BOOST

Better Oyster Outplacement & Seeding Techniques

BOOST REPORT

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TKI PROJECT 2023 - 2024

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ABSTRACT

The BOOST project aims to support the restoration of European flat oyster (*Ostrea edulis*) reefs in the North Sea by developing a method for large-scale reef restoration. This method focuses on BlueLinked's ReefBooster in combination with automated observing techniques such as the autonomous underwater vehicle (AUV) developed by Lobster Robotics and artificial intelligence models developed by Wageningen University & Research. For underwater observations in the field, the use of AUVs like the Lobster Scout has improved large-scale reef imaging, offering more accurate data for assessments after deployment. AI-based spat detection models, particularly Cascade Mask R-CNN, have proven effective in tracking oyster spat in a hatchery setting. However, detecting ReefBoosters with spat once deployed on the seabed still requires more work for it to be applicable in the field. Furthermore, small design modifications to the ReefBooster, such as weight distribution, can significantly impact its functionality. With regards to material composition of the ReefBoosters, some results were inconclusive, but main observations suggest that calcium-rich materials, such as lime and seashells, promote oyster settlement and foster marine biodiversity. BlueLinked has demonstrated the ability to successfully cultivate flat oysters in their hatchery, from broodstock to new oyster spat. Despite challenges in assessing long-term stability, the ReefBooster method in combination with automated techniques such as an AUV and AI, is shown to be effective for nearshore restoration and offers promising scalability for large-scale projects.

DEFINITIONS

Check Table 1 and Table 2 for the definitions of the used abbreviations and terms in this report. Table 3 shows an overview of the BOOST project partners and their roles.

TABLE 1. OVERVIEW OF ABBREVIATIONS AND THEIR DEFINITIONS.

Abbreviation	Definition
AI	Artificial Intelligence
AUV	Autonomous Underwater Vehicle
BL1 / BL2 / BL3	BlueLinked material compositions 1, 2 and 3
BOOST	Better Oyster Outplacement & Seeding Techniques
DSA	Dedicated Spat Area
OCR	Optical Character Recognition
(O)PC	(Ordinary) Portland Cement
QGIS	Quantum Geographic Information System
ROV	Remotely Operated Vehicle
TO	TinyOcean

TABLE 2. OVERVIEW OF TERMS AND THEIR DEFINITIONS.

Term	Definition
Bonamia	Parasitic protozoans that affect shellfish, particularly oysters, often resulting in mortality. A Bonamia-free compartment is a designated area that is certified free from Bonamia parasites.
broodstock	The sexually mature flat oysters obtained from a Bonamia-free compartment used for breeding.
nearshore	Refers to the areas of the sea (North Sea in this project) that are close to the coastline, typically within a few kilometres.
offshore	Refers to the areas of the sea (North Sea in this project) that are farther from the coast, usually beyond the nearshore zone, often in deeper waters.
ReefBooster	The small (relative to other structures on the market) pyramid structure developed by BlueLinked for flat oyster and other epibenthic marine organism restoration.
Scout	The autonomous underwater vehicle (AUV), developed by Lobster Robotics, used to test oyster restoration observing techniques.
TinyOcean	TinyOcean is a 6.000-liter, self-cleaning seawater tank designed by BlueLinked to recreate a marine-like ecosystem on land. This innovative, closed system supports a fully circular water process, reducing water waste and energy consumption and ensures biosecurity.

TABLE 3. OVERVIEW OF PROJECT PARTNERS AND THEIR ROLES IN THE BOOST PROJECT.

Partner	Role
Advanced Tower Systems	Since 2003, Advanced Tower Systems B.V. (ATS) has been developing and producing prefabricated concrete wind turbine towers. ATS holds several patents for segmented towers, and more than 1,400 towers (typically 1,000 tons per tower) have been constructed worldwide onshore by ATS and its licensees. In Northwest Europe, new wind turbines are now being built offshore, and our business and product development is focused on this shift. ATS is eager to be involved at an early stage to contribute to the initial research steps needed to achieve ecological enhancement in wind farms. ATS's expertise in intellectual property, certification, production, quality assurance, and integrated project and cost management is essential to apply the ambitions of the BOOST project on the industrial scale of offshore wind farms.
BlueLinked	BlueLinked's (BL) core value is its commitment to sustainable food production and ocean rewilding. Restoring the health of the North Sea is therefore a high priority for BL. Since 2011, BL has been working on the design of the ReefBooster for reef restoration. What began as reef tiles for tropical coral reef restoration was further developed in 2022—thanks to funding from The Rich North Sea and a collaboration with TU Delft—for the restoration of flat oyster reefs in the North Sea. Additionally, through its years of experience in cultivating various marine organisms, BL has developed an innovative and circular cultivation system (*TinyOcean*). BL is eager to start research on the attachment of oysters to these reef tiles within its innovative cultivation systems to enable large-scale application.
Boskalis	Boskalis (BK) has been closely involved in developing and scaling up solutions for biodiversity and climate adaptation through its Artificial Reefs Program (ARP) since 2017. Under the ARP, Boskalis supports key players in the market who possess the technology to enable disruptive scaling of positive impacts. The new ideas and techniques from BlueLinked can strategically contribute to unlocking the offshore wind market, creating substantial (nature) value in the execution of current and future offshore projects.

The Rich North Sea

The Rich North Sea (*De Rijke Noordzee*, DRN) is currently managing 6 offshore projects focused on nature restoration in wind farms. The team has extensive experience and is capable of combining state-of-the-art ecological knowledge with the challenging realities of working at sea. With experience from oyster projects in Gemini, Luchterduinen, and Blauwwind, DRN is keenly aware that large-scale oyster restoration depends on a thorough understanding of the ecosystem and its interaction with artificial structures, as well as a deployment method suited to this system. For DRN, it is crucial to develop an efficient method, with minimal materials and costs, for restoring or strengthening populations of biogenic reef builders in the North Sea, such as the flat oyster.

Lobster Robotics

Lobster Innovations (LI) envisions revolutionizing underwater exploration technology, with the ultimate goal of making the world's oceans transparent to researchers and conservationists. Current observation methods for oyster restoration are too costly due to the large number of man-hours required. Automation is challenging because underwater currents are difficult to predict. Additionally, creating overlapping photos to form a mosaic requires high precision and accuracy. LI has the expertise to develop a control method for robots that enables them to autonomously collect data for high-quality photo mosaics in strong currents. LI has a test platform equipped with an underwater camera, which can be used to research new methods for scalable visual data collection for oyster restoration. BOOST aligns perfectly with LI's vision, and it is crucial that reliable and scalable data collection methods are developed that are suitable for mapping oyster populations.

Wageningen University & Research

Wageningen Marine Research (WMR) is the Dutch institute for applied marine ecological research, contributing to more sustainable and careful management, use, and protection of the natural resources in marine, coastal, and freshwater areas through knowledge, independent scientific research, and advice. WMR has been involved in the restoration of flat oysters in the North Sea since the first feasibility studies in 2014. In addition, in collaboration with Wageningen Food and Biobased Research (WFBR), WMR has worked on automating the analysis of underwater images from four sand grounds in the North Sea. WMR has a vested interest in applying and expanding its expertise in this field by conducting research into automating oyster recognition through the BOOST project.

INTRODUCTION

The BOOST project was established to address the urgent need for large-scale restoration of European flat oyster (*Ostrea edulis*) reefs in the North Sea. Historically, these native oyster reefs were widespread along European coasts, providing essential ecological functions such as water filtration, nutrient recycling, and habitat creation for various marine species. (Thurstan et al., 2024) However, due to overfishing and diseases, their populations have drastically declined till the current point of IUCN Red Listing. (zu Ermgassen et al., 2024) *O. edulis* plays a key ecological role by filtering water, recycling nutrients, and providing habitat for marine species. Recognizing the ecological value of these reefs and the potential benefits of their restoration, the Netherlands has initiated several projects to explore effective restoration methods. (Bos et al., 2023) BOOST aims to overcome key challenges that have limited current restoration efforts, including issues related to economic feasibility, the cultivation of flat oysters, effective observing, and decommissioning.

Restoring *O. edulis* through the active cultivation has as main benefit that thousands to millions of oysters can be produced and reintroduced at once. However, with regards to scaling the cultivation of *O. edulis*, there is a particular complication due to the disease *Bonamia ostreae*. This protozoan parasite greatly effects the health of flat oysters and can lead to high mortality rates. Therefore, strict regulations are in place within Europe to prevent the spreading of this disease. (Bennema et al., 2020; Pogoda et al., 2019) BlueLinked has developed a hatchery with a closed water circulation and can cultivate *O. edulis* without the risk of getting a *Bonamia* infection.

O. edulis starts it life cycle as free swimming larvae. After 14 to 26 days the larvae undergo a metamorphosis and are ready for settlement on a hard substrate (Helmer et al., 2019). BlueLinked has developed a specific structure for settlement that aids in active restoration, namely the ReefBooster (Figure 1). This structure allows for efficient settlement in the hatchery, as well as efficient transport and deployment. This way the ReefBoosters with spat can be ‘seeded’ in the ocean covering acres at a time.

Restoration at such as large scale, however, requires innovative observing techniques. Observing oyster reef restoration in the North Sea in a cost-efficient and accurate manner remains challenging, primarily due to the absence of automated observing equipment capable of withstanding the harsh weather conditions and low underwater visibility typical of the region. The Lobster Scout developed by Lobster Robotics is an underwater vehicle that is specialized in underwater photogrammetry in poor underwater visibility environments like the North Sea (Figure 2). The Scout is equipped with underwater positioning sensors that

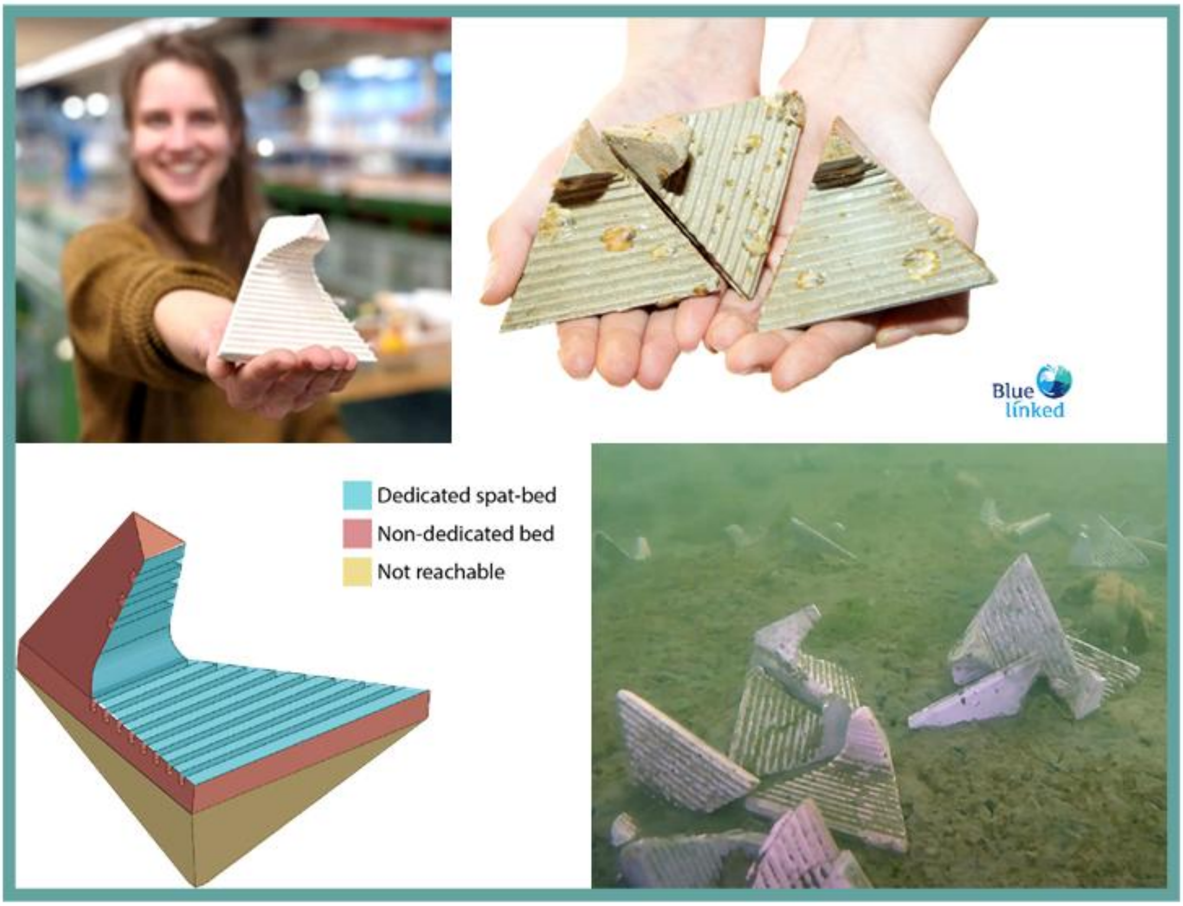


Figure 1. From top left to bottom right: 1) ReefBooster held for scale, 2) ReefBoosters with spat, 3) schematic depiction dedicated spat area of ReefBooster, and 4) installed ReefBoosters in the Port of Rotterdam. Dedicated spat area is the surface of the ReefBooster where spat would preferably settle; the non-dedicated spat area refers to the area of the ReefBooster that is still in contact with the water column of the TO and as such could be used as a settlement place; the not reachable area is the surface of the ReefBooster that is not in contact with the water column in the TO and cannot be reached by the oyster larvae.

work together with an integrated camera and strobe lights, allowing it to take pictures at a specific underwater position. The Scout follows seabed slopes of up to 40 degrees and will always precisely fly at a certain attitude. In this project, the Scout was used to experiment with various observing techniques in combination with the ReefBooster method for flat oyster restoration. Another aspect to consider is efficient analysis. Having to analyse the settlement success and deployment over time of thousands of ReefBoosters with spat is time consuming and labour intensive. Therefore, the BOOST project also focuses on developing Artificial Intelligence programs that are capable of supporting data analysis on a hatchery level, as well as imaging data retrieved by the Lobster Scout. Wageningen University & Research has experience with addressing complex challenges with their extensive expertise in AI and image analysis, and within BOOST this was further developed to make an object recognition model specifically for flat oyster cultivation and restoration.

This project takes some of the first steps in working towards a long-term goal of restoring oyster reefs on a scale of at least acres in offshore areas, considering that these reefs once covered thousands of square kilometres in the Dutch North Sea. The primary objectives of the BOOST project are to establish foundational knowledge and methods in a nearshore setting to enable large-scale reef restoration offshore. To this end, BOOST's research focuses on two main parts: 1) Flat Oyster Settlement Research on the ReefBooster in a Hatchery Setting, and 2) Hydro-morphological and Ecological Characteristics of the ReefBooster in a Nearshore Setting.

Through this research, BOOST aims to lay the groundwork for a scalable and sustainable approach to oyster reef restoration, providing a model for future large-scale restoration projects in the North Sea. This project report outlines the research activities, findings, and progress made in pursuit of these objectives.

FIGURE 2. THE LOBSTER SCOUT AUV NEXT TO AN OPERATOR FOR SCALE.

Part I: Flat Oyster Settlement Research on the ReefBooster in a Hatchery Setting

- **Bio-receptiveness**: Investigate various material compositions suitable for promoting flat oyster settlement on the ReefBooster.
- **Analysis of Settlement Rate**: Apply AI to analyse settlement rates within a controlled hatchery environment.

Part II: Hydro-morphological and Ecological Characteristics of the ReefBooster in a Nearshore Setting

- **Data Collection**: Develop and assess the use of an autonomous underwater vehicle (AUV) for efficient observing of ReefBooster deployments.
- **ReefBooster Performance Nearshore**: Examine hydro-morphological behaviour in relation to abiotic factors, including:
  - Orientation of the ReefBooster on the seabed
  - Stability and sedimentation of the ReefBooster on the seabed
- **Bio-receptiveness**: Evaluate biological activity on ReefBoosters in response to biotic factors in the nearshore environment.

PART I

FLAT OYSTER SETTLEMENT RESEARCH

ON THE REEFBOOSTER IN A HATCHERY SETTING

1.1 BLUELINKED'S HATCHERY

Part I of this project focuses on flat oyster cultivation in the BlueLinked hatchery in BlueLinked's TinyOcean (TO) cultivation system on land (Figure 3). The TO is precisely what its name suggests: a mini ocean containing 6,000 litres of seawater with a fully circular water treatment system due to the purifying seabed established by BlueLinked. The circular and closed design of the TO ensures the highest level of biosecurity, creating a controlled environment that effectively prevents contamination and safeguards against external biological threats. This is especially important for rearing larvae with the intent of active restoration.

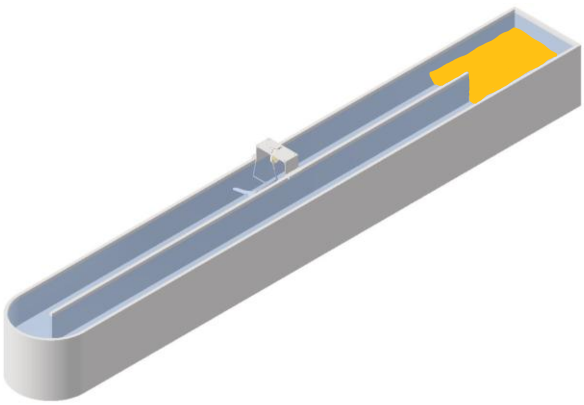


FIGURE 3. SCHEMATIC OF THE TO DESIGNED BY BLUELINKED. IN YELLOW IS THE LOCATION OF THE LIVING SOIL THAT BEHAVES AS THE FILTER FOR PURIFYING THE CIRCULATING WATER. IN WHITE IN THE MIDDLE OF THE LENGTH OF THE TO THERE IS AN UNDERWATER FIN CREATING THE DESIRED FLOW IN THE WATER COLUMN.

The larval rearing period refers to the collection the mobile larvae released by the brooding female oysters and transferring them to a rearing tank. There they will be provided with a complete and nutritious diet to enable them to complete their metamorphosis into settlement competent larvae. Female oysters filter sperm from the water and fertilise their eggs in the pallial cavity. The larvae are released into the water by the female after 8 to 10 days, depending on the temperature. The mobile larvae spend an additional 10 to 14 days in the water column before settling on a suitable surface. This period of development from mobile larvae to the sessile spat stage is called metamorphosis. It is thought that metamorphosis and its timing are largely dependent on food availability and can take up to two weeks to complete. During metamorphosis different life stages can be distinguished. The classification used is the veliger and umbo stage and the settlement competent pediveliger stage, which is characterised by an eye spot and an active foot (Figure 4).

Part I will elaborate on how the TO was utilised for rearing larvae and the settlement success of competent larvae on the various ReefBoosters produced. *Chapter 1.2 Settlement research on various material compositions* focuses on the investigation of various material compositions suitable for promoting flat oyster settlement on the ReefBooster. *Chapter 1.3 Analysis of settlement rate in a hatchery using AI* focuses on the analysis of the settlement rate of the larvae on the ReefBoosters by applying artificial intelligence.

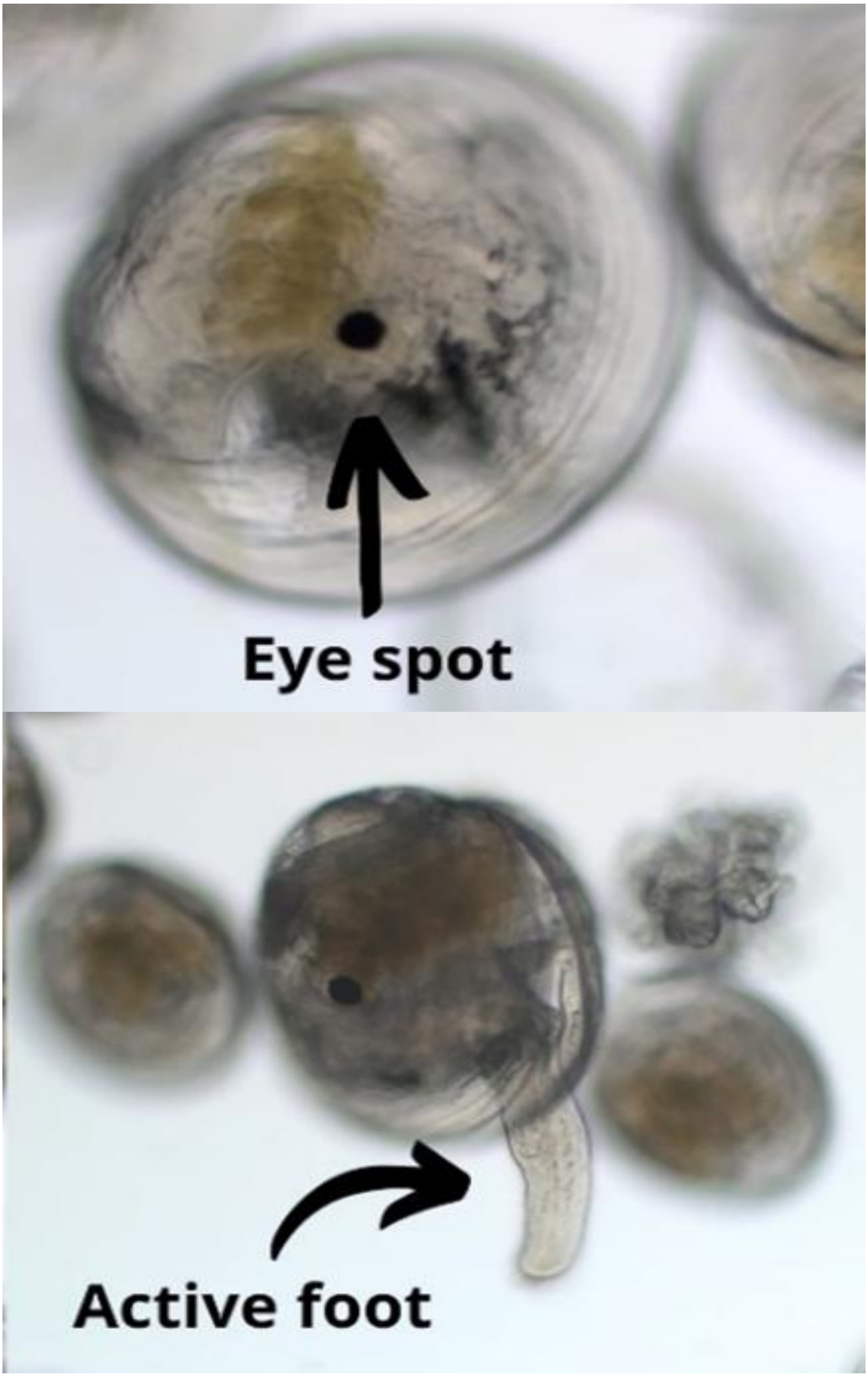


Figure 4. Settlement competent pediveliger larvae, top picture with eye-spot and bottom picture indicating active foot. (source: BlueLinked)

1.2 SETTLEMENT RESEARCH ON VARIOUS MATERIAL COMPOSITIONS

1.2.1 Introduction

The ReefBooster method aims to actively restore flat oyster populations through a structured process of cultivation. A key step in this process is promoting the successful settlement of oyster larvae onto the ReefBoosters within a hatchery environment. To ensure the effectiveness of this settlement, it is essential to assess the bio-receptiveness of different material compositions of the ReefBoosters based on their ability to attract larval attachment. Certain substrate characteristics, like higher calcium content, are hypothesised to attract more larvae, making compositions with shell powder, lime, or gypsum promising candidates for successful colonisation by oyster spat (Soniat T. M. & Burton G.M., 2005). However, practical testing is essential to confirm these effects, as the complex interactions between larvae and substrate materials are difficult to predict theoretically.

Given the ecological and environmental implications, the selection of materials was guided by a specific set of criteria aimed at ensuring the long-term sustainability and effectiveness of the ReefBooster, namely sustainability, locality, degradability and bio-receptivity.

Sustainability

To minimize environmental impact, the project focused on identifying alternatives to traditional Portland cement. Portland cement production is a major source of CO_2 emissions, with cement-related CO_2 emissions, constituting around 7% of total annual energy and industry emissions (Fennell et al., 2021). One promising alternative identified is Tras, a natural pozzolanic material. Tras has cement-like properties that, when mixed with lime or other binding agents, forms a durable and more environmentally friendly concrete. Unlike Portland cement, which has a high carbon footprint due to the energy-intensive clinker production process, Tras requires lower processing temperatures and utilizes natural materials. The incorporation of Tras into the material compositions might offer a solution that balances structural integrity with environmental responsibility. The integration of Tras was tested in terms of settlement and bio-receptivity (*Chapter 1.2 Settlement research on various material compositions* and *Chapter 2.5 Bio-receptiveness nearshore*).

Locality

To ensure minimal environmental impact and promote regional compatibility, the project prioritized the use of local materials. North Sea sand emerged as a key component, providing a locally abundant resource that reduces transportation-related emissions. Using North Sea sand ensures that the material is naturally compatible with the local marine environment, enhancing the integration and stability of the ReefBooster within the ecosystem. North Sea sand was used in all experiments conducted (*Chapter 1.2 Settlement research on various material compositions* and *Chapter 2.5 Bio-receptiveness nearshore*).

Degradability

BlueLinked's vision is to leave behind a natural self-sustaining reef without any manmade structures. Therefore, the aim is to create a material composition which naturally erodes and degrades over time, giving the ReefBoosters a lifetime of a minimum of 5 years and a maximum of 20 years. Gypsum, a naturally occurring mineral rich in calcium sulphate, was found to dissolve in saltwater in previous experiments conducted at BlueLinked. This introduced the potential to use gypsum as an eco-friendly additive in cement mixtures, allowing natural erosion up to the point of full degradation in marine environments. The effect of using gypsum was explored specifically in section *2.5.4 Discussion and Conclusion*.

Bio-receptivity

For the reef units to be effective in promoting oyster growth, the material must be conducive to oyster attachment and colonization. To enhance bio-receptivity, the material mix was enriched with crushed shells and additional calcium. The crushed shells provide a familiar substrate for oysters to settle on, while the calcium content promotes the growth and health of oyster shells. This combination ensures that the ReefBoosters are not only structurally supportive but also biologically attractive to the oysters. Bio-receptivity of different compositions was tested in terms of settlement in Chapters 1.2 and 2.5. The objective of this research is to identify and evaluate materials suitable for flat oyster settlement on the ReefBooster.

The settlement experiments in the hatchery focused on the following research question: Does increasing the calcium content and/or reducing Portland cement content in the composition enhance flat oyster larvae settlement rates on the ReefBooster?

1.2.2 Methodology

Settlement experiments were performed twice. Once in November/December 2023, and once in August/September 2024. Two repetitions were done in 2023, and one in 2024. They will be referred to as experiments S1, S2, and S3 respectively.

ReefBooster production

In both experiments the same 4 different composition were tested (Table 4). They were produced using a pouring method at the facility of BlueLinked.

Broodstock conditioning

The flat oysters (*O. edulis*) broodstock were sourced from Tralee Bay, Ireland, which has a Bonamia-free status. Two different batches of broodstock were used for this research. Adult Batch 1 was received on 13 April 2022 and the adult Batch 2 was received on 25 August 2023. The oyster broodstock size was 30 adults for each batch. They were conditioned at the hatchery from a cold hibernation period of two months to a gradual warming period until summer spawning. To control the water temperature in the system, a climate-controlled cool-heater was used, which allowed a gradual increase of 0.1 °C per step reaching 20 °C during the conditioning period before transport to the TinyOcean holding set-up. For adult Batch 1, the temperature was kept below 7 °C for the winter period 2022 to 2023. For the winter period of 2023 to 2024 both batches were kept between 9 and 10 °C for two months.

Separate systems were installed to allow gradual temperature increases while maintaining optimum water quality. A protein skimmer, mechanical band filter and bacterial floating sand bed were installed as life support systems. The systems were placed in a climatised room to control the air temperature and light regime. Daily water quality control was performed for salinity, oxygen, temperature and pH. Weekly quality control was carried out for ammonium, nitrite, nitrate and phosphate levels (Table 5). Additionally, the health of the oysters was observed weekly according to BlueLinked protocols.

TABLE 5. WATER QUALITY VALUES DURING CULTURE PERIOD.

Salinity	30‰ ± 1
Temperature	20 °C ± 5
Oxygen	100% ± 10
pH	8,0 ± 0,5
Ammonium (NH_4^+)	≤ 0,1 mg/L
Nitrite (NO_2^-)	≤ 0,01 mg/L
Nitrate (NO_3^-)	≤ 8 mg/L
Phosphate (PO_4^{3-})	≤ 3 mg/L

They were fed 10.000 to 20.000 algae cells per millilitre for 16 hours per 24-hour period. During the warm-up period before spawning, the feed was increased to 50.000 algae per millilitre on a 24-hour cycle and to 70,000 algae per millilitre during the spawning period. The distribution of species fed was 50% diatoms and 50% ciliates in terms of numbers. Species fed were *Isochrysis galbana* and *Pavlova lutea* for ciliate algae and *Skeletonema costatum* and *Chaetoceros calcitrans* for diatoms. The algae fed to the oysters were cultured on site and stored in a refrigerator by 8 °C for a maximum period of 4 days. Algal densities were calculated for a feeding density of 20.000 cells per millilitre for 16 hours a day during the hibernation period. Algal cell densities were gradually raised to 50.000 cells per millilitre up to the spawning period. Algae

TABLE 4. OVERVIEW OF MATERIAL COMPOSITIONS TESTED (W=WEIGHT, V=VOLUME).

Mixture	Sand	Portland cement	Tras	Lime	Gypsum	Shell powder
PC (%w/w)	66	33				
BL1 (%w/w)	27	10	63			
BL2 (%w/w)	60	30			10	
BL3 (% v/v)	47	24		24		5

were fed daily to the oyster in the system from a storage tank using positive displacement pumps. The cell density of the algae delivered to the oysters (20.000 – 50.000 cells/ml) was calculated taking into account the flow rate and the density of the algae delivered. Daily nutrient requirements could be calculated automatically using the Blue Linked data system, BlueLinked EcoSystem or BLES.

Larval rearing

Once the adult oysters were able to release mobile larvae, they were transferred from the stand-alone conditioning system to a flow-through system connected to the TO. The outlet of the tank holding the adult oysters was connected to a conical vessel for collection, the nursery tank. These tanks have a capacity of 140L (Figure 5). The nursery tanks are equipped with aeration and a constant flow of water from the TO system to ensure optimal water quality. To retain larvae in the nursery tank, a large 125-micron sieve is used for the outlet. The larvae collected in a nursery tank may remain in these tanks for up to 5 days before being transferred to a rearing tank. These tanks have the same set-up as the nursery tanks, but are disconnected from the tanks with adult oysters, making it possible to rear a specific batch. Algal cell densities were maintained at a minimum of 50.000 cells per millilitre during the metamorphic period. Algae were fed into the culture tanks daily from a storage tank using positive displacement pumps. The cell density of the algae supplied to the larval tank (50.000 – 70.000 cells/ml) was calculated taking into account the flow rate and the density of the algae culture supplied. Daily nutrient requirements are automatically calculated using the Blue Linked data system (BLES).

FIGURE 5. OVERVIEW OF LARVAL REARING SETUP IN THE HATCHERY OF BLUELINKED. THE WHITE CONICAL TANKS ARE THE NURSERY TANKS.

The larvae in the rearing tank were checked daily to follow development. During metamorphosis, different life stages can be distinguished. The classification used is the veliger and umbo stage and the settlement competent pediveliger stage, characterised by an eye spot and an active foot. Once a minimum of 50% of a batch had developed an eyespot and an active foot (Figure 4), the larvae were considered settlement competent and transferred to the settlement tanks.

Experimental setup

In all experiments, a settlement tank was set up with 20 ReefBoosters of each material composition: PC, BL1, BL2 and BL3 (Figure 6 and Figure 7). This resulted in 80 ReefBoosters in total in each tank. Although all setups (S1-3) were quite similar, they differed slightly in filtration and certain abiotic factors. In all three setups, the ReefBoosters were placed in a crate of 90L on top of a TO in BlueLinked facilities. The water from the TO would flow through the crate continuously, and algae was fed directly within the crate. A filter of 150 µm was present between each settlement tank and the outflow, which connected again to the TO.

In all set ups, the larvae were fed a diet of 50.000 algae per ml of the following algae species: *Chaetoceros calcitrans* (Chca), *Isochrysis galbana* (Isga) and *Pavlova lutheri* (Palu) in a ratio of 3:1:1. The environmental conditions were kept as stable as possible with a salinity of 30‰, dissolved oxygen above 95%, and a pH above 7.9. However, for S3 the salinity and temperature were slightly higher, namely 31‰ and 24°C, respectively.

Nutrient content was also measured weekly to ensure safe levels were maintained. Ammonia was kept below 0.05 mg NH_3-N/L, nitrite below 0.1 mg NO_2-N/L, nitrate below 10.0 mg NO_3-N/L and phosphate was maintained below 5.0 mg PO_4-P/L.

Experiment S1

This set up contained an active carbon filter between the water inlet from the TO and the larval tank (Figure 6). Settlement competent larvae were introduced in the tanks at 23-11-2023. A concentration of 0,044 larvae / ml was introduced (~4000 oyster larvae in 90L). Spat was counted on 11-01-2024, 49 days after introduction to the tank.

Experiment S2

This setup, larvae number, and timing were the same as for S1 (Figure 6). However, within this set up there was no filter before the water inlet and the same.

Experiment S3

The filter set up of the water outlet was improved for the third experiment by increasing the total filter area (Figure 7). A concentration of 0,053 settlement competent larvae per ml was introduced (~4800 larvae in 90L) in the tank at 20-08-2024. Spat was counted on 17-09-2024, 28 days after introduction to the tank.

Data analysis

The non-parametric Kruskal-Wallis H Test was performed to check for a significant difference between the 4 groups of materials. For a post-hoc pairwise comparison, a Dunn's Test with a Bonferroni correction was performed to determine which specific groups differed from each other.

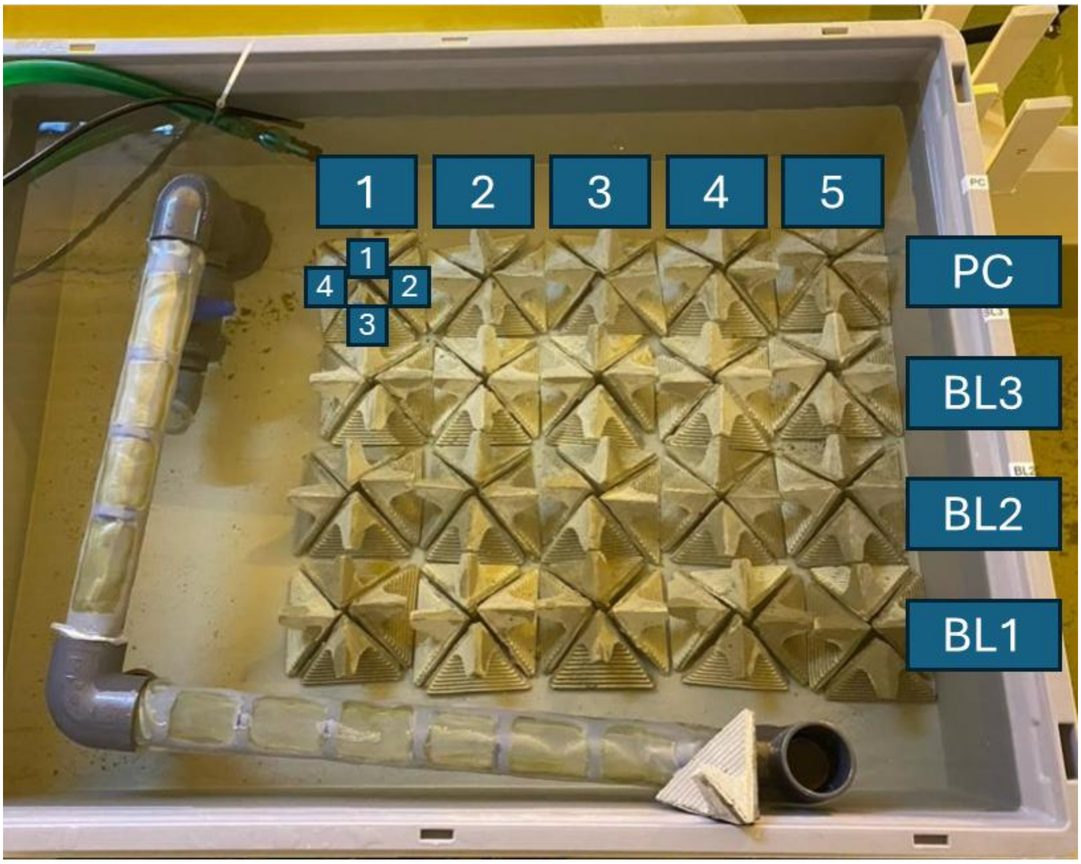


FIGURE 6. SETUP OF EXPERIMENT S1 AND S2 WITH THE REEFBOOSTERS OF DIFFERENT MATERIAL COMPOSITIONS IN THE CRATES ON TOP OF A TO CULTIVATION SYSTEM.

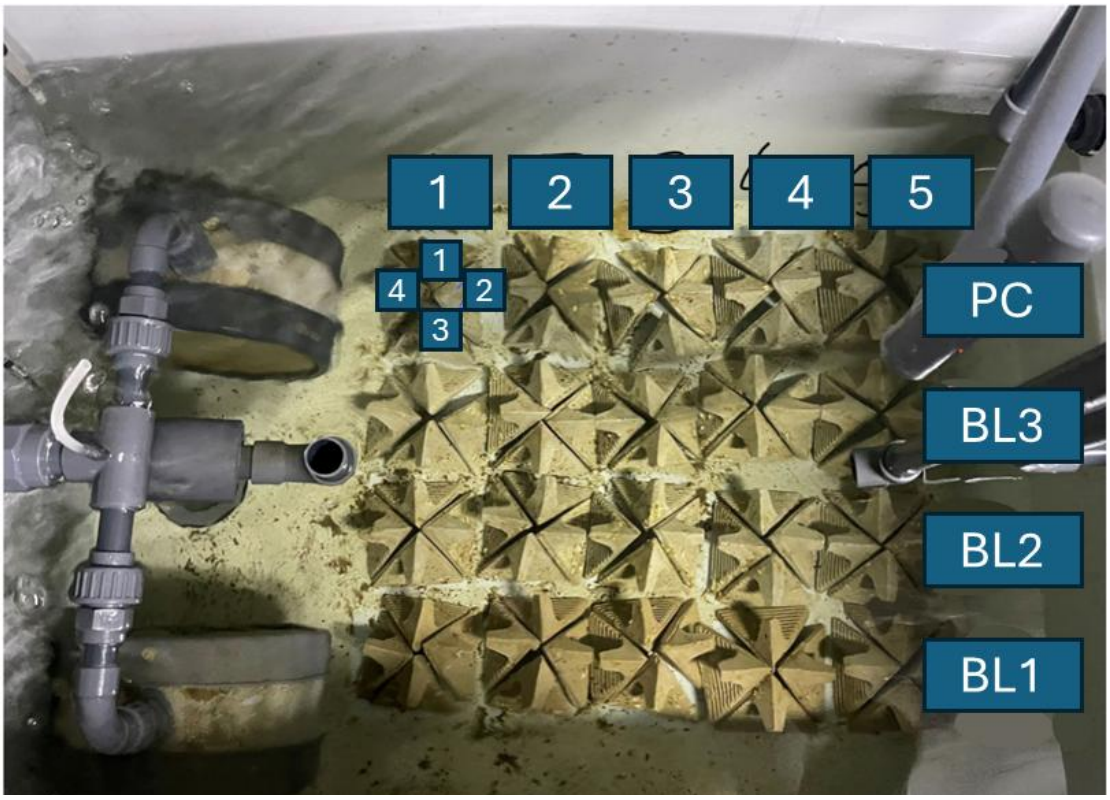


FIGURE 7. SETUP OF EXPERIMENT S3 WITH THE REEFBOOSTERS OF DIFFERENT MATERIAL COMPOSITIONS IN THE CRATES ON TOP OF A TO CULTIVATION SYSTEM.

1.2.3 Results

Experiment S1

There was no overall significant difference between the compositions and number of settled spat (p-value = 0.4975) (Figure 8). However, the Dunns pairwise tests uncovered some significance between specific compositions. PC and BL2 exhibit a significant difference from each other at the alpha level of 0.05 (p-value = 0.00827), with PC experiencing a significantly higher number of settled spat. Similarly, BL2 and BL1 show a significant difference (p-value = 0. 00532), with BL1 showing significantly higher settlement success. Regarding BL3, despite not exhibiting any considerable differences in settlement numbers compared to the other compositions, it does seem to show the highest median (~4 spat per ReefBooster) alongside BL1 (Figure 8). As such, the lack of significance could be due to high variation in the data recorded for this composition.

Experiment S2

Similarly to S1, S2 showed no overall significant difference in the effect of the different compositions on the number of settled spat in each ReefBooster (p-value = 0.9549).

Additionally, again, although there was no overall significance in the number of spat settled per composition, the Dunns test showed some significant pairwise comparisons. However, the results of this experiment showed that BL3 had significantly more settled spat per ReefBooster in comparison to PC (p-value = 0.000068), BL2 (p-value = 0.0031) and BL1 (p-value = 0.0046) (Figure 9).

Experiment S3

The final settlement experiment again confirmed the lack of statistical significance in the number of spat settled per ReefBooster per composition (p-value of 0.08545) (Figure 10). There was also no statistically significant differences between any of the pairs of compositions after adjusting performing the Dunns test. However, the effect size of the material composition was 0.5166447 (ε^2 value), indicating that about 51.7% of the variance in the number of spat per ReefBooster can be explained by the material composition.

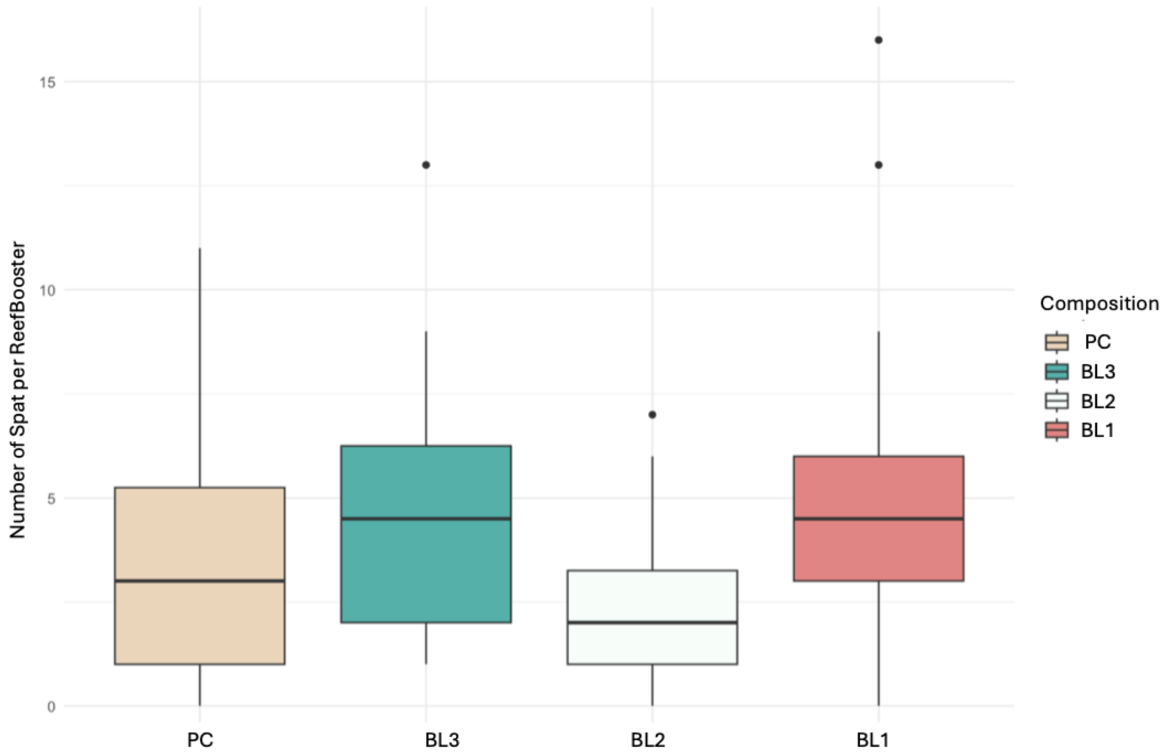


FIGURE 8. BOX PLOT ILLUSTRATING THE RESULTS OBTAINED IN THE S1 EXPERIMENT WITH THE ACTIVE CARBON FILTER SET-UP BETWEEN THE TO AND THE LARVAL TANK. P-VALUE = 0.4975.

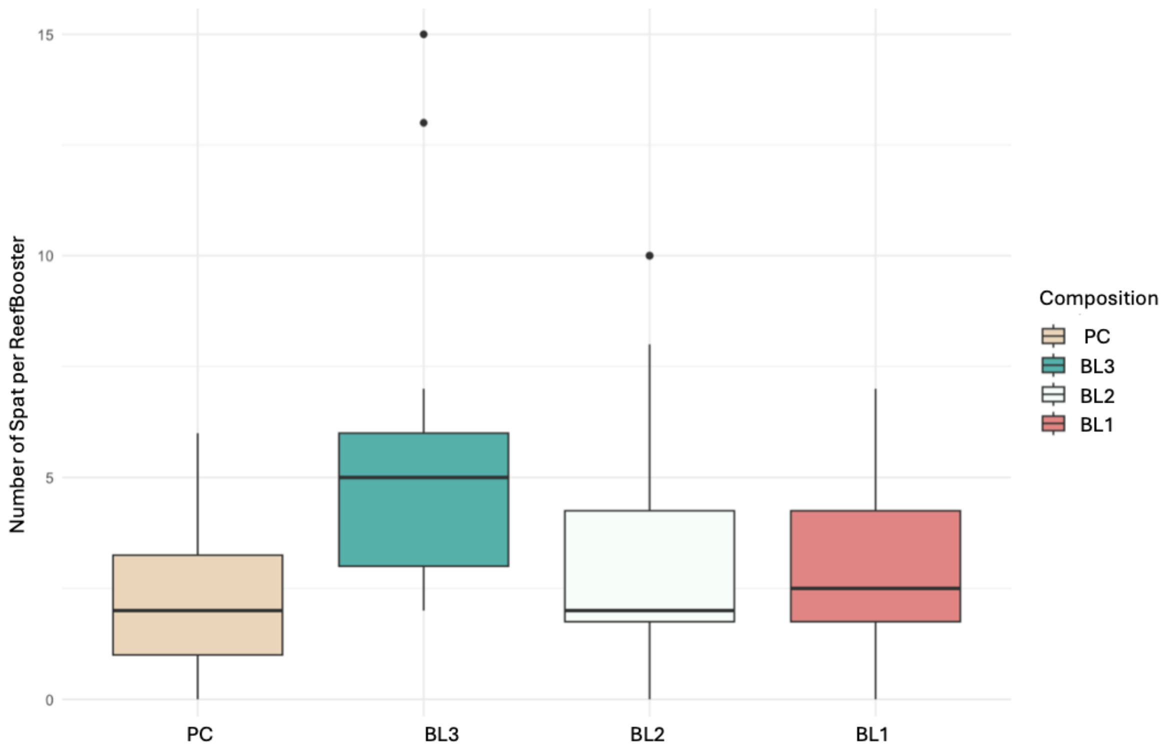


FIGURE 9. BOX PLOT ILLUSTRATING THE RESULTS OBTAINED IN THE S2 EXPERIMENT WITH WATER FLOWING DIRECTLY FROM THE TO, WITHOUT THE ACTIVE CARBON FILTER. P-VALUE = 0.9549).

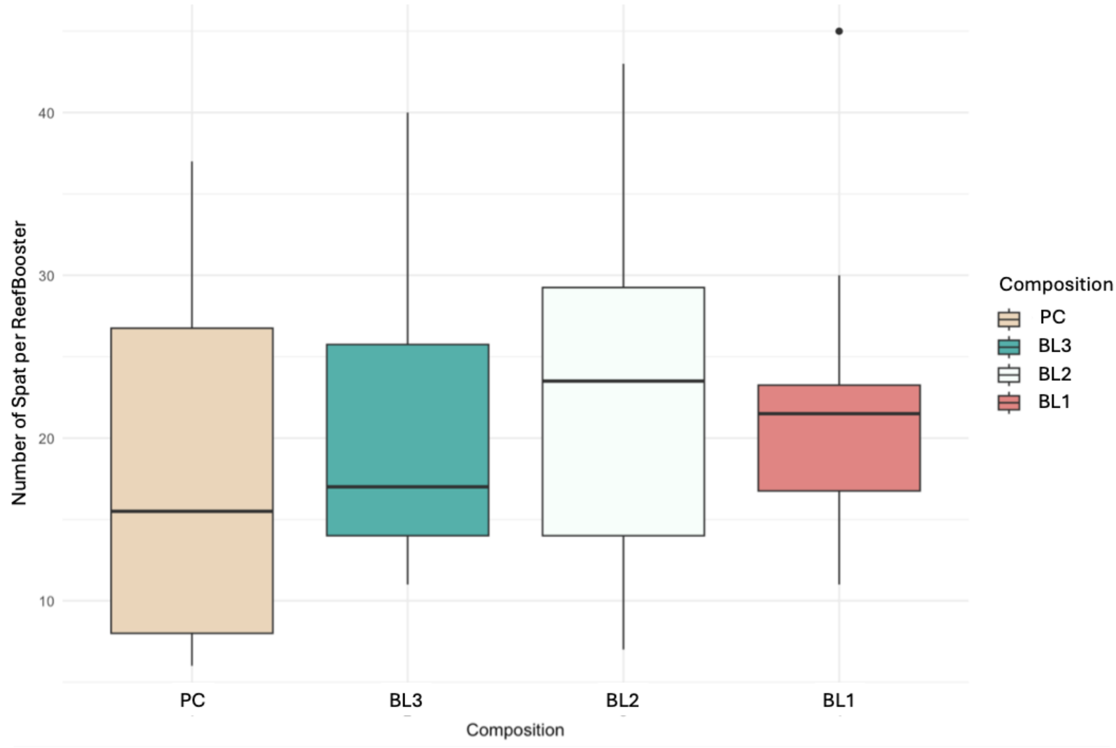


FIGURE 10. BOX PLOT ILLUSTRATING THE RESULTS OBTAINED IN THE S3 EXPERIMENT WITH WATER FLOWING DIRECTLY FROM THE TO INTO THE LARVAL TANK (P-VALUE = 0.08545).

1.2.4 Discussion and Conclusion

Compositions with higher Calcium content and lower Portland cement were expected to attract more larvae due to their reduced surface pH compared to the control of solely ordinary Portland cement compositions. Preliminary results from experiments S1 and S2 suggest that compositions with higher calcium content (BL3) or using Tras cement as a binder (BL1) generally exhibit greater bio-receptivity to oyster larvae compared to those with higher Portland cement content (BL2 and PC).

In S1, while no significant settlement differences were observed, BL3 and BL1 showed the highest median settlement numbers. In S2, pairwise comparisons (Dunn's test) revealed that BL3 was significantly more bio-receptive than other compositions, confirming its potential for enhancing larval settlement. These findings align with existing literature indicating that flat oysters favour calcium carbonate-rich substrates like BL3 over PC, BL1, and BL2 (Fitt et al., 1990; Smyth et al., 2018).

The results from the S3 experiment were somewhat contradictory, with BL2 showing greater settlement success. An explanation for the discrepancy of the performance of BL2 in the different experiments could be the different curing times. The same batch of

ReefBoosters were used for all three experiments, however, seeing as S1 and S2 happened almost 1 year prior to S3, the ReefBoosters produced had less time to cure before they were placed in the water for the S1 and S2 experiments. This reduced curing time could have led to lower chemical stability, thus becoming an unattractive substrate for oyster larvae. Nevertheless, there were no statistically significant differences between compositions in either the overall or pairwise comparisons at the 0.05 significance level. Additionally, the effect size was large (51.7%), indicating a notable impact of material composition. As such, the lack of significance could be attributed to the high variability within the data (particularly within PC and BL2) that likely obscured any clear statistical significance (Figure 10). This variation may have contributed to the lack of clarity in interpreting settlement trends. The high effect size and lack of significance also suggest that while material composition might influence settlement, other factors, may have contributed to the lack of clear results. This is consistent with available literature that has observed that other than chemical properties such as calcium content, physical characteristics like surface texture play an important role on larvae recruitment (Potet et al., 2021).

All in all, the results from experiments S1, S2, and S3 indicate that while compositions with higher calcium content (BL3) generally showed better performance in attracting oyster larvae, the trend was inconsistent. This was expected to some extent given the fact that the material compositions did not differ very significantly and that past experiments have shown that flat oyster larvae are able to settle on different types of hard substrate (Potet et al., 2021; Shelmerdine & Leslie, 2010). Nevertheless, consistency was observed with PC showing the lowest settlement success in all experiments.

1.3 ANALYSIS OF SETTLEMENT RATE IN A HATCHERY USING AI

1.3.1 Introduction

The objective of this research, as presented in this chapter, is to detail the process of developing an AI model for the detection and counting of oyster spats settled on a ReefBooster in a hatchery conditions. This process involved several key steps, from data collection, annotation, preprocessing to model selection, training, and evaluation. Images were captured in a controlled lab environment (BlueLinked) and of good quality and visibility, with a ruler included in the frame for reference (Figure 11 and Figure 12).

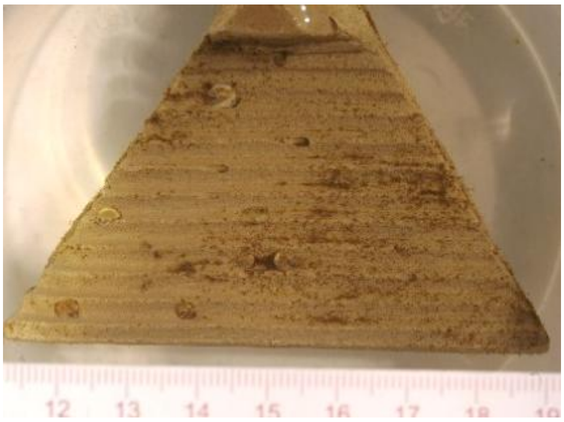


FIGURE 11. REEFBOOSTER WITH OYSTER SPAT IN A HATCHERY.

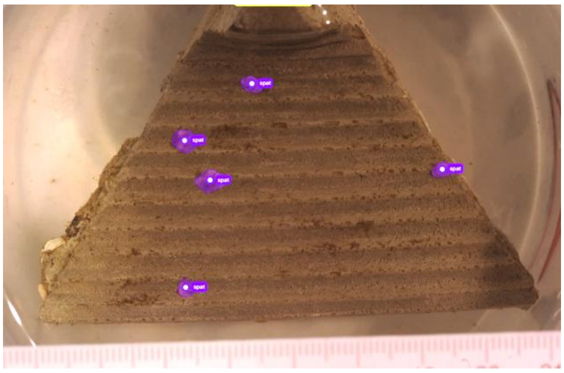


FIGURE 12. EXAMPLE WITH INSTANCE MASKS FROM DARWIN ANNOTATION TOOL.

1.3.2 Methodology

Data Preparation

Each image containing the spat was manually annotated with instance masks (polygons) to accurately outline the objects of interest using the Darwin V7 tool. This type of annotation provides precise boundary information, which is necessary for detecting irregularly shaped objects as spats. The annotation files were then exported in the COCO (Common Objects in Context) format, a widely used format for object detection tasks due to its structured and detailed labelling system. An example of the annotated image is given in Figure 12, where the instance masks of spats are outlined in purple.

Model Selection: Cascade Mask R-CNN

For this task, the Cascade Mask R-CNN model, an advanced deep learning architecture known for its high precision in both object detection and instance segmentation tasks, was chosen. This choice was made by the model's ability to achieve high accuracy through its multi-stage refinement process. As the backbone of the model, a ResNeXt-101 network is selected, which is a powerful architecture pre-trained on large datasets to extract features from images. Cascade Mask R-CNN is particularly effective for tasks requiring precise instance segmentation. It was implemented using the MMDetection toolbox, an open-source object detection toolbox based on PyTorch. MMDetection offers a variety of pre-built models and a highly modular design that facilitates customization. It provides a comprehensive framework for training and testing object detection models. It includes a range of tools for data preparation, model configuration, training, and evaluation.

Training

The model was trained using the MMDetection framework on an NVIDIA RTX 3090 GPU, which enabled efficient processing of the high-resolution images. The learning rate was initialized at 0.0005, with the optimizer set to SGD (Stochastic Gradient Descent) and configured with a momentum of 0.9 and a weight decay of 0.0001 to prevent overfitting.

The original image size is 4256 x 2832 pixels. Given the number of images, 70% is used for training, 15% for testing, and 15% for validation. The distribution is shown in Table 6. Only one class is considered: *oyster spat*. Input images are resized to 2333 x 1800 pixels due to hardware limitations. During training, images are randomly resized and flipped to help the model generalize better.

TABLE 6. DATA DISTRIBUTION.

Subset	Images	Annotations (nº of oyster spat)
train	151	333
test	33	96
validation	33	80

Area estimation

A ruler was included in the images as a reference, allowing pixel measurements to be converted to physical units. Optical Character Recognition (OCR) was used to read the ruler's markings, from which a pixel-to-centimetre conversion factor was calculated and subsequently applied to the object masks. This allowed for area estimation in real-world units.

1.3.3 Results

The R-squared (R^2) metric was calculated to assess the model's accuracy in predicting spat counts, with results of 73% for the test set and 92% for the validation set. These values represent the proportion of variance in the actual object counts that the model successfully explains. The actual versus predicted counts of detected spats are visualized in Figure 13, providing a comparison of the model's performance in accurately estimating object quantities. The results indicate that the model's accuracy decreases as the object count in the image rises, especially when detecting smaller objects. Figure 14 illustrates an example of missed detections (highlighted in red circle), showcasing some of the challenges in accurately identifying smaller items in dense scenes. A sample image with spat detections and their estimated areas is shown in Figure 15.

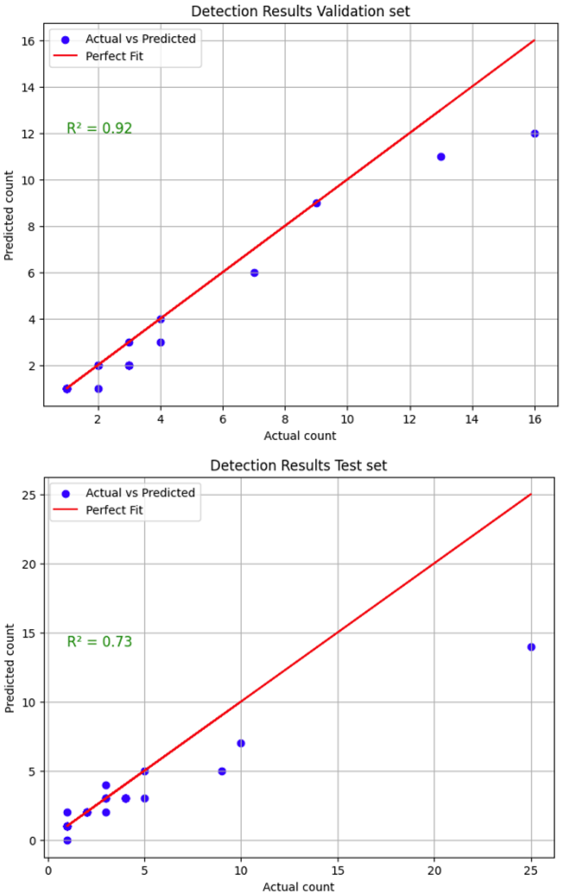


FIGURE 13. THE ACTUAL VERSUS PREDICTED COUNTS OF DETECTED SPATS.

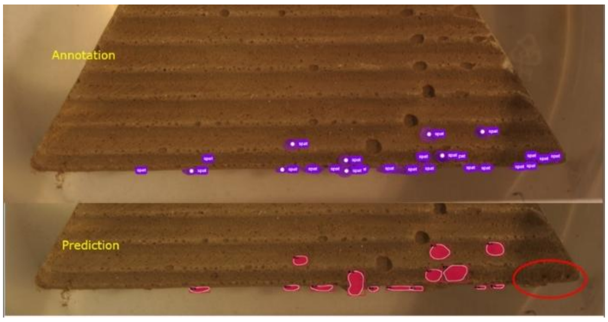


FIGURE 14. AN EXAMPLE OF MISSED DETECTIONS (HIGHLIGHTED IN RED CIRCLE).

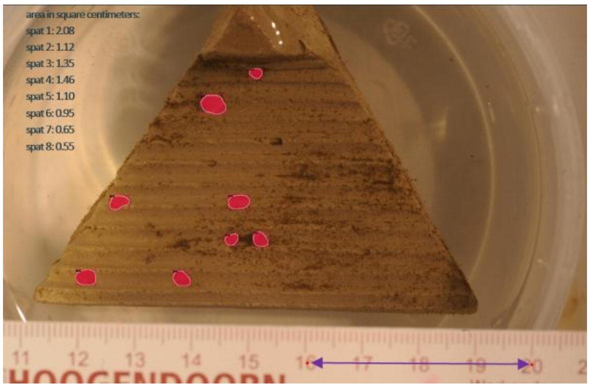


FIGURE 15. EXAMPLE IMAGE OF ESTIMATED AREAS OF DETECTED SPATS USING PIXEL-TO-CENTIMETRE CONVERSION.

1.3.4 Discussion and Conclusion

This task focused on developing an object detection model to identify and quantify spats within a dataset of images from the hatchery. The Cascade Mask R-CNN model was chosen due to its high accuracy and multi-stage refinement approach, which allowed it to capture object details and handle complex boundaries. The model demonstrated strong performance on the validation set, accurately identifying objects with a high degree of precision. However, a slight drop in accuracy was noted on the test set, suggesting that further improvements in generalization may be beneficial.

In addition to detection, area estimation of each detected spat was one of the objectives. Despite its effectiveness, this approach faced several limitations. First, the ruler was not consistently visible in all images, making the conversion method inapplicable for some cases. Furthermore, OCR accuracy was occasionally affected by variations in image quality and lighting, which sometimes resulted in misinterpretations of the ruler markings. Lastly, the distance between the camera and the ruler is unknown, and the ruler and objects are not positioned on the same plane. This introduces errors in calculated areas. These limitations suggest that alternative calibration techniques or supplementary references could improve accuracy and ensure more consistent area measurements.

The trained object detection model has been made accessible through an online interface using Hugging Face and Gradio, available at https://unaibai-boost.hf.space/. This deployment allows users to interact with the model directly from their web browser without needing specialized software or technical knowledge. Through the interface (Figure 16), users can upload images and see the model's detections in real-time, with objects automatically identified and highlighted with their bounding boxes. This setup makes it easy for users of all backgrounds to explore and test the model's capabilities in a simple, user-friendly way. However, as the deployment is hosted on the free version of Hugging Face, occasional stability issues were observed. The limitations of this free-tier deployment highlight the need to consider alternative hosting options if long-term or high-volume usage is expected.

In summary, the results are promising, demonstrating the feasibility of the pipeline for the detection of objects as small as spats and their area estimation. With refinements to calibration, deployment stability, and model configuration, this approach can be enhanced and expanded for further applications.

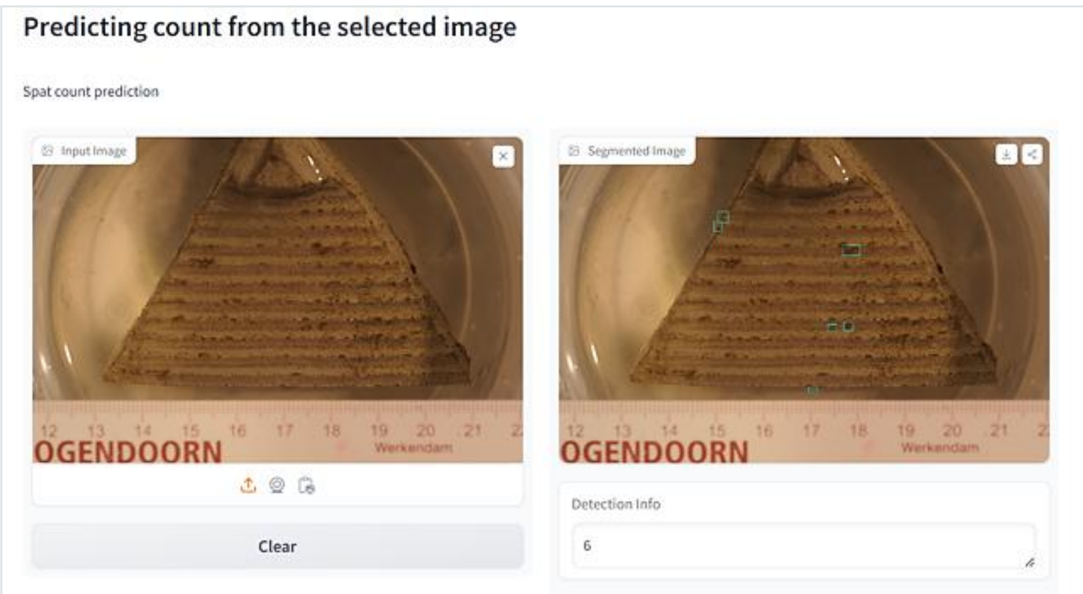


FIGURE 16. THE WEB-BASED INTERFACE THAT ENABLES THE MODEL USAGE.

PART II

Hydro-morphological and Ecological Characteristics of the ReefBooster in a Nearshore Setting

2.1 THE NEARSHORE MARGRIETHAVEN

Part II of this report examines the hydro-morphological and ecological characteristics of the ReefBooster within a nearshore environment. The experiments investigated both the interaction of the ReefBooster with abiotic factors and its bio-receptivity. Additionally, methods for scalable data collection on deployed ReefBoosters with spat were optimized and evaluated based on the Lobster Scout and the development of an artificial intelligence program for data analysis. The initial project plan included long-term stability testing over the winter, which would have offered valuable data on the ReefBooster's performance in harsher conditions. However, delays in production and adjustments to the observing schedule led to stability testing being conducted over a three-month period in the summer of 2024. Because all experiments were conducted using the same 3 plots, in Figure 17, first an overview is given of the location and installation of the different

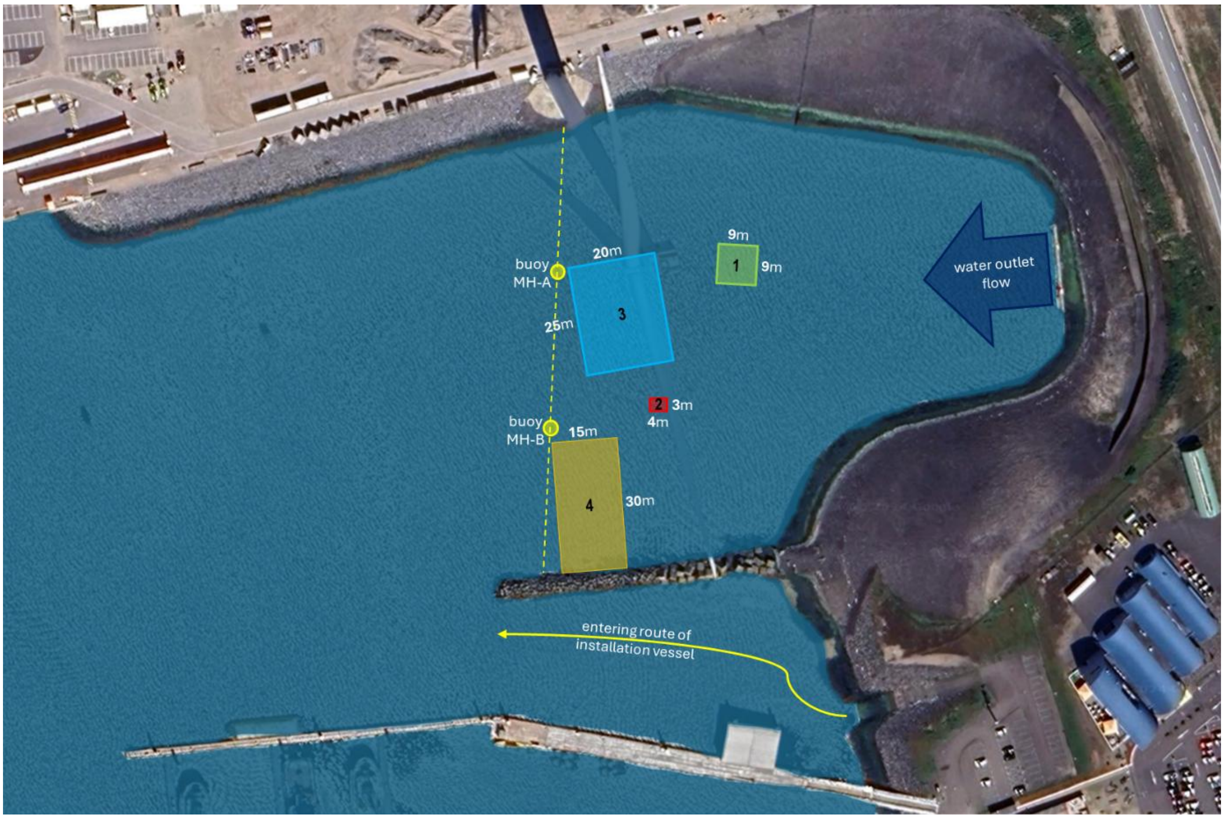


FIGURE 17. OVERVIEW OF EXPERIMENTAL AREA IN THE MARGRIETHAVEN. THE RIGHT SIDE OF THE YELLOW DOTTED LINE IS A NO SHIPPING ZONE. FOR INSTALLATION THE ROUTE INDICATED WITH THE YELLOW ARROW WAS FOLLOWED. THE BLUE ARROW INDICATES THE COOLING WATER OUTLET FLOW OF THE ENERGY PLANT ACROSS THE ROAD. PLOT 1 (GREEN), PLOT 2 (RED) AND PLOT 3 (BLUE) ARE THE LOCATIONS OF THE DEPLOYED REEFBOOSTERS FOR THE DIFFERENT EXPERIMENTS (BASED ON OBSERVING IMAGERY AFTER INSTALLATION). PLOT 4 (ORANGE) CONTAINS THE REEFBOOSTER ATTACHED TO LINES FOR THE BIO-RECEPTIVENESS EXPERIMENT.

plots. In *Chapters 2.2 Data collection – Using an AUV for the observation of ReefBoosters, 2.3 Analysis of ReefBoosters with spat after deployment using AI, 2.4 ReefBooster performance nearshore* and *2.5 Bio-receptiveness nearshore* the experiments are described in more detail.

Location of experiments

Three plots with ReefBoosters were installed in the Prinses Margriethaven in the port of Rotterdam. The Prinses Margriethaven was chosen based on accessibility to the site and safety; it is closed off with a fence, the pier provides easy access for a vessel and there is no boat traffic allowed behind the two buoys MH-A and MH-B, ensuring safe working conditions for the operation of the AUV. The specific plot locations within the Margriethaven were chosen because they did not hamper pontoons/vessels from coming in and out of the port to the pier (Figure 17). On top of that, the locations were as far from an energy plant cooling water outlet as possible, as the outlet creates underwater currents which can influence the underwater visibility and quality of the observing.

These plots were assessed using two different methods, either a Remotely Operated Vehicle (ROV) from The Rich North Sea programme (QYSEA Fifish V6), or the Lobster Scout (AUV) designed by Lobster Robotics.[1] The ReefBoosters were installed in the four selected plots over the course of one year. An overview of the different plots can be found in Table 7. Due to the different dimensions of the plots, a different number of ReefBoosters was added to each plot. The first installation happened in July 2023 of plot 1 and 4, and the last installation of plots 2 and 3 was completed in May 2024.

Table 7. Overview of the 4 plots as shown in Figure 17. With design A referring to the original design, and design B being an adapted design to facilitate production.

Plot	Plot size (m)	ReefBooster quantity	ReefBooster design	Observing technique	Installation date (T0)	T1	T2
1	9x9	500	A	ROV / AUV	26-07-2023	30-10-2023	28-02-2024
2	4x3	85	A	AUV	14-05-2024	13-06-2024	12-08-2024
3	25x20	3300	B	AUV	14-05-2024	13-06-2024	12-08-2024
4	30x15	7 lines with 50 per line	B	Manual	26-07-2023	28-08-2024	n/a

[1] Due to a fire at the facility of Lobster Robotics, their AUV (also referred to as Scout) obtained serious damage. For this reason, plot 1 was assessed using an ROV.

ReefBooster production and material

Different type of ReefBoosters were used for the different experiments. Since plot 4 focused on the bio-receptiveness of materials, different material compositions were tested. Additionally, the design did not play an important role in these experiments, and therefore an alternative design was used which facilitated production, referred to as design B (Figure 18). The experiments are elaborated on in *Chapter 2.5 Bio-receptiveness nearshore*. For plot 1 and 3, composition BL2 was used which included gypsum to ensure degradability (Table 8). ReefBoosters of design A were deployed in plot 1 and 2 and were produced by BlueLinked using a pouring method. However, to facilitate the production of a larger quantity, ReefBoosters for plot 3 were made from design B by Advanced Tower Systems. Compared to design A, design B allows for a production method which is less labour-intensive, not restricted by a set number of available moulds, and requires less curing times due to the lower fluidity of the mixture needed.

TABLE 8. MATERIAL COMPOSITION USED IN THE PRODUCTION PROCESS.

Material	Amount
Sand	50 kg (60%)
Portland cement	25 kg (30%)
Plaster	8 kg (10%)
Water (tap)	8.5 L

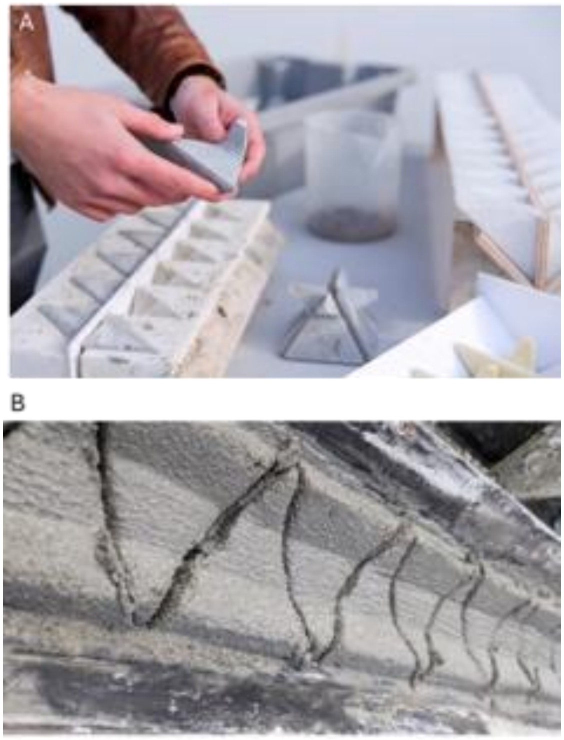


FIGURE 18. PICTURES OF THE PRODUCTION PROCESS OF THE DIFFERENT REEFBOOSTER DESIGNS. A SHOWS DESIGN A, AND B SHOWS DESIGN B.

Installation of experiments

Plot 1 and 4 were installed using a small workboat. This was still possible due to the small quantity of ReefBooster used within the experiments. The installation of plot 2 and 3 happened simultaneously and required a larger vessel. The ReefBoosters were placed in stackable crates as shown in Figure 19. In total, 32 crates with inner dimensions of 56x36,5x10cm were transported by a small sized truck directly from Advanced Tower Systems (production facility) to the Margriethaven in Rotterdam. A crate of this size can transport about 60 ReefBoosters per crate.

A

B

FIGURE 19. REEFBOOSTERS IN CRATES READY FOR TRANSPORT. A SHOWS DESIGN A, AND B SHOWS DESIGN B.

All crates were stacked onboard of the Falcon, a MultiCat vessel from Boskalis, and the vessel sailed to the installation plot (Figure 20). Upon arrival, the MultiCat reduced its speed and slowly but steadily advanced into plot 3. As it advanced, the crates were emptied by two people standing on each side of the vessel holding a crate each and shaking it with the aim to drop between 5 and 10 ReefBoosters per square meter (Figure 20, middle image).

Full crates were handed to them by two other people while one extra person removed the empty ones. The plot area was indicated by buoys (Figure 20, right figure). The installation vessel sailed through the plot once as its width was approximately the same as that of the plot.

2.2 DATA COLLECTION – USING AN AUV FOR THE OBSERVATION OF REEFBOOSTERS

2.2.1 Introduction

Observing of oyster restoration projects focuses on the survival, growth, reproduction, and recruitment of the oysters. Essentially, the primary task of

FIGURE 20. LEFT: CRANE ON MUTLICAT FALCON HANDLING CRATES, MIDDLE: DROPPING CRATES FROM MULTICAT INTO PLOT 3, RIGHT: BUOYS INDICATING INSTALLATION AREA.

observing is to map a section of the seabed where the reef structures are expected to be located and to do so in such detail that the development of the oysters can be tracked through high-quality photography/videography. Cost-efficient and accurate observing of oyster reef restoration in the North Sea remains a challenge due to a lack of automated observing equipment that can deal with the bad weather conditions and poor underwater visibility of the North Sea.

Currently, ROVs are used for these types of observing operations. One of the main drawbacks of these contemporary observing methods is that they rely on manual labour. Skilled labourers are difficult to come by, and observing tends to be a repetitive and dull task, yet often still require significant technical expertise. This is one of the reasons why staff shortages in the survey industry are so severe at the moment. Furthermore, currents in the North Sea can be significant which limits the use of small inexpensive ROVs or divers, as they are not equipped to deal with these currents. Current observing efforts are therefore often limited to a small operational window during a change of tides. When sizable areas have to be observed, large and expensive ROVs are required with an appropriately sized (read large) suitable support vessel, further increasing the observing costs.

A third problem for oyster restoration observing results from the limited underwater visibility in the North Sea. Light is a great signal carrier for observing small details, such as oyster spat. The tiniest details can be observed by using a camera system, rather than sonar. There is one immense challenge however, and even more so in the North Sea: turbidity. This both decreases the quality of the image and the exposure of natural light. Fine particulate matter can be modelled as attenuation of the optical signal, but larger particles, including marine snow, form occlusion and require different approaches. The reflectivity of the pollutant in the water column also plays a large role. Then, there are the physical properties of light spreading, explained in Figure 21.

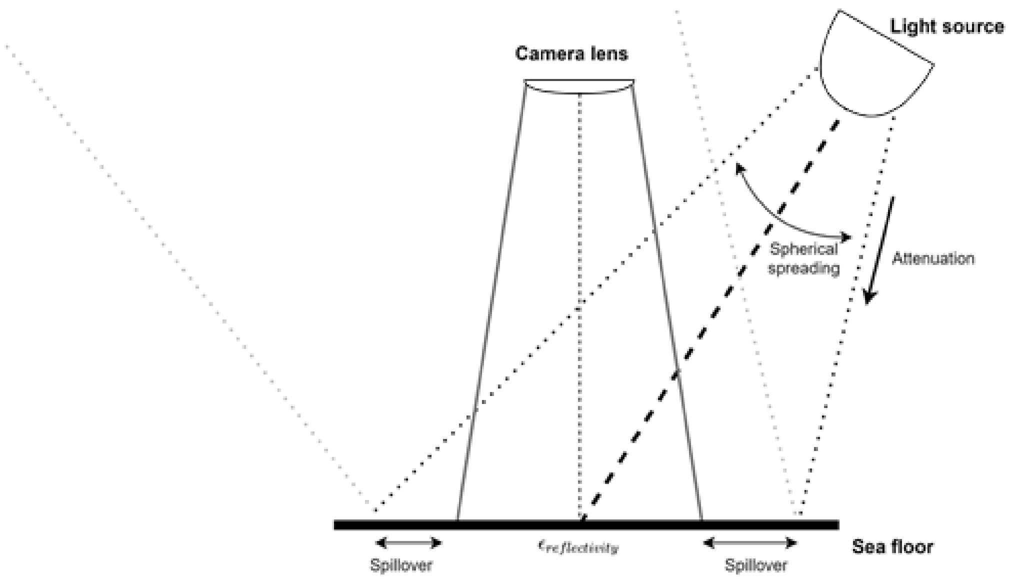


FIGURE 21. EXPLANATION OF THE IMAGING SYSTEM TO COLLECT VISUAL DATA ON THE REEFBOOSTERS.

In the BOOST project, a new automated method has been researched as an industrial research project, and validated to achieve large-scale oyster reef restoration observing in the future. In this method, an AUV is used to take high-resolution georeferenced pictures of the seabed autonomously while following the contours of the seabed in close proximity. These pictures are then automatically post-processed in a single large georeferenced visual map that provides a global overview and local high-resolution features of the observed area.

The following research question was asked to access the new method:

“To what extent is it possible to optically map an oyster reef restoration area in dynamic currents and poor underwater visibility in an automated fashion?”

Dissecting this research objective, a solution needs to be found for an AUV to:

1. Correctly overcome water current from an unknown direction;
2. Take sufficiently high-quality images of the oyster reef restoration effort despite poor visibility conditions;
3. And do so without human intervention.

2.2.2 Methodology

As discussed, the ReefBoosters were deployed in a test site, the Princess Margriethaven located at the Maasvlakte in the port of Rotterdam (Figure 22). It should be noted that due to an energy plant cooling water outlet, there were significant underwater currents at the test site which both decreased underwater visibility and challenged the new observing method. In a period of 1 year, the ReefBoosters were deployed on the seabed of the test site in three plots, as described in Table 7 Plot 1, 2 and 3.

Plot 1 was first observed with a conventional, but not professional, ROV (QYSEA Fifish V6) which established a reference for comparison of the new scalable observing method. Then all plots were periodically observed using the Lobster Scout AUV (Figure 2) over one year with the new observing method which can be used to assess ReefBooster stability, distribution, and spat growth over time. The observation moments took place across different seasons and numerous intervals, providing exposure to a wide range of environmental conditions. This variability offered opportunities to refine and re-test the observation method and experimental equipment, while also enabling a thorough evaluation of the navigational accuracy and the robustness of data quality over time.

Figure 22. The deployment site of the different plots in the Prinses Margriethaven.

Overcoming underwater currents

Underwater currents exert forces on the Scout, causing it to deviate from its intended path. Although the Scout has actuation capabilities in all directions, strong lateral currents exceeding approximately 0.3 m/s can overwhelm its thrusters, rendering it unable to complete its mission. A viable solution is to ensure that currents never act from the side. In the forward or backward direction, the Scout has sufficient thrust to counteract the forces.

To address this, the system estimates the direction of the currents relative to the Scout. The mission plan is then adjusted by rotating it to align with the current, effectively eliminating the lateral velocity component. Once realigned, the mission proceeds as normal. The algorithm used to estimate current direction leverages a function of the Scout's Doppler Velocity Log (DVL) sensor. This sensor measures the Scout's velocity relative to the seafloor and provides a relative current velocity profile beneath the vehicle.

Overcoming poor water visibility

The Scout was already equipped with artificial lights, to provide additional illumination of the sea floor. A number of solutions were researched to overcome the problems of turbidity affecting the image quality. First, the most effective one, was to reduce the distance from the object (seafloor) to the observer (camera). The second solution was to develop a way to have a semi-live feed of the images, such that an operator could check whether the image quality is sufficient. If not, the Scout could be instructed to decrease altitude or postpone the survey to a more suitable moment. The infrastructure for evaluating the image provided a solid foundation for automation of this process at a later stage. Then, the Scout could adaptively change its survey altitude based on the actual visibility.

Automation to enable large scale visual mapping

One of the research objectives of this project was to find a way to automate the visual mapping capability required of oyster reef restoration, to avoid the staffing bottleneck in scaling up restoration efforts (Figure 23). The challenges that were focused on in this project were executing an autonomous lawnmower mission reliably and position repeatably over the three plots.

Additionally, the settings for the exposure control of the camera system need to be tweaked based on the visibility and reflectivity of the seabed. If this is done incorrectly, it could lead to under- or over-expose. Finally, a set of reliability metrics was developed to evaluate the performance of the autonomous system.

FIGURE 23. STAFF ON STANDBY AT THE PLOT SITE, OPERATING THE AUV. THE AUV PERFORMS THE SURVEY AUTONOMOUSLY, AS SUCH, THE STAFF CAN DO OTHER TASKS IN THE MEANTIME IN THE FUTURE.

2.2.3 Results

ROV observing baseline

Figure 24 and Figure 25 show some frames of the ROV footage collected while observing Plot 1. Figure 24 displays a frame on a day with good visibility right after deployment of the ReefBoosters, while the image quality is good, it is difficult to say anything about the distribution of the ReefBoosters. In Figure 25, the visibility was significantly worse and analysis of the data was tedious, taking over 4 hours to check whether footage had ReefBoosters or not. It was only possible to do a couple of transects during each of these observation moments and as such the scale of observation was only 10ths of square meters in total which took a long time to do as controlling the ROV was very challenging. ReefBooster has partially sunken into the seabed. It is difficult to get any more information from this data.

Overcoming underwater currents

There were a number of challenges to overcome in getting the proof of concept working. At first, a different current estimation method was used. The body forces on the Scout were estimated based on the additional thrust that was required to hold position. This was then converted in an estimated current direction and speed. However, it took several minutes for this estimate to converge properly and the estimated current vector could have a large error compared to the true currency vector due to inaccuracies in the dynamic model of the Scout. Another issue was found, that if the current changes after the initial estimate (during the mission), it negatively affects the performance of the system. The new current estimation method overcomes these issues by directly measuring the current vector continuously using the DVL, as such, a dynamic model of the Scout is not required and the estimates are much more accurate. In the future, the mission plan could also be adapted during the execution of the mission to deal with changing current vectors in the future as the current estimation can be done in seconds rather than minutes.

FIGURE 24. ROV OBSERVING OF THE REEFBOOSTERS IN PLOT 1 RIGHT AFTER DEPLOYMENT.

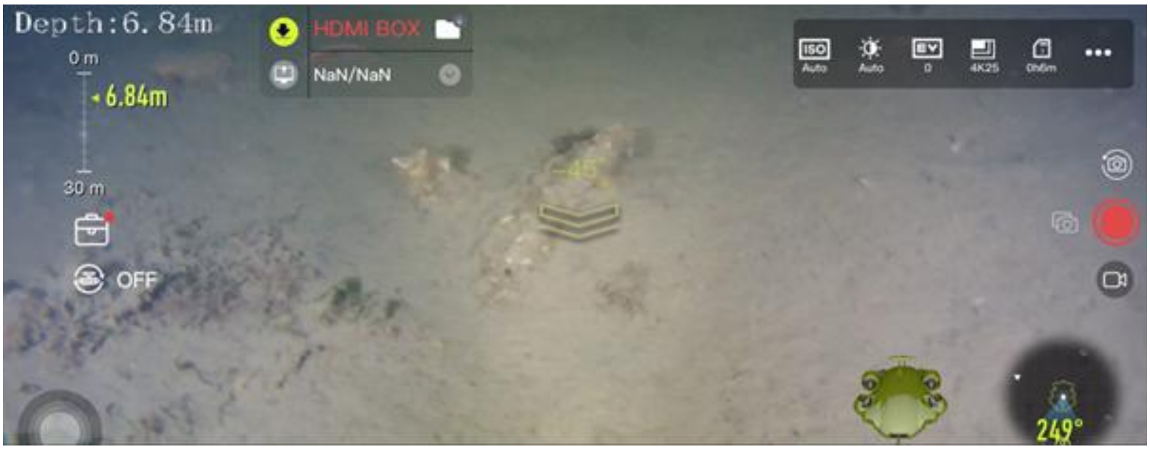


FIGURE 25. ROV OBSERVING OF THE REEFBOOSTERS IN PLOT 1 THREE MONTHS AFTER DEPLOYMENT.

Overcoming poor water visibility

Over the course of several trials, it was observed that the Scout could fly as close as 50cm to the seabed without disturbing the sediment. Below that, agitated sediment would locally increase the turbidity to a point that nothing was visible anymore. To get this close to the seabed, some of the control systems had to be adapted to decrease overshoot. Also, the DVL had to be configured in a different way in order for its internal algorithms to provide usable values.

The biggest challenge of the observation moments was the poor visibility in the Princess Margriethaven. Due to the energy plant, it was almost impossible to plan for good visibility. Even with an advanced camera system and flying at 50 cm, it was sometimes difficult to capture intricate details required for the observing of the ReefBoosters. Visibility was sometimes measured using a Secchi disk, which indicated visibility less than 60 cm at various observing moments. Some examples of good, medium and poor visibility are shown in

Figure 26. However, on a day with good visibility, it was possible to see oyster spat on the ReefBoosters, as shown in Figure 27. This indicates that the image quality is high enough to capture intricate details required for future oyster restoration project observing.

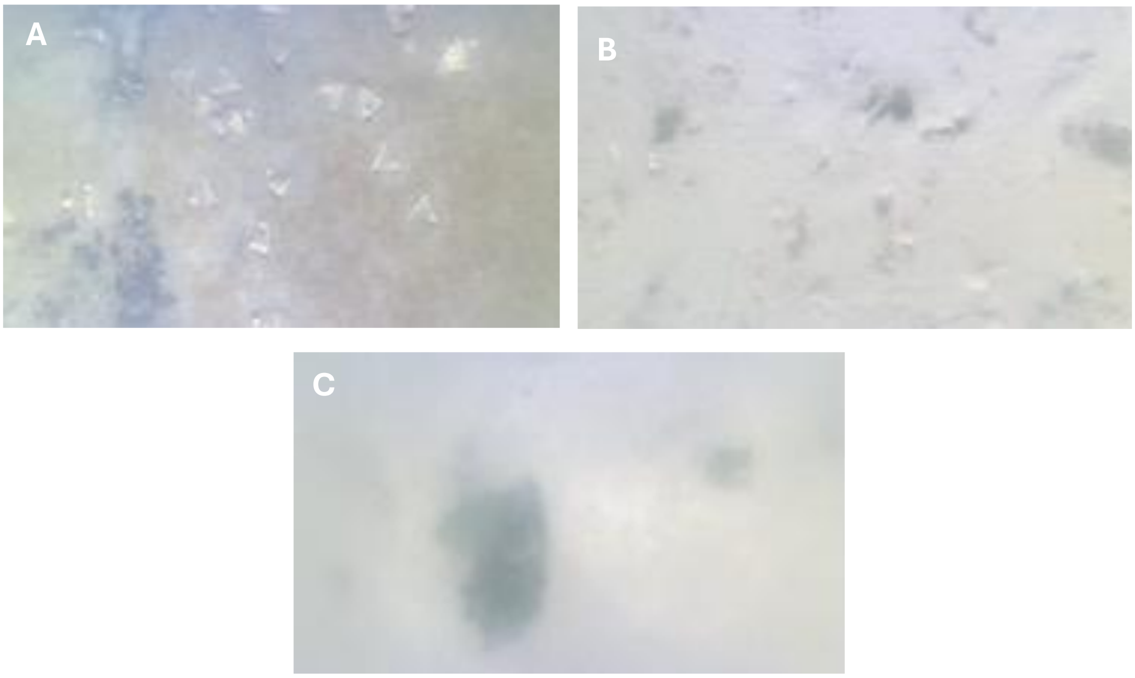


FIGURE 26. THE VARIETY OF UNDERWATER VISIBILITY ACROSS DIFFERENT SURVEYING DAYS AT THE SITE. A. GOOD VISIBILITY (14-05-24) B. MEDIUM VISIBILITY (28-02-2024) C. POOR VISIBILITY (13-06-2024)

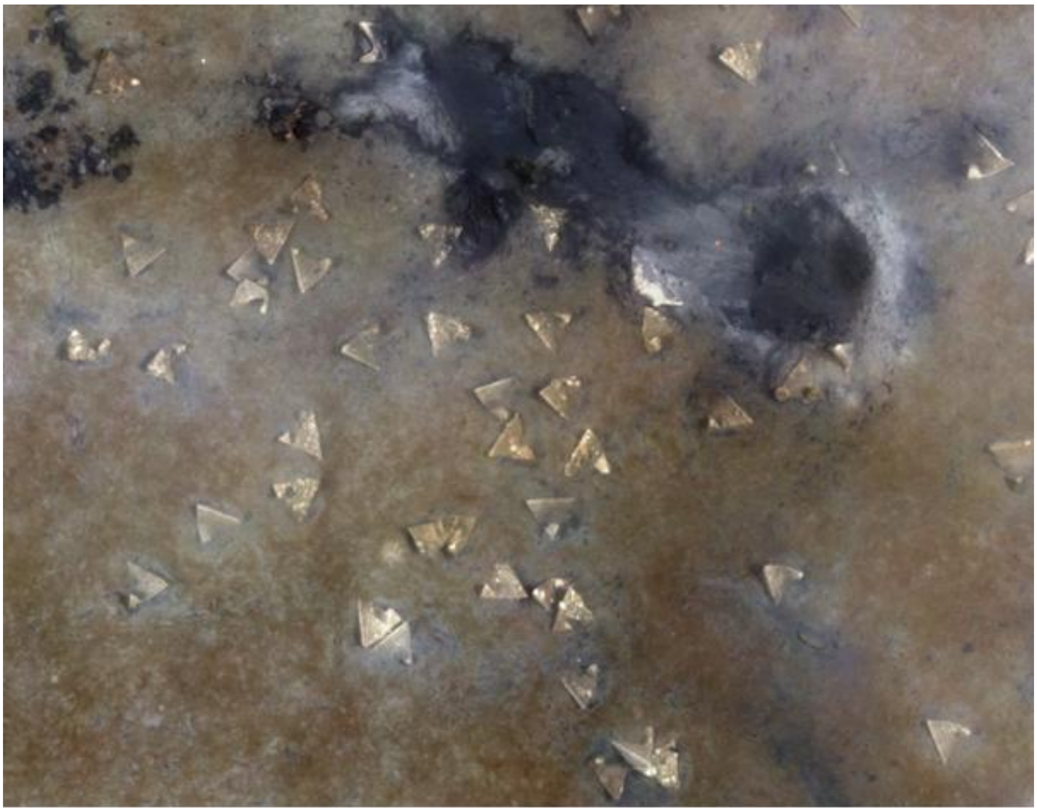


FIGURE 27. OBSERVING ON MAY 14TH 2024. UNDERWATER VISIBILITY WAS 3+ METERS. IMAGE QUALITY IS HIGH ENOUGH TO CONFIRM THAT OYSTER SPAT WAS PRESENT ON THE REEFBOOSTERS.

Automation to enable large scale observing

Initially, the underwater positioning did not work at the test site: the soft sediments dampened the DVL acoustic signals and combined with background noise from nearby dredging and shipping activity, navigation was error prone. This resulted in having to manually reset the navigation software frequently. A different DVL sensor was acquired and integrated, and after some tuning the performance improved greatly. Now visual maps could be created automatically and an example is shown in Figure 28. This figure displays an orthomosaic map created of Plot 3 one day after deployment of the ReefBooster. The map provides a clear overview of the placement/distribution of ReefBoosters.

A second issue was discovered in that the magnetometer was influenced by the thruster magnetic field and resulted in curved maps as shown in Figure 28.

FIGURE 28. GLOBAL DISTRIBUTION OF THE REEFBOOSTERS A DAY AFTER DEPLOYMENT OF PLOT 3. WHITE DOTS ARE REEFBOOSTERS.

This was solved by placing the magnetometer further away from the thrusters. Further reliability increases were obtained throughout the project by fixing a number of bugs in the camera and control software. The set of reliability metrics that was developed consisted of the following:

1. Mission success rate (%)
2. Stitch Quality (1-10)
3. Unscheduled repairs (#)
4. Operational efficiency: On-site time / Mission time (%)
5. Did the data answer the question?

This is a mix of quantitative and qualitative metrics, to be evaluated shortly after the operations. This model helps to understand the effectiveness of the autonomous system, which may otherwise be difficult to measure. This framework can be used for future developments as well.

2.2.4 Discussion and Conclusion

The use of a conventional ROV with video footage proved inadequate for the large-scale quantitative observing of oyster restoration, as they fall short in systematism and data representation. It has become evident that data suitable for orthomosaic processing is vital for effective observing. To address this, a new approach has been developed and validated, leveraging a dynamic underwater drone like the Lobster Scout, that can autonomously survey a site of interest while being able to effectively navigate currents near the seabed and has the adaptability to deal with to poor underwater visibility. This innovation is promising for large-scale oyster restoration observing in difficult environments like the North Sea.

A proof of concept of a current estimator was tested during the observing tests and was found to be promising. It allows the Scout to negate the effects of lateral currents on the mission success, thereby automating an important aspect of the observing process. A future improvement that was identified is to estimate and correct for changing currents during the mission as well, to ensure smooth operations during the change of tides.

The most effective way to overcome turbidity is to reduce the distance between the camera and the seafloor. This brings its own set of challenges, including issues with the control systems and the DVL. These were solved and proven in the observing tests. Also, a method for the operator to review the images was devised, which proved very useful in an operational setting. This could be automated on the Scout in a next project to increase the level of autonomy and result in automatic adaptation of the underwater visibility.

Additionally, the successful deployment of an automatic observation system using underwater drones in silt-heavy seabed areas, such as Margriethaven, requires the use of spread spectrum acoustic navigation for precise location and positioning.

The learnings of this industrial research project suggest that a simple but effective automated observing method can be used to observe oyster reef restoration projects at scale. The challenges of observing underwater using conventional methods are tackled with using autonomous underwater technology. This results in a simple three step method:

1. The area within the reef restoration site to be observed and the observing time interval are chosen.

2. An AUV with the required capabilities is deployed within range of the designated site. The following key capabilities are required:

a. Being able to survey the designated site in a systematic manner automatically makes high quality georeferenced pictures of the seabed.
b. Being able to follow the contours of the seabed at 1 meter altitude or less to deal with bad underwater visibility.
c. The AUV is able to deal with large currents present in the North Sea.

3. The georeferenced underwater photos made by the AUV are downloaded and combined to a single georeferenced visual map of the sea floor using photogrammetry software.

The resulting map provides instant insights, such as a global overview of the reef structures as well as providing intricate details of, for example oyster spat when zoomed in. In this map the size of the structure can precisely be measured.

All in all, the Margriethaven has posed a challenging environment to conduct the ReefBooster observing. It's expected that visibility conditions offshore are significantly better in most places, which will result in data quality consistent with that seen during the observing in May 2024. The newly developed method is promising for observing large scale oyster reef restoration projects or other benthic surveys. The technology required to do so is more complex than contemporary methods and will need more time and resources to sufficiently mature for large scale deployment. This project has shown there are no major roadblocks however, and when a representative environment is chosen, the results can be stunning.

2.3 ANALYSIS OF REEFBOOSTERS WITH SPAT AFTER DEPLOYMENT USING AI

2.3.1 Introduction

This chapter focuses on analysing field images captured by the Scout Autonomous Underwater Vehicle (AUV) to identify the ReefBoosters and the oyster spats in an underwater setting. The primary objective is to detect oysters on ReefBoosters, to aid in observing habitat conditions and assessing the distribution of oysters on ReefBoosters in the surveyed area. Underwater imaging presents unique challenges, such as variations in lighting, limited visibility, and large image scales, require specialised processing techniques. This analysis is performed on images spanning a 10x10 meter area, with specific preprocessing and object detection techniques applied to achieve reliable results. Additional information about the field data collection process, including equipment specifications and sampling methods, can be found in *Chapter 2.1 The nearshore Margriethaven* and *Chapter 2.2 Data collection – Using an AUV for the observation of* ReefBoosters.

2.3.2 Methodology

Image Preprocessing and Tiling

The field image captured by the AUV of plot 2 covers a large area of approximately 10x10 meters, resulting in a high-resolution image with a dimension of 44249 x 42191 pixels across four channels (RGBA) (Figure 29). Only the first three channels (RGB) were utilised for object detection task, as the fourth (alpha) channel was not required. Given the large image size, processing the entire image at once would be computationally intensive and impractical for the object detection model. Therefore, the entire image was divided into smaller, manageable tiles of 1200 x 1200 pixels. This tiling approach not only reduced memory requirements but also allowed the model to process sections at an original resolution, preserving the details necessary for detecting smaller objects. The visualization of the tiled image is shown in Figure 30.

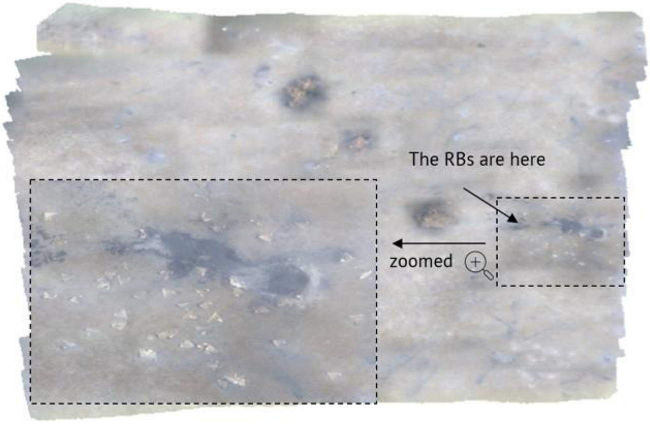


Figure 29. Monitored underwater area image. ReefBoosters are highlighted with a dotted rectangle.

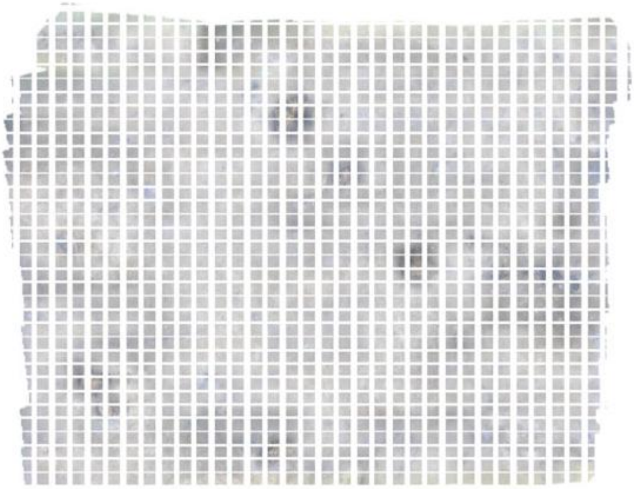


Figure 30. Example of the large image tiled into smaller areas.

Dataset Preparation

Only the images containing ReefBoosters were included in the dataset:

- 33 images containing ReefBoosters, and
- 25 images containing oysters (spats) on ReefBoosters.

The images were manually annotated using Darwin V7 tool, with each target object carefully labelled. A sample annotated image is shown in Figure 31.

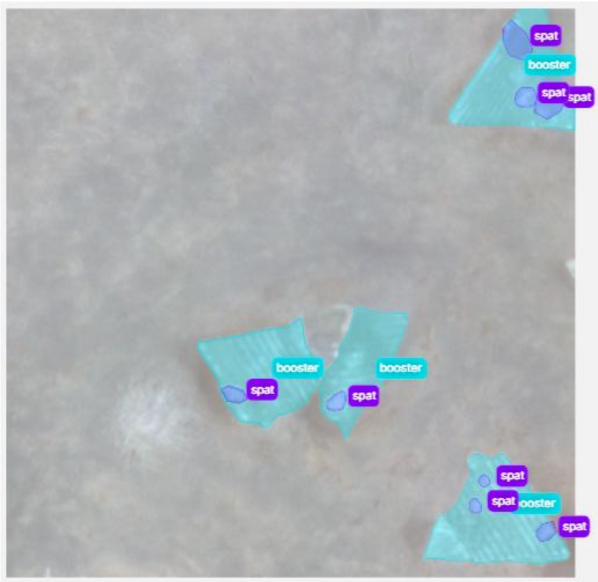


Figure 31. Example with instance masks from Darwin annotation tool.

These annotations included segmentation masks and bounding boxes to accurately define the objects' boundaries, which were then exported in the COCO JSON format for compatibility with the object detection model.

Despite the limited number of images with target objects, the dataset was split to maximize model training effectiveness: 70% of the images were allocated for training, 15% for validation, and 15% for testing. The distribution is shown in Table 9. This division was designed to ensure the model could generalize well while still providing a robust validation and testing framework.

Object Detection Model

The Cascade Mask R-CNN model was selected for object detection due to its high accuracy and effectiveness in capturing object boundaries and shapes. This model uses a multi-stage refinement approach, which is particularly suitable for detecting objects with complex edges. The model was configured to focus on detecting and segmenting objects within each tile.

Training

The model was trained using the MMDetection framework on an NVIDIA RTX 3090 GPU. The learning rate was initialised at 0.0005, with the optimiser set to SGD (Stochastic Gradient Descent) and configured with a momentum of 0.9 and a weight decay of 0.0001 to prevent overfitting. Two classes were considered: *'booster'* and '*spat'*. The size of the input images used was 1200 x 1200 pixels. During training, images are randomly resized and flipped to help the model generalise better.
The model was trained and evaluated using standard object detection metrics, such as mean Average Precision (mAP) and Intersection over Union (IoU), to quantify its performance across the training, validation, and test sets.

TABLE 9. DATA DISTRIBUTION. THE NUMBER OF INSTANCES REFERS TO THE NUMBER OF MASK POLYGONS FOR EACH OBJECT: BOOSTER AND SPAT.

File	Category	Number of Images	Number of Instances
train	ReefBoosters	23	102
	Oyster spat	23	49
test	ReefBoosters	5	23
	Oyster spat	5	14
validation	ReefBoosters	5	19
	Oyster spat	5	9

2.3.3 Results

The trained object detection model achieved a mean Average Precision (mAP) of 0.48 for the "booster" category. The evaluation results are given in Table 10. This performance indicates moderate accuracy in detecting and localizing ReefBoosters within the images. Notably, the Average Precision (AP) peaked at 0.77, suggesting that the model is effective when a less stringent overlap between predicted and actual bounding boxes is allowed.

TABLE 10. EVALUATION RESULTS.

Category	mAP	mAP_50
Booster	0.48	0.772
Spat	0.1	0.286

In contrast, the model's performance was considerably lower for the "oyster spat" category, with a mean Average Precision (mAP) of only 0.1. This lower score reflects the difficulty in accurately detecting and segmenting "oyster spat" category, which are smaller in size and often less visible within the images. The size and visibility differences between the two categories explain the disparity in model performance, as smaller objects like "oyster spat" pose a greater challenge for detection models.

Images are shown side by side in Figure 32, with the left side displaying the ground truth annotations and the right side showing the model's detections. In this example, some instances of the "spat" category are missed, illustrating the model's difficulty in detecting smaller objects. A fragment of the field image containing ReefBoosters, with visualized detections, is shown in Figure 33 (and Figure 34 for more detail without software layers). In this visualization, the green colour represents detected "booster" objects, while the blue colour indicates detected "spat" objects.

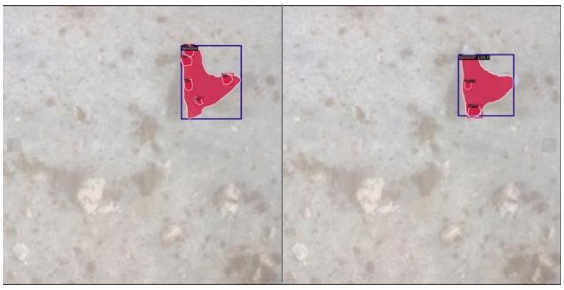


FIGURE 32. ON THE LEFT - ANNOTATION AND ON THE RIGHT- DETECTION RESULTS.

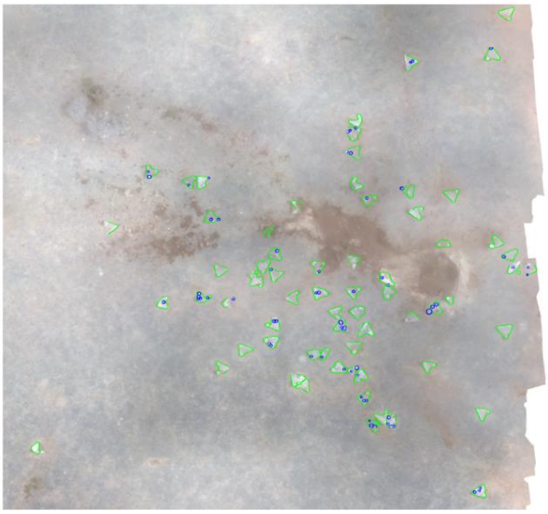


FIGURE 33. FRAGMENT FROM THE OBSERVED AREA WITH THE DETECTED BOOSTERS AND SPAT.

FIGURE 34. GENERAL EXAMPLES OF OBSERVED REEFBOOSTERS WITH SPAT ON THE SEABED.

These results suggest that while the model performs reliably for larger, more visible objects, improvements are needed to enhance detection accuracy for smaller objects.

2.3.4 Discussion and Conclusion

The current results indicate that the model's performance is not yet optimal, particularly for the "Spat" category, which has a mean Average Precision (mAP) of just 0.1. The "Booster" category performs slightly better, with an mAP of 0.48. These results suggest that there is significant room for improvement.

To enhance the accuracy of the model and overall detection process, the following steps are recommended:

1. Increase the number of training images. More training data will help the model learn better representations, particularly for the underperforming "Spat" category.
2. Explore other State-of-the-Art networks like EfficientDet, YOLOv5, or DetectoRS. Experimenting with more advanced neural networks could yield better results, especially for detecting small objects.
3. Adjust the material colour of the booster. The current light-coloured tips on the ReefBoosters often reflect light (Figure 34), appearing as bright spots in the images. This reflection can interfere with object detection algorithms. Changing the material, particularly at the tips of the ReefBooster, to a darker shade could minimize glare, improve image contrast, and enhance the visibility of "Spat" in the images.
4. Increase image resolution. Higher-resolution images are crucial for accurately detecting and analysing small objects like "Spat." If feasible, increase the resolution of the images captured during field observing to ensure that finer details are preserved and accurately detected.

2.4 REEFBOOSTER PERFORMANCE NEARSHORE

2.4.1 Introduction

Two critical factors were considered in assessing the effectiveness of the ReefBooster: landing position and stability of the position over time. The orientation of the ReefBooster after landing, and over time, plays a significant role in the survival of spat. However, external factors, such as currents, may cause the ReefBooster to roll or flip, potentially altering its position and reducing spat survival chances. Additionally, minimizing siltation is crucial in maintaining a favourable environment for spat. Excessive sediment accumulation on the structure can negatively impact the growth and survival of the spat. Silt and clay particles typically have a particle size less than 62.5 micrometres. These are considered fine-grained sediments. Sand has a larger particle size up to 2 mm. (Alden,

2023) The ease of sediment upwelling and blocking visibility and covering hard substrate depends on the sediment's grain size and the water flow.

The ReefBooster has been designed with several factors in mind. With other restoration methods, like spat on shell, a large part of the spat can get buried under the sediment because the spat, for example, settled on the wrong side of the shell, or because sand builds up within the shell. This decreases overall survival chances of the spat. In order to increase survival, the ReefBooster has a 'dedicated spat area' (DSA) as part of its design (Figure 1). The optimal orientation is one in which the DSA faces upwards. This leads to the research questions: "What is the likelihood of a ReefBooster landing with the DSA facing upward after deployment? How stable does the ReefBooster remain in this position? Does siltation on the ReefBooster happen over time?" The tests discussed in this section focus on the orientation and presence of the ReefBoosters in plot 1 and plot 2 and 3 (Figure 17) directly after installation and after 3 months.

2.4.2 Methodology

Orientation of ReefBooster designs

Two different designs of ReefBoosters were tested, namely Design A and B (Figure 35). The relative dimensions and centre of gravity of these ReefBooster designs were different. To analyse the orientation positions, a distinguishment was made between different positions: "Side", "DSA up", "Standing", "Face Down", "Upside Down", and "Unclear" (where Upside Down was defined as the opposite position of standing).

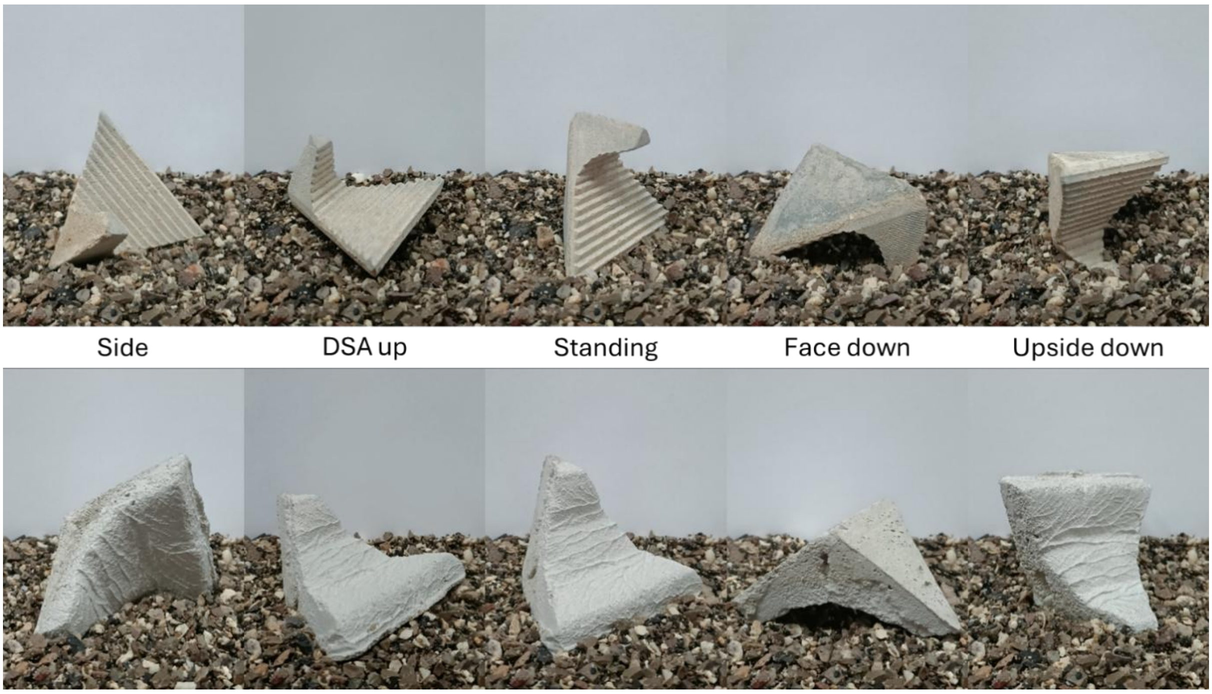


FIGURE 35. LANDING POSITIONS OF REEFBOOSTER DESIGN A (UPPER ROW PICTURES) AND REEFBOOSTER DESIGN B (LOWER ROW PICTURES) ON A COARSE SEDIMENT SURFACE WITH THE DEFINITIONS (SIDE, DSA UP, STANDING, FACE DOWN, UPSIDE DOWN).

Analysis of the different types of footage (ROV/AUV)

Plot 1

Different observing methods were used for the data collection. For plot 1 (Figure 17), the quantity and orientation of the ReefBoosters was assessed per frame of the ROV video footage. Figure 36 shows an example of such a frame. This was done for video footage right after installation on 26-07-2023 (T0) and after 3 months on 30-10-2023 (T1). Unfortunately, due to the limitations of the ROV method and the absence of GPS coordinates, only the orientation could be analysed for plot 1, and stability analysis was not possible.

Figure 36. Screenshot of a video frame from ROV footage of ReefBoosters installed in plot 1.

Plot 2

The AUV method was used for plot 2 and 3, and made top view images which are GPS linked and stitched together to create a map of the seabed. To analyse this stitched image, a grid was put on top of the image of 1x1m in QGIS (Figure 37). The quantity and orientation of the ReefBooster were catalogued for T0, but unfortunately, no data was available for T1 or T2 (Figure 38).

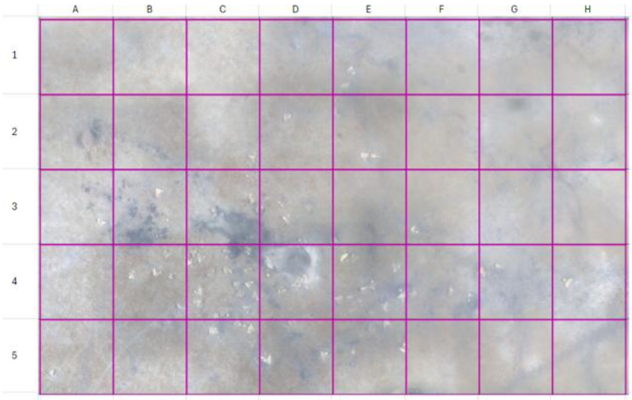


Figure 37. Part of the stitched images from AUV observing of plot 2, with a grid of 1x1m in QGIS.

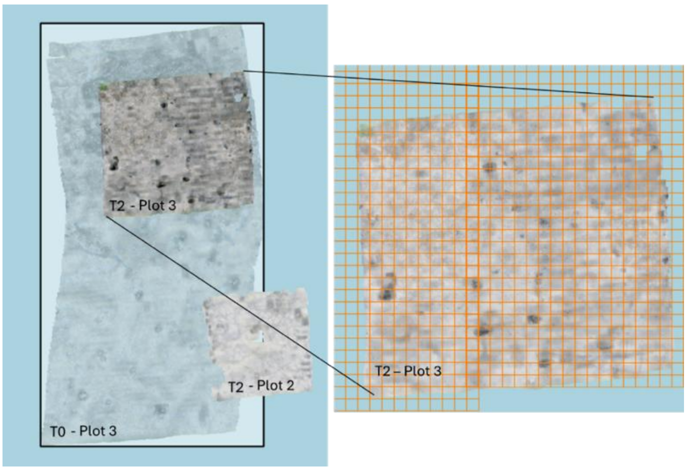


Figure 38. *Left*: Screenshot of data in QGIS, with correctly GPS alignment of T2 of plot 3 (13x15) and plot 2 (9x9) overlapping on transparent T0 40x20 (covering Plot 3 and Plot 2). *Right*: Zoomed in version of the partial observed area of Plot 3 of 13x15

Plot 3

For plot 3 a grid was made as well of 1x1m in QGIS. The orientation of the ReefBoosters was analysed using a selective counting method designed to streamline data collection while ensuring representativeness, assuming the ReefBoosters were equally divided on the chosen seabed surface. Within the "A" series, only odd-numbered samples were included (e.g., A1, A3, A5), while even-numbered samples such as

A2, A4, and A6 were skipped. For column B this was then reversed, including B2, B4 etc. and skipping B1, B3 etc. This method provided an efficient way to gather data while focusing on 50% of the samples across the entire plot.

The ReefBoosters in plot 3 were installed on 14-05-2024 (T0) and observed again on 13-06-2024 (T1) and 12-08-2024 (T2). Unfortunately, the conditions during T1 and T2 were not good enough to create a high quality map of the whole area. The weather conditions were unfavourable, and a technical issue with the AUV midway through the observing led to the mission being cut short. For this reason, only a part of plot 3 (of 13x15m) was mapped out during T2. Due to a margin of error on the GPS accuracy, a point of reference was used to accurately align T0 and T2 (Figure 38). On the basis of these reference points, the coordinates were matched and the grid was correctly aligned (Figure 39). This way a part of the grid of T0 could be compared to T2.

Figure 39. Top: Reference point T0 (proper vision). Bottom: Reference point T2 (poor vision). The grid of T0 and T2 overlaps at the exact GPS coordinates, using multiple non-moveable reference points like rocks visible in these screenshots.

2.4.3 Results

Orientation and stability of the ReefBoosters

Plot 1

As can be seen in Table 11, of the 500 ReefBooster (of design A) installed, 238 were counted right after installation (T0) and only 20 remained visible after 3 months (T1). Overall, the predominant orientation right after installation (T0) was with the DSA up (51%), followed by a side-ways orientation (34%). Over time, no ReefBoosters were observed in a "Face down" or "Upside down" position (with 1 exception).

Plot 2

Of all ReefBoosters analysed in Plot 2, the majority landed DSA up (59%), followed by a side-ways orientation (38%) (Table 12). No ReefBoosters were found to have landed "Standing" or "Face down". Unfortunately, there is no representative data from T1 and T2.

TABLE 11. OVERVIEW OF THE LANDING POSITIONS THE REEFBOOSTERS OF DESIGN A IN PLOT 1 BASED ON ROV FOOTAGE. (PERCENTAGES ARE ROUNDED)

	Side	DSA up	Standing	Face Down	Upside Down	Unclear	Total
T0 (absolute count)	81	122	4	1	-	30	**238**
T0 (percentage)	34%	51%	2%	0%	-	13%	100%
T1 (absolute count)	9	5	5	-	-	1	**20**
T1 (percentage)	45%	25%	25%	-	-	5%	100%

TABLE 12. OVERVIEW OF LANDING POSITIONS FROM REEFBOOSTERS OF DESIGN A WITH SPAT IN PLOT 2. (PERCENTAGES ARE ROUNDED)

	Side	DSA up	Standing	Face Down	Upside Down	Unclear	Total
T0 (absolute count)	32	50	-	-	3	-	**85**
T0 (percentage)	38%	59%	-	-	4%	-	100%

TABLE 13. OVERVIEW OF LANDING POSITIONS FROM REEFBOOSTERS OF DESIGN B BASED ON 50% OF A PARTIAL PLOT 3. (PERCENTAGES ARE ROUNDED)

	Side	DSA up	Standing	Face Down	Upside Down	Unclear	Total
T0 (absolute count)	145	280	193	119	244	49	**1030**
T0 (percentage)	14%	27%	19%	12%	24%	5%	100%
T2 (absolute count)	13	17	33	8	-	201	**272**
T2 (percentage)	5%	6%	12%	3%	-	74%	100%

Plot 3

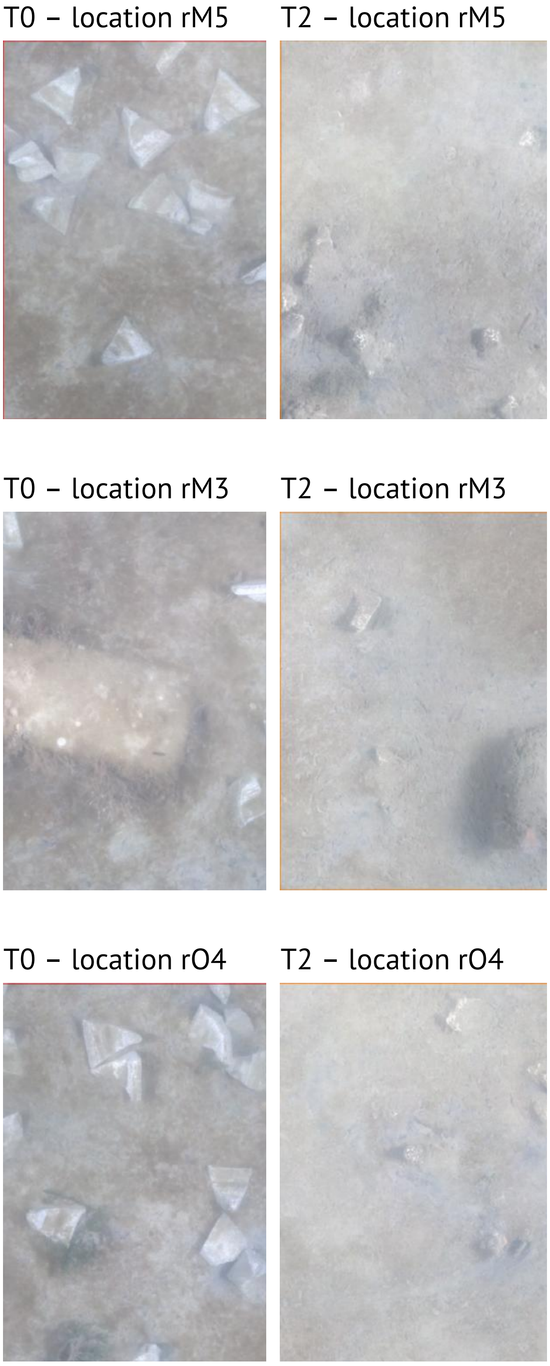
The majority of the ReefBoosters analysed in Plot 3 landed with DSA up (27%) (Table 13). A large percentage was observed to be "Face down" and "Upside down", namely 36%. Unfortunately, there is no representative data from T1. The majority of the ReefBoosters after 3 months (T2) was in an unclear position. For plot 3, the total amount of ReefBoosters at T0 and T2 was observed for 50% of the entire and partial plots (Table 14). When comparing T0 and T2 for the partial plot the amount of ReefBoosters decreased from 1030 to 272.

TABLE 14. REEFBOOSTERS OF DESIGN B IN PLOT 3 OF EITHER THE TOTAL PLOT OR THE PARTIAL PLOT. BECAUSE OF LIMITATIONS OF THE T2 IMAGES, ONLY THE PART WITH HIGH QUALITY WAS USED TO COMPARE T2 WITH T0.

Plot 3	Total
Total plot, T0	**1439**
Partial plot, T0	**1030**
Partial plot, T2	**272**

Siltation of the ReefBoosters

The high silt and clay environment of the nearshore test location makes it difficult to assess the ReefBoosters' stability as the fine particles block the top-view on the seabed with ReefBoosters. To illustrate this and give some examples, square frames of the GPS aligned grid (with a margin of accuracy) of Plot 3 in QGIS are compared between T0 and T2 (Figure 40).



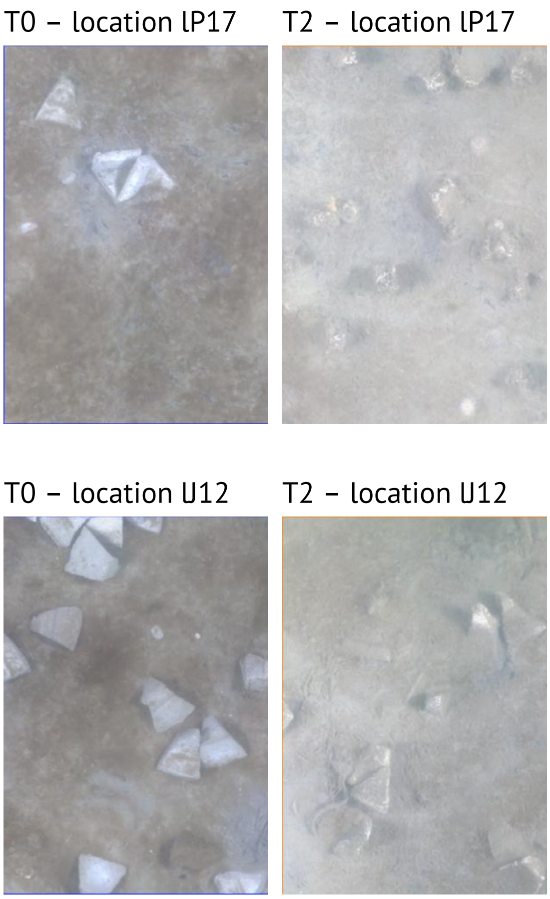


FIGURE 40. COMPARISONS BETWEEN T0 AND T2 ON THE SAME GRID IN PLOT 3 IN QGIS. THE FINE SEDIMENT PARTICLES BLOCKING THE VIEW ON THE REEFBOOSTERS MAKE THE IMAGES HARD TO ANALYSE.

2.4.4 Discussion and Conclusion

Design A demonstrated a higher proportion of Dedicated Spat Area (DSA) in the upright orientation after deployment compared to Design B. This was confirmed by consistent results from both Plot 1 and Plot 2 in comparison with Plot 3, with 51% and 59% respectively compared to 27% (Table 11, Table 12 and Table 13). Furthermore, Design A showed significantly fewer ReefBoosters landing in unfavourable orientations, such as "Face down" or "Upside down," with less than 5% affected, in contrast to 36% for Design B. Additionally, the "Side" orientation observed in Design B is more ‘closed’, potentially hindering the DSA's effectiveness, and thus spat survival in cases of siltation (for orientations see Figure 35). Overall, these findings suggest that Design B may be less favourable for spat survival due to its post-deployment orientation. This difference between Design A and B is most likely influenced by a shift in the weight's centre point. A balance must be struck between optimizing the ReefBooster's production method and ensuring its functionality after deployment. It is important to note that this study did not account for orientation outcomes when deployed on alternative substrates, such as gravel beds or scour protection. Additionally, this research unfortunately could not assess a change in orientation over time as a results of for example flipping of rolling, due to the limited data obtained in T1 and T2 observing.

Plot 1 was observed using an ROV which made it harder to quantify the ReefBoosters before and after since no GPS coordinates were linked to the video footage. Of the 238 ReefBooster observed in the T0 observing, only 20 were observed after 3 months in 2023. Plot 3 was observed using an AUV which followed a course based on GPS coordinates. This made it possible to compare ReefBoosters in exactly the same grid at T0 and after 3 months (T2). The stitched images of T2, however, were not of high quality, making it hard to distinguish ReefBoosters from other objects. Within this partial plot, only 272 of the original 1030 could be observed (Table 13). Because plot 3 was only partially observed during T2, it is unclear whether this is the result of ReefBoosters having moved outside of the plot or if the low observation is due to siltation.

Figure 40 shows several examples of ReefBoosters that were (partly) covered in sediment. The cause is likely to be location specific, and 3 main factors were observed that could have played a role, namely 1) the high content of silt and clay, 2) dredging and maintenance activities within the port, and 3) a cooling water outlet. Extensive dredging in the Rotterdam harbour is necessary to maintain navigability, as sediment from freshwater and tidal processes accumulates. This results in silt and clay dominating over sand in the Margriethaven. Unlike sand, which settles quickly due to its size and weight, silt and clay remain suspended longer. Between May and August 2024, several factors contributed to the suspension of fine sediments. Strong winds (e.g., 10 m/s on June 10, 15, and August 9) likely stirred sediments via wave action. Ship activity also created turbulence, with a surveyor ship and a container ship near the ReefBooster area potentially disturbing the seabed. Ongoing Yangtzekanaal construction since September 2023 may have further influenced sediment dynamics near the testing site. And lastly, the Uniper Benelux power plant's cooling water outlet most likely generated strong currents, resuspending sediment which could have contributed to high sediment accumulation that likely covered significant portions of the ReefBoosters.

These findings highlight the importance of site-specific conditions, such as sediment dynamics and human activities, in influencing visibility and stability, rather than inherent weaknesses in the ReefBooster design. Future experiments should be conducted in locations more representative of offshore environments, with reduced human impact (e.g., dredging and maintenance) and coarser sediment types. It is also crucial to consider that the ReefBooster concept targets large-scale application, focusing on acre-level restoration rather than precise micro-scale predictability. While it is anticipated that a significant portion of ReefBoosters may become silted or displaced, the success of even a small percentage (e.g., 10%) remaining stable can provide a foundation for initiating reef development.

2.5 BIO-RECEPTIVENESS NEARSHORE

2.5.1 Introduction

In addition to evaluating the settlement success of flat oysters on various ReefBooster material compositions, it is equally important to assess how these materials interact with local marine biodiversity. Understanding this interaction is crucial not only for validating the results obtained under controlled hatchery conditions but also for gaining insight into the ReefBooster's potential bio-receptiveness to species beyond flat oysters.

To explore this aspect, an additional experiment was conducted in the port of Rotterdam to observe the biological activity around the ReefBooster in a dynamic, nearshore marine environment. The results of this experiment will not only help assess short-term interactions between the materials and local species but also allow for long-term observing of the materials' durability and ecological performance in the marine environment. Therefore, the research question of focus for the nearshore bio-receptivity experiment is: Does increasing the calcium content/ reducing Portland cement content in the composition enhance the biodiversity of and frequency of organisms present in the ReefBooster? The hypothesis is that there will be extensive biological growth in all the ReefBoosters but that the BL1 and BL3 compositions will house a greater level of biodiversity.

2.5.2 Methodology

ReefBooster production

Advanced Tower Systems B.V. (ATS) was responsible for the production of the ReefBoosters used in this experiment. 650 ReefBoosters in total were produced of the 4 different material compositions (Table 4). The ReefBoosters were produced with a hole in the middle, so they could be attached to a nylon line Figure 41.

FIGURE 41. DESIGN B OF THE REEFBOOSTER, PRODUCED BY ATS, WITH A HOLE FOR THE NYLON LINE TO PASS THROUGH.

Experimental set-up

The ReefBooster were attached to a nylon line (77kg), with 25 cm between each ReefBooster. Of each material, 50 ReefBoosters of were attached to a line. Each material was tested in duplicate, and thus with two lines and a total of 100 ReefBoosters. The ReefBoosters made of the conventional Portland cement mix were considered to be the control in this experiment. In total, 7 lines were installed in the Prinses Margriethaven next to a stone pier on the 26 of July 2023 (Figure 42). The ReefBoosters of different compositions were transported in separate coolers to mitigate the challenges of quantifying mobile species like starfish and sea.

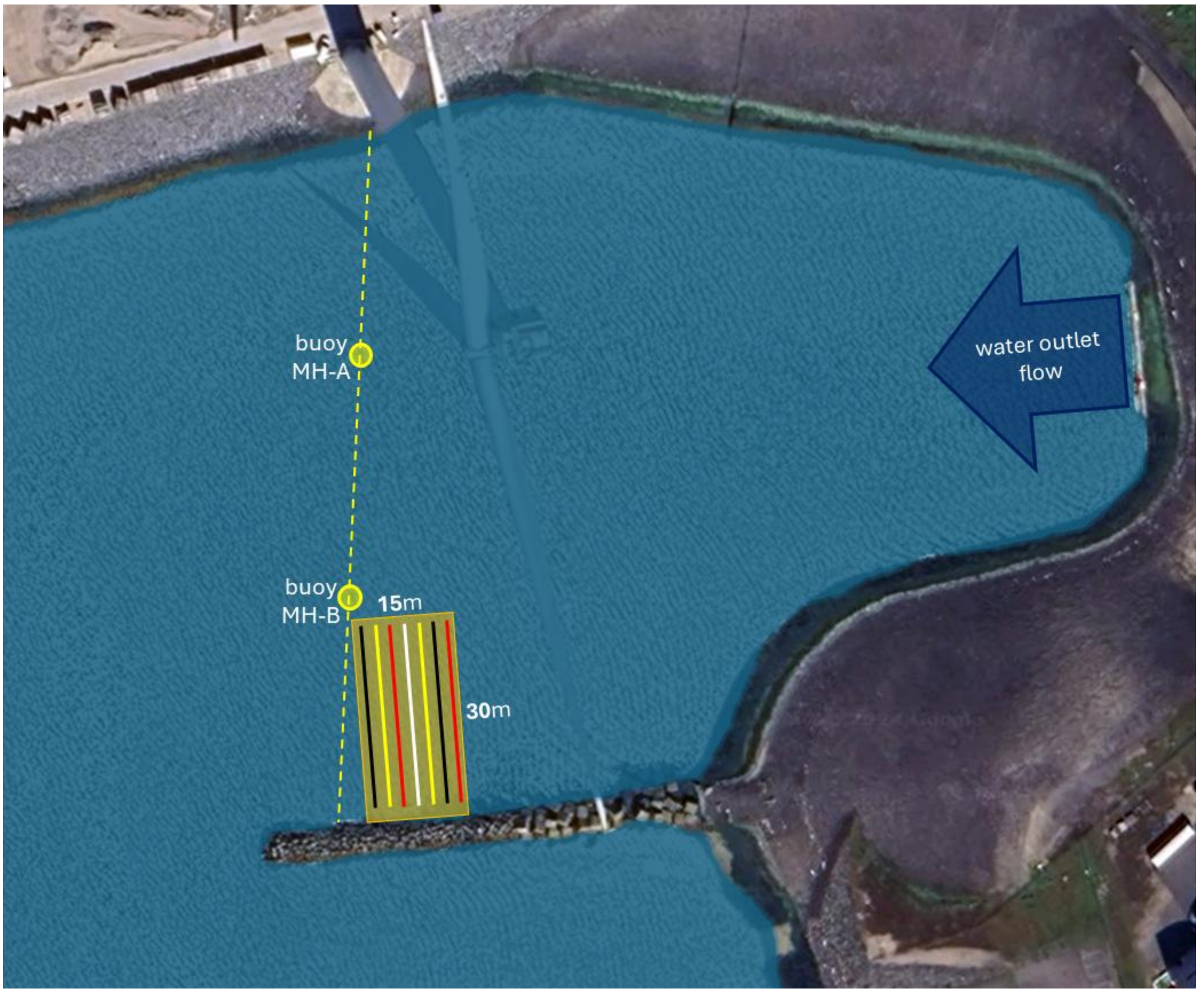


FIGURE 42. OVERVIEW OF THE LINES WITH REEFBOOSTERS INSTALLED IN THE PRINSES MARGRIETHAVEN OF THE DIFFERENT COMPOSITIONS. BLACK = BL1, RED = BL2, YELLOW = BL3, AND WHITE = PC.

Data collection

For data collection, each ReefBooster was carefully inspected on a phylum level (Table 15). Because it was too difficult to accurately quantify each individual, the different organisms encountered on each ReefBooster were marked as present or absent. The relative abundance of the different phyla per material composition was assessed based on the total presence/absence data of the phyla per material composition PC, BL1, BL2, or BL3. In this context, 'relative abundance' refers to how frequently a particular species is found among the ReefBoosters. Specifically, it means how many ReefBoosters have organisms of that phylum in question present, rather than the total number of individual organisms.

Table 15. Overview of different phyla analysed.

Phylum	Examples on class level
Mollusca	Gastropoda (Sea Snails) and Bivalvia (Mussels and oysters)
Arthropoda	Maxillopoda (Barnacles)
Bryozoa	Ectoprocta (Bryozoans)
Porifera	Demospongiae, Calcarea, or Hexactinellida (commonly known as sponges)
Annelida	Polychaeta, Oligochaeta, or Hirudinea (commonly known as annelids)
Chordata	Ascidiacea (Ascidia, or commonly known as Sea Squirts)
Cnidaria	Anthozoa (anemones)
Macro Algae	-----
Echinodermata	Asterioidea (starfish)

2.5.3 Results

The phyla diversity and relative abundance was relatively similar across the different compositions with the exception of BL2, for which Chordata, Porifera, or Cnidaria were absent. In general, the phyla Annelida, and Arthropoda seemed to dominate across all 4 compositions, whereas phyla such as Mollusca, Bryozoans, and Porifera showed extremely low relative abundances.

Despite a similar phyla diversity across all ReefBooster compositions, a difference could be seen in the total amount of presence of phyla observed across the different compositions. When considering all phyla, in total, BL3 had more ReefBoosters with organisms attached compared to BL1, BL2 and PC (Figure 43). BL3 had a total of 171 ReefBoosters colonised by various organisms, whilst BL1 and PC had about 150 and BL2 only 42. Although there is only a slight distinction between BL3, BL1 and PC in total, BL3 more consistently had the most colonised ReefBoosters per phylum, then BL1 followed by PC. However, BL2 consistently ranked lowest in terms of the frequency of presence of different phyla. Finally, PC was remarkably successful with species like Sponges (40% Porifera) but contributes less to all other phyla (20-30%), and did not house a single mussel or oyster. Although only 40 ReefBoosters in total contained organisms from the Mollusca phylum, just 7 of these included Pacific oyster. due to their large size (Figure 44).

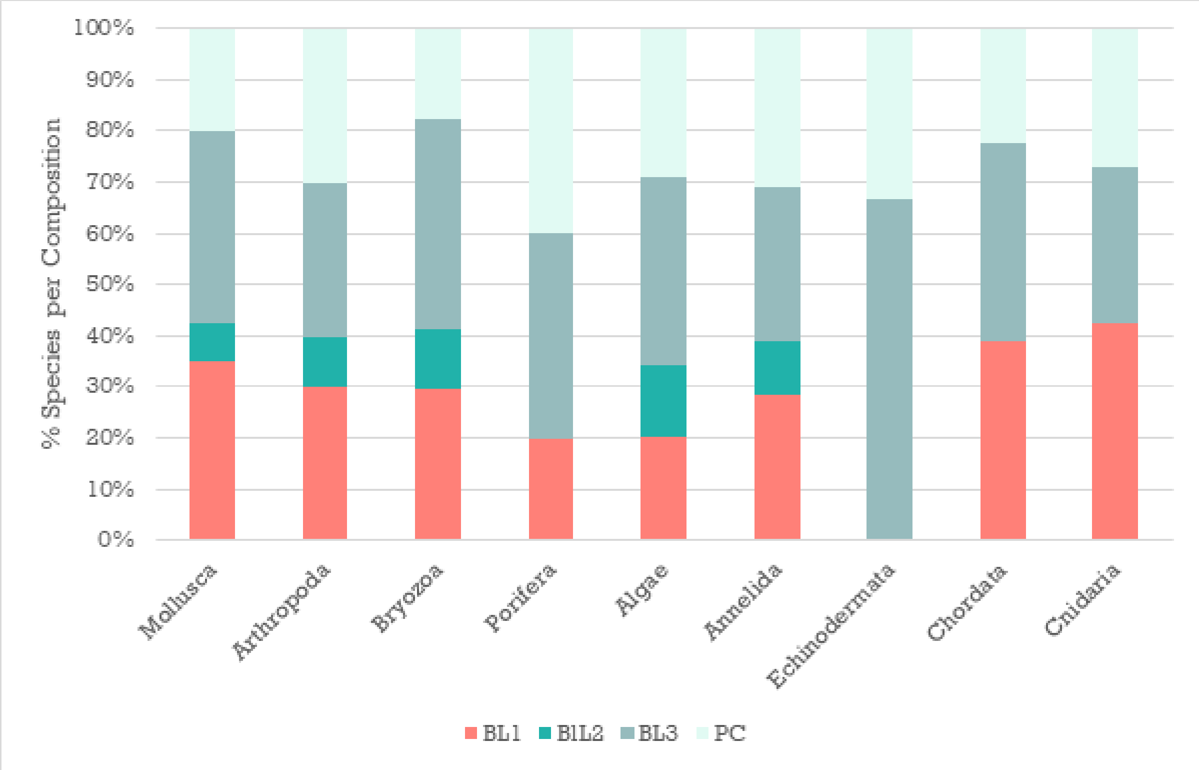


FIGURE 43. STACKED PLOT SHOWING THE RELATIVE ABUNDANCE OF PHYLA DISTRIBUTION ACROSS THE DIFFERENT COMPOSITIONS. PERCENTAGES REPRESENT THE PROPORTION OF REEFBOOSTERS WITHIN EACH MATERIAL COMPOSITION THAT CONTAINS THE RESPECTIVE SPECIES. CALCULATED BY DIVIDING THE TOTAL NUMBER OF REEFBOOSTERS WITH A GIVEN PHYLA (REGARDLESS OF COMPOSITION) BY THE TOTAL NUMBER OF REEFBOOSTERS FOR EACH COMPOSITION.

FIGURE 44. PICTURES OF THE REEFBOOSTER RETRIEVED FROM THE PORT WITH OYSTERS ATTACHED. THE OYSTERS ARE SURROUNDED IN YELLOW AND THE REEFBOOSTER IS SURROUNDED IN BLUE (THIN LINES).

2.5.4 Discussion and Conclusion

It was expected that the compositions with a higher calcium content (such as BL2 and BL3) would attract more Molluscs, but this was not observed. The material composition did not noticeably influence phyla diversity (Figure 43), with the exception of BL2. Contrary to what was expected, BL2 underperformed. This was likely due to its calcium source being gypsum, which accelerated the ReefBoosters' degradation to the point of only partial ReefBoosters being left at the moment of observation. Premature degradation reduced the number of ReefBoosters and their surface area for organism attachment, most likely leading to lower biodiversity and abundance. This observation, alongside laboratory observations (see Appendix) suggest gypsum levels should remain below ~2% to ensure degradation aligns with the desired 5-20 year timeframe.

Despite the phyla diversity not greatly varying between the compositions, a difference could be observed between the different compositions with regards to the total presence/absence of the phyla. BL3 showed an overall higher number of ReefBoosters colonised by different phyla. BL1 and PC however performed similarly, suggesting that an increase in calcium in the composition could be the cause rather than a decrease in Portland cement. This suggests calcium is an important factor in attracting marine life (Fitt et al., 1990; Smyth et al., 2018).

Finally, considering that this project was focused on flat oyster cultivation, the presence of oysters attached to the ReefBoosters was a positive result (Figure 44). One that was especially surprising due to the size of the (Pacific) oysters present. Oysters, being sessile filter feeders, typically require specific conditions for successful attachment and growth. In this context, the timing of the introduction of the substrates could have matched the natural oyster spawning season and caused the presence of oysters attached to the ReefBooster. However, these were likely at an advanced life stage since it does not explain their size within one year time (Figure 44). Likely, the oysters had been growing on a nearby substrate and grew onto the ReefBooster, becoming attached to it.

OVERALL DISCUSSION AND CONCLUSIONS

Material

Both hatchery and nearshore bio-receptivity results suggest calcium-rich compositions are more promising for enhancing oyster settlement and supporting marine life. Calcium content from lime and seashells showed higher bio-receptivity for oyster larvae, and the ability to attract and support a greater diversity and abundance of marine organisms. Unfortunately, calcium content from gypsum (10%) resulted in inconclusive results in both the hatchery and nearshore experiments. This could be attributed to the time frame of the different experiments (1 month in the hatchery vs 1 year nearshore) resulting in premature degradation of the ReefBoosters in the wild, and thus reduced surface area for attachment. Differences in curing time across the hatchery experiments likely explain the more favourable results observed in the most recent trial, where the gypsum composition showed the highest spat count instead of the lowest. Longer curing times likely increased the chemical stability of the ReefBoosters, preventing a rise in surrounding pH. Elevated pH can hinder shell formation and other metabolic activities in bivalves, which may account for the lower bio-receptiveness observed in the first two experiments (Mayrand & Benhafid, 2023).

Overall, these observations highlight that gypsum is a promising component for accelerating the desired natural degradation process of the ReefBooster. However, the percentage used in these experiments (10%) was too high. Combined with data from the BlueLinked hatchery (see Appendix),the gypsum content should stay probably stay below 2% to avoid premature degradation and the curing time should be long enough to achieve chemical stability.

While the hatchery experiments provided promising insights, they also showed high variability and lack of significance, supporting the idea that material composition is not the biggest influencer of settlement success. It also highlights the need for more significantly different compositions studies and larger sample sizes. Nonetheless, the observations from both experimental settings show that Portland cement compositions, tras cement compositions, and Portland cement and gypsum compositions can quite consistently show lower performance compared to compositions with reduced Portland cement and crushed shells and lime, reinforcing the idea that calcium-rich materials (Table 4) are more bio-receptive for marine life, as long as they do not significantly alter the surrounding pH.

Considering these conclusions, further research should be made on the impact of calcium content on bio-receptiveness before being able to draw more confident conclusions. It could be interesting to explore replacing traditional binders such as PC and Tras cement with more calcium-rich materials. It could be interesting to explore replacing traditional binders such as PC and Tras cement with more calcium-rich materials. Adding crushed shells could help increase calcium content while maintaining chemical stability, offering a sustainable and potentially bio-compatible alternative. It would also be interesting to investigate the effects of curing times on the chemical stability of the ReefBoosters to ensure optimal bio-receptiveness. Thus achieving a more sustainable and effective composition for fostering a diverse marine ecosystem and creating an environment conducive to oyster growth and success. Future research should also investigate the microstructure of the different material compositions and further understand its impact on bio-receptiveness.

Observing technique

This project has demonstrated the viability of a new approach to large-scale oyster reef observing by using an autonomous underwater drone like the Lobster Scout. Lobster Scout offers a significant advancement over conventional ROVs, which lack systematic coverage and produce subpar data for quantitative reef assessment. The integration of spread-spectrum acoustic navigation proved essential for precise positioning in challenging environments, while the current estimator showed promise in countering lateral currents, paving the way for further refinements.

While reducing the camera's distance to the seabed addresses turbidity issues, it presents trade-offs, including longer observing times, as evidenced during nearshore trials at the Prinses Margriethaven. Automation of image review could mitigate these challenges and enhance efficiency. However, having done the observing with the aim of covering more ground in a shorter time resulted in poor visibility of some areas of the plots. This affected the accuracy of the investigation of the ReefBooster performance nearshore.

Although the technology requires additional development to achieve maturity for widespread deployment, the results—particularly in representative environments with improved visibility—highlight its potential. The ability to capture data suitable for orthomosaic processing enables detailed visual maps that combine reef overviews with intricate insights, such as oyster spat distribution.

Ultimately, this method represents a promising step forward for large-scale oyster reef restoration and benthic surveys in environments like the North Sea. Its adaptability, precision, and ability to produce high-quality data make it a groundbreaking tool in ecosystem observing, setting the stage for future innovation and application.

Artificial intelligence

AI has demonstrated significant potential for tracking spat both in hatchery environments and in the field. The development and deployment of spat detection models, particularly the Cascade Mask R-CNN, have proven effective in detecting and quantifying spats with high precision during validation. However, the model's accuracy decreased slightly on test data, highlighting the need for better generalization through more diverse image datasets. Nevertheless, the model was successfully deployed via an accessible online interface, enabling real-time spat detection. Transitioning to stable, high-capacity hosting platforms will be critical to support broader usage.

Challenges in area estimation arose due to inconsistent ruler visibility, OCR errors, and variable camera distances, affecting measurement accuracy. This could potentially be addressed by creating a more standard photo set-up which includes a consistent object for scale which is present at the same height as the spat.

For AI applications in nearshore environments, the ReefBoosters model requires substantial improvement, especially for detecting "Spat" (achieving a low mAP of 0.1). Strategies to enhance performance include expanding the training image dataset, testing advanced networks such as EfficientDet or YOLOv5, reducing glare by using darker booster materials, and capturing higher-resolution images to improve the detection of small objects.

These advancements will collectively enhance AI's effectiveness in spat observing and promote its broader adoption in marine research and aquaculture.

ReefBooster nearshore performance

The study highlights that small design changes, such as weight distribution, can significantly affect functionality. Particularly the orientation of ReefBoosters after deployment, which can directly impact oyster spat survival. Therefore it is important to optimize the production process fitted for large scale without compromising on essential design features such as the central mass. Unfortunately, due to limitations in observing footage, no conclusions could be drawn about the long-term stability or orientation changes over time of the ReefBooster nearshore. Additionally, considering the amount of siltation observed, the overall sediment type most likely plays an important role in the effectiveness of the ReefBooster method. The nearshore port location in which thousands of ReefBoosters were tested, had a high presence of silt and clay which is made up of fine particles. To decrease the effect of siltation, it is recommended to perform pilots in locations with more coarse sediments. This most likely increases the total amount of ReefBoosters which result in sexually mature adult oysters, and with that increases the chances of successfully kickstarting reef formation.

Overall, the method relies on utilizing the power of numbers, with potentially only small areas within a plot of acres succeeding. To enable restoration at such scale, it is therefore also important to focus on developing transport and installation methods that allow for efficient deployment. This would involve the use of specialized, stackable crates designed for transport in refrigerated containers, ensuring the safe handling of live organisms. Additionally, for large-scale deployment with a vessel, mechanized systems such as conveyor belts should be considered to streamline the process. This development in combination with the continuous development of automated processes such as AUVs and AI will be crucial for scaling up oyster reef restoration efforts.

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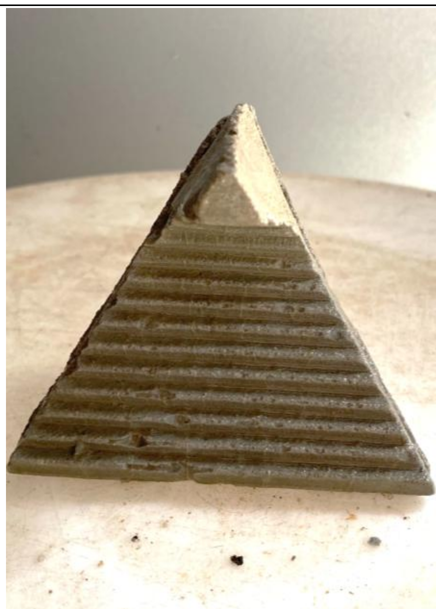
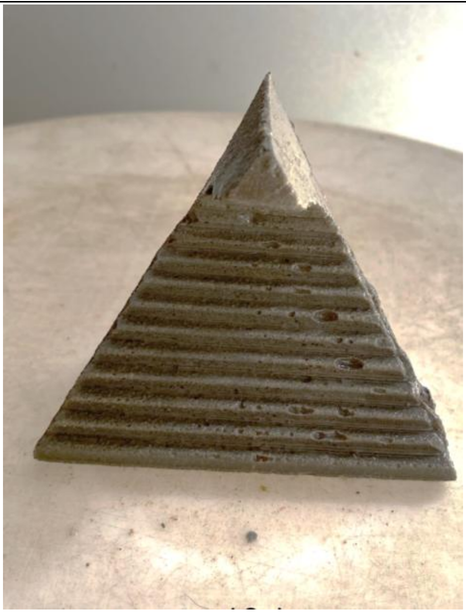
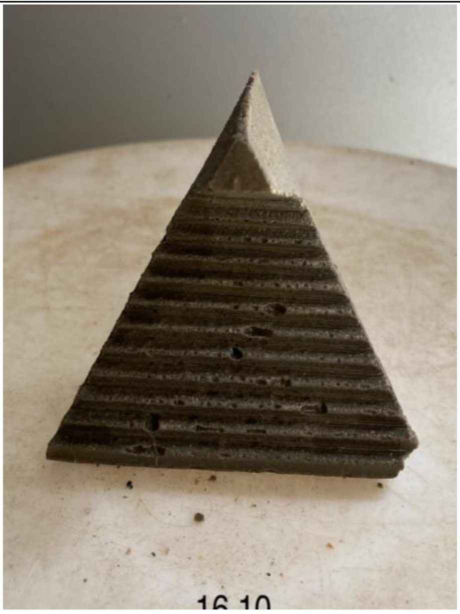
APPENDIX

RESULTS TABLE SHOWING THE STATE OF THE REEFBOOSTERS BEFORE BEING PLACED IN THE TANK AND AFTER 9 MONTHS SUBMERGED AND SUBJECTED TO A STEADY FLOW OF 0.6M/S.

Gypsum (%)	View	Fri 21 10 22	Thu 20 07 23
1	Right Side		
1	Front Side		

1	Right Side		
1	Front Side		

5	Right Side		
5	Front Side		

5	Right Side		
5	Front Side		

10	Right Side		
10	Front Side		

10	Right Side		
10	Front Side		

20	Right Side		
20	Front Side		

20	Right Side		
20	Front Side