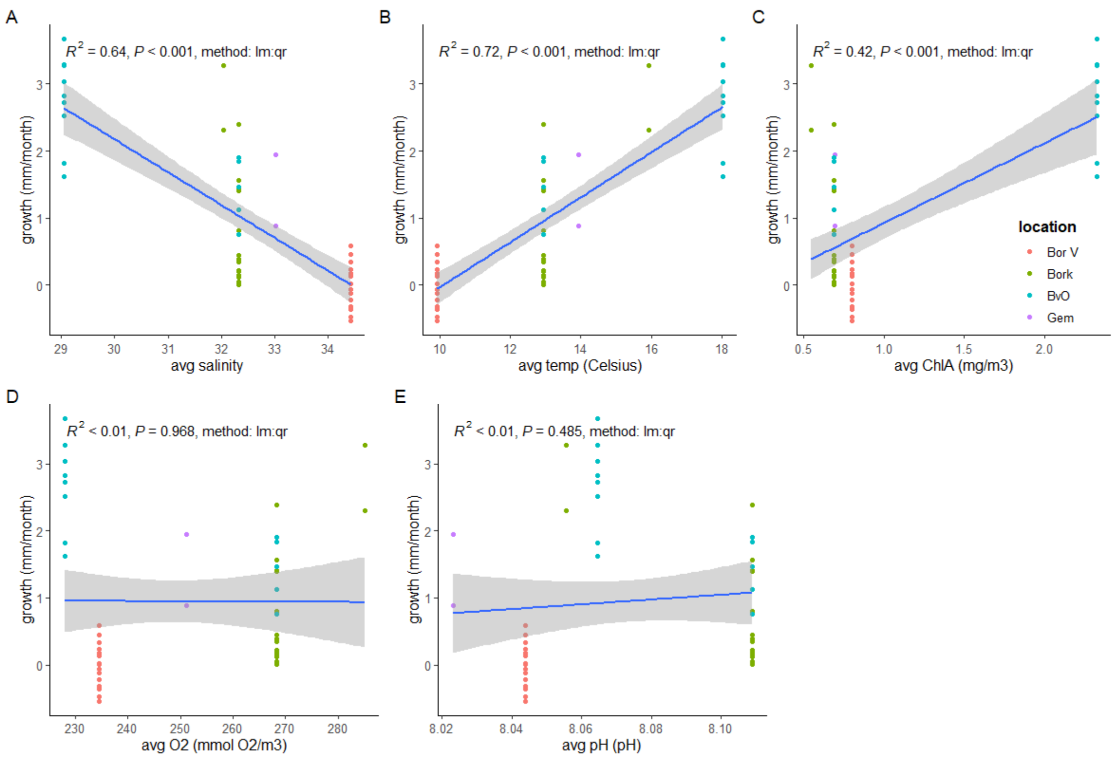


Figure 4. Oyster growth (mm/month) in relation to abiotic parameters in different oyster restoration pilots (Bos et al. submitted).

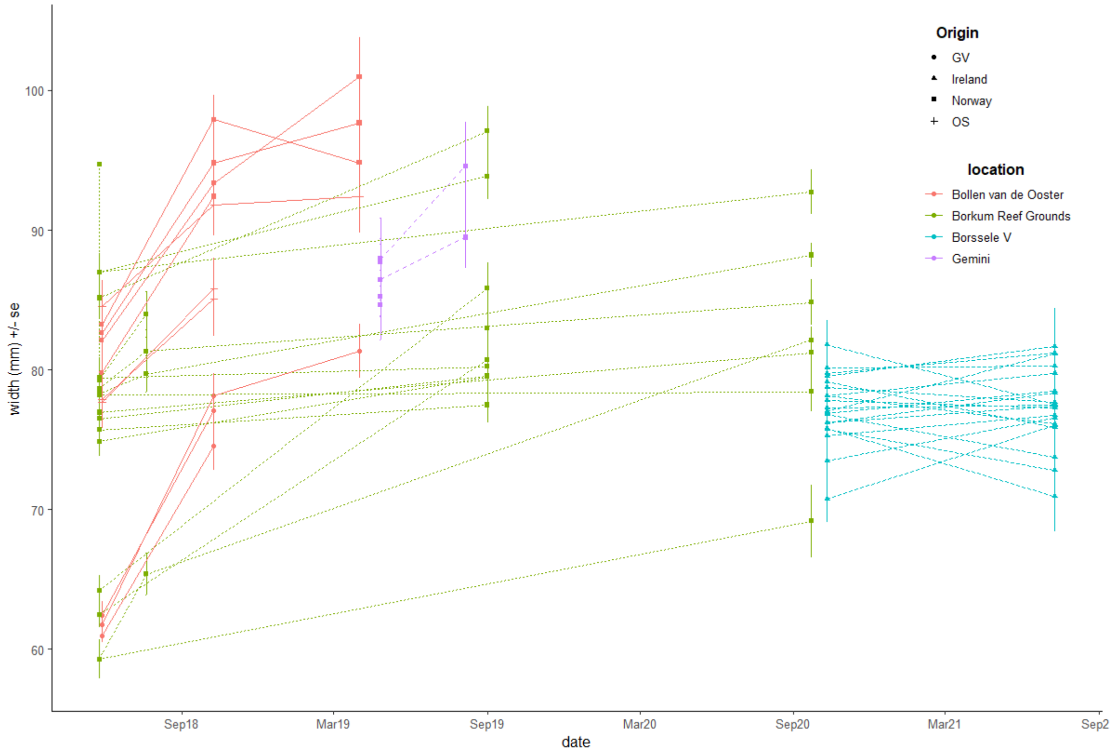


Figure 5. Oyster size (width) through time at different pilot locations (Gemini = blue) (Bos et al. submitted).

Figure 6. Oyster survival in different oyster restoration pilots (Bos et al. submitted). Gemini=purple.

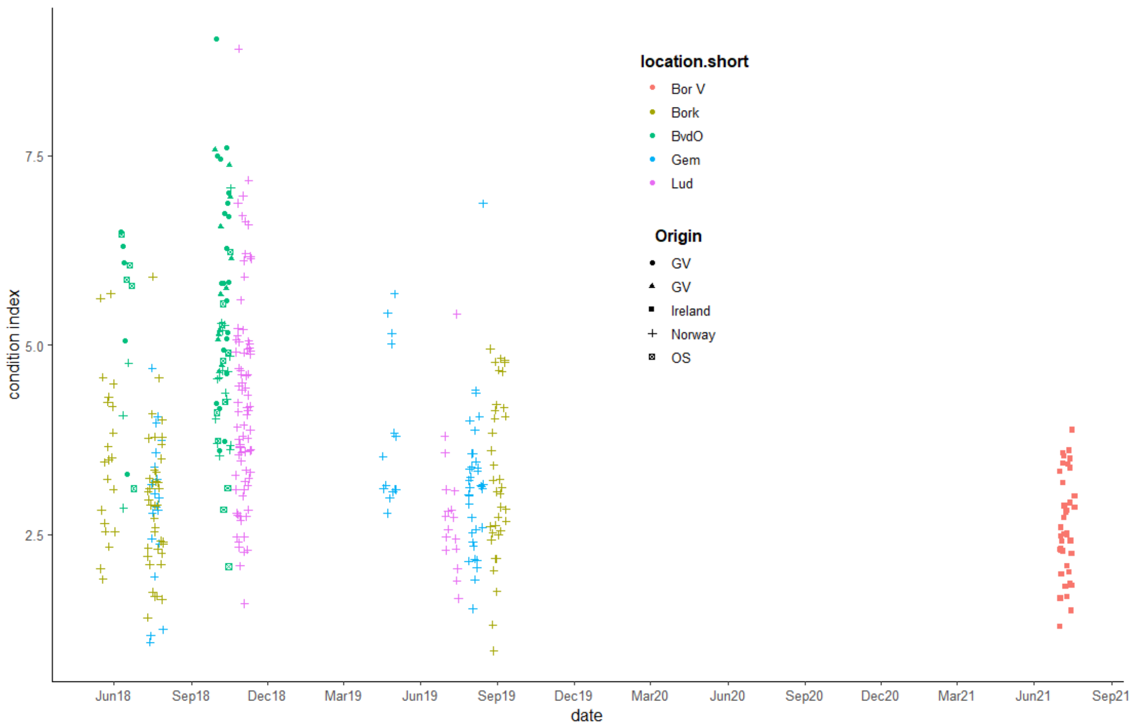


Figure 7. Oyster condition in different oyster restoration pilots (Bos et al. submitted). Gemini=light blue.

Take home messages:
- Flat oyster populations sourced abroad (Norway) can survive in the Dutch North Sea.
- These flat oysters show healthy condition. Larval settlement detection techniques need to be developed.
- Positive relationships between growth and temperature were observed.
- However, growth rate alone is not a comprehensive enough indicator to illustrate project performance.
- There is an urgent need for a standardized monitoring, so more data become available (e.g. individual growth rate per oyster) and inter-site comparison can be done more accurately.

2.4 Testing cost effective monitoring methods for oyster bed restoration activities

2.4.1 Tagging, measuring and photographing oysters

We tested how oysters can be best documented and measured by improving existing methods. Prior to deployment in the sea, oysters were photographed with a ruler on a neutral background (10 oysters per tray) (Figure 8). This serves to have a visual archive. Also, in case of loss of data, lengths and width can still be determined. The photos are stored for future Machine learning objectives.

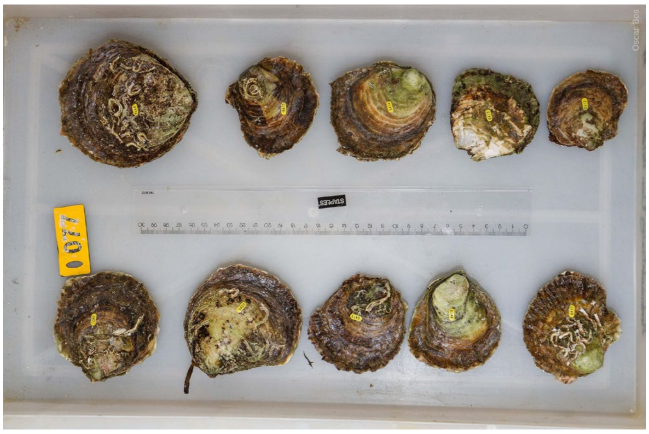


Figure 8. Numbered oysters for deployment in oyster cubes, April 2019.

Individual oysters that were placed in cages were tagged with a plastic number (Figure 8). This allows for measurement of parameters on the individual oysters (such as weight, length, sex and gonad development) (*Figure 10*, Figure 11, Figure 12). Thin (150 micrometer), 4x8 mm flexible polyethylene tags (Hallprint glue-on-shellfish-tags: www.hallprint.com) were glued with an ethyl-based general purpose instant adhesive (Loctite 422: www.loctite.com) on the shells. This adhesive even allows for firm attachment to wet salty surfaces. After half a year of deployment in the field, oysters were successfully retrieved (Figure 14) and the tags were still present.

The tags proved to work very well. Nonetheless, somewhat broader tags could be used, since 4x8 mm is somewhat hard to handle. In other pilot projects such as the Borkum Reef pilot by WWF, oysters were stacked in racks to be able to individually recognize them, but their growth may have hampered due to the construction.

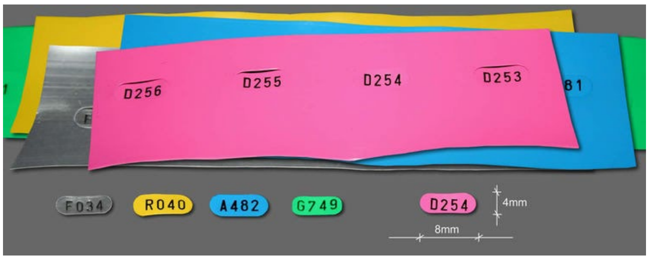


Figure 9. Glue-on-shellfish tags used for tagging oysters (source: https://hallprint.com).

Figure 10. Measuring tagged oysters before deployment.

Figure 11. Determination of oyster sex and gonad weight (photo: Oscar Bos, WMR).

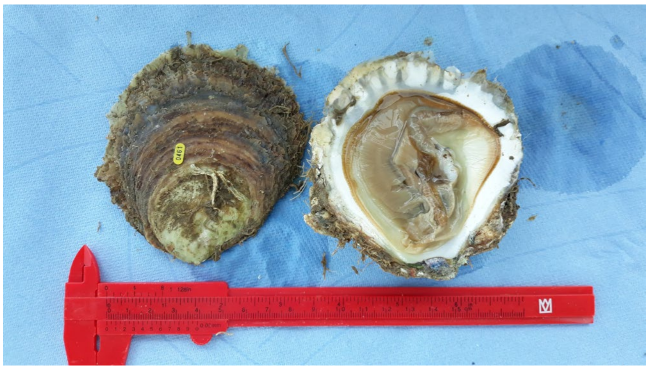


Figure 12. Oysters measured in the laboratory in Yerseke: outer part is newly grown shell (photo: Pauline Kamermans, WMR).

2.4.2 Oyster cubes

To test how well oysters survive and grow in offshore wind farm locations, oysters have in earlier projects been placed on heavy metal or concrete 'tables' or reef constructions, which require large vessels for installation. In the ECOFRIEND project we tested if lightweight 'oyster cages' would be usable as well. In offshore windfarms Gemini, oyster cubes were deployed (1x1x1 m x 18 mm steel)

(Figure 13), and oysters were stored in plastic oyster cages (ca 60x15x15 cm, 1 cm mesh), which are commercially used in the oyster industry. However, the light construction also caused these cages to start moving over the sea floor. Of the deployed cages, only 1 out of 3 could be found back. These oysters were taken to the laboratory for measurements, as described above. The recovered oysters all appeared to have survived and showed growth (Bos et al., in prep) (see 2.3).

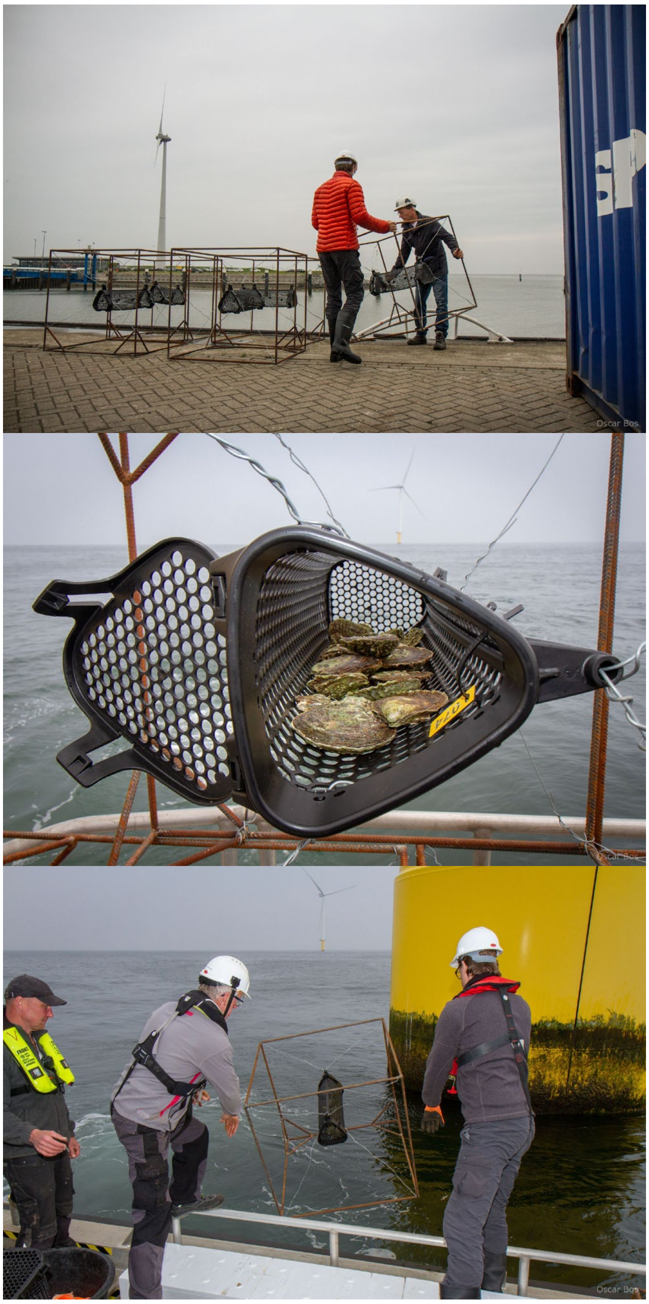


Figure 13. Oyster cubes with plastic cages as deployed in 2019 (photos Oscar Bos, WMR).

Figure 14. Marine growth on oyster cubes (predominantly the hydroid Tubularia larynx and also the bryozoan Electra pilosa).

The results on oyster cubes will be incorporated in a manuscript on oyster performance across 5 different pilot oyster restoration projects, including Gemini and Borkum Reef Grounds (Bos et al., in prep).

2.4.3 Bait cam

A way of assessing fish and other mobile species biodiversity is the use of a baitcam: a frame with a installed camera, which is placed on the seafloor to attract fish and benthic species using bait (e.g. pieces of mackerel). We have deployed the camera a few times in between eDNA sampling to check if the species detected by eDNA analysis would also be the species seen on camera. In general, this is the case.
The bait cam used consists of a 13 kg steel frame, equipped with an eye for attachment of rope, to which 40 m rope (Tendon Static 10.5) was attached. At the surface, 2 buoys (red, circa 25 cm diameter) make sure the rope would float and could be found back easily. Two GoPro Hero7-black cameras were attached to the frame. Bait was attached to the frame to attract fish, and mobile benthic species such as starfish, crabs and shrimp.

Video footage was analyzed by recording any species observed in the footage. A first quickscan of the footage of 2019 showed a few velvet swimming crabs (*Necora puber*, fluwelen zwemkrab) being attracted to the bait, as well as a few sand stars (*Astropecten irregularis*, kamster). A small school of red mullets (*Mullus surmuletus*) passes by, and about 3 to 4 times, large schools (10-100s of individuals) of a non-identifiable fish species (probably gadids, kabeljauwachtigen) pass by. Around the steel frame, 5-10 gobies (*Pomatoschistus* sp., grondels) are swimming around throughout the period. No flatfish were seen. Since baitcam was not applied throughout the project, the results only serve to demonstrate a proof-of-concept.

The trials resulted in these recommendations for future bait-cams:

- Add a 3rd camera directed towards the outside of the frame, not to detect fish attracted to bait, but for other species in the water column.
- Redesign the location of the bait, so that all predators are visible on the cam
- Reduce the size of the bait cam or change gearing of buoys, so that the frame does not fall over in higher wave condition.

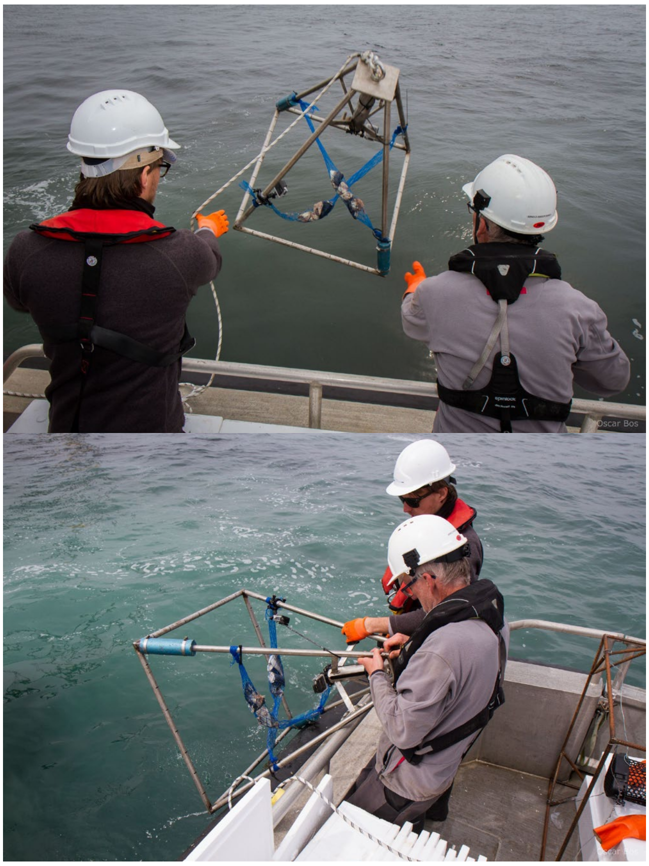


Figure 15. Deployment and recovery of the bait cam, April 2019 (photos: Oscar Bos, WMR).

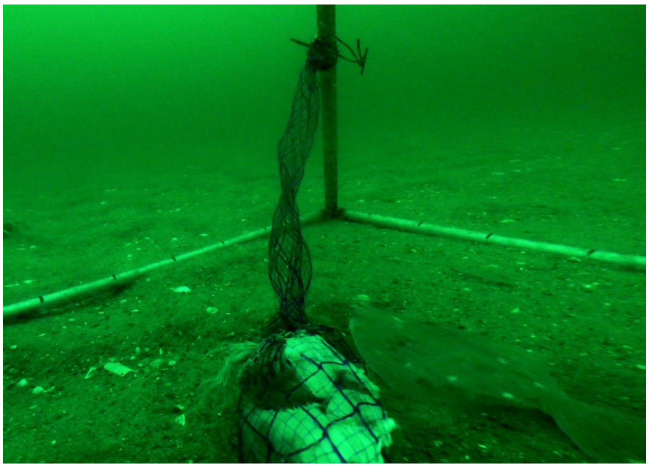


Figure 16. Plaice and other fish attracted to the bait, April 2019 (photos: Oscar Bos, WMR).

2.4.4 Commercial ROV vs small ROV

During the annual inspection survey of the offshore windfarm (In August 2019) a large industrial ROV operated by Bluestream (www.bluestreamoffshore.com) on board of an offshore vessel was used to retrieve oyster cubes and perform a small oyster bed survey,. ECOFRIEND prepared instructions for the ROV operator, which were communicated by Gemini (see paragraph 2.7: Gemini ROV inspection 5 Aug 2019). In the year before, 2018, a large ROV was used to retrieve 1 oyster cage and locate the other 5 oyster cages.

Every year, the Gemini offshore windfarm is inspected by a large surveyor, to inspect the scour protection and other assets. The yearly inspection took place in August 2019 and Gemini offered to dedicate part of the last survey day to retrieve oyster cubes and bring them to the harbour. ECOFRIEND was invited to join the survey, but since it was not possible to enter or leave the survey vessel at sea (time span constraints), it was decided that providing instructions to the ROV operators would be more efficient. The instructions were sent to Gemini, who instructed the operators of the large ROV to retrieve 2 of the oyster cubes deployed in April 2019 and to perform a small survey of the oyster bed. The inspection at the site took place on 5 Aug 2019. The survey vessel returned to Den Helder at 6 Augustus 9:30h AM after an overnight crossing. The oysters that had been stored in a fridge on board were then transported to Yerseke by train, where they arrived at 14:00h. The oyster cubes were covered by marine growth, predominantly the hydroid *Tubularia larynx* and also the bryozoan *Electra pilosa* (Figure 14).

2.4.5 Cost effective use of vessels

Figure 17. Crew tender vessel in 2019 used for monitoring in Offshore Windfarm Gemini (photo Oscar Bos, WMR).

Costs of fieldwork are largely determined by ship costs. Usually, expensive DP2 offshore vessels meet the minimum requirements for performance and safety, and are therefore used to install e.g. oyster cages or artificial reefs. The advantage of such big ships is that these constitute stable platforms even under harsh weather circumstances, and that they can perform complicated monitoring tasks using e.g. industrial ROVs under relatively high current velocities. The disadvantage is their high daily rate (e.g. 40-60kE/day). In the ECOFRIEND project we aimed at using low cost vessels that need relatively good weather to be of use, and using mainly hand operable monitoring techniques (weighing <25kg). As a small vessel we could use the CTV (crew transfer vessel) 'BMS Vrijheid', a high speed (max 35 kts) 2 jet engine aluminium vessel, length: 14 m, width: 5 m. Working with CTVs proved to be fast and efficient. The distance to Gemini offshore windfarm was 60-80 km, and could easily be covered using this type of hydrofoil vessel.

3 Develop and test innovations for oyster restoration monitoring

3.1 Develop easy-to-monitor adult oyster cage system, combined with ROV retrieval system

Introduction
Flat oyster restoration pilots aim to initiate the recovery of a self-sustaining population. The success parameters of restoration are survival, growth, reproduction and recruitment of flat oysters. These parameters can be measured with live oysters in baskets. These baskets are fixed to the cages, which are deployed on the sea floor. The cages are an alternative for monitoring flat oysters on the seafloor by scientific divers.

Design requirements
Oysters are filter feeders and forage on phytoplankton. This implies that the water flow through the baskets and research cages should be as large as possible to provide enough food and oxygen. After deployment, epibenthic organisms will cover the baskets and cages (Figure 14, Figure 23) which will diminish the flow through. This implies that the mesh size of the baskets and cages should be as large as possible, but small enough to contain the oysters.

The oyster cages are deployed on the seafloor or scour protection. This implies that the cages should be stable during the monitoring program. In addition, the oyster cages should be hoistable for deployment and recovery during the program.

As a result of these design requirements, the oyster cages as applied in pilot studies in offshore wind farms are oversized, to prevent them from moving due to wave-induced bottom shear during storms. Displacement of a cage could potentially lead to damage to the assets present in wind farms. The disadvantage of oversized cages is that the cages become (very) heavy and only a large vessel with sufficient crane capacity is able to deploy and hoist the cage. In addition, the large size of the oversized oyster cages can result in scouring which can make them instable and lead to coverage by sand. The starting point for the required design (called the 'WERC-dock') was to create a structure that has a high stability and could also be deployed by smaller vessels and hoisted up by light equipment (even by hand) from the seafloor.

The design
The structure that contains the oysters needs to be stable on the sea floor, but small and light enough to be placed and collected by small vessels and light equipment. We carefully scrutinized the basis of the design, and it appeared that the basic design of WERC-dock should not be a cage, but a rather different structure: three layers stacked on top of each other over a vertical pole which is stably fixed to the sea floor (Figure 18, Figure 19). On each layer, several baskets (with oysters) can be attached. The different layers are relatively light and can be collected using a ROV to attach a line with which the layer is lifted from the vertical pole. The line is attached to the layer through a loop on the tube that is placed over the vertical pole. The layers are horizontal and thin to minimize drag. The vertical pole has a loop on top for lowering the whole construction to and collecting it from the sea floor.

The base of the WERC-doc consists of four widespread thin arms, to keep the pole vertical. All construction tubes are open, so no air gets trapped inside. The legs are detachable for transport. The vertical pole is flexible at the bottom, in all directions to absorb wave-induced bottom shear impact on the whole WERC-dock.

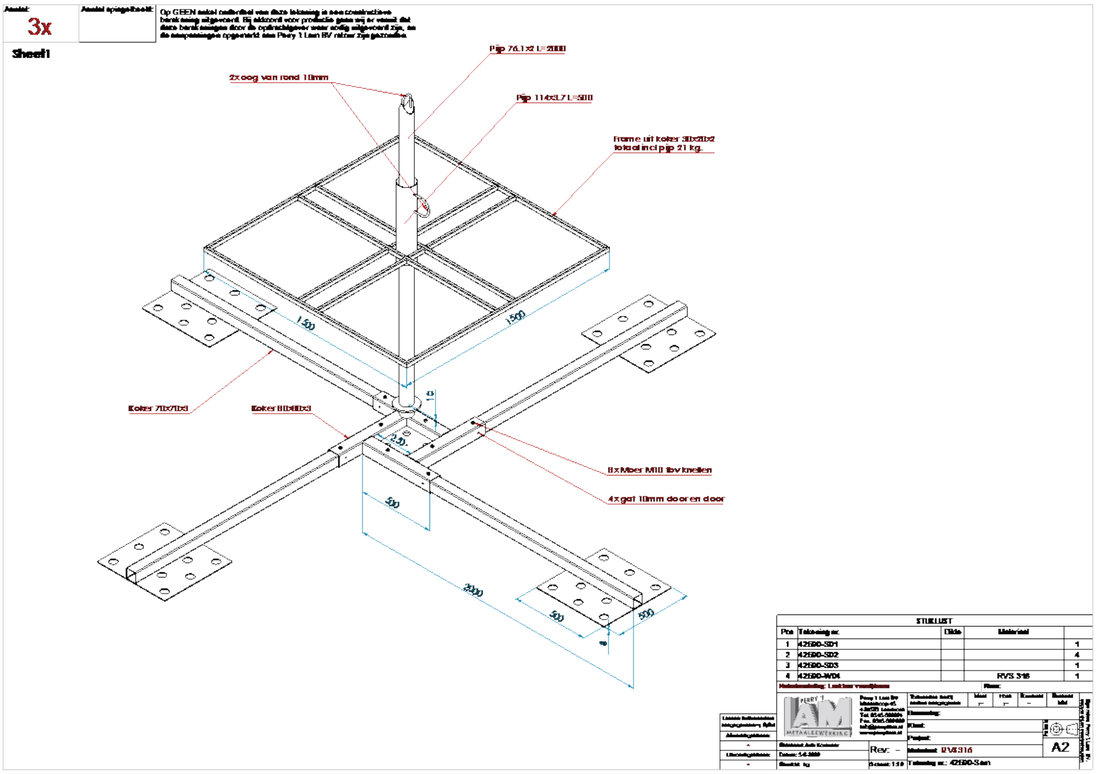


Figure 18. Design of WERC-Dock for oyster cages.

Figure 19. Prototype WERC-Dock before deployment.

Flume test

We investigated the stability of the WERC-dock for different wave conditions; from a once-a-year storm (heavy) to a once-a-100-year storm (severe). Two potential failure mechanisms are relevant: sliding and overturning. We also looked at the orientation of the structure in the flow, both in flume tests to determine the resistance and in the calculations to find the most likely direction for the structure to overturn/slide. These tests resulted in small adjustment to the first design. The legs need to be extended outward by 1,75 m with three layers and 1,0 m with two layers. This causes the structure not to tilt in all storm conditions. Because the structure is very light, sliding needs to be prevented too. Pins on the underside of the legs will perform that function. Once on the seafloor, even if it is sandy, the pins should create sufficient horizontal resistance to remain in place.

These design considerations led to the use of maximal two layers in the WERC-dock. In addition, the legs were all lengthened by 50 cm and provided with plates on the ends and pins that hook into the ground (see figures below). The plate at end of each leg keeps the structure from sinking too far into the sea floor and gives it resistance once there is some sediment covering them.

Experiences with the WERC-dock

In February 2021, the prototype WERC-dock was deployed at the Offshore test site (North Sea Innovation Lab) in Scheveningen in cooperation with the Rich North Sea (De Rijke Noordzee) program (Figure 20, Figure 21).

Figure 20. Final design of WERC-dock just before deployment in February 2021.

Figure 21. Deployment of the WERC-dock at the offshore test site in February 2021.

In September 2021, the first monitoring was carried out. The structure was standing upright and in place as intended. Some marine growth was already forming. A school of young fish (pout) stayed around the structure during the diving research (Figure 22).

Two years after deployment, in April 2023, the second monitoring was carried out. This inspection occurred after three intense storm events (9-12 bft) in February 2022. The structure was still standing upright and in place as intended. The pole was upright and flexible. The layers were above the sand and could be moved. The legs were just under the sand. Now the structure was fully covered by epibenthic organisms (mainly giant plumose anemone *Metridium dianthus* and Oaten Pipes Hydroid, *Tubularia indivisa*) (Figure 23). Oyster baskets were still present on the lower layer. The oyster baskets on the upper layer were removed during the first monitoring in September 2021.

Conclusion

The WERC-dock performed as expected and is a cost-effective alternative for scientific diving and the large, oversized cages used in previous oyster restoration pilots.

Figure 22. The WERC-dock after deployment during monitoring in September 2021.

Figure 23. Epibenthic organisms on the WERC-dock two years after deployment during monitoring in April 2023.

3.2 Detection of flat oyster larvae in field samples

Figure 24. Sampling of oyster larvae by filtering seawater from above the seafloor.

An easy-to-use larval sampling technique was developed by which larvae were sampled by filtering 200 L (2 x 100L) of seawater per sample from 0,5 – 1 m above the seafloor with the help of a 30 m long hose and a pump over a 100 µm plankton net (Figure 24). This functioned very well.
The residue of one 200 L sample was stored in formaldehyde and used for microscopic counts. The other was stored in ethanol and used for qPCR (DNA-based technique to identify specific species). Samples within the ECOFRIEND project were taken at the offshore locations Gemini windfarm, Borkum Reef Ground and "Halfweg" (a location halfway the two locations, as a reference), in 2019, 2020, 2021. At all locations, larvae were found, even at the reference location. As a follow up project, a genetic determination is required to identify the origin of the larvae at all sites. To put the results in context, the Gemini and Borkum Reef Ground results were compared to other pilot oyster restoration sites (Figure 25) (Bos et al., submitted).

In addition, samples were collected at the near-shore location Blokkendam in the Voordelta in 2022. Oyster larvae were detected in the samples with two methods: microscopically (the traditional and reliable method) and with qPCR. The majority of the samples showed a some agreement between the two methods, but in some cases either the microscope or the qPCR method did not detect *O. edulis* larvae**.** Hence, the DNA-technique needs further improvement.

Main findings:

- Larvae were successfully sampled at oyster pilot locations in summer using a pump and filtering 200 L of water from near the seafloor.
- During the ECOFRIEND sampling, also at a reference site in between oyster pilot locations Gemini and Borkum Reef Ground oyster larvae were found. This needs further investigation with Deltares models (can the larvae theoretically end up there?) and genetic research (are they genetically the same as the oysters in the oyster pilots?).
- Larvae can be detected by microscope, and by DNA (qPRC).
- The majority of the samples showed some agreement between the two methods, but in several cases either the microscope or the qPCR method did not detect *O. edulis* larvae. It is recommended to further improve the DNA-technique.

The larval counts will be incorporated in a manuscript on oyster performance across 5 different pilot oyster restoration projects, including Gemini and Borkum Reef Grounds (Bos et al., in prep). The larval from the reference site need further investigation. This will be done in follow up project.

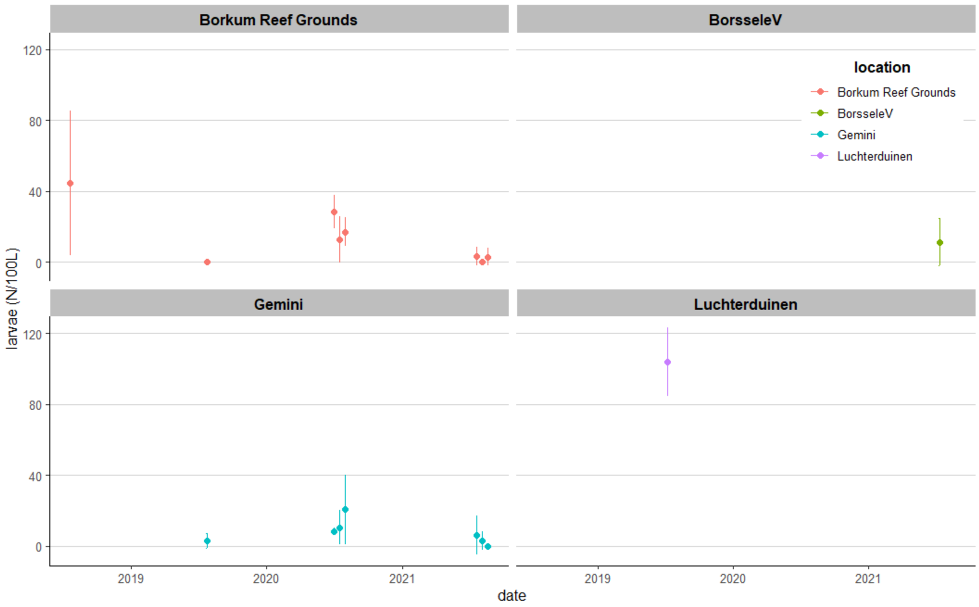


Figure 25. Oyster larvae (N/100 liter) in different oyster restoration pilots (Bos et al. submitted).

3.3 Detecting fish with eDNA

The restoration of native oysters is expected to result in biodiverse biogenic reefs that are attractive to many fish species, because the reef can serve as a shelter and provide food. In the ECOFRIEND project we have further progressed the use of eDNA as a tool to standard monitor fish and benthic biodiversity in oyster restoration studies. eDNA is collected by sampling seawater in which small particles of fish DNA are present. The eDNA is compared to species specific ‘barcodes' in DNA databases.


Figure 26. Collecting eDNA by filtering seawater from above the seafloor.

In the ECOFRIEND project, water samples were collected from 0,5 – 1 m above the seafloor. First using 30 m long tubes and a pump (2019), later (2020-2022) with a Niskin water bottle (1.7 L) at the locations Gemini windfarm, Borkum Reef Ground and Halverwege, which is halfway in between these two locations in 2019, 2020, 2021. eDNA metabarcoding showed that specific fish species generally found over all sample years together, Raitt's sand eel (*Ammodytes marinus*), horse mackerel (*Trachurus trachurus*) and mackerel (*Scomber scombrus*) are the most common at Gemini, while Lesser sand eel (*Ammodytes* tobianus), herring (Clupea harengus) and Lozano's goby (*Pomatoschistus lozanoi*) are the most common at Borkum and at Halfway, sand goby (*Pomatoschistus minutus*), Lozano's goby (*Pomatoschistus lozanoi*) and scaldfish (*Arnoglossus laterna*) are the most commonly found fish species. There seems to an increase in species richness in all sample locations over the years 2019 to 2021. This is probably caused by improved laboratory work and bioinformatics analysis rather than an true increase in the number of species at all the sample sites.

With the eDNA method it appears to be feasible to detect significant differences in species composition between sample sites and/or timepoints. Looking at relative abundances of specific traits for fish species, a clear distinction between Halverwege and both Borkum Reef Ground and Gemini can be made. This indicates that eDNA metabarcoding can be used to identify (changes in) habitat types based on the specific traits of the detected fish. Or vice versa, giving an indication of missing species that can be expected based on the known habitat.

A scientific manuscript on the eDNA results was produced by Doorenspleet et al. (2021, preprint) and is now under revision, and new data will be added. The manuscript focuses on the fish composition, and on performance of new eDNA analysis techniques. The aim was to develop and validate a method in which longer parts of the DNA are analysed ('long read nanopore sequencing'), compared to older methods in which shorter DNA parts were analyses. This improvement has great potential for future rapid and accurate fish species monitoring. However, currently, incomplete reference databases still form a major bottleneck in further developing this high resolution long read metabarcoding technique.

<u>Main findings:</u>

- The new eDNA analysis method 'high resolution long read metabarcoding' has great potential for rapid and accurate fish monitoring. However, reference databases are still incomplete.
- Methods and DNA barcodes are yet to be further developed, and offer opportunities for in situ and laboratory analysis.
- eDNA metabarcoding can be used to identify (changes in) habitat types based on the specific species composition of the detected fish.

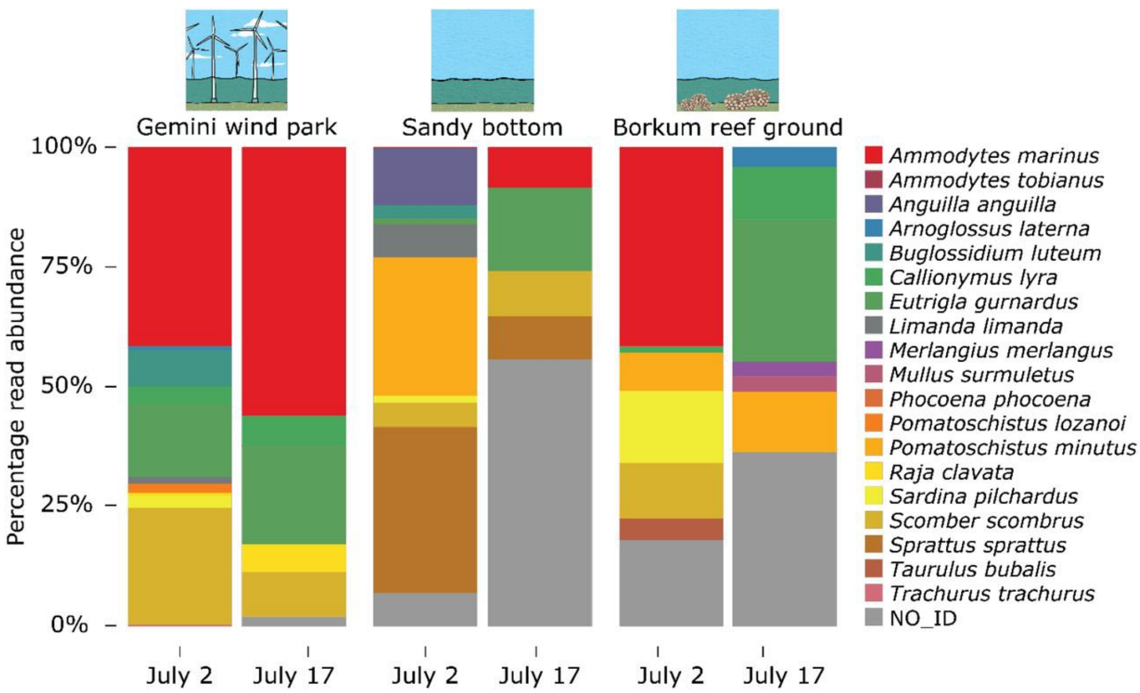


Figure 27. Percentage read abundance plot of the marine vertebrate species in the locations Gemini wind park, at sandy bottom halfway Gemini wind park and the Borkum reef grounds and at the Borkum reef grounds. Bars indicate at which date the water samples were taken. Colors indicate the proportion read abundance of the identified fish species. (Doorenspleet et al., 2021, preprint).

3.4 Develop and test valve gape monitor

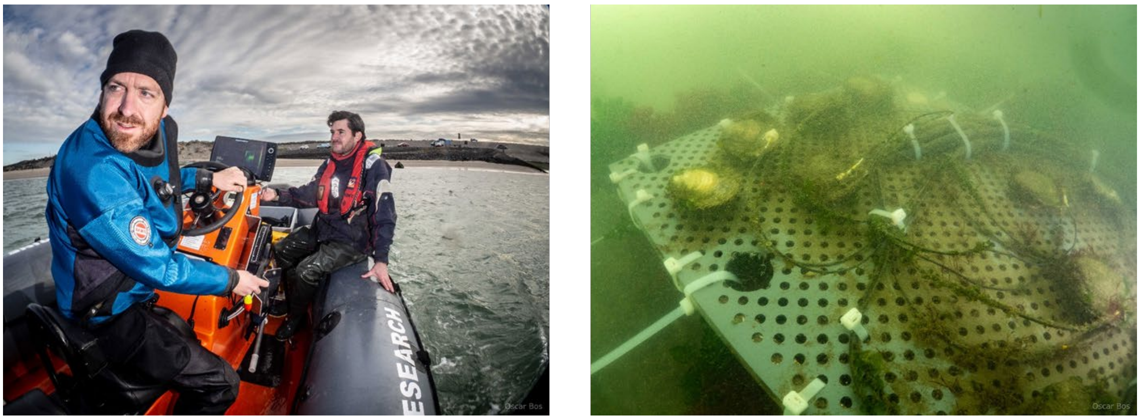


Figure 28. Placing the valve-gape monitor with flat oysters in the Voordelta.

The valve gape monitor study is published by Tonk et al. (2023). A valve gape monitor, a device that continuously measures opening and closing of live bivalves, can potentially be used as an effective method to determine survival and behaviour of the European flat oyster *Ostrea edulis*. In ECOFRIEND, we have tested and further developed this device. Electrodes are glued on both valves of the oyster, so that the width between the both valves can be measured. Whenever an oyster opens to feed, or closes due to stress, this can be detected, and this can be useful information to understand the success or failure of an oyster restoration project.

In the ECOFRIEND study, eight individual O. edulis were equipped with valve gape sensors in order to relate gape to environmental conditions such as food availability. Valve gape activity was monitored under controlled laboratory conditions, with and without food, in a concrete basin in the Oosterschelde and in the field (Voordelta, Dutch North Sea). Under controlled laboratory conditions, oysters clearly responded to changes in food availability. Starved oysters closed their valves significantly longer than oysters that received food, and the relative gape width in fed oysters were larger. In the concrete basin (Oosterschelde), a positive correlation between valve opening and Chlorophyll-a was found. Additionally, valve gape activity and tidal movement appeared to be linked. When exposed to a full tidal cycle (Voordelta), a negative correlation between valve opening and Chlorophyll-a was found. However, there was no correlation between valve gape and current velocity. In autumn, longer periods of inactivity were seen, but when valves opened, the valve gape was larger.

These data indicate that valve gape can provide valuable information on behaviour (gape frequency and gape width), but also show that it is not necessarily a good proxy for feeding rate. Nevertheless, these results show that the gape monitor can be used to determine the natural behaviour of flat oysters under field conditions, and that gape opening provides information on behaviour and the stress response of bivalves to environmental conditions.

Take home message:

- Valve gape monitoring can provide valuable information on behaviour (gape frequency and gape width),
- Valve gape monitoring does not necessarily provide a good proxy for feeding rate.
- The gape monitor can be used to determine the natural behaviour of flat oysters under field conditions,
- Valve gape opening provides information on behaviour and the stress response of bivalves to environmental conditions.
- Valve monitoring can be combined with other methods (eg. hart beat sensors) to overcome knowledge gaps.

3.5 Using dummy oysters for modelling and traceability

Figure 29. Dummy oysters being prepared to be released just above the seafloor.

One important question is: what happens to the source material (adult oysters, spat on shell) after being deployed in the field. In the past years it was noted that in the WWF oyster restoration site 'Borkum Reef Grounds' (*Figure 1*) oysters which were deployed loosely on the sea floor became dispersed strongly, so that they could not be found back in their high initial densities. A widely dispersed oyster population cannot function as starting point/incubator for a reef. It was hypothesized that this could be due to effects of winter storms and therefore it was investigated whether the Deltares 3D model would be able to predict the hydrodynamic effects on oysters in general. This would then help to predict were oyster restoration would be successful and where oysters may be 'blown away'.

As a first step, a lab experiment was conducted in July 2021 in a Deltares flume tank. About 200 dummy oysters were used that were 'made' from empty shells by WMR in Yerseke, by gluing matching flat oyster doublets together. Different sizes were used, and different weight classes were made within the size classes, by adding metal parts inside in some of the shells. Each doublet was numbered. Also a few oyster clumps were made by gluing 2 to 4 dummy oysters on top of each other. The dummy shells had been measured and weighed by WMR and were again weighed (under water) by Deltares before being systematically tested under different hydrodynamic regimes. The results are reported in par. 4.4.

The next step was to release these dummy oysters 'in the field'. This was done on September 25th, 2021 on the Borkum Reef Grounds (see Figure 27). In September 2022, another visit was paid to the area to investigate how these had dispersed from their deployment location over time. However, none of the dummy oysters could be found back by divers or by ROV. This suggests strongly that the oyster motion on the seafloor is strong. See par. 4.4 for the discussion on near-seafloor turbulence and its effect on loosely strewn oysters.

We therefore strongly recommend developing deployment methods by which the oyster population remains together on the seafloor.

3.6 Using seal data to validate models

During the ECOFRIEND project, DELTARES was constantly looking for environmental data to improve models that can predict temperature and current on local scales. Within other projects of Wageningen Marine Research, seals have been equipped with transmitters that record position, depth and temperature. These seals were tagged in Eems-Dollard region and frequently visit the Borkum Reef Areas. To improve the models, we have extracted the relevant abiotic data from the seals and added them to the Delft model. This is an example of combining very different data sources, cost-efficiently to obtain data that otherwise are difficult to obtain.

4 Modelling as a tool to support oyster restoration

4.1 Introduction

In this chapter the results of the modelling framework employed by Deltares to assess various aspects of oyster restoration are reported. The actual oyster restoration activities took place at Gemini offshore wind farm and in Borkum Reef Grounds (see par. 2.2). The modelling is described in detail in Van Duren et al. (2023).

4.2 Prediction of oyster spawning period

To monitor the success of a flat oyster restoration project, it is essential to be able to observe whether the seed population produces larvae and therefore to predict the period when the seed population produces these.

Therefore, a model was developed by which this period can be predicted by using data that are included in standard North Sea monitoring. On an annual basis, oyster spawning is assumed to occur once oysters exceed a certain temperature sum. This is the number of days that the water temperature near the seafloor is above a certain threshold times the temperature in degrees Celsius above that threshold per relevant day (Maathuis et al., 2020).

The input data for this model were generated by the 3D Dutch Continental Shelf Model in Flexible Mesh (3D DCSM-FM). Among others, it can predict water temperature development in a given year at any location. At the time of this research, it is the best available hydrodynamic model for the North Sea, outperforming similar models in simulating processes such as stratification.

The temperature development prediction in this study is based on a hindcast of seabed temperature at Gemini with the 3D-DCSM-FM model. Following certain assumptions concerning the site-specific conditions controlling oyster spawning and swarming, the results demonstrate a 40-day variation in the spawning onset for the 17 years considered, i.e., between 19 July and 28 August.

Within each year of prediction however, this variation appears to have a constant uncertainty of only 7 days. This means that if a temperature hindcast is made for the year that offshore activities related to oyster restoration are planned, such as monitoring of oyster larvae in the water column or deployment of suitable substrate for its settlement, the predicted optimal time window to execute those may be narrowed down significantly. This works as follows: for example, monitoring needs to take place somewhere in August/September and the optimum week is sought. Early July, one could use the model to hindcast the development of the temperature up to June and thereby get a good estimate for the temperature build-up up to that point. The prediction of the period of larval swarming then can be made either using expert judgement or statistical analysis.

4.3 Larval dispersal modelling

During ECOFRIEND fieldwork, larvae were sampled at 2 locations where oysters restoration pilots were located (Gemini wind farm and the Borkum Reef Ground), as well as at a location halfway between these sites (the 'Halfweg' location) (see 3.2). Surprisingly, in two consecutive years the Halfweg location yielded higher larval concentrations than at the restoration sites. Using two different particle tracking models (Julia and D-Part; both based on the DCSM-FM hydrodynamic model) we assessed the most likely location of the source population for this halfway site. The models yielded fairly similar results and neither model approach indicated that the larvae at the Halfweg location were likely to originate from either of the two restoration sites. The most likely origin of these larvae is an area west to southwest of the monitoring site at less than 40 km from the site. This would indicate to the presence of a relic population, capable of reproduction, that is currently not identified. Genetic analyses on the larvae and comparison with genetic profiles of the mother populations at Gemini and Borkum Reef Ground should yield more insight into their potential origin.

4.4 Oyster mobility modelling

A series of experiments was undertaken to investigate the mobility of loose oysters on the seabed, see par. 3.5 for the motivation and set-up of the experiments. Basin tests were executed in a controlled environment at Deltares' Atlantic Basin which is employed with a test section consisting of a mobile sandy seabed, waves and currents.

Figure 30. Basin test with oysters at Deltares.

This experiment highlighted the strong dependence of oyster mobility on oyster density. Moreover, glued oysters representing oyster clusters were seen to remain fairly stable. In terms of hydrodynamic conditions, the near-bed wave action was found to control oyster instability, whereas under steady currents oysters showed limited to no movement.

Based on the experimental data obtained, a first attempt is made to use a formulation to predict the onset of motion for loose oysters given certain hydrodynamic conditions, and oyster properties. By applying this formulation with wave and current hindcasted conditions from numerical modelling, it is concluded that the oyster models deployed at Borkum Reef Grounds would be mobile and potentially displaced under severe wave events within a few months after deployment. This provides a possible explanation for the unsuccessful attempt to locate them in a monitoring survey executed one year after deployment, as reported in par. 3.5.

4.5 Oyster cage stability modelling

Finally, for oyster cages, an analytical model is set-up that can predict the stability of oyster cages depending on their weight and dimensions. This model is based on certain hydrodynamic forcing from waves and currents, thereby providing a useful tool for evaluating various designs. From this model, it is concluded that it is the wave action that has the largest potential to destabilize oyster cages, like for example in Gemini (see par. 2.4.2). Currents on the other hand, are the main driver for transporting an oyster cage, once unstable, far from its original position. Based on this analytical model, and using hindcast of current and wave conditions, the potential location of a dislodged oyster cage can be approximated, but only very roughly. This means that in practise it’s wiser to use acoustic release systems or GPS/AIS to retrieve equipment or trace lost equipment.

5 Recommendations for result application in restoration projects and future research

The ECOFRIEND project’s objective was to develop and test a variety of monitoring techniques which can be applied in the - usually harsh - offshore conditions, to follow several aspects of flat oyster reef development. We recommend employing the following guidelines and innovations in future projects:

- Use standard monitoring protocols, to enable better comparison between various flat oyster restoration pilots.
- Use suitable, demonstrated and validated monitoring cages (such as the in this project demonstrated the WERC-dock), as replacement of the much more cumbersome traditional oyster monitoring cages.
- Measure oyster activity with the valve gape monitor, since this apparatus monitors oyster condition continuously and *in situ*.
- Continue the exploration of novel, adapted, and applied techniques to support oyster bed, and reef development
- Sampling and analysing larvae: employ the developed easy-to-use larval sampling technique. Combination with eDNA-techniques to identify flat oyster larvae is promising but needs further development to increase accuracy. Use the developed temperature model to predict the period that larvae are in the water, to plan the sampling visit at the right time.
- Oyster motion: avoid the loose deployment of oysters on the sea floor in water depths of less than 40 meters, since the oysters tend to disperse widely in these conditions. However, an adequate alternative deployment technique still has to be developed.
- Biodiversity monitoring: a variety of techniques may be employed, ranging from the use of bait cams to eDNA to detect species.

Recommendations for further research and development are:

- Develop a practically applicable standard monitoring protocol for flat oyster restoration projects and stimulate its broad usage.
- Develop a detection technique for settlement of young oysters.
- Further improve the eDNA identification of flat oyster larvae.
- Develop techniques to deploy an initial flat oyster population in such a way that it does not get dispersed on the sea floor.
- Investigate genetic pools for differences, genetic fitness and indigenous characteristics.
- Implement the larval and sea bed monitoring in future broader and associated monitoring programs (such as MONS and other programs to be developed)

An open question resulting from the ECOFRIEND project is where the larvae detected at the ‘Halverwege’ location originate. Most probably, they do not stem from the oyster pilots at Gemini or Borkum Reef Ground, so it may be that there is a, so far undetected, flat oyster population nearby. Further research, among others by comparing genetic profiles of these larvae with the populations in Gemini and Borkum Reef Ground, is recommended.

These results and recommendations have already been disseminated widely, via various communication methods and channels (see Chapter 6).

6 Outreach

6.1 Contribution to standardizing monitoring

The ECOFRIEND team has contributed to the Native Oyster Restoration Alliance (NORA) handbook for native oyster restoration projects in European waters. In this handbook all of the ECOFRIEND monitoring methods have been described. In 2023, ECOFRIEND joined a follow-up to standardize monitoring data. This initiative was also organised by NORA.

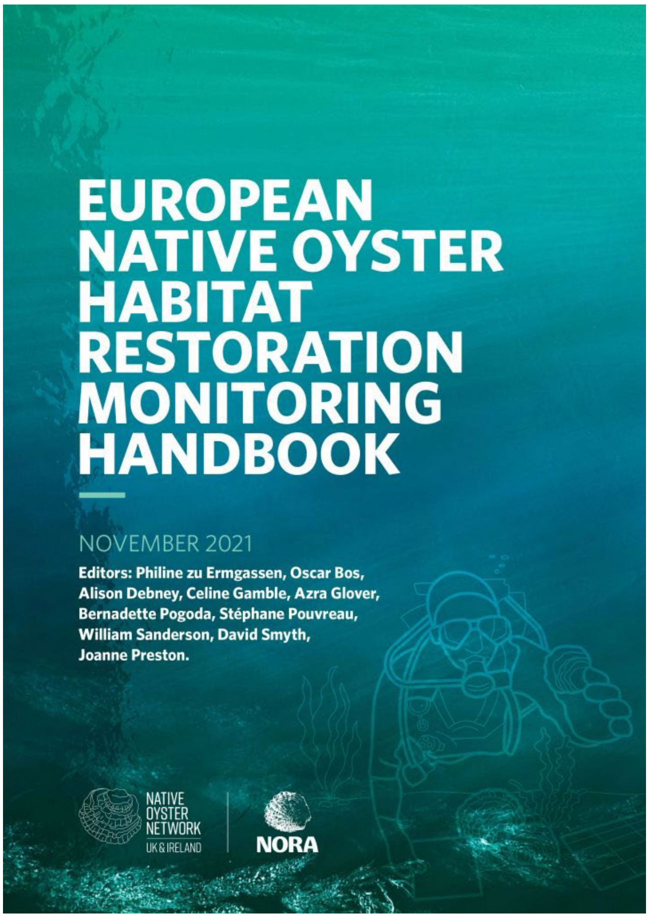


Figure 31. NORA Handbook for monitoring oyster restoration projects (Zum Ermgassen et al., 2021) (https://noraeurope.eu/wp-content/uploads/other-publications/European-Native-Oyster-Habitat-Restoration-Monitoring-Handbook.pdf).

6.2 Contribution to science

The ECOFRIEND contributions to science were five scientific papers and four presentations to conferences (Table 1).

Table 1. List of ECOFRIEND contributions to science.

Date	Scientific papers and reports	Publisher
2020	Maathuis, M. A. M., Coolen, J. W. P., van der Have, T., & Kamermans, P. (2019). Factors determining the timing of swarming of European flat oyster (Ostrea edulis L.) larvae in the Dutch Delta area: Implications for flat oyster restoration. Journal of Sea	Elsevier (Journal of Sea Research).

	Research, December, 101828. https://doi.org/10.1016/j.seares.2019.101828	Peer reviewed paper
Under revision	Doorenspleet et al. (2021 preprint) High resolution species detection: accurate long read eDNA metabarcoding of North Sea fish using Oxford Nanopore sequencing. https://www.biorxiv.org/content/10.1101/2021.11.26.470087v1.full (scientific paper on eDNA)	To be decided
2023	Tonk, L., Witbaard, R., van Dalen, P., Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (Ostrea edulis) feeding behaviour. Aquatic Living Resources, 36(6), [6]. https://doi.org/10.1051/alr/2022021	Aquatic Living Resources
2023	Van Duren, L., Emmanouil. A., Gerritsma, I., Kleissen, F., Ballas, G., & Raaijmakers, T. C. (2023). *JIP ECO-FRIEND-GEMINI oyster restoration pilot Experimental and numerical modelling*. Report 11203476-000-HYE-0003	Deltares
Submitted	Bos OG, J Bergsma, S Duarte-Pedrosa, S Heye, K Didderen, P Kamermans. (submitted) Performance of European oysters (*Ostrea edulis* L.) in the Dutch North Sea, across five restoration pilots	Submitted to Frontiers in Marine Science
	Presentations at conferences	
2021	Bos, O. G., Kamermans, P., van der Have, T., Bergsma, J., Raaijmakers, T., van Duren, D., Sas, H., & Folkerts, L. (2021). Oyster monitoring challenges in an offshore windfarm (ECOFRIEND project). In NORA 4 Reconnecting across Europe https://noraeurope.eu/wp-content/uploads/2021/11/NORA-4-Abstracts.pdf#page=32	NORA 4 conference abstract
2021	van Duren, L., Ballas, G., Raaijmakers, T., Zijl, F., Bos, O. G., Kamermans, P., van der Have, T., Bergsma, J., Sas, H., & Folkerts, L. (2021). Modelling tools to support successful offshore oyster restoration. In NORA 4 Reconnecting across Europe https://noraeurope.eu/wp-content/uploads/2021/11/NORA-4-Abstracts.pdf#page=19	NORA 4 conference abstract
2023	WIND ENERGY AND BIODIVERSITY IN PRACTICE OYSTER RESTORATION IN WIND FARMS; WILL THEY STAY OR WILL THEY GO? Antonios Emmanouil, Isabel Gerritsma, Luca van Duren, Pauline Kamermans, Oscar G. Bos, Joost H. Bergsma, Hein Sas, Peter M.J. Herman	CWW conference abstract
	Posters	
2022	Van Duren, L., Raaijmakers, T., Emmanouil, A., Bos, O. G., Kamermans, P., Bergsma, J., Sas, H., Schutter, M., Tonk, L., Alexandrova, N., & Cohn, N. (2022). Offshore wind farms – modelling tools for nature inclusive design in a dynamic sand wave area. Poster session presented at State of the Science Workshop on Wildlife and Offshore Wind Energy, Tarrytown, New York, United States. https://edepot.wur.nl/576078	

6.3 Social media

ECOFRIEND featured in social media on six occasions (Table 2).

Table 2. List of ECOFRIEND publications

Date	Publications	Publisher
	ARTICLES	
22-12-2021	Waar blijven de oesters? (https://edepot.wur.nl/559074) NL https://edepot.wur.nl/559090 (UK)	WUR
02-02-2022	https://www.naturetoday.com/intl/nl/nature-reports/message/?msg=28736	Nature Today
03-09-2021	Oesters in 't golfslagbad https://www.resource-online.nl/index.php/2021/09/03/oesters-in-t-golfslagbad/ Oysters in the wave pool https://www.resource-online.nl/index.php/2021/09/03/oysters-in-the-wave-pool/?lang=en	WUR
06-03-2023	https://www.topsectorenergie.nl/spotlight/eco-friend-restoring-oyster-reefs-north-sea	TKI Wind op Zee
	VIDEOS	
25-09-2021	https://youtu.be/1aq5PCky5Q8	WUR
06-03-2023	https://youtu.be/-XPpSoG67yQ Video made by TKI Wind op Zee	TKI Wind op Zee

6.4 Contribution to stakeholders

The ECOFRIEND contributions to stakeholders on six occasions (Table 3).

Table 3. List of ECOFRIEND activities

Date	Activity	Publisher
2023	Transfer of experiences and knowledge to develop a tool for stakeholders, organised by De Rijke Noordzee.	De Rijke Noordzee
02-17-2022	Nature inclusive building in OWFs in the Netherlands. Opportunities for nature recovery within offshore wind farms around the UK. Blue Marine Foundation. Online presentatie voor UK.	WMR

03-28-2022	Nature inclusive building in OWFs in the Netherlands. MuSSeL Foresight Workshop. Online presentation for German scientists and stakeholders	WMR
12-10-2022	Nature inclusive building in OWFs in the Netherlands Week van de Noordzee, 12 okt 2022, Scheveningen. For Energiebeheer Nederland (energy sector)	WMR
07-09-2022	Offshore beurs Den Helder, 7 september 2022. Demonstration mini ROV	WMR
23-05-2023	Co-organisation of and participation in the first meeting of the NORA offshore flat oyster restoration working group (NL/D/B/Dn/UK)	Sas Consultancy, WMR

6.5 Knowledge transfer to other projects

ECOFRIEND was among the first projects in the Netherlands that specifically focussed on monitoring oyster restoration in offshore windfarms. The project started to transfer knowledge and experiences to other initiatives right from the start after the first year of fieldwork, such as to the Rijke Noordzee programme. For example, the use of eDNA monitoring and larval sampling was quickly adopted. In 2020 the knowledge on offshore restoration was brought to the Native Oyster Restoration Alliance (NORA) and incorporated in a monitoring handbook. In 2022 the first offshore wind tender procedure started in which ecology was important (Hollandse Kust West). The knowledge obtained in the ECOFRIEND project is transferred to the science community by writing or contributing to scientific articles on eDNA, oyster performance, oyster monitoring using valve monitors, etc. Altogether, we assess that the economical, ecological and scientific impacts can be claimed.

ECOFRIEND has been actively liaising with similar projects that work on oyster pilots to exchange experiences and offer advice:

- Native oyster restoration projects, undertaken by The Rich North Sea in wind farms (Luchterduinen, Borssele) and by ARK/WWF NL in Borkum Reef and Voordelta
- WOZEP (Modelling)
- North Sea Reef Vitalization For Ecosystem Services (North Sea ReViFES): A project supported by NWO to develop new techniques to restore native oyster reefs and to quantify harvestable ecosystem services provided by the reefs.
- Scour protection design for biodiversity enhancement in North Sea Offshore Wind Farms (BENSO): A TKI joint Industry Project to create economical value by developing, testing and implementing smart ecological design for scour protection in offshore wind farms and to implement smart techniques in monitoring programmes and maintenance operations.
- The Native Oyster restoration Alliance (NORA) to produce a monitoring handbook for native oyster restoration projects in European waters. The specific contribution by ECOFRIEND is to advise on adequate and cost-effective monitoring methods in offshore waters. It involved Participation in NORA workshop (Portsmouth, UK, 11 & 12 December 2019)

7 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

If the quality cannot be guaranteed, appropriate measures are taken.

References

Bos, OG, Glorius S, Coolen JWP, Cuperus J, Weide BE van der, Aguera Garcia A, van Leeuwen PW, Lengkeek W, Bouma S, Hoppe M, Van Pelt H (2014) Natuurwaarden Borkumse Stenen Project Aanvullend beschermde gebieden.

Coolen JWP, Bos OG, Glorius S, Lengkeek W, Cuperus J, Van der Weide BE, Agüera A (2015) Reefs, sand and reef-like sand: A comparison of the benthic biodiversity of habitats in the Dutch Borkum Reef Grounds. J Sea Res 103:84–92.

Didderen K, Lengkeek W, Kamermans P, Deden B, Reuchlin-Hugenholtz E, Bureau Waardenburg Culemborg, WWF Zeist (2019) Pilot to actively restore native oyster reefs in the North Sea (https://www.ark.eu/sites/default/files/media/Schelpdierbanken/Report_Borkumse_Stenen.pdf). BuWa.

Doorenspleet et al. (2021 preprint) High resolution species detection: accurate long read eDNA metabarcoding of North Sea fish using Oxford Nanopore sequencing. https://www.biorxiv.org/content/10.1101/2021.11.26.470087v1.full

Kamermans, P., van Duren, L. and Kleissen, F. (2018). European flat oysters on offshore wind farms: Additional locations : opportunities for the development of European flat oyster (*Ostrea edulis*) populations on planned wind farms and additional locations in the Dutch section of the North Sea Report C053/18. Wageningen Marine Research. doi: 10.18174/456358

Maathuis, M. A. M., Coolen, J. W. P., van der Have, T., & Kamermans, P. (2019). Factors determining the timing of swarming of European flat oyster (Ostrea edulis L.) larvae in the Dutch Delta area: Implications for flat oyster restoration. Journal of Sea Research, December, 101828. https://doi.org/10.1016/j.seares.2019.101828

OFL (2020). Het Akkoord voor de Noordzee. Extra mijlen voor een gezonde Noordzee. https://www.noordzeeloket.nl/publish/pages/180789/onderhandelaarsakkoord_voor_de_noordzee_juni_2020.pdf

Olsen OT (1883) The piscatorial atlas of the North Sea, English and St. George's Channels, illustrating the fishing ports, boats, gear, species of fish (how, where, and when caught), and other information concerning fish and fisheries. Taylor and Francis, London.

OSPAR (2008). OSPAR List of Threatened and/or Declining Species and Habitats. OSPAR Agreement 2008-06. https://www.ospar.org/work-areas/bdc/species-habitats/list-of-threatened-declining-species-habitats.

OSPAR BDC (2020). Status Assessment 2020 - European flat oyster and Ostrea edulis beds. https://oap.ospar.org/en/ospar-assessments/committee-assessments/biodiversity-committee/status-assesments/european-flat-oyster/.

Smaal AC, Kamermans P, Have TM Van Der, Engelsma M, Sas HJW (2015) Feasibility of Flat Oyster (*Ostrea edulis* L.) restoration in the Dutch part of the North Sea.

Tonk, L., Witbaard, R., van Dalen, P., Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (Ostrea edulis) feeding behaviour. Aquatic Living Resources, 36(6), [6]. https://doi.org/10.1051/alr/2022021

Van Duren, L., Emmanouil. A., Gerritsma, I., Kleissen, F., Ballas, G., & Raaijmakers, T. C. (2023). JIP ECO-FRIEND-GEMINI oyster restoration pilot Experimental and numerical modelling. Report 11203476-000-HYE-0003.

Justification

Report C032/23
Project Number: 4312100095 - 4312100095

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Marnix Poelman
researcher

Signature:

Date: 09-06-2023

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Date: 09-06-2023

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