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Settlement success of European flat oyster (*Ostrea edulis*) on different types of hard substrate to support reef development in offshore wind farms

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ABSTRACT

The native European flat oyster (*Ostrea edulis*) is an ecosystem engineer providing important ecosystem services, but became nearly extinct from the North Sea due to diseases and overfishing. There's a growing interest to restore these oyster reefs for their valuable contribution in re-establishing a rich ecosystem in the North Sea. In order to reintroduce the flat oyster population, the availability of hard substrate is crucial for initial settlement and reef development. Such substrate is offered by the infrastructure in offshore wind farms, by means of quarried rock placed at the base of the wind turbine foundations and on top of cable crossings to prevent scouring of the seabed. Further anthropogenic disturbances of the seabed are largely restricted, making wind farm areas promising sites for oyster reef restoration.

For successful oyster reef initiation, offering a suitable type of substrate for larvae settlement is important. Here, we assess the settlement preference of flat oysters on 9 different types of substrate, by comparing total settlement, spat densities and spat survival. Oyster larvae settlement preference based on the total number of spat per surface area of the substrate was the highest for granite, a rock type conventionally used as scour protection in offshore wind farms. The lowest settlement preference was observed for steel and the biodegradable polymer BESE. The experiments were performed in a spatting pond and in a natural bay to be able to compare spat collection under controlled and natural conditions. Settlement rates in the spatting pond were much higher than in the natural environment, though survival rates were lower. Our results provide insight in the settlement preference of the European flat oyster for different types of substrate under controlled and natural conditions. Knowing these favorable substrates and conditions for oyster larvae settlement allows for the selection of pro-active measures that contribute to flat oyster reef restoration in the North Sea.

1. Introduction

European flat oysters (*Ostrea edulis*) form biogenic reefs that contribute to a heterogeneous seabed and a biodiverse ecosystem (Bouma et al., 2009; Smyth and Roberts, 2010; Thrush et al., 2008). These oyster reefs improve water quality through filtration (Dolmer, 2000; Newell, 2004) and provide a habitat for a diverse associated community by offering settlement substrate, food and shelter (Coen and Luckenbach, 2000; Lown et al., 2021). Oyster reefs can counterbalance physical and biological stresses in a dynamic marine environment, creating a hospitable habitat for organisms that would otherwise be unable to tolerate severe conditions (Crain and Bertness, 2006).

Flat oyster reefs were abundant in the North Sea until late 19th century (Olsen, 1883), but became nearly extinct due to human disturbances such as overfishing, introduction of diseases and habitat destruction (e.g. Gross and Smyth, 1946; Korringa, 1952). In recent years, there has been growing interest in restoring these native oyster reefs for their valuable contribution to a rich marine ecosystem (e.g.

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Pogoda et al., 2019; Preston et al., 2020). An opportunity to restore these once abundant ecosystem engineers arises from the rapidly growing offshore wind energy industry. In the southern North Sea alone, 62 windfarms have been installed during the first two decades of this millennium covering a total area of 3388 km^2, with a projected tenfold increase due to further development of offshore wind energy production (Ter Hofstede et al., 2023a). These wind farm areas are largely closed for bottom-disturbing activities such as bottom-trawl fisheries or sand extraction, providing an undisturbed seabed needed for oyster reef development. A small part of the seabed in a windfarm area (~0.0005%) offers hard substrate, usually the quarried rock granite, placed at the base of the wind turbine foundations and on top of cable crossings to prevent scouring of the seabed (Ter Hofstede et al., 2023a). This scour protection is generally composed of a flat filter base layer consisting of small-sized rock, topped with an armour layer of larger rocks at the wind turbine foundations, or topped with a sprinkler layer of gravel at cable crossings (Ter Hofstede et al., 2023a). The deployment of scour protection modifies the seascape, by changing a sandy seabed to rocky substrates, creating a heterogeneous seabed (Krone et al., 2013). Furthermore, the three-dimensional hard-substrate provides a habitat on which marine life can settle, forage and find shelter, leading to a local increase in species abundance and species diversity (Coolen et al., 2020; Degraer et al., 2020; Ter Hofstede et al., 2022). Scour protection in wind farms offers the potential for flat oyster reef restoration providing hard substrate that is crucial for the settlement of oyster larvae (Wieczorek and Todd, 1998). The type of hard substrate used for scour protection affects oyster settlement rates and thereby the success of potential reef development (Tamburri et al., 2008; Smyth et al., 2018; Chuku et al., 2020).

Despite the availability of hard substrate in offshore wind farms in the North Sea, spontaneous establishment of oyster reefs has not yet been reported. European flat oyster larvae have a pelagic stage of several weeks and their behavior is aimed at self-recruitment (Rodriguez-Perez et al., 2020). The remaining absence of oyster settlement in offshore windfarms could be therefore be due to a lack of connectivity between existing oyster beds and the newly developed wind farms (Kamermans et al., 2018; Rodriguez-Perez et al., 2020). Hence, the development of oyster reefs in offshore wind farms likely requires the active introduction of oysters to initiate settlement (Ter Hofstede et al., 2023a), allocated at sites where high self-recruitment is expected (Stechele et al., 2023). In the Dutch part of the North Sea, adult oyster broodstock has been introduced in offshore wind farms, aiming to locally produce larvae that can settle and develop into thriving reefs on the available substrates (e.g. Didderen et al., 2019; Schutter et al., 2021). Alternatively, deploying substrate pre-settled with oyster spat could also be an option to initiate reef development (Preston et al., 2020). Both oyster deployment methods of either broodstock or spat-on-substrate have their advantages. For instance, the benefit of using broodstock is that they can reproduce in the first spawning season after deployment for fast reef initiation. Using spat has the advantage that it can be produced in hatcheries or ponds without affecting natural populations for collection of source material and limited competition with other fouling organisms. To select the preferred strategy for actively initiating oyster reef development in offshore wind farms, it is required to consider the differences between spat yield in a natural environment (after deployment of broodstock) and in a controlled environment (using pre-settled substrate).

In this study we evaluate the settlement success of flat oyster larvae on different types of substrate, allowing us to determine their suitability for use in offshore wind farms to facilitate the initiation of oyster reef development. Our experiments were conducted in a spatting pond and in a natural bay to determine differences in spat yield on the substrate types under both controlled and natural conditions. Knowing the favorable substrates and conditions for oyster larvae settlement contributes to allowing governments and wind farm developers to select appropriate measures that support oyster reef restoration. Optimizing the infrastructure of offshore wind farms for flat oyster reef restoration purposes will greatly improve the involvement of wind energy production to increasing the nature values of the North Sea.

2. Material & methods

2.1. Substrate material

An experiment was conducted to assess settlement success of flat oyster larvae on nine different types of hard substrate (Fig. 1; Table 1). These substrate types were selected based on their application as scour protection in offshore windfarms (granite, sandstone and flint), as substrates that are used in shellfish reef restoration (conventional concrete, concrete with natural adhesives (ECOncrete; http://econcretetech.com), galvanized steel, and circular biodegradable reef blocks (Biodegradable EcoSystem Engineering elements – BESE; htttp://bese-products.com) and as substrates that are commonly used for spat collection in oyster farms (mussel shell and clay roof tiles).

2.2. Spat collection locations

The experiment took place at two different locations in Ireland (Fig. 2). To assess settlement success under controlled conditions, an oyster spatting pond was selected, located in New Quay, County Clare, (53°09′25.9"N 9°04′00.2"W) (Fig. 2). The spatting pond is a square pond of 25 by 25 m with a depth of 2 m. Brood stock was placed in the ponds and once the oysters started spawning, water refreshment was kept to a minimum to prevent the oyster larvae from washing out. To observe settlement under natural conditions, a site with a resident wild population of oysters in the natural environment was selected, located on the west coast of Tralee Bay, County Kerry (52°16′18.8"N 9°51′43.3"W) (Fig. 2). Tralee Bay is known for its natural reproduction capability of flat oysters and sustains one of the few self-seeding wild flat oyster fisheries found in Europe. The substrates were deployed in the water column using longlines of approximately 60 m length, in a relatively shallow part of the bay (6-8 m) near a resident population of flat oysters.

2.3. Deployment of substrate baskets

The substrates were contained in polyethylene baskets (diameter 15 cm, height 40 cm) with a 2x2cm mesh size. Weight and volume of the content in each basket was determined prior to deployment. At both locations, the substrate baskets ($n = 5$ per substrate type) were suspended 20-30 cm below the water surface, approximately 30 cm apart. The sequence of the substrate baskets was randomly assigned. To limit biofouling that could potentially interfere with settlement of oyster larvae, the substrate baskets were deployed shortly before the expected peak in larvae settlement. The settlement peak of flat oyster larvae generally occurs about two weeks after a peak in larvae numbers is observed (Maathuis et al., 2020; Van den Brink et al., 2020). Peaks in larvae numbers were determined through daily monitoring of free-swimming larvae numbers from water samples in the spatting pond from June 9th until August 23rd 2019, providing an indication of expected peaks in larvae settlement. Peaks in settlement were determined by counting spat on standard settlement plates, in the spatting pond on a daily basis over the same period, and in the natural bay on three days (July 15th, August 18th, September 2nd). Several peaks in larvae settlement were observed in the spatting pond, starting from June 19th with the highest peak in settlement on July 2nd. Oyster larvae settlement in the natural bay was confirmed on all three monitoring occasions. The substrate baskets were deployed in the spatting pond on June 25th and all were retrieved on September 23rd. The substrate baskets were deployed in the natural bay on July 1st and retrieved on September 25th. Some of the baskets in the natural bay were lost due to severe weather conditions, resulting in <5 replicates for some substrate types (Table 1).

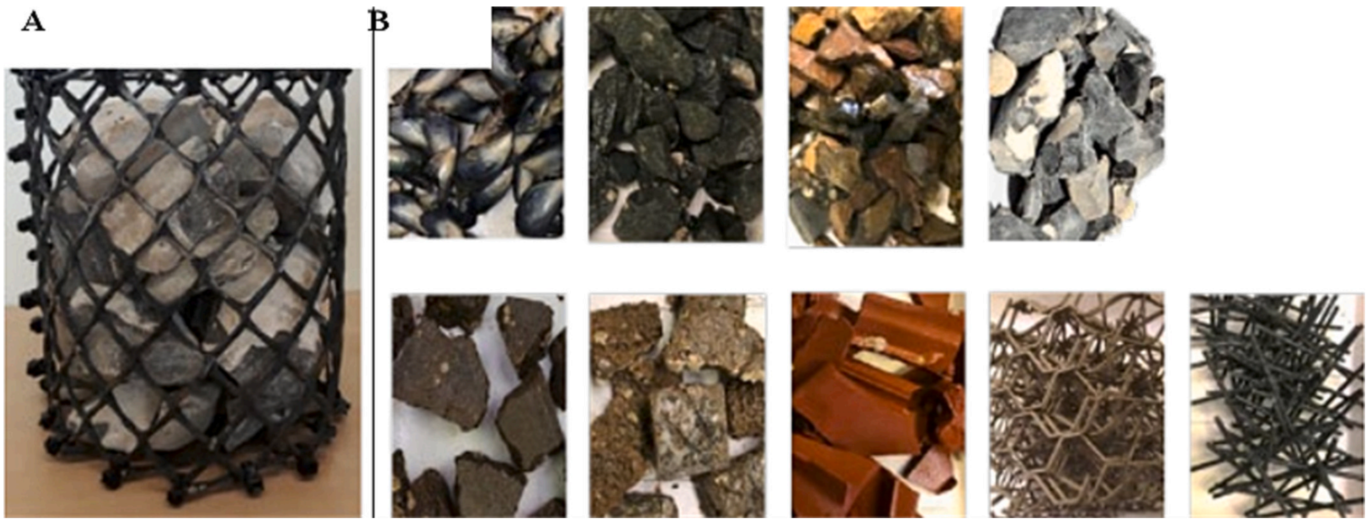

Fig. 1. A) basket used to hold the substrates, in this example filled with silex. B) overview of the different substrates used in the field experiments. From top left to bottom right: mussel shells, granite, sandstone, silex, concrete, ECOncrete, roof tile, BESE, steel.

Table 1
Overview of the number and weight of the substrates used in the experiment.

Substrate	Spatting pond			Natural bay		
	# baskets	Mean weight/ basket (g) (SD)	Mean surface/ basket (cm2) (SD)	# baskets	Mean weight/ basket (g) (SD)	Mean surface/ basket (cm2) (SD)
Mussel	5	753.4 (47.7)	6795.6 (430.0)	2	1010.0 (41.0)	9110.2 (366.9)
Granite	5	4313.2 (208.8)	3306.6 (160.1)	4	4347.0 (138.5)	3332.5 (106.1)
Sandstone	5	3461.2 (120.0)	5396.2 (187.1)	5	3596.0 (262.1)	5606.3 (408.7)
Silex	5	3951.4 (251.6)	2461.0 (156.7)	5	4465.2 (156.8)	2781.0 (97.6)
Concrete	5	4498.2 (311.7)	2990.6 (207.2)	4	4653.8 (244.3)	3094.1 (162.5)
ECOncrete	5	3108.4 (491.3)	2170.8 (343.1)	4	3293.5 (343.8)	2300.1 (240.1)
Rooftile	5	3309.8 (150.0)	3196.6 (144.9)	3	3491.3 (62.4)	3371.9 (60.3)
BESE	5	152.6 (3.6)	1742.5 (40.9)	5	292.6 (44.6)	3341.1 (509.4)
Steel	5	1512.6 (31.5)	1768.6 (36.8)	3	1582.3 (93.4)	1850.2 (109.2)

2.4. Counting spat

After retrieval of the substrate baskets, the substrates in each basket were weighed, biofouling was removed, and if necessary, the substrate was cleaned using filtered seawater. Then, the total number of oyster spat on the substrate was counted. In order to assess the initial settlement preference, the total number of spat included both living and dead spat, which was recorded separately. If the total number of spat was estimated to be over 250 individuals per basket before counting, a subsample was taken by spreading out the substrate evenly and splitting it into equal parts. The numbers of spat were then counted in the subsample, while ensuring that subsamples always contained a minimum of 100 spat. The substrate in the subsample was weighed and the total number of spat in the basket was estimated by multiplying the number of spat counted in the subsample by the fraction of the total weight in the subsample.

2.5. Determining the surface area

In order to compare the spat densities on the different substrate types, the three-dimensional surface area of the different substrates was estimated using a combination of double wax dipping and 3D scanning. Double wax dipping involves dipping a substrate in melted paraffin wax twice, and the increase in weight between the first and second dip is taken as an indication for the surface area (Stimson and Kinzie, 1991; Holmes, 2008). In order to determine the available settlement surface, a representative subsample of a random size mix of pieces of each substrate type was used for double wax dipping. Five different sized pieces of every substrate type that were used for wax dipping were also scanned with a 3D scanner (Artec Eva Handheld scanner). The surface area of these pieces of substrate was calculated using 3D models created with Artec Studio (V14). A calibration curve was then calculated based on the 3D models to determine the available surface area from the weight difference between the first and second wax dip:

$$3D\ surface\ area = 3.41 + 27.48 * weight\ difference$$

Where, 3.41 mm^2 is the minimum possible surface where there is no substrate but just a drop of wax, and the *weight difference* is the difference between the first and second wax layer. Based on the subsample that was dipped in wax, the surface area in cm^2 per kg was calculated using the above formula. This was then multiplied by the weight of the substrates in each basket to estimate the available surface in cm^2 for settlement in each basket.

2.6. Data analysis

To determine which substrate collected the highest numbers of spat, the total numbers of spat were compared between the two locations and between different substrate types. Spat density was taken as an indicator for settlement preference, calculated by dividing total spat by the available settlement surface in cm^2. Spat survival was calculated as the fraction of living spat out of the total spat counted after retrieval, and also compared between locations and substrate types. Because the variance of the settlement differed greatly between the substrate types and locations, statistical analyses were performed using non-parametric Kruskal-Wallis tests. If the Kruskal-Wallis test indicated significant differences between substrates, Conover-Iman post-hoc tests were performed to determine which substrates differed significantly in terms of spat survival, total spat or spat density. In all cases, the results presenting variability refer to the standard deviation of the mean.

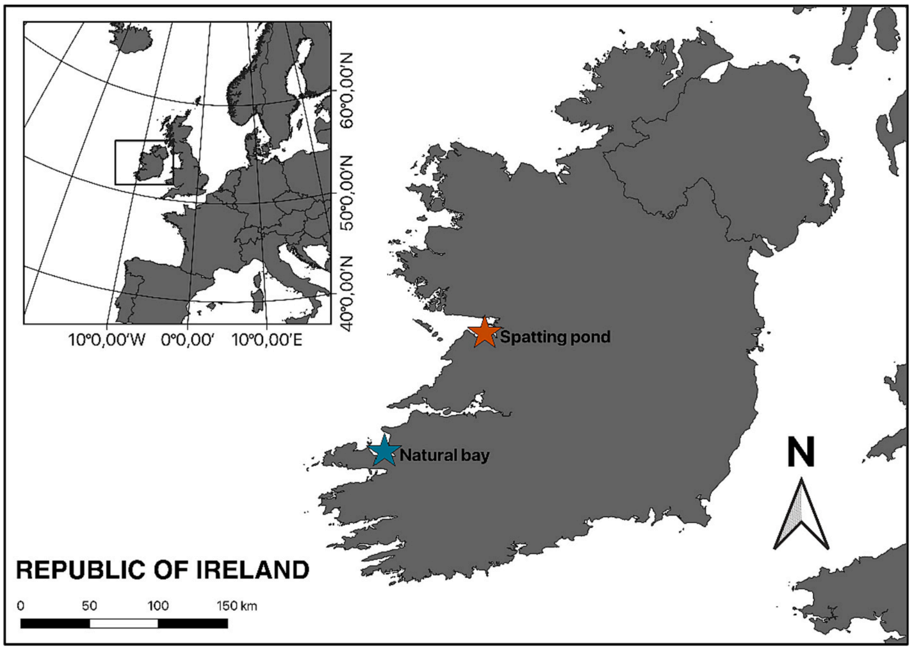

Fig. 2. Map indicating the two locations where the field experiments were conducted, the spatting pond in New Quay (orange star) and the natural bay in Tralee (green star). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Data analysis was done in R (version 4.3.1, R Core Team, 2021) with the Tidyverse package (Wickham et al., 2023). Kruskal-Wallis tests were performed using the Stats package (R Core Team). Conover-Iman post-hoc tests were performed using the conover.test package (Dinno, 2017). The maps were created in QGIS (version 3.30) and all other plots were made using ggplot2 (Wickham, 2016). For ease of understanding the plots, compact letter displays generated with the rcompanion package were added to plots (Mangiafico, 2023).

3. Results

After retrieving the baskets with substrates, there was no biofouling observed on the substrates deployed in the spatting pond, while those deployed in the natural bay contained soft-bodied fouling organisms such as *Ectopleura larynx* (ringed tubularia), different species of anemones, sponges and bryozoa, as well as *Spirobranchus triqueter* (brushworm), saddle oysters (*Anomiidae*), scallops (*Pectinidae*) and other molluscs. Sizes of oyster spat collected on the substrates differed from several mm to 1.5 cm due to the occurrence of multiple settlement peaks during the experiment.

The settlement success rate in terms of average total spat (both living and dead) per basket was significantly higher on the substrates placed in the spatting pond (469.1 ± 517.5) than in the natural bay (98.7 ± 74.4) (Kruskal-Wallis, $H = 13.31$, $df = 1$, $p < .01$). There were significant differences between the total numbers of spat on the different types of substrates in both locations (Kruskal-Wallis: spatting pond $H = 36.49$, $df = 8$, $p < .01$; natural bay $H = 26.26$, $df = 8$, $p < .01$). On average the most spat was found on granite, both in the spatting pond (1120.8 ± 796.7) and in the natural bay environment (206.8 ± 32.1) (Fig. 3). The substrate BESE had the lowest settlement in the spatting pond (2.8 ± 2.4), and collected no spat in the natural bay environment. The average spat survival (fraction living spat of total spat) significantly differed

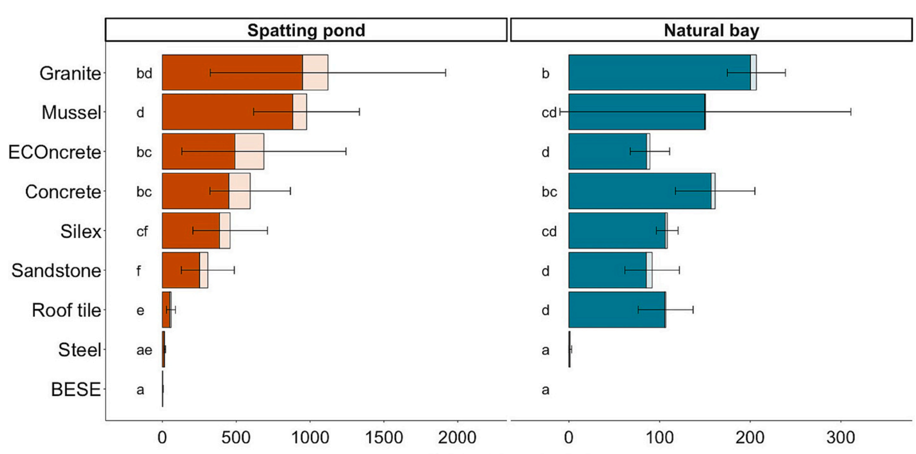

Fig. 3. Total spat per basket (mean and SD) per substrate for the two locations. To illustrate survival total spat is divided into living (dark colour) and dead spat (light colour). Significant difference was observed between the locations per substrate type. Letters indicate the effect of substrate type on total spat per basket; if substrate types have letters in common, they do not significantly differ from each other. Note the difference in magnitude of the x-axes for the Spatting pond (in orange) and the Natural bay (in green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between the spatting pond (0.79 ± 0.16) and natural bay environment (0.77 ± 0.39) (Kruskal-Wallis: $H = 14.70$, $df = 1$, $p < .01$; Fig. 3). Spat survival per substrate type was generally higher in the natural bay. The highest average spat survival was on mussel shells, both in the spatting pond (0.90 ± 0.05) and in the natural bay (0.99 ± 0.01). The lowest average spat survival was observed on steel, both in the spatting pond (0.68 ± 0.18) and in the natural bay (0.33 ± 0.58).

Based on the total number of spat per surface area (cm^2), settlement preference differed for specific types of substrate at both locations (Kruskal-Wallis: spatting pond $H = 37.79$, $df = 8$, $p < .01$; natural bay $H = 30.61$, $df = 8$, $p < .01$; Fig. 4). Oyster larvae preferably settled on granite, both in the spatting pond and in the natural bay, on average 0.35 ± 0.26 and 0.06 ± 0.01 spat per cm^2 respectively. In the spatting pond, a group of five substrate types (granite (0.35 ± 0.26), mussel shells (0.15 ± 0.06), ECOncrete (0.31 ± 0.22), concrete (0.20 ± 0.10), silex (0.19 ± 0.12)) had significantly higher settlement rates than the other substrate types (sandstone (0.06 ± 0.03), roof tile (0.02 ± 0.01), steel (0.01 ± <0.01), BESE (<0.01 ± <0.01)) (Fig. 4). In the natural bay, settlement preference between substrates was more pronounced, as only two substrate types, i.e. granite (0.06 ± 0.01) and concrete (0.05 ± 0.01), showed significantly higher settlement preference than all other substrates (Fig. 4). Steel and BESE had very low settlement rates compared to the other substrates, both in the spatting pond and the natural bay.

4. Discussion

4.1. Settlement preference

Overall, a variable settlement of spat across multiple substrates was observed, with some distinct outcomes. Settlement rates in the spatting pond were higher than in the bay area hosting natural oyster reefs, which was to be expected as the oyster larvae are restricted to the confined space of the pond and their main settlement opportunity was on the provided substrate types. Also, the average spat survival until retrieval of the substrates differed significantly between the spatting pond and natural environment, being higher in the natural bay. Our finding that the survival in an uncontrolled natural environment was higher than in a confined spatting pond could be considered remarkable, since in the natural bay the spat is exposed to external stressors such as predators and fouling organisms competing for space. However, the higher settlement densities in the spatting pond could also lead to higher mortality of the spat, as also observed by Zorita et al. (2021) when comparing survival of *O. edulis* spat between different stocking densities, for example due to competition for food and space. Furthermore, our experimental setup of placing the substrates in baskets and hanging them on a long-line off-bottom has likely severely reduced predation pressure, in particular from benthic organisms like crabs and starfish.

Settlement of *O. edulis* was generally the highest on granite, in total spat per basket as well as in numbers per surface area (cm^2), both in the spatting pond and in the natural bay. Granite rock material is commonly used as scour protection in offshore wind farms (Ter Hofstede et al., 2023a), which implies that wind farms generally offer favorable settlement substrate for oyster larvae. Settlement densities (per cm^2) of *O. edulis* were also observed particularly high on concrete in the natural bay. Concrete has been observed previously as an even more preferable settlement substrate than natural materials like rock and shell for oyster larvae of the species *Crassostrea virginica* (Graham et al., 2017). Total settlement per basket was also high for mussel shells, which is not unexpected as shell material generally attracts high numbers of oyster larvae for settlement (Levine et al., 2017; Smyth et al., 2018; Van den Brink et al., 2020). On the contrary, the spat densities (per cm^2) on mussel shells were low. In our study mussel shells had the highest surface area/weight ratio compared to the other substrate types used. Therefore, even if spat densities were low, the total spat in a basket filled with shells was high because of the large total surface area offered for settlement. This implies that offering substrate with a large surface area such as shells, could be an efficient way for spat collection compared to more compact substrates (Kuykendall et al., 2015).

Oyster larvae settlement was observed to be the lowest on the steel and BESE substrates, though both materials have shown to be successfully colonized by bivalve larvae in previous studies (e.g. Pouvreau et al., 2021; Nauta et al., 2023; Temmink et al., 2022). Experiments by Pouvreau et al. (2021) indicate high colonization rates by *O. edulis* on untreated steel. This is in contrast to our results and might be explained by the fact that the steel used in our experiment was smooth and galvanized, therefore likely less suitable for larvae settlement. Also BESE appears unsuitable as settlement substrate for *O. edulis* larvae, at least in its grid shape as used in our experiments. However, BESE has shown to be a suitable habitat modifier in other shellfish reef restoration projects (e.g. Nauta et al., 2023; Temmink et al., 2022).

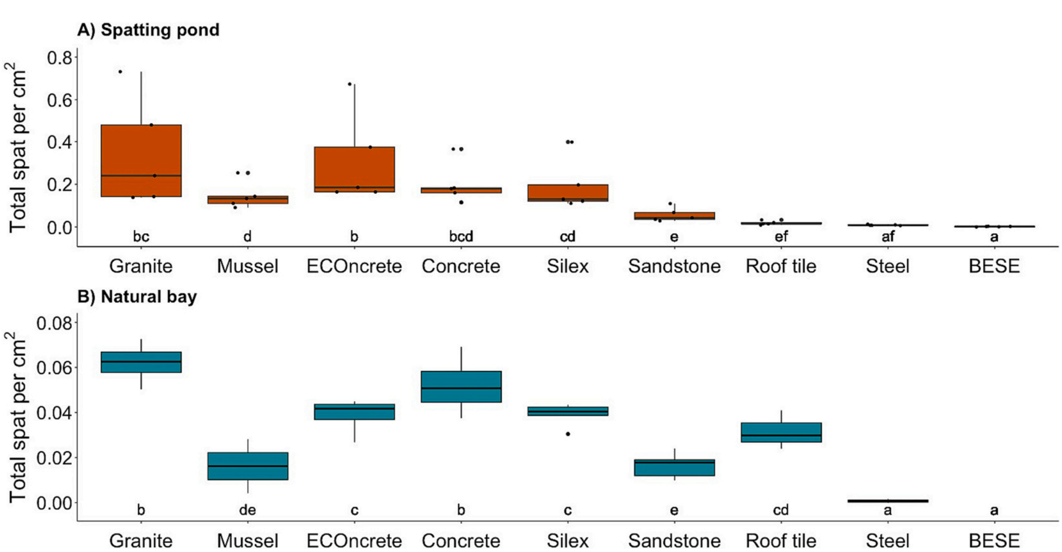

Fig. 4. Total spat per cm^2 indicating settlement preference per substrate type for the A) Spatting pond (in orange) and B) Natural bay (in green). Boxplots depict the median, quantile, outliers and distribution of the spat per cm^2 in the baskets. Letters indicate the effect of substrate type on spat density; if substrate types have letters in common, they do not significantly differ from each other. Note the difference in magnitude of the y-axes between the Spatting pond and the Natural bay graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Implementation in offshore wind farms

The deployment of favorable settlement substrate could be an adequate intervention to support oyster reef development in offshore wind farms. The selection of substrate material highly depends on its application, whether it is merely used for nature enhancement including oyster development, such as artificial reef structures, or whether it should have a function as part of the infrastructure of the wind farm, such as scour protection. Artificial reef structures are commonly installed to provide the hard substrate required for oyster reef restoration (Baine, 2001; La Peyre et al., 2014). Concrete is often used as the main construction material for artificial reefs (Baine, 2001), which according to the outcome of our experiments appears to be a suitable substrate for oyster larvae settlement. A material like concrete easily allows formation into shapes that are optimal for oyster larvae settlement, for example by the inclusion of specific surface roughness and richness in calcium carbonate (Cuadrado-Rica et al., 2016; Potet et al., 2021), making it a potentially preferable settlement substrate. However, the downside of concrete is it being toxic as the cement mortars often leach trace metals over time (Hillier et al., 1999; Wilding and Sayer, 2002). ECOncrete partly compensates for this, as it contains nature-friendly adhesives (Perkol-Finkel and Sella, 2014), thereby notably reducing its toxicity compared to conventional concrete. Still, the manufacturing process of artificial reef structures made of concrete or ECOncrete causes substantial emissions of carbon dioxide (Blankendaal et al., 2014; Fennell et al., 2021). Furthermore, these structures would need to be produced in large amounts to achieve impact at scale (Bohnsack and Sutherland, 1985).

Instead of installing artificial reef structures in offshore wind farms to provide substrate for oyster reef development, our study implies that it would be more advantageous to achieve the desired impact by using and enhancing the infrastructure of the wind farm itself. Marine infrastructures inherently provide artificial habitat at large scale: its long-term presence allows nature development, and designs can be optimized to target certain species (Ter Hofstede et al., 2023b). The scour protection in offshore wind farms can be made of the natural rock material granite, observed in our study as the most favorable substrate for oyster larvae settlement. It can even be designed to further increase opportunities for oyster larvae settlement. Oyster larvae benefit from reduced flow velocities at the seabed (Korringa, 1940), and these conditions can be created within the scour protection through more irregular extensions in both vertical and horizontal directions. Incorporating such microhabitats with reduced flow velocities in the design of a scour protection, would enhance settlement opportunities for oyster larvae in offshore wind farms (Ter Hofstede et al., 2023a). It yet remains to be determined how exactly the various elements of scour protection in offshore wind farms can be used or attuned to positively influence oyster reef development, and only by putting interventions into practice one can study their effects. Fact is, the presence of stable hard substrate by means of scour protection provides settlement opportunities for the flat oyster larvae (Smyth et al., 2018), and it is to be expected that an increase in its habitat complexity by bringing in more variety in use of materials, shapes and dimensions (Ter Hofstede et al., 2023a), will result in a higher oyster abundance, as is the case for epibenthic biodiversity in general (Lapointe and Bourget, 1999; Firth et al., 2014).

Merely deploying favorable settlement substrate and creating suitable settlement conditions is likely not sufficient to initiate oyster reef development in offshore wind farms due to the absence or low abundance of flat oysters (Ter Hofstede et al., 2023a). There's often a lack of connectivity between existing oyster beds and the newly developed wind farms (Kamermans et al., 2018; Rodriguez-Perez et al., 2020). This results in a lack of recruitment to initiate oyster reef development, despite the presence of hard substrate for settlement. Currently, the focus lies on deploying oyster broodstock in offshore wind farms, to serve as local larvae pumps for initiation of oyster reefs (Didderen et al., 2019; Schutter et al., 2021). However, the observed higher settlement rates in a spatting pond could support decision-making in setting an alternative strategy to pro-actively introduce oysters in offshore wind farms. Deploying substrate that is already pre-settled with oyster spat could become the preferred strategy to kickstart oyster reefs, knowing that spat densities on the used substrate will be high when settlement occurs in a controlled environment such as a spatting pond.

The selection of the type of substrate for pre-settlement can also be made based on cost-efficiency and suitability for the offshore environment. Making use of the infrastructure of the wind farm is the most cost-effective (Ter Hofstede et al., 2023b), as it is part of the construction process and existence of the wind farm itself without additional costs, which is even feasible with optimizations such as calciferous rock material as scour protection. Another cost-effective measure relates to the use of pre-settled spat on substrate. High settlement of oyster larvae was observed on mussel shells, a substrate with a high surface:volume ratio. The high surface:volume ratio of shells takes less volume of substrate to host a higher number of spat, during both spat collection and transportation to the wind farm. It is also for these reasons that mussel shells are commonly applied in oyster cultivation practices as spat collectors (Van den Brink et al., 2020). On the other hand, the high surface:volume ratio of shell material leads to a high chance of the shells to wash away by currents, once deployed in wind farms. A heavier material with a lower surface-volume ratio such as rock will be more stable once deployed, and provides hard substrate for oyster reef development over a longer period of time. The final selection of suitable interventions however needs to be based on a case-by-case assessment, making a trade-off between desired impact and costs.

5. Conclusions

The reinstatement of large European flat oyster reefs in the North Sea could benefit from the rapid increase in offshore wind farms. The use of hard substrate as scour protection in the infrastructure of the wind farms provides suitable settlement conditions for oyster reefs to develop. Our results show that oysters preferentially settle on stony substrates such as granite and concrete. Granite would be the most favorable substrate for use as (additional) substrate to facilitate oyster reef development in offshore wind farms, being a material from natural origin and already commonly applied as scour protection, simplifying its implementation. The initiation of oyster reef development in offshore windfarms likely requires the pro-active introduction of oysters, either spat pre-settled on substrate or adults, due to the lack of connectivity with existing oyster beds. Settlement rates in the spatting pond were much higher than in the natural bay, implying that deploying substrate pre-settled with spat under controlled conditions, could be an efficient strategy worth to consider for kickstarting oyster reefs.

Our results provide insight in the settlement preference of the European flat oyster for different types of substrate under both controlled and natural conditions, and allow for the selection of measures to initiate oyster reef development in offshore wind farms. Implementation of these findings can contribute to establishing the return of a large flat oyster population in the North Sea.

Author contributions

First authors RH, SW contributed equally to the manuscript; RH, LT, PK designed the project; RH, SW, LT wrote the manuscript; LT, SW performed the experiments; SW analyzed the data; PK, MK provided substantial edits; all authors contributed to the final version of the manuscript.

CRediT authorship contribution statement

Remment ter Hofstede: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Sterre**

Witte: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Pauline Kamermans:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Mark van Koningsveld:** Writing – review & editing, Supervision, Funding acquisition. **Linda Tonk:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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