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Effects of material composition and face block exposure on the long-term (2014-2018) colonisation of an intertidal RECIF Artificial Reef in the Bay of Seine

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Abstract. An experimental artificial reef (AR) with the incorporation of crushed seashells of the queen scallop *Aequipecten opercularis* was initiated on the intertidal zone of the Bay of Seine in the framework of the INTERREG IVa RECIF project for a everyone year monitoring observations from March 2014 to April 2018. Three block types were operated: eco-friendly material with 20% or 40% of crushed queen scallop shells, and ordinary concrete made from natural aggregates. On the seven blocks analysed at the end of the experiment in 2018, 74 taxa including 32 sessile and 42 motile fauna have been accounted; no difference of taxonomic richness was observed between material and immersion time. The sessile fauna was identified for the six external faces of two blocks aged of four years: horizontal above face (FAB), horizontal below Face (FBE), face oriented offshore (FOF), face oriented inshore (FIN), vertical face oriented west (FEW) and vertical face oriented east (FEA). FBE showed higher taxonomic richness and abundance than the other block faces. FAB exposed to the light showed the lowest abundances. Our observations showed the efficiency of such AR on the intertidal zone accessible at each spring tide, which was easier to survey than in the subtidal zone, where divers must be mobilised to sample blocks.

1. Introduction

Artificial Reefs (ARs) have been used for decades around the world to create, protect or restore a rich and diverse ecosystem [1-5]. Furthermore, recent deployment on ARs were focused on community structure or composition of ARs, showing a shift from improving fisheries as a resource to marine ecosystem rehabilitation and marine structure increasing the biodiversity [4, 6-8].

One of the scientific goals regarding ARs are to identify the species colonizing a reef, i.e. the biodiversity, the temporal succession of species after its deployment and the growth of existing species [1, 9-10]. Many motile taxa were associated with the sessile fouling organism, which increases the attractiveness of such ARs for predators [11]. Nevertheless, most of the monitoring's of ARs considered only the species composition and succession of main groups of macroalgae or macrofauna, mainly sessile organisms, and during a short period frequently one year [7, 12-13]. Only few ARs were surveyed during a long-term period (> 3 years) [7, 14-15].

Nowadays, the use of shells derived from oyster culture or king scallop fisheries, which were considered as waste, was also a request of sustainable development in material construction of ARs [16]. In this perspective, the 2013-2015 RECIF project proposed the incorporation of crushed seashells of the queen scallop *Aequipecten opercularis* (Linnaeus, 1758) into the substrate of concrete blocks as an innovative building materials development for ARs [16]. In this project, an experimental structure was initiated in March 2014 on the intertidal zone of the Bay of Seine (eastern part of the English Channel) [9-10, 17]. The short-term colonisation during the first year of monitoring until February 2015 was described in [9]. At the end of the one-year experiment (2014-2015), it was chosen to extend the monitoring every year until April 2018, for a four-year temporal monitoring of the colonisation and succession of such innovative intertidal ARs in the English Channel [18].
The objectives of this paper are: 1) to provide the inventory of sessile and motile invertebrates found in April 2018 four years after the beginning of the monitoring; and 2) to identify the effect of the material composition of the motile and sessile fauna on block colonization efficiency, and 3) to identify the colonisation pattern of the sessile fauna on the six external faces of the blocks according to their position and their location on the oyster tables.

2. Materials and Methods

2.1. Experiment site and design of blocks

At the beginning of the experiment (19-20 March 2014), 75 blocks (20 x 20 x 40 cm) were deployed on eight oyster culture tables on the intertidal zone of Luc-sur-Mer (49°19′15″N-0°20′55″W) [9-10, 17] (Figure 1). At high tide, the water depth is 6.5 m and the concrete blocks were emerged about 44% of the time [9].

Figure 1. Location of the site at Luc-sur-Mer (Calvados coast, southern part of the Bay of Seine) [9].

Three types of block have been studied: 60 blocks of eco-friendly material for each with 40% (30 blocks) and 20% (30 blocks) of crushed queen scallop *Aequipecten opercularis* (L., 1758) shells and, and 15 blocks of ordinary concrete made from natural aggregates [15]. Then in a view to a yearly temporal monitoring, the blocks were collected once a year until April 2018. On the seven blocks collected in 2018 (Figure 2), one corresponded to ordinary concrete, two with 20% and four with 40 % of crushed queen scallop shells. Two blocks had resided at sea during four years, one during three years, two during two years and the two last blocks only during one year.

Figure 2. Blocks on oyster tables at the end of the experiment in April 2018.

2.2. Laboratory analyses of the blocks

The sessile and motile fauna were examined in the laboratory with two distinguished protocols. The sessile fauna was observed under a binocular microscope. Sub-sampling was carried out over scraping unit areas delimited by a mask for each of the six external faces: horizontal above face (FAB), horizontal below Face (FBE), face oriented offshore (FOF), face oriented inshore (FIN), vertical face oriented west (FWE) and vertical face oriented east (FEA) of each block (Figure 3). This process was used for the two four years blocks sampled in April 2018, i.e. natural and 20% of crushed queen scallop shells.

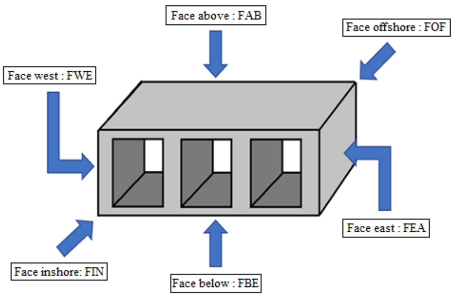

Figure 3. Designations of the six faces of the experimental blocks.

On horizontal above face (FAB) and below (FBE): 8 x 0.0025 m^2 for a total of 0.02 m^2 corresponding to 1/4 of the total surface of each horizontal face had been analysed. For the vertical faces oriented respectively to the east (FEA) and the west (FEW): 4 x 0.0025 m^2 for a total surface of 0.01 m^2, corresponding to 1/4 of the total surface of each vertical face were analysed. On the two vertical faces (not full) oriented offshore (FOF) and inshore (FIN): 2 x 0.003 m^2 and 1 x 0.004 cm^2 corresponding to 1/3 of the total surface of each vertical face had been analysed.

Moreover, each face was photographed in its entirety as well as each sub-sample. Countable organisms (barnacles, mussels, some solitary ascidians) had been considered for quantitative analyses. For each face, an inventory of all present taxa was carried out, as well as counted where it was possible. All organisms were stored in ethanol 96°.

After having placed the blocks in bowls in anoxic conditions at least 24h, the seawater used to keep the fauna alive during the laboratory observations was then filtered on a 0.5-mm mesh sieve to collect the motile fauna. The retained material was fixed with 96% alcohol, and then it was identified to the more precise level of taxonomy and count as motile fauna living associated with the blocks. For polychaetes and nemerteans, only the head of the individuals were counted.

All the abundances of sessile and motile organisms had been normalized to 0.6 m^2.

2.3. Statistical analyses
To test the differences of colonisation between block faces, taxonomic richness (TR) and total abundances (0.6 m²) for the sessile and motile, and all taxa combined were compared by ANOVAs. For the sessile fauna, the comparison was based on block faces (FAB, FBE, FOF, FEW, FIN, and FEA). For motile fauna, only the total fauna (taxonomic richness and abundances) between blocks have been tested. The null hypotheses H_0 stated that there was no effect of a factor or no effect of an interaction between factors on any of the variables. The alternative assumptions H_1 stated that there were effects of the date, or the faces. A post-hoc test (Tukey) was performed if the H_0 null hypothesis was rejected in favour of the H_1 hypothesis. The normality and homogeneity of the variances in the distribution of the data or residuals were tested with a Shapiro test and Bartlett tests, respectively. When one of these conditions was not meet, a non-parametric Scheirer-Ray-Hare test was applied instead of the ANOVA, followed by a post-hoc Dunn test.

3. Results

3.1. *Composition of the fauna in 2018*
Within the seven blocks analysed in 2018, the sessile fauna accounted 32 taxa while the motile fauna accounted 42 taxa for a total of 74 taxa. Among the sessile fauna, the most diversified groups were the polychaetes, ascidians, demosponges, and cnidarians mainly belonging to the group of hydrozoans (Table 1). However, in terms of abundance, the most numerous countable sessile species were the crustacean barnacle *Balanus crenatus*, *Perforatus perforatus*, the bivalve *Mytilus edulis*, the ascidia *Perophora japonica*, *Corella eumyota* and *Molgula* spp. as well as the polychaetes *Spirobranchus* (Table 1). Among the non-countable sessile species, the hydrozoans *Kirchenpaueria pinnata*, *Dynamena pumila* and *Obelia longissima* as well as the sponges *Halichondria panicea* and *Hymeniacidon* sp. were presented in all the faces of the blocks and covered in some cases the totality of a face. Among the motile fauna, polychaetes remained the most diversified group, followed by malacostraca (mainly amphipods, decapods and isopods). There were few differences between the TR of the two kinds of blocks (Table 1). The most abundant motile taxa were the Nematoda, the polychaete Nereidae and Phyllodocidae *Eulalia*, and the tanaid *Zeuxo holdichi* (Table 1).
The total number of taxa per blocks varied from 31 for the four year block with natural aggregates and 44 for a one year block with 40% of crushed queen scallop shells (Table 1). The number of sessile taxa was on the same order of magnitude on the seven blocks: i.e. from 16 to 19 without significant differences between years (p=0.343), while the number of motile taxa varied strongly from 16 to 29 but without significant difference (p=0.352). It was notable that the number of taxa of the four years blocks was among the lowest values (Table 1). The number of taxa on the seven blocks analysed in 2018 showed no significant differences between blocks (p=0.819 for sessile taxa and p=0.304 for motile taxa)
In summary, they were no significant differences on temporal and nature of aggregates patterns. The colonisation was fast in one year. Then low changes on sessile taxa number were observed while those of motile taxa varied highly from one block to another without link to the immersion time (Table 1).

3.2. Colonisation pattern according to the faces of the blocks
The figure 4A showed the number of sessile taxa found on each face of both 4 year blocks. The number of taxa varied from a minimum of two to a maximum of 12; but there were no significant differences between faces (p=0.249).

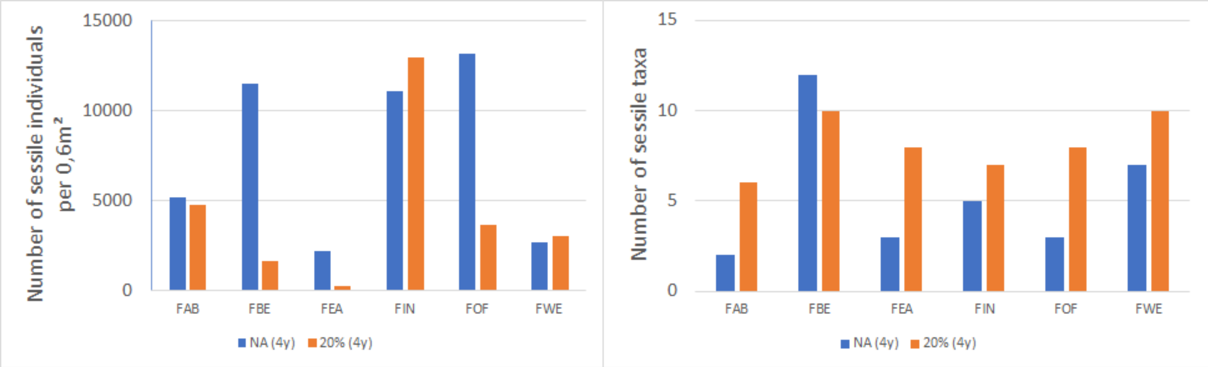

Figure 4. Number of individuals (left) and number of taxa (right) of sessile taxa accounted in the six faces of the two 4 year blocks (NA: Natural aggregate and 20%: 20% of crushed queen scallops). FIN: face oriented inshore; FOF: face oriented offshore, FBE: horizontal below Face; FAB: horizontal above face; FWE vertical face oriented west (FEW) and FEA: vertical face oriented east.

The figure 4B showed the abundance per 0.6 m² of sessile and motile fauna in the six faces of the two 4 year blocks. The western and eastern faces showed low abundances while the face oriented inshore and offshore showed high abundances in the same order of magnitude than of the below face. The horizontal above face showed intermediate numbers of individuals between maximal and minimal values; but there were no significant differences between faces ($p=0.235$).
The figure 5 illustrated the difference of colonisations of two main faces the above and the below faces for both 4-year blocks with the presence of algae on the above faces while the below faces were mostly colonised by sessile taxa.

4. Discussion

Analyses of the short-term colonization (2014-2015) showed temporal difference in species abundance with a succession of macroalgae and macrofauna colonizers along the first year of the experiment, but there were no difference in colonization according to the block composition [10]. Moreover, during the first year of the experiment, the main colonizing organisms were barnacles, hydrozoans, tunicates, mussels and sessile annelids with a biological succession over time: a first assemblage mainly dominated by barnacles then a richer assemblage with the dominance of ascidians [10]. Our monitoring confirmed the permanence and dominance of the barnacles mainly the species *Balanus crenatus* and *Perforatus perforatus*, the mussel *Mytilus edulis*, the Polychaeta *Spirobranchus*, and the ascidian - among them *Corella euryota* and *Perophora japonica* were Non-Indigenous Species in Normandy. Among the motile fauna, the blocks were colonized by small species such as Nematoda, the polychaeta Nereidae, Cirratulidae, Syllidae, *Eulalia* spp., and crustacean such as the isopod *Dynamene bidentata*, *Gnathia* spp., the amphipod *Monocorophium* and the tanaid *Zeuxo holdichi*. This tanaid was very abundant during the first year of the experiment with several thousands of individuals collected and until 2,500 ind.m^{-2} in 2015 [10]. The species could be classified as pioneer and invasive species, with dramatically decreased along the time on artificial structures. The reason for the high abundances in the first stage of block colonisation at this location remained enigmatic.

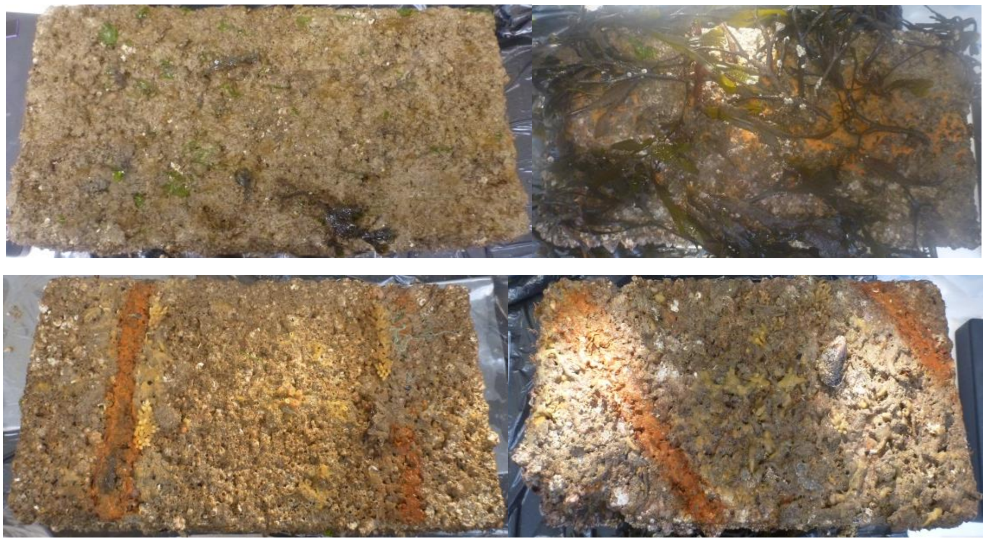

Figure 5. Photos of the four year block. On left 20% crushed queen scallop and on right Natural aggregates. Above FAB: horizontal above face and below FBE: horizontal below Face.

During the first year of immersion, the main environmental factors controlling settlement, colonization and competition between species were : speed of tidal currents, wind force and direction, sunshine hours and rainfall during emergence with species settled preferentially on certain faces according to environmental parameters (light, hydrodynamics) and larval behaviour [10, 17]. A significant taxonomic colonisation was observed mainly on the above and below faces of the blocks. It was clear that the light, the desiccation of the exposed above face of the block and the protection of the below faces on the block played an important role in the colonisation of the blocks, which is amplified in our intertidal experiment with the alternation of immersion and emersion phase.
Our four years experiment showed that the colonisation of such blocks deployed on oyster table on the intertidal zone of the Bay of Seine was very fast; in one year the taxonomic richness underpasses 40 and remained stable along the time. Butler and Connolly (1995) [19] surveyed long term dynamics of sessile fauna, in the Spencer Gulf, South Australia, during six years after initial immersion of piles and showed that the taxonomic composition were stable after three years of immersion. For a long-term (1981-2001) experiment, Nicoletti et al. (2007) [14] identified five phases of colonisation for ARs deployed in the mid Tyrrhenian Sea, Italy, from pioneer species during the first months of immersion to Bryozoans bioconstruction dominance at the end of the experiment, and with phase of dominance, regression and absence of the mussel *Mytilus galloprovincialis*. Recently, Taormina et al. (2020) [20] had monitored epibenthic colonization of artificial structure in the subtidal North-Brittany (France) coast using image-based underwater monitorings and showed a mature stage in four years.
Finally, our experiment showed the efficiency of such AR on the intertidal zone accessible at each spring tide, which was easier to survey than in the subtidal zone, where divers must be mobilised to sample blocks, and can be easily deployed to test for example different aggregates used in the ARs construction and design.

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Table 1. List of the taxa recorded in the seven blocks studied in April 2018. NA: natural aggregates, 20% and 40% aggregates with 20% or 40% of crushed queen scallop shells. Y: number of immersion year. Black case: presence of the taxon and number corresponding to the abundance per 0.6 m². * Non-Indigeneous Species.

Motile fauna		40% (1y)	20% (1y)	40% (2y)	40% (2y)	40% (3y)	NA (4y)	20% (4y)
Ammothella longipes	Hodge, 1864	■	■		■	■	1	1
Amphipholis squamata	Delle Chiaje, 1828						-	2
Arenicolides ecaudata	Johnston, 1835			■			-	1
Cancer pagurus	Linnaeus, 1758	■		■		■	-	
Carcinus maenas	Linnaeus, 1758	■	■	■	■	■	-	1
Chironomidae					■		-	-
Cirratulidae	Ryckholt, 1851	■				■	6	43
*Monocorophium**	Latreille, 1806			■			10	-
Dynamene bidentata	Adams, 1800	■	■	■	■	■	2	10
Eulalia	Savigny, 1822	■	■	■	■	■	36	-
Eumida sanguinea	Örsted, 1843	■		■		■	1	-
Eunicida					■		-	-
Fecampia erythrocephala	Giard, 1886	■		■		■	-	-
Gnathia	Leach, 1814	■	■	■	■	■	27	5
Golfingia vulgaris	de Blainville, 1827						-	2
Harmothoe	Kinberg, 1856	■				■	-	-
Lekanesphaera monodi	Arcangeli, 1934	■				■	-	-
Lepidonotus squamatus	Linnaeus, 1758	■				■	-	6
Lineus	Sowerby, 1806	■		■		■	14	5
Marphysa sanguinea	Montagu, 1813	■				■	-	-
Microdeutopus anomalus	Rathke, 1843	■				■	-	-
Nematoda		■	■	■	■	■	61	287
Nemertea		■	■	■	■	■	11	5
Nereididae	Blainville, 1818	■	■	■	■	■	112	5
Nototropis swammerdamei	Milne Edwards, 1830				■	■	-	-
Nucella lapillus	Linnaeus, 1758	■				■	-	-
Ostracoda	Latreille, 1802	■	■	■		■	-	3
Peringia ulvae	Pennant, 1777						-	1
Pholoe inornata	Johnston, 1839	■		■		■	11	12
Phoxichilidium femoratum	Rathke, 1799						-	-
Phyllodoce mucosa	Örsted, 1843		■	■			-	-
Phyllodocidae	Örsted, 1843		■		■		-	-
Pilumnus hirtellus	Linnaeus, 1761	■	■	■	■	■	-	-
Pinnotheres pisum	Linnaeus, 1767				■		-	-
Polititapes rhomboides	Pennant, 1777	■		■	■	■	-	-
Polynoidae	Kinberg, 1856	■	■		■	■	-	-
Porcellana platycheles	Pennant, 1777	■	■	■	■	■	-	-
Schistomeringos	Jumars, 1974			■			-	-
Syllidae	Grube, 1850	■	■	■		■	10	16
Tryphosa nana	Krøyer, 1846	■				■	-	-
Venerupis corrugata	Gmelin, 1791		■				1	1
Zeuxo holdichi	Bamber, 1990	■		■		■	54	-
Total of motile fauna		**28**	**16**	**32**	**17**	**29**	**15**	**18**
Sessile fauna								
Balanus crenatus	Bruguière, 1789	■	■	■	■	■	4505	2064
Bispira polyomma	Giangrande & Faasse, 2012	■	■	■		■	6	2
Botrylloides	Milne Edwards, 1841						-	■
Botryllus schlosseri	Pallas, 1766						-	■
Branchiomma bombyx	Dalyell, 1853	■	■	■	■	■	9	21
Ciona	Fleming, 1822	■	■	■	■	■	-	-
*Corella eumyota**	Traustedt, 1882	■	■	■	■	■	4	80
Diadumene cincta	Stephenson, 1925						9	

Diphasia fallax	Johnston, 1847						■	■
Dipolydora	Verrill, 1881						-	1
Dynamena pumila	Linnaeus, 1758				■	■	-	-
Flustrellidra hispida	Fabricius, 1780						-	4
Halichondria	Fleming, 1828		■	■	■	■	■	■
Hymeniacidon	Bowerbank, 1858	■	■	■	■		■	■
Jasmineira elegans	Saint-Joseph, 1894	■		■		■	-	-
Kirchenpaueria pinnata	Linnaeus, 1758		■	■	■	■	■	-
Lanice conchilega	Pallas, 1766	■				■	2	-
Molgula	Forbes, 1848	■	■	■	■	■	40	72
Musculus discors	Linnaeus, 1767				■		-	-
Mytilus edulis	Linnaeus, 1758	■	■	■	■	■	29	106
Obelia longissima	Pallas, 1766						■	■
Perforatus perforatus	Bruguière, 1789	■	■	■	■	■	71	52
*Perophora japonica**	Oka, 1927	■					64	84
Pista mediterranea	Gaillande, 1970			■			-	-
Polyclinum	Savigny, 1816						-	-
Psamathe fusca	Johnston, 1836			■			-	-
Sabellidae	Latreille, 1825	■	■	■	■	■	-	-
Serpulidae	Rafinesque, 1815	■	■			■	-	-
Spirobranchus	Blainville, 1818	■	■	■	■	■	56	31
Suberites	Nardo, 1833		■	■	■	■	-	-
Terebellidae	Johnston, 1846	■	■	■	■	■	-	1
Urticina	Ehrenberg, 1834						-	2
Total of sessile taxa		**16**	**16**	**18**	**16**	**18**	**17**	**19**
Total of taxa		**44**	**32**	**40**	**33**	**47**	**31**	**37**