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Which concrete substrate suits you? *Ostrea edulis* larval preferences and implications for shellfish restoration in Europe

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Abstract :

The European flat oyster, *Ostrea edulis*, is an important ecosystem engineer that has been progressively disappearing from European coasts over the last century mainly due to overexploitation, habitat degradation and disease. It is now the subject of many conservation and restoration programs throughout Europe, including the Flat Oyster REcoVERy (FOREVER) project in France.

Protecting and managing the remaining populations has become a nature conservation priority because this species is able to build biogenic reefs, very specific habitats that provide many ecosystem functions and services. The availability of suitable hard substrates for larval fixation is a critical factor during this reef-building process. Although natural substrates are in short supply, production and deployment is an easy step to help oyster reef restoration.

The present study was carried out to improve artificial reef design and the composition of the concrete used to build them, focusing on the impact of concrete formulation and surface texture on larval settlement in the field. Nine bio-sourced concrete formulations and ten surface textures were evaluated. The number of settled larvae counted on each concrete substrate reflected their preferences, and results demonstrated that microscale surface texture has a greater impact on recruitment than concrete formulation, with larvae preferring to settle in depressions on a rough rock-like texture and avoiding flat, horizontal and exposed areas.

Physical and mechanical properties of the different formulations were also evaluated. Although they had almost no impact on recruitment, these results could be helpful for artificial reef-building (in terms of 3D design, durability, manufacture and deployment) and guide choices of materials that should be used and the proportion and granulometry of incorporated shells to obtain the best surface texture.

Highlights

► *Ostrea edulis* larval preferences for different substrates were evaluated in situ. ► Various bio-sourced concrete mix formulations and surface textures were tested. ► Incorporation of seashell by-products influence mechanical properties of concrete. ► Substrate texture has a greater impact on larval settlement than its formulation. ► Substrate micro-topography has direct implications for flat oyster restoration.

Keywords : *Ostrea edulis*, Larval recruitment, Artificial reef, Concrete, Seashell by-products, Restoration

1. Introduction

47 The flat oyster *Ostrea edulis* is a European native species that once covered vast
48 areas in the North Sea, on the Atlantic coast and in other European coastal waters
49 including the Mediterranean region (zu Ermgassen et al., 2020; Pogoda et al., 2019).
50 All these populations have been heavily fished by dredging over the last three
51 centuries. More recently, the development of parasitic diseases (due to the emergence
52 of *Marteilia* and *Bonamia*) combined with the proliferation of various predators
53 dangerous to the species (especially sea stars, sea bream and oyster drills) and many
54 human-induced stressors (pollutants, terrestrial outputs, coastal development) have
55 caused a dramatic decrease in the last remaining flat oyster populations (Duchêne et
56 al., 2015; Thurstan et al., 2013). Today, this species has disappeared from many
57 locations in Europe and is registered on the OSPAR (Oslo-Paris Convention for the
58 Protection of the Marine environment of the North-East Atlantic) list of threatened
59 and/or declining species (Pogoda et al., 2019). In France, the flat oyster is confined to
60 only a few localised environments, notably in Brittany and Normandy (Duchêne et al.,
61 2015). However, these residual populations continue to be subjected to a range of
62 threats that limit them still further, to the point that if no conservation and/or restoration
63 actions are taken soon, the species and its associated habitats could disappear
64 completely from French coasts.

65 On an ecological level, oysters are 'engineer species'. Like corals in tropical waters,
66 they build calcareous biogenic habitats (from clusters and aggregates to massive
67 reefs) that are favourable for many other organisms and thus increase the biodiversity
68 of the surrounding environment (other invertebrates, algae, fishes, etc.; e.g., Beck et
69 al., 2011). These reefs also provide many other ecosystem services, such as
70 promoting sedimentation, reducing turbidity and eutrophication and helping to prevent
71 coastal erosion by acting as breakwaters (Borsje et al., 2011; Meyer et al., 1997;

72 Salvador de Paiva et al., 2018). Finally, the flat oyster contributes to the local economy
73 through aquaculture, fishing and recreational activities. For all these reasons, many
74 projects are underway in Europe to preserve this species (Pogoda et al., 2019).

75 The European flat oyster is characterised by a biological cycle with a pelagic larval
76 stage. The reproductive period that includes a brooding phase starting generally in
77 June and ending in September (Bayne, 2017; Martin et al., 1995). Larvae can be
78 observed throughout the summer, but are generally more abundant in July on the
79 French Atlantic coast. After release by the brooding mothers, they develop in the water
80 column for 2 weeks. Then, at the end of their pelagic life, they swim to the bottom
81 searching for a substrate on which to settle. Oysters are able to settle on different types
82 of hard substrate such as rocks, gravel or muddy sand with cultch (Shelmerdine and
83 Leslie, 2009), but still have a preference for conspecific shells, especially those of living
84 congeners already present on the seabed. Indeed, motile spat have been observed to
85 settle preferentially on the growth rim of these shells (Kennedy and Roberts, 1999;
86 Korrynga, 1946). This behaviour offers some fitness advantages as it favours the
87 formation of aggregates that, after a long period without major disturbances, enlarge
88 to become new biogenic reefs, known as 'oyster beds'. The proximity of individuals
89 and cumulative recruitment conferred by the reef are important for reproductive
90 success, individual growth and survival, thus ensuring the self-sustainability of the reef
91 over time (Guy et al., 2018; Schulte et al., 2009).

92 Thus, the disappearance of flat oyster shells or other hard substrates from the
93 bottom due to dredging activities combined with the effects of other stressors
94 (environmental degradation, especially chemical contaminants and soil leaching
95 causing increased sedimentation in estuaries, diseases, predation, etc.) leads to a
96 progressive disappearance of the habitat favourable to the oyster (Beck et al., 2011;

97 Pogoda et al., 2019). It is now understood that one of the limiting factors for *O. edulis*
98 stock recovery on which we can easily act, is the availability of suitable hard substrate
99 material for oyster larval settlement (Smyth et al., 2018).

100 Since 2018, the Flat Oyster REcoVERY project (FOREVER) has been promoting
101 the reestablishment of native oysters in Brittany (France). This multi-partner project is
102 led by CRC (*Comité Régional de la Conchyliculture*) Bretagne Sud and involves
103 IFREMER (*Institut Français de Recherche pour l'Exploitation de la Mer*), ESITC (*Ecole*
104 *Supérieure d'Ingénieurs des Travaux de la Construction*) Caen, *Cochet*
105 *Environnement* and *CRC Bretagne Nord*. The project consists of (1) inventorying and
106 evaluating the status of the main wild flat oyster populations across Brittany, (2) making
107 detailed analysis of the two largest oyster beds in the bays of Brest and Quiberon to
108 improve understanding of flat oyster ecology and recruitment variability and to suggest
109 possible ways of improving recruitment, and (3) proposing practical measures for the
110 management of wild beds in partnership with members of the shellfish industry and
111 marine managers. In this last action, the development of artificial substrates and reefs
112 that preserve the seabed and promote the settlement of oyster larvae is an important
113 goal for restoration.

114 Many studies have already been conducted on settlement cues and the search by
115 pelagic marine invertebrate larvae for suitable substrates. Since larval settlement
116 constitutes a critical bottleneck in the life cycle of marine invertebrates, more insight
117 into these processes could significantly improve marine ecological restoration efforts.
118 It has been demonstrated that planktonic larvae are sensitive to a wide range of
119 environmental factors over their biological development: water temperature, salinity,
120 gravity and pressure (see reviews by Hidu and Haskin, 1978; Mann et al., 1991; Young,
121 1995), current and turbulence (e.g., Fuchs et al., 2015; Knights et al., 2006), sound

122 (e.g., Lillis et al., 2016), light (e.g., Vazquez and Young, 1998), chemical molecules
123 (Tamburri et al., 2008, 1996) and, finally, substrate nature at settlement (see review by
124 Hodin et al., 2018). These different cues are used partially or totally by larvae at
125 different ontogenic stages to assist them in feeding, protecting themselves, swimming
126 or diving, but also for settlement and habitat selection. Obviously, these cues differ
127 according to species: for instance, bivalves that adhere via a byssal thread tend to be
128 less discerning in their substrate preference compared with those that adhere with a
129 cement glue (Tamburri et al., 2008). In some cases, settlement can be very highly
130 specific, e.g., *Ostrea denselamellosa* larvae only use veneriid clam shells as their
131 settlement substrate (Noseworthy et al., 2016).

132 In the mid 20th century many studies were conducted on *O. edulis* settlement in an
133 attempt to sustain oyster culture (e.g., Korringa, 1941, and see Bayne, 2017, for a
134 recent exhaustive review). It was clearly shown that *O. edulis* larvae are able to attach
135 themselves to a wide range of hard substrates, but that they have a strong preference
136 for shells or coralline algae (e.g., Smyth et al., 2018). The reasons for this preference
137 are thought to include surface roughness and chemical composition (calcium
138 carbonate) (e.g., Cuadrado-Rica et al., 2016). Limestone, tiles or plates coated with
139 lime and other calcareous materials (sand and shell fragments), therefore constitute
140 efficient alternative substrates (e.g., Lok and Acarli, 2006). In contrast, smooth
141 surfaces such as very smooth pebbles, glass or seaweed are intrinsically unsuitable
142 for this species (Cole and Jones, 1939). Chemical cues are also involved in settlement,
143 especially molecules emitted by conspecific or prey species (Bayne, 1969; Rodriguez-
144 Perez et al., 2019) or by biofilms composed of bacteria, micro-algae and
145 exopolysaccharides (Hadfield, 2011; Tritar et al., 1992). Concerning artificial
146 substrates, Graham et al. (2017) showed that concrete is the most effective substrate

147 for *Crassostrea virginica*, being better than limestone rocks, oyster shells and river
148 pebbles. Anderson (1995) explained that the phenomenon of calcium hydroxide
149 leaching, which increases the surface alkalinity of concrete, combined with the
150 presence of a biofilm, would allow better recruitment of oyster larvae, while this
151 alkalinity is detrimental to the development of other invertebrate organisms (Lukens
152 and Selberg, 2004). In addition, Nguyen et al. (2013) and Cuadrado-Rica et al. (2016)
153 emphasised the potentially useful role of seashells in the conception of artificial
154 substrates.

155 In this context and with the aim of restoring native oyster beds, the goal of our study
156 was to find an optimal artificial substrate that would be both very attractive to *O. edulis*
157 larvae and easy to produce (e.g., Theuerkauf et al., 2015). Considering the settlement
158 preferences of the species for shell and calcareous material, it was decided to use
159 limestone concretes into which different amounts and grain sizes of oyster shells were
160 incorporated.

161 Then, taking the most attractive concrete-shell formulation, different moulds were
162 tested to identify larval preferences in terms of surface texture. Ultimately, the optimal
163 concrete formulation and surface texture will be used for artificial reef building, taking
164 into account that concrete also makes it possible to build massive and complex shapes
165 (Baine, 2001) without significant environmental consequences that other materials
166 may cause (Risso-de Faverney et al., 2010).

167 **2. Materials and Methods**

168 Our study was based on two complementary experiments carried out in 2018 and
169 2019 in two natural sites (bays of Brest and Quiberon, see 2.1) where there are still
170 living flat oyster beds. The first experiment (summer 2018) was designed to

171 characterise the most attractive concrete formulation for *O. edulis* larvae. It was
172 conducted in both sites, by submerging pavers composed of different formulations of
173 shell concrete. The second experiment (summer 2019) was designed to characterise
174 the most attractive surface texture for *O. edulis* larvae. It was conducted in the bay of
175 Brest only (where flat oyster recruitment is higher), by exposing concrete pavers
176 moulded with different surface textures. The same overall testing method was used in
177 both cases: (1) fabrication of pavers to be tested in spring, (2) testing pavers on-site
178 during the flat oyster reproductive season (which occurs in July) using a random
179 experimental design operated by scientific divers and (3) evaluating the performance
180 of each paver by counting larvae settled on the surface after 15 days of immersion (this
181 value is called 'recruitment density').

182 **2.1. Experimental sites**

183 Experiments to test substrates were conducted in two coastal environments in
184 Brittany (France) where *Ostrea edulis* is still present and reproduces each year in
185 summer: the bay of Brest (North Brittany) and the bay of Quiberon (South Brittany).

186 **Bay of Brest:** Located in north-western France, the bay of Brest is a semi-enclosed
187 macrotidal coastal ecosystem influenced by both freshwater inputs from rivers to the
188 east and fast-mixing exchanges with the Atlantic Ocean to the west. One of the most
189 productive areas within the bay of Brest is Daoulas bay, a very shallow embayment
190 (maximum depth 8 m) that is home to various aquaculture and fishing activities and is
191 also a protected marine area managed by Natura 2000. In the south-east part of this
192 bay, there is still a residual wild population of *Ostrea edulis* (Le Roz oyster bed) at 3–
193 4 m depth, which covers less than 10 hectares at low density (< 5 individuals/m²).
194 Experiments for the bay of Brest were conducted in the heart of this bed.

195 **Bay of Quiberon:** Quiberon bay, which is located on the south coast of Brittany,
196 France, is a semi-circular bay bordered on the west and south-west by a peninsula
197 extending approximately 12 km offshore. This bay is an important location for shellfish
198 aquaculture (around 80 shellfish growers with about 2500 hectares of shellfish farms).
199 Oyster production (6000 t Pacific oysters and 500 t flat oysters) is carried out in deep
200 water. On the west side of this bay, there are two beds of flat oysters managed by the
201 oyster farming profession to produce the spat necessary for farming. In these two beds,
202 oysters cover around 20 hectares at 2–6 m depth, at densities between 0 and 10
203 oysters/m².

204 **2.2. Raw materials**

205 Two families of formulations were used for the substrates: concretes in experiment
206 1 and a mortar (the formulation chosen based on the results of experiment 1) in
207 experiment 2. All the formulations were made up of the following compounds. The base
208 material was cement CEM III/A 52.5N, adapted to the marine environment, with a slag
209 content of more than 60%. Four types of aggregate were used: a limestone sand, a
210 siliceous sand, a limestone gravel and, as a substitute for some gravel and sand, oyster
211 shells (*Crassostrea gigas*) of different grain sizes. The density and water absorption
212 (at 24 hours; WA₂₄) of the various aggregates were determined according to standard
213 NF EN 1097-6 (2014) (see Table 1).

214 **Table 1. Characteristics of the main materials**

Aggregates	Density (ton/m³)	WA₂₄ (%)
Limestone sand < 4 mm	2.52 ± 0.02	2.80 ± 0.05
Siliceous sand < 4 mm	2.64 ± 0.05	2.50 ± 0.05

Limestone gravel 4–10 mm	2.49 ± 0.03	3.00 ± 0.03
Oyster Shell < 1 mm	2.27 ± 0.02	20.94 ± 0.06
Oyster Shell < 6 mm	2.56 ± 0.03	14.70 ± 0.04
Oyster Shell 6–10 mm	2.74 ± 0.04	4.50 ± 0.04

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2.3. Experiment 1: concrete formulation comparison

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In this first experiment, nine concrete formulations were developed and tested by adjusting the proportions of cement, sand, gravel and shell. The mixtures were made in a mixer with volume of 50 L and then poured into rectangular moulds of 28 x 24 x 3 cm. A vibrating table ensured the distribution and compaction of the concrete. A diamond saw was then used to cut the samples into 9 cm squares. All formulations are summarised in Table 2 and described below.

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– **Reference (without shell):** A reference concrete (called REF) with only limestone sand (60%, grain size < 4 mm) and gravel (40%, grain size 4–10 mm) and without shell was formulated to obtain an optimal granular skeleton (with a water/cement ratio of 0.5).

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– **Influence of oyster shell (grain size and proportion):** Based on this reference, oyster shells were incorporated by partly replacing the limestone sand and gravel. Three grain sizes of shell pieces were selected to replace the limestone aggregates: size class S1 consisted of powdered shells, with particles up to 1 mm, size class S2 consisted of shells fragments up to 6 mm and finally, size class S3 consisted of large shell fragments between 6 and 10 mm. They were incorporated at a fixed proportion of 20% by replacing the limestone (sand and gravel) according to the grain size, thus obtaining the formulations 20Shell-S1, 20Shell-S2 and

235 20Shell-S3, respectively. In order to determine the influence of shell proportion,
 236 three substitution rates were also compared: 20%, 35% and 50%. These
 237 formulations were made with the S2 size class (up to 6 mm), which gave the
 238 formulations 20Shell-S2, 35Shell-S2 and 50Shell-S2, respectively.

239 – ***Influence of siliceous sand:*** It was also decided to test whether the presence of
 240 silica in the formulation could have an impact on recruitment, since it is known that
 241 this compound plays a role during larval adhesion and fixation (in *Crassostrea*
 242 *virginica*, 6% of the adhesive is composed of silicon; Metzler et al., 2016). To study
 243 the effects of silica, a common siliceous sand (up to 3 mm) was used at a proportion
 244 of 15% by replacing the limestone sand in the reference concrete, giving the
 245 formulation REF-15Si, and in the formulation containing the greatest proportion of
 246 shell (50Shell-S2), giving the formulation 50Shell-15Si.

247 **Table 2. List of cement formulations tested in Experiment 1; W/C corresponds to the**
 248 **water/cement ratio for each formulation**

Study of:	Formulation name	Oyster shell (various granulometry)			W/C ratio
		S1 <1 mm	S2 <6 mm	S3 6–10 mm	
Reference	REF	-	-	-	0.5
Variation of proportion and oyster shell grain size	20Shell-S1*	20%	-	-	0.5
	20Shell-S2	-	20%	-	0.5
	20Shell-S3	-	-	20%	0.5
	35Shell-S2	-	35%	-	0.5
	50Shell-S2	-	50%	-	0.5
	50Shell	-	-	50%	0.5
Influence of siliceous sand	REF-15Si	-	-	-	0.5
	50Shell-15Si	-	50%	-	0.5

*20Shell-S1: '20Shell' correspond to the 20% proportion of shell and S1 to the shell grain size

249 These different formulations created a roughness gradient. Overall roughness
250 measurements were performed on an area of 8 x 8 cm² of each sample. A Keyence
251 VHX-6000 optical microscope equipped with a confocal measurement system was
252 used, allowing the reconstruction of relief images. Several parameters of the
253 microscope were calibrated: in-plane resolution, depth fields, brightness, numerical
254 aperture and wavelength for white light. To calibrate these parameters, we did a
255 repeatability test four times, with variation under 1%. Roughness was expressed by
256 the arithmetic mean height (Sa; eq.1), calculated as follows:

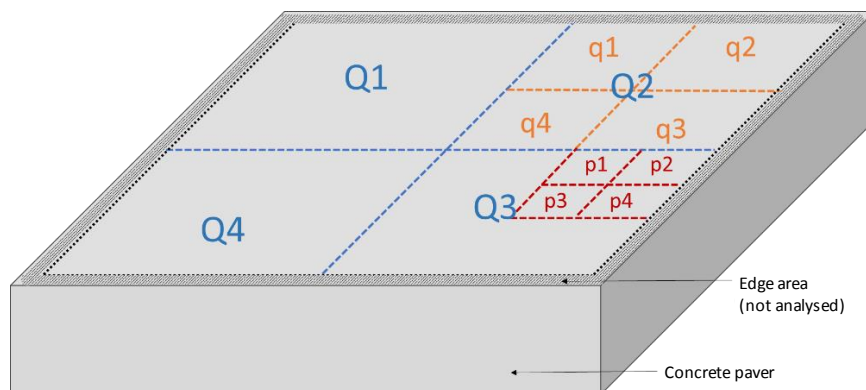
$$257 \quad Sa = \frac{1}{A} \iint Z(x, y) dx dy \quad (\text{Eq.1})$$

258 With Sa: Total surface area analysed; x, y = length and width of part of the study
259 area; A: area of the analysed surface with dimensions (x, y), and Z: mean height of the
260 analysed surface with dimensions (x, y).

261 For each concrete formulation obtained, the physical (water porosity and gas
262 permeability) and mechanical (compressive quality) properties were first tested in
263 triplicate. The water porosity test was realised according to EN 206 NF P18-459 (2010).
264 Gas permeability tests were realised with a Cembureau cell device according to EN
265 206 XP P18-463 (2011). Compression tests (results expressed as compressive
266 strength, σ_c) were realised according to EN 206 NF EN 12390-3 (2019).

267 These nine concrete formulations were then tested in the field for their biological
268 efficiency in terms of larval settlement. Testing was performed from 10 to 23 July 2018
269 in the bay of Brest and from 28 June to the 13 July 2018 in bay of Quiberon. On each
270 site, the 9 tested formulations (pavers of 9 x 9 x 2 cm³) were randomly positioned on
271 duplicate grids. See section 2.5 for details of the recruitment density assessment.

272 A relationship between roughness and recruitment was observed visually. It was
 273 therefore decided to carry out a detailed roughness analysis on the 50Shell formulation
 274 which had the highest recruitment density (see 3.1.3) and was also the roughest
 275 (supplementary data 1). To this end, the sample was divided into four zones measuring
 276 $4 \times 4 \text{ cm}^2$ called Q_i ($n = 4$). Each Q_i zone was then divided into four sub-areas of 2×2
 277 cm, called q_i ($n = 16$). Finally, the q_i sub-areas were also divided into four fractions of
 278 $1 \times 1 \text{ cm}^2$ called p_i ($n = 64$; Figure 1). Settled larvae were then counted in these pre-
 279 defined areas. Roughness measurements (expressed as S_a) were made using the
 280 Keyence microscope to reconstruct a 3D image by moving the working distance of the
 281 lens. Repeatability tests were performed with different lens zooms by varying the
 282 brightness and analysis step. The x50 zoom was chosen to obtain sufficient accuracy
 283 and repeatability despite variations in light intensity. In order to avoid edge effects, the
 284 periphery of the concrete paver samples was excluded from the analysis.



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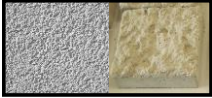
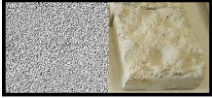



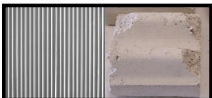

286 **Figure 1. Scheme representing the different scales of roughness analysis on the surface of a**
 287 **sample**

288 **2.4. Experiment 2: surface texture preference**

289 In the second experiment (conducted in 2019), it was decided to focus on surface
 290 texture of one specific formulation. Based on the results of experiment 1, the chosen

291 formulation was composed of a cement base (587 kg/m^3) to which two aggregates
292 were added: 1057 kg/m^3 of limestone sand and 762 kg/m^3 of crushed oyster shell
293 sieved at $500 \mu\text{m}$ (these two ingredients represented 58% and 42% of the aggregates,
294 respectively). The formulation was made in a 5-L mixer, to reach a water/cement ratio
295 (W/C) = 0.5, and then poured into moulds measuring $7 \times 7 \times 2 \text{ cm}^3$.

296 These moulds were then covered with ten different matrices, selected for their
297 differences in surface texture, with a gradient from the smoothest to the waviest. These
298 reusable elastic structural matrices are manufactured by Reckli®
299 (<https://www.reckli.com/fr/>). The ten selected matrices and their structural features, as
300 well as the substrates obtained with them are presented in Figure 2. The resulting
301 substrates made it possible to test larval settlement preferences for (1) more or less
302 smooth surface textures and (2) positions relative to the surface texture micro-topology
303 (on the tops, valleys or sidewalls).

- | | | |
|----|---|--|
| 1 |  | 2/81 Plafond: a smooth formliner without a pattern to create an absolutely smooth concrete surface. |
| 2 |  | 2/200 Sodingen: the functional formliner creates a smooth concrete surface with a fine sand look without noticeable elevations. |
| 3 |  | 2/157 Fichtelberg: a rock pattern with slight irregularities. Viewed as a whole, it still appears very even though. |
| 4 |  | 2/69 Marne: a pattern with a surface reminiscent of coarse roughcast. The granulation amounts to 16 mm. |
| 5 |  | 2/104 Sambesi: a pattern with a coarse roughcast look with a granulation of up to 8 mm. |
| 6 |  | 2/108 B Indus: vertically running ribs with a rough fractured pattern. Separated by u-shaped joints with a diameter of 10 mm. |
| 7 |  | 2/63 Wisla: vertically aligned u-shaped elevations with a smooth surface and a diameter of 10 mm. |
| 8 |  | 2/190 Ardenne: an abstract pattern with indented small plates that are stacked behind each other, creating a scale-like texture. |
| 9 |  | 2/94 Orinoco: a wave pattern with a wavy steel roof look, which is enlivened by the interplay of light and shadows. The distance between the elevations is 35 mm. |
| 10 |  | 1/36 RIB Type H: a clear and smooth rib pattern with vertically aligned ribs, separated by 15-millimeter wide joints. |

304

305 **Figure 2. Selected matrices for Experiment 2. Each matrix (left picture) and the resulting paver**
 306 **(right picture) are shown. Each matrix is then described, using the designation and features**
 307 **mentioned on the manufacturer's website.**

308 For each of the ten textures, nine square pavers were made. These were placed in
 309 seawater for 3 days to release any impurities, then fixed *in situ* on the grids by scientific
 310 divers. This experiment was conducted in the Bay of Brest only, with substrates left in
 311 place from 9 to 23 July, 2019. The substrates were randomly positioned on three grids,
 312 each grid supporting 30 pavers (3 pavers for each texture per grid). To resume, on
 313 each grid there were n = 3 pavers for a given texture, and n = 9 all grids combined.

314 In section 3.2.2, we paid particular attention to the geographical distribution of
315 settlement at two scales: between two juxtaposed pavers and within each individual
316 paver (micro-topography effects).

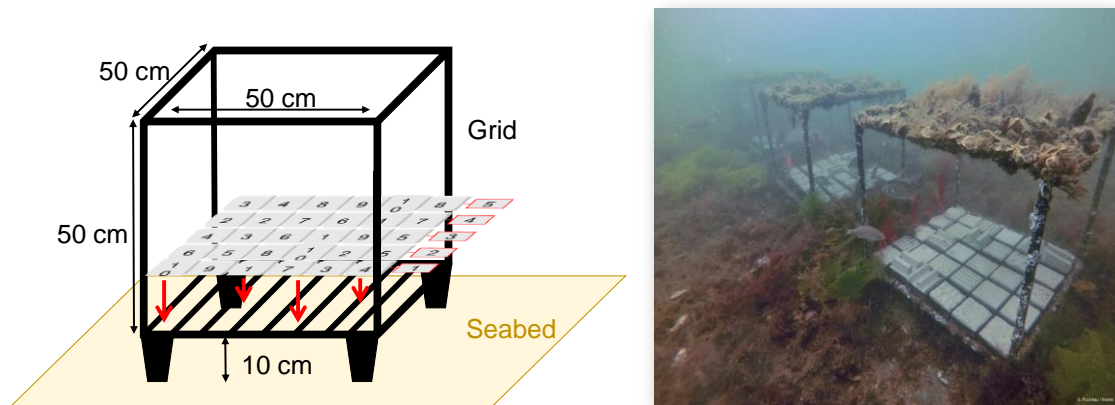
317 Besides classical recruitment, we also looked at the distribution of settled larvae on
318 textures with marked relief (n°7, 9 and 10). Here, three zone types were defined: top
319 (for the tops of the bumps), pit (for the valleys) and sidewall (for the slopes). The
320 number of settled larvae was assessed relative to the area of each of these zone types
321 on the pavers.

322 **2.5. Recruitment, larval counting and statistics**

323 Recruitment of flat oysters is known to be greatly affected by larval supply,
324 environmental conditions, transport processes, habitat selectivity and substrate
325 characteristics (Rodriguez et al., 1993), but also by competition, predation and other
326 post-settlement processes (Michener and Kenny, 1991).

327 In our study, we paid specific attention to standardising the protocol for assessing
328 recruitment. For both experiments, the tested substrates were handled by scientific
329 scuba divers who systematically placed them horizontally, just above the sea floor and
330 near adult oyster beds within each site (bays of Brest and Quiberon). In both
331 experiments, the pavers were randomly positioned on underwater grids using the R
332 'sample' function (Figure 3). Concerning exposure time, it is known that biogenic
333 modification of the substratum surface (especially the build-up of bacterial films) occurs
334 on new substrates within some days of their immersion and this step is a preliminary
335 phase essential for oyster settlement. Conversely, after a long period (> 1 month)
336 substrates accumulate too much biofouling and sediment deposits and thus become
337 progressively less suitable for oyster settlement. A period of 15 days of immersion

338 therefore constitutes an optimum compromise, allowing enough recruitment to
339 evaluate substrate differences. Substrates were immersed at the time when larval
340 concentration was around its maximum (from the end of June to the end of July,
341 depending on location and year; Pouvreau et al., in press) and seawater temperature
342 was around 20 °C (+/- 1 °C).



343

344 **Figure 3. Experimental design with pavers positioned on underwater grids**

345 After 15 days of immersion, the substrates were collected by scientific divers, gently
346 rinsed and dried at ambient temperature at the laboratory. Newly-settled recruits were
347 identified, counted and marked on the top sides of each paver by visual inspection
348 under the microscope (Keyence VHX 6000). This species is easily distinguishable and
349 confusion with other bivalve species can be avoided using morphological criteria.
350 *Ostrea edulis* post-larvae have a symmetrical umbo with two nearly identical shells.
351 The potential confusion with *Crassostrea gigas* post-larvae can be avoided because
352 the umbo of this other species has a 'twisted' asymmetry (Trimble et al., 2009).
353 Confusion with *Anomia ephippium* can be avoided because the inferior shell of this
354 species is almost glued on the substrate and thus invisible. For data analysis,
355 recruitment values were standardised to the number of individuals settled per cm² (total

356 area) within 15 days of exposure, which we refer to in this study as 'recruitment
357 density'.

358 After checking normality and homoscedasticity, the effect of experimental factors
359 was tested using permutational multivariate analysis of variance (PERMANOVA) or
360 Kruskal Wallis tests, depending on homoscedasticity and the number of experimental
361 factors considered (site and/or formulation in experiment 1; texture and/or grid/zone
362 type in experiment 2). Comparisons between conditions were made using pairwise
363 Student t test; *p-values* were corrected using the False Discovery Rate (Benjamini &
364 Hochberg method) and a *p-value* < 0.05 considered significant. The relationship
365 between roughness and recruitment was tested using linear models (adjusted R²).
366 Statistical analyses were made using R (R Development Core Team, 2005) software
367 version 3.5.3.

368 **3. Results**

369 **3.1. Experiment 1: concrete formulation comparison**

370 ***3.1.1. Microstructural characterisation***

371 The different formulations created a roughness gradient (see Supplementary Data
372 1). Roughness varied from Sa 362 to 1417 µm depending on formulation.

373 Incorporation of shell material is thought to significantly modify the properties of
374 concrete. The influence of shell grain size and proportion on porosity is shown in
375 Supplementary Data 2 for the six formulations containing shell.

376 Firstly, at the same rate of substitution (20%), we can observe a decrease in porosity
377 as the shell grain size increases from S1 (< 1 mm), through S2 (< 6 mm) to S3 (6–10
378 mm) (Supp. Data 2a). This phenomenon is due to the granular arrangement. The

379 compactness of the formulation increases with decreasing shell size, the finest
380 particles filling the porosity. Organisation of the aggregates of the largest shells does
381 not seem optimal and generates porosity within the materials.

382 Secondly, we studied the influence of shell substitution rate at 20%, 35% and 50%
383 for the same shell grain size (S2). We chose S2 because it was the intermediate
384 aggregate size. The porosity increases with increasing shell substitution rate (Supp.
385 Data 2b).

386 After studying the porosity, we also examined the gas permeability of the six shell-
387 containing formulations (Supplementary Data 3). The shell grain size seems to have
388 an impact on porosity as air permeability is ten times higher for the larger shell grain
389 sizes, S2 and S3 (Supp. Data 3a). The air permeability value for the smallest grain size
390 (S1), was the same as that of ordinary concrete. The greater the proportion of shell
391 incorporated into the formulation, the higher the permeability becomes (Supp. Data
392 3b).

393 Supp. Data 2b shows how porosity increases with the proportion of shell in the
394 concrete, and Supp Data 5b suggests that increased porosity leads to higher
395 permeability of the material.

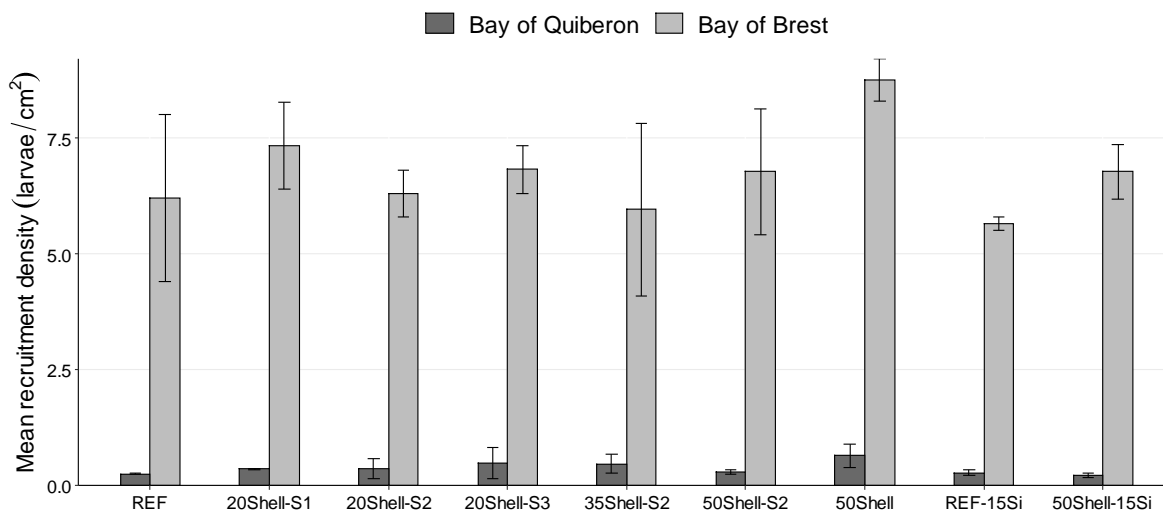
396 ***3.1.2. Mechanical properties***

397 Addition of shells to concrete significantly reduces its mechanical strength
398 (Supplementary Data 4). This fall appears to occur quite linearly with the increase in
399 shell proportion. It can be seen that with 50% of Shell-S2, the compressive strength of
400 the concrete is divided by almost three compared with the reference without shell.

401 The grain size of the shells also affected mechanical strength. While there was no
402 change in resistance between the S1 and S2 shell classes ($\sigma_c \approx 25$ MPa), the strength
403 with S3 was about 34 MPa, representing a relative gain of more than 30%.

404 **3.1.3. Formulation effect on larval settlement**

405 Flat oyster recruitment was systematically higher in the bay of Brest than in the bay
406 of Quiberon (PERMANOVA, $p = 0.001$; $F = 604.5$; $df = 1$), with a four-fold difference
407 between sites (Figure 4). However, there was no significant impact of formulation ($p =$
408 0.19 ; $F = 1.69$; $df = 8$), or of the interaction between site and formulation ($p = 0.38$; $F =$
409 1.16 ; $df = 8$) on recruitment.



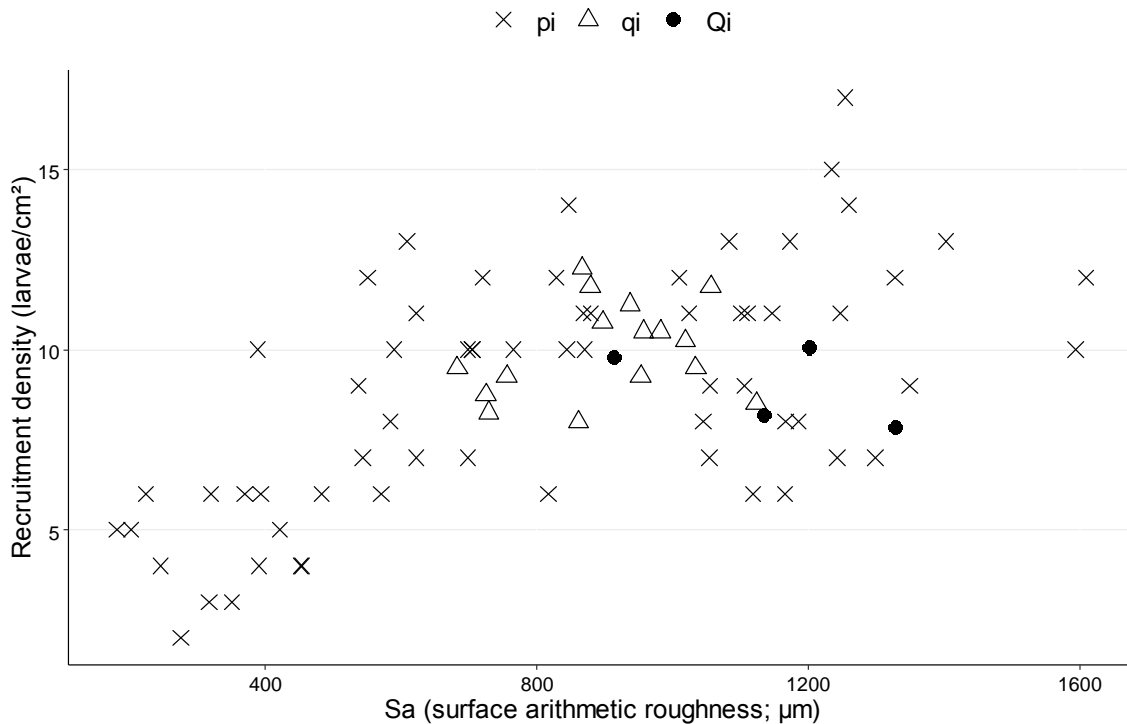
410
411 **Figure 4. Recruitment results (n° of newly settled flat oysters per cm²) after 15 days of**
412 **immersion at the sites (mean ± SD, n = 2)**

413 Whatever the source of these differences, the results per site are the same for the
414 overall ranking of the formulations despite significant variation between replicates. The
415 impact of concrete formulation on recruitment was non-significant according to
416 Kruskal-Wallis tests, whether we consider both sites simultaneously ($p = 0.88$; $\chi^2 = 3.7$,
417 $df = 8$), bay of Brest only ($p = 0.36$; $\chi^2 = 8.8$, $df = 8$) or bay of Quiberon only ($p = 0.38$;

418 $\chi^2 = 8.6$, $df = 8$). Shell grain size had no apparent effect on recruitment for 20Shell-S1,
419 20Shell-S2 and 20Shell-S3 and there was no significant relationship between the
420 proportion of shell in the concrete (20Shell-S2, 35Shell-S2 and 50Shell-S2) and the
421 recruitment observed. The REF and REF-15Si samples had similar recruitment, so the
422 presence of siliceous sand did not appear to have a significant influence. Finally,
423 50Shell stood out among the formulations, showing the highest recruitment values at
424 both sites.

425 **3.1.4. Small scale roughness**

426 A gradual roughness analysis was performed on the 50Shell sample by analysing
427 the first the four Q_i zones (largest scale), then the 16 q_i zones (intermediate scale) and
428 64 p_i zones (smallest scale; Figure 5). We chose the 50Shell formulation to perform
429 this analysis because it showed the highest roughness values ($S_a = 1417 \mu\text{m}$) and
430 reached the greatest recruitment values (Figure 4). For the largest areas (Q_i), all the
431 values are located randomly in the centre of the scatterplot, suggesting an absence of
432 any trend in the relationship between roughness and recruitment at this largest scale
433 (adjusted $R^2 = -0.05$). The values corresponding to the q_i zones are also very clustered,
434 with a roughness between 650 and 1100 μm and a corresponding recruitment density
435 between 8 and 12 individuals/cm² (adjusted $R^2 = 0.003$). However, the values
436 corresponding to the p_i zones form an extended scatterplot both in terms of roughness
437 (from 200 to 1600 μm) and in terms of recruitment (from 2 to 17 individuals/cm²),
438 suggesting an effect of roughness on recruitment that becomes observable at this
439 small scale as we approach dimensions similar to those of larvae (adjusted $R^2 = 0.36$).
440 This key result highlights that the surface texture of the substratum is probably a major
441 factor in settlement and which we then made the focus of our second experiment.



442

443

Figure 5. Recruitment density as a function of roughness (Sa) on the 50Shell sample at different analytical scales (pi = 1 x 1 cm²; qi = 2 x 2 cm²; Qi = 4 x 4 cm²)

444

445

3.2. Experiment 2: surface texture preference

446

In view of the results from experiment 1, we sought to understand the preferences of the larvae by reasoning on their scale by providing substrates with different moulded surface textures in experiment 2. The formulation used for this second experiment was only mortar, although the raw materials were the same than for experiment 1. This mortar formulation was used to assess the surface texture preferences for larval settlement.

451

452

3.2.1. Recruitment: general effects

453

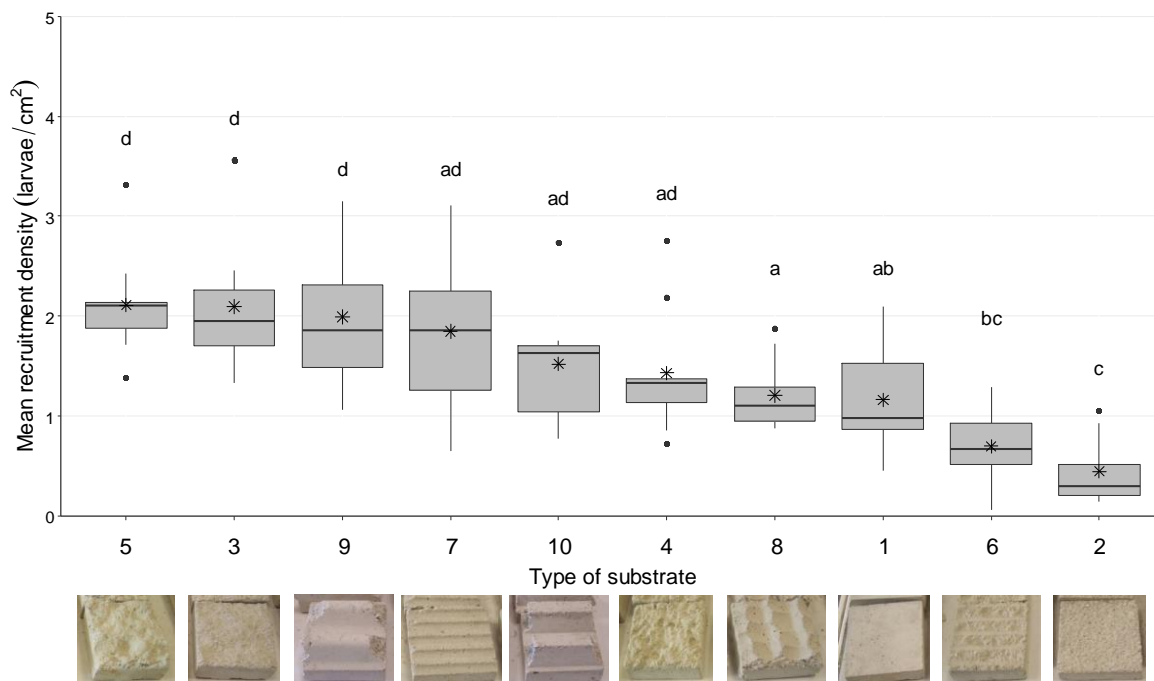
Surface texture had a significant effect on larval settlement, as shown by the comparative recruitment density (Figure 6; $p = 0.001$; $F = 9.43$; $df = 9$; all grids combined). The highest recruitment density values were obtained on textures n°5 and

455

456 n°3, with 2.1 larvae/cm², and the lowest on texture n°2 with 0.45 larvae/cm². The effect
 457 of surface texture alone could result in differences of up to a factor of 5.

458 Some of the textures (n°9, 7, 1 and 6) showed a high variance compared with the
 459 others (n°5, 3, 10, 4, 8 and 2).

460 The more wavy textures (n°9, 7 and 10) had a similar mean recruitment density (2,
 461 1.8 and 1.5 larvae/cm², respectively) and were grouped, as were the flattest textures
 462 (n°1, 6 and 2), which showed the lowest densities (1.2, 0.7 and 0.45 larvae/cm²,
 463 respectively).

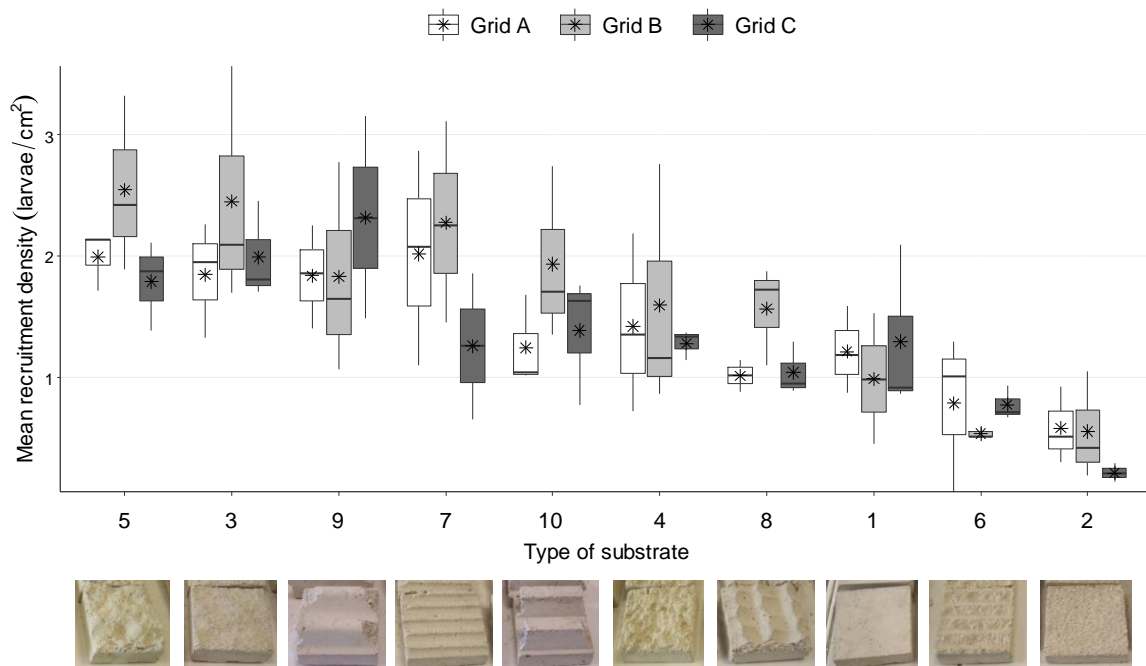


464

465 **Figure 6. Boxplot representing recruitment density for each texture (n = 9). The mean for each**
 466 **substrate is shown by a black star (n = 9). Outliers are indicated by circles. Substrates that do**
 467 **not share a common letter have a significantly different recruitment ($p < 0.05$; PERMANOVA**
 468 **followed by a pairwise Student t test for multiple comparisons)**

469 Figure 7 shows the results obtained by grid, for which there was no effect ($p = 0.13$;
 470 $F = 2.15$; $df = 2$) and no interactive effect of texture*grid ($p = 0.79$; $F = 0.71$; $df = 18$).
 471 Thus, there were no significant differences in recruitment density between

472 experimental grids, even though grid B seems to have slightly (though not significantly)
 473 higher values.



474

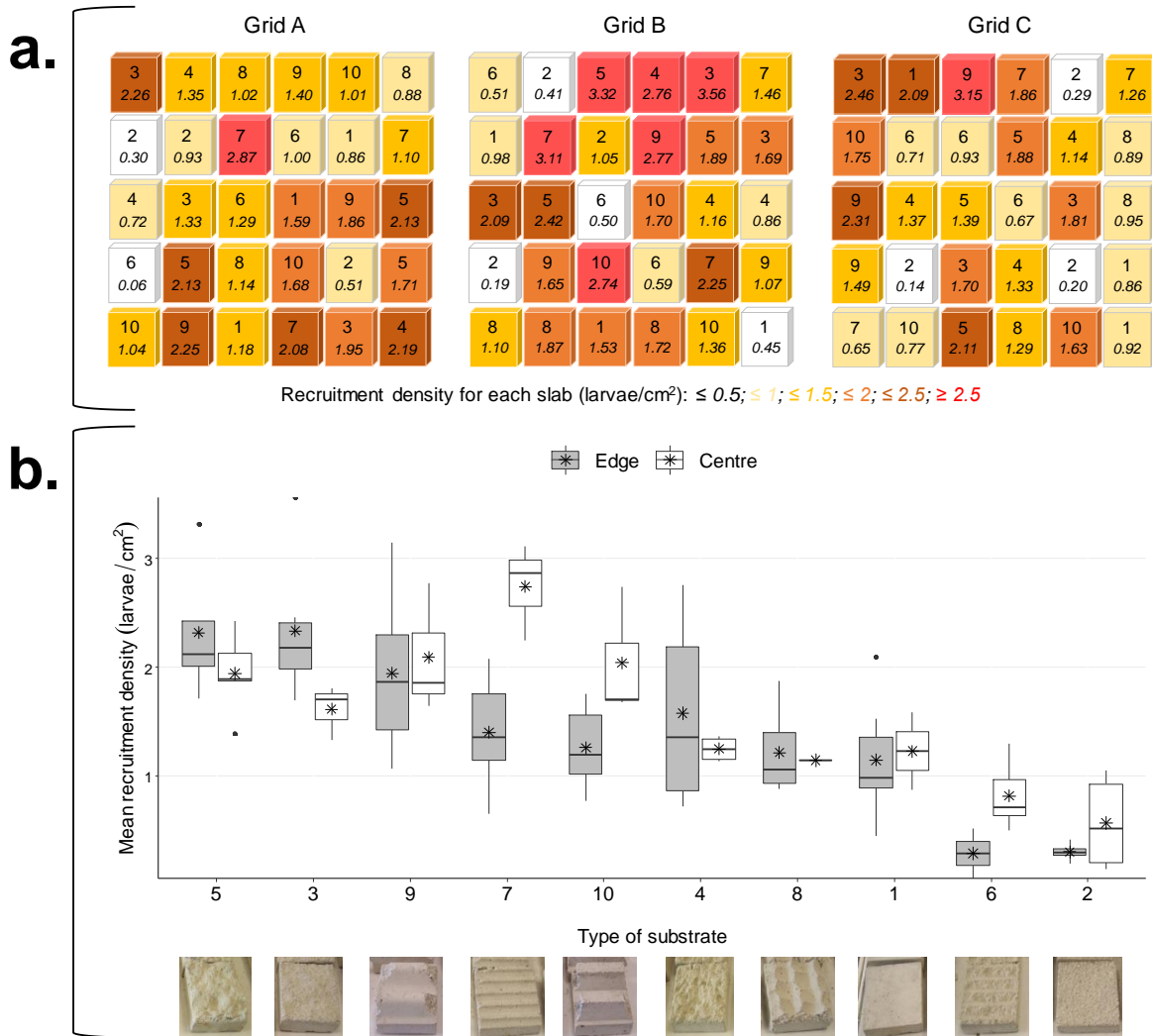
475 **Figure 7. Boxplot representing recruitment density for each texture (n = 3) on each grid (A in**
 476 **white, B in light grey, or C in dark grey). The mean value for each substrate is indicated by a**
 477 **black star**

478

3.2.2. Recruitment: larval distribution at different scales

479 Figure 8a shows strong inter-paver variability: two neighbouring pavers could have
 480 huge difference in recruitment density. When considering the position of the pavers on
 481 the grids (Figure 8b), no differences were observed between the pavers positioned on
 482 the perimeter and those positioned in the centre of the grids (Student t-test; $p = 0.98$;
 483 $t = -0.02$; $df = 88$). If both texture and paver position factors were integrated
 484 simultaneously, PERMANOVA indicated no influence of the position ($p = 0.18$, $F =$
 485 1.84 , $df = 1$), but a significant effect of texture ($p = 6.3910 \cdot 10^{-11}$, $F = 11.6$, $df = 9$) and of
 486 the interaction of both factors ($p = 0.01$, $F = 2.76$, $df = 9$). When looking at these
 487 combinations in detail, there was only a significant interaction between position and

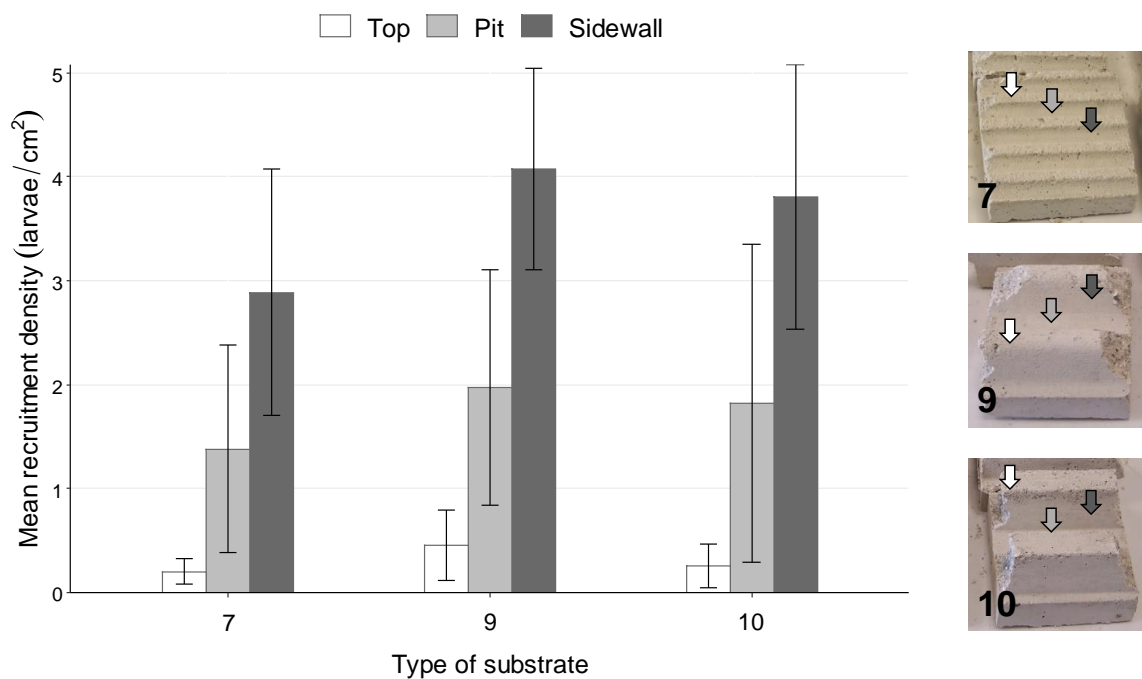
488 texture n° 7 ($p = 0.04$), with a higher recruitment density when this texture was
 489 positioned in the centre of the grid. This interaction did not appear with the other
 490 textures.



491
 492 **Figure 8. a) Random distribution of pavers on experimental grids. For a given paver, the top**
 493 **number is the texture reference and the bottom number the recruitment density. b) boxplot**
 494 **representing, for each texture, mean recruitment for pavers that were positioned on the edge**
 495 **(the perimeter) or in the centre of the grids**

496 The results also revealed intra-texture variability, with both high and low recruitment
 497 values for some textures, which explains the higher variance observed for some of
 498 them, especially for pavers with a highly wavy texture (n° 7, 9 and 10). For these

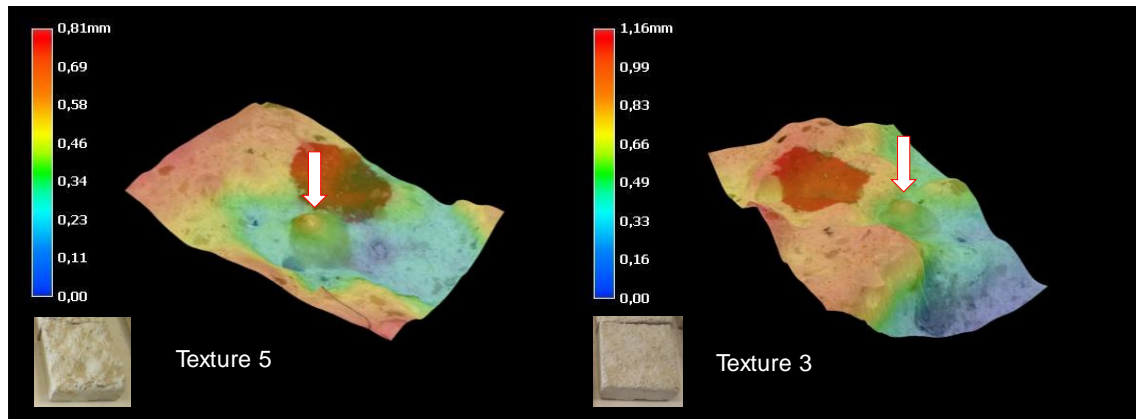
499 textures, a specific analysis was conducted on the repartition of larvae among three
 500 pre-defined zone types: top, pit and sidewall. Figure 9 shows this repartition: there is a
 501 significant effect of zone type on recruitment density ($p = 0.001$; $F = 75.38$; $df = 2$), with
 502 a strong larval preference for settlement on sidewalls (3.6 larvae/cm²) and in pits or
 503 valley bottoms (1.7 larvae/cm²), whereas settlement on exposed flat areas on the tops
 504 is very low (0.3 larvae/cm²). These results again demonstrate the effect of texture at a
 505 small scale (closer to oyster larva size).



506

507 **Figure 9. Barplot representing recruitment density (mean ± SD; n = 3) on each defined zone**
 508 **type (top, pit or sidewall) for pavers with highly wavy textures (n°7, 9, 10)**

509 Figure 10 illustrates this key observation. When larvae settle, they obviously avoid
 510 light exposition by fixing on more shadowed and protected areas, even with non-wavy
 511 textures such as n°5 and 3.



512

513 **Figure 10. 3D pictures made with a Keyence VHX-6000, showing larval preference for 'hiding in**
 514 **a hole'. The arrow indicates the position of the larva and the colour gradient indicates the**
 515 **depth (in mm) within the sample. Two textures were chosen for this illustration: texture n°5 (on**
 516 **the left) and texture n°3 (on the right)**

517 **4. Discussion**

518 Oyster bed restoration is mainly dependent on the availability of natural hard
 519 substrates and the creation of artificial ones (Kerckhof et al., 2018; Pogoda et al., 2019;
 520 Smyth et al., 2018). The selection of an efficient substrate is important both for larval
 521 fixation in open water and for commercial oyster culture (zu Ermgassen et al., 2020).

522 Oyster shells are used extensively as substrates for restoration but are in limited
 523 supply, so a variety of alternative substrates have been tested (George et al., 2015;
 524 Manning et al., 2019). For instance, Graham et al. (2017) evaluated recruitment
 525 efficiency on different materials for *Crassostrea virginica* larvae. They showed that
 526 concrete is the most efficient, ahead of limestone, oyster shells and finally river rocks
 527 (granite). A similar study was recently conducted for *O. edulis* (Colsoul et al., 2020) in
 528 the field and showed that settlement rates were significantly higher on baked clay,
 529 followed by slaked lime and bivalve shells, whereas wooden materials did not perform.

530 **4.1. Weak effects of formulation but strong effects of micro-**
531 **topography**

532 The formulations we tested were all based on a standard cement, to which different
533 grain sizes of aggregates (limestone sand, limestone gravel, siliceous sand) and/or
534 oyster shells were incorporated in variable proportions (20%, 35% and 50%).

535 Due to their different compositions, the nine formulations tested showed differences
536 in terms of porosity, air permeability and mechanical strength. We observed that
537 porosity and air permeability increased with the proportion of shell incorporated into
538 the concrete. As shell grain size increased, porosity decreased, whereas air
539 permeability increased. Shell proportion and grain size may thus influence the ability
540 of a concrete substrate to retain water and minerals, and to release compounds into
541 its surrounding environment. A higher granulometry provides a better mechanical
542 resistance, whereas a higher shell proportion tends to decrease mechanical
543 resistance.

544 Although testing differences between sites was not an objective of this study, one
545 explanation for the difference in recruitment between sites could be related to the
546 preparation of the samples. For the bay of Brest, the samples were 'conditioned' in
547 seawater by immersion in a tank for 7 days, while those from Quiberon bay were
548 directly tested at the site, due to time constraints. The significant differences in
549 recruitment between the two sites could partly be linked to differences in the release
550 of repellent substances by the concrete substrates over the first days of immersion
551 and/or the colonisation of their surfaces by favourable microorganisms (biofilm effects,
552 see below). Another explanation could be the reduced flushing time in the bay of

553 Quiberon, which could explain the lower recruitment despite higher larval densities for
554 this site.

555 Despite these differences in mechanical properties, the impact of formulation on
556 larval settlement was quite limited in our study, irrespective of the location of the test.

557 Several previous studies (e.g., Graham et al., 2017) have, in contrast, shown more
558 of an impact of substrate type on recruitment. Theuerkauf et al. (2015) showed that
559 juvenile recruitment of *C. virginica* was three times higher on Oyster Castles® made
560 with concrete and unconsolidated shell than on embedded shell. For *O. edulis* in the
561 field, Colsoul et al. (2020) showed a settlement preference for baked clay and slaked
562 lime over shell and wood substrates. In some other cases, results differed less among
563 substrates, e.g., George et al. (2015), who found no significant difference in spat
564 density between concrete, porcelain, limestone, river rock, oyster shell and bare
565 sediment, and Lok and Acarli (2006), who found no differences in recruitment between
566 oyster and mussel shell collectors.

567 In our case, the different formulations did not differ significantly from one another.
568 Some trends can nevertheless be observed (see Figure 4), and the lack of significance
569 might be due to the low number of replicates ($n = 2$). We must also recall that our
570 formulations all had the same cementitious base. Differences in terms of settlement
571 preference were consequently very low compared with those of Colsoul et al. (2020),
572 for example, who tested shell vs river rock or wood. In our study, even without
573 incorporating oyster shells (e.g., REF-15SI), recruitment remained high. The most
574 important compound appears to be cement, rather than the proportion of incorporated
575 shells or their granulometry. The work done here on concrete formulation can be useful
576 for human-made artificial substrates. It clearly shows that artificial substrates made of
577 concrete are suitable for flat oyster recruitment, even if the precise formulation varies

578 a little. The integration of limestone and oyster shells in variable proportions and grain
579 sizes into the cement should rather be considered in terms of mechanical
580 characteristics and practical optimisation (3D design, weight, durability, resistance,
581 etc.).

582 Previous studies have mostly used limestone aggregates and shells, but some have
583 also been done with siliceous materials. Metzler et al. (2016) showed a very high silica
584 content (6%) in the adhesive of *Crassostrea virginica* oysters compared with their shell
585 composition. However, in our case, we found that silica in the concrete formulation had
586 no influence on *O. edulis* larval fixation and so we did not consider this compound
587 further.

588 Of the formulations tested, best compromise seemed to be the 50Shell, for which
589 the recruitment density was the highest. Furthermore, this formulation offers good
590 mechanical resistance, which is a useful property for reef building. It is also one of the
591 roughest formulations, a parameter which is known to increase larval fixation in many
592 marine invertebrates (Kohler et al., 1999; Skinner and Coutinho, 2005; Su et al., 2007).
593 Surface characteristics, i.e., topography at a microscale, can play an important role in
594 larval settlement (Coombes et al., 2015; Hanlon et al., 2018) and we revealed a
595 positive correlation between roughness over 1 cm² (millimetre-scale) and recruitment
596 for this specific formulation. From a biological point of view, this roughness effect on
597 settlement demonstrated at a small scale (closer to the oyster larvae size) is
598 presumably linked to a 'protection' mechanism, i.e., camouflage, against predators
599 during the sensitive period of metamorphosis (George et al., 2015; O'Beirn et al.,
600 2000).

601 The 50Shell formulation was then modified slightly to maximise both recruitment and
602 mechanical properties before being used in the texture preference experiment

603 (experiment 2) and for future artificial reef building. The proportion of concrete was
604 increased to enhance surface alkalinity which is also known to play a role in recruitment
605 based on Anderson's work (1996). The proportion of shell was slightly reduced to
606 approximately 40% and grain size was reduced ($< 500 \mu\text{m}$); the remaining aggregate
607 was composed of limestone sand. This formulation made it possible to maximise both
608 porosity and mechanical resistance.

609 Contrary to the small differences observed for recruitment between formulations,
610 our second experiment revealed a significant impact of surface texture on recruitment,
611 demonstrating a micro-topography effect. Indeed, substrate texture produced up to 5-
612 fold increased settlement ($n^{\circ}5 = 2.1 \text{ larvae/cm}^2$; $n^{\circ}2 = 0.45 \text{ larvae/cm}^2$). Differences in
613 recruitment among the different surface textures demonstrate high larval selectivity, a
614 conclusion that is also supported by the heterogeneous distribution on experimental
615 grids: although the pavers were arranged very close to each other, differences could
616 be highly pronounced between neighbouring pavers. The preferred surface texture,
617 with a low variability, was $n^{\circ}5$. It is also interesting to note that this texture is the one
618 that looks most like natural stone.

619 The repartition of larvae on the waviest textures ($n^{\circ}7, 9$ and 10) revealed that larvae
620 prefer to settle on the sidewalls, but systematically avoid the tops. Being on the tops
621 would expose them to stronger water currents and presumably to predation; in the pits
622 and on sidewalls they are more protected. Moreover, several studies have shown a
623 negative phototaxis for oysters (Cole and Jones, 1939) and other invertebrate larvae
624 (Ells et al., 2016), which is in total agreement with our results.

625 Another explanation lies in the hydrodynamical properties of the substrate. The
626 shape, orientation and texture of the substrate are known to influence water flows over its
627 surface and thus larval attachment (Whitman and Reidenbach, 2012). Johnson (2017)

628 showed that the best profile for *C. virginica* larval settlement is intermediate concavity. On
629 the one hand, if the profile is too flat, water flows smoothly over it and particles (i.e., larvae)
630 are not retained. This might explain why, in our case, the flattest substrates (n°2, 6 and
631 1) were also those avoided by settling larvae. On the other hand, if the profile is highly
632 concave, particles make several circuits before being ejected. Rough textured
633 substrates, such as concrete, create more surface turbulence and increase settlement
634 compared with smooth pebbles, for example (Fuchs and Reidenbach, 2013). This
635 difference highlights the interest of such materials for artificial reef building.

636 It is also known that larval fixation is enhanced when substrates are vertical. For
637 instance, when collector lines are laid horizontally, so that the collectors are in a vertical
638 position, the attached *C. gigas* spat is significantly greater than in other orientations
639 (Lagarde et al., 2016). Oyster reefs tend to form vertically, which might increase long-
640 term survival of oysters by protecting them from predation and sedimentation (Soniati et
641 al., 2004) and could explain the preference for vertical settlement observed in the present
642 study.

643 In conclusion, we can say that the effects of water movement and larval behaviour
644 combine to influence settlement on different substrates. Besides chemotaxis, larvae may
645 also be subject to phototaxis and geotaxis, since they prefer to settle vertically in sheltered
646 concavities. All of these microscale properties are important to consider in restoration. In
647 our study, changing only the surface texture could increase *O. edulis* settlement by up to
648 five times.

649 **4.2. Perspectives for further research**

650 Although some major optimal characteristics for artificial reef building were identified
651 in this study (i.e., formulation and texture), other aspects could also be improved in the

652 future. Many questions on larval preferences at settlement remain to be answered that
653 would help to develop more efficient and ecologically-friendly artificial substrates for
654 oyster restoration. Nevertheless, it has been shown that the larvae actively select their
655 substrate by testing different areas before finding the right place to settle (Fuchs et al.,
656 2015; Zimmer-Faust and Tamburri, 1994), a finding also supported by our study.

657 Close relationships exist between the materials used for substrate formulation,
658 surface reactivity, biofilm formation and larval fixation (Anderson, 1996; Hadfield,
659 2011). As the aim of this study was to see to what extent different concrete formulations
660 and textures were favourable to oyster settlement, we did not evaluate the biofilm
661 communities here. We thus cannot conclude whether the larval preferences resulted
662 from (1) direct influences (Anderson and Underwood, 1994; Bavestrello et al., 2000)
663 such as chemical cues released from the formulations (experiment 1) or the paths
664 followed by larvae on different surface textures (experiment 2), or (2) effects mediated
665 via the development of biofilm communities (Keough and Raimondi, 1995), which may
666 also have differed depending on formulations (different components) or surface
667 textures (hollows, bumps, areas more or less exposed to light or water movement).
668 This aspect could be examined in greater depth in a future study.

669 Beyond the nature and micro-topography of the substrate, *O. edulis* larvae have
670 gregarious behaviour and settle according to cues representative of their habitat
671 requirements as adults (Rodriguez-Perez et al., 2019). Larval fixation particularly
672 occurs in response to chemical signals, which can be compounds that will induce a
673 specific behaviour or metamorphosis (reviewed by Pawlik, 1992). Signals can also be
674 linked to a response to the presence of conspecifics, with chemical signals inducing
675 fixation close to adults (for review see Burke, 1986; Zimmer-Faust and Tamburri,
676 1994). As mentioned above, bacterial and algal biofilms are also settlement cues

677 (Tamburri et al., 1992) and presumably aid settlement physically. Larvae using these
678 signals show active habitat selection. There is much evidence of the influence of
679 chemical signals on larvae from laboratory studies, but relatively little from the field,
680 and results can be contradictory between laboratory and field (Anderson, 1996). It
681 would be useful to clarify such behaviours in the field in order to identify optimal
682 restoration conditions, particularly concerning the choice of site locations.

683 Concerning chemical formulation, Manning et al. (2019) recently tested a new
684 formulation called NEC (Nutrient Enriched Concrete) on *Crassostrea virginica* larvae. They
685 integrated nutrients into concrete to stimulate biofilm and larval growth; they also added
686 pine sawdust, which helps to control predation. Such material is very attractive to larvae,
687 although attention must be paid to the proportion of each compound to avoid weakening
688 the structure.

689 Substrate colour also plays a role in larval fixation. Studies have shown larval phototaxis
690 and a preference for darker substrates (Wang et al., 2017) and for less bright areas (Ells
691 et al., 2016). This aspect should also therefore be tested in further studies as it is an easy
692 feature to modify on artificial substrates. We should perhaps consider using natural dyes,
693 e.g., cuttlefish ink, carbonised wood or even oyster-shell ashes that can replace lime and
694 simultaneously enhance the substrate's mechanical strength (Li et al., 2015).

695 Another aspect that should be addressed concerns the 3D shape, form, size and
696 height of an artificial reef. It is known that *O. edulis* physiological performances
697 increase when their distance from the seabed increases (Sawusdee et al., 2015),
698 related to increased food availability and water renewal increase with height due to
699 boundary layer properties, which improve growth and reproduction. Moreover, height
700 could also increase avoidance from predation, sedimentation and disease. Although
701 concrete substrates are a good alternative to the use of oyster shells, they are

702 generally large and heavy structures that require specialist transport, installation by
703 barge, and which occupy a large area on the seabed. To facilitate the widespread
704 distribution and installation of artificial substrates in coming years, it would be
705 interesting to conceive smaller supports that could just be thrown in the water, and
706 would work regardless of the way in which they fell. These would have a low footprint
707 on the bed, and could be made from eco-friendly materials through the development
708 of 3D printing, another promising avenue for native oyster restoration (e.g. Li et al.,
709 2020).

710 **Conclusion**

711 This study demonstrates that *Ostrea edulis* larval recruitment is influenced more by
712 substrate surface texture and micro-topography than by substrate formulation (within
713 the ranges tested here). The best formulation of the nine formulations tested was that
714 containing the highest proportion of shell (50%), although this trend was not significant.
715 This concrete provides good mechanical resistance and is also rougher than the other
716 formulations. Our results suggest that, overall, to enhance recruitment, the ideal
717 surface texture must have irregularities and slight concavities at a microscale.
718 Substrates should also be oriented so that the larvae can settle vertically. The optimal
719 support to maximise both larval recruitment and mechanical properties would be made
720 from a formulation containing aggregate made up of 60% limestone sand and 40%
721 crushed oyster shells sieved at 500 μm , with an irregular and rough texture, and should
722 be fixed vertically.

723 The formation of a natural reef, capable of resisting natural environmental stresses,
724 takes several years. For the moment, solitary oysters, fixed on small stones, are easily
725 displaced by currents, which precludes or slows down the reformation of dense banks.

726 Setting up protected areas in which a large number of small, stable, eco-friendly
727 supports could be deployed could significantly help to rebuild more resilient
728 populations. Restoration efforts must be maximised by deploying such substrates at
729 the right time and on the most favourable sites in terms of parental density, but also
730 considering hydrodynamic and physico-chemical parameters.

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737 **References**

- 738 Anderson, M.J., 1996. A chemical cue induces settlement of Sydney Rock Oysters,
739 *Saccostrea commercialis*, in the laboratory and in the field. *Biol. Bull.* 190, 350–
740 358. <https://doi.org/10.2307/1543027>
- 741 Anderson, M.J., 1995. Variations in biofilms colonizing artificial surfaces: seasonal
742 effects and effects of grazers. *J. Mar. Biol. Assoc. U. K.* 75, 705–714.
743 <https://doi.org/10.1017/S0025315400039114>
- 744 Anderson, M.J., Underwood, A.J., 1994. Effects of substratum on the recruitment and
745 development of an intertidal estuarine fouling assemblage. *J. Exp. Mar. Biol.*
746 *Ecol.* 184, 217–236. [https://doi.org/10.1016/0022-0981\(94\)90006-X](https://doi.org/10.1016/0022-0981(94)90006-X)
- 747 Baine, M., 2001. Artificial reefs: a review of their design, application, management and
748 performance. *Ocean Coast. Manag.* 44, 241–259.
749 [https://doi.org/10.1016/S0964-5691\(01\)00048-5](https://doi.org/10.1016/S0964-5691(01)00048-5)

750 Bavestrello, G., Bianchi, C.N., Calcinai, B., Cattaneo-Vietti, R., Cerrano, C., Morri, C.,
751 Puce, S., Sarà, M., 2000. Bio-mineralogy as a structuring factor for marine
752 epibenthic communities. *Mar. Ecol. Prog. Ser.* 193, 241–249.
753 <https://doi.org/10.3354/meps193241>

754 Bayne, B.L., 2017. *Biology of oysters*, Developments in aquaculture and fisheries
755 science. Academic Press, an imprint of Elsevier, London, United Kingdom ; San
756 Diego, CA, United States.

757 Bayne, B.L., 1969. The gregarious behaviour of the larvae of *Ostrea edulis* L. at
758 settlement. *J. Mar. Biol. Assoc. U. K.* 49, 327.
759 <https://doi.org/10.1017/S0025315400035943>

760 Beck, M.W., Brumbaugh, R.D., Airoidi, L., Carranza, A., Coen, L.D., Crawford, C.,
761 Defeo, O., Edgar, G.J., Hancock, B., Kay, M.C., Lenihan, H.S., Luckenbach,
762 M.W., Toropova, C.L., Zhang, G., Guo, X., 2011. Oyster reefs at risk and
763 recommendations for conservation, restoration, and management. *BioScience*
764 61, 107–116. <https://doi.org/10.1525/bio.2011.61.2.5>

765 Borsje, B.W., van Wesenbeeck, B.K., Dekker, F., Paalvast, P., Bouma, T.J., van
766 Katwijk, M.M., de Vries, M.B., 2011. How ecological engineering can serve in
767 coastal protection. *Ecol. Eng.* 37, 113–122.
768 <https://doi.org/10.1016/j.ecoleng.2010.11.027>

769 Burke, R.D., 1986. Pheromones and the gregarious settlement of marine invertebrate
770 larvae. *Bull. Mar. Sci.* 39, 9.

771 Cole, H.A., Jones, E.W.K., 1939. Some observations and experiments on the setting
772 behaviour of larvae of *Ostrea edulis*. *ICES J. Mar. Sci.* 14, 86–105.
773 <https://doi.org/10.1093/icesjms/14.1.86>

774 Colsoul, B., Pouvreau, S., Di Poi, C., Pouil, S., Merk, V., Peter, C., Boersma, M.,
775 Pogoda, B., 2020. Addressing critical limitations of oyster (*Ostrea edulis*)
776 restoration: identification of nature-based substrates for hatchery production
777 and recruitment in the field. *Aquat. Conserv.* in press.

778 Coombes, M.A., La Marca, E.C., Naylor, L.A., Thompson, R.C., 2015. Getting into the
779 groove: Opportunities to enhance the ecological value of hard coastal
780 infrastructure using fine-scale surface textures. *Ecol. Eng.* 77, 314–323.
781 <https://doi.org/10.1016/j.ecoleng.2015.01.032>

782 Cuadrado-Rica, H., Sebaibi, N., Boutouil, M., Boudart, B., 2016. Properties of ordinary
783 concretes incorporating crushed queen scallop shells. *Mater. Struct.* 49, 1805–
784 1816. <https://doi.org/10.1617/s11527-015-0613-7>

785 Duchêne, J., Bernard, I., Pouvreau, S., 2015. Vers un retour de l’huître indigène en
786 rade de Brest - PERLE. *Espèces* 51–57.

787 Ells, V., Filip, N., Bishop, C.D., DeMont, M.E., Smith-Palmer, T., Wyeth, R.C., 2016. A
788 true test of colour effects on marine invertebrate larval settlement. *J. Exp. Mar.*
789 *Biol. Ecol.* 483, 156–161. <https://doi.org/10.1016/j.jembe.2016.07.011>

790 EN 206 NF EN 12390-3, 2019. Essais pour béton durci - Partie 3 : résistance à la
791 compression des éprouvettes.

792 EN 206 NF P18-459, 2010. Béton - Essai pour béton durci - Essai de porosité et de
793 masse volumique.

794 EN 206 XP P18-463, 2011. Bétons - Essai de perméabilité aux gaz sur béton durci.

795 Fuchs, H.L., Gerbi, G.P., Hunter, E.J., Christman, A.J., Diez, F.J., 2015. Hydrodynamic
796 sensing and behavior by oyster larvae in turbulence and waves. *J. Exp. Biol.*
797 218, 1419–1432. <https://doi.org/10.1242/jeb.118562>

798 Fuchs, H.L., Reidenbach, M.A., 2013. Biophysical constraints on optimal patch lengths
799 for settlement of a reef-building bivalve. PLoS ONE 8, e71506.
800 <https://doi.org/10.1371/journal.pone.0071506>

801 George, L.M., De Santiago, K., Palmer, T.A., Beseres Pollack, J., 2015. Oyster reef
802 restoration: effect of alternative substrates on oyster recruitment and nekton
803 habitat use. J. Coast. Conserv. 19, 13–22. [https://doi.org/10.1007/s11852-014-](https://doi.org/10.1007/s11852-014-0351-y)
804 [0351-y](https://doi.org/10.1007/s11852-014-0351-y)

805 Graham, P.M., Palmer, T.A., Pollack, J.B., 2017. Oyster reef restoration: substrate
806 suitability may depend on specific restoration goals. Restor. Ecol. 25, 459–470.
807 <https://doi.org/10.1111/rec.12449>

808 Guy, C., Smyth, D., Roberts, D., 2018. The importance of population density and inter-
809 individual distance in conserving the European oyster *Ostrea edulis*. J. Mar.
810 Biol. Assoc. U. K. 1–7. <https://doi.org/10.1017/S0025315418000395>

811 Hadfield, M.G., 2011. Biofilms and marine invertebrate larvae: what bacteria produce
812 that larvae use to choose settlement sites. Annu. Rev. Mar. Sci. 3, 453–470.
813 <https://doi.org/10.1146/annurev-marine-120709-142753>

814 Hanlon, N., Firth, L.B., Knights, A.M., 2018. Time-dependent effects of orientation,
815 heterogeneity and composition determines benthic biological community
816 recruitment patterns on subtidal artificial structures. Ecol. Eng. 122, 219–228.
817 <https://doi.org/10.1016/j.ecoleng.2018.08.013>

818 Hidu, H., Haskin, H.H., 1978. Swimming speeds of oyster larvae *Crassostrea virginica*
819 in different salinities and temperatures. Estuaries 1, 252.
820 <https://doi.org/10.2307/1351527>

821 Hodin, J., Ferner, M.C., Heyland, A., Gaylord, B., 2018. Chapter 13 - I feel that! Fluid
822 dynamics and sensory aspects of larval settlement across scales, in: Carrier,

823 T.J., Reitzel, A.M., Heyland, A. (Eds.), Evolutionary Ecology of Marine
824 Invertebrate Larvae. Oxford University Press, pp. 190–207.

825 Johnson, K.B., 2017. Laboratory settlement of the eastern oyster *Crassostrea virginica*
826 Influenced by substratum concavity, orientation, and tertiary arrangement. J.
827 Shellfish Res. 36, 315–324. <https://doi.org/10.2983/035.036.0203>

828 Kennedy, R.J., Roberts, D., 1999. A survey of the current status of the flat oyster
829 *Ostrea edulis* in Strangford Lough, Northern Ireland, with a view to the
830 restoration of its oyster beds. Biol. Environ. Proc. R. Ir. Acad. 99B, 79–88.

831 Keough, M.J., Raimondi, P.T., 1995. Responses of settling invertebrate larvae to
832 bioorganic films: effects of different types of films. J. Exp. Mar. Biol. Ecol. 185,
833 235–253. [https://doi.org/10.1016/0022-0981\(94\)00154-6](https://doi.org/10.1016/0022-0981(94)00154-6)

834 Kerckhof, F., Coolen, J.W.P., Rumes, B., Degraer, S., 2018. Recent findings of wild
835 European flat oysters *Ostrea edulis* (Linnaeus, 1758) in Belgian and Dutch
836 offshore waters: new perspectives for offshore oyster reef restoration in the
837 southern North Sea. Belg. J. Zool. 148. <https://doi.org/10.26496/bjz.2018.16>

838 Knights, A.M., Crowe, T.P., Burnell, G., 2006. Mechanisms of larval transport: vertical
839 distribution of bivalve larvae varies with tidal conditions. Mar. Ecol. Prog. Ser.
840 326, 167–174. <https://doi.org/10.3354/meps326167>

841 Kohler, J., Hansen, P.D., Wahl, M., 1999. Colonization patterns at the substratum-
842 water interface: how does surface microtopography influence recruitment
843 patterns of sessile organisms? Biofouling 14, 237–248.
844 <https://doi.org/10.1080/08927019909378415>

845 Korringa, P., 1946. A revival of natural oyster beds? Nature 158, 586–587.
846 <https://doi.org/10.1038/158586d0>

847 Korringa, P., 1941. Experiments and observations on swarming, pelagic life and setting
848 in the European flat oyster *Ostrea edulis* L. Archives Néerlandaises de zoologie,
849 Amsterdam.

850 Lagarde, F., Fiandrino, A., Richard, M., Bernard, I., 2016. Déterminisme du
851 recrutement larvaire de l'huître creuse *Crassostrea gigas* dans la lagune de
852 Thau. IFREMER.

853 Li, G., Xu, X., Chen, E., Fan, J., Xiong, G., 2015. Properties of cement-based bricks
854 with oyster-shells ash. J. Clean. Prod. 91, 279–287.
855 <https://doi.org/10.1016/j.jclepro.2014.12.023>

856 Lillis, A., Bohnenstiehl, D., Peters, J.W., Eggleston, D., 2016. Variation in habitat
857 soundscape characteristics influences settlement of a reef-building coral. PeerJ
858 4, e2557. <https://doi.org/10.7717/peerj.2557>

859 Lok, A., Acarli, S., 2006. Preliminary study of settlement of flat oyster (*Ostrea edulis*
860 L.) on oyster and mussel shell collectors. Isr. J. Aquac. - Bamidgeh 58, 105–
861 115.

862 Lukens, R.R., Selberg, C., 2004. Guidelines for marine artificial reef materials. (No.
863 121). Gulf and Atlantic States Marine Fisheries Commissions.

864 Ly O., Yoris-Nobile A.I., Sebaibi N., Blanco-Fernandez E., Boutouil M., Castro-Fresno
865 D., Hall A.E., Herbert R.J.H, Deboucha W., Reis B., Franco J.N., Teresa
866 Borges M., Sousa-Pinto I., van der Linden P., Stafford R., 2020. Optimisation
867 of 3D printed concrete for artificial reefs: Biofouling and mechanical analysis.
868 Constr. Build. Mater. <https://doi.org/10.1016/j.conbuildmat.2020.121649>

869 MacDonald, J., Freer, A., Cusack, M., 2010. Attachment of oysters to natural substrata
870 by biologically induced marine carbonate cement. Mar. Biol. 157, 2087–2095.
871 <https://doi.org/10.1007/s00227-010-1476-7>

872 Mann, R., Campos, B.M., Luckenbach, M.W., 1991. Swimming rate and responses of
873 larvae of three mactrid bivalves to salinity discontinuities. *Mar. Ecol. Prog. Ser.*
874 68, 257–269. <https://doi.org/10.3354/meps068257>

875 Manning, T.J., Lane, W., Williams, R.D., Cowan, M., Diaz, M., Slaton, C.A., MacKey,
876 K., Patel, P., Plummer, S., Butler, B., Baker, T., 2019. The use of microbial
877 coatings, nutrients and chemical defense systems in oyster restoration. *Mar.*
878 *Technol. Soc. J.* 53, 39–54. <https://doi.org/10.4031/MTSJ.53.4.2>

879 Martin, A.-G., Littaye-Mariette, A., Langlade, A., Allenou, J.P., 1995. Cycle de
880 reproduction naturelle de l’huître plate *Ostrea edulis*, in: *La reproduction*
881 *naturelle et contrôlée des bivalves cultivés en France*. Nantes (France), pp. 21–
882 33.

883 Metzler, R.A., Rist, R., Alberts, E., Kenny, P., Wilker, J.J., 2016. Composition and
884 structure of Oyster adhesive reveals heterogeneous materials properties in a
885 biological composite. *Adv. Funct. Mater.* 26, 6814–6821.
886 <https://doi.org/10.1002/adfm.201602348>

887 Meyer, D.L., Townsend, E.C., Thayer, G.W., 1997. Stabilization and erosion control
888 value of oyster cultch for intertidal marsh. *Restor. Ecol.* 5, 93–99.
889 <https://doi.org/10.1046/j.1526-100X.1997.09710.x>

890 Michener, W.K., Kenny, P.D., 1991. Spatial and temporal patterns of *Crassostrea*
891 *virginica* (Gmelin) recruitment: relationship to scale and substratum. *J. Exp.*
892 *Mar. Biol. Ecol.* 154, 97–121. [https://doi.org/10.1016/0022-0981\(91\)90077-A](https://doi.org/10.1016/0022-0981(91)90077-A)

893 NF EN 1097-6, 2014. Essais pour déterminer les caractéristiques mécaniques et
894 physiques des granulats - Partie 6 : détermination de la masse volumique réelle
895 et du coefficient d’absorption d’eau.

896 Nguyen, D.H., Boutouil, M., Sebaibi, N., Leleyter, L., Baraud, F., 2013. Valorization of
897 seashell by-products in pervious concrete pavers. *Constr. Build. Mater.* 49,
898 151–160. <https://doi.org/10.1016/j.conbuildmat.2013.08.017>

899 Noseworthy, R.G., Lee, H.-J., Choi, S.-D., Choi, K.-S., 2016. Unique substrate
900 preference of *Ostrea denselamellosa* Lischke, 1869 (Mollusca: Ostreidae) at
901 Haechang Bay, on the south coast of Korea. *Korean J. Malacol.* 32, 31–36.
902 <https://doi.org/10.9710/kjm.2016.32.1.31>

903 O’Beirn, F.X., Luckenbach, M.W., Nestlerode, J.A., Coates, G.M., 2000. Toward
904 design criteria in constructed oyster reefs: oyster recruitment as a function of
905 substrate type and tidal height. *J. Shellfish Res.* 19, 387–395.

906 Pawlik, J.R., 1992. Chemical ecology of the settlement of benthic marine invertebrates.
907 *Oceanogr. Mar. Biol. - Annu. Rev.* 30, 273–335.

908 Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P.,
909 Sanderson, W., von Nordheim, H., 2019. The Native Oyster Restoration
910 Alliance (NORA) and the Berlin Oyster Recommendation: bringing back a key
911 ecosystem engineer by developing and supporting best practice in Europe.
912 *Aquat. Living Resour.* 32, 13. <https://doi.org/10.1051/alr/2019012>

913 Pouvreau S., Cochet H., Fabien A., Arzul I., Lapègue S., Gachelin S., Salaun, B. (in
914 press). Inventaire, diagnostic écologique et restauration des principaux bancs
915 d’huitres plates en Bretagne : le projet FOREVER. Rapport Final. RBE/PFOM/PI
916 2021-1

917 R Development Core Team, 2005. R: A language and environment for statistical
918 computing. R Foundation for Statistical Computing, Vienna, Austria.

919 Risso-de Faverney, C., Guibbolini-Sabatier, M.E., Francour, P., 2010. An
920 ecotoxicological approach with transplanted mussels (*Mytilus galloprovincialis*)

921 for assessing the impact of tyre reefs immersed along the NW Mediterranean
922 Sea. Mar. Environ. Res. 70, 87–94.
923 <https://doi.org/10.1016/j.marenvres.2010.03.007>

924 Rodriguez, S.R., Ojeda, F.P., Inestrosa, N.C., 1993. Settlement of benthic marine
925 invertebrates. Mar. Ecol. Prog. Ser. 97, 193–207.
926 <https://doi.org/10.3354/meps097193>

927 Rodriguez-Perez, A., James, M., Donnan, D.W., Henry, T.B., Møller, L.F., Sanderson,
928 W.G., 2019. Conservation and restoration of a keystone species: Understanding
929 the settlement preferences of the European oyster (*Ostrea edulis*). Mar. Pollut.
930 Bull. 138, 312–321. <https://doi.org/10.1016/j.marpolbul.2018.11.032>

931 Salvador de Paiva, J.N., Walles, B., Ysebaert, T., Bouma, T.J., 2018. Understanding
932 the conditionality of ecosystem services: The effect of tidal flat morphology and
933 oyster reef characteristics on sediment stabilization by oyster reefs. Ecol. Eng.
934 112, 89–95. <https://doi.org/10.1016/j.ecoleng.2017.12.020>

935 Sawusdee, A., Jensen, A.C., Collins, K.J., Hauton, C., 2015. Improvements in the
936 physiological performance of European flat oysters *Ostrea edulis* (Linnaeus,
937 1758) cultured on elevated reef structures: Implications for oyster restoration.
938 Aquaculture 444, 41–48. <https://doi.org/10.1016/j.aquaculture.2015.03.022>

939 Schulte, D.M., Burke, R.P., Lipcius, R.N., 2009. Unprecedented restoration of a native
940 oyster metapopulation. Science 325, 1124–1128.
941 <https://doi.org/10.1126/science.1176516>

942 Shelmerdine, R.L., Leslie, B., 2009. Restocking of the native oyster, *Ostrea edulis*, in
943 Shetland: habitat identification study. Scottish Natural Heritage.

944 Skinner, L.F., Coutinho, R., 2005. Effect of microhabitat distribution and substrate
945 roughness on barnacle *Tetraclita stalactifera* (Lamarck, 1818) settlement. Braz.

946 Arch. Biol. Technol. 48, 109–113. <https://doi.org/10.1590/S1516->
947 89132005000100014

948 Smyth, D., Mahon, A.M., Roberts, D., Kregting, L., 2018. Settlement of *Ostrea edulis*
949 is determined by the availability of hard substrata rather than by its nature:
950 Implications for stock recovery and restoration of the European oyster. *Aquat.*
951 *Conserv. Mar. Freshw. Ecosyst.* 28, 662–671. <https://doi.org/10.1002/aqc.2876>

952 Soniat, T.M., Finelli, C.M., Ruiz, J.T., 2004. Vertical structure and predator refuge
953 mediate oyster reef development and community dynamics. *J. Exp. Mar. Biol.*
954 *Ecol.* 310, 163–182. <https://doi.org/10.1016/j.jembe.2004.04.007>

955 Su, Z., Huang, L., Yan, Y., Li, H., 2007. The effect of different substrates on pearl
956 oyster *Pinctada martensii* (Dunker) larvae settlement. *Aquaculture* 271, 377–
957 383. <https://doi.org/10.1016/j.aquaculture.2007.02.039>

958 Tamburri, M.N., Finelli, C.M., Wethey, D.S., Zimme-Faust, R.K., 1996. Chemical
959 induction of larval settlement behavior in flow. *Biol. Bull.* 191, 367–373.
960 <https://doi.org/10.2307/1543009>

961 Tamburri, M.N., Luckenbach, M.W., Breitburg, D.L., Bonniwell, S.M., 2008. Settlement
962 of *Crassostrea ariakensis* larvae: Effects of substrate, biofilms, sediment and
963 adult chemical cues. *J. Shellfish Res.* 27, 601–608.
964 [https://doi.org/10.2983/0730-8000\(2008\)27\[601:SOCALE\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[601:SOCALE]2.0.CO;2)

965 Tamburri, M.N., Zimmer-Faust, R.K., Tamplin, M.L., 1992. Natural sources and
966 properties of chemical inducers mediating settlement of Oyster larvae: A re-
967 examination. *Biol. Bull.* 183, 327–338. <https://doi.org/10.2307/1542218>

968 Theuerkauf, S.J., Burke, R.P., Lipcius, R.N., 2015. Settlement, growth and survival of
969 eastern oysters on alternative reef substrates. *J. Shellfish Res.* 34, 241–250.
970 <https://doi.org/10.1101/010793>

971 Thurstan, R.H., Hawkins, J.P., Raby, L., Roberts, C.M., 2013. Oyster (*Ostrea edulis*)
972 extirpation and ecosystem transformation in the Firth of Forth, Scotland. J. Nat.
973 Conserv. 21, 253–261. <https://doi.org/10.1016/j.jnc.2013.01.004>

974 Trimble, A.C., Ruesink, J.L., Dumbauld, B.R., 2009. Factors preventing the recovery
975 of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864.
976 J. Shellfish Res. 28, 97–106.

977 Tritar, S., Prieur, D., Weiner, R., 1992. Effects of bacterial films on the settlement of
978 the oysters, *Crassostrea gigas* (Thunberg, 1793) and *Ostrea edulis*, Linnaeus,
979 1750 and the scallop *Pecten maximus* (Linnaeus, 1758). J. Shellfish Res. 11,
980 325–330.

981 Vazquez, E., Young, C.M., 1998. Ontogenetic changes in phototaxis during larval life
982 of the Ascidian *Polyandrocarpa zorritensis* (Van Name, 1931). J. Exp. Mar. Biol.
983 Ecol. 231, 267–277. [https://doi.org/10.1016/S0022-0981\(98\)00094-X](https://doi.org/10.1016/S0022-0981(98)00094-X)

984 Wang, Q., Li, J., Liang, F., Xie, S., Du, X., Deng, Y., 2017. Effects of different substrates
985 on settlement and growth of pearl oyster (*Pinctada maxima*) larvae in
986 hatcheries. Aquac. Eng. 77, 15–19.
987 <https://doi.org/10.1016/j.aquaeng.2017.02.001>

988 Whitman, E.R., Reidenbach, M.A., 2012. Benthic flow environments affect recruitment
989 of *Crassostrea virginica* larvae to an intertidal oyster reef. Mar. Ecol. Prog. Ser.
990 463, 177–191. <https://doi.org/10.3354/meps09882>

991 Young, C.M., 1995. Behavior and locomotion during the dispersal phase of larval life,
992 in: McEdward, L. (Ed.), Ecology of Marine Invertebrate Larvae. CRC Press,
993 Boca Raton, Florida, pp. 249–278.

994 Zimmer-Faust, R.K., Tamburri, M.N., 1994. Chemical identity and ecological
995 implications of a waterborne, larval settlement cue. *Limnol. Oceanogr.* 39,
996 1075–1087. <https://doi.org/10.4319/lo.1994.39.5.1075>

997 zu Ermgassen, P.S.E., Bonacic, K., Boudry, P., Bromley, C.A., Cameron, T.C.,
998 Colsoul, B., Coolen, J.W.P., Frankic, A., Hancock, B., Hauton, C., an der Have,
999 T., Holbrook, Z., Kamermans, P., Laugen, A.T., Nevejan, N., Pogoda, B.,
1000 Pouvreau, S., Preston, J., Ranger, C., Sanderson, W.G., Sas, H., Strand, A.,
1001 Sutherland, W.J., 2020. Forty questions of importance to policy and practice of
1002 oyster restoration in Europe. *Aquat. Conserv.* in press.

1003 zu Ermgassen, P., Gamble, C., Debney, A., Colsoul, B., Fabra, M., Sanderson, W.G.,
1004 Strand, A., Preston, J., 2020. European Guidelines on Biosecurity in Native
1005 Oyster Restoration. The Zoological Society of London, UK., London, UK.

1006

1007