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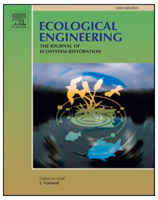

Novel settlement substrates for European flat oyster (*Ostrea edulis*) restoration

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ABSTRACT

The flat oyster *Ostrea edulis* L., once common in the North Sea, declined rapidly due to intensive fisheries in the late 19th century and disease outbreaks at the beginning of the 20th century and is now listed as 'threatened' or 'declining,' with restoration of oyster beds now included in European directives and national plans. For oyster restoration, availability of suitable substrate is required to ensure successful settlement of oyster larvae. Off-shore windfarms are good candidates for restoration as bottom disturbance is not allowed and hard substrate is present in the form of so-called scour protection. This can provide settlement substrate for oyster larvae. In addition to the rock material that currently makes up the scour protection, studies focus on finding alternative and moldable materials that stimulate settlement. The aim of this study was to identify flat oyster larvae settlement preferences for different substrate materials. Oyster settlement on conventional scour protection rock (granite and eclogite), and currently used sandstone and concrete were compared to new types of scour protection rock (marble and limestone). In addition, three new substrates were included in the tests: a coating based on fine ground oyster shells (BESE-reef paste), substrate made of sandy dredged sediment (Geowall) and a bioinspired glue that binds crushed oyster shell fragments together (SeaCrete). Flat oyster larvae were exposed to the substrates in two hatchery experiments as well as under realistic, challenging field conditions. Flat oyster larvae settled on all substrates, with the lowest spat density on eclogite, granite and Geowall and the highest spat density on the two novel substrates SeaCrete and BESE-reef paste. These results promise to enhance native European oyster bed restoration with limited environmental impact as the novel substrates have low CO_2 footprints and make use of wasted shells from the seafood industry.

1. Introduction

Oyster beds are considered an ecologically important habitat, as oysters are ecosystem engineers which provide multiple ecosystem services such as water filtration resulting in reduced eutrophication (Newell, 1988), capturing sediment (Walles et al., 2015), settlement substrate for epibenthic flora and fauna, food supply, and benthic-pelagic coupling, thereby enhancing biodiversity and fishery conditions (Beck et al., 2011; Christianen et al., 2018; Coen et al., 2007; Kellogg et al., 2013; Lee et al., 2020; Smyth and Roberts, 2010). Furthermore, oyster reefs offer shelter from predation to mobile species making the reefs important nursing grounds for fish species (zu Ermgassen et al., 2016). Until about a century ago the flat oyster *Ostrea edulis* L. was a common species in the North Sea (Gercken and Schmidt, 2014; Houziaux et al., 2008; Olsen, 1883), and more broadly along European coasts (Thurstan et al., 2024). However, the oyster population declined

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rapidly as a result of intensive flat oyster fishery at the end of the 19th century and disease outbreaks at the beginning of the 20th century (Gercken and Schmidt, 2014; Houziaux et al., 2008; Pouvreau et al., 2023). At present, European oyster beds are absent or rare in most of their natural range (OSPAR BDC, 2020), with only a few individuals remaining in the North Sea (Kerckhof et al., 2018). The species is listed as 'threatened' or 'declining' by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2008). And a recent study showed that *O. edulis* reefs are 'Collapsed' using IUCN criteria (zu Ermgassen et al., 2024). Therefore, the return of biogenic reefs is mentioned as part of the European Marine Strategy Framework Directive (https://environment.ec.europa.eu/topics/marine-environment_en), and regionally, restoring flat oyster beds are often included in national plans, e.g. Dutch Beleidsnota Noordzee 2016–2021 (Ministerie van Infrastructuur en Milieu and Ministerie van Economische Zaken, 2015), Noordzee 2050 Gebiedsagenda, Natuurambitie Grote Wateren 2050 (Ministerie van Economische Zaken, 2013a, 2013b) and Uitvoeringsagenda Natuurlijk Kapitaal (Ministerie van Economische Zaken, 2013a, 2013b) in the Netherlands or French national plans (Pouvreau et al., 2021).

Given their ecological importance, and degradation in the North Sea region (Gercken and Schmidt, 2014), flat oysters are now the subject of many restoration programs. Throughout Europe, at least 30 projects with oyster bed restoration activities are taking place within 13 countries (website of the Native Oyster Restoration Alliance https://noraeurope.eu). Once the suitability of the local habitat is established (Kamermans et al., 2018), several options for restoration exist, making use of options the life cycle of the species offers. Flat oysters are brooding bivalves where female oysters release one to two week old larvae that have already developed shells. The timing of maximum larval release can be predicted based on the temperature sum expressed in degreedays (Maathuis et al., 2020). Next, they spend another 8–17 days as free-swimming larvae (Korringa, 1940; Walne, 1974). Metamorphosis, from swimming larvae into sessile spat, depends on the growth rate of the larvae which is in turn largely dependent on temperature and food availability for the larvae. Settlement, or spat fall, occurs when a suitable location is detected. A drop of biogenic cement is produced, and the left valve is glued to the surface, where they will stay for the rest of their life (Walne, 1974).

Depending on the local situation there are different options for oyster restoration. When populations are absent or too depleted adult oysters can be introduced as broodstock (Ter Hofstede et al., 2023). Under appropriate environmental conditions, these oysters will produce larvae. When larval abundance in the wild is still sufficient, broodstock is not the limiting factor (Pouvreau et al., 2023). Then availability of suitable substrate may be required to facilitate successful settlement (Smyth et al., 2018). Availability of substrate has been known for decades as a constraint in the expansion of natural or restored oyster beds (Korringa, 1946; Möbius, 1877). Thus, in case suitable substrate is not present at the restoration location anymore, it is most effective to introduce this. And finally, when both broodstock and substrate are not available, the development of an oyster bed or reef can be kick-started by introducing hatchery-produced spat settled onto substrate. This may facilitate further natural development of a healthy reef in the field by attracting further natural settlement. Numerous substrates have been tested for settlement preferences of *O. edulis* larvae (reviewed in Colsoul et al., 2021; Korringa, 1952; Potet et al., 2021; Ter Hofstede et al., 2024). These tests showed that larval settlement was especially high on substrates composed of calcareous material and with a high rugosity, (e.g. calcarous rock, bivalve shells, a mix of shells and concrete, or tiles coated with lime).

The European oyster is an important focal species for nature inclusive building and restoration projects in offshore wind farms (Bos et al., 2023). Off-shore windfarms are potentially suitable locations for oyster restoration because bottom trawling is not allowed. In addition, the offshore wind farm infrastructure generally includes layers of rock material for scour protection, installed at the base of wind turbines or covering cable crossings to prevent the seabed from scouring providing a stable geogenic base for oyster beds. These scour protections generally resemble a pancake, composed of a filter layer of small-sized quarried rock, such as granite, topped with an armour layer of larger rocks (Ter Hofstede et al., 2022). The rock sizes and dimensions used in the scour protection depend on local water depth, geomorphological and hydrodynamical conditions, and diameter of the wind turbine foundation. For illustration, in the Southern North Sea the filter layer is generally composed of rock with a size range between 22/90 mm and 45/180 mm and has an average diameter of 33.4 (±8.5) m and thickness of 0.5 (±0.1) m, and the armour layer consists of rock with a size range between 5 and 40 kg and 60–300 kg and has an average diameter of 26.0 (±6.5) m and thickness of 0.9 (±0.3) m (Ter Hofstede et al., 2023). The rocky material acts as an artificial reef, hosting a broad range of marine taxa such as algae, invertebrate species and fish (Coolen et al., 2020; Ter Hofstede et al., 2022). Conventional scour protection can be adjusted to increase the habitat complexity by bringing in more variety in use of materials and their texture, shape and dimensions (Ter Hofstede et al., 2023), which is expected to result in a higher biodiversity (Firth et al., 2014; Lapointe and Bourget, 1999). The use of calcareous rock such as limestone or marble increases settlement by shellfish (Hidu et al., 1975; Soniat et al., 1991). Irregular extensions in both vertical and horizontal directions by making heaps and berms will increase surface area and provide leesides for shelter. Reducing the size range of the rocks results in more crevices, and variation in rock size at different locations increases habitat complexity, serving a wide range of rock-dwelling species (Ter Hofstede et al., 2023). Such changes can easily be incorporated into the project design to ecologically enhance marine infrastructure.

In several restoration projects the effectiveness of various substrates for oyster restoration was tested. A recent study using floating baskets filled with different types of scour protection stones showed that *O. edulis* larvae prefer settlement on granite (Ter Hofstede et al., 2024). At Borkum Reef Ground in the Dutch North Sea, 3D-printed reefs of sandstone were introduced to stimulate spat settlement (Bos et al., 2023). Also, concrete that was molded to provide different surface textures was studied for its suitability for larval settlement in the field (Potet et al., 2021). However, the use of concrete (except for repurposed concrete that would otherwise be waste) is debated because of its high CO_2 footprint and large amounts of fresh water and sand needed for its production (Blankendaal et al., 2014). For oyster larvae, oyster shells are a good substrate to settle. Individual shells, however, do not support a steady reef; they must be held together. Adhesive technology is infrequently used in wet environments as water is detrimental to the performance by promoting swelling of the material or weakening the contact with the adherend. Current underwater adhesives, however, are based on reactive two-part epoxide chemistry that is unnatural, expensive, and potentially harmful (Ilioni et al., 2019). Therefore, new adhesive design principles are needed for sustainable reef substrate solutions. Nature, by means of marine sandcastle worms (*Phragmatopoma californica*) has solved these issues using proteinaceous complex coacervate glues to form extremely durable underwater structures (Stewart et al., 2011). Inspired by these natural solutions SeaCrete was developed using a bio-inspired complex coacervated-based alternative for cement that binds fragmented oyster shells together and which upon immersion in seawater creates a high strength and long-lasting bond (Stewart et al., 2017). SeaCrete structures are intended to last like concrete would. Another recently developed application is a coating based on ground shells called BESE-reef paste (Witte et al., 2024).

The current research aimed to determine the suitability of both the novel materials and conventional materials for European oyster settlement as a basis to ultimately restore and enhance native oyster beds at a large scale. To this end, equally-sized substrates of different materials were made available to flat oyster larvae in two different locations: in tanks (hatchery, Zeeschelp, Kamperland, The Netherlands) and in the field (Roz Flat, Bay of Brest, France). Resulting settlement densities (number/cm ^{2}) on the wide range of exposed settlement substrates were compared as a measure of flat oyster settlement preferences. This can inform decision makers in the selection of scour protection materials in windfarms that also will enhance oyster reef development.

2. Materials and methods

2.1. Selection of settlement substrates

Substrate selection was based on four criteria: (1) Processable: easy to incorporate in structures; (2) Structural integrity: meet its purpose, either durable or degradable; (3) Scalable: easy to obtain in high quantity and (4) Acceptable: avoid objections from society. Eleven different substrate materials were included in the settlement tests (Table 1). Granite and eclogite served as controls as they are already commonly used as scour protection material in offshore windfarms. Marble and limestone could serve as a potential nature-benefiting alternative. Additional substrates were selected that can be used for making add-ons, conventional materials with specific structures (3D-printed sandstone and concrete with a smooth and roughened surface) and substrates with eco-friendly quality – meaning causing little harm to the environment - (concrete with a coating of BESE-reef paste, hereafter referred to as reef paste, Geowall made of sandy dredged sediment, and bio-inspired SeaCrete). Details about the materials are provided in Table 1 and the text below.

Table 1
Specifications of the eleven different substrate materials tested for oyster settlement rates.

Usage	Material	Details
Conventional rock for scour protections	Granite (GN)	Norwegian quarry, density 2.6–2.7 c/cm^3
	Eclogite (EC)	Norwegian quarry, density 3.20 g/cm^3
Alternative calcareous rock for scour protections	Marble (MA)	Norwegian quarry, density 1.5–1.6 g/cm^3; >98 % $CaCO_3$
	Limestone (LI)	Belgian quarry, density 2.7 g/cm^3; 90–95 % $CaCO_3$
Conventional settlement add-ons	3D-printed sandstone (3D)	Mixture of 0.5 mm Alpine sand (70 %) and pozzalanic cement (30 %), and water (www.D-shape.com), density 2.4 g/cm^3
	Concrete-smooth (CS)	Mortar Weber Beamix 100, strength class C30/37 (www.nl.weber), density 2.1 g/cm^3
	Concrete-rough (CR)	Mortar Weber Beamix 100, strength class C30/37(www.nl.weber), density 2.4 g/cm^3
Eco-friendly settlement add-ons	Reef paste (RP)	Mixture of fine ground oyster shells (80 %) and binding adhesives (20 %) (www.bese-products.com), density 1.5 g/cm^3, pasted on smooth concrete
	Geowall – smooth (WS)	Sandy dredged sediment from the Western Scheldt (Netherlands) together with clay, gravel and a small proportion of cement (III/b) as binder, up to a density of 2.4 g/cm^3
	Geowall – rough (WR)	Sandy dredged sediment from the Western Scheldt (Netherlands) together with clay, gravel and a small proportion of cement (III/b) as binder, up to a density of 2.4 g/cm^3
	SeaCrete (SC)	Mixture of fine ground flat-oyster shell (75 wt%) and biobased binding adhesive (25 wt%) inspired on the sand castle worm glue, density 1.95 g/cm^3

2.2. Production of settlement substrates

Similar settlement tiles of 5x5x2 cm were fabricated for all materials, each having a 1 cm in diameter opening in its center for attachment purposes. Tiles of this size and shape are commonly used to determine settlement rates of larvae (e.g. Doropoulos et al., 2014; Hughes et al., 1999), and a similar shape allows for comparisons between the different materials. Tiles of granite, eclogite, limestone, and marble were made of quarried rocks, sliced into tiles using a mitre saw. Tiles of concrete were made of sieved (0.5 mm) concrete mortar, air-dryed in a smooth-surfaced wooden mold. The concrete-rough tiles were roughened during the drying process using a steel brush.

Reef paste tiles were made by coating concrete tiles with a layer of reef paste (BESE, Culemborg, the Netherlands) composed of fine ground oyster shells and binding material with the cured material consisting of >80 % calcium carbonate ($CaCO_3$; E170). The tiles of 3D-printed sandstone were fabricated using a binding jetting 3D printer.

In an attempt to re-use material dredging waste was used to produce settlement tiles. The tiles of GEOWALL® were made of sandy dredged sediment originated from the Western Scheldt (the Netherlands), pressed into blocks together with gravel, clay and a small proportion of cement as binder, up to a density of 2.4 g/m^3. The surface texture of the Geowall-rough tiles was more rough than the Geowall-smooth tiles due to a higher proportion of gravel in its mixture.

SeaCrete was produced by combining a complex coacervate underwater adhesive (similar to Wang and Schlenoff, 2014) and a 75 wt% ground aggregate, as flat oyster, *O. edulis,* shells. The adhesive consisted of two polymers, cationic poly(diallyldimethylammonium) chloride (Mw = 450 kDa) and anionic sodium poly(styrene sulfonate) (Mw = 70 kDa) at a molar charge ratio of 1.1: 1. More details about SeaCrete production can be found in Kodger et al., in prep).

In the field test in Bay of Brest, a scientific reference substrate was added consisting of aragonite sand coated by lime. The composition, the shape, the colour of this reference substrate is totally controlled and consequently very constant over time. It is used by Ifremer for several years to check if spatfall occurred during the experimental period and to compare recruitment with experiments carried out in other years (Pouvreau et al., 2024). The recruitment index on this reference must be greater than 50 individuals to validate the success of the experiment.

2.3. Experimental set up hatchery tests

In 2021, tiles of eight different substrates were attached to a structure of PVC tubes and secured with tie-wraps (Fig. 1A, Table 2) and in 2022 they were strung on ropes (Fig. 1B). The PVC frame and 4 ropes were suspended in 2000 L circular tanks in the hatchery of Zeeschelp ($51^0$35′50” N $3^0$41′06″ E) (Fig. 1C). The tanks were filled with filtered seawater (0.2 μm) and 3 million ready to settle *O. edulis* larvae. Air was supplied and seawater (temperature 20 °C and salinity 32) was exchanged continuously during the settlement period keeping the larvae in the tank with a banjo filter. In 2021 one tank was used and in 2022 two tanks (Table 2). Per experimental unit, the tiles were replicated for each substrate type to minimize idiosyncratic effects of flow/current or side of the tank. These were considered pseudo-replicates because they were all in the same tank, and statistical analyses were done at the level of the experimental unit ($n = 1$ in 2021, $n = 2$ in 2022).

2.4. Experimental setup field test in Bay of Brest

A protocol for testing a material for flat oyster recruitment in the field was developed as part of the FOREVER project (Pouvreau et al., 2021) and has since been validated and published (Colsoul et al., 2020; Potet et al., 2021). To ensure the best possible representativeness and complement studies carried out in the laboratory, the test protocol favors in situ trials (Colsoul et al., 2020). Environmentally and ecologically monitored for almost 10 years, the test site used in this study is

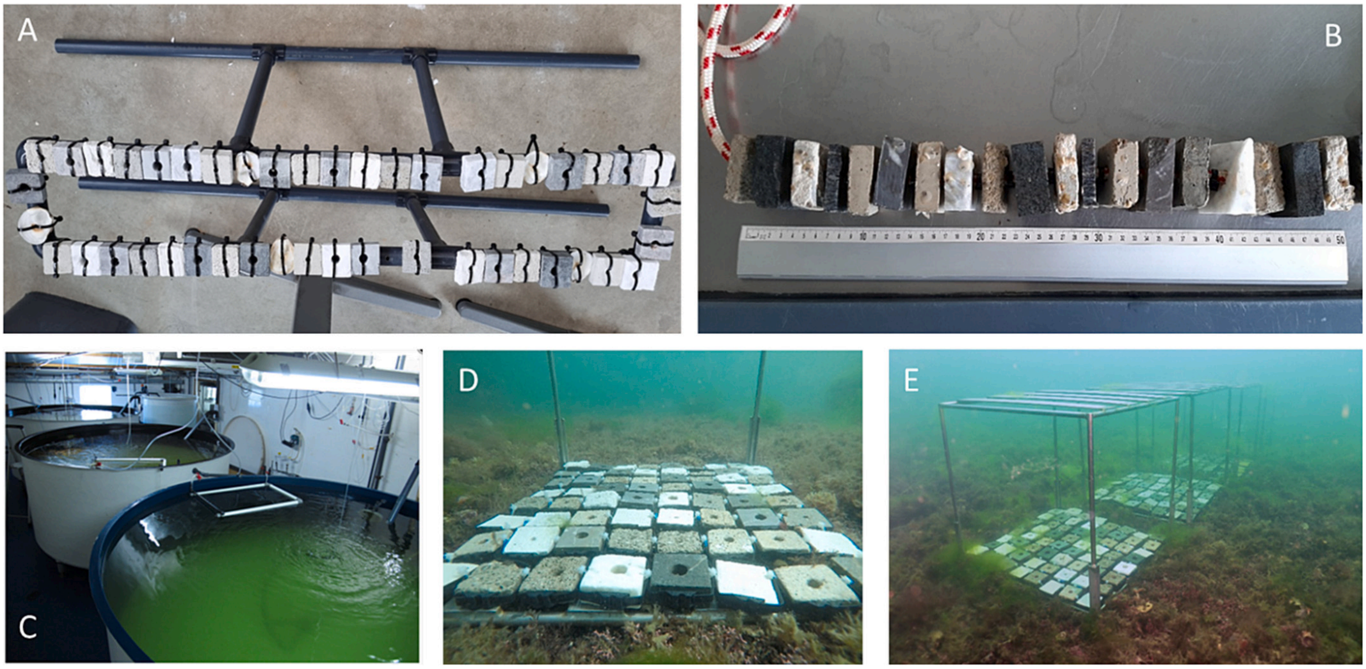

Fig. 1. Placement of substrates on PVC frame (A) and rope (B) for exposure in hatchery tank (C) and underwater view of the rack with substrates (D & E) in Bay of Brest of which two were used for the test.

Table 2
Overview settlement test. See Table 1 for substrate codes.

Date of introduction of the tiles	Date of retrieval of the tiles	Number of experimental units	Number of replicates per substrate	Type of substrates included
12 August 2021	28 September 2021	1 tank with 1 PVC structure	5	3D; CR; CS; EC; GN; LI; MA; RP
22 July 2022	24 August 2022	2 tanks with 2 ropes each	16 in tank 1 and 12 in tank 2	3D; CR; CS; EC; GN; LI; MA; RP
03 July 2023	26 July 2023	2 frames	5 per frame except for 9 REF, 6 CR, 4 3D, 6 EC frame #1, 4 GN frame #1	3D; CR; CS; EC; GN; LI; MA; RP; SC; WS; WR: REF

located in the Bay of Brest on a site dedicated to ecological restoration on the Banc du Roz (48^{0}19′29” N 4^{0}19′26” W). On this site, there is still a small population of flat oysters covering less than 10 ha at low density ($<$ 5 individuals/m^2) and 3–4 depth, that, by reproducing each year at the beginning of summer, provides a useful larval source for the field experiment such as this study.

The experimental protocol was as follows: the substrates to be tested were constructed to a similar size and placed horizontally on grids protected by metal frames, just above the seabed and close to the adult oysters present on the site (Fig. 1D). Each sample of material was tested in replicates ($n = 10$) and all samples were randomly positioned on a 50 cm square checkerboard grid containing 64 tiles, replicated in 2 units (Fig. 1E and Table 2). An additional substrate (REF) serving as a reference was also added to the substrates pool.

All these substrates were immersed on 3 July 2023 at the peak of the breeding season and left in place for three weeks. During this period the average water temperature was 19.6 °C and salinity 34.5. The precise timing of placement and retrieval was determined by monitoring water temperature and larval abundance. Prior to immersion in the field, the samples were allowed to develop a biofilm through soaking for a week in a seawater tank at the nearby Ifremer laboratory. In this tank, water temperature and salinity were kept very constant and close to field conditions: temperature was 20 °C and salinity 35 PSU.

2.5. *Counting of spat*

After each experiment, the substrates were transported to the laboratory for counting of the oyster spat. In the hatchery tests, all six sides of each tile were visually inspected and counted under microscope. In the field test only the top sides of the tiles were counted, as that was the only side exposed to seawater. After counting the numbers they were adjusted to numbers per cm^2 by dividing the total number by the surface area of the tile.

2.6. *Statistical analysis*

R version 4.2.2 was used for all statistical analyses and visualization (R Core Team, 2022). Graphs were plotted via the *ggplot2* package (Wickham, 2016). Normality and homogeneity of the residuals were checked via Shapiro-Wilk's and Levene's tests, respectively. As assumptions of these tests were violated, non-parametric variants were used. The datasets were split by year, generating a 2021 dataset (replicates: n = 1), a 2022 dataset and a 2023 dataset (for both, replicates: $n = 2$). As the 2021 dataset had only one replicate, the distribution of corrected spat numbers per substrate type was compared to a distribution if the chance of settlement was equal for all substrates. The comparison was done via a multinomial goodness-of-fit test with fixed probabilities by Monte-Carlo simulations via the *xmonte* function in the *Xnomial* package in R (Engels, 2015). The equal distribution was defined as a theoretical equal proportion of 1/8th of spat per substrate type, since there were eight substrate types in total.

For the 2022 and 2023 datasets, two replicate tanks or frames were available, and we could more explicitly test for differences between corrected spat numbers over the different substrates. As the assumption of homogeneity of variances was violated, a non-parametric permutation test was performed using the function *independence_test* as implemented in the *coin* package (Holthorn et al., 2008). Here, the main effect of type of substrate was tested. The *pairwisePermutationTest* function in the *rcompanion* package was used to perform of pairwise comparisons of substrate types (Mangiafico, 2015). Here, a false discovery rate (fdr) correction of the *p*-values was used to correct for multiple testing.

3. Results

Settlement of flat oyster larvae took place on all types of substrates but varied in density (Fig. 2 & 3). The distribution of spat numbers on the eight different substrates in the first hatchery test of 2021 did not significantly differ from an equal distribution (Log-likelihood ratio *p*-value = 0.084 ± 0.0009, Fig. 3 A). However, the substrate with the highest spat density (Reef paste, RP) had about four times as many spat as the substrate with the lowest number of spat (Eclogite, EC), with 2.42 spat/cm^2 versus 0.64 spat/cm^2, respectively. In the second hatchery test of 2022, the number of spat differed significantly between substrate types (maxT = 3.26, *p*-value = 0.009) (Fig. 3B). After correcting for multiple testing no pairwise comparison was significantly different. However, the trend differences between substrates were similar to the results of 2021 with Reef paste (RP) having the highest number of spat (0.52 spat/cm^2). Concrete Smooth (CS), Eclogite (EC) and Granite (GN) had the lowest number of spat (0.11–0.12 spat/cm^2). In the field test of 2023 (Fig. 3C), four new substrates were added (a reference tile (REF), SeaCrete (SC) and two types of Geowall (rough WR and smooth WS). In general, for all tiles densities of spat were higher than in the hatchery tests. The number of spat differed significantly between substrate types (maxT = 3.39, *p*-value = 0.008). After correcting for multiple testing, the test lacked sufficient power to show which substrates were significantly different from each other. SeaCrete seemed to be the best performing substrate with an average of 5.86 spat/cm^2, which was 6–10 times higher than the lowest spat density found on both Geowall substrates (0.53 spat/cm^2 for rough and 0.88 for smooth spat/cm^2). In contrast to the hatchery tests, concrete rough performed better than 3D printed sandstone and concrete smooth, while the order of the spat densities on other substrates was the same as in the hatchery tests.

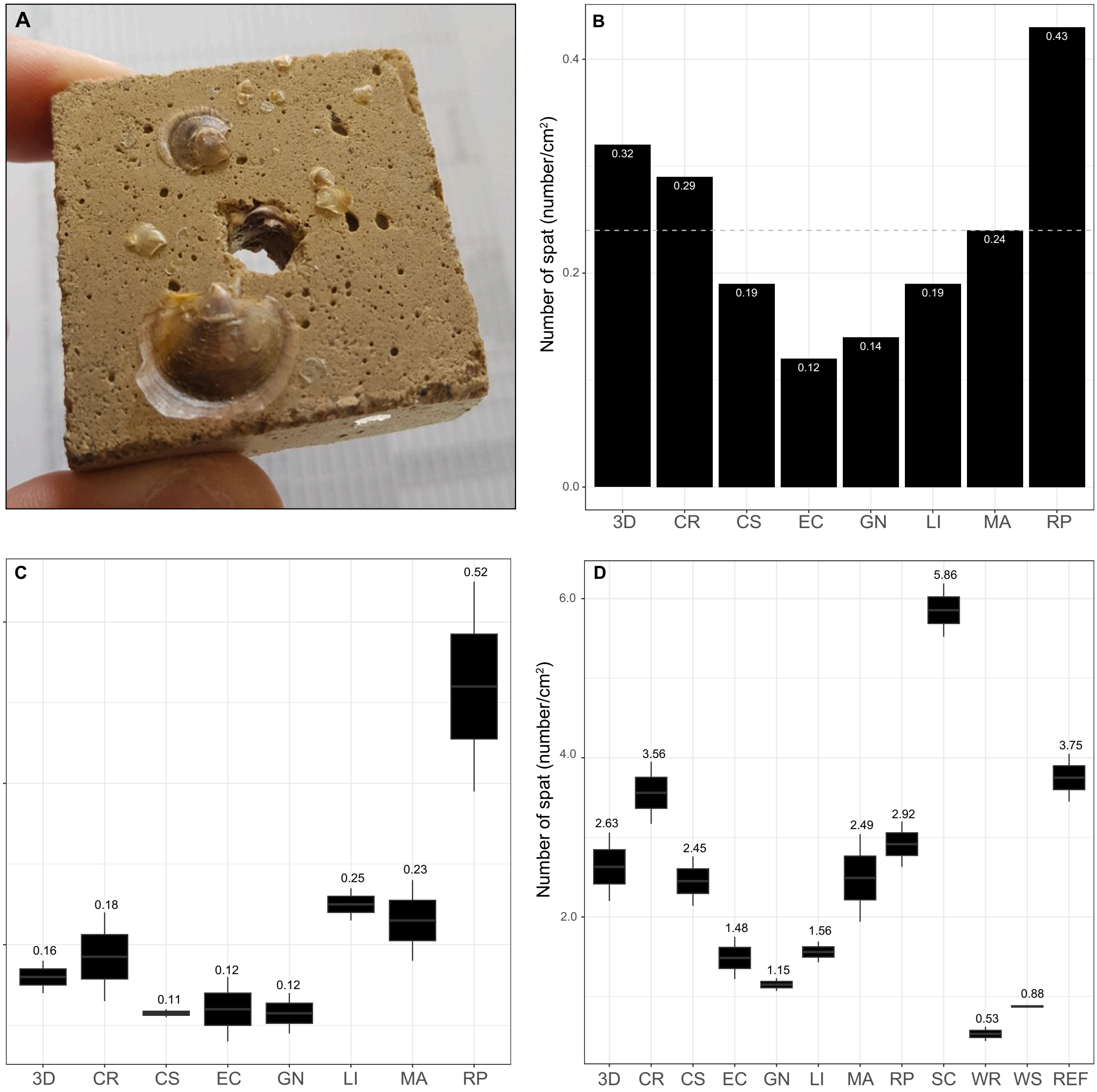

Fig. 2. (A) Concrete tile with settled flat oyster spat. B–D shows bar graph and box plots with number of *O. edulis* spat (#spat/cm^2) measured for different substrate types in (B) the hatchery test of 2021, (C) the hatchery test of 2022, and (D) the field test of 2023. Values display total number of spat (B) or mean number of spat (C and D). 3D = 3D printed sandstone; CR = concrete rough; CS = concrete smooth; EC = eclogite; GN = granite; LI = limestone; MA = marble; REF = scientific reference (2023 only), RP = reef paste, SC = SeaCrete (field only), WS = Geowall smooth (field only), WR = Geowall rough (field only).

4. Discussion

The different substrates tested in the present study showed significant differences in spat settlement density. Spat densities observed in the field test in Bay of Brest (0.5–6 spat per cm^2) were in the same range as earlier studies on that location (Colsoul et al., 2020: 2–4 spat per cm^2; Potet et al., 2021: 0.5–3 spat per cm^2). Comparison of the spat density on smooth and rough concrete of the present field test carried out in 2023 with the results obtained by Potet et al. (2021) with similar concrete substrates in the same area, in 2019, showed slightly higher spat densities in 2023 (2.5–3.5 spat/cm^2) compared to 2019 (1–2 spat/cm^2). A field test in Ireland with granite and concrete in 2019 yielded much lower spat densities (around 0.06 spat/cm^2) compared to the present field test in Brittany with 1–2.5 spat per cm^2. Comparison of spat settlement on the same substrates between locations and years is however not straightforward as the outcome heavily depends on the larval abundance present. The main purpose of the tests is to compare between substrates at the same location. Densities obtained in the hatchery tests were much lower. This is surprising as one would expect that exposure in a hatchery setting would be more effective than under field conditions. Larval abundance at Roz Bank has been monitored since 2012 using the protocol of Pouvreau (2015) and larval densities in the hatchery were much higher than in the field (1.5 million/m^3 and between 500 and 1000/m^3 respectively). However, when taking into account the continuous new supply of larvae in the field during the full exposure period of 3 weeks, the cumulative spat densities may have been higher in the field.

The best performing substrates were reef paste and SeaCrete, while eclogite, granite and Geowall had the lowest spat abundance. Both reef paste and SeaCrete contain fragments of oyster shell. Oyster shells are known to be a good settlement substrate for flat oyster larvae (e.g. van den Brink et al., 2020). Geowall was made from sandy dredged sediment from a highly urbanised estuary (Western Scheldt). The tiles may have contained toxic compounds, yet were not tested for this. Why eclogite and granite were less successful is unclear, though it should be noted that these stony substrates (as well as limestone and to a certain extent marble) had an extremely smooth surface texture (Fig. S2, Supplementary material) due to the slicing process when fabricating the tiles, which is generally considered unfavorable for oyster larvae settlement (Potet et al., 2021). It may also be harder for freshly settled larvae to maintain themselves on smooth substrate under the dynamic conditions in the Bay of Brest.

To effectively aid recruitment on oyster reefs, a detailed understanding of habitat selection and settlement cues for pelagic larvae is required. It is known that microhabitat selection is mediated by substrate colour (Herman, 1937) and micro-structure (Potet et al., 2021). Therefore, colour and rugosity of the substrates used in the field experiment was determined as a preliminary investigation whether these may have played a role in the results of the present experiment (see Supplementary material). Significant correlations with spat density were found for colour (Fig. S3) and rugosity (Fig. S4). However, a significant interaction effect between substrate colour and rugosity was also observed (Fig. S5). The experiment was not designed to study the effect of colour or rugosity, as a proper test of those factors would have required treatments in which the material of the substrate would be the same and only the colour or rugosity varied. However, the analysis suggests that material type has a greater impact on spat density than colour or rugosity.

Other factors that can enhance settlement of oysters are biofilms (Rodriguez-Perez et al., 2019) and chemical compounds such as glycoprotein (Vasquez et al., 2014). The present study did not include characterisation of differences in biofilms on the substrates and chemical composition of the substrates. Nevertheless, the clear preference for materials produced with oyster shell fragments indicates that chemicals in oyster shell are important. These should be fixed enough in a structure to prevent being easily resuspended and carried away by the currents or covered by sand. In addition, in restoration projects often the shapes of the substrate also play a role in reef functioning as these can provide shelter to relevant organisms. Reef paste can be used to coat any other substrate making it more attractive during the first 1–5 years until it dissolves. SeaCrete can be used to produce more permanent structures as it is not a coating but the biobased polymer glues shell fragments together. Both of these substrates are made with ocean-based waste materials from the food industry, giving them a lower environmental impact and making them suitable for use in marine protected environments such as Natura 2000 areas. The results of the present experiments show the short-term (3 weeks) suitability of settlement substrates. It is important to establish whether the tested substrates remain suitable over a longer periods (several month, several years). E.g. the novel substrates fundamentally differ in their purpose as reef paste is degradable and made to disappear in 1–5 years while SeaCrete is designed to stay. In addition, the focus of the present experiments was on comparing substrates of different materials and thus all substrates had the same two-dimensional shape. A three-dimensional shape of the materials is important for oyster survival in the long term. How both degradability and shape of the tested substrates will affect the persistence of oysters at a restoration location is still unknown.

4.1. Conclusion

The availability of suitable hard substrates for larval settlement is crucial for kick-starting and maintaining an oyster population. As natural substrates are in short supply in many locations, production and deployment of substrates is a good solution to help oyster reef restoration. Two novel settlement substrates (reef paste and SeaCrete) showed higher abundance of oyster spat in short term settlement experiments than more conventional materials. These results are promising and experiments should be conducted to test over at least a one-year period before selecting a substrate for large-scale European oyster restoration activities. It also is important to assess the cost-effectiveness of the materials including the carbon footprint and overall environmental impact of the substrates.

CRediT authorship contribution statement

Pauline Kamermans: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Conceptualization. **Fleur Anteau:** Writing – review & editing, Investigation. **Karin Didderen:** Writing – review & editing, Resources. **Remment ter Hofstede:** Writing – original draft, Methodology, Investigation, Conceptualization. **Yanhua Zhao:** Resources. **Awen Le Graet:** Investigation. **Diede Maas:** Writing – original draft, Visualization, Formal analysis. **Stephane Pouvreau:** Writing – original draft, Methodology, Investigation, Funding acquisition. **Sophie Valk:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Tim Wijgerde:** Writing – review & editing. **Abel Zempleni:** Investigation. **Thomas E. Kodger:** Writing – review & editing, Resources. **Tinka Murk:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Tinka Murk reports financial support was provided by Dutch Research Council. Stephane Pouvreau reports financial support was provided by French Office for Biodiversity. Thomas Kodger has patent pending to Thomas Kodger. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2025.107532.

Data availability

Data will be made available on request.

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