



WINOR oyster spat collection experiment in wind park Gemini Zee-Energie

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Wageningen University &
Research report: C046/24

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2 Waardenburg Ecology (WE)

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Wageningen Marine Research
Yerseke, July 2024

Wageningen Marine Research report C046/24

Keywords: flat oyster, *Ostrea edulis*, restoration, spat collection

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This report can be downloaded for free from <https://doi.org/10.18174/669522>
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Photo cover: Renate Olie

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KvK nr. 09098104,
WMR BTW nr. NL 8113.83.696.B16.
Code BIC/SWIFT address: RABONL2U
IBAN code: NL 73 RABO 0373599285

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A_4_3_2 V33 (2023)

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Summary

At present, European flat oyster beds are rare or absent in most of their natural range and only a few individuals remain in the North Sea. The goal of is to establish a self-sustaining bed in terms of recruitment. As part of the Gemini oyster bed restoration project of De Rijke Noordzee (DRN) an initial population of flat oysters (*Ostrea edulis*) was introduced in Gemini ZeeEnergie in 2021 and 2022. Substrate, such as cleaned Pacific oyster shells, can be introduced to facilitate expansion of the bed. Timing of the introduction of shells is crucial. An earlier study showed that covering substrates with Reefpaste enhanced larval settlement. To study this at larger scale oyster shells were coated with Reefpaste. To test the timing deployment of settlement shells larvae were sampled and oyster shell substrate (with and without Reefpaste) was introduced during the larval period in 2023 (year 3) of the Gemini project. It was envisioned to install substrate in four research trips: during the peak in larvae, two weeks after the peak in larvae, four weeks after the peak in larvae and six weeks after the peak in larvae. Results of the study can be used to decide on the type of shells (with or without Reefpaste) and to time the introduction of the shells with the purpose of expanding off-shore oyster beds.

Finding a suitable weather time-window proved to be a challenge. Sometimes the waves looked good three days ahead, but then at the planned date they were too high again, or the window was too short to do the work. Finding a suitable and approved vessel was also challenging. Because of this, field work started later than anticipated. Only three trips to install the frames during the larval season were carried out. This resulted in placing two frames on the same date. Retrieval of the frames was planned for October 2023. However, after 13 unsuccessful attempts (where in two cases the park was actually visited) the frames are still in the park.

The effect of temperature on larval abundance could not be determined since there were no oyster larvae detected in the larvae (water filtrates) samples. Sampling may have been too late in the season. The effect of deployment week of the spat collectors on spat abundance and the effect of addition of Reefpaste to the shells on spat abundance could not be determined because the frames were not retrieved from the field.

The WINOR oyster spat collection experiment required frequent field trips to be able to follow larval abundance over time and introduce settlement substrates at different times during the larval season. Next to that, the frames should be retrieved before the winter. For this, the use of small vessels like CTV's were chosen as they are flexible in time and cheaper than larger ships. Furthermore, frames to hold the bags with substrates were designed in such a way that they could be deployed from a small vessel. The use of small vessels required a calm sea with waves of 1 m or less. The year 2023 was characterised by long periods with a lot of wind and very few weather windows in which the wave height was less than 1m. Locating the experiment in a wind farm came with extra measures which sometimes delayed the process of accessing the site. In conclusion, under the present circumstances testing the timing of oyster spat collection is demonstrated to be too complicated in practice to be studied in a windfarm.

1 Introduction

Until about a century ago the European flat oyster *Ostrea edulis* L. was a common species in the North Sea (Gercken & Schmidt, 2014; Houziaux et al., 2008; Olsen, 1883). However, the oyster population declined rapidly as a result of intensive flat oyster fishery at the end of the 19th century (Gercken & Schmidt, 2014; Houziaux, 2008). At present, European oyster beds are rare or absent in most of their natural range (OSPAR BDC, 2020), e.g. only a few individuals remain in the North Sea (Kerckhof et al., 2018). The species is listed as 'threatened' or 'declining' by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2008). In addition, the return of biogenic reefs is mentioned as part of the European Marine Strategy Framework Directive (https://environment.ec.europa.eu/topics/marine-environment_en). And restoring flat oyster beds are often part of national plans, e.g Dutch Beleidsnota Noordzee 2016-2021 (Ministerie van Infrastructuur en Milieu and Ministerie van Economische Zaken, 2015), Noordzee 2050 Gebiedsagenda, Natuurambitie Grote Wateren 2050 (Ministerie van Economische Zaken, 2013) and Uitvoeringsagenda Natuurlijk Kapitaal (Ministerie van Economische Zaken, 2013).

In The Netherlands, several flat oyster restoration activities are taking place (Bos et al., 2023 and [De Rijke Noordzee | Home](#)). Restoration is the action of returning the flat oyster population in the North Sea to a former condition. Once it has been established that the habitat is suitable (Kamermans et al., 2018), several options for oyster restoration exist. These options make use of the natural life cycle of the species. Mature flat oysters can switch between sexes to be male or female (Walne 1974). Generally, flat oysters start as males and become females as they grow older. Older oysters can spawn twice during one season, once as a male and once as a female. Sperm cells are expelled through the exhalant siphon. Eggs remain in the mantle cavity of the female, where they are fertilized and develop into larvae with two shells in a period of one to two weeks. After that period the larvae are released. The timing of maximal larval release can be predicted based on temperature and takes place in July and August (Maathuis et al., 2020). Next, they spend another 8–17 days as free-swimming larvae (Walne 1974). Metamorphosis, from swimming larvae into sessile spat, depends on temperature and food availability for the larvae. Settlement, or spat fall, occurs when a suitable location is detected. A drop of cement adhesive is produced, and the left valve is glued to the surface (Walne, 1974). Oyster spat settles on hard substrates, such as stones, shell fragments, or oyster shells (Korringa, 1952).

For restoration, adult oysters can be introduced as broodstock (Ter Hofstede et al., 2023). Provided that appropriate environmental conditions are present, these oysters will produce larvae. Availability of suitable substrate is required to ensure successful settlement (Smyth et al., 2018). Limitation of substrate has been identified as a constraint in the expansion of natural or restored oyster beds (Möbius, 1877; Korringa, 1946). Substrate can be introduced when this is not present at the restoration location. Numerous substrates have been tested for settlement preferences of *O. edulis* larvae and oyster shell performs well (reviewed in Korringa, 1952 and Colsoul et al., 2020). Reefpaste has been shown to enhance larval settlement (Kamermans et al., in prep). Reefpaste coating consisted of BESE reef paste (BESE, Culemborg, the Netherlands) composed of grinded shell and binding material with the cured material consisting of >89% calcium carbonate (CaCO₃).

Oyster restoration in offshore windfarm Gemini

In close cooperation with the wind and hydraulic engineering sector and science, de Rijke Noordzee (DRN) works on underwater nature enhancement. This is done by placing and fostering living reefs and artificial structures. As part of the Gemini Zee-Energie project of DRN initial populations of flat oysters (*Ostrea edulis*) were introduced in 2021 and 2022 (Figure 1). The goal of oyster bed restoration is to establish a self-sustaining bed in terms of recruitment. Results of the Ecofriend project in neighbouring windfarm Gemini Buitengaats showed that introduced oysters produced free-swimming larvae in summer (Bos et al., 2023). Substrate, such as (empty) (Pacific) oyster shells, can be introduced to facilitate expansion of the bed (Baggett et al., 2014). The larvae settle on the shells and become part of the bed. Timing of the introduction of shells is crucial to optimise between recruitment of fouling organisms and oyster spat. Recent near-shore

studies showed that release of the larvae is mainly driven by the temperature sum (Maathuis et al., 2019) and that introduction of the substrate can best be done 2 weeks after the peak in larval abundance (van den Brink et al., 2020).

2 Assignment

2.1 Aim

The aim of this research was to test if the flat oysters that were introduced in offshore windfarm Gemini Zee-Energie in 2021 and 2022, would produce oyster spat in the subsequent year 2023. This was tested by deploying 4 spat collectors in summer 2023 with 2 different types of oyster shell material as settlement substrate. To verify if the timing of larval release and settlement was the same at an off-shore location as at an earlier studied near-shore location, larvae were sampled and oyster shell substrate was introduced at different times during the larval period in year 3 of the Gemini project.

Results of the present study are relevant for future projects with the purpose of expanding offshore oyster beds to decide on which type of shells to use (with or without Reefpaste) and on the timing of the introduction of shells.

2.2 Research questions

This study addresses the following research questions:

1. What is the effect of temperature sum on larval abundance?
2. What is the effect of deployment week of the spat collectors on spat abundance on substrates?
3. What is the effect of addition of Reefpaste to the shells on spat abundance on substrates?

3 Materials and Methods

3.1 Location

The field work was carried out at Gemini ZeeEnergie windfarm (red dot in Figure 1A). A 5-ha test area was located in between pilon J7 and G7 (red circle in Figure 1B, coordinates in Table 1). The research was conducted at the central oyster deployment hectare (red square in Figure 1C, coordinates in Table 2). At this location, 4 spat collectors were deployed (Figure 1C, Table 2) and water samples were taken for larval counts and DNA detection.

At the request of the Alfred Wegner Institute water samples were collected for larval counts at Borkum Reef for their RESTORE project (Figure 2) at 11 August 2023.

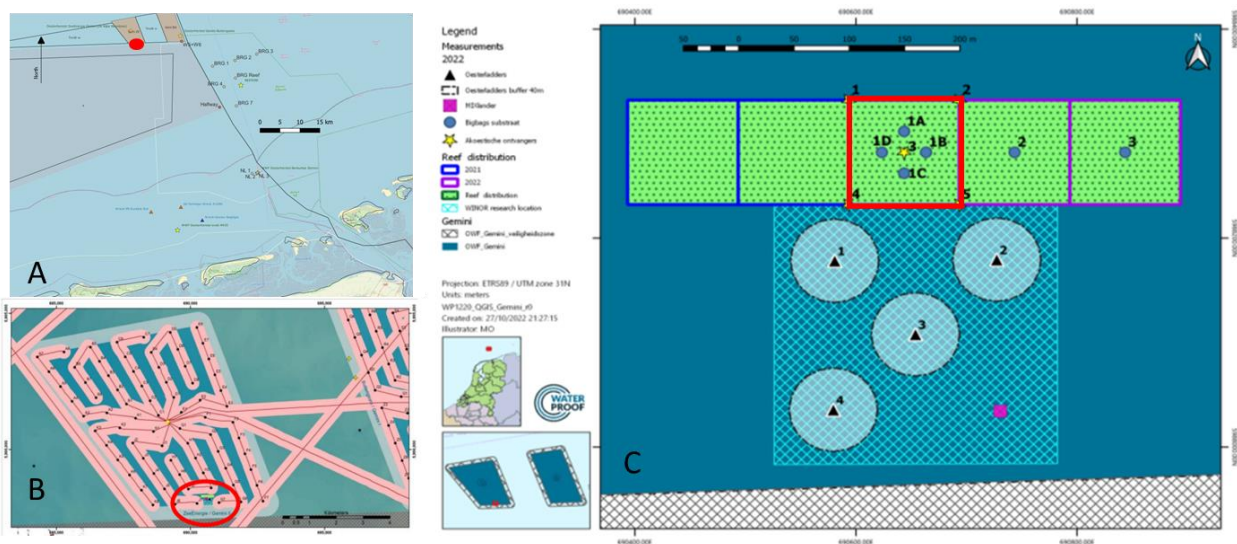


Figure 1: (A, B, C): Location at Gemini ZeeEnergie (red dot in A and red circle in B) with 5 ha area with introduced oysters in green and location of spat collectors indicated with a red square (C). Numbers 1A, 1B, 1C, 1D and outside red square in C are not relevant.

Table 1. Coordinates of the study area (red square) (1 = NW, 2 = NE, 5= SE, 4 = SW, 3 = middle):

Label	X (ETRS89 UTM31N)	Y (ETRS89 UTM31N)	Long (WGS84 - dec)	Lat (WGS84 - dec)	Long (WGS84 - dm)	Lat (WGS84 - dm)
1	690593.40	5988332.04	5.9085700	54.0080425	5°54.5142'	54°0.48255'
2	690693.40	5988332.04	5.9100940	54.0080055	5°54.60564'	54°0.48033'
3	690643.39	5988282.04	5.9093005	54.0075752	5°54.55803'	54°0.45452'
4	690593.40	5988232.04	5.9085073	54.0071448	5°54.51044'	54°0.42869'
5	690693.40	5988232.04	5.9100313	54.0071079	5°54.60188'	54°0.42647'

Table 2. Location of the frames after deployment in summer 2023. All frames were located within a square of about 100x100 m.

Location	WGS84 (degr)
Frame 1	N 54.0072 E 05.9097
Frame 2	N 54.0072 E 05.9094
Frame 3	N 54.0078 E 05.9092
Frame 4	N 54.0078 E 05.9087

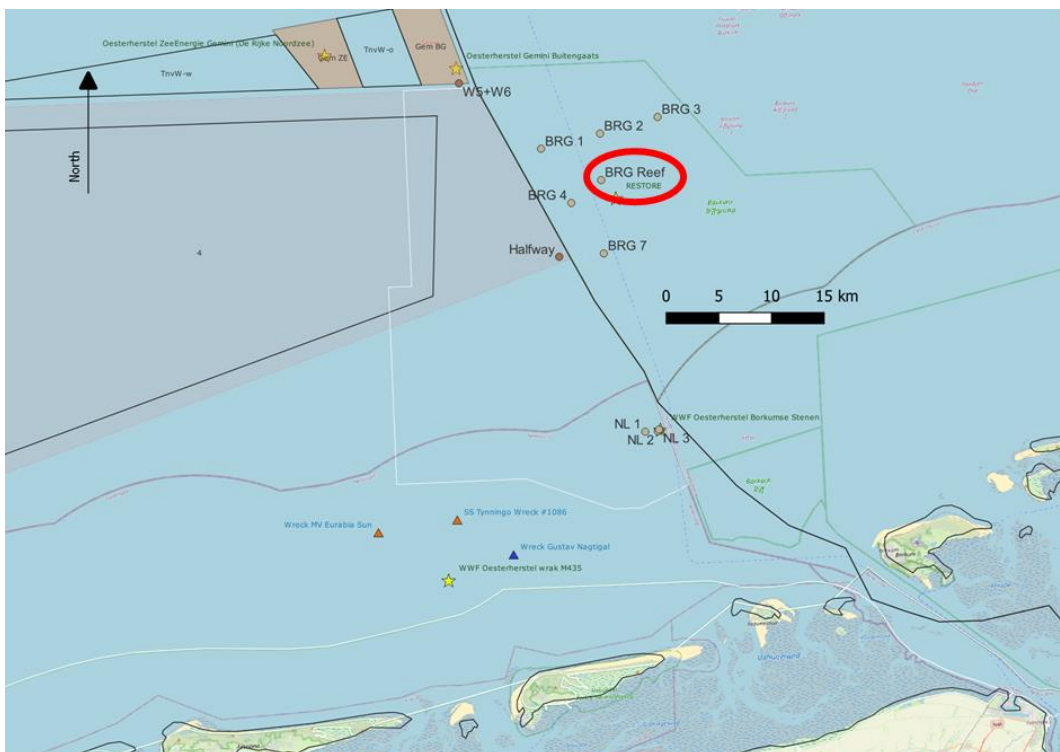


Figure 2: Location of the German oyster restoration project 'RESTORE' at the Borkum Riffgrund (BRG Reef).

3.2 Experimental setup

In 2021 and 2022 oysters were introduced at five ha of the pilot (Figure 1) by The Rich North Sea. In 2023 it was expected that most oysters would have become mature and would be able to produce larvae. Therefore both larval abundance and spat settlement were monitored in July and August 2023. The timing of sampling was planned to be around neap tide. During each fieldtrip, triplicate water samples were taken for analysis for the presence of flat oyster larvae both microscopically and with DNA analysis (qPCR). And spat collectors were deployed for oyster spat collection.

To verify if the timing of larval release and settlement is the same at an offshore location as at a near-shore location, it was envisioned to install substrate during the larvae peak, two weeks after the peak in larvae, four weeks after the peak in larvae and six weeks after the peak in larvae (Table 3). At each spat collector, 4 bags of Pacific-oyster shell material were attached: 2 bags with clean Pacific-oyster shell material (by letting them dry on land for at least 6 months) and 2 bags with Pacific-oyster shells coated with Reefpaste (see

introduction). Reefpaste was added by mixing the paste and shells manually and subsequently air dry the shells.

Table 3. Planned experimental setup to deploy and retrieve spat collectors in Gemini Offshore windfarm Zee-energy in 2023.

	At larval peak Summer 2023	2 w after larval peak	4 w after larval peak	6 w after larval peak	Retrieval After summer
Spat collector #1 + water samples	x				X
Spat collector #2 + water samples		x			X
Spat collector #3 + water samples			x		X
Spat collector #4 + water samples				x	x

3.3 Prediction of larval peak

Maathuis et al. (2020) developed a formula in which the development of the water temperature can be used to predict the moment of highest abundance of flat oyster larvae (larval peak):

$$\text{Temperature Sum} = \sum_{1 \text{ st of January}}^{\text{end date}} (T_i - T_{th}) * \Delta d$$

This temperature sum is in *degreedays* ($^{\circ}\text{C} * d$), T_i is the water temperature ($^{\circ}\text{C}$) on day i , T_{th} is the threshold temperature above which gonad development takes place ($\sim 7^{\circ}\text{C}$) and Δd is the time step of one day. The study of Maathuis et al. (2020) showed that most larvae were observed at a temperature sum of 576. To test the predictive value of the formulas surface temperature data provided by Gemini were used to calculate the day with most larvae present.

In 2023, the larval peak was predicted to occur at 8 July 2023.

3.4 Timing of fieldwork

The optimal sampling period and time for fieldwork was determined by focussing on the days around neap tide (twice per month) (<https://waterinfo.rws.nl/#!/kaart/astronomische-getij/>).

The suitable periods around neap tide were 14-16 July, 28 July-1 Aug, 11-14 Aug and 27-29 Aug 2024 (Figure 4). During neap tide, the sampling window around slack tide, defined as periods with predicted currents $< 0.5 \text{ kn}$ are very long and last several hours (Table 4). Next, the weather conditions had to be favourable for safe and reliable fieldwork.

Waterhoogte Astronomisch t.o.v NAP

Huibertgat

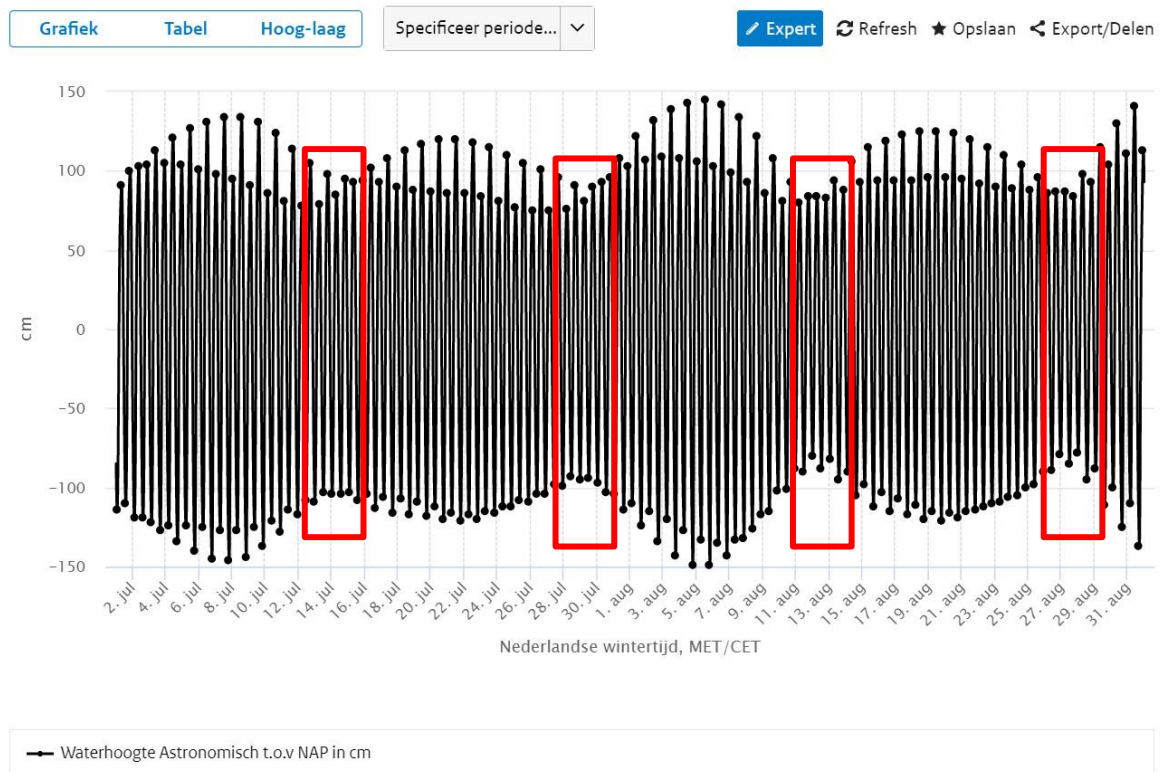


Figure 4: predicted tides for Huibertgat (Schiermonnikoog). Optimal sampling periods (neap tide) are indicated with the red boxes (source: <https://waterinfo.rws.nl/#!/kaart/astro-nische-getij/>)

Table 4. Days and times at which currents were predicted to be <0.5 knots (software: Noordzeestroom, Harrold Bataram)

	06:30	7:00	7:30	8:00	8:30	9:00	9:30	10:00	10:30	11:00	11:30	12:00	12:30	13:00	13:30	14:00	14:30	15:00	15:30	16:00	16:30	17:00	17:30	18:00	18:30	19:00	19:30	20:00			
GEMINI																															
11-07-23	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1						1	1	1	1	1	1	1		
12-07-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					1	1	1	1	1	1	1		
13-07-23	1	1	1	1	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1								1	1	
14-07-23			1	1	1	1	1	1	1	1						1	1	1	1	1	1	1							1	1	
July 26-29																															
26-07-23	1	1						1	1	1	1	1	1									1	1	1	1	1	1	1	1		
27-07-23	1	1	1	1				1	1	1	1	1	1	1	1							1	1	1	1	1	1	1	1		
28-07-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1											1	1	
29-07-23		1	1	1	1	1	1	1	1						1	1	1	1	1	1									1	1	
August 9-11																															
09-08-23				1	1	1	1								1	1	1	1	1	1											
10-08-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1						1	1	1	1	1	1	1	
11-08-23	1	1	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1	1	1								1	1	
August 26-29																															
26-08-23	1	1						1	1	1	1	1	1									1	1	1	1	1	1	1	1	1	
26-08-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1							1	1	1	1	1	1	
27-08-23		1	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1	1	1									1	1
28-08-23			1	1	1	1	1	1	1	1									1	1	1	1	1							1	1
29-08-23					1	1	1	1	1	1	1	1										1	1	1	1					1	1

3.5 Spat collector frames and substrate

A spat collector consists of a stainless steel frame (see Figure 4) (208 x 208 cm x 40 cm) on which four spat collector bags are strapped with plastic straps (Figure 5). The total weight of the frame above water is approx. 32 kg. Spat collector are designed to be deployed by hand from a small survey vessel. In the centre of the frame, the hoisting / lifting point is constructed from where the frame will be lowered to the seafloor (Figure 6). The spat collectors were equipped with an acoustic release for retrieval (Figures 5) and a

temperature datalogger. An extra ROV (remote Operated Vehicle) hook has been added to the frame as backup option for retrieval (Figure 6: yellow hook).

The stability of the frames was tested by Waterproof and approved by Gemini for deployment during the period July to October 2023 (Annex 1, stability assessment memo, Waterproof 2023). After the larval settlement period all spat collectors would be retrieved and analysed for abundance of spat. Data on larval abundance, spat abundance and temperature were analysed to reveal if a similar pattern as at near-shore locations was present offshore.

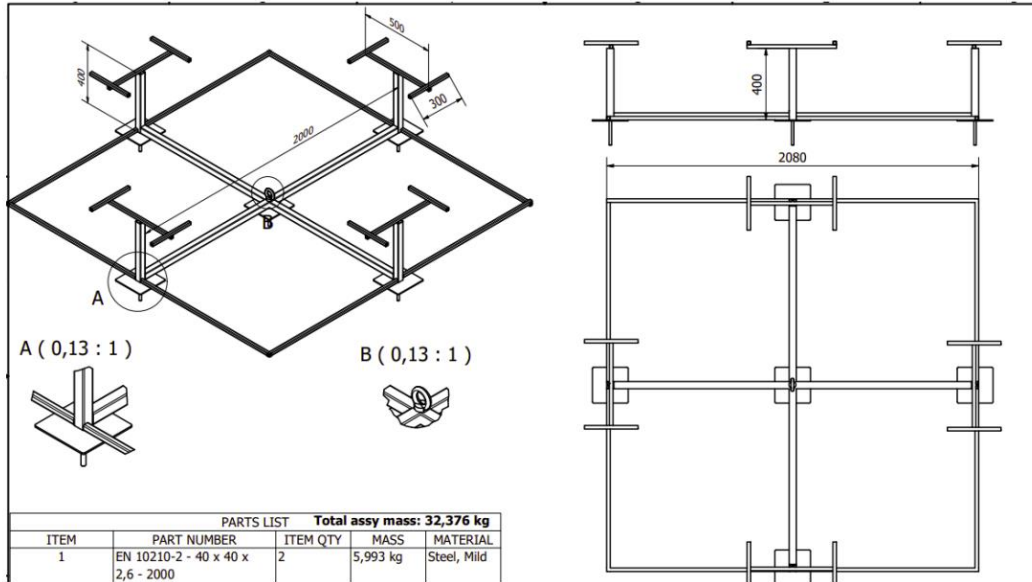


Figure 4. Oyster spat collector (design: Waterproof).

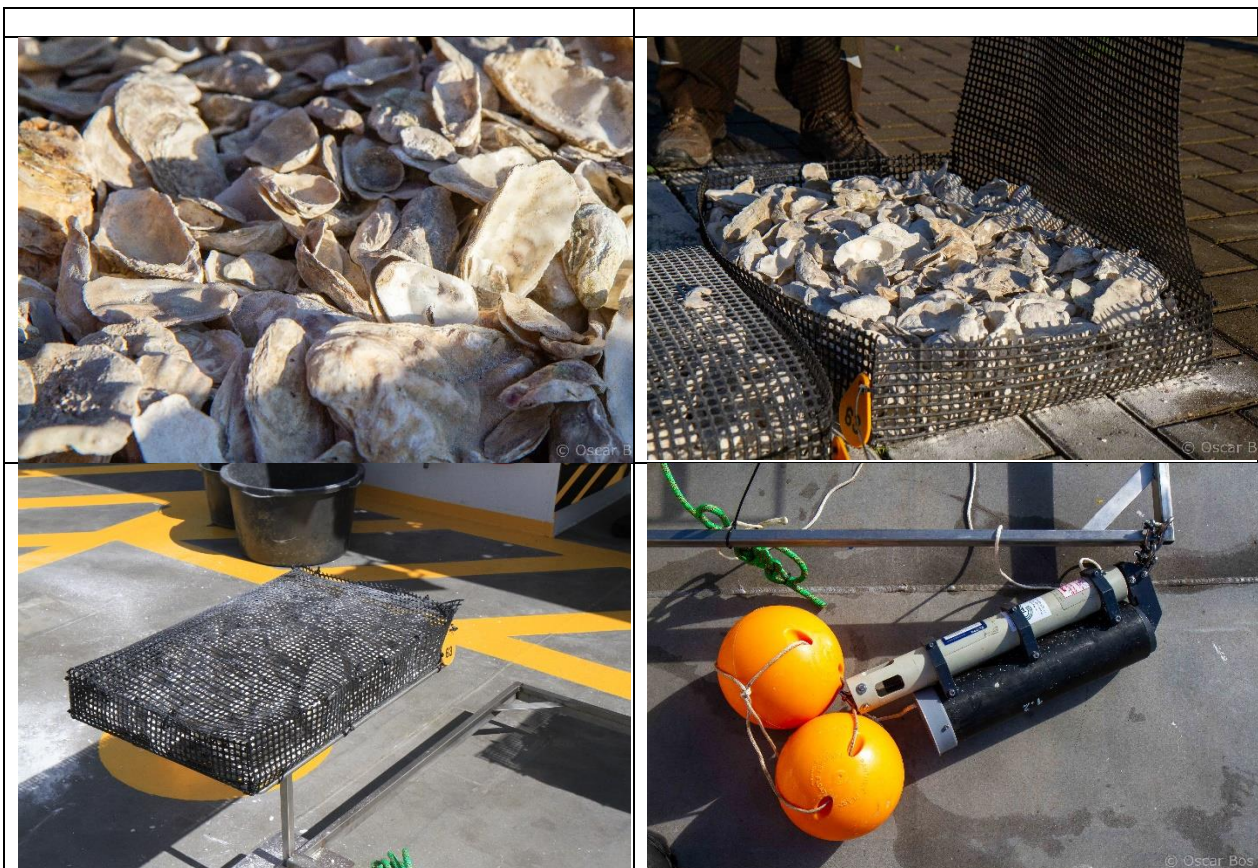


Figure 5. Pacific oyster shell material (upper left); filling of a bag (upper right), bag on the oyster spat collector (bottom right) and the acoustic release (bottom left) (28 Aug 2023, photo: Oscar Bos, WMR).



Figure 6. Deployment of oyster spat collector (photo: Renate Olie, DRN).

3.6 Larval abundance and oyster DNA detection

Depending on the current speed one or two samples were collected on one transect. Sampling took place near the seafloor, by lowering the pump by hand. Water was pumped using an electrical pump running on 220V. For each 200 L sample, 2 x 100 L water was filtered with a plankton net (see Figure 7) 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 7). In between, the container was emptied. Samples were labelled, fixed with ethanol and stored for further processing in the laboratory at WMR in Yerseke and Wageningen Environmental Research (WEnR) in Wageningen.

The residue of each 200 L sample was stored in 96% ethanol and used for microscopic counts and ddPCR analysis (droplet digital PCR, a DNA-based technique to identify specific species). In the lab, the samples of larvae were filtered using a 30 µm plankton gauze. The volume of the samples was reduced to 20 – 60 mL, depending on the amount of suspended matter. From the concentrated samples subsamples were taken for counting numbers of larvae. A Hensen plunger-sampling pipette was used to take subsamples. Bivalve larvae were identified and counted using an inverted microscope. Three subsamples of each sample were analysed. Depending on the density of the samples, subsamples of 1 to 2.5 mL were counted. Larvae were identified according to Loosanoff et al. (1966) and Hendriks et al. (2005) combined with data obtained from cultured larvae. WEnR developed OED-WENR primers and performed the ddPCR analysis. ddPCR is more accurate than qPCR because it divides the reaction volume in >20000 drops in which separate reactions occur. Concentration is estimated through dividing the number of drops in which *Ostrea edulis* DNA is detected by the total number of drops.



Figure 7: Sampling of oyster larvae by filtering seawater pumped from above the seafloor.

3.7 Field trip procedures

For each fieldwork trip a Risk Assessment and Method Statement (RAMS) was prepared. Each RAMS was reviewed by Gemini offshore wind farm. After approval of the RAMS, the ship and the crew, permission is given by Gemini Operation Control Centre (OCC) to enter the site. Ship and crew have to present the right certificates. In addition, a client representative of Gemini had to be on board. More details can be found in the RAMSs. Below we only describe the fieldwork procedures.

3.7.1 Planning fieldwork

The following steps were taken to plan the fieldwork:

- WMR checks if wave height is less than 1 m at windy.com and windfinder.com for a suitable weather window
- WMR finds available ship
- WMR arranges people and hotel for scientific crew and, in case of the frame retrieval, people to analyse samples upon return of the ship
- WMR provides description of work to all parties involved
- DRN arranges Gemini client representative
- DRN arranges charterers' liability insurance (dependant on vessel)
- WP arranges access permit

3.7.2 Deployment of spat collectors

Deployment

- Vessel is positioned at the deployment location, during slack tide. If still currents occur, the vessel is positioned slightly up-stream of the deployment location (drifting (or ground) speed to be verified by captain prior to deployment).
- The required crew for deployment consists of 3 persons.
- Central deployment line guided through a descender (PETZL GRIGRI BLEU 23), which is connected with a sling to a bollard (mooring post) at the vessel. The deployment line is looped through the lifting eye to easy retrieve the line after deployment (see Figure 6).
- The rest of the deployment line is stored in a bucket, to prevent loose lines over the deck. One crewmember is responsible for the deployment line and guards all processes regarding this line.
- A GO signal is given by the captain of the vessel, engine / propulsion is shut off.
- The frame is lifted sideways over the side of the vessel by two crewmembers and is slowly slid over board into the water.
- Position is verified with the captain, engines / propulsion is to be shut off.
If still in position, the frame is lowered to the seabed by slowly releasing the line with the descender.

- Once the frame is at the seabed, the line will be pulled through the lifting eye and retrieved to the surface.
- Upon retrieval of deployment line, the crew will give a COMPLETED notification to the captain on which engines / propulsion can be restarted.

Deployment can only be executed in very calm weather conditions (Hs max 1m).

The following descender is used (see Figure 8). The line is lowered controlled by pushing the lever of the descender. Once let go, the descender will block automatically and lowering is stopped.

The deployment and lifting rope that will be used is a new climbing rope with a diameter of 10mm and breaking strength of 25kN (~2500kg). This is sufficient for the weight that is being lowered, taking into account dynamic forces due to waves that move the vessel up and down.



Figure 8: Descender Petzl GriGri Blue 23.

3.7.3 Water sampling

Six times 200 L were sampled, taking approx 1 hour in total. Slack tide is preferred. Engines are off and the vessel will drift along transects. The position was logged at the start and end of each transect. The transects were near the location of spat collector.

3.7.4 Retrieval of spat collectors

Pre-checks

- Test ping system in harbour
- Put pulley in crane
- Retrieval can only be executed in very calm weather conditions (Hs max 1m).

Retrieval

- Vessel is positioned within 200 m of the deployment location, preferably during slack tide. If there is still current, vessel is positioned slightly down-stream of the deployment location (drifting speed to be verified by captain prior to deployment).
- A **GO** signal is given by the captain of the vessel, engine / propulsion is shut off.
- Acoustic release is dropped overboard and pings the frame. Make sure that the transponder is sufficiently deep in the water. Use entire cable and attach lead with tie-rip when current prevents sinking of the cable.
- If ping comes back positive, release is triggered
- Two observers look out for yellow/orange buoy. Buoy is small and might be submersed partially or fully depending on weather conditions.
- If buoy is found, appoint one person to point to buoy at all times.
- Bring transponder back on deck, check nothing in the water, crew will give a **OK** notification to the captain on which engines / propulsion can be restarted.
- If buoy is visible, give captain **GO** to motor slowly towards it until buoy can be taken by dredge/boat hook. Use person pointing at buoy as navigational aid.
- Put line into pulley.
- Hoist frame up.
- The frame is lifted sideways over the side of the vessel by the small davits crane or A-frame of the vessel.

-
- Remove spat collector bags from frame and place each in a separate plastic bag. Add yellow label for bags from frame 2 and 3. Place bags in upright position in crates and keep crates in shade.
 - Upon retrieval of frame, the crew will give a **COMPLETED** notification to the captain on which engines / propulsion can be restarted.
 - Repeat process until all four frames are on deck.
 - If acoustic releases do not trigger, inform Gemini so that they can plan to retrieve them with the inspection ROV expeditions. Try next frame.

In harbour

- Remove temperature dataloggers from frames.
- Use quay crane or vessel crane to lift frames to trailer
- Secure frames to trailer for Den Helder
- Place spat collector bags and temperature dataloggers in van for Yerseke

4 Results

4.1 Logistics of field trips

Finding a suitable weather window proved to be a challenge. Sometimes the waves looked good three days ahead, but then at the planned date they were too high again, or the time window was too short to do the work. Finding a suitable ship was also difficult. Many ships preferred a longer assignment and were therefore not available. Other ships did not get permission to enter the wind park. The successful trips and attempts are summarised in Table 5.

Table 5. Field trips to Gemini in 2023 and 2024. The peak of the larvae was predicted to occur at 8 July 2023.

Date	Waves	Ship	Permission	Comments	Successful
12 July 2023	OK	BMS Vrijheid	Certificates expired	Cancelled	No
18 July 2023	OK	Eems Wave	OK	Motor problem	No
28 July 2023	OK	Sapphire	OK	Placed collector frame #1	Yes
11 August 2023	OK	Eems Wave	OK	Placed collector frame #2 and #3	Yes
28 August 2023	OK	Eems Wave	OK	Placed collector frame #4	Yes
3 October 2023	Waves too high	Eems Wave		Cancelled	No
10 October 2023	Waves too high	Eems Wave		Cancelled	No
23 October 2023	Window too short	Eems Wave		Cancelled	No
26 October 2023	Waves too high	Eems Wave		Cancelled	No
4 November 2023	OK	No ship		Cancelled	No
12 November 2023	OK	No ship		Cancelled	No
22 November 2023	Window too short	Jade		Cancelled	No
28 November 2023	Waves too high	Jade		Cancelled	No
1 December 2023	Window too short	Jade		Cancelled	No
7 December 2023	OK	Eems Wave	No client rep	Cancelled	No
12 December 2023	OK	Terschelling	Certificates not supplied in time	Sailed to Gemini, but no permission to enter windfarm	No
10 January 2024	OK	Eems Wave	OK	All four frames were still there. Retrieval of first one did not succeed due to entanglement of rope	Partly

				but acoustic release surfaced and was collected, others were not tried because of risk identified by client rep.	
21 May 2024		Northern Maria and ROV of Blue Stream	OK	Three frames found. One upside down covered in sand and not in original location. Two covered in sand. Retrieval failed, not able to hook onto lifting eye.	Partly

Because of the late start only three trips to install the frames during the larval season were carried out. This resulted in placing frame #2 and #3 on the same date. Retrieval of the frames was planned for October 2023. However, after 13 attempts the frames are still in the park. In January 2024 it was confirmed that all four frames were still on the bottom (Table 5). In May 2024 only three frames were visually found by ROV and those were all covered in sand. In July 2024 DRN went to the windfarm for another survey. Frames #1, 3 and 4 responded to ping, but no action was taken. Since the acoustic release of frame #2 was already collected in January, that one did not respond.

4.2 Larval abundance

No oyster larvae were observed in the Gemini larvae samples (Table 6). Both detection methods (microscope in larvae/100L and ddPCR in molecules/ μ l) did not show any oyster larvae. The eDNA samples taken on the 28th of July 2023 by DRN did show presence of *O. edulis* DNA, but that could also originate from the adult oysters that were introduced in 2021 and 2022. A low concentration of *O. edulis* DNA was detected with ddPCR at the German RESTORE site. Whether this is from larvae or from adults is unclear as there was no eDNA sample taken at that location.

Table 6. Flat oyster larvae detected in water samples in 2023.

Week number	Date	Sample code	Average # larvae/100L	Sample code	Average concentration (molecules/ μ l)	eDNA samples DRN
30	28-jul-23	Gemini 1	0	Gemini 2	0	positive
30	28-jul-23	Gemini 4	0	Gemini 4	0	positive
30	28-jul-23	Gemini 5	0	Gemini 6	0	positive
32	11-aug-23	Restore 1	0	Restore 2	3.8	no sample
32	11-aug-23	Restore 4	0	Restore 3	0	no sample
32	11-aug-23	Restore 5	0	Restore 6	0	no sample
32	11-aug-23	Gemini 1	0	Gemini 2	0	no sample
32	11-aug-23	Gemini 4	0	Gemini 3	0	no sample
32	11-aug-23	Gemini 5	0	Gemini 6	0	no sample
35	28-aug-23	Gemini 1	0	Gemini 2	0	no sample
35	28-aug-23	Gemini 3	0	Gemini 4	0	no sample
35	28-aug-23	Gemini 5	0	Gemini 6	0	no sample

4.3 Spat abundance

Spat abundance could not be determined because the frames were not retrieved.

5 Conclusions

5.1 Logistics of field trips

The WINOR oyster spat collection experiment required frequent field trips to be able to follow larval abundance over time and introduce settlement substrates at different times during the larval season. Next to that, the frames should be retrieved before the winter storms. For this, the use of small vessels like CTVs (Crew Transfer Vessels) were chosen as they are flexible in time and cheaper than larger ships. Furthermore, frames to hold the bags with substrates were designed in such a way that they could be deployed from a small vessel. The use of small vessels required a calm sea with waves of 1 m or less. Because of these circumstances rapid responses were needed when an advantageous weather window occurred.

The year 2023 was characterised by long periods with a lot of wind and very few weather windows in which the wave height was less than 1m (see Figure 9 for an example; note that both locally-generated waves ('Golven') and swell ('Deining') determine the total wave conditions encountered). Even when windows seemed to arise, the weather changed rapidly. Offshore work is prone to adverse weather conditions. Locating the experiment in a wind farm came with extra measures which sometimes delayed the process of accessing the site. In conclusion, under the present circumstances testing the timing of oyster spat collection is too complicated to be studied in a windfarm.



Figure 9. Wave height forecast of windy on Tuesday 5 December 2023.

5.2 Effect of temperature on larval abundance

The **effect of temperature on larval abundance** could not be determined since there were no larvae detected. In addition, the temperature dataloggers were not retrieved. Thus, verification of the bottom temperature could not be carried out. The temperature sum (see paragraph 3.3 for definition) based on surface temperature data was reached on the 8th of July 2023. The first opportunity to visit the offshore windfarm Gemini was three weeks after this date (Table 5). Accordingly, sampling started too late in the season. In 2022 water samples were also collected and analysed. Then, no larvae were detected microscopically (Table 7). Also, for that occasion, sampling may have been too late in the season. For that period, *O. edulis* DNA was observed with the ddPCR and eDNA analyses (Table 7). This may suggest that we picked up DNA of the adults, rather than larval DNA

Table 7. Flat oyster larvae detected in water samples in 2022 at Gemini ZeeEnergie.

Week number	Date	Sample code	Average # larvae/100L	Average concentration (molecules/ul)	eDNA samples DRN
32	8/8/2022	Gemini 1	0	6.2	negative
32	8/8/2022	Gemini 2	0	1.3	positive
32	8/8/2022	Gemini 3	0	2.7	negative

In the Ecofriend project larval abundance was determined at Gemini Buitengaats in the period 2019-2021. In these years, larvae were detected microscopically and occasionally with qPCR (Table 8). This shows that the reason for not observing larvae in 2023 cannot be found in the methods used. Few larvae were detected after week 31. This suggests that the sampling carried out in 2023 was at the end of the season and therefore did not yield any larvae.

Table 8. Flat oyster larvae detected in water samples in 2019-2021 at Gemini Buitengaats in the framework of the Ecofriend project (Bos et al., 2023).

Week	Date	Sample code	mic (larvae/100L)	qPCR (DNA)
30	7/24/2019	Gemini #1	0	no
30	7/24/2019	Gemini #2	6	no
27	7/2/2020	Gemini #1	9	no
27	7/2/2020	Gemini #2	6	yes
27	7/2/2020	Gemini #3	10	no
29	7/17/2020	Gemini #1	0	no
29	7/17/2020	Gemini #2	19	no
29	7/17/2020	Gemini #3	13	no
31	7/31/2020	Gemini #1	13	no
31	7/31/2020	Gemini #2	6	no
31	7/31/2020	Gemini #3	43	no
29	7/22/2021	Gemini #1	0	yes
29	7/22/2021	Gemini #2	19	no
29	7/22/2021	Gemini #3	0	no
31	8/5/2021	Gemini #1	0	no
31	8/5/2021	Gemini #2	0	no
31	8/5/2021	Gemini #3	9	no
33	8/20/2021	Gemini #1	0	no
33	8/20/2021	Gemini #2	0	no
33	8/20/2021	Gemini #3	0	no

5.3 Effect of deployment week of the spat collectors on spat abundance on substrates

The effect of deployment week of the spat collectors on spat abundance could not be determined, since the frames were not retrieved from the field.

5.4 Effect of addition of Reefpaste to the shells on spat abundance on substrates

The effect of addition of Reefpaste to the shells on spat abundance could not be determined because the frames were not retrieved from the field. The spat-collector frames were designed for deployment under summer circumstances. In spring of 2024, three of the four frames were observed by the ROV, but one was upside down and all were covered in sand. This shows that the stability of the current design is not suitable for winter conditions.

Acknowledgements

We would like to thank Hans Verdaat (WMR), Enzo Kingma (WMR), Quirijn Schürmann (WE), Renate Olie (DRN), Jetze van Zwol (WMR) and Dirk Spruijt (WE) for being stand-by or going on field trips; Frank Jacobs (DRN), Marjolein Kelder (DRN), Margot van Aalderen (DRN), Roelant Snoek (WP) and Marijke Olivierse (WP) for their role in arranging the field trips; Joost Bergsma (WE) for support in the design of the spat collector frames; Pim van Dalen (WMR), Arjen de Groot & Ivo Laros (WEnR), Reindert Nijland (WU-MAE) for analysis of water samples; Babeth van der Weide (WMR) and Pim van Dalen (WMR) for being stand-by for the analysis of shell samples. We would like to thank the crews and the Gemini reps at the various vessels for their support. And we would like to thank Gemini Offshore Windfarm for their support.

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.

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Justification

Report C046/24

Project Number: 4312100135

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: Ing. M. Poelman MSc
 Colleague scientist

Signature: 

Date: July 23rd, 2024

Approved: Dr. Ir. T.P. Bult
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Annex 1 Memo Stability assessment Oysterspat Collector



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WP1230R2r0

Date:
07/06/2023

MEMO

Stability assessment Oysterspat Collector

BACKGROUND

De Rijke Noordzee (DRN) is going to place oysterspat collector frames on the seabed. Since the frame will be deployed and left on the seafloor for a number of weeks / months during summer 2023, the frames need to be stable and able to withstand storm conditions at the OWF location during summer conditions.

WaterProof has assessed the stability of the oysterspat structure and provided advice on optimizations to the frame to be made. This memo describes the results of the stability assessment during 1/1 year storm conditions.

SUMMARY

WaterProof assessed the stability of the proposed structure, at this location. To this end, first the extreme MetOcean boundary conditions (waves, flow) were identified. Subsequently, using analytical calculations the stability of the frames was determined, both for the failure mechanism (1) turning over and (2) sliding. The result of the study show that additional measures needed to be taken to prevent the structures against turning over / sliding, as the stability of the structure is not sufficient to be able to withstand a 1/1 year storm. With the proposed optimizations, the frame is concluded to be stable.

DESIGN OYSTERSPAT COLLECTOR

The design of the structure is shown in Figure 1. The dimensions of the system are as follows:

- The system consists of an x-shaped frame with a length of 2 m and 4 feet;
- On each feet a vertical 0.5 m high beam holds the oysterspat collectors
- 5 pins penetrate into the seabed underneath the frame



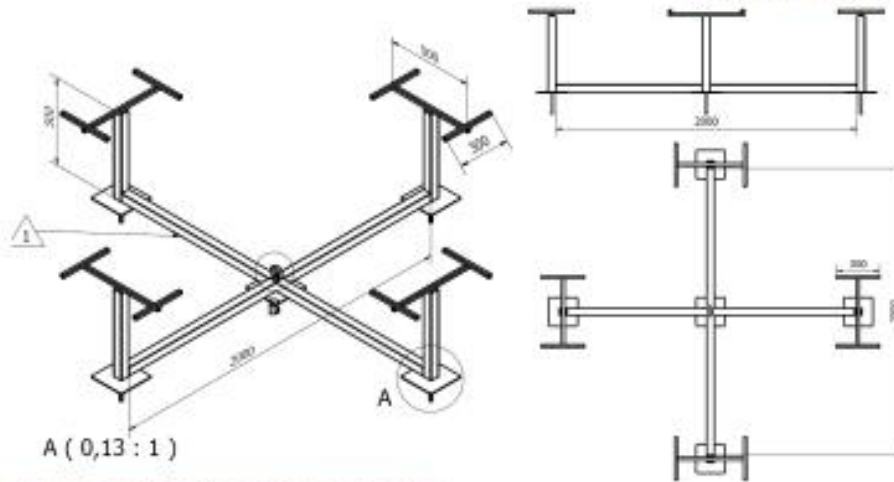


Figure 1: Technical design of the Oysterspat collector

On the frame oysterspat collector bags are going to be placed, see Figure 2 .

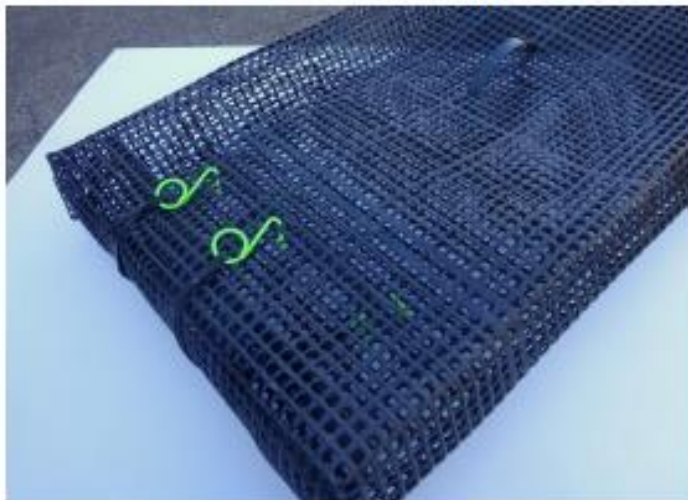


Figure 2: Oysterspat collector bags

HYDRO- AND MORPHODYNAMICS AT GEMINI

Introduction

The Gemini windfarm area is located several kilometres offshore in northern direction from the Wadden Sea, in the North Sea. The projected location for the measurement frame is shown in Figure 1.

For the construction of this windfarm, an extensive MetOcean study has been performed by DHI-WASY (2012). The hydrodynamic conditions that are used for determining the stability of the structure are the result of this extensive MetOcean study from 2012. The output location of this site is shown in Table 1.

The research area where the structure is going to be placed (see Figure 1) lies relatively close to this output point (approx. 6.5 km), and has a similar water depth, thus allowing the use of the MetOcean study without adjusting.

Table 1: Coordinates location MetOcean data OWP Gemini

Site	Latitude	Longitude	Depth [m +MSL]
Gemini NW	54.058 N	5.857 E	-35.1

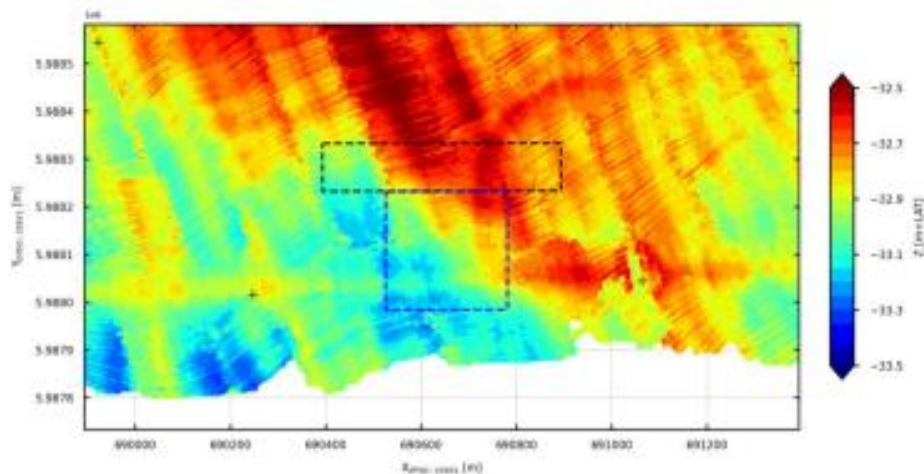


Figure 3: Oyster reef location (black), research location where the frame will be placed (blue) and the MetOcean output location (+) at the Gemini OWP area.

Water levels

Based on the MetOcean data, the following conditions on the local extreme total water levels (see also Table 2) are extracted:

- The 1-year return value of the extreme high total water level is 1.55m and of the extreme low water level is -1.30m.

Table 2: Extreme high and low total water levels (HWL and LWL) at the OWP Gemini, for several return periods.

Return period [years]	Extreme high water levels, relative to MSL [m]	Extreme low water levels, relative to MSL [m]
1	1.55	-1.30
5	1.80	-1.45
10	1.95	-1.55
50	2.40	-1.70
100	2.65	-1.80

Wave and wind climate

Table 3 shows the extreme sea states at the OWP Gemini location, for several return periods. From the MetOcean study, the following conditions are extracted:

- The most extreme waves come from the West-Northwest sectors. The less extreme waves come from the Northeast-Southeast sectors. The omni-directional estimate of the 1-year return value of the significant wave height is of 7.3 m, with a peak period of 11.5 s.

Table 3: Wave conditions (significant wave height H_s , peak period T_p), for several return periods. The wave conditions are for the maximum still water levels. Wave directions are given as coming from, clockwise from North.

Values for maximum still water level						
Sector		Return period				
		1-yrs	5-yrs	10-yrs	50-yrs	100-yrs
OMNI	H_s [m]	7.3	8.2	8.6	9.5	9.9
	T_p [s]	11.5	12.2	12.4	13.1	13.3

Currents

The extreme currents are based on the combined effect of tidal currents and wind. The following conditions are extracted (see Table 4):

- The most dominant flow directions are in West and East direction (going to), due to the dominance of the tidal currents.
- The 1-year return value estimates of the depth-averaged current for the main directional sectors West and East at the OWP Gemini are 0.9m/s respectively 0.7m/s.

The current profile can be estimated with the following formula and the depth average currents from table Table 4:

$$U_{c,sub}(z) = \frac{8}{7} U_{mean} \left(\frac{z}{d} \right)^{\frac{1}{7}}$$



Table 4: Depth-averaged currents the two governing directions (including wind effects) for several return periods. Currents are defined as going towards direction, clockwise from North. Values are rounded up to 0.05 m/s.

Sector	Return Period [m/s]				
	1-yrs	5-yrs	10-yrs	50-yrs	100-yrs
90°	0.90	1.00	1.05	1.15	1.20
270°	0.70	0.80	0.80	0.90	0.95

Bed composition

Based on core data from the DINOLoket, the following conditions are extracted (see Figure 4):

- The bed is composed of sandy materials;
 - There is almost no silt material present at the surface (<4%)
- The average grainsize, D_{50} , is in the order of 250 μm

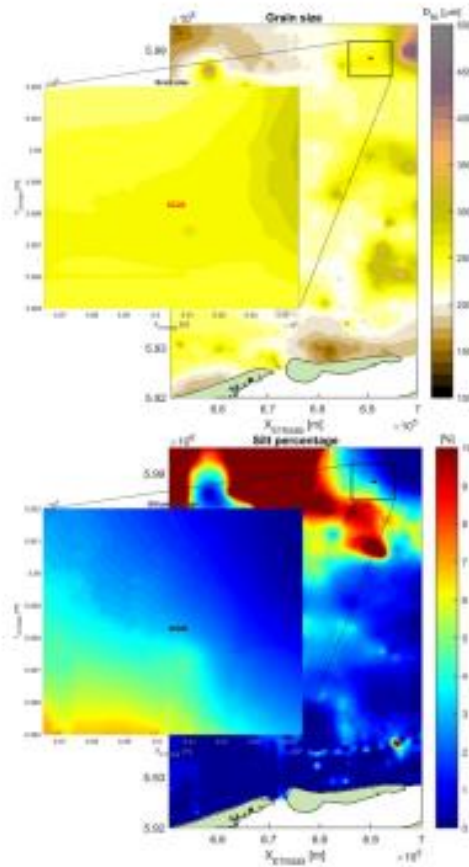


Figure 4: Bed composition based on core data of the DINDLaket

STABILITY OYSTERSPAT COLLECTOR

In this section the stability of the structure is assessed. The interaction between the hydraulic forces and structures can result in 2 failure mechanisms (see Figure 5):

- 1) Failure due to turning over;
- 2) Failure due to movement out of foundation.

For mechanism 1, the moments (Force x distance) on the structures are of importance. Whether or not the structure turns over depends on the balance of the acting moment by the flow/waves, and the counteracting moment due to the weight of the structure itself. The acting moment is the product of

the force and its application point as determined by the Morison equation. The counteracting moment depends on the weight of the structure and the distance a_2 : this is the distance from the centre of the structure to the turning point (see Figure 5).

Apart from structure turning over, the structures can be pushed out of the foundation area (mechanism 2, see right panel in Figure 5). Here, the balance between the horizontal force against the construction and the shear stresses between foundation and construction is of importance. The maximum shear stress (just before sliding occurs) depends on the weight of the structure, size and roughness of the structure, and roughness of the foundation layer.



Figure 5: Failure mechanisms considered (from WaterProof, 2020).

Forces on the structures

A worldwide used method to calculate forces under a combination of currents and waves is the (adapted) Morison equation. The Morison equation is the sum of two force components: an inertia force in phase with the local flow acceleration and a drag force proportional to the (signed) square of the instantaneous flow velocity.

$$F = \rho C_M V a + \frac{1}{2} \rho C_D A u |u|, \text{ where:}$$

ρ = density of water,
 C_M = inertia coefficient,
 V = volume of the structure,
 a = acceleration at the structure,
 C_D = drag coefficient,
 A = Surface area that blocks the flow,
 u = flow velocities approaching the structure

It can be said that the stability of the structures depends on:

- The fluid flow velocities approaching the structure;
- The fluid accelerations at the structure due to waves;
- The effective surface area normal to the flow;
- The volume of the structure;
- The hydraulic drag coefficient (C_D) and the inertia coefficient (C_M);
- The distance of the centre of the structure to the turning point (a_2), only relevant for mechanism 1;
- The friction of the seabed.

The first four points are dependent on the design and placement alone. The drag coefficient can be determined with laboratory experiments. The inertia and seabed friction coefficients are based on literature.



The highest uncertainties in the equations lie in the drag coefficient of the structure. In previous studies, WaterProof has determined the drag coefficients using several flume experiments. For this study, the drag characteristics of a similar structure (cage type) are taken and applied. Hence, in this study, the drag coefficient is taken as 1.1 (lower limit) – 1.6 (upper limit), with the upper limit forming the basis for the stability calculations.

Stability calculations

The methodology and assumptions are applied as follows:

- **Drag coefficient, C_D**

Earlier studies for similar structures show a value between 1.1 – 1.6. For conservative reasons, a C_D of 1.6 is used for the the Oysterspat collector. It is important to mention that the application of additional attachments to the structure or marine growth can further increase the value of this parameter. In order to take marine growth still into account, it is assumed that marine growth will increase the width of the structure by 2 cm at all sides.

- **Distance to turning point, a_2 :**

The distance a_2 equals the distance between the location of the centre of gravity and the turning point. This value is maximum when the turning point is at the corner of the structure. Therefore, the maximum value equals half of width parallel to flow. However, if structure placed on top of scour protection, uneven stone distribution or angled stones can lower this distance significantly by moving the turning point closer to the centre of the structure. Additionally, if the combined weight of the structure and the protection exceed bearing capacity, (uneven) settling could induce additional slope, which also lowers the distance. For the calculations performed in this section, the distance is assumed to be maximum. But, in order to account for the uncertainties, a safety factor is applied (see further).

- **Inertia coefficient, C_M :**

This coefficient depends on the added mass to the structure; the extra inertia the structure will feel when water is accelerated (or decelerated). This dependence is expressed as (Bai, 2005):

$$C_M = C_a + 1$$

The presence of a fixed boundary has a pronounced effect on the inertia (and the added mass) coefficient; the smaller the distance to the bed, the larger this parameter becomes. As the structure is placed at the seabed, C_a becomes equal to 2. With the aforementioned dependence between the inertia coefficient and the added mass coefficient C_a , C_M therefore becomes equal to 3.

- **Friction of bed, friction parameter μ_d**

The bed friction is an important parameter that determines the moment when sliding is initiated. The friction resulting from the weight of the structure is as follows:

$$\begin{aligned} \text{Friction} &= F_{\text{weight}} \cdot \mu_d \text{ with,} \\ F_{\text{weight}} &= (\rho_{\text{structure}} - \rho_{\text{water}}) \cdot \text{Volume structure} \cdot g \\ \rho_{\text{structure}} &= \text{depending on materials used [kg/m}^3\text{]} \\ \rho_{\text{water}} &= 1025 \text{ [kg/m}^3\text{] (salt water)} \\ g &= 9.81 \text{ [m/s}^2\text{]} \end{aligned}$$

μ_d = dynamic friction coefficient

The friction coefficient can be interpreted as follows; the lower the value is, the lower the friction force becomes, the easier it is for the structure to start sliding. Several studies have looked into the friction coefficient for sand. Depending on the sand type and its moisture contents (Fall, 2014) this value can vary. For wet sand, the friction value equals between 0.4 and 0.5. Our flume for example has a friction coefficient equal to 0.46. It is expected however, that in reality the structure will not slide smoothly, but will push itself into the sand / the scour protection layer. Additionally, rocks have a higher friction coefficient than sand. Therefore, this study assumes the seabed friction coefficient to be equal to 0.6.

- **Safety factor**

As explained above, there are a number of uncertainties; especially with respect to the drag parameter and the distance to the turning point. Based on these uncertainties a total safety factor of 1.2 is applied in the stability calculation for conservatism reasons.

Forces and moments

Table 5 summarizes the forces on the structure for the orientation where forces are largest.

Table 5: Resulting forces (F) and moments (M) on the structure considered. The numbers are red if the forces are larger than the resistance of the structure (requirement is not met) and green if the forces are smaller than the resistance of the structure, based on the resistance as mentioned in Table 6.

Structure	Return period [years]									
	1		5		10		50		100	
	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]
Oysterspat	1.0	0.23	1.2	0.31	1.3	0.35	1.6	0.46	1.8	0.52

It can be seen that for current structure design:

- The resistance against sliding is sufficient until a 1/1 year storm (M), and against tipping over not sufficient for any storm (F).

In the conclusions we give recommendations how to increase the stability of the structure.

Resistance

The resistance of the structure (as-is) is a combination of 2 aspects; the dimensions and weight of the structure (for both failure mechanisms). The 'above-water' weight of the structure is estimated to be 15 kg.

The total resistance against sliding and against tipping over is shown in Table 6.

Table 6: Estimated resistance of the original Oysterspat collector

Structure	Resistance	
	Against sliding [kN]	Against tipping over [kNm]
Oysterspat	0.82	0.20

In the following section, recommendations are given to increase the stability of the structure.

Based on the calculations in this report, it can be said that under all conditions the resistance against sliding and the resistance against tipping over are not met.

For this reason, the structure needs to be optimized in order to meet the stability criteria. This is described below.

RECOMMENDATIONS FOR DESIGN OPTIMIZATION

Recommendations for design optimization

The structure should be optimized to enhance the ability to withstand the acting forces. Most optimal optimization would be to increase the weight of the collector because that increases the resistance both on forces as well as moments. However, the idea of this cage is to be able to place this on the seabed by hand, a lightweight collector is this preferred.

Therefore we chose to optimize the collector as follows (see Figure 6):

- We increased the base width of the structure in order to enlarge the resisting moment the structure can have (we enlarge a_2). We did this by changing the x-type structure into a squared structure.
- We slightly lowered the vertical beams of the structure from 0.5 m to 0.4 m. This reduces the acting moment on the vertical beams
- We increased the resistance against sliding by adding a metal strip under the horizontal beams which rest on the seabed. In this way the resistance against sliding is increased.

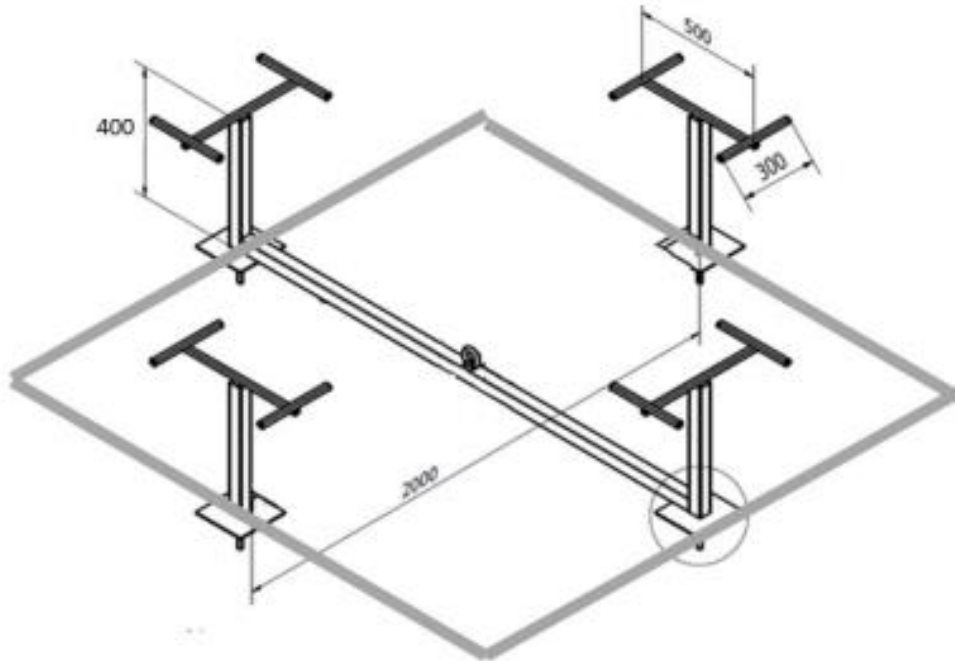


Figure 6: Proposed optimizations on oysterspat collector design

Final results and Conclusions

In Table 7 and Table 8 respectively the estimated resistance and acting forces and moments are presented. It can be seen that due to the adaptations to the frame the resistance has been increased and the acting forces / moments have decreased.

With the improved structure layout the frame is stable in flow and wave conditions with a return period of 1 per year.

Given the fact that the frames are only placed in summer 2023, we expect that the probability the frames will move or tip over are negligible small.

Table 7: Estimated resistance of the original Oysterspat collector

Structure	Resistance	
	Against sliding [kN]	Against tipping over [kNm]
Oysterspat	1.0	0.24

Table 8: Acting forces (F) and moments (M) for the optimized structure

Structure	Return period [years]									
	1		5		10		50		100	
	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]	F [kN]	M [kNm]
Oysterspat	0.8	0.2	1.0	0.28	1.1	0.32	1.4	0.4	1.5	0.48

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