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RESEARCH ARTICLE

The epibiotic community associated to the European flat oyster: a function of the state of development of the reef

Abel Zempléni^{1,2,3} , Stéphane Pouvreau¹, Nicolas Desroy²

When healthy, *Ostrea edulis* populations are capable of creating remarkable biogenic reefs, providing a unique habitat for marine biodiversity. At present, the biotic assemblages associated with flat oysters remain poorly defined. This study aims to analyze biodiversity associated with the reef's development stage by focusing on its epibiotic community. The studied oyster population is an old remnant bed located in the Bay of Brest, which has been undergoing restoration for 5 years. The epibiotic communities (>500 µm) of scattered individual living ($n = 10$) and dead ($n = 9$) oysters, as well as of aggregates from the ground ($n = 10$) and from a restored reef ($n = 3$) were compared. 137 species associated with oysters were found, among which 22 were specific to individual oysters and 55 to aggregates and reefs. Although reef samples formed a distinct group in the SIMPER analysis, Shannon's (between 2.25 and 2.52) and Simpson's (between 0.84 and 0.86) diversity indices remained constant for each reef development stage. Pielou's indices (between 0.71 and 0.80) were significantly lower for aggregates displaying a less evenly distributed community. Mean abundance and species richness per sample were higher for aggregated structures. However, when standardized to abundance per centimeter square of shell surface, both parameters were significantly higher for individual oysters. Therefore, while reefs may support a higher species richness at a larger spatial scale, at a smaller scale, single oysters have a higher species richness per unit area of shell. Seemingly, despite the poor state of their remnant populations, flat oysters are still hosting important macrofaunal biodiversity.

Key words: biodiversity indices, ecosystem services, habitat, macrofaunal invertebrates, *Ostrea edulis*, restoration ecology

Implications for Practice

- The flat oyster and its habitat are collapsing, and this type of study is helping to promote its ecological restoration at the European scale.
- The high biodiversity found in this study demonstrates the urgent need to restore this marine habitat.
- By implementing a metric included in the European Native Oyster Restoration Alliance restoration handbook, this study proposes a precise methodology that can be used in future restoration actions.
- This study will also contribute to define more precisely the reference ecosystem for this lost habitat.

Introduction

Oyster reefs are among the most degraded marine habitats in the world (Beck et al. 2011; Fitzsimons et al. 2020; Thurstan et al. 2024). Around 85% of native oyster reefs disappeared or are severely degraded (Beck et al. 2011). Many native oyster species (especially *Crassostrea virginica* (zu Ermgassen et al. 2013), *C. rhizophorae*, *C. hongkongensis* (Lau et al. 2020), *Ostrea angasi* (Gillies et al. 2020), *O. edulis* (Thurstan et al. 2024), *O. lurida* (zu Ermgassen et al. 2013), and *Saccostrea glomerata* (Gillies et al. 2020)) have been specifically investigated, and all site-specific studies confirmed this alarming tendency.

The flat oyster, *O. edulis*, the only oyster native to the European Atlantic coasts, and its biogenic habitat were once dominant in most coastal ecosystems. Its natural geographic range extends from 65°N in Norway, along the coasts of western Europe and the British Isles, to North Africa and into the western Mediterranean and the Black Sea (Thurstan et al. 2024). *Ostrea edulis* is now highly threatened due to centuries of overexploitation, especially by dredge fishing, habitat destruction, pollution, river run-off, pathogen infections, and biotic interaction imbalance (Pogoda 2019; Pouvreau 2023; zu Ermgassen et al. 2023). Consequently, the disappearance of its biogenic habitat caused an important loss in biodiversity as oyster reefs, thanks to their complex three-dimensional structures, are a hosting habitat for many marine species (Smyth & Roberts 2010; Guy et al. 2018; Pouvreau et al. 2021).

Authors contribution: all authors contributed equally to the study design, sampling, data collection, and analyses; all authors have revised the manuscript.

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In France, *O. edulis* now survives only in limited environments, particularly in Brittany (Pouvreau et al. 2021). After several centuries of exploitation, these last remaining populations are subject to a range of pressures. If no targeted conservation and ecological restoration actions are taken quickly, these could lead to their extinction (Pogoda 2019; Pouvreau 2023). Very recently, a number of studies and reviews conducted all over Europe have provided a solid framework for the species' restoration ecology (Smyth & Roberts 2010; Zwerschke et al. 2016; zu Ermgassen et al. 2020; Pouvreau et al. 2021). These works have shown that the reduction and/or suppression of certain pressures can significantly help the species' return to the wild. Consequently, active restoration would make it possible to reform fairly dense colonial reef constructions, which are essential for the health and resilience of individuals, populations, and the associated biodiversity.

Based on these works and within the framework of the European Native Oyster Restoration Alliance (NORA), oyster restoration and conservation have become a key biodiversity issue in the European context (zu Ermgassen et al. 2020; Pouvreau et al. 2021). *Ostrea edulis* is listed as a Critically Endangered Species by the European Environment Agency (EUNIS 2016). Flat oyster reefs are identified as a threatened, declining, or collapsed habitat in all OSPAR regions where it occur (2020) and are likely to enter the World Conservation Union Red List of Ecosystems (zu Ermgassen et al. 2023). Whatever the species or habitat targeted, ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed. This definition has been set by the Society for Ecological Restoration (SER 2023) as a common international standard. After identifying the top 40 questions that need to be tackled for an efficient restoration strategy (zu Ermgassen et al. 2020), the European Native Oyster Restoration Monitoring Handbook has been compiled by the NORA community to serve as a common basis for monitoring restoration in Europe (zu Ermgassen et al. 2021). It provides a list of 31 monitoring metrics, among which the assessment of ecosystem services takes an important place. Indeed, as all ecosystem engineer species when they are healthy, wild *O. edulis* reefs deliver several ecological functions and services. They primarily provide a habitat for a wide range of biodiversity, along with life cycle maintenance and gene pool protection (Pogoda et al. 2020; Pouvreau et al. 2021; Thomas et al. 2022). However, at present, the biotic assemblages associated with flat oysters and their habitat remain poorly defined: Do *O. edulis* reef aggregations shelter a community that is relatively unique to this habitat, or is this community highly specific to each study site? And therefore, is it possible to define a general reference ecosystem for *O. edulis* reefs? Characterizing such a reference ecosystem would be mandatory for future restoration projects.

Some studies have already investigated the biodiversity associated with wild or farmed European flat oysters (Ellis 1753; Möbius 1877; Philpots 1891; Shodduyn 1931; Smyth & Roberts 2010; Deane et al. 2015; Green 2016). Besides giving a list of epibiota living on the oysters, most of these studies also showed that biodiversity on *O. edulis* shells is significantly higher than on the surrounding substratum. Recently,

Christianen et al. (2018) identified 14 species with a special conservation status in Germany, associated with flat oysters. Their abundance was significantly higher on the oysters than on the surrounding substrate, suggesting that flat oyster reefs, when they are healthy, can be considered as biodiversity "hotspots," hosting sometimes more than a hundred different epibiotic species (Shodduyn 1931; Smyth & Roberts 2010).

The elements of the biotic assemblage in oyster reefs can be classified into four groups: infaunal invertebrates (in the surrounding sediment), epifaunal invertebrates (this study), small resident fish and mobile invertebrates, transient fish, and crustaceans (zu Ermgassen et al. 2021). The aim of this study is to survey epibionts and endobionts (epifaunal invertebrates) on flat oyster reefs from the Bay of Brest at different reef development stages (individual oysters, aggregates, and reefs). This study is a first contribution to a broader work, aiming to define a reference community linked to the flat oyster reef habitat. Pooled with other similar studies (Smyth & Roberts 2010; Zwerschke et al. 2016; Thurstan et al. 2024), our results will progressively serve as a baseline for monitoring restoration trajectories, allowing us to perform Ecological Trajectory Analyses (ETAs) that quantify the evolution and the functional changes of the epibiotic community in the targeted ecosystem (Sturbois et al. 2021).

Methods

Study Site

This study was conducted on a remnant oyster bed locally called "Banc du Roz" (48.31842°N/−4.33571°W), what used to be a large oyster reef occupying most of the Bay of Daoulas in the Bay of Brest (Fig. 1). This bay is a shallow inlet, with a depth varying between 2 and 10 m depending on the tide. It benefits from oceanic incursions at each tidal flow and a regular supply of freshwater from the river "La Mignonne." Water currents remain moderate (<1 m/s), allowing water masses to reside for several days (Petton et al. 2020). The last remaining oysters are found in a 30 ha area, scattered over a seabed consisting mainly of maerl (*Lithothamnium corallioides*), and attach themselves to the remaining oyster shell fragments. For the past 10 years, this bed has been part of a Natura 2000 zone with a moratorium on all dredging and bottom-trawling activities. According to Pouvreau et al. 2021, the natural oyster density is generally below 1 individual/m², but some clumps of oysters can be occasionally found, which ranks this population in a critical but restorable state. The restoration of this population began 5 years ago, so denser artificial reefs are now present in a part of the former bed, on its south-eastern edge. Since 2018, many environmental and biological parameters have been continuously monitored at this site. Summer temperature, salinity, phytoplankton concentration, and currents are favorable for *Ostrea edulis* reproduction and larval life. Recruitment dynamic is now well known, showing a regular spatfall each summer with a peak in July (Pouvreau et al. 2021). On the other hand, the presence of *Martelia* and *Bonamia* as well as high densities of predators such as sea breams, oyster drills, and spider crabs

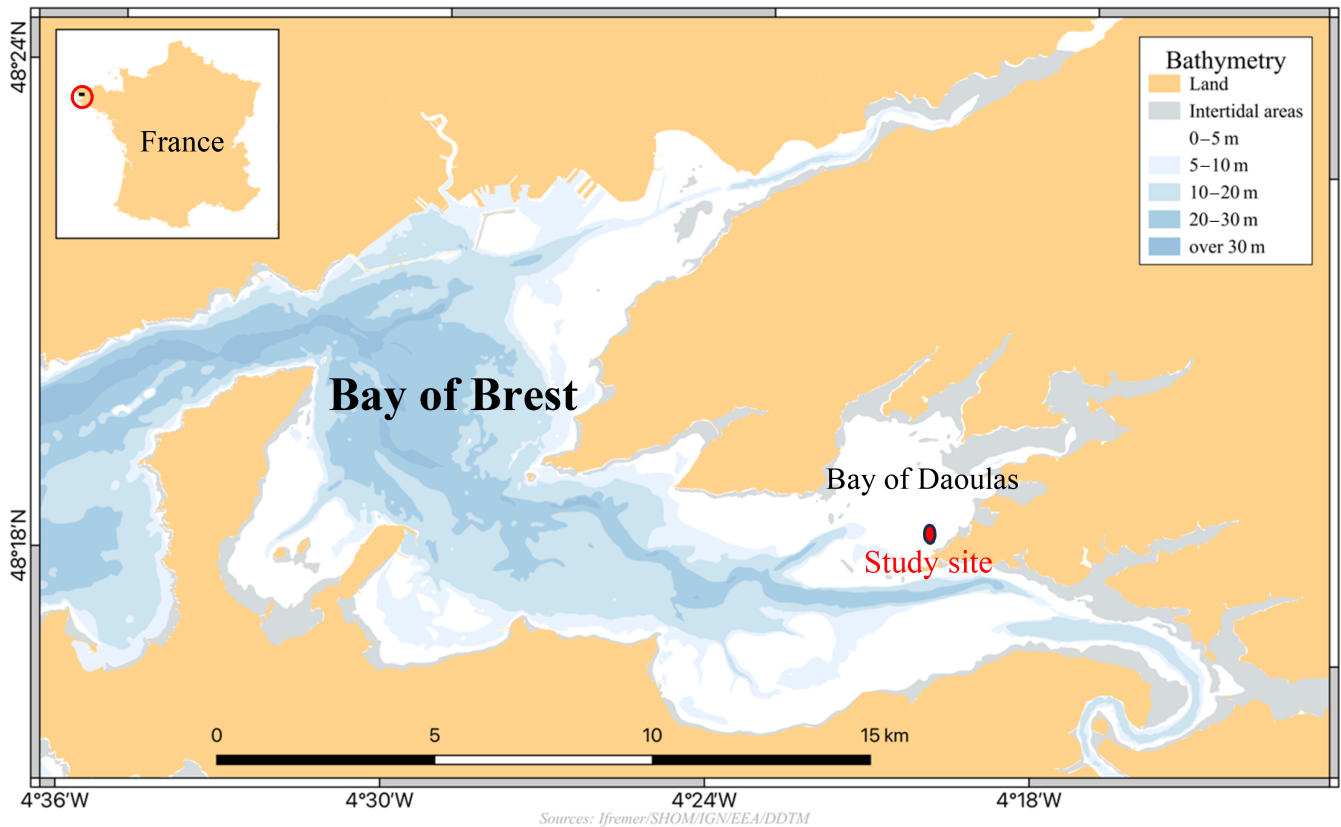


Figure 1. Localisation of our study site, the “Banc du Roz,” in Bay of Brest (North Brittany, France).

constitutes significant pressures on the oyster population (Pouvreau et al. 2021).

Sampling Method

To evaluate the relationship between biodiversity and the developmental stages of the reef (see conservation scale in zu Ermgassen et al. 2021), we sampled each of four stages (Fig. 2): Stage 0 (scattered individuals that are not fixed, further categorized as dead or alive), stages 1–2 (aggregated individuals forming an initial clump of oysters), and stages 3–4 (significant reef structures that are under formation with a density of more than 20 individuals/m²). Concerning stage 0, 10 individual living (“Living” group) and nine individual dead (“Dead” group) oysters were collected. For stages 1–2 (Fig. 2), 10 replicates (“Aggregate” group) composed of four to six living and dead aggregated oysters were taken. All these samples were picked directly from the seabed. For stages 3–4, only three samples (“Reef” group) with more than 12 living or dead aggregated oysters were collected. As on our study site stages 3 and 4 were only available on an elevated metallic restoration structure (30–40 cm above the seabed), taking more (Reef) samples would have permanently degraded the restored oyster reef.

All samples were collected by scientific divers on the 4th of April 2023. Each sample was collected in a sealed plastic bag

underwater. On shore, after filtering on a 500 µm sieve, they were fixed in formol (4%) for transport and storage.

Data Collection

Each sample was rinsed to remove the formaldehyde and washed over a 500 µm square mesh size sieve. The surface area of the oyster shells (given in cm²) of each replicate was determined by measuring the length and width of each oyster shell and calculated by assimilating oysters to an ellipse. Each sample was examined under a binocular magnifying glass, and all epibionts on and inside shells were collected. Shells were gently broken to collect all the endobionts that occur inside the shells and between the shell layers. Organisms were then identified to the species level when possible and counted. Only macroinvertebrates were identified. Each species' name was then updated according to the latest version of the Word Register of Marine Species (WORMS 2024) and some of their main functional traits were determined through literature analysis. The following trait categories were used, based on the definitions given by Robert et al. (2021): feeding mode (suspension-feeders, deposit-feeders, carnivorous and grazers), mobility (highly mobile, mobile, sedentary, and sessile), and average size (small [<2 cm], medium [between 2 and 10 cm], and large [>10 cm]).

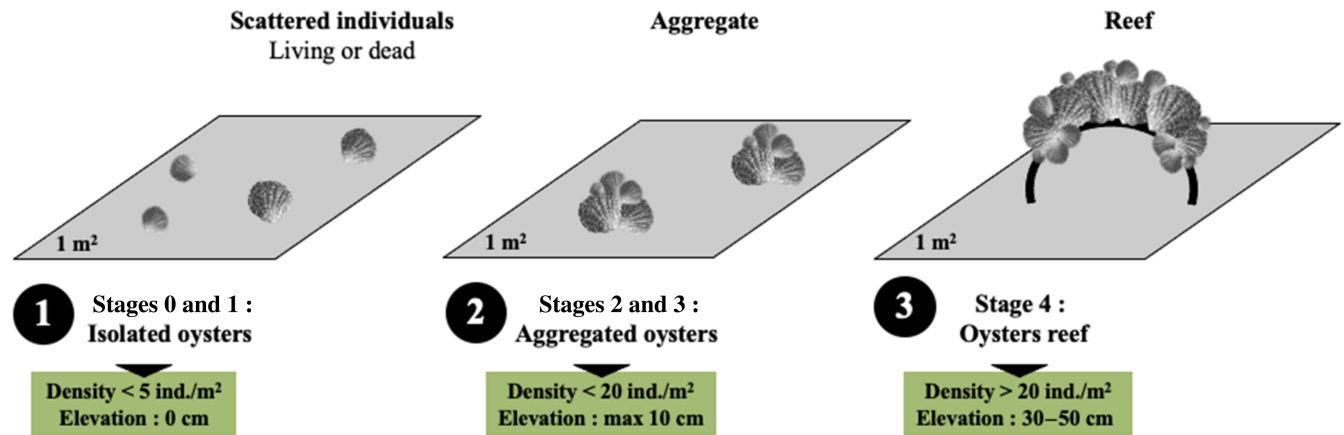


Figure 2. The different reef development stages sampled in this study with a brief description of their respective ecological stage. For more information, see zu Ermgassen et al (2021).

Statistical Analysis

For each sample, abundance and species richness were calculated and standardized by the surface area of the oyster shells (per cm²). Species and functional trait diversity (Shannon's and Simpson's) and equitability (Pielou's) indices were then determined. As normality (Shapiro–Wilk test) and homoscedasticity (Levene test) were verified for all variables, one way analysis of variance (ANOVA) models with four factors (Living, Dead, Aggregate, and Reef) were built. A Tukey's range test was then applied to each model. A nonmetric multidimensional scaling (NMDS) was performed based on the abundance per centimeter square of each species in order to visualize differences between the epibiotic community of each aggregation state. The contribution to dissimilarities between aggregation states of the defining taxa was evaluated through a similarity percentage (SIMPER) analysis. A principal component analysis (PCA) was built with all previously listed functional traits to investigate functional diversity for each substrate type. The shell surface area was not used for building the PCA, but is represented as a supplementary variable. A correlation matrix was used for identifying the main drivers of the PCA axis. All statistical analyses were performed on R (R Core Team 2023).

Comparing Our Results to Data From Different Sites

A bibliographic review was carried out in order to compare our list of epibionts to other existing lists. For each list, only epibionts were selected, and comparisons were only made at a Phylum and Class level, since higher taxonomic levels were not always well-identified. In order to have standardized data, the phyla in each study are expressed as a percentage.

Results

Global Description of the Associated Biodiversity of Flat Oysters

A total of 137 species were found across all samples (see Table S1). Annelida was the most commonly represented group ($n = 69$), followed by Arthropoda ($n = 34$), Mollusca ($n = 14$),

Chordata ($n = 7$), Echinodermata ($n = 5$), Bryozoa ($n = 2$), Cnidaria ($n = 2$), Porifera ($n = 1$), Phoronida ($n = 1$), and Platyhelminthes ($n = 1$). The list of species and their abundance were different according to the reef development stage. In absolute terms, abundance and species richness increased with the reef development stage (Table 1), however, when the available surface area was accounted for, the trend was reversed: per unit area of available surface, the shell of an isolated individual (Living or Dead) was more species rich than a clustered fraction (Aggregate or Reef). When comparing abundance per shell surface area, Reef substrates showed significantly lower values than scattered individuals (Living and Dead) and Aggregate substrates (Table 2). When comparing species richness per shell surface area, Aggregates and Reefs were significantly lower than Living and Dead oysters (Table 2). These results highlighted the relative decrease in abundance and species richness per unit area of shell with the increase of the reef development stage. In other words, the more structured habitats supported a lower species richness and abundance per unit area of shell surface relative to less structured habitats.

Shannon's and Simpson's diversity and Pielou's evenness indices (mean \pm SD) were calculated for each reef development stage (Table 1). Diversity indices showed no significant differences according to the level of aggregation. Pielou's indices were significantly lower for Aggregates compared to Living (degrees of freedom [df] = 12, p value = 0.03) and Dead (df = 13, p value = 0.004) oyster samples (Table 2). Also, the lowest Pielou's indices were measured for the highest Shannon's indices (Aggregates and Reefs), while Simpson's indices remained between 0.84 and 0.86 for all reef development stages (Table 1).

Each reef development stage had its own diversity since only 31 species (22%) were common to all levels of aggregation (Fig. 3). These 31 species were also the most abundant ones, representing more than 75% in terms of abundance of all samples considered. Thirty-seven species (27%) were exclusively found on Aggregates (Fig. 3). These species were relatively rare and were found on a small number of samples (they represent 0.9% of total abundance).

Table 1. Main ecological parameters and diversity indices (mean ± SD) for the epibiotic community on all the different reef development stages (Living, Dead, Aggregate, and Reef). *Significantly lower values according to Tukey's post hoc test. Detailed in Table 2.

	Scattered individuals			
	Living	Dead	Aggregate	Reef
Main ecological parameters				
Samples (n)	10	9	10	3
Mean oyster shell surface (cm ²)	320.8 (±66.2)	295.5 (±72.2)	1015.2 (±318.7)	2725.1 (±881.7)
Mean abundance	84.50 (±28.4)	77.56 (±31.1)	246.40 (±73.9)	190 (±31.2)
Mean number of species	19.20 (±6.2)	19 (±6.5)	35.60 (±4.0)	33 (±5.2)
Mean abundance/cm ²	0.27 (±0.08)	0.26 (±0.08)	0.26 (±0.1)	0.07 (±0.01)*
Mean number of species/cm ²	0.06 (±0.02)	0.06 (±0.02)	0.04 (±0.01)*	0.01 (±0.003)*
Main diversity indices				
Shannon	2.25 (±0.29)	2.33 (±0.27)	2.52 (±0.25)	2.52 (±0.30)
Simpson	0.85 (±0.04)	0.86 (±0.04)	0.84 (±0.06)	0.84 (±0.06)
Pielou	0.78 (±0.05)	0.80 (±0.05)	0.71 (±0.05)*	0.72 (±0.06)

Table 2. Detailed results of Tukey's post hoc tests on two ecological parameters (abundance and species richness per area of oyster shell) and Pielou's evenness indices for all reef development stages. Only significant differences (p value <0.05) are displayed in this table.

The two compared reef development stages		Estimate	95% CI	Degrees of freedom (df)	p Value
Abundance/cm ²					
Living	Reef	-0.19	(-0.36; -0.03)	19	0.013
Dead	Reef	-0.19	(-0.35; -0.03)	20	0.018
Aggregate	Reef	-0.19	(-0.35; -0.03)	19	0.015
Number of species/cm ²					
Living	Aggregate	-0.02	(-0.04; -0.003)	12	0.015
	Reef	-0.05	(-0.07; -0.02)	19	0.0003
Dead	Aggregate	-0.03	(-0.05; -0.007)	13	0.005
	Reef	-0.05	(-0.08; -0.02)	20	0.0001
Pielou's evenness indices					
Living	Aggregate	-0.07	(-0.14; -0.004)	12	0.03
Dead	Aggregate	-0.1	(-0.17; -0.027)	13	0.004

The five most abundant species were the same for each reef development stage, namely the polychaetes *Polydora ciliata*, *Platynereis dumerilii*, *Syllidia armata*, *Spirorbis spirorbis*, and the tanaid *Hexapleomera wombat*. Their relative abundance was around 70% (between 66.3 and 73.5%) of the epibionts on the samples. According to the SIMPER analysis, for all pairwise comparisons, these species were also the most influential contributing to the differences between reef development stages. Their cumulative contribution was between 0.51 and 0.52 for comparisons between scattered individual Living and Dead oysters and between scattered oysters (Living or Dead) and Aggregates. Cumulative contributions were between 0.59 and 0.63 in comparison between Reefs and all the other reef development stages.

The NMDS (Stress = 0.158) showed that the scattered individual group (Living and Dead oysters) overlapped and covered a large part of the factorial plan (Fig. 4). Aggregates, although slightly grouped together, remained mixed with the scattered oysters' group. The only clearly distinct, homogenous group was Reefs. When looking at the five most influential species in the SIMPER analysis, they contributed significantly (p value <0.05) to the differences between the Reef group and all the

others. *S. spirorbis*, *S. armata*, *H. wombat*, and *P. dumerilii* were significantly different between Living and Reef (with respective p values of 0.023; 0.021; 0.001; 0.001) and Dead and Reef (with respective p values of 0.003; 0.001; 0.001; 0.003) groups and *P. ciliata* was significantly different between Aggregate and Reef groups (p value = 0.006).

Functional Traits

There were no significant differences in functional diversity (Shannons' and Simpsons') between the different reef development stages (Table 3). However, when comparing the equitability index on functional traits, Aggregates had a significantly lower value (Table 3). As opposed to the other substrates, all 11 functional groups were systematically represented on the Aggregates but with very different numbers of individuals in each functional group.

The PCA on functional traits (Fig. 5) showed that no functional traits appear to be particularly linked to a reef development stage. Still, the scattered group (individual Dead and Living oysters) supported a greater variability in functional traits, whereas aggregated substrates (Aggregate and Reef) were

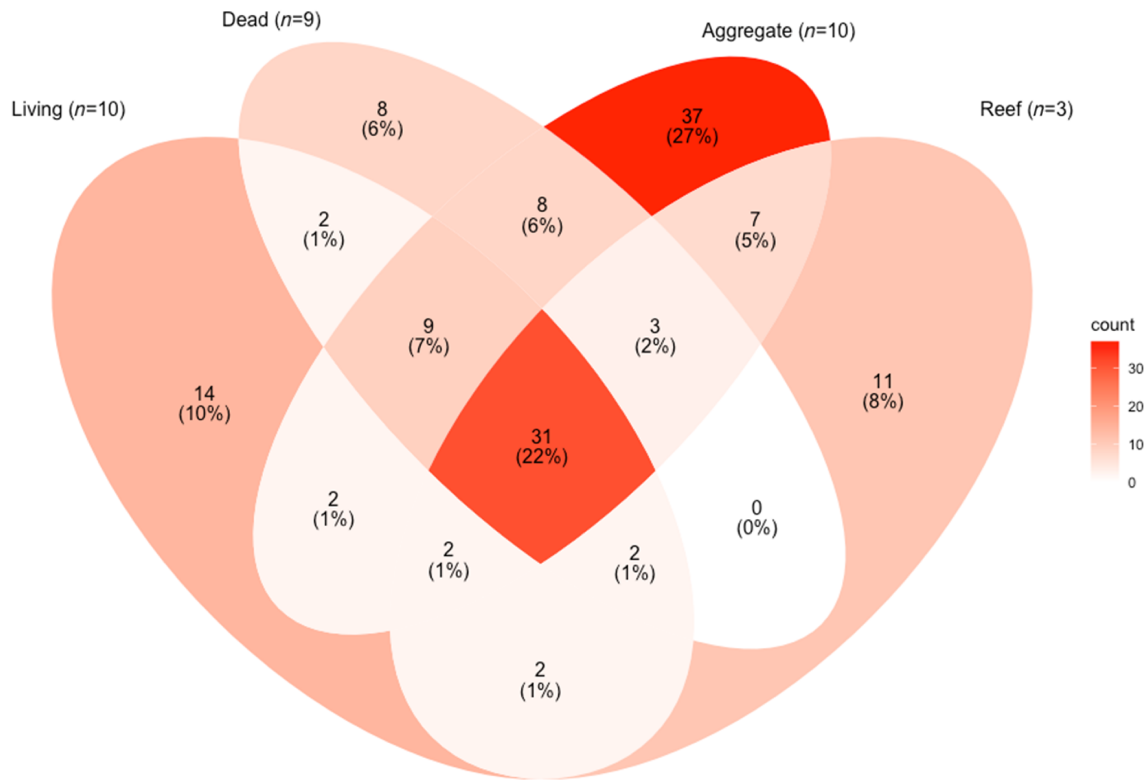


Figure 3. Venn diagram of the distribution of the 137 found epibionts among the different reef development stages.

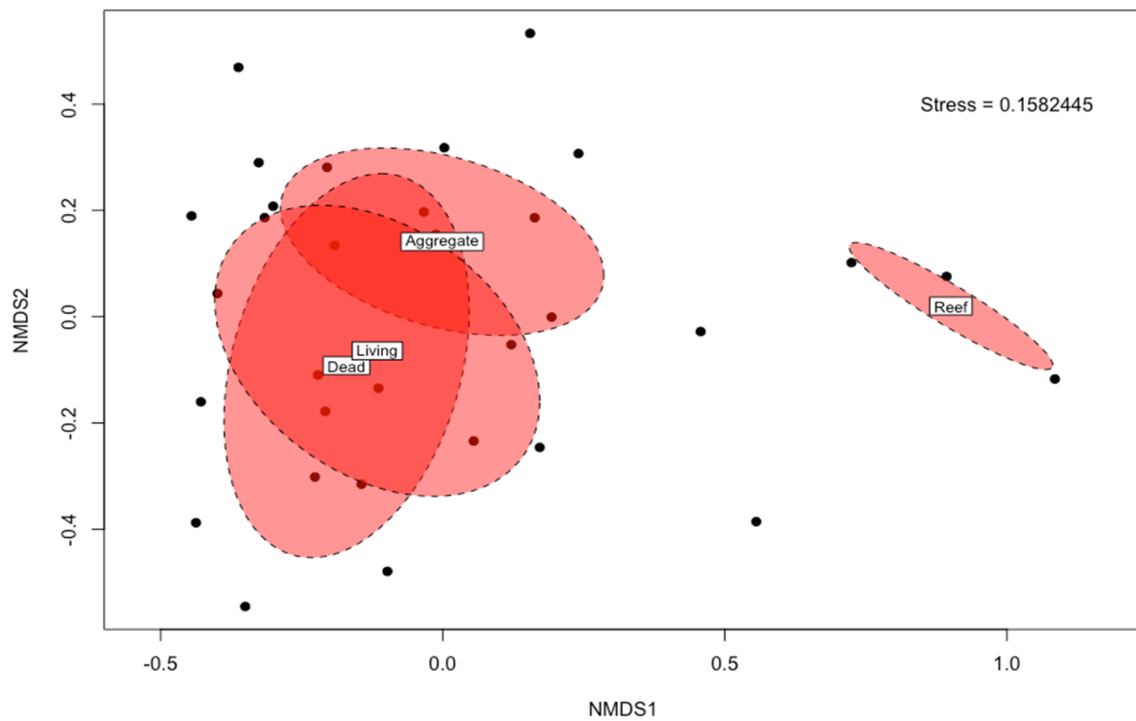


Figure 4. Nonmetric multidimensional scaling based on the abundance per shell surface area (cm²). Number of samples for each reef development stage: Living (n = 10), Dead (n = 9), Aggregate (n = 10), and Reef (n = 3).

Table 3. Main functional diversity and evenness indices (mean ± SD) for the epibiotic community of each reef development stage. (*) Tukey post hoc test: Significantly lower than Living ($df = 12, p \text{ value} = 0.01$), Dead ($df = 13, p \text{ value} = 0.01$), and Reef ($df = 19, p \text{ value} = 0.008$).

	Scattered individuals			
	Living (n = 10)	Dead (n = 9)	Aggregate (n = 10)	Reef (n = 3)
Mean number of functional groups	9.8 (±1.03)	9.77 (±0.44)	11 (±0.00)	10 (±0.00)
Shannon _{func}	1.98 (±0.05)	1.99 (±0.05)	1.99 (±0.07)	2.06 (±0.03)
Simpson _{func}	0.85 (±0.01)	0.85 (±0.01)	0.84 (±0.01)	0.86 (±0.01)
Equitability index	0.87 (±0.04)	0.87 (±0.02)	0.83 (±0.03)*	0.89 (±0.01)

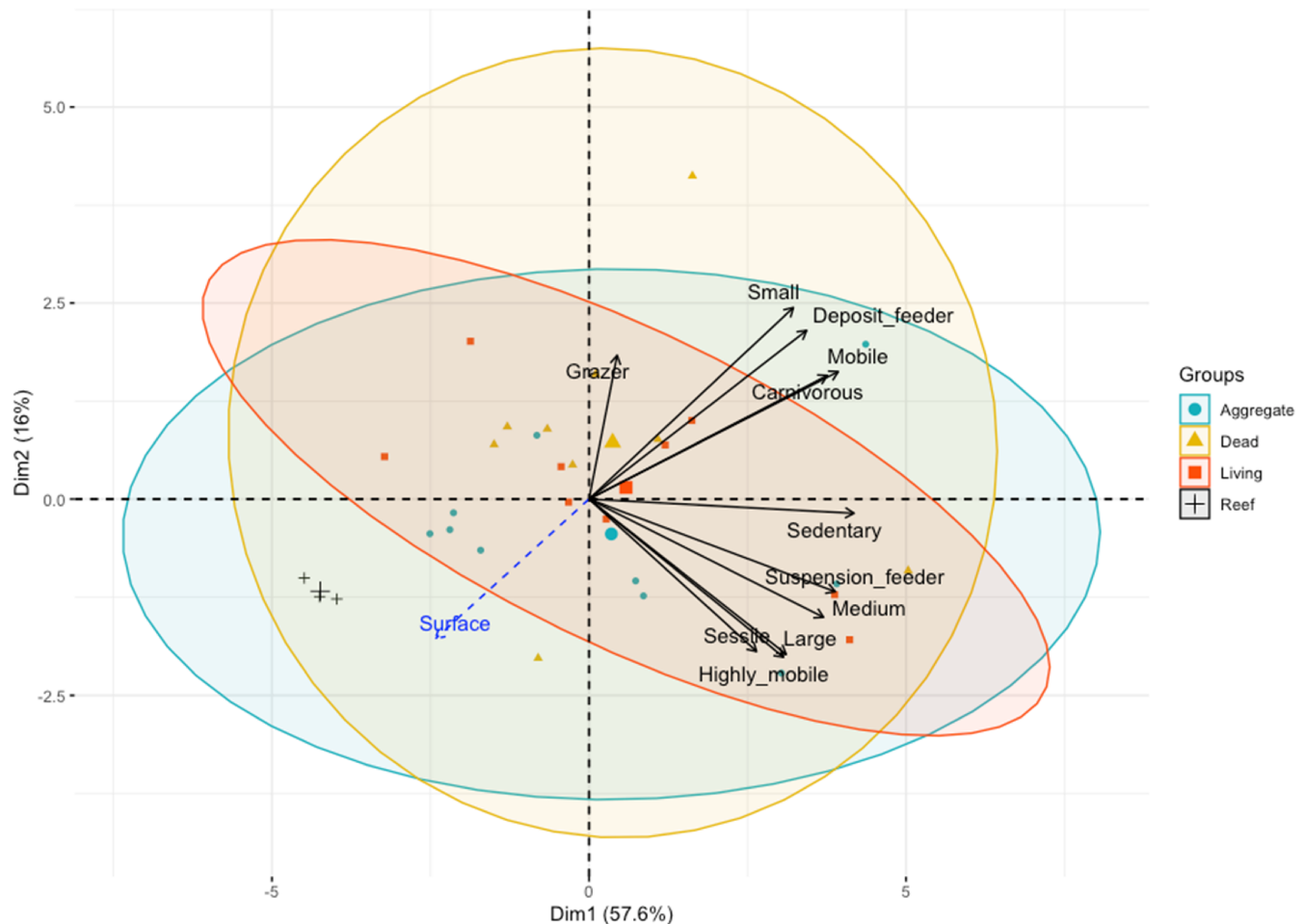


Figure 5. Principal component analysis of functional traits of taxa found, standardized by the oyster shell surface area of the samples. Shell surface area (Surface) was not used in building the PCA and is only represented as a supplementary variable. Number of samples for each reef development stage: Living ($n = 10$), Dead ($n = 9$), Aggregate ($n = 10$), and Reef ($n = 3$).

more clustered. Only Reef substrates were negatively correlated to Dim1. The correlation matrix showed that Dim1 was mainly driven by mobility traits, especially sedentary (quality of representation [QR] = 0.88) and mobile (QR = 0.81) organisms. Suspension-feeders (QR = 0.74) and carnivores (QR = 0.73) were feeding traits that were also well represented. Dim2 was mainly driven by the highly mobile group, but its QR was low (QR = 0.24).

Comparison to Literature

In order to identify possible similarities and contribute to the definition of a reference ecosystem, our results were compared to four other articles that investigated the associated biodiversity of *Ostrea edulis*. The first one, written by Smyth and Roberts (2010), had very similar objectives and methods to this study, as they investigated the epibiota of natural *O. edulis* reefs of different ages in Strangford Lough (Northern Ireland) in order to

assess their contribution to biodiversity. They identified 64 epibionts belonging to nine phyla. Zwerschke et al. (2016) compared benthic assemblages associated with native (*O. edulis*) and non-native (*Crassostrea gigas*) oysters in Strangford Lough (Northern Ireland). Experiments were carried out in situ next to natural oyster reefs, but on experimental plates, colonized by juvenile oysters from hatcheries. They identified 146 epibionts belonging to 10 phyla. Shodduyn (1931) investigated the epibiota of landed, commercial flat oysters coming from the Atlantic Ocean, the Irish Sea, or the English Channel. As these oysters had already been manipulated and transported before investigation, the list of epibionts given by the author is restricted. He identified 31 epibionts belonging to 10 phyla. In their report on historical flat oyster reef location and their ecological importance, Thurstan et al. (2024) provides a list of all the organisms related to flat oyster reefs from the Wadden Sea the Baltic Sea, and the North Sea, mentioned in different texts written by naturalists from the eighteenth and nineteenth century. They identified 161 epibionts belonging to 12 phyla.

Compared to these four studies (see Table 4), the total number of species found in our study ($n = 137$) is relatively high. Note that, as only strict epibionts and endobionts were determined, the total number of oyster reef associated species (considering all vagile species) might be even higher.

Thus, in addition to our results, the comparison of all these studies enabled us to give a description of the average biodiversity that an *O. edulis* reef structure can host. At a Phylum level, we usually found Annelida (between 13 and 51%), Mollusca (between 11 and 30%) and Arthropoda (between 13 and 27%) to be the most abundant (Fig. S1), especially for studies with relatively high numbers ($n > 80$) of identified epibionts (Smyth & Roberts 2010; Zwerschke et al. 2016; Thurstan et al. 2024). When comparing at a Class level, Polychaeta (between 13 and 50%) and Malacostraca (between 4 and 25%) were found to be the most abundant in most of the studies, including ours (Fig. S2). Mollusca had a well-balanced distribution between Bivalvia and Gastropoda. In Zwerschke et al. (2016) and Thurstan et al. (2024) a significant number of Cnidaria and Chordata were identified, whereas in our study there were very few. At a Class level, Hydrozoa were dominant for Cnidaria and Ascidiacea were largely dominant for Chordata.

Discussion

Oyster Reef: A Hotspot of Biodiversity?

This study demonstrated that, despite the poor state of their remnant populations, flat oysters are still hosting an important macrofaunal biodiversity. This is illustrated by the values of Shannon's diversity indices (H') ranging from 2.25 and 2.52 depending on the reef development stage. These values are comparable to those of other foundation species' associated fauna present on the French Atlantic coast. They are higher than the ones recorded for the tube-building polychete *Lanice conchilega* on the French side of the English Channel (H' between 1.55 and 1.83) (De Smet et al. 2015) and the bivalve *Mytilus edulis* ($H' = 0.99 \pm 0.24$) (Markert et al. 2010), but lower than for the polychete *Sabellaria alveolata* from the bay of Mont Saint-Michel (H' between 2.50 and 5.80) (Dubois 2014) and the eelgrass (*Zostera marina*) from the English Channel (H' between 3.74 and 4.27) (Pezy et al. 2019). As in all the others cited above, *O. edulis*' associated fauna is dominated by polychetes (50%), followed by malacostraca (27%) and bivalves (7%). There is a strong dominance of some species, such as the polychetes *Polydora ciliata* or *Syllidia armata*. *Polydora ciliata* has a strong preference for infesting *O. edulis* compared to the non-native Pacific oyster (*Crassostrea gigas*), also present in the Bay of Daoulas (Lemasson & Knights 2019). Interestingly, *P. ciliata* has not been found among the fauna associated to the neighboring maerl habitat described by Tauran et al. (2020). Besides, these maerl habitats, while hosting very high macrofaunal diversity, share only 16% of their species with flat oyster reefs (Tauran et al. 2020). This low value of common species between the two habitats suggests that the flat oyster fosters its own biodiversity.

Compared with other studies carried out on *O. edulis* reefs, this list of epibionts shows many similarities at phylum level, characterized by a strong dominance of Annelida, Mollusca, and Arthropoda. The main difference is that very few cnidaria were observed on our samples. Literature data shows that hydrozoans, and particularly hydroids, are largely dominant for cnidaria. Hydroids are mostly sessile species, with sometimes no planktonic (free-living) stage (Maronna et al. 2016). The hypothesis for hydrozoans other than hydroids is that during

Table 4. The number of epibionts associated with *Ostrea edulis* in this study and four scientific articles (Shodduyn 1931; Smyth & Roberts 2010; Zwerschke et al. 2016; Thurstan et al. 2024).

Number of identified species	Localization	Aim of the study	Source
31	English Channel-North sea-Irish sea	Identification of epibionts on landed oysters	Shodduyn (1931)
64	Strangford Lough (Northern Ireland)	Investigate intra- and inter-site differences for epibionts	Smyth and Roberts (2010)
161	Wadden Sea, North Sea, and Baltic Sea	Literature review on epibionts of 12 documents from 1743 to 1893	Thurstan et al. (2024)
146	Strangford Lough (Northern Ireland)	Comparison of <i>Ostrea edulis</i> ' and <i>Crassostrea gigas</i> ' epibionts	Zwerschke et al. (2016)
137	Bay of Brest	Evaluating the reefs' associated biodiversity	This study

our sampling period they were in a planktonic stage (medusae). For hydroids, additional surveys are necessary to confirm their presence in the neighboring ecosystems.

Aggregation Effect

The initial hypothesis was that, compared to isolated scattered oysters, aggregates on the seabed and from the reefs would host greater species richness and abundance. Our results show that epifaunal abundance is twice as high and species richness is higher for aggregated oysters as for single oysters, if the available surface area for settlement is not corrected for. However, if data are expressed per surface area of shells, results show, for both abundance and species richness, a decrease with the level of aggregation. These preliminary findings contrast, for instance, with the results presented by Deane et al. (2015) showing that their *O. edulis* reefs had greater species richness per oyster shells than oysters lying on the seabed. However, Deane et al. (2015) mimicked an oyster reef by assembling 1200 dead oyster shells in a box with 20 living oysters added after deployment. Although they emphasize that their reef design was a close replicate of natural reefs, their reef samples were very different from ours, taken from a reef formed by the natural colonization of an artificial substrate. Also, Deane et al. (2015) tracked the colonization of cleaned and dried oyster shells, whereas our samples were from a 4-year old oyster reef. Nevertheless, the artificial (metallic) structure hosting the oyster reef might have an effect on the epibiotic community.

This negative correlation between species richness (standardized by the available area for colonization) and the density of the bioengineer species is somewhat counter-intuitive but has already been observed for other reef-building organisms, such as *S. alveolata* (Dubois 2014). To explain this result, Dubois (2014) argues that the higher the density of the reef building organism is, the less place there is available for epibionts to settle. This explanation could also be applied to our results.

When it comes to diversity indices in our study, Shannon's and Simpson's indices remain similar for each aggregation stage. The main reason is that structures with more oysters (such as aggregates and reefs) host more species specific to them that have low abundances per centimeter square, resulting in a more unbalanced epibiotic assemblage. This statement is supported by the values of Pielou's evenness index, which is significantly lower for aggregates and low for reefs (the lack of significance differences between reefs and individual oysters might be due to the low number of studied reef samples [$n = 3$]). Further investigations in other sites would be useful to support these first results and verify this hypothesis of gradual self-reduction in biodiversity with the increase of reef complexity.

Other Sources of Variation of Biodiversity

In our study, isolated oysters rest directly on the bottom, aggregates allow a vertical rise of a few centimeters above the bottom, and the structure of the reef allows the oysters on the reef crest to rise 30–40 cm. Oyster reef elevation might be an important factor for determining species composition, as some seabed-

associated species might not be able to easily access elevated structures. By contrast, some demersal organisms would rather settle on elevated structures. Vertical elevation measurement was not considered in the present study, but we recommend it for future studies. Another parameter that is likely to have a significant effect on reef colonization is the oyster populations' age. Although it is difficult to precisely age an oyster, and even more so an aggregate, a precise measurement of the size of each oyster could make it possible to give an average age of the reef formations to the nearest 1 or 2 years. Considering the size of the isolated oysters and in the aggregates, the age of our samples can be approximated to around 5–7 years old, substantially older than the restored reef samples, aged of 3 years old. The younger age of reefs can partly explain the lower species richness and abundance observed. However, for keystone species, quite the opposite is expected: usually restoration initiates a rapid increase in macrofaunal biodiversity (species richness and abundance) until reaching an asymptote (Dillon et al. 2015; Hemraj et al. 2022; Fariñas-Franco et al. 2023).

Seasonality is also a source of variation. Boudreaux et al. (2006) and Deane et al. (2015) observed a significant effect of the sampling season on *Crassostrea virginica* reefs' epifaunal species richness. In our study, species richness may be possibly underestimated, as sampling took place in the beginning of April (spring). This hypothesis is coherent with the in situ observations made while diving during summer periods that show large numbers of ascidians on reef structures, even though very few of them were observed on the studied samples. Nevertheless, Grall (2018) showed that the macrofauna associated with the neighboring maerl habitat presented very little to no variations according to the sampling season.

When it comes to functional traits, there were no differences between the functional diversity of the epifauna of the reef development stages. These results were to be expected, as for all stages, the same five species were largely dominant. Hence, on each aggregation stage, the same functional traits (sedentary, mobile, suspension-feeder, and carnivores) were the most represented.

Toward the Definition of a Reference Ecosystem for *O. edulis* Reefs

Identifying infaunal and epifaunal invertebrates is a mandatory but only the first step of characterizing oyster reef associated biodiversity. Biggs et al. (2020) confirmed through a meta-analysis that functional redundancy is positively correlated to resilience. Therefore, tracking biodiversity on restored reefs could be an effective way of evaluating the success of a sustainable restoration project. In order to have a complete evaluation of this ecosystem, all small resident mobile organisms and transient fish and crustaceans should also be identified and quantified (zu Ermgassen et al. 2021). For that, zu Ermgassen et al. (2021) advocate underwater and/or remote video surveys to monitor restoration trajectories over time. This technique should be coupled to eDNA analysis to have a better understanding of *O. edulis* biodiversity in the ecosystem. Underwater video

surveys will be conducted on our restoration site in the coming years.

Tracking biodiversity over time in the context of restoration on a complex oyster reef is rather time-consuming when identification is undertaken to a species level, especially for infaunal and epifaunal macroinvertebrates. Nevertheless, some simplifications can be introduced. In a review on taxonomic sufficiency, Terlizzi et al. (2003) concluded that identifying organisms to a family level seems to be sufficient for monitoring purposes. However, the authors emphasize that lowering taxonomic resolution is only compatible with well-known ecosystems. Indeed, for a new restoration project on an unknown site, a complete identification of the species seems mandatory initially to validate or reject the use of lower taxonomic resolution.

Overall, our study shows a rich and diverse, but relatively unbalanced, associated epibiotic fauna with the domination of some species (*P. ciliata*, *P. dumerilii*, and *S. armata*). This high biodiversity offered by native oyster reefs constitutes a key reason for supporting and scaling up restoration projects for this species in Europe, particularly within the framework of NORA, the European Alliance for the Restoration of the Flat Oyster. More specifically, completing our analysis with the identification and quantification of all the other biodiversity groups presented by zu Ermgassen et al. (2021) will give us an extensive overview of this *O. edulis* reef's associated biodiversity. Having such a complete dataset will serve as a baseline for restoration projects in this area and will allow to track the evolution of biodiversity over time (Sturbois et al. 2021; zu Ermgassen et al. 2021). In addition, combining these results with other similar studies from the European coast will contribute to define a reference ecosystem for flat oyster reefs that would serve as a baseline for monitoring biodiversity in forthcoming oyster reef restoration projects.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. List of all the species found on all the samples (cumulated sum).

Figure S1. Comparison of our list of epibionts to literature at a Phylum level.

Figure S2. Comparison to literature of the repartition of the Classes of *Ostrea edulis* associated epibionts.

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