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## Changes in benthic macrofauna in oyster parks during an OsHV-1 $\mu$ Var oyster spat mortality outbreak

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### Abstract :

In intertidal areas, oyster farming creates a crosshatching pattern between oyster tables and aisles. Tables provide a refuge from the current and solar irradiance and the oysters facilitate the accumulation of OM, thereby structuring the spatial organization of the associated macrozoobenthic community at mesoscale. The aim of this study was to describe the quality of the oyster table environment at small scale and the response of the macrozoobenthic community to OsHV-1  $\mu$ var oyster mortality. The species assemblage was dominated by *Golfingia vulgaris*, *Tubificoides benedii*, *Capitella capitata* and *Scoloplos armiger*. The table habitat appeared to be in a bad ecological state throughout the 2-month survey (May and June 2017), whereas in the aisle, eutrophication occurred lately and was clearly related to be due to the massive stranding of dead seaweed at the end of the survey (in early July). So, this disturbance of the species assemblage seemed to occur in two phases: 1) after oyster spat mortality and 2) after seaweed stranding resulted in a bad ecological status, as revealed by macrofaunal indicators. Large quantities of OsHV-1 DNA were also found in some species, including small crabs and amphipods, one week after the mortality crisis, but there is no apparent virus reservoir found in the benthic species.

### Highlights

► Macrofauna was more diversified and more abundant under the oyster table than in the aisles. ► A shift from a good to a bad ecological status occurred during this summer field survey. ► Benthic macrofaunal assemblages responded to oyster spat mortality then to dead seaweed. ► Eutrophication indicators showed a high organic pollution under oyster tables first and in aisle after. ► Amphipods, barnacles, cockles and crabs were passively contaminated with of OsHV-1  $\mu$ Var particles after mortality outbreak.

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**Keywords** : Benthic macrofauna, Benthic indicators, Oyster farming, *Crassostrea gigas*, OsHV-1?var mortality

## **1. Introduction**

Estuaries are a particular type of environment located at the interface between the land, a river and the sea. These ecotones are among the most productive biotopes in the world (Whittaker and Likens, 1975). Characterization of the ecological status of estuaries is crucial

for integrated coastal management and protection. Benthic macrofauna is a well-recognized biological indicator of the quality of coastal ecosystems (Dauvin, 2007; Dutertre et al., 2013). Many studies aimed at designing management plans that are usually based on benthic macrofaunal diversity (Keeley et al., 2013; Dutertre et al., 2013; Carstensen et al., 2014) and, for this purpose, benthic indicators are routinely measured to assess the environmental status of the ecosystem and water quality. These indicators have been measured within the scope of the Marine Strategy Framework Directive (MSFD) Descriptor I (Biodiversity). As reported by Dauvin et al. (2007), soft-sediment macrozoobenthic organisms are considered to be good indicators because they (i) are relatively sedentary and consequently unable to escape deteriorated water/sediment quality, (ii) have relatively long life spans, (iii) include diverse species with different tolerances to stress, and (iv) play a vital role in biogeochemical and material cycling between the underlying sediment and the overlying water column. Macrozoobenthic assemblages can vary in response to changes at the water-sediment interface including enrichment in organic matter (OM) related to eutrophication (All references cited herein, e.g. Borja et al., 2000; Ysebaert and Herman, 2002; Dauvin et al., 2007). Algal blooms can typically lead to benthic enrichment in OM because of wreck seaweeds.

The introduction of the cultivated Pacific oyster *Crassostrea gigas* (Bayne et al., 2017) can provoke an ecological unbalance, since this bivalve is known to modify its environment (Padilla, 2010) in several ways and can be considered now as an invasive species (introduced in France for cultivation in the early seventies). One of the direct effects of *C. gigas* is trophic competition with other suspension-feeders (e.g. *Cerastoderma edule*, see Ubertini et al., 2012).

Oyster farming is known to create particular environmental conditions beneath or adjacent to cultivation areas (Matisson and Lindén, 1983; Ubertini et al., 2012). These disturbances can elevate the topographic level (Forrest and Creese, 2006) through the accumulation of biodeposits (Haven and Morales-Alamo, 1966; Hily, 1976; Sornin et al., 1983; Mitchell, 2006; Nugues et al., 1996) thereby increasing the OM content of sediment beds (Nugues et al., 1996; Forrest and Creese, 2006; Echappé et al., 2018). Such sediment processes can then induce an increase of anoxic compounds (Van Duyl et al., 1992; Forrest and Creese, 2006) triggering bacterial remineralization (Baines and Pace, 1991; Azam et al., 1994; Nagata, 2008). These changes in sedimentary properties also lead to the development of microphytobenthic biofilms (Orvain et al., 2012; Echappé et al., 2018). In addition to the

biological impact of the oysters themselves, oyster farming metallic structures also play a role in reshaping the sedimentary environment. Indeed, the elevated tables, on which oysters are grown, attenuate friction velocity and increase habitat muddification (Kervella et al., 2010; Nugues et al., 1996; Sornin, 1981). For all these reasons, the modification of the sediment characteristics directly in oyster parks affects the benthic macrofaunal structure and may lead to the rarefaction of filtering bivalves and a strong colonization by annelids (Sylvand, 1995; Nugues et al., 1996; Dubois et al., 2007, Ubertini et al. 2012). Indeed, it is recognized that oyster tables determine species assemblages and associated trophic pathways, like the higher presence of carnivores, even at the small scale of one oyster table (Dubois et al., 2007).

Since the first reports of oyster spat mortality in France in 2008 (Segarra et al., 2010), noticeable episodes have been observed worldwide (Lynch et al., 2012; Jenkins et al., 2013; Hwang et al., 2013) causing massive losses of stock for oyster farmers. When the water temperature exceeds 16 °C, summer mortalities have mainly been attributed to the Ostreid herpes virus  $\mu$ variant (OsHV-1  $\mu$ Var) (Pernet et al., 2012) which can kill 80% of oyster spats but with many variations depending on the site, the batch and the year. During a mortality outbreak, the flesh of the dead oyster spat can deposit locally or be dispersed in the environment by tidal currents. Through this dissemination mechanism, the virus associated with dead oyster spat flesh could also contaminate the benthic compartment. These spat mortality can also have an ecological impact (Forrest et al., 2009) including a flux of organic matter downward the sediment. This sudden organic enrichment can modify the sediment dynamics and the growth rates of microphytobenthic biofilms that can in turn disturb local benthic communities. The fate of this pathogen agent and its resilience outside of the host organism (i.e. once the virus is transferred to the bottom sediment) is poorly described in literature. some macrozoobenthic species could also specifically interact with viral resilience outside the host and be considered as virus reservoirs. The present study was developed to give some new insights in the interaction between this virus contamination and the macrozoobenthic community in relation to eutrophication, by using a sampling strategy integrating the spatial patterns, at the very small spatial scale of the oyster table

## 2. Material & method

### *Study site*

The Bay of Veys is located in the south-western part of the Seine Bay where 40% of the inputs originate from the River Vire (Jouenne et al., 2007). This bay is an estuarine and shellfish ecosystem that stretches over an intertidal zone of 1.60 km<sup>2</sup>. During spring tide, a tidal range of 8 meters with currents of 3 m.s<sup>-1</sup> can be reached (Orvain et al., 2012). Within this area, oyster farms are mainly located on soft sediment with a natural tendency to slow silting due to the modification of the field of the tidal currents related to the installation of oyster parks (Kopp et al., 1991). Although oyster growth in this shellfish basin is the fastest in France, it only comes in second in terms of production (25 000 tons in 2016; source: *Comité National de la Conchyliculture*).

### *Experimental design*

The study was conducted along one single row of oyster tables located at G fosse-Fontenay in the Bay des Veys (Normandy, France) on the foreshore (from 49°22'53.2" N; 001°05'44.2" W to 49°22'54.6" N; 001°05'43.4" W) from the 22<sup>nd</sup> of May 2017 to the 1<sup>st</sup> of July 2017. The sampling strategy was developed to take advantage from the particular aspect of the local spatial pattern of oyster cultures, with alternating structure between one culture table and the adjacent aisle, where oyster farmer tractors pass through. Dubois et al. (2007) observed some differences in terms of benthic habitat and macrofauna assemblages in this oyster park, between one oyster table and the proximate aisle. We thus investigated the community and virus detection at this very small scale, by repeated sampling of sediment, macrofauna determination and virus quantification in the two habitats: under the oyster table and in the proximate aisle. We paid a special attention on the local difference of the benthic community in order to minimize the bias by uncontrolled factors (like the oyster culture condition that could be very different from one site to the other, or the differences in terms of sedimentary and microphytobenthic factors that could be very strong in this sector, as already described by the kriged maps shown in Orvain et al.2012 and Ubertini et al. 2012).

One single oyster table was divided into three proximate areas (A, B & C) (Figure 1), to divide the entire table in three 50-meter long areas. Each area was composed by 20 oyster bags each containing smaller bags containing two hundred 3-month old oyster spats for a good control of oyster culture conditions history on the site. These oyster spats were produced using standard hatchery procedures (Petton et al., 2015).

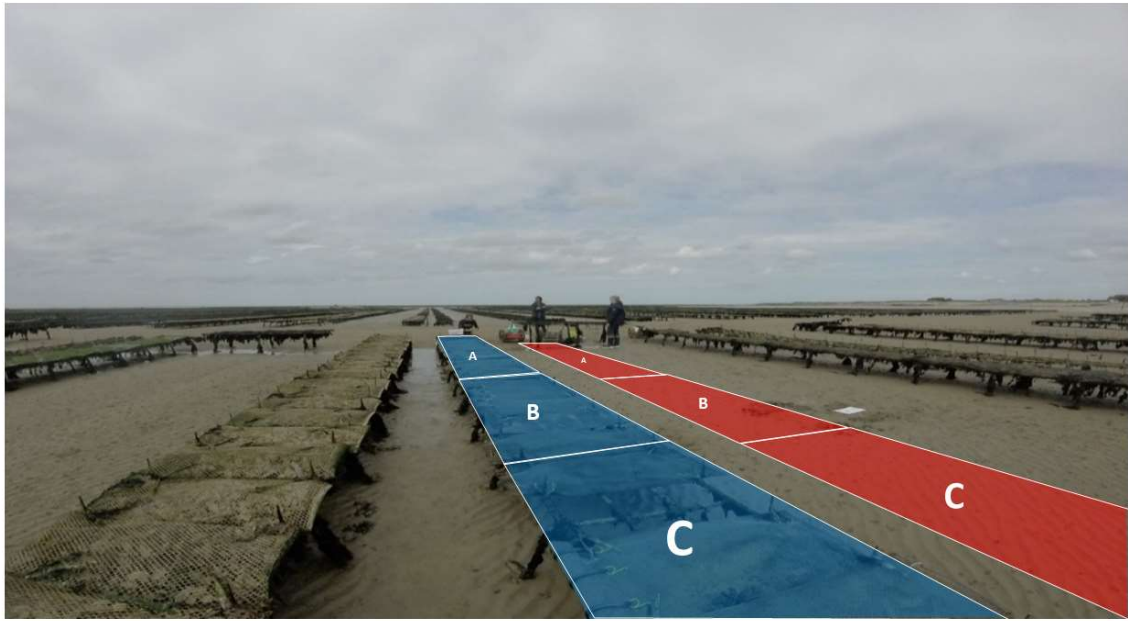


Figure 1 : Experimental field design. The oyster table sample areas are in blue (A, B & C) and the aisle sample areas are in red (A, B & C)

### *Macrofaunal sampling*

The sampling dates are expressed as the relative Julian days with reference to the 1<sup>st</sup> January 2017 (Day 1 = 1<sup>st</sup> January). During the 40-days survey, the chosen dates were the following ones: Day 143 (23 May), 152 (2 June), 159 (9 June), 172 (22 June), 179 (29 June) and 182 (2 July). On each occasion, one bag out of the 20 oyster bags were randomly selected in each of the 3 areas (A, B and C) and faunal samples were taken in the two closed habitats studied i.e. (i) directly under the oyster table and (ii) at a distance of 2 meters in the adjacent aisle. This sampling was spatially repeated three times in the 3 areas A, B and C. For each sample, benthic macrofauna was sampled in a 0.2 m diameter PVC core (surface = 0.0314 m<sup>2</sup>) inserted to a maximum depth of 20 cm. Samples were then sieved through a 1 mm mesh size on the field and fixed in a 4% formaldehyde solution before being transferred to ethanol for conservation. The species were then counted and identified using (Lincoln and British Museum, 1979) identification keys for amphipods, (Fauvel, 1927, 1923) identification keys for annelids, and (Hayward and Ryland, 1995) identification keys for decapods and bivalves. On each sampling day, pictures were taken of the surface of the sediment to assess the extent of algal coverage.

### *OsHV-1 sampling*

The most abundant and visible macrozoobenthos species on each sampling date were sampled (maximum 5 individuals per species) and conserved at -20 °C for quantification of

their OsHV-1 charge. After thawing, sampled organisms were crushed and their DNA was extracted and purified using the NucleoSpin® 96 Blood Kit (Macherey-Nagel). Total extracted DNA was quantified using a Nanodrop 200 (Thermoscientific) for standardization purposes. OsHV-1 DNA in the samples was quantified via real-time qPCR CFX 96™ C1000™ (Biorad).

#### *Data analysis*

The habitat diversity was estimated using species richness and the biodiversity index of Shannon & Weaver (H'). This index is maximum when all individuals are distributed equally among all species and vice versa. It is expressed by the following equation:

$$H' = - \sum_{i=1}^{n-1} \left( \frac{ni}{N} \right) \times \log(ni/N)$$

where H' is the diversity index; n is the number of individuals of taxon i; and N is the total number of individuals. The species evenness was calculated using the index of Piélou (J') which makes it possible to compare the structures between the studied table and the adjacent aisle. This index varies between 0 and 1; it tends to 0 when diversity is low and tends to 1 when all species show the same abundance. This index is calculated as follows:

$$J' = \frac{H'}{H'_{max}}$$

In addition, rank-frequency diagrams were calculated to describe variations in the structure of macrofauna assemblages between table and aisle habitats over time. When the curve is hyperbolic, it reflects very strong disparities between species (dominance relationships, rare species) and potentially highly disturbed ecosystems (Frontier, 1985). On the other hand, when the diversity and abundance of species are strong the curve will be more convex (Frontier et al., 2008)

The Benthic Opportunistic Polychaetes - Amphipods (BOPA) ratio index (Dauvin and Ruellet, 2007) was used to measure the ratio between the frequency of amphipods (the latter being very sensitive to the presence of organic matter) and that of opportunistic polychaete annelids belonging to groups IV and V. This index is determined on a scale from 0 to 0.301 with a log (base = 10).

$$BOPA = \log \left( \frac{f_P}{f_{A+1}} + 1 \right)$$



The AZTI Marine Biotic Index (AMBI) index (Borja et al., 2000) establishes the ecological quality of a particular site, determined on a scale from 1 to 5 with the calculation of the benthic coefficient (BC) representing the quality of the benthic conditions in ranks. from 0: unpolluted to 7: highly polluted (Hily 1984, Majeed 1987). This index was calculated according to the following formula:

$$AMBI = [(0 * \%GI) + (1,5 * \%GII) + (3 * \%GIII) + (4,5 * \%GIV) + (6 * \%GV)] / 100$$

In continuity with the AMBI, the BENTIX index (Simboura and Zenetos, 2002) was calculated to describe the response of soft substrate benthic communities to different natural and man-made disturbers by reducing the number of ecological groups involved in the formula to limit errors due to the grouping of species. This index is calculated according to the following formula:

$$BENTIX = \{(6 \times \%GI) + 2 \times (\%GIII + \%GV)\} / 100$$

The ecological groups II and IV of the AMBI index can respectively be associated with ecological groups I and III of the BENTIX index due to their low significance. This index ranges from 2 (poor environmental quality) to 6 (good environmental quality). Characterization of the ecological status according to each index is reported in Table 1.

*Table 1: Classification of soft bottom benthic habitats based on BOPA, AMBi and BENTIX indexes*

<b>Ecological quality status</b>	<b>BOPA</b>	<b>AMBI</b>	<b>BENTIX</b>
High	0 – 0.04576	0 – 1.2	4.5 – 6.0
Good	0.04576 – 0.13966	1.2 – 3.3	3.5 – 4.5
Moderate	0.13966 – 0.19382	3.3 – 4.3	2.5 – 3.5
Poor	0.19382 – 0.26761	4.3 – 5.5	2.0 – 2.5
Bad	0.26761 – 0.30103	> 5.5	0

Pictures were also taken to estimate algal coverage during the survey by calculating the percentage of recovered surface using ImageJ software. Data were analyzed using multivariate methods using “vegan”, “MASS”, “ggplot2”, “dplyr”, “tidyr”, “grDevices”, “FactoMineR”, “factoextra”, “corrplot”, “RColorBrewer” and “ade4” packages via the R-studio software. Multivariate analysis (Canonical Analysis, factorial analysis of correspondence) was performed on the 10 most abundant species with supplementary

variables described in a parallel study (Vanhuysse et al., submitted). Rare amphipods and polychaetae were added to the CA as additional variables because of their scarcity.

### 3. Results

#### *Abundance, diversity and specific richness*

A total of 389 individuals belonging to 19 species were identified. The 19 species corresponded to 12 annelids, 5 arthropods, 1 bivalve and 1 Sipuncula. The number of taxa was not evenly distributed between the two habitats since 19 species were observed under the table and only 11 in the aisle. Analysis of the fauna showed that the oyster table habitat had a higher density ( $26.88 \text{ ind.m}^{-2}$ ;  $p\text{-value} < 0.0001$ ) than that the aisle habitat ( $7.52 \text{ ind.m}^{-2}$ ). This difference did not apply to biomass ( $p\text{-value} > 0.05$ ) due to a wide standard deviation (oyster table biomass mean =  $49.96 \pm$  standard deviation 152 aisle biomass =  $12.2 \pm 20.3$ ). Abundance increased by a factor of 2.6 between day 179 and day 182 under the oyster table, while it increased by a factor of 2.8 in the aisle.

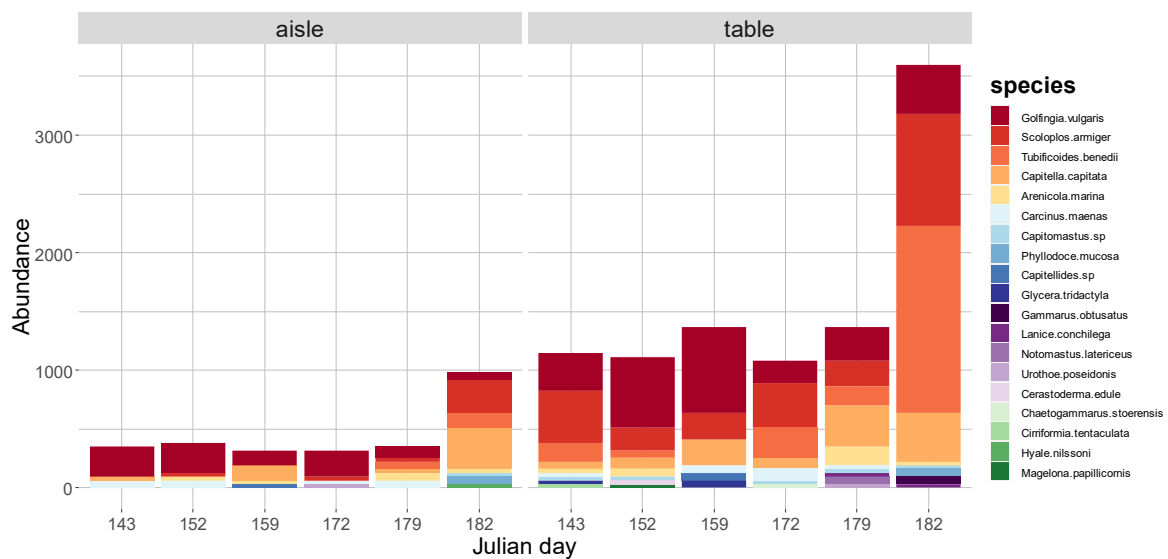


Figure 2: Temporal evolution of species abundance observed according to habitats and days (see Annex 1 for detailed tables with the list of species for each local habitat).

The top rank species was the Sipuncle *Golfingia vulgaris* that is present homogeneously in the 2 conditions (under oyster tables as well as in aisles). The second and third top rank species in terms of density were the Polychaete *Scoloplos armiger* and the Oligochaete *Tubificoides benedii*, but these 2 species were only present under tables. The Polychaete *Capitella capitata* ranked at the 4<sup>th</sup> position, but like the Sipuncle *Golfingia*, these worms were present in both environments indifferently (

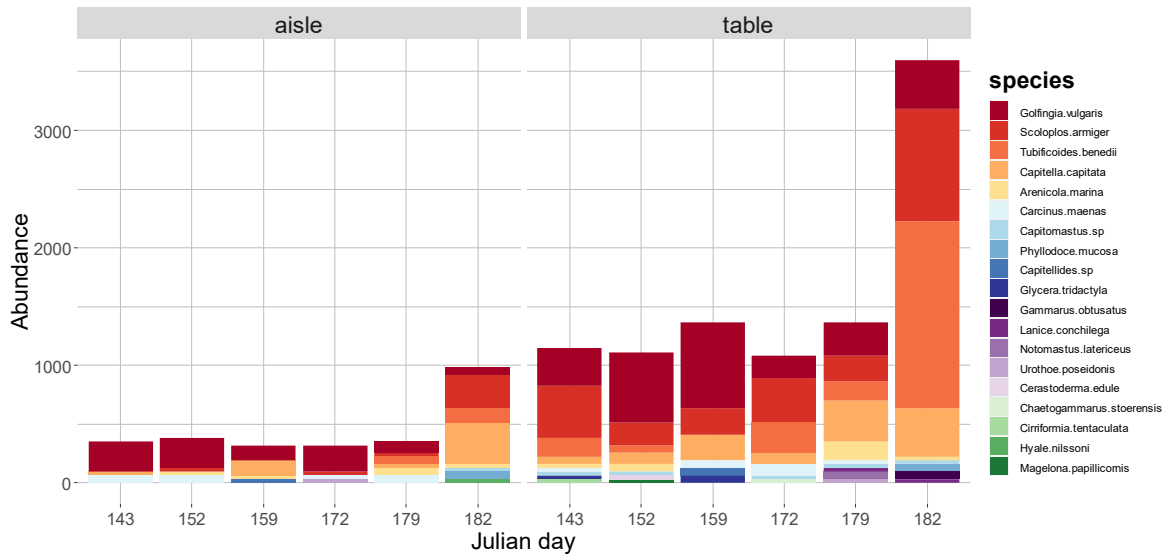


Figure 2). The level of species richness remained more or less constant in each habitat (Table 2) but almost doubled under the table between day 172 and day 179, with values shifting from 7 to 11 species. Consequently the highest density values were observed at day 182 in both habitats: respectively, 3597 and 987 ind. m<sup>-2</sup> were found under the oyster table and in the aisle. The level of patchiness was always very high and there were numerous species sometimes very high standard deviation (annex 1) for all species under tables as well as in aisles, and when densities (ind.m<sup>-2</sup>) were at the minimum (table : 583 +/-546 on day 152 , aisle 167 +/-163 on day 159) or at the maximum (table : 1867 +/- 1881 , aisle 517 +/- 705).

Table 2: Shannon index, Species Richness and Pielou index

Julian Day	Habitat	Shannon (H')	Species richness	Pielou (J')
143	aisle	0,76	3	0,69
143	table	1,66	9	0,75
152	aisle	0,98	4	0,71
152	table	1,48	8	0,71
159	aisle	1,19	4	0,86
159	table	1,35	6	0,76
172	aisle	0,94	4	0,68
172	table	1,65	7	0,85
179	aisle	1,72	6	0,96
179	table	2,00	11	0,83
182	aisle	1,68	8	0,81
182	table	1,48	9	0,67

The species richness shifted from the number of 3 (aisle, day 143) to the value of 11 (table, day 179). The averaged richness was globally higher under the oyster table (8.33) than in the aisle (4.83). From a temporal view, species richness was always higher under the oyster table, but at the end of the survey, tended to increase in the aisle to almost reach the table values. In terms of species diversity, the Shannon index values (Table 2) ranged from 0.76 (aisle, day 143) to 2.00 (table, day 179). The index was higher under the oyster table (H' =

1.60) than in the aisle ( $H' = 1.21$ ). The Pielou index (Table 2) ranged from 0.67 (table, day 41) to 0.96 (aisle, day 179). This index averaged 0.762 under the oyster table and 0.78 in the aisle. The Pielou index remained quite stable around 0.75 under the oyster table except on day 172 and 179. More variations were recorded in the aisle specially at the end of the survey.

### Frequency rank chart

Rank-frequency distribution was used to characterize species diversity (Figure 3).

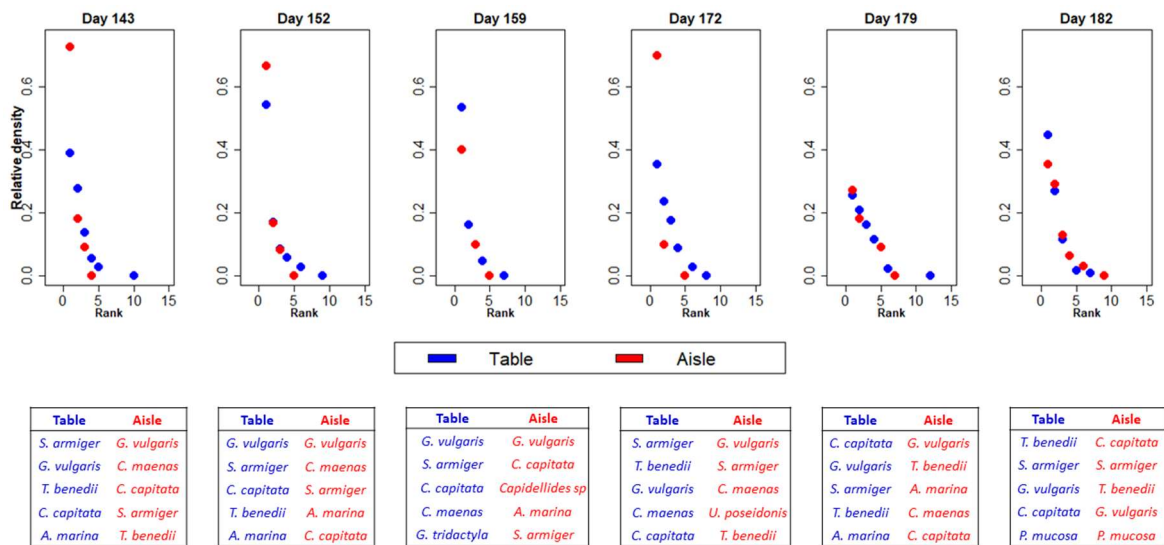


Figure 3: Rank frequency diagrams for sampling days. Blue points correspond to table samples and red points to aisle samples. The table under each diagram corresponds to the five most abundant species for each habitat.

Globally, relative densities under the table tended to be higher than the ones in the aisle except on day 159. There did not seem to be a clear difference in the shape of the curves between the two environments, even though the dominant species were not the same ones between the 2 contrasted environments. The aisle habitat was less diversified compared to the oyster table. Nevertheless, a three-phase turnover was observed in terms of dominance. During the first phase (days 143, 150 and 159), the species *Golfingia vulgaris* dominated the species assemblage, with a notable presence of *Scoloplos armiger*. The second phase, day 172, was a transitional state before the third phase, when *G. vulgaris* and *S. armiger* were still dominant but a stronger dominance of *Tubificoides benedii* was observed, especially under the table. The third phase (days 179 and 182) was characterized by a decline in *G. vulgaris* density and a massive presence of species such as *T. benedii* under tables, *Capitella capitata* in aisles and *S. armiger*, highly present in both habitats

### *Ecological indicators*

The BOPA index (Figure 4. A) ranged from 0.001 to 0.035. The mean index under the oyster table was estimated to be 0.0178 and 0.00475 in the aisle. The BOPA index was higher under the oyster tables. There was a gradual increase in the BOPA index over time whatever the habitat., but the ecological status remained high, in all cases.

The AMBI index (Figure 4. B) ranged from 0.6 to 4.35 indicating a contrasted environmental quality ranging from high to poor. The average AMBI index was 2.98 under the table and 2.22 in the aisle. Except on day 159, the calculated AMBI index was higher under the oyster table. The AMBI index tended to increase over time whatever the habitat. The AMBI index reached maximum in the two habitats on day 182. In terms of functional groups, the aisle habitat was mostly composed of group II species (species that are not affected by a slight enrichment in organic matter) and group III (species that tolerate organic matter enrichment) while more group V species (very opportunistic species that characterize disturbed environments) were observed under the oyster table particularly at the end of the survey. Indeed, a gradual dominance of group V species was observed from day 143 to day 182 under the table mainly due to species such as *Capitella capitata*, *Scoloplos armiger* and *Tubificoides benedii*.

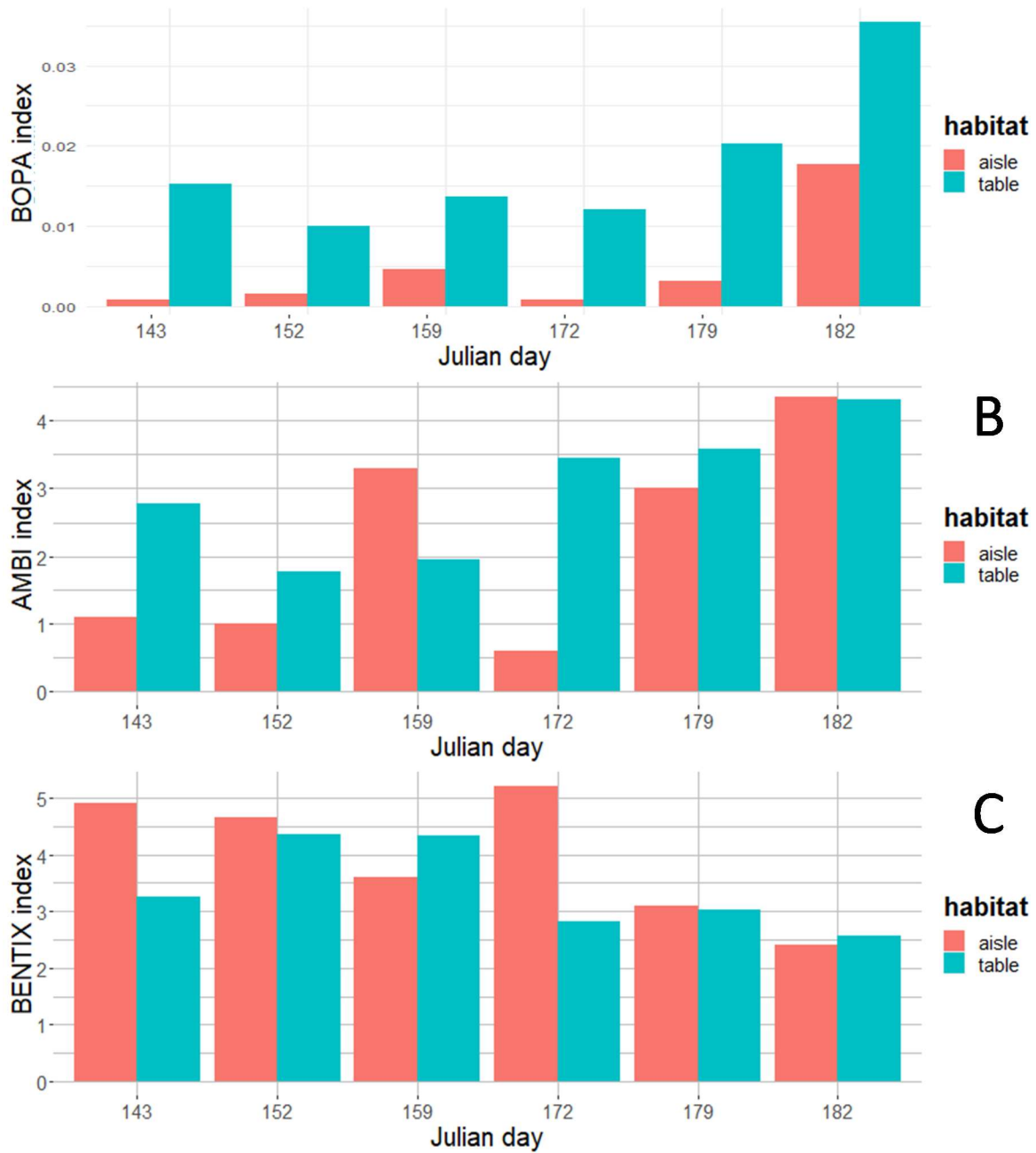


Figure 4 : benthic indexes with A. BOPA index. B. AMBI index. C. BENTIX index

The BENTIX index (Figure 4. C.) ranged from 2.41 to 5.20, with an average of 3.39 under the table and 3.98 in the aisle. Except on day 159, the calculated BENTIX index was higher under the oyster table, but decreased over time in both habitats to reach a minimum value of 2.5 on day 182, confirming the poor quality already detected by the AMBI index.

### Multivariate analysis

Axis 1 of the Analysis of Correspondence (CA) accounted for more than 49% of total variation, axis 2 accounted for more than 18%, and axis 3 for more than 12% of total variation (Table 3).

Table 3: Contribution of species to the first 3 axes of the correspondence analysis

	Dim.1 (49.18%)	Dim.2 (18.87%)	Dim.3 (12.1%)
<i>Golfingia.vulgaris</i> (I)	<b>38.84</b>	5.5	1.74
<i>Scoloplos.armiger</i> (III)	6.43	1.33	2.81
<i>Tubificoides.benedii</i> (V)	<b>37.49</b>	10.15	0.02
<i>Capitella.capitata</i> (V)	0.29	<b>50.29</b>	0.11
<i>Arenicola.marina</i> (III)	1.07	9.42	40.33
<i>Carcinus.maenas</i> III	7.65	10.26	18.19
<i>Capitomastus.sp</i> (V)	0.22	0.47	0.5
<i>Phyllodoce.mucosa</i> (III)	2.76	4.27	0.08
<i>Capitellides.sp</i> (V)	3.55	8.32	19.08
<i>Glycera.tridactyla</i> (II)	1.68	0	17.16

The fluctuations in the abundances of the two species *Golfingia vulgaris* and *Tubificoides benedii* were the best represented on Dim. 1 which together accounted for more than 76% of the variation. On the positive part of this axis, *G. vulgaris* was associated with the beginning of the study (Figure 5). On the negative side of the axis 1, the Oligochaeta *T. benedii* was more associated with the data at the end of the survey (day 182). In the middle of the cloud of individuals, the species *S. armiger*, rare amphipods, rare polychaeta and other polychaeta (e.g. *Capitomastus sp*, *Phyllodoce musoca*) were found more on the negative side of the axis 1. *Arenicola marina*, *Glycera tridactyla* and *Carcinus maenas* were found on the positive side of the cloud of individuals.

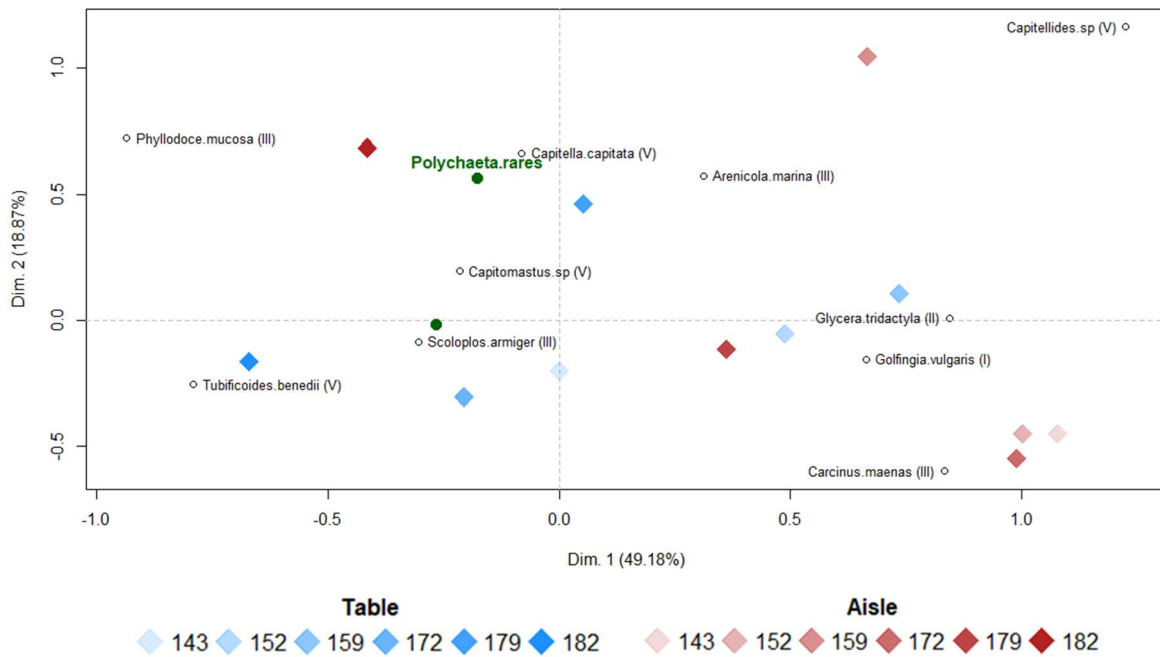


Figure 5: Analysis of Correspondence. Roman numerals in parentheses after the species name correspond to the AMBI ecological groups.

The fluctuation in the abundance of *Capitella capitata* was the best represented on Dim. 2 with more than 50% of variation explained by this species. *C. capitata* was associated with positive values on this axis at the end of the study. The species *Golfingia vulgaris* and *Carcinus maenas* were found on the negative part of axis 2. There was no clear difference in habitat (table vs aisle), *Tubificoides benedii* was present in table samples on the last day of the survey while *C. capitata* and *G. vulgaris* were found in both habitats.

Table 4: Contribution of variables to the first 4 axes of the principal correspondence analysis

	Dim.1 (27.92%)	Dim.2(26.33%)	Dim.3 (16.1%)	Dim.4 (11.1%)
<i>Water content</i>	<b>25.8</b>	0.62	0.01	12.28
<i>chla biof</i>	<b>22.19</b>	3.03	3.25	3.34
<i>chla sed</i>	0.12	<b>24.13</b>	10.47	1.79
<i>NH4</i>	1.64	9.27	3.95	<b>28.13</b>
<i>OM</i>	4.85	3.55	<b>16.48</b>	<b>19.92</b>
<i>Mud content (inf63)</i>	<b>17.15</b>	7.81	10.36	0.37
<i>I</i>	<b>25.96</b>	0.69	0.07	0.92
<i>II</i>	1.67	<b>20.6</b>	<b>12.58</b>	11.6
<i>III</i>	0.13	<b>15.64</b>	<b>32.46</b>	0.63
<i>V</i>	0.48	<b>14.68</b>	10.38	<b>21.03</b>

Following the CA, a Principal Component Analysis (PCA) was performed on quantitative variables using sedimentary data and species biomass ordered according to AMBI ecological



groups. The algal coverage percentage was included as an additional variable (Figure 6), since this is a semi-quantitative variable.

Axis 1 (27.92% of total inertia) of the PCA contained the biomass of AMBI group I species and three sedimentary variables: mud content (named “inf63” on the diagram), water content of the sediment and biofilm chl *a* (Table 4). More precisely, AMBI group I species biomass, were on the negative part of the axis with biofilm chl *a* and water content of the sediment opposed to fine sediment particles (Figure 6). Axis 2 (26.33% of total variance) of the PCA contained the sediment chl *a* and group II, III and V AMBI species. These AMBI groups were together on the positive part of the axis in opposition to sediment chl *a*. Axis 3 (16.1% of total variance) of PCA contained organic matter content (OM) and group II and III AMBI species. These variables were positively correlated together (not shown in Figure 6). Axis 4 (11.1% of total variance) of the PCA contained NH<sub>4</sub><sup>+</sup> concentrations, organic matter content (OM) and the group V AMBI species. These species and NH<sub>4</sub><sup>+</sup> concentrations variables were negatively correlated with OM content and therefore opposite on axis 4.

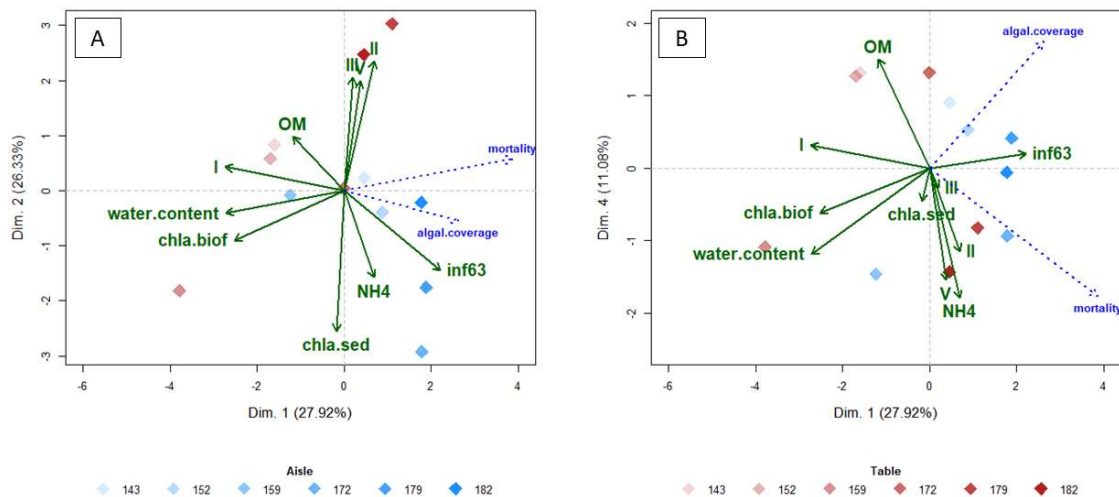


Figure 6: PCA analysis with A. dimensions (Dim) 1 and 2. Dim 1 contained biomass for AMBI II and III species, fine sediment particles (inf63) and organic matter content (OM) variables. Dim 2 contained the sediment water content, the biofilm and sediment chl *a* and the AMBI I species. B. axis 1 and 4. Dim 4 contained NH<sub>4</sub><sup>+</sup> concentrations and the species of the AMBI V groups

Algal coverage added as an additional variable, which seemed to be the most associated with mud content (% of sedimentary particles < 63 μm) on Dim 1 (Figure 6. A.), but not to other variables not macrofaunal groups. On the second graph of the PCA (Figure 6. B.), the algal

coverage variable contributed strongly to the variation and was positively associated both with the OM fraction and mud content (Dim. 4) but was clearly opposed to  $\text{NH}_4^+$  concentration. Oyster spat mortality was also included as an additional variable. This variable seemed to behave like algal coverage on Dim 1 and was opposed to water content and chl *a* concentration within the surface microphytobenthic biofilm (Figure 6. A). The oyster spat mortality (Figure 6. B) was associated with both  $\text{NH}_4^+$  concentration and with the AMBI groups II and V species (Dim. 4) and opposed to organic matter content (OM).

#### *Oyster mortality episode*

After the structure of the macrozoobenthic community was investigated in detail, the link with the context of oyster activity was examined to relocate the oyster spat mortality crisis (OsHV-1  $\mu$ Var) in time. The first oyster spat mortalities were recorded on day 157 (Figure 7). Mortalities continued for 20 days until they stabilized at around 40% of survivors on day 177. No obvious correlation was observed between stranding of dead seaweed and oyster spat mortality. However, the dead seaweed stranding happened in the second period (from day 172).

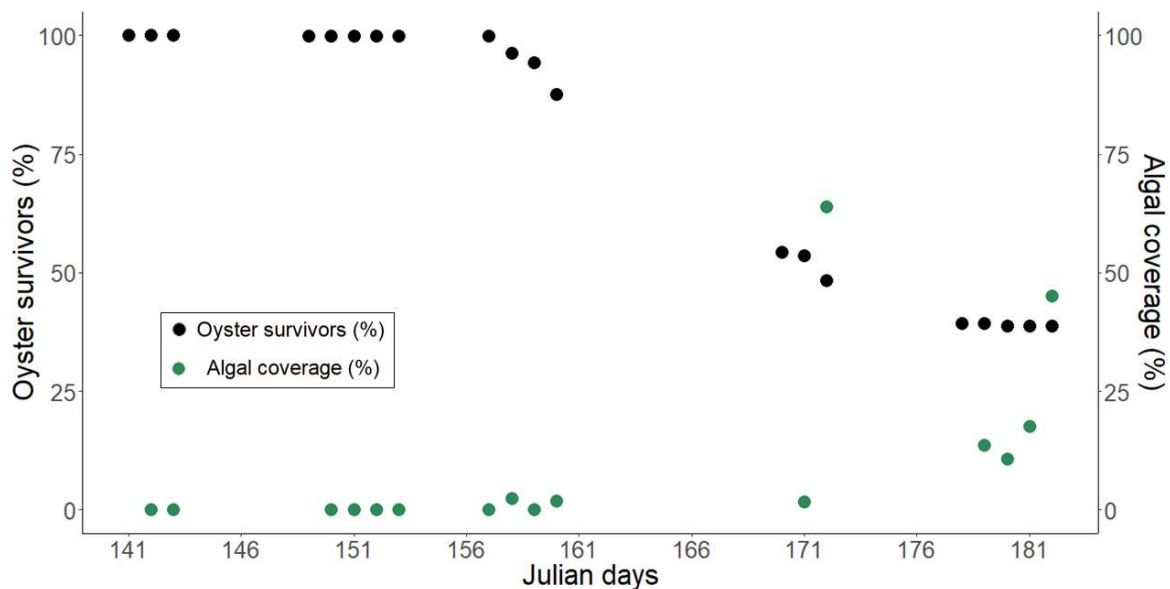


Figure 7: Percentage of survival and algal coverage during the oyster spat mortality in summer 2017

#### *OsHV-1 DNA detection and quantification*

The biggest quantities of viral DNA were found in the tissues of crustaceans (amphipods *Gammarus obtusatus*, and epibenthic barnacles *Balanus sp.* and the epibenthic crab *C. maenas*) regardless of the day. A trend was observed after oyster spat mortality: the heaviest

OsHV-1 DNA copy numbers were found in these organisms after the mortality period, despite the fact that some standard deviations overlapped. A significant increasing gradient of viral detection was observed over time (p-value < 0.001). Significant differences were observed between species on day 159 (p-value < 0.001) and day 179 (p-value < 0.01). On day 159, *Littorina littorea* showed the highest viral contamination (768.54 UG/ng DNA) but with only one individual. In second place came the species *S. armiger* (209.44 ± 319.77 UG/ng DNA). Conversely, no viral DNA was detected in the mussel *Mytilus edulis* on day 159. On day 179, *Balanus sp* had the highest viral load with (563.74 ± 597.64 UG/ng DNA) followed by the common cockle *Cerastoderma edule* (393.10 ± 399.93 UG/ng DNA). The lowest viral load was associated with the crab *C. maenas* as no OsHV-1  $\mu$ Var was detected in this organism on that day.

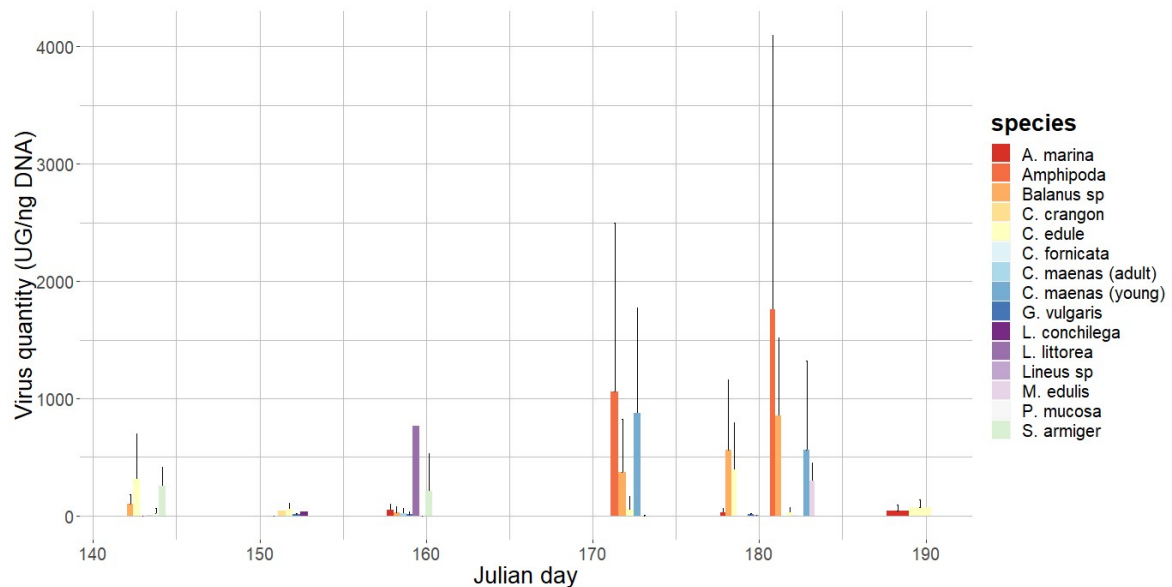


Figure 8: OsHv1  $\mu$ Var viral loads in macrozoobenthic fauna during the oyster spat mortality episode of summer 2017

#### 4. Discussion

The observed community can be considered as a single one but with clear differences and local specificities between the investigated table and aisle habitats, in terms of species hierarchy and density / biomass. In this study, the most important taxonomic group of soft-sediment macrofaunal community was polychaeta, as usually observed in this estuarine oyster parks (Sylvand, 1995; Ropert and Dauvin, 2000a). This marine worm taxa is considered to be one of the one of the most important in coastal and marine environments, in terms of diversity, abundance and functional role (Fauchald and Jumars, 1979). Their preference for fine or very fine sediment is a common feature in many estuaries (Ysebaert and Herman, 2002). The absence of species such as *Cerastoderma edule* illustrates the

trophic competition between filtering organisms induced by the massive presence of *Crassostrea gigas*. Indeed *C. edule* has gradually disappeared from this site but was still very abundant and dominant in the rest of the bay in 2012 (Ubertini et al., 2012). Regarding the history of this ecosystem deeply impacted by oyster production, *Lanice conchilega*, took advantage of the impact of oyster biodeposition and underwent very strong proliferation in the 1970s and until the beginning of the 2000s (Ropert and Dauvin, 2000) before almost disappearing in 2003, likely before the massive mortality caused by a heat wave that occurred that summer.

During the present study, amphipods remained scarce and were mainly found at the end of the survey in the form of the genus *Gammarus*. These organisms are mainly detritivorous (Guerra-García et al., 2014) and were found feeding on dead oyster spat flesh. Carnivorous species can be very sensitive to OM inputs (Borja et al., 2000) but they were poorly represented in both habitats, in contrast to the results obtained in another study in the same oyster park more than one decade ago (Dubois et al., 2007). Four species dominated the populations under tables: *Golfingia vulgaris*, *Scoloplos armiger*, *Tubificoides benedii* and *Capitella capitata*.

The species *Golfingia vulgaris* (at the 1<sup>st</sup> top rank) is a Sipunculid deposit-feeder that can be favored by a small increase in organic matter content, which may explain its widespread presence in oyster parks. This species was one of the most abundant species in the present study, and its presence is characteristic of coasts with a soft bottom. *Golfingia vulgaris* was recently observed on the western coast of Cotentin Peninsula (Normandy) in another oyster park (Pezy et al., 2019). When we compared the results of the different studies carried out on the macrozoobenthos in the Bay of Veys (Dubois et al., 2007; Sylvand, 1995), it was surprising to note the absence of *G. vulgaris* in previously published reports. This species may have appeared in this ecosystem in the last decade. This Sipunculid species is also present in Rade de Brest (Afli and Glemarec, 2000) and the appearance of the species of the genus *Golfingia* on the British coasts of the English Channel was already reported by Gibbs (1973).

The species *Capitella capitata* (at the 4<sup>th</sup> top rank) is also known to proliferate in organically polluted sediments and can be observed during early recolonization of azoic areas (Tsutsumi, 1987) due to its tolerance of anaerobic conditions and its high growth potential (R-strategy). Like *C. capitata*, the Oligochaete *Tubificoides benedii* can proliferate in anoxic conditions (Giere et al., 1999). These worms can live in dense populations in these stressed habitats,

which are often characterized by high levels of hydrogen sulfide (Dubilier et al., 1995). These authors also highlighted the fact that *T. benedii* is one of the most successful opportunistic and pioneering inhabitants of ecologically sulfidic benthic environments (Dubilier et al., 1997, 1994; Giere et al., 1988). Thus the massive proliferation of this species on the last date of the study must reflect the occurrence of an episode of pollution that caused environmental conditions favoring the emergence of these two species, hence penalizing species sensitive to pollution. Indeed, after the disappearance of species that cannot tolerate eutrophic environmental conditions, a recolonization phase by group V pioneer species was observed. These group V species are the best adapted to survive in such a degraded ecosystem. In addition, because of their reproductive R strategy, these species have shorter generation times and adaptation to eutrophication.

Due to its wide range of tolerance, its high reproductive coefficient and its tolerance to salinity stress, the species *Scoloplos armiger* (at the 2<sup>nd</sup> top rank) is one of the most common species in the eastern North Atlantic (Kruse et al., 2004). This species is also known to have hypoxic adaptation capacities (Schöttler and Grieshaber, 1988) and potential tolerance to the presence of reduced organic matter when it is exposed to diminished oxygen supplies during low tide under detritical seaweed mats (Schöttler, 1980).

These species proliferated particularly at the end of the survey at the beginning of July, during a massive local stranding of dead drifting seaweed. In terms of organic eutrophication dynamics, the oyster spat mortality crisis due to the occurrence of the pathogen agent OsHV-1  $\mu$ Var occurred first at the beginning of June, while the benthic indicators started to shift to a moderately bad ecological status. In second stage, the massive stranding of dead seaweed occurred on day 172 (*i.e.* the 21<sup>st</sup> of June) was followed by a new shift of the macrozoobenthic community with the emergence and hyper-dominance of *Tubificoides*, indicating a bad ecological status at the end of the survey (end of June and beginning of July). These two events (spat mortality and dead seaweed stranding) represented an input of OM to the benthic habitat. This OM occurred in two steps and disturbed the macrozoobenthic assemblages.

It appeared that the table habitat hosted more species and better diversity equilibrium (Shannon index) than the aisle. However, these ecological indicators tended to decline during the last part of the survey because the values of richness and Shannon indicators in the aisle sediments progressively reached those in the table sediments during the second period of the survey. In both cases, this could indicate a tendency to a uniformity of the

environmental conditions in the two habitats due to a global increase in organic matter content (eutrophication) and a shift from a good to a bad ecological status.

The Pielou index was mostly higher in the aisle than in the sediments under the oyster table. This suggests that the table habitat is dominated by a few opportunistic species whereas in the aisle, there is better equilibrium in the distribution of species. This dominance of a few species (i.e. *C. capitata*, *T. benedii*, *S. armiger*) points to potential eutrophication of the environment due to inputs of OM under the oyster tables at the end of the survey (in July).

The BOPA index (Benthic Opportunistic Polychaete/Amphipod) is a common indicator that makes it possible to determine the ecological status of an environment (Dauvin and Ruellet, 2007). The absence of Amphipods is a good pollution bioindicator because they are very sensitive to eutrophication (Dauvin and Ruellet, 2007; Gesteira and Dauvin, 2000). A large quantity of OM leads to a reduction in the abundance of amphipods, while, on the contrary some polychaetes are particularly resistant to excessive intake of OM and associated reduced compounds (e.g. sulfides, methane) typical of fine sediments (e.g. *C. capitata*, *T. benedii*). Overall, the BOPA index reflected a high ecological status under oyster tables as well as in aisles. In terms of changes in the quality of the environment, the classification of the environment according to the AMBI index shows that the two habitats at the beginning of the sampling were globally good and they were qualified as moderate and almost poor at the end of this survey. These changes were better detected by the AMBI than the BOPA. Overall, according to the AMBI index the environment was in a better health in the aisle than under the oyster table. This change in the ecological status is hypothesized to be the result of the variations of environmental parameters and hence, the species assemblages. Indeed, an important increase in group V species was observed while the abundance of group I species (sensitive to pollution) tended to decrease increasing the AMBI index in both habitats at the end of the survey (in July). The BENTIX index tended to decrease over the survey period. Indeed, the environment ecological status went from good to moderate. Overall, the ecological status was better in the aisle than under the oyster table, but on the last two sampling days, no difference was observed between habitats with minimum values of the survey for both, with a uniformization of the impact of the organic pollution

Calculated benthic indexes show that group I species (sensitive to hypertrophication), were present at the beginning of the survey but decreased over time (particularly after the mortality episode). Under normal conditions, these species are usually dominant in any environment and first disappear during OM enrichment of the environment (Hily, 1984). Conversely,

group V species (deposit-feeders) increased over time due the OM input. Moreover, when we focused on the response of the two habitats to a variation in environmental conditions, the oyster habitat seemed to be more affected by organic stress but more stable over time. Indeed, due to oyster biodeposition, this habitat would already be somewhat polluted with OM hosting species adapted to these conditions (AMBI group V species). On the other hand, the aisle habitat would be more sensitive to these environmental variations finally resulting in a modification of the macrozoobenthic assemblages in favor of species that are more tolerant to OM. Temporal trends appeared to matter more than small-scale effects during this summer survey.

The Analysis of Correspondence was mainly structured by the most abundant species including *Golfingia vulgaris*, *Tubificoides benedii* and *Capitella capitata*. *G. vulgaris* was mostly observed on the early dates of the survey, with correlation to water content of the sediment and biofilm chl *a*, whereas *T. benedii* and *C. capitata* species were mainly observed on late dates, with correlation with  $\text{NH}_4^+$ . This major change in species dominance could be an evidence for an environmental shift following a disturbance of environment parameters. Indeed, as can be seen on the PCA, a succession of AMBI groups from group I (e.g. *G. vulgaris*) to group V (e.g. *T. benedii* and *C. capitata*) was observed. This succession of species occurred in three phases of successive dominance, from the initial phase with good ecological status and dominance of *Golfingia* and/or *Scoloplos armiger*) followed by degradation of the benthic habitat in June as a result of the progressive enrichment in organic matter likely due to the oyster spat mortality (from day 156 to 177) and massive dead seaweed stranding on day 172, before the final shift of the benthic habitat to almost poor quality (dominance of AMBI group V). The environment considered in this study was strongly enriched in organic matter, as shown by the specific composition of benthic communities, dominated by species as *Tubificoides benedii* or *Capitella capitata*. The overall trend is to the degradation of the habitat quality, as shown by the three indices used, with a clear shift around day 172 for AMBI and BENTIX. Indices tested are all based on the tolerance of species on organic matter enrichment.

AMBI group V species are deposit feeders, which proliferate in reduced sediments including first-order opportunistic species. The presence of these opportunistic species linked to enhanced microphytobenthic production can lead to the rapid removal of pollutants in the sediment and improve remineralization processes (Rossi, 2006). In the present study, this group mainly comprised *Tubificoides benedii* and *Capitella capitata*. These group V species

were associated with  $\text{NH}_4^+$  concentrations and opposed to OM content (Dim. 4). The increase in the abundance of *C. capitata* and in the concentration of  $\text{NH}_4^+$  are two indicators of a degradation of the environment. Indeed, we hypothesize that the increase in OM enhanced  $\text{NH}_4^+$  concentrations that would have facilitated the proliferation of opportunistic organisms such as *T. benedii* and *C. capitata* species. Indeed, it is known that the accumulation of drifting seaweed can reduce richness and abundance of macrofauna but promote a few small opportunistic species (Thomsen and McGlathery, 2006). The link with algal mat coverage was not straightforward, but we observed a succession of phases: first, patches of dead seaweed were retained by the oyster table at our study site, and these seaweeds were deposited on, or buried in the sediment, with the accumulation of reduced compounds causing major stress for the benthic community, related to eutrophication. In the second step, decomposition and release of algal-derived nutrients influenced the distribution of benthic organisms, responsible for the uptake of ammonium  $\text{NH}_4^+$  in sediment porewater. A huge load (more than 30 cm) of seaweed-derived organic matter probably profoundly affected the assimilation and flux pathways. The recycling pathways and the bacterial remineralization rate can be fast after such an event, and the resulting degraded material can be then used by microphytobenthic biofilms. However, on the PCA (Principal Correspondence Analysis), the episode of oyster spat mortality was correlated with AMBI group V species and with  $\text{NH}_4^+$  concentrations. So, in parallel with the seaweed-related OM input, dead oyster flesh appeared to contribute to the concentrations of  $\text{NH}_4^+$  as well as AMBI group V species biomass.

As revealed by the PCA, OM and fine grain-size sediments were not directly correlated together indicating that the origin of the OM was not sediment dynamics but another source (probably first by the oyster mortality episode, and then by the dead seaweed). As it can be seen on the PCA (Figure 6. A.), the additional variable dead seaweed coverage could explain this input of OM (Ansell et al., 1998; Corzo et al., 2009; Garcia-Robledo and Corzo, 2011). Indeed, in the field, a massive stranding of dead seaweed was clearly visible during the second sampling period. The oyster parks consist of alternating rows of tables and aisles that can succeed for hectares. These tables consist of trestles whose feet crisscross the foreshore and extend over large areas. These structures therefore represent an obstacle to water flow and can retain drifting seaweed carried by the tide. So instead of being stranded at the top of the foreshore on the sea leash, these macroalgae are trapped in the oyster parks where they accumulate. This massive addition of organic matter is mineralized by the microbial loop in



$\text{NH}_4^+$  and also increases the anoxia of the sediment thereby altering community structure (Lyons et al., 2014) and reducing populations of AMBI I, II and III group organisms in favor of group V organisms. The OM observed in this study was thus mainly related to the stranding of dead seaweed, then in background to the production of biodeposits by oysters. The mortality episode may have played a preliminary role in the OM flux to the benthic compartment but it appeared to be negligible compared to the later input of OM from the dead seaweed.

First, it should be noted that the detection of OsHV-1 DNA does not imply the presence of infective viruses (enveloped virus capsids), but could be due to the persistence of naked (inactivated) viral DNA fragments (Schikorski et al., 2011). Moreover, OsHV-1 is known to infect a wide range of host species belonging to the class of bivalve mollusks (Arzul et al., 2017) but it is highly unlikely that it will be able to infect organisms from other classes. The detection of OsHV-1 DNA in Cockles, annelid, Sipunculid worms and Crustaceans thus indicates that these organisms can be considered as healthy passive carriers due to the passive adsorption of virions, or OsHV-1 DNA fragments, or diet-related mechanisms.

The detection of OsHV-1 DNA increased significantly over the course of this study starting on day 159 and peaked on day 179, then dropped in the last days of sampling (early July). Differences in the quantity of OsHV-1 DNA were found depending on species on days 159 and 179. On day 172, viral loads were also high, corresponding to a significant increase in the AMBI index (environmental degradation). Thus, the oyster spat mortality episode probably induced a first alteration of the environment, but which was less significant than the stranding of dead seaweed, which clearly provoked a strong ecological disturbance (AMBI group V dominance).

Regarding the macrofauna species found in the core samples, some species were missing in the OsHV-1 analysis including the very small worms *Tubificoides benedii* and *Capitella capitata*. This bias was the consequence of the method of sampling used, which was of the species most visible to the naked eye in the field, which were not necessarily the most abundant species observed after sorting the specimens in the bulk sediment in the cores. The high OsHV-1 DNA detections found for these species appeared to be related to their diet. Amphipods, whelks and crabs are indeed scavenger species, and *Carcinus maenas* and amphipods (especially those belonging to the *Gammarus* genus) were found in oyster bags feeding on the flesh of dead oysters, in direct contact with viral particles. Filter feeder species (e.g. barnacles) would have also been in contact with OsHV-1  $\mu\text{Var}$  but indirectly by the

infected water. The OsHV-1  $\mu$ Var viruses derived from the flesh of dead oysters would have spread into the environment and adsorbed onto the surrounding particles in suspension in the water column. This OsHV-1  $\mu$ Var-enriched seston would then have been filtered by barnacles thus becoming positive to OsHV-1 detection. Barnacles were the suspension feeders with the highest quantity of OsHV-1  $\mu$ Var for two main reasons. First, these species are found on hard substrates such as oyster tables and are thus in the close vicinity of the reared oysters. Second, due to trophic competition with *Crassostrea gigas*, other filter organisms such as *Cerastoderma edule* are strongly excluded. The cockle *Cerastoderma edule* showed high levels of viral DNA. The high prevalence of pathogens in such bivalves may contribute to its low density in oyster parks. Indeed, our taxonomy results showed suspension feeders to be very uncommon at this location. Among the scavenger species that were not in direct contact with the oysters, *Scoloplos armiger* can be seen to carry OsHV-1  $\mu$ Var but in small amounts. This species could be a transmitter of the virus to the benthic habitat because it is a ground deposit feeder (Wolff, 1973).

## 5. Conclusion

Overall, benthic macrofaunal assemblages were more diverse and abundant under the oyster table than in the aisle. However, these assemblages changed in response to a massive input of organic matter that caused degradation of the benthic habitat. First This alteration of environmental conditions may be due to the intake of the degraded flesh from dead spats in June. Second, the massive stranding of drifting dead seaweed also stressed the environment at the end of June. The table habitat appeared to be in a bad ecological state throughout the 2-month survey (May and June), whereas in the aisle, eutrophication appeared to be due to the massive stranding of dead seaweed, but only at the end of the survey (in early July). This micro-ecosystem appeared as destabilized as the one under the oyster tables at the end of the survey. Under the oyster culture tables, massive stranding reinforced the disturbances but the responses of the benthic assemblage were less pronounced over time. Indeed, under the oyster table, benthic macrofauna were already characteristic of a softly eutrophic environment in May and June. In this way, the benthic environment under the oyster tables appeared to be more rapidly subjected to eutrophication mainly because it is already richer in organic matter than the aisle habitat. Benthic macrofauna may have been in contact with OsHV-1  $\mu$ Var for a few days following the oyster mortality episode, but it seems unlikely that macrofauna would be a reservoir that enabled the persistence of the virus in the environment. The local accumulation of dead seaweed trapped by the metal structures

of oyster parks may also affect local water and sediment quality, thereby reinforcing eutrophication and increasing the risk of oyster mortality.

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### References

- Afli, A., Glemarec, M., 2000. Fluctuation à long terme des peuplements macrobenthiques dans le golfe du Morbihan (Bretagne, France). *Cah. Biol. Mar.* 41, 67–90.
- Ansell, A., Gibson, R., Barnes, M., Press, U., 1998. Ecological impact of green macroalgal blooms. *Oceanogr. Mar. Biol. Annu. Rev.* 36, 97–125.
- Arzul, I., Corbeil, S., Morga, B., Renault, T., 2017. Viruses infecting marine molluscs. *J. Invertebr. Pathol.* 147, 118–135.
- Azam, F., Smith, D.C., Steward, G.F., Hagström, A., 1994. Bacteria-organic matter coupling and its significance for oceanic carbon cycling. *Microb. Ecol.* 28, 167–179.
- Baines, S.B., Pace, M.L., 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnol. Oceanogr.* 36, 1078–1090.
- Bayne, B.L., Ahrens, M., Allen, S.K., D'auriac, M.A., Backeljau, T., Beninger, P., Bohn, R., Boudry, P., Davis, J., Green, T., Guo, X., Hedgecock, D., Ibarra, A., Kingsley-Smith, P., Krause, M., Langdon, C., Lapègue, S., Li, C., Manahan, D., Mann, R., Perez-Paralle, L., Powell, E.N., Rawson, P.D., Speiser, D., Sanchez, J.-L., Shumway, S., Wang, H., 2017. The Proposed Dropping of the Genus *Crassostrea* for All Pacific Cupped Oysters and Its Replacement by a New Genus *Magallana*: A Dissenting View. *J. Shellfish Res.* 36, 545–547. <https://doi.org/10.2983/035.036.0301>
- Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* 40, 1100–1114.
- Carstensen, J., Conley, D.J., Bonsdorff, E., Gustafsson, B.G., Hietanen, S., Janas, U., Jilbert, T., Maximov, A., Norkko, A., Norkko, J., Reed, D.C., Slomp, C.P., Timmermann, K., Voss, M., 2014. Hypoxia in the Baltic Sea: Biogeochemical Cycles, Benthic Fauna, and Management. *AMBIO* 43, 26–36. <https://doi.org/10.1007/s13280-013-0474-7>
- Corzo, A., Van Bergeijk, S.A., Garcia-Robledo, E., 2009. Effects of green macroalgal blooms on intertidal sediments: net metabolism and carbon and nitrogen contents. *Mar. Ecol. Prog. Ser.* 380, 81–93.
- Dauvin, J.-C., 2007. Paradox of estuarine quality: Benthic indicators and indices, consensus or debate for the future. *Mar. Pollut. Bull.*, Implementation of the Water Framework

- Directive in European marine waters 55, 271–281.  
<https://doi.org/10.1016/j.marpolbul.2006.08.017>
- Dauvin, J.-C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. *Mar. Pollut. Bull.* 55, 215–224.
- Dauvin, J.-C., Ruellet, T., Desroy, N., Janson, A.-L., 2007. The ecological quality status of the Bay of Seine and the Seine estuary: Use of biotic indices. *Mar. Pollut. Bull.* 55, 241–257. <https://doi.org/10.1016/j.marpolbul.2006.04.010>
- Dubilier, N., Giere, O., Grieshaber, M.K., 1995. Morphological and ecophysiological adaptations of the marine oligochaete *Tubificoides benedii* to sulfidic sediments. *Am. Zool.* 35, 163–173.
- Dubilier, N., Giere, O., Grieshaber, M.K., 1994. Concomitant effects of sulfide and hypoxia on the aerobic metabolism of the marine oligochaete *Tubificoides benedii*. *J. Exp. Zool.* 269, 287–297.
- Dubilier, N., Windoffer, R., Grieshaber, M.K., Giere, O., 1997. Ultrastructure and anaerobic metabolism of mitochondria in the marine oligochaete *Tubificoides benedii*: effects of hypoxia and sulfide. *Mar. Biol.* 127, 637–645.
- Dubois, S., Marin-Léal, J.C., Ropert, M., Lefebvre, S., 2007. Effects of oyster farming on macrofaunal assemblages associated with *Lanice conchilega* tubeworm populations: A trophic analysis using natural stable isotopes. *Aquaculture* 271, 336–349. <https://doi.org/10.1016/j.aquaculture.2007.03.023>
- Dutertre, M., Hamon, D., Chevalier, C., Ehrhold, A., 2013. The use of the relationships between environmental factors and benthic macrofaunal distribution in the establishment of a baseline for coastal management. *ICES J. Mar. Sci.* 70, 294–308. <https://doi.org/10.1093/icesjms/fss170>
- Echappé, C., Gernez, P., Méléder, V., Jesus, B., Cognie, B., Decottignies, P., Sabbe, K., Barillé, L., 2018. Satellite remote sensing reveals a positive impact of living oyster reefs on microalgal biofilm development. *Biogeosciences* 15, 905–918. <https://doi.org/10.5194/bg-15-905-2018>
- Fauchald, K., Jumars, P., 1979. The diet of worms : a study of polychaete feeding guilds 92.
- Fauvel, P., 1927. Faune de France 16, Polychètes-sédentaires [WWW Document]. URL [http://www.faunedefrance.org/bibliotheque/docs/P.FAUVEL\(FdeFr16\)Polychetes-sedentaires.pdf](http://www.faunedefrance.org/bibliotheque/docs/P.FAUVEL(FdeFr16)Polychetes-sedentaires.pdf) (accessed 1.15.18).
- Fauvel, P., 1923. Faune de France 5, Polychètes errantes [WWW Document]. URL <http://sci-hub.bz/http://www.sciencedirect.com/science/article/pii/S0044848607002797> (accessed 11.24.17).
- Forrest, B.M., Creese, R.G., 2006. Benthic Impacts of Intertidal Oyster Culture, with Consideration of Taxonomic Sufficiency. *Environ. Monit. Assess.* 112, 159–176. <https://doi.org/10.1007/s10661-006-0359-3>
- Forrest, B.M., Keeley, N.B., Hopkins, G.A., Webb, S.C., Clement, D.M., 2009. Bivalve aquaculture in estuaries: Review and synthesis of oyster cultivation effects. *Aquaculture* 298, 1–15. <https://doi.org/10.1016/j.aquaculture.2009.09.032>
- Frontier, S., 1985. Diversity and structure in aquatic ecosystems. *Ocean. Mar Biol* 23, 253–312.
- Frontier, S., Pichod-Viale, D., Leprêtre, A., Davoult, D., Luczak, C., 2008. Ecosystèmes. Structure, fonctionnement, évolution.
- García-Robledo, E., Corzo, A., 2011. Effects of macroalgal blooms on carbon and nitrogen biogeochemical cycling in photoautotrophic sediments: an experimental mesocosm. *Mar. Pollut. Bull.* 62, 1550–1556.

- Gesteira, J.L.G., Dauvin, J.-C., 2000. Amphipods are Good Bioindicators of the Impact of Oil Spills on Soft-Bottom Macrobenthic Communities. *Mar. Pollut. Bull.* 40, 1017–1027. [https://doi.org/10.1016/S0025-326X\(00\)00046-1](https://doi.org/10.1016/S0025-326X(00)00046-1)
- Gibbs, P.E., 1973. On the genus *Golfingia* (Sipuncula) in the Plymouth area with a description of a new species. *J. Mar. Biol. Assoc. U. K.* 53, 73–86. <https://doi.org/10.1017/S0025315400056642>
- Giere, O., Preusse, J.-H., Dubilier, N., 1999. *Tubificoides benedii* (Tubificidae, Oligochaeta)—a pioneer in hypoxic and sulfidic environments. An overview of adaptive pathways, in: *Aquatic Oligochaetes*. Springer, pp. 235–241.
- Giere, O., Rhode, B., Dubilier, N., 1988. Structural peculiarities of the body wall of *Tubificoides benedii* (Oligochaeta) and possible relations to its life in sulphidic sediments. *Zoomorphology* 108, 29–39.
- Guerra-García, J.M., Tierno de Figueroa, J.M., Navarro-Barranco, C., Ros, M., Sánchez-Moyano, J.E., Moreira, J., 2014. Dietary analysis of the marine Amphipoda (Crustacea: Peracarida) from the Iberian Peninsula. *J. Sea Res.* 85, 508–517. <https://doi.org/10.1016/j.seares.2013.08.006>
- Haven, D.S., Morales-Alamo, R., 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnol. Oceanogr.* 11, 487–498.
- Hayward, P.J., Ryland, J.S., 1995. *Handbook of the Marine Fauna of North-West Europe*. OUP Oxford.
- Hily, C., 1984. Variabilité de la macrofaune benthique dans les milieux hyper-trophiques de la rade de Brest.
- Hily, C., 1976. *Ecologie benthique des pertuis charentais*.
- Hwang, J.Y., Park, J.J., Yu, H.J., Hur, Y.B., Arzul, I., Couraleau, Y., Park, M.A., 2013. Ostreid herpesvirus 1 infection in farmed Pacific oyster larvae *Crassostrea gigas* (Thunberg) in Korea. *J Fish Dis* 36, 969–972.
- Jenkins, C., Hick, P., Gabor, M., Spiers, Z., Fell, S.A., Gu, X., Read, A., Go, J., Dove, M., Connor, W.O., 2013. Identification and characterisation of an ostreid herpesvirus-1 microvariant (OsHV-1  $\mu$ -var) in *Crassostrea gigas* (Pacific oysters) in Australia. *Dis. Aquat. Organ.* 105, 109–126.
- Jouenne, F., Lefebvre, S., Véron, B., Lagadeuc, Y., 2007. Phytoplankton community structure and primary production in small intertidal estuarine-bay ecosystem (eastern English Channel, France). *Mar. Biol.* 151, 805–825.
- Keeley, N.B., Forrest, B.M., Macleod, C.K., 2013. Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management. *Mar. Pollut. Bull.* 66, 105–116.
- Kervella, Y., Germain, G., Gaurier, B., Facq, J.-V., Cayocca, F., Lesueur, P., 2010. Experimental study of the near-field impact of an oyster table on the flow. *Eur. J. Mech. - BFluids* 29, 32–42. <https://doi.org/10.1016/j.euromechflu.2009.09.002>
- Kopp, J., Joly, J.-P., Moriceau, J., Legagneur, E., Jacqueline, F., 1991. *La Conchyliculture en Baie des Veys*.
- Kruse, I., Strasser, M., Thiermann, F., 2004. The role of ecological divergence in speciation between intertidal and subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae). *J. Sea Res.* 51, 53–62.
- Lincoln, R.J., British Museum, 1979. *British marine amphipoda, gammaridea*. London : British Museum (Natural History).
- Lynch, S.A., Carlsson, J., Reilly, A.O., Cotter, E., Culloty, S.C., 2012. A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the Pacific oyster, *Crassostrea gigas*, in Ireland. *Parasitology* 139, 1526–1532.

- Lyons, D.A., Arvanitidis, C., Blight, A.J., Chatzinikolaou, E., Guy-Haim, T., Kotta, J., Orav-Kotta, H., Queirós, A.M., Rilov, G., Somerfield, P.J., 2014. Macroalgal blooms alter community structure and primary productivity in marine ecosystems. *Glob. Change Biol.* 20, 2712–2724.
- Matisson, J., Lindén, O., 1983. Benthic macrofauna succession under mussels, *Mytilus edulis* L. (Bivalvia), cultured on hanging long-lines. *Sarsia* 68, 97–102. <https://doi.org/10.1080/00364827.1983.10420561>
- Mitchell, I.M., 2006. In situ biodeposition rates of Pacific oysters (*Crassostrea gigas*) on a marine farm in Southern Tasmania (Australia). *Aquaculture* 257, 194–203. <https://doi.org/10.1016/j.aquaculture.2005.02.061>
- Nagata, T., 2008. Organic matter-bacteria interactions in seawater. *Microb. Ecol. Oceans* 2, 207–241.
- Nugues, M.M., Kaiser, Spencer, Edwards, 1996. Benthic community changes associated with intertidal oyster cultivation.
- Orvain, F., Lefebvre, S., Montepini, J., Sébire, M., Gangnery, A., Sylvand, B., 2012. Spatial and temporal interaction between sediment and microphytobenthos in a temperate estuarine macro-intertidal bay. *Mar. Ecol. Prog. Ser.* 458, 53–68. <https://doi.org/10.3354/meps09698>
- Padilla, D.K., 2010. Context-dependent Impacts of a Non-native Ecosystem Engineer, the Pacific Oyster *Crassostrea gigas*. *Integr. Comp. Biol.* 50, 213–225. <https://doi.org/10.1093/icb/icq080>
- Pernet, F., Barret, J., Le Gall, P., Corporeau, C., Dégremont, L., Lagarde, F., Pépin, J., Keck, N., 2012. Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France. *Aquac. Environ. Interact.* 2, 215–237. <https://doi.org/10.3354/aei00041>
- Petton, B., Bruto, M., James, A., Labreuche, Y., Alunno-Bruscia, M., Le Roux, F., 2015. *Crassostrea gigas* mortality in France: the usual suspect, a herpes virus, may not be the killer in this polymicrobial opportunistic disease. *Front. Microbiol.* 6. <https://doi.org/10.3389/fmicb.2015.00686>
- Pezy, J.-P., Delecun, C., Baffreau, A., Basuyaux, O., Dauvin, J.-C., 2019. Anthropogenic impact of oyster farming on macrofauna biodiversity in an eelgrass (*Zostera marina*) ecosystem of the English Channel. *Ecol. Indic.* 106, 105480. <https://doi.org/10.1016/j.ecolind.2019.105480>
- Ropert, M., Dauvin, J.-C., 2000a. Renewal and accumulation of a *Lanice conchilega* (Pallas) population in the baie des Veys, western Bay of Seine. *Oceanol. Acta* 23, 529–546.
- Ropert, M., Dauvin, J.-C., 2000b. Renewal and accumulation of a *Lanice conchilega* (Pallas) population in the baie des Veys, western Bay of Seine. *Oceanol. Acta* 23, 529–546. [https://doi.org/10.1016/S0399-1784\(00\)00143-2](https://doi.org/10.1016/S0399-1784(00)00143-2)
- Rossi, F., 2006. Small-scale burial of macroalgal detritus in marine sediments: effects of *Ulva* spp. on the spatial distribution of macrofauna assemblages. *J. Exp. Mar. Biol. Ecol.* 332, 84–95.
- Schikorski, D., Faury, N., Pepin, J.F., Saulnier, D., Tourbiez, D., Renault, T., 2011. Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*: Kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. *Virus Res.* 155, 28–34. <https://doi.org/10.1016/j.virusres.2010.07.031>
- Schöttler, U., 1980. Der Energiestoffwechsel bei biotopbedingter Anaerobiose: Untersuchungen an Anneliden. *Verh Dtsch Zool Ges* 1980, 228–240.
- Schöttler, U., Grieshaber, M., 1988. Adaptation of the polychaete worm *Scoloplos armiger* to hypoxic conditions. *Mar. Biol.* 99, 215–222. <https://doi.org/10.1007/BF00391983>

- Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res.* 153, 92–99. <https://doi.org/10.1016/j.virusres.2010.07.011>
- Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. *Mediterr. Mar. Sci.* 3, 77–111.
- Sornin, Feuillet, Heral, Deslous-Paoli, 1983. Effet des biodépôts de l’huître *Crassostrea gigas* (Thunberg) sur l’accumulation de matières organiques dans les parcs du bassin de Marennes-Oléron.
- Sornin, J.-M., 1981. Processus sédimentaires et biodéposition liés à différents modes de conchyliculture: Baie de Cancale, Anse de l’Aiguillon et Bassin de Marennes-Oléron (PhD Thesis). université de Nantes.
- Sylvand, B., 1995. La baie des Veys (Littoral occidental de la baie de Seine, Manche), 1972-1993: structure et évolution à long terme d’un écosystème benthique intertidal de substrat meuble sous influence estuarienne (PhD Thesis). Caen.
- Thomsen, M.S., McGlathery, K., 2006. Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. *J. Exp. Mar. Biol. Ecol.* 328, 22–34.
- Tsutsumi, H., 1987. Population dynamics of *Capitella capitata* (Polychaeta; Capitellidae) in an organically polluted cove. *Mar. Ecol. Prog. Ser.* 36, 139–149. <https://doi.org/10.3354/meps036139>
- Ubertini, M., Lefebvre, S., Gangnery, A., Grangeré, K., Le Gendre, R., Orvain, F., 2012. Spatial Variability of Benthic-Pelagic Coupling in an Estuary Ecosystem: Consequences for Microphytobenthos Resuspension Phenomenon. *PLoS ONE* 7, e44155. <https://doi.org/10.1371/journal.pone.0044155>
- Van Duyl, F.C., Kop, A.J., Kok, A., Sandee, A.J.J., 1992. The impact of organic matter and macrozoobenthos on bacterial and oxygen variables in marine sediment boxcosms. *Neth. J. Sea Res.* 29, 343–355. [https://doi.org/10.1016/0077-7579\(92\)90074-O](https://doi.org/10.1016/0077-7579(92)90074-O)
- Vanhuyse, C., Normand, J., Lepoittevin, M., Rakotomalala, C., Mallet, C., Lelong, C., Pernet, F., Orvain, F., submitted. Drivers of the epipelagic microphytobenthic photobiology and growth in oyster farm. Submitted to *Estuaries and Coasts*.
- Whittaker, R.H., Likens, G.E., 1975. The biosphere and man, in: *Primary Productivity of the Biosphere*. Springer, pp. 305–328.
- Wolff, W.J., 1973. The estuary as a habitat : an analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse, and Scheldt. Communication nr. 106 of the Delta Institute for Hydrobiological Research 240.
- Ysebaert, T., Herman, P., 2002. Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Mar. Ecol. Prog. Ser.* 244, 105–124. <https://doi.org/10.3354/meps244105>

ANNEX 1 : Density results with averaged values and standard deviation (ind.m<sup>-2</sup>) under oyster tables (A) and in the adjacent aisle (B) for days 143 (23 May), 152 (2 June), 159 (9 June), 172 (22 June), 179 (29 June) and 182 (2 July).

A)

TABLE HABITAT Species	Day factor					
	143	152	159	172	179	182
Arenicola marina	17 +/- 29	33 +/- 29	0 +/- 0	0 +/- 0	83 +/- 29	17 +/- 29
Capitella capitata	33 +/- 29	50 +/- 50	117 +/- 161	50 +/- 87	<b>183 +/- 176</b>	217 +/- 208
Notomastus latericeus	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	33 +/- 58	0 +/- 0
Capitomastus sp	17 +/- 29	17 +/- 29	0 +/- 0	17 +/- 29	17 +/- 29	17 +/- 29
Capidellides sp	0 +/- 0	0 +/- 0	33 +/- 29	0 +/- 0	0 +/- 0	0 +/- 0
Lanice conchilega	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29	0 +/- 0
Scoloplos armiger	<b>233 +/- 252</b>	100 +/- 100	117 +/- 104	<b>200 +/- 304</b>	117 +/- 58	500 +/- 391
Cerastoderma edule	0 +/- 0	17 +/- 29	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Magelona papillicornis	0 +/- 0	17 +/- 29	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Glycera tridactyla	17 +/- 29	0 +/- 0	33 +/- 58	0 +/- 0	0 +/- 0	0 +/- 0
Phyllodoce mucosa	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	33 +/- 58
Cirriformia tentaculata	17 +/- 29	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Golfingia vulgaris	167 +/- 104	<b>317 +/- 252</b>	<b>383 +/- 76</b>	100 +/- 132	150 +/- 0	217 +/- 161
Tubificoides benedii	83 +/- 104	33 +/- 29	0 +/- 0	133 +/- 231	83 +/- 76	<b>833 +/- 878</b>
Carcinus maenas	17 +/- 29	0 +/- 0	33 +/- 58	50 +/- 50	17 +/- 29	0 +/- 0
Chaetogammarus stoerensis	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29	0 +/- 0	0 +/- 0
Gammarus obtusatus	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	33 +/- 58
Urothoe poseidonis	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29	0 +/- 0
Hyale nilssoni	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Total (ind/m <sup>2</sup> )	600 +/- 633	583 +/- 546	717 +/- 486	567 +/- 862	717 +/- 512	1867 +/- 1811

B)

AISLE HABITAT Species	Day factor					
	143	152	159	172	179	182
Arenicola marina	0 +/- 0	17 +/- 29	17 +/- 29	0 +/- 0	33 +/- 58	17 +/- 29
Capitella capitata	17 +/- 29	0 +/- 0	<b>67 +/- 76</b>	0 +/- 0	17 +/- 29	<b>183 +/- 275</b>
Notomastus latericeus	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Capitomastus sp	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29
Capidellides sp	0 +/- 0	0 +/- 0	17 +/- 29	0 +/- 0	0 +/- 0	0 +/- 0
Lanice conchilega	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Scoloplos armiger	0 +/- 0	17 +/- 29	0 +/- 0	17 +/- 29	17 +/- 29	150 +/- 180
Cerastoderma edule	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Magelona papillicornis	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Glycera tridactyla	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Phyllodoce mucosa	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	33 +/- 58
Cirriformia tentaculata	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Golfingia vulgaris	<b>133 +/- 189</b>	<b>133 +/- 104</b>	<b>67 +/- 29</b>	<b>117 +/- 126</b>	<b>50 +/- 87</b>	33 +/- 29
Tubificoides benedii	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	33 +/- 58	67 +/- 76
Carcinus maenas	33 +/- 58	33 +/- 29	0 +/- 0	17 +/- 29	33 +/- 29	0 +/- 0
Chaetogammarus stoerensis	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Gammarus obtusatus	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0
Urothoe poseidonis	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29	0 +/- 0	0 +/- 0
Hyale nilssoni	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	0 +/- 0	17 +/- 29
Total (ind/m <sup>2</sup> )	183 +/- 276	200 +/- 191	167 +/- 163	167 +/- 212	183 +/- 289	517 +/- 705